Diversity and Characterisation of a few *Curcuma* genetic resources

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By

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CERTIFICATE

This is to certify that the thesis entitled "**Diversity and Characterisation of a few *Curcuma* genetic resources**" submitted by **Ms. Sajitha P.K**., to University of Calicut for the award of degree of **Doctor of Philosophy** in **Biotechnology** is the result of research work carried out by her in the Division of Crop Improvement and Biotechnology, ICAR-Indian Institute of Spices Research, Kozhikode, Kerala, India under my supervision and guidance during the period, February 2012 to November 2017.

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Place: Kozhikode

Date :

DECLARATION

I hereby declare the thesis entitled "Diversity and Characterisation of a few *Curcuma* genetic resources." submitted for the award of the degree of Doctor of Philosophy in Biotechnology to University of Calicut contains the results of bonafide research work done by me at ICAR-Indian Institute of Spices Research, Kozhikode, Kerala under the guidance of Dr. B. Sasikumar, Head and Principal Scientist, Division of Crop Improvement and Biotechnology, ICAR-Indian Institute of Spices Research. This thesis has not been submitted for the award of any other degree or diploma of this or any other University.

(Sajitha P.K)

Place: Kozhikode

Date:

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Sajitha P.K

CONTENTS

List of Tables

List of Figures

ABBREVIATIONS

%	Per cent
ºC	Degree centigrade
µ	Micro
µg ml^{-1}	Microgram per millilitre
µM	Micromolar
µl	Microlitre
3’	Hydroxyl-terminus of DNA molecule
5’	Phosphate-terminus of DNA molecule
β	Beta
AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of Molecular Variance
ASTA	American Spice Trade Association
bp	Base pairs
Cl	Chloride
cm	centimetre
CTAB	Cetyl trimethyl ammonium bromide
CV	Coefficient of Variation
CuMiSat	*Curcuma* MicroSatellite
DAMD	Directed Amplification of Minisatellite-region DNA
DAP	Days After Planting
DNA	Deoxy ribonucleic acid
dNTP	Deoxy nucleotide triphosphate
EDTA	Ethylene diamine tetra acetic acid
g	Gram
g	Relative centrifugal force
GA	Genetic Advance
GAM	Genetic Advance as percentage of Mean
GC	Gas Chromatography

GC-MS	Gas Chromatography- Mass Spectrometry
GCV	Genotypic Coefficient of Variation
h^2	Heritability
HPLC	High Performance Liquid Chromatography
ISSR	Inter Simple Sequence Repeats
Kb	Kilo base pair
Kg/ha	Kilogram per hectare
LSD	Least Significant Difference
M	Molar
m	Metre
mg	Milligram
$MgCl_2$	Magnesium chloride
MI	Marker Index
min	minutes
ml	Millilitre
mm	Millimetre
mM	Millimolar
NaCl	Sodium chloride
NS	Not Significant
ng	Nanogram
nm	Nanometre
PAGE	Poly acrylamide gel electrophoresis
PIC	Polymorphism Information Content
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PCV	Phenotypic Coefficient of Variation
pmole	Picomole
pmol μl^{-1}	Picomole per microlitre
PVP	Polyvinylpyrrolidone

RAPD	Random Amplified Polymorphic DNA
RNase	Ribonuclease
rDNA	Ribosomal DNA
SAHN	Sequential Agglomerative Hierarchical Non-overlapping
SCAR	Sequence Characterised Amplified Region
SDS	Sodium dodecyl sulphate
SEM	Scanning Electron Microscopy
SM	Simple Matching
SNP	Single Nucleotide Polymorphism
SRAP	Sequence-Related Amplified Polymorphism
SSR	Simple Sequence Repeat
Taq	*Thermus aquaticus*
T_a	Annealing temperature
TBE	Tris Borate EDTA
TEMED	Tetramethyl-ethylene diamine
TLC	Thin Layer Chromatography
U	Units
UPGMA	Unweighted Pair Group Method
UV	Ultra violet
V	Voltage

Chapter 1

Introduction

Genetic diversity is the fundamental source of biodiversity and there are three layers for this genetic diversity; genetic variation among population, genetic variation among individuals within a population and genetic variation within individuals (Das, 2011).

Impact of environmental changes on plant populations mainly depend on factors such as genetic diversity of the population and gene flow. Differences observed for the morphological traits between populations growing under same environment are most likely due to the genetic differentiation of the plants. Genetic diversity influences productivity, stability and growth of plants (Hughes *et al*., 2008) and thus diversity studies are important in the development, conservation and utilization of plant resources (Shah *et al*., 2008; Ding *et al*., 2013).

Genetic diversity act as a template for the adaptation and sustainability of plant species and individuals, especially under constantly changing and evolving environmental conditions. Natural genetic variability has been exploited within various crop species to meet subsistence of food requirement from the very beginning of agriculture (Govindaraj *et al*., 2015). Low genetic diversity seriously affects the ability of plants to adapt to the new environment and thus results in deterioration of quality and yield (Ludwig *et al*., 2013). Thus assessment on inter and intra-specific variation provides a better understanding of systematics, which have diverse applications in conservation and utilization of genetic resources as well as in taxonomic studies (Kavitha *et al*., 2010). Assessment of the distribution and extend of genetic diversity in a species are imperative in understanding the evolutionary relationships, conservation and breeding techniques among species and their accessions.

Several factors influence the genetic diversity of plant population. Mutation, chromosomal variation, hybridization, polyploidy etc. could be the main events behind the genetic variation (Soltis *et al*., 2009; Robertson *et al*., 2010). Studies have also suggested that human interference and environmental pressures (industrial pollution, insecticides, global warming and agricultural pests) can also play a major role in influencing the genetic diversity (Zheng *et al*., 2015). Several plant traits like floral morphology, method of pollination, mechanism of pollen dispersal, phenological variation, population size and

breeding systems are reported to influence the genetic diversity in a plant population (Loveless and Hamrick, 1984).

Genetic variations are believed to be minimum in the clonally propagated plants, although an increasing number of literature on this matter reveal a different scenario, that considerable amount of genetic diversity exists in the population of clonal plants (Widen *et al*., 1994). In such cases environmental gradients (climatic changes, soil, temperature, precipitation, latitude and altitude) are believed to be influencing variation (Hulshof *et al*., 2013) among and within species (Huang *et al*., 2016).

Zingiberaceae, is one of the largest and important families of plant kingdom predominantly asexually reproduced and propagated through underground rhizomes. However, studies have reported considerable genetic variation in the family (Jatoi *et al*., 2007).

Zingiberaceous plants are widely distributed in tropical Asia and are usually known for their aromatic properties, in all or at least one of their plant parts. The family is a well-known natural sources of spices, natural dyes, herbal medicines, perfumes while some species are cultivated for their beautiful ornamental flowers (Sirirugsa, 1999). *Alpinia, Amomum, Curcuma, Zingiber, Boesenbergia, Kaempferia, Elettaria, Etlingera* and *Hedychium* are some of the most important genera under the family. Among these genera, three species *viz*., *Curcuma longa*, *Elettaria cardamomum* and *Zingiber officinale* are the commercially important species. Various authors have pointed out the discrepancies pertaining to the number of genera and species in the family, the main reason behind this ambiguity is that Zingiberaceae family is still under an active stage of evolution (Larsen *et al*., 1999). This confusion and controversies can be resolved only by comprehensive and integrated approach which is to be carried out systematically.

Genus *Curcuma* belonging to the family Zingiberaceae is believed to be originated in the Indo-Malayan region and has a widespread distribution in the tropics of Asia, Africa and Australia (Purseglove, 1968). At present the crop is distributed in India, Indonesia, Malaysia, Myanmar, Vietnam, Thailand, Philippines, Japan, China, Korea, Sri Lanka, Nepal, South Pacific Islands, East and West African nations, Malagasy, Caribbean islands, Central America and Pakistan (Sabu, 1991; Apavatjrut *et al*., 1999). Out of the one hundred species reported in the genus about forty are of Indian origin (Velayudhan *et al*., 1999; Sasikumar, 2005).

Original descriptions of many *Curcuma* species are vague and inaccurate and type specimens are often lacking or fragmentary (Skornickova *et al*., 2007). In addition, high intra and inter-specific variation have led to debate concerning species concepts and

boundaries, as a result, one species has often been mentioned under different names whereas the same name has been given to different taxonomic entities (Skornickova *et al*., 2007).

Curcuma species thrives well in diverse tropical and sub-tropical climatic conditions which ranges from sea level to a height of 2000 meters in the hilly slopes of the Western Ghats, Himalayas etc. (Sasikumar, 2005). They spread out well in loose, friable loamy or alluvial soil (Islam *et al*., 2005) and expanded themselves in a wide range of tropical and subtropical forests, broad-leaved evergreen forests, open grass lands, plantations etc. In India, the genus has been distributed in North Eastern India, South India and Andaman Nicobar Islands. The combination of wide geographical range, varying climatic and soil conditions and well spread population enables the genus to generate high genetic variability.

Curcuma are well known for its multifarious uses as spice, medicine, cosmetics, dyes, flavouring, starch and ornamentals, which coronate them as a very important genus in the family Zingiberaceae. The medicinal uses of different species of *Curcuma* are innumerable and very ancient. The genus is credited with molecules having anti-cancerous, anti-hepatotoxic, anti-diabetic, anti-viral, anti-venomous, cholerectic, anti-microbial, anti-fibrotic, anti-inflammatory and anti-rheumatic properties (Sasikumar, 2005).

Earlier studies based on morphological traits suggested considerable variation in the species of *Curcuma* collected from various geographical locations (Velayudhan *et al*., 1994; Velayudhan *et al*., 1999; Pinheiro *et al*., 2003; Sasikumar, 2005; Hussain *et al*., 2008). However, some studies also reported a different scenario wherein there was no significant correlation between their geographical and genetic distance (Zheng *et al*., 2015).

A number of methods are currently available and widely used for the analysis of genetic variation, such as morphological, biochemical and molecular (DNA based) data. Since the genetic composition are not affected or influenced by external environmental factors unlike morphological and biochemical parameters, molecular markers are frequently used for the analysis of genetic relationship, characterization, phylogenetic relationships, population diversity in different plants, cultivars and varieties (Mohanty *et al*., 2014).

Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR), Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeats (SSR) markers are used by various authors (Xiao *et al*., 2000; Sreeja, 2002; Nayak *et al*., 2006; Jatoi *et al*., 2006; Syamkumar and Sasikumar, 2007; Angel *et al*., 2008; Donipati and

Sreeramaulu, 2015) to analyze genetic diversity within and among species and populations of the *Curcuma* genus.

Though *C. longa* is the most commonly utilized species in the genus *Curcuma,* there are many other economically and medicinally important species under the genus, such as *C. aromatica, C. amada, C. caesia, C. zedoaria, C. xanthorrhiza, C. comosa, C. malabarica, C. angustifolia, C. montana, C. alismatifolia, C. decipiens* etc.

In the current study we have considered four economically important *Curcuma* species, *viz*., *C. amada* Roxb, *C. aromatica* Salisb, *C. caesia* Roxb and *C. xanthorrhiza* Roxb. *C. amada* rhizomes, which have characteristic smell of fresh green mango, used as a flavouring spice in Asian cooking, for the preparations of pickles, chutney etc. and in traditional and tribal medicines (Gupta *et al*., 1999; Srivastava *et al*., 2006; Policegoudra *et al*., 2007). In case of *C. amada* Roxb., pharmacological studies revealed presence of a wide spectrum of chemical constituents which can be exploited medicinally for various ailments (Hussain *et al*., 1992; Warrier *et al*., 1994; Policegoudra and Aradhya, 2008).

Curcuma caesia Roxb. is commonly known as black turmeric due to bluish-black rhizomes which emits a characteristic sweet smell. *C. caesia* is a rich source of essential oil, flavonoids, phenols, alkaloids, proteins. Presence of these bioactive secondary metabolites are responsible for its medicinal uses. Rhizomes are used in the treatment of hemorrhoids, epilepsy, leprosy, wound, asthma, menstrual disorder, cancer, fever, vomiting, muscle relaxant activity (Pandey and Chowdhury, 2003; Sasikumar, 2005; Sarangthem and Haokip, 2010; Karmakar *et al*., 2011).

C. aromatica Salisb., the wild turmeric (Anoop, 2015), is well known for its multifaceted properties and thus used in traditional systems of medicines (Ayurveda and Unani). The plant is extensively used as an aromatic medicinal cosmetic in India. In traditional system it is mentioned as a remedy for various diseases related to skin, cardiovascular and respiratory system. For the last few decades' extensive studies have been done to establish the pharmacological potential of *Curcuma aromatica* and its extracts (Al-reza *et al*., 2011; Sikha and Harini, 2015; Anoop, 2015). *Curcuma xanthorrhiza* Roxb. is a medicinal plant indigenous to and widely used in East Asia. It has a round tuber with a yellow outer skin and orange-yellow rhizome inner core colour. Plant is widely used in folk medicine to treat various disorders (Rukayadi *et al*., 2006; Devaraj *et al*., 2010; Mangunwardoyo and Usia, 2012; Lew *et al*, 2015).

Variation for morphological and biochemical characters have been reported in *Curcuma* Spp. collected from different regions (Pandey and Choudhary, 2003; Jatoi *et al*., 2007).

Wide variability for characters like curcumin, oleoresin, essential oil content and dry recovery are reported in the collections of *C. longa* and *C. aromatica* (Ratnambal,1986), likewise natural variation for morphological traits is observed in population of *C. amada* (Rao *et al*., 2008). *C. amada, C. aromatica, C. xanthorrhiza* and *C. caesia* showed variation for essential oil constituents according to the location from where it is collected (Kuroyanagi *et al*., 1987; Zwaving and Bos, 1992; Bandyopadhay, 1993; Kojima *et al*., 1998; Behura *et al*., 2002; Pandey and Choudhary, 2003; Jarikasem *et al*., 2005; Paliwal *et al*., 2011; Behar *et al*., 2014). Inter and intraspecific diversity in genus *Curcuma* (Jatoi *et al*., 2006), such as *C. longa* (Nayak *et al*., 2006; Syamkumar and Sasikumar, 2007), *C. zedoaria* (Islam *et al*., 2005), *C. caesia, C. amada, C. aromatica* (Das *et al*., 2011) *C. xanthorrhiza, C. angustifolia* (Apavatjrut *et al*.,1999; Paisooksantivatana *et al*., 2001a; Kavitha *et al*., 2010) have been carried out by several researchers. The present work is an attempt to study intra and inter-specific variations among four species of *Curcuma* such as *Curcuma amada*, *Curcuma aromatica*, *Curcuma caesia* and *Curcuma xanthorrhiza* using morphological, molecular and biochemical parameters with following objectives.

1. Collection and multiplication of different accessions of *C. caesia, C. amada, C. aromatica, C. xanthorrhiza* and their field evaluation for aerial features and underground rhizome characters.
2. Biochemical characterization of primary metabolites and secondary metabolites including GC-MS analysis of essential oils and separation and distribution pattern of total curcuminoids using HPLC.
3. Quantitative and qualitative analysis of starch from the four species of *Curcuma.*
4. Phenological variation in two species of *Curcuma viz., C. amada* and *C. aromatica.*
5. Molecular characterization of the four *Curcuma* species using RAPD, ISSR and SSR markers.
6. Inter and intra specific diversity in four *Curcuma* species using morphological, biochemical and molecular parameters.
7. Population diversity study in four *Curcuma* species.

Chapter 2

Review of Literature

2.1. General Introduction

The genus *Curcuma* belongs to the family Zingiberaceae has a widespread occurrence in the tropics of Asia and extends to Africa and Australia. The genus contains many economically important species of rhizomatous annual or perennial herbs besides the most important entity, *Curcuma longa* L. syn. *Curcuma domestica* Val., the common turmeric (Purseglove, 1974; Sirirugsa, 1999). Linnaeus coined the name '*Curcuma*' in his '*Species Plantarum'* in 1753. The earliest description of turmeric was found in '*Hortus Malabaricus'* (Rheede, 1678-1693) which described it under the local name 'Manjellakua', which was later established as a lectotype of *Curcuma* (Burt,1977). The word is believed to be derived from the Arabic word 'kurkum' which means 'yellow' (Purseglove *et al*., 1981; Sirirugsa, 1999; Sasikumar, 2005). The genus *Curcuma* is well known for its various uses as spice, medicine, dyes, cosmetics, food, starch, perfume, for flavouring as well as for decorative (ornamental) purpose (Wilson *et al*., 2005; Baghel *et al*., 2013). The genus has been divided into two subgenera, *Paracurcuma* and *Eucurcuma* by many taxonomists based on their different morphological traits such as presence/absence of anther spur (Valeton, 1918). *Eucurcuma* was further divided into three sections based on the presence or absence of tubers or stolons namely tuberosa, non tuberosa and stolonifera (Valeton, 1918, Velayudhan *et al*., 1999).

The genus *Curcuma* is placed in tribe Zingibereae within subfamily Zingiberoideae as suggested by Kress *et al*. (2002). Earlier it was included in the tribe Hedychieae (Purseglove, 1974). The previous classification of the genus by Roxburgh (1820 – 1824) was merely based on the morphological description. In his "*Flora Indica*", Roxburgh mentioned 65 species of Zingiberaceae under the class Monandria monogymia. In '*The Flora of British India'*, Baker, (1890-1892) described 27 species which he subdivided into three sections namely *exantha*, *mesantha* and *hitcheniopsis*. Turmeric and other economically important species such as *Curcuma angustifolia* Roxb. (Indian arrowroot), *Curcuma aromatica* Salisb, and *Curcuma zedoaria* (Christm.) Roscoe. are included in the section *exantha*.

Burtt and Smith (1972) proposed a new classification by modifying the classification suggested by Schumann (1904). They divided Zingiberaceae into two subfamilies *viz.*, Zingiberoideae and Custoideae. In Burtt and Smith's classification, Zingiberaceae is split into four tribes *viz.* Zingibereae, Hedychieae, Globbeae and Alpinieae. The genus *Curcuma*

is included in the tribe Hedychieae. The current classification of the Zingiberaceae, which is based on both vegetative and floral characteristics, comprises four tribes as suggested by Burtt and Smith (Larsen, 1998; Kress *et al*., 2002).

A lot of discrepancies have been reported for the number of genera and species in the family (Jatoi *et al*., 2007). According to Ghazanfar and Smith (1982) Zingiberaceae comprised of 45 genera and 1000 species, whereas Chen (1989) reported 52 genera and 1500 species and Kress (1990) reported 53 genera and over 1200 species. The reason for this ambiguity is attributed to the active stage of evolution of the family (Larsen *et al*., 1999).

In a recent phylogeny study on *Curcuma* using plastid regions (*trnL-trnF, psbA-trnH, matK*) and the internal transcribed spacer (ITS) of nuclear ribosomal DNA, Zaveska *et al*. (2012) proposed three subgenera *viz.*, subgen. *Curcuma*, subgen. *Ecomata* and subgen. *Hitcheniopsis.* The study also suggested inclusion of *Curcuma* like genera to the genus *Curcuma*. Later Skornickova *et al*. (2015) sunk *Curcuma* like genera in to genus *Curcuma*.

The current taxonomical hierarchy of genus *Curcuma* is as follows (Kress *et al*., 2002).

Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Zingiberidae
Order	Zingiberales
Family	Zingiberaceae
Subfamily	Zingiberoideae
Tribe	Zingibereae
Genus	*Curcuma*

2.1.1. Origin and distribution

India is considered as one of the centres of diversity for the genus as out of the hundred or so species reported in the genus, about 40 are of Indian origin (Velayudhan *et al*., 1999). Genus *Curcuma* contains economically and medicinally important species (Sasikumar, 2005; Jatoi *et al*., 2006). *C. longa* is the most popular species within the genus because of its yellow pigmentation and valued for its culinary importance in most of the Asian cuisines (Purseglove, 1974; Apavatjrut *et al*., 1999). Many studies have established its importance in the medicinal field too (Chandarna *et al*., 2005; Lobo *et al*., 2009; Beevers and Huang, 2011;

Samant, 2012; Tripathi *et al*., 2013; Samsudin and Panigaro, 2013; Jose and Thomas, 2014; George and Britto, 2016).

Species belonging to the genus *Curcuma* thrives well in diverse tropical and sub-tropical climatic conditions which range from sea level to a height of 2000 meters in the hilly slopes of the Western Ghats, Himalayas, etc. (Sasikumar, 2005).

The genus expanded themselves into a wide range of tropical forests, subtropical forests, broad-leaved evergreen forests, open grasslands and plantations. Geographically the genus is distributed from India to Thailand, Indo-China, Indo-Malayan, Indonesia and northern Australia. In India, *Curcuma* species are distributed in North-Eastern India, South India and Andaman Nicobar Islands (Sabu, 1991; Apavatjrut *et al*., 1999). Table 1 represents the *Curcuma* species occurring in India. The list is revised as new species are added recently, and some of the existing species are now treated as synonyms.

Table 1. *Curcuma* species occurring in India

Sl.No	Species	Sl No	Species
1	*Curcuma aromatica* Salisb.	23	*Curcuma longa* L.
2	*Curcuma amada* Roxb.	24	*Curcuma mangga* Val. And Van Zijp.
3	*Curcuma aeruginosa* Roxb.	25	*Curcuma montana* Roxb.
4	*Curcuma amarissima* Rosc.	26	*Curcuma mutabilis* Skornickova *et al*.
5	*Curcuma angustifoila* Dalz & Cibs.	27	*Curcuma neilgherrensis* Wight.
6	*Curcuma aurantiaca* Van Zijp.	28	*Curcuma oligantha* Trim.
7	*Curcuma bhatii* (R.M.s.m) Skornick & M. Sabu.	29	*Curcuma petiolata* Roxb.
8	*Curcuma caesia* Roxb.	30	*Curcuma picta* Roxb. *ex* Škornick.
9	*Curcuma caulina* J. Graham.	31	*Curcuma prakasha* S.Tripathi
10	*Curcuma codonantha* Skornickova *et al.*	32	*Curcuma pseudomontana* J.Graham.
11	*Curcuma comosa* Roxb.	33	*Curcuma raktakanta* Mangaly and Sabu
12	*Curcuma cordifolia Roxb.*	34	*Curcuma reclinata* Roxb.
13	*Curcuma coriaceae* Mangaly & Sabu	35	*Curcuma roscoeana* Wall.
14	*Curcuma decipiens* Dalz.	36	*Curcuma rubescens* Roxb.
15	*Curcuma ferrugenia* Roxb.	37	*Curcuma rubrobracteata* Skornickova *et al.*
16	*Curcuma haritha* Mangaly and Sabu	38	*Curcuma scaposa* (Nimmo) Skornick. & M. Sabu.
17	*Curcuma inodora* Blat. Syn. *Curcuma purpurea* Blatt	39	*Curcuma strobilifera* Wall.
18	*Curcuma karnatakensis* Amalraj, Velay. & Mural.	40	*Curcuma sulcata* Haines
19	*Curcuma kudagensis* Velay, Pillai & Amalraj.	41	*Curcuma vamana* Mangaly & Sabu
20	*Curcuma latifolia Rosc.*	42	*Curcuma xanthorrhiza* Roxb.
21	*Curcuma leucorrhiza* Roxb.	43	*Curcuma zedoaria* (Christm.) Roscoe.
22	*Curcuma mukhraniae* R. Kr. Singh & Arti Garg	-	-

Sources: Velayudhan *et al*. (1999); Sasikumar (2005); Škorničková *et al*. (2010); Singh and Garg, (2014).

2.1.2. Morphology of Genus *Curcuma*

Genus *Curcuma* shows a wide range of variability for various taxonomically important characters (Apavatjut *et al*., 1999) and considered as a taxonomically confusing genus, problematic for turmeric breeders, herbarium technicians and taxonomists (Mangaly and Sabu, 1993).

Genus is characterised with either erect or semi-erect plant types with plant height ranging from 50 to 200 cm. Leaf sheath colour varies from purple – brown, purple – green, light to dark purple. Leaves are characterised by the presence of hair on the dorsal or ventral side of the leaf. Leaves are hairy or glabrous in some species. Another peculiar character of the genus is their variation in leaf midrib colour. Colour varies from green, light purple green to light purple-brown (Velayudhan *et al*., 1999; Sasikumar, 2005). Leaves are mostly basally positioned with leaf blade lanceolate or oblong, or in some cases it is linear to narrow (Sabu, 2006). Another discriminating character of the genus is the presence/absence of terminal bracts which forms into a sterile cluster called a 'coma'. They are often brightly coloured e.g., white, yellow and purple flowers (Sabu, 2006).

Genus is blessed with a large compound spike inflorescence bearing spiral bracts, which vary in colours such as white, pale yellow, orange, red purple spot, blue, red and purple (Sasikumar, 2005). Spiral bracts are often fused to form parches containing 2 to 10 flowers that have a single versatile anther (Islam *et al*., 2005). Some species of the genus have anther spur whereas in some species it is absent. Likewise, fertile bract colour, size and shape also show variation among the species of the genus (Sasikumar, 2005).

The predominant mode of reproduction in the family is asexual and propagation occurs mainly through underground rhizomes. However, viable seed set is also reported in some species like *C. longa* and *C. aromatica* (George, 1981; Sasikumar *et al*.,1996; Jatoi *et al*., 2007). Rootstocks (rhizome) of the genus are branched, fleshy, either oblong or cylindrical in shape. Colour of the rhizomes varies from yellow, reddish yellow, orange-yellow, blue cream to blue-black. Some of the rhizomes of the genus are characterised by camphoraceous, mango or turmeric aroma (Velayudhan *et al*., 1999; Sabu, 2006).

Two types of the inflorescence are reported in the genus, lateral or terminal. Early flowering species (April – May) have lateral inflorescence which is developed from rhizomes. Terminal inflorescence usually appears in late flowering species (August – September) which is produced terminally from the leafy shoots (Sirirugsa, 1999). Flowers are arranged in cincinnus on the axils of bracts with thin, elliptic bracteoles in which sides are inflexed (Apavatjrut *et al*., 1999). Calyx is unequally toothed, as well as short and split nearly half way down on one side. Corolla tube plus staminal tube is tubular at the base, the upper half cup-shaped, and the corolla lobes are inserted on the edges of the cup with above them. The ovary is binocular, fruit is ellipsoid, thin walled, and dehiscing seeds in the bract pouch. Seeds are ellipsoid, with a lacerate aril (Sabu, 1991, Ravindran *et al*., 2007; Nirmal Babu *et al*., 2011).

2.1.3. Species diversity

Curcuma species are reported to display diversity in habitat, morphology, biochemical and ethnomedicinal use (Jatoi *et al*., 2007). The habitat expanded from sea level to hilly slopes of Western Ghats and Himalayas. Highest species diversity is reported in India and Thailand followed by Bangladesh, Vietnam, Myanmar, China and Indonesia (Sirirugsa,1999; Sasikumar, 2005; Krishnamoorthy *et al*., 2012). In India, south, northeast part and Andaman and Nicobar Islands are having a rich species diversity (Sasikumar, 2005).

Species like *Curcuma longa* L., *Curcuma aromatica* Salisb., *Curcuma amada* Roxb. and *Curcuma zedoaria* (Christm.) Roscoe. are predominantly found in plains whereas species like *Curcuma neilgherrensis* Wight, *Curcuma vamana* Mangaly & Sabu, *Curcuma angustifolia* Roxb., *Curcuma kudagensis* Velay., Pillai & Amalraj, *Curcuma coriacea* Mangaly&M. Sabu and *Curcuma pseudomontana* J. Graham. are prevalent in mountainous areas (Krishnamoorthy *et al*., 2012). Species like *C. haritha, C. ecalcarata, C. coriaceae, C. kudagensis, C. neilgherrensis, C. oligantha* var *lutea, C. vamana, C. raktakanta, C. mutabilis, C. bhatti* etc. are endemic to peninsular India (Velayudhan *et al*., 1999; Sabu, 1991, Sabu, 2006).

Taxonomically, *Curcuma* is a complex genus of morphologically similar species and cannot be easily segregated into definite groups based on their gross morphology. The early flowering group displayed much similarities among them which led to confusion in their identification (Apavatjrut *et al*., 1999). This confusion has led to the taxonomic revision of the genus as many of the existing species are now treated as synonyms (Sasikumar, 2005). Chinese species, *C. albicoma* S.Q.Tong and *C. chuanyujin* C.K. Hsieh & H. Zhang are synonyms of *C. sichuanensis* X.X. Chen and *C. kwangsiensis* S.G.Lee & C.L.Liang. Chinese species *C. wenyujin* Y.H. Chen & C. Ling is now treated as a synonym of *C. aromatica*. *C. caesia* was misidentified as *C. aeruginosa* in the past (Liu and Wu, 1999). *C. aeruginosa*, and *C. caesia* shared unique morphological trait of blue colour of the rhizome. However, *C. aeruginosa* has blue circle only in the central portion. Both the species also grouped together in molecular based dendrogram (Syamkumar and Sasikumar, 2007). Likewise, *C. phaeocaulis* Valeton was misidentified as *C. zedoaria* (Liu and Wu, 1999). According to Syamkumar (2008), *C. zedoaria* and *C. malabarica* in India may be synonyms as they showed very high similarity in morphological, biochemical and molecular clustering pattern. Senan (2011) also suggested delimitation of *C. zedoaria* and *C. malabarica* in to single species as they showed very high similarity in the clustering pattern using microsatellite

markers. *C. amada* and *C. mangga* closely resembles for a number of quality attributes (Sasikumar, 2005; Krishnamoorthy *et al*., 2012).

Similarly, the morphological resemblance between *C. montana* and *C. raktakanta* for plant type, floral, vegetative and rhizome characters (Velayudhan *et al*., 1999) was further confirmed by molecular marker based analysis (Syamkumar and Sasikumar, 2007). Syamkumar and Sasikumar (2007) reported clustering of two *Curcuma* species namely *C. decipiens* and *C. ecalcarata*, belonging to two different subgenera (*Eucurcuma* and *Paracurcuma*) under the same group.

Position of spikes (lateral / terminal), presence or absence of coma bract and bract colour are generally used as discriminating traits in *Curcuma* species. However, reports suggested that position of spikes is seasonal, as early flowering results in lateral spike position whereas late flowering ones are central (Roxburgh,1910; Larsen and Smith,1978). Floral characters of some of the *Curcuma* species tend to vary as two types of spikes (lateral and central) are reported in *C. pseudomontana* (Santapau, 1952) besides bract colour variation within the species of *C. ecalcarata* (Santapau, 1952; Sabu,1991; Sasikumar, 2005). *C. ecalcarata* and *C. decipiens* shared many floral, vegetative and rhizome characters with each other (Sabu, 1991). Rhizome shape, size, colour and aroma showed variation among the different *Curcuma* species (Angel *et al*., 2008). The variation may be arising out of intraspecific genetic differentiation, geographical or seasonal effects. And thus identification of *Curcuma* species merely relying on the appearance of rhizome or on aerial morphological characters may not be accurate.

Recent studies have reported several new species of *Curcuma* from Asia such as *C. pambrosima* Skornick. & N. S. Ly. from central Vietnam (Skornickova and Ly, 2010); *C. bella* Maknoi, K. Larsen & Sirirugsa from Thailand (Maknoi *et al*., 2011); *C. arracanensis* W. J. Kress & V. Gowda from Myanmar (Gowda *et al*., 2012); *C. roxburghii* Rahman et Yusuf, *C. wallichii* Rahman et Yusuf and *C. wilcockii* Rahman et Yusuf. from Bangladesh (Rahman and Yusuf, 2012); *C. leonidii* Škorničk. & Luru (Skornickova and Luu, 2013), *C. newmanii* Škorničk. and *C. xanthella* Škorničk. from southern Vietnam (Skornickova and Tran, 2013); *C. gulinqinensis* N. H. Xia & J. Chen from China (Chen and Xia, 2013), *C. pygmaea* Škorničk. & Šída f. from Vietnam (Skornickova *et al*., 2014), *C. peramoena* Souvann. & Maknoi from Laos (Souvannakhoummane and Maknoi, 2014), *Curcuma mukhraniae* R. Kr. Singh & Arti Garg from India (Singh and Garg, 2014), *C. arida* Škorničk. & N. S. Lý and *C. sahuynhensis* Škorničk. & N. S. Lý from Vietnam (Skornickova *et al*., 2015), *C. woodii* N. H. Xia & J. Chen from Thailand (Chen *et al*., 2015), *C. prasina*

Škorničk. from Thailand (Skornickova *et al*., 2017) and *C. cotuana* Luu, Škorničk. & H. D. Tran. from Central Vietnam (Luu *et al*., 2017).

Velayudhan *et al*. (1999) used numerical taxonomy tools to characterise thirty-one *Curcuma* species which was carried out in sessile and non-sessile tuber-bearing species. The data were based on distribution, habitat, flowering time, floral traits, qualitative and quantitative features of aerial and rhizome characters of the 31 *Curcuma* species. Dendrogram showed that 31 species were clustered into nine groups in which species with sessile tubers showed a distinct status from species with non-sessile tubers.

Essential oil profile in different *Curcuma* species were reported to be varying with respect to species, explant, methodology and locations from where they were collected (Zwaving and Bos, 1992; Bordoloi *et al*., 1999; Srivastava *et al*., 2001; Behura *et al*., 2002; Leela *et al*., 2002; Pandey and Chowdhury, 2003; Singh *et al*., 2003; Behura and Srivastava, 2004; Raina *et al*., 2005; Mustafa *et al*., 2005; Paliwal *et al*., 2011; Angel *et al*., 2014).

Tang *et al*. (2008) explored genetic relationships of the 39 materials in six species of *Curcuma* (*C. phaeocaulis, C. wenyujin, C. kwangsiensis, C. chuanhuangjiang, C. longa* and *C. sichuanensis*) using peroxidase isozyme and esterase isozyme. In the analysis, the species generated species-specific zymogram. The study also revealed that genetic relationships were not associated with the geographical distributions and a close genetic relationship existed between *C. sichuanensis* and *C. longa*. The study suggested that *C. sichuanensis* as the cultivated mutation species of *C. longa* by isozyme patterns of POD (peroxidase) and EST(esterase). Deng *et al*. (2011) also suggested the close relationship of *C. longa* and *C. sichuanensis* using four isozymes (superoxide dismutase, polyphenol oxidase, malate dehydrogenase and cytochrome oxidase). Similarly, Zou *et al*. (2011) reported closer relationship of *C. longa* and *C. sichuanensis* using RAPD markers. They also share similar vegetative, floral and rhizome characters. The study concluded that *C. sichuanensis* was the cultivated variety of *C. longa*.

The *matK* gene sequence and the intron spacer region of the *trnK* gene showed great diversity among six medicinal *Curcuma* species (*C. longa, C. phaeocaulis, C. sichuanensis, C. chuanyujin, C. chuanhuangiiang* and *C. chuanezhu*) from Sichuan Province of China (Cao and Komatsu, 2003).

2.1.3.1. Economically important *Curcuma* species

The species belonging to the genus *Curcuma* are well known for their multiple uses. They are widely used as spices, dyeing agents, medicines, cosmetics, flavouring, starch and

ornamentals since Vedic age (Salvi *et al*., 2000; Shirgurkar *et al*., 2001; Sasikumar, 2005). A number of *Curcuma* species in the genus are blessed with beautiful inflorescence and rich foliage that have immense commercial value as the flowers have a natural dormancy which makes it a versatile ornamental crop. It can be used as a cut flower, pot and landscape plant (Paisooksantivatana *et al*., 2001a and 2001b; Skornickova *et al*., 2007; Velayudhan, 2015) even as a starch source. Yams, cassava, aroids, sweet potato and arrowroot are some of the well documented starch sources. Recent studies in some of the starchy *Curcuma* species like *C. amada* (Policegoudra and Aradhya, 2008), *C. malabarica*, *C. zedoaria* (Jyothi *et al*., 2003), *C. longa* (Braga *et al*., 2006), *C. angustifolia* (Rani and Chawhaan, 2012), *C. caesia, C. aromatica, C. xanthorrhiza* and *C. amada* (Sajitha and Sasikumar, 2015) have also shed some light towards exploring *Curcuma* species as potential starch sources for food and industries. Studies on physiochemical properties of *Curcuma* starch indicated that it has stable viscosity, a good resistant gel, swelling power and more importantly an easily digestible starch resembling arrowroot starch that will find application in infant food as well as starch based industries (Jyothi *et al*., 2003). Moreover, *Curcuma* starches are believed to have medicinal properties with the presence of trace amounts of oleoresin and curcuminoids in them (Braga *et al*., 2006). Jamir and Seshagirirao (2017) reported high amylose content in starch isolates from *C. caesia, C. amada, C. aromatica* and *C. aeruginosa* which determines the property of starch and its application in food and pharmaceutical industries. A list of some economically important *Curcuma* species is given in the Table 2.

Table 2. List of economically important *Curcuma* species

Species	Use
C. longa L. syn. *C. domestica* Val.	Spice, dye, medicine, local religious delicacies, insect repellent, perfume and aromatherapy
C. amada Roxb. and *C. mangga* Val. & Zijp.	Pickles, salads, medicine, spice
C. aromatica Salisb.	Medicine, toiletry articles, insect repellent
C. caesia Roxb.	Medicine and spice
C. zedoaria Roxb.	Folk medicine, arrowroot industry
C. ochrorhiza Val & Van Zijp	Malayan traditional medicine
C. pierreana Gagnep.	Vietnamese traditional medicine
C. kwangsiensis S. G. Lee & C. F. Liang syn. *C. chuanyujin* and *C. phaeocaulis* Val.	Chinese traditional medicine
C. comosa Roxb.	Traditional medicine of Thailand
C. angustifolia Roxb, *C. zedoaria, C. caulina* F.Grah., *C. montana* Roxb. *C. pseudomontana* F.Grah., *C.rubescens* Roxb. *C. leucorrhiza, C. xanthorrhiza, C. decipiens* Dalz., *C. malabarica* Vel *et al., C. haritha* Mangaly & Sabu , *C. raktakanta* Mangaly & Sabu, *C. amada* Roxb. *and C. aeruginosa* Roxb.	Arrowroot industry
C. cordata Wal., *C. alismatifolia* Gagnep., *C. gracillima* Gagnep. *C. roscoeana* Wall., *C. pseudomontana* F.Grah., *C. auranticaca* Van Zijp.,*C. bicolor* Mood & K.Larsen, *C. petiolata* Roxb.,*C. thorelii* Gagnep., *C. parviflora* Wall. and *C. australasica* Hook.F. *C. oligantha* Trimen	Ornamental (cut flower)

Sources: Sasikumar (2005); Policegoudra and Aradhya (2008); Velayudhan (2015)

2.1.4. Cultivar/Varietal diversity

India is rich in cultivar/varietal diversity of turmeric and other *Curcuma* species and they are often known by their place of origin, cultivation or collection. Orissa, north-eastern and southern part of India is found to be rich in cultivar diversity (Sabu, 1991). Other than India, countries like Nepal, Vietnam, Myanmar, Pakistan, Bangladesh, Thailand, Malagasy, China, South Pacific Islands etc. are rich in cultivar/varietal diversity of *Curcuma* (Islam, 2004). Popular cultivars/varieties of turmeric grown in India are given in the Table 3.

Table 3. Popular cultivars/varieties of turmeric grown in India

Sl. No	Cultivar/Variety	Sl. No	Cultivar/variety	Sl. No	Cultivar/variety
1	' Alleppey'	34	‘Guntur'	66	‘Punjabi Haldi 1’
2	'Alleppey' Supreme	35	‘Erragunturu’	67	‘Punjabi Haldi 2’
3	'Amalapuram'	36	'Ethamukkala'	68	'Rajapuri'
4	'Amrithapani'	37	'Gorakpur'	69	‘Pragati’
5	'Armoor'	38	‘Jabedi'(G-67)	70	'Prathibha'
6	Avanigadda'	39	'Kanthi'	71	'Rajendra Sonia'
7	‘Azad haldi-1’	40	'Kasturi'	72	'Ranga'
8	'Balaga'	41	'Kasturi Tanaka'	73	‘Rasmi'
9	‘Bangalore local’	42	'Katpadi Local'	74	‘Renuka'
10	‘Barua sagar’	43	'Kedaram'	75	‘Roma'
11	‘Belgaum local’	44	‘Kodur’	76	‘Sadashiv peth’
12	‘Bidar-1’	45	'Kothapetta'	77	‘Shimla'
13	‘Bidar-4’	46	'Krishna'	78	‘Sobha'
14	'Bilaspur'	47	'Lekadong'	79	‘Sona'
15	‘Brahmani’	48	'Lokhande'	80	‘Soni'
16	'BSR-1'	49	‘Madras’	81	‘Sudarsana'
17	'BSR-2'	50	'Megha turmeric'	82	‘Sugandham'
18	'Bullapura'	51	'Mundage'	83	‘Suguna'
19	'Ca-12'	52	'Mydukkur'	84	‘Suranjana'
20	‘Ca-72 Udayagiri'	53	'Nandyal'	85	‘Suroma'
21	‘Chayapaspu'	54	‘Narendra Haldi 1’	86	‘Suvarna'
22	'Chinnanadan'	55	‘Nizamabad bulb’	87	‘Thalachira'
23	‘CIM-Pitamber’	56	'Pakistan'	88	‘Thekurpetta'
24	‘CLL- 324'	57	‘Palam Pitambar’	89	‘Tsundar'
25	‘CLL- 328' '	58	‘Palam Lalima’	90	‘Vandse’
26	‘CO-l'	59	‘Pant Peetabh'	91	‘Varna'
27	‘Deshi'	60	‘Panamalur'	92	‘Vellanikkara’
28	‘Duggirala'	61	‘Parravona’	93	‘Vallabh Priya’
29	‘Dughi'	62	‘Pattani'	94	‘Vombinitta'
30	'Dindigam'	63	‘Perianadan’	95	‘Waigon’
31	'Erode Local'	64	'Perumnadan'	96	‘Wynadan'
32	‘GLPuram'	65	‘Prabha'	97	‘Yelachage'
33	‘GN Turmeric 1’				

Source: Syamkumar (2008).

More than 80 cultivars of turmeric are popular in India, majority of them belonging to *C. longa* and few of them belongs to *C. amada* (‘Amba’, ‘Pundibari Col-1’, ‘Pundibari Col-2’, ‘Daspur’, ‘Krishnanagar Col-1’, ‘Krishnanagar Col-2’, ‘Kesinga’, ‘Sargiguda’, ‘Brahmapur’, ‘Cochin’) (Chatterjee *et al*., 2012). Some of the popular cultivars like ‘Armoor’, ‘Alleppey’, ‘Bhavanisagar', ‘Duggirala', Sugandham', ‘Nandyal', ‘Rajapuri', GL Puram’ are known by the places where they are grown (Nair *et al*., 1980; Krishnamurthy *et al*., 2012).

Cultivars are mainly categorised into three types; short duration ‘kasturi' types which are characterized with thick, long rhizome and low curcumin; medium duration ‘kesari’ types are having thick rhizome, medium long with close internode; and long duration type are characterized with long, stout, smooth and hard rhizomes (Krishnamurthy *et al*., 2012). The onset of flowering in turmeric is found to vary according to the cultivars and climatic conditions as reported by many authors (Pathak *et al*., 1960; Nambiar *et al*., 1982).

Velayudhan *et al*. (1999) reported the existence of 21 morphotypes of *C. longa* which in turn got grouped into six taxonomic varieties *viz.*, *C. longa* var. *typica*, *C. longa* var. *spiralijolia*, *C. longa* var. *camphors*, *C. longa* var. *musacijolia*, *C. longa* var. *paltijolia*. and *C. longa* var. *atypica.*

Varieties ‘Krishna', ‘Rajendra Sonia', Prathibha, Prabha, Alleppey Supreme, Pragathi, Suvarna' etc are high yielding ones. ‘Armoor’, ‘Kasturi Tanaku’ GL Puram-2 and ‘Mannuthy Local are tolerant to leaf blotch disease whereas turmeric variety ‘Vallabh Priya’ is resistant to leaf blight disease (Singh *et al*., 2007). Likewise, various workers reported wide variability in cultivars for growth parameters, yield attributes and quality traits (Table 4). Salient features some released turmeric varieties in India are given in Table 5.

Table 4. Variability for growth parameters, yield and quality traits in some cultivar/variety of turmeric and mango ginger.

Material used	**Trait studied**	**Remark**	**Author**
One hundred and eighty four accessions of *Curcuma* including both *longa* and *aromatica* types along with exotic and wild collections	Dry recovery, oleoresin, oil and curcumin	Wide variability for curcumin, essential oil, oleoresin and dry recovery were observed among the collections. Cultivar 'Konni' reported with maximum oleoresin. Curcumin content varied from 2.3% (‘Hahim’) to 10.9% (‘Edapalayam’). *C. aromatica* recorded with maximum essential oil content.	Ratnambal, 1986
VK-116, PTS-10, VK-31, Vontimitta, PTS-24, VK-11, VK-114, VK-77, VK-70, VK-55, VK-47, YK-82, Chayapasupu, Sugandham, Pilicode local	Yield and yield attributes	Cultivars exhibited significant variation for various yieldand yield attributing traits under open and partially shaded conditions. Highest yield was recorded in cultivar ‘Chayapasupu’ and ‘VK-31’, whereas cured rhizome yield was recorded maximum in ‘VK-116’.	Latha *et al*., 1995
Suvarna, Suguna, Sudarsana, Suroma, BSR-l, CO-I, Lakodong	yield and growth parameters	Varieties showed variation among themselves with regard to yield and yield attributing characters. Rhizome yield was recorded maximum in ‘BSR-1’, followed by ‘Suvarna’ and ‘Suroma’.whereas ‘Lakodong’ recorded with highest curing percentage and Curcumin content.	Patil *et al*., 1995
Armoor, Duggirala, CLI-317, CLI-330, PCT-13 (Suguna) and PCT-14 (Sudharshana)	Yield, curing percentage and curcumin content	Fresh yield, curing percentage and curcumin content varied among genotypes at different growth stages. Yield and curing percentage were found highest in ‘Duggirala’ and ‘Armoor’ whereas curcumin content was highest in ‘PCT-14’ and ‘PCT-13’.	Rao *et al*., 2006
Suvarna, Rajendra Sonia, Suguna and Sudarshana	Yield and yield attributes	Significant variation was observed among the cultivars for fresh and cured yield. Yield attributing character like number and size of rhizomes varied among the cultivars. Variety 'Krishna' excelled in performance compared to rest of the varieties.	Chaudhary *et al*., 2006
CO-1, Krishna, Suvarna, Parravona, Azad haldi-1, Rajendra sonia, Barua sagar	Yield and yield attributing characters	Significant variation for yield and growth attributes were observed among the varieties grown under same agro-climatic conditions which can be attributed to genetic factors. ‘Azad haldi-1’ and ‘Barua sagar’ excelled in yield and growth parameters at all seasons.	Chaturvedi *et al*., 2009
Salem, Krishna, Rajapuri and Prathibha	Curcumin	Curcumin percentage ranged from 3.584 to 7.730% in ‘Pratibha’; ‘Salem’ -2.169 to 5.932%; ‘Rajapuri’ -2.812 to 4.366% and ‘ Krishna’- 1.599 to 3.520%.	Kamble *et al*., 2011
Megha Turmeric-1, Suranjana, Narendra Haldi-1, IISR Allepy Supreme, IISR Kedaram, IISR Pratibha, Duggirala, BSR-2, Rajendra Sonia, Rasmi, Roma	Growth, yield and quality parameters of varieties at three planting dates.	Significant variation was observed for different characters at different planting dates. ‘Megha Turmeric-1’, ‘Duggirala’, ‘IISR Pratibha’ and ‘Roma’ were performed well with respect yield, dry recovery and curcumin content. Planting of turmeric in last week of April resulted in a better yield and quality parameters.	Singh *et al*., 2013

Table 4. (Conti…) Variability for growth parameters, yield and quality traits in some cultivar/variety of turmeric and mango ginger.

Material used	Trait studied	Remark	Author
Duggriala Red, IISR Alleppey supreme, IISR Kedaram, IISR Prathibha, Roma, Rasmi, Suranjana, Rajendra Sonia, BSR-2, Megha turmeric, Narendra haldi	Fresh yield, dry recovery, curing percentage and curcumin	Study showed significant variation among cultivars, environment and cultivar by environment interaction for fresh and dry yield, curing percent and curcumin content. 'Megha turmeric' was most stable for fresh yield whereas a consistent performance was observed in 'IISR kedaram' across different environments.	Anandaraj *et al*., 2014
P36 (Potangii) cv. Suroma, P37 (Potangii) cv. Roma, P38 (Potangii) cv. Ranga, P39 (Potangii) cv. Rasmi, P40 (Potangii) cv. Lakadong.	Growth parameters, curcumin, essential oil, oleoresin.	Yield attributing characters varied significantly among the genotypes from different agro-climatic regions. Higher content of oleoresin was recorded in cultivars. Curcumin content varied from 5.2% (Rasmi-P39) to 8.8% (Surama-P36); Leaf essential oil from 0.5% ('Surama', 'Lakadong') to 0.7% ('Roma') and rhizome essential oil from 0.9% ('Roma') to 1.2% ('Lakadong').	Singh *et al*., 2014
Co-1, Salem, Prabha, Krishna, Rajapuri, Prathibha, PTS-24, Cuddapah, Alleppey, Bidar-1, Bidar-4, CLI-327, CLI-14, CLT-325, Belgaum local, Erode local	Growth parameters, yield and yield attributes	Significant variations were observed in growth parameters, yield and yield attributes for different cultivars. 'Salem', 'Rajapuri', 'Prathibha' and 'CLT-325' were excelled for growth, yield and yield attributes.	Venkatesha and Siddalingayya, 2014
Duggriala Red, IISR Alleppey supreme, IISR Kedaram, IISR Prathibha, Roma, Rasmi, Suranjana, Rajendra Sonia, BSR-2, Megha turmeric, Narendra haldi	Yield and yield attributes	Turmeric varieties exhibited variation for yield and growth characters. The varieties with tall plants ('Megha Turmeric', 'Duggriala Red', 'Rasmi') were recorded with more tillers, leaves, leaf area and Leaf area index, whereas varieties with short plants ('Rajendra Sonia', 'Narendra Haldi 1' and 'Suranjana') had few tillers and less leaves and Leaf area index.	Kandiannan *et al*., 2015
Roma, Suroma, Rajendra Sonia, Prabha, Pratibha, Kedram	Yield, growth characters and curcumin content	Significant variation was observed for yield, growth characters and curcumin among the varieties. 'Suroma' and 'Roma' were the best varieties for the growth characters and yield whereas, 'Pratibha' and 'Roma' contained highest amount of curcumin.	Singh *et al*., 2015
Kanti, Cuddapah, Kedram, Prabha, Suguna, CLT-325, Salem, Rajapuri, Varna, Alleppey supreme, Alleppey, PTS-24, swarna, Bidar-1, Prathibha, Bidar-4,Belgaum local,Sona and Sobha.	Yield and yield attributes	All the varieties showed significant variation for yield and yield attributes. Curcumin content varied from 2.19% ('Cudadapah') to 7.23% (PTS-24). 'Kanti', 'CLT-325', and 'PTS-24' found promising for rainfed condition under hill zone of Karnataka.	Hanchinamani *et al*., 2016
C. amada Pundibari Col-1, Pundibari Col-2, Daspur, Krishnanagar Col-1, Krishnanagar Col-2, Kesinga, Sargiguda, Brahmapur, Cochin	Yield, curcumin, oleoresin, crude protein, total sugar and starch	Germplasms of *C. amada* showed a distinct variation among them with regard to fresh yield, curcumin, oleoresin, total sugar, starch and crude protein content. The highest crude protein content was recorded in 'Pundibari Col.-1' (7.85%) and lowest in 'Kesinga' (5.37%), whereas total sugar and starch content was recorded highest (5.24%) in 'Kesinga' and lowest (3.53%) in 'Sargiguda' and 'Pundibari Col.-2' (39.26%), highest curcumin content was noted in 'Kesinga' (0.43%) and lowest in 'Pundibari Col.-1' (0.22%)	Chatterjee *et al*., 2012

Table. 5. Salient features of some turmeric varieties released in India

Variety	Avg. yield (kg/ha, fresh)	Salient feature	State
Co.1	30.5	Bold and bright orange yellow rhizomes, curcumin 3.2%, oleoresin 6.7%, essential oil3.7%,dry recovery 19.5%,suitable for drought prone areas, saline and alkaline areas. crop duration 270 days.	Tamil Nadu
BSR.1	30.7	Bright yellow rhizome, curcumin 4.2%,oleoresin 4.0%,essential oil 3.7%,dry recovery 20.5%,crop duration285 days, suitable for drought prone areas of Tamil Nadu	Tamil Nadu
BSR.2	32.7	A high yielding short duration variety(245 days)with bigger rhizomes, resistant to scale insects, curcumin 2.5%	Tamil Nadu
Krishna	9.2	Plumby rhizomes, curcumin 2.8%,oleoresin3.8%, essential oil 2.0%,dry recovery16.4%,duration 240days.Moderately resistant to pests and diseases.	Maharashtra
Sugandham	15.0	Thick, round rhizomes with short internodes. curcumin 3.1%, oleoresin11.0%, essential oil 2.7%, dry recovery 23.3%, duration 210 days. Moderately resistant to pest and diseases.	Gujarat
Roma	20.7	Suitable for both rained and irrigated condition. Suitable for hilly areas and late season planting. Curcumin 6.1%,Oleoresin13.2%,essential oil 4.2% and dry recovery 31.0%,duration 250 days.	Orissa
Suroma	20.0	Round and plumby rhizome, curcumin 6.1%,oleoresin13.1%,essential oil 4.4% and dry recovery 26.0%,duration 253 days field tolerance to leaf blotch, leaf spot and rhizome scale.	Orissa
Ranga	29.0	Bold and spindle shaped mother rhizome, suitable for late planting and low lying areas, curcumin 6.3%, oleoresin 13.5%, essential oil4.4% and dry recovery 24.8%, duration 250 days. Moderately resistant to leaf blotch and scales.	Orissa
Rasmi	31.3	Bold rhizomes, suitable for both rainfed and irrigated condition, early and late sown reason, curcumin 6.4%,oleoresin 13.4%,essential oil 4.4% and dry recovery 23.0%,duration 240 days.	Orissa
Rajendra sonia	42.0	Bold and plumby rhizome, grows widely under all north Indian conditions. Curcumin 8.4%,essential oil 5.0% and dry recovery 18.0%, duration 225 days	Bihar
Megha turmeric-1	23.0	Bold rhizomes, high curcumin content 6.8% and dry recovery 16.37%, duration 300-315 days. Suitable for the North East hill and North West Bengal.	Meghalaya
Pant Peethabh	20.0	Long attractive fingers, curcumin 7.5%,essential oil 1.0%,dry recovery 18.5%,resistant to rhizome rots.	Uttar Pradesh and Uttaranchal
Suranjana (TCP-2)	29.0	Suitable for open and shaded conditions, sole or inter crop, suitable for rainfed as well as rain fall areas. curcumin 5.7%,oleoresin 10.9%,essential oil 4.1%,dry recovery 21.2%,duration 235 days, tolerant to leaf blotch and rhizome rot. Resistant to rhizome scales and moderately resistant to shoot borer.	West Bengal
Suguna	29.3	Short duration type(190 days),curcumin 4.9%,oleoresin 13.5%,essential oil 6.0% and dry recovery 20.4%,field tolerance to rhizome rot.	Kerala
Suvarna	17.4	Bright orange coloured rhizome with slender fingers. Maturity 200 days, Curcumin 4.3%,oleoresin13.5%,essential oil7.0% and dry recovery 20.0%.field tolerant to pest and diseases.	Kerala
Sudharsana	28.8	High yield variety, short duration type(190 days).Curcumin 5.3%, oleoresin 15.0%,essential oil 7.0% and dry recovery 20.6%.Field tolerant to rhizome rot.	Kerala

Table. 5. (Conti…) Salient features of some turmeric varieties released in India

Variety	Avg. yield (kg/ha, fresh)	Salient feature	State
IISR Prabha	37.47	High yielding variety, Curcumin content 6.5% ,oleoresin 15.0%,essential oil 6.5% and dry recovery19.5%,crop duration 205 days.	Kerala
IISR Prathiba	39.12	High quality line,6.2% curcumin content with high yield,16.2% oleoresin,6.2% essential oil,18.5% dry recovery, crop duration 225 days.	Kerala
IISR Alleppy	35.4	Rhizomes contain 5.55%curcumin,16.0% Oleoresin,19.0% dry recovery, crop duration 210 days. Shows tolerance to leaf blotch disease.	Kerala(rainfed)
IISR Kedaram	34.5	Rhizomes contain 5.5% curcumin,13.6% oleoresin, maturity 210 days and 18.9% dry age. Tolerant to leaf blotch diseases.	Kerala(rainfed)
Kanthi	37.65	Big mother rhizomes with medium bold fingers and closer internodes. Medium duration. Curcumin 7.18%, oleoresin 8.25%, essential oil 5.15%,dry recovery 20.15%duration 240-270 days.	Kerala
Sobha	35.88	Mother rhizome big with medium bold and closer internodes. Inner core of rhizomes is dark orange like Alleppey. More territory rhizomes. Dryage 19.38%,curcumin content7.39%,oleoresin9.65%,essential oil4.24%,medium duration 240-270 days.	Kerala
Sona	21.29	Orange yellow rhizome, medium bold with no territory fingers. Best suited for central zone of Kerala. Rhizome medium bold, Curcumin 7.12%, essential oil 4.4%, oleoresin 10.25%.18.88% dry recovery, medium duration 240-270 days. Field tolerant to leaf blotch.	Kerala
Varna	21.89	Bright orange yellow rhizome, medium bold with closer inter nodes, territory fingers present. Suited to central zone of Kerala. Field tolerant to leaf blotch, curcumin 7.87%,essential oil 4.56%,oleoresin 10.8%.19.05% dry recovery, medium duration 240-270 days.	Kerala
GN turmeric 1 (Gujarat Navsari Turmeric 1)	33.60	maturity in 252-260 days. compact rhizomes, high number of fingers per rhizomes, higher curcumin (2.84 %) as well as higher oleoresin (8.68 %), fibreless rhizome and non-lodging habit	Gujarat
Punjab Haldi 1	27.2	Maturity 215days, Curcumin content is 3.33 % and, oleoresin 6.76 %.	Punjab
Punjab Haldi 2	30.0	Maturity 238 days, Curcumin content in this clone is 2.91% and, oleoresin 7.61%	Punjab
Palam Pitambar	33.2	High yielding, curcumin 5%, essential oil 7 %	Himachal Pradesh
Palam Lalima	35.7	High yielding, curcumin 7%	Himachal Pradesh
CIM-Pitamber	60-65	The general duration of the crop is 180-190 days, 12.5 % curcumin, tolerant to common leaf botch disease.	Uttar Pradesh
IISR-Pragati	38.0	High yielding variety with crop duration of 180 days, high curcumin variety (5.02%), moderately resistant to root knot nematode infestation, suitable for cultivation in Kerala, Tamil Nadu, Andhra Pradesh, Telangana, Karnataka and Chhattisgarh states.	Kerala

Sources: Ravindran *et al*. (2007); Prasath *et al*.(2011)

Shamina *et al*. (1998) studied isozyme polymorphism in a germplasm collection of *C. longa* using acid phosphatase, superoxide dismutase, esterase, polyphenol oxidase, peroxidase and catalase, which showed good polymorphism in the 15 accessions studied.

Nayak *et al*. (2006) reported the existence of variation among 17 cultivars of *C. longa*, which was quantified through rhizome yield; the observed intraspecific variation was proved to have a genetic basis as confirmed using 4C DNA content and RAPD marker polymorphism. The inter cultivar polymorphism ranged from 35.6% (PTS 51) to 98.6% (ACC. 31). Although 17 cultivars possessed same chromosome number (2n= 48), significant variation was observed among the cultivars as the 4C DNA content varied from 4.3pg to 8.83pg. This variation might be due to the addition/deletion of repeats in the genome which is ultimately attributed to the varying micro and macro climatic condition of growing habitats.

Turmeric varieties/ cultivars like 'Alleppey', 'Alleppey Supreme', 'Lekkadong', 'Prabha', 'Prathibha', 'Kedaram', 'Roma', 'Sugandham', 'CIM-Pitamber', IISR Pragati'etc. are well known for their curcumin content (above 5%). Likewise, high heritability coupled with appreciable genetic advance was observed for *Curcuma* rhizome yield, number of tillers per plant, number of leaves, leaf width, leaf length, number of primary rhizomes, height of plant, yield of secondary rhizomes, dry rhizome weight, curcumin content and crop duration (Philip and Nair, 1986; Sinkar *et al*., 2005; Singh *et al*., 2012; Rajyalakshmi *et al*., 2013; Prajapati *et al*., 2014; Gupta *et al*., 2016; Hanchinamani *et al*., 2016).

Syamkumar (2008) studied relationships of 36 Indian turmeric cultivars using RAPD and ISSR markers. The majority of the improved varieties clustered distinctly from the landraces/cultivars. Most of the land varieties collected, based on its vernacular names, from a particular geographical region, were grouped together along with released varieties which were improved through germplasm selection of material obtained from the same region. The popular varieties such as 'Alleppey Supreme' and 'Prathibha' showed maximum similarity within the group and clustered together in all the dendrogram. Cultivars 'Amruthapani' and 'Armoor' and/or 'Amalapuram' clustered together with maximum similarity. The study suggested that these cultivars are genetically similar or it might be collected as distinct accessions based on their vernacular names.

Singh *et al*. (2012) evaluated genetic diversity among five cultivars and 55 accessions of *C. longa* collected from 10 different agro-climatic regions using RAPD and ISSR primers. A high level of significant polymorphism was observed among the population which might be due to the intraspecific variation. Among the various agro-climatic zones, hilly areas showed low genetic variability probably because the populations in hilly areas are comparatively

undisturbed whereas population from plain and plateau land exhibited highest genetic diversity as these areas are affected by disturbances due to intensive agricultural practices.

Relatively high genetic diversity was detected among five varieties of *C. alismatifolia;* 'Chiang Mai Red', 'Chiang Mai Pink', 'Doi Tung 554', 'Sweet Pink' and 'Kimono Pink' cultivated in Malaysia using 16 ISSR and 8 SSR markers. Cluster analysis based on ISSR markers separated the five varieties into two clusters, 'Doi Tung 554' variety formed a distinct cluster, showing least similarity with rest of the varieties. Whereas in case of SSR analysis, out of the two clusters formed, 'Kimona pink' formed the first cluster and rest of the varieties included in the second cluster. Cluster analysis and Principal Component Analysis demonstrated the considerable variation in five varieties of *C. alismatifolia* (Taheri *et al*., 2012; Taheri *et al*., 2014).

Prashanth *et al*. (2016) used 20 ISSR primers to study the diversity in 18 popular varieties of turmeric from Telangana region. The study indicated presence of high variability among the varieties. Low yielding and high yielding varieties are clustered separately in the dendrogram. The ISSR profile showed a polymorphic index value of 87.27% across all the genotypes, in which all the primers were able to distinguish 18 genotypes of turmeric with distinct profile successfully.

Verma *et al*. (2015) used Directed Amplification of Minisatellite DNA (DAMD) and Inter-Simple Sequence Repeats (ISSR), methods to estimate the genetic variability in indigenous turmeric germplasm including cultivars like 'Rajendra Sonia', 'Prabha', 'Roma', 'Pant Peetabh', 'Azzad 1' etc. The 29 genotypes were separated into two main clusters. Although the genotypes did not exclusively group according to their geographical location, subclusters showed location specific grouping from Uttar Pradesh. Most diverse sub clusters were sub cluster I(b) (RH-5, 'Prabha', 'Roma', NBH17 and NBH 18) and sub cluster II (a) ('Azaad-1', 'Pant peetabh', KTS-2, NBH-16 and NBH-20) comprising of cultivars. Cluster analysis showed that there was considerable diversity amongst the genotypes; moreover, the study highlighted the potential of DAMD and ISSR markers in genetic diversity studies.

Singh *et al*. (2015) investigated genetic diversity in 10 turmeric genotypes ('IISR Alleppey', 'Suguna', 'Roma', 'Rasmi', 'Suroma', 'Ranga', 'Rajendra Sonia', 'Sugandham' and 'BSR-1') using SSR markers. The cluster analysis formed two major clusters with high level of genetic variation among the genotypes ranging from 0.60 to 0.98. Cluster I, the major cluster was further divided into two groups. Group I was formed by 'Ranga', 'Rasmi', 'Suguna', 'BSR-1' and 'IISR Alleppey' whereas group II was formed by 'Rajendra Sonia', 'Sugandham' and 'Suroma'. Cluster II formed by 'Krishna' and 'Roma'. 'Ranga' and

'Suguna' showed 98% of similarity among them. The higher polymorphism observed in the study was due to the intraspecific variation among the turmeric cultivars.

2.1.5. Intraspecific diversity

There is wide variability present between seed setting species and those which propagate through the vegetative mode of reproduction, which ultimately resulted in the dispute concerning species concepts between taxa (Škorničková *et al*., 2010). In addition, some *Curcuma* species tend to hybridise in the wild and thus result in the progenies which may develop into new genotype (Škorničková and Sabu, 2005; Škorničková *et al*., 2007) which make the species concept even more challenging. Various species of *Curcuma* are reported to exhibit morphological variation at the intraspecific level (Apavatjrut *et al*., 1999) for growth, yield, and quality parameters (Pandey and Chowdhury, 2003; Angel *et al*., 2008; Hanchinamani, 2012; Angel *et al*., 2014). Floral characters of some of the *Curcuma* species showed intraspecific variation as two types of spikes were reported in the same species. *C. pseudomontana* exhibited larger spike coming out from the side of the leaves at the beginning of the rainy season but gradually by the beginning of August the lateral spike decayed and central spike appeared surrounded by leaves, which ultimately resulted in the presence of two types of spikes in the same plant (Santapau, 1952). Likewise, bract colour variation within the species is also reported by many authors (eg. *C. ecalcarata*) (Santapau, 1952; Sabu,1991; Sasikumar, 2005).

Curcumin, oleoresin, essential oil content, starch etc. are reported to be varying within the species with respect to explant, methodology, place of collection, agro-climatic conditions and also by genotypes (Zwaving and Bos, 1992; Srivastava *et al*., 2001; Behura *et al*., 2002; Paliwal *et al*., 2011).

Pothitirat (2006) reported variation in volatile oil and curcumin content in dried powder of *C. longa* collected from ten locations of South, Central, North and Northeast parts of Thailand. The study concluded that essential oil and curcumin content varied from location to location. Plants growing under cool climate produced highest essential oil and those growing in rainfed regions had the highest curcumin content.

Bahl *et al*. (2014) studied variation in morphological features, leaf and rhizome essential oil content, yield and quality, curcumin content in the rhizome, curcumin yield and the potential for curcumin extraction from rhizomes of 84 accessions of *C. longa*, collected from various geographical locations of Northern India (Bihar, Haryana, Uttarakhand and Uttar Pradesh). All the assessed characters showed higher variation. Number of leaves (2 to 84); rhizome

yield (21g to 3.5kg); essential oil content of fresh leaves (0.05% to 0.83%) and curcumin content (0.33 to 1.55%) varied among the accessions. The accessions displayed wide variation for the contents of leaf essential oils (terpene, 1,8-cineole and cymene) and the rhizome (myrcene, pinene, Ar-curcumene and turmerones) essential oils.

Cintra *et al*. (2005) evaluated genetic divergence among 21 accessions of *C. longa L*. based on morpho-agronomical traits. Multivariate analysis grouped 21 accessions in to five clusters. The genetic divergence was mainly contributed by two traits; dry weight and curcuminoids content. Accessions 20 and 21 formed separate group in the multivariate analysis owing to its high dry weight and low curcuminoids content. The study suggested potential use of accessions 20 and 21 as an alternative starch source due to its dry weight. Accession 19 showed most favourable traits in terms of dry weight and curcuminoids content.

Soontornchainaksaeng and Jenjittikul (2010) reported variation in rhizome morphology for Wan-chak-motluk (Thai local name for native *Curcuma* species) including *C. comosa*, *C. elata* and *C. latifolia*. Chromosome numbers, floral and leaf morphology along with inflorescence, were used to distinguish wan-chak-motluk into five cultivars belonging to three species: *C. comosa, C. elata,* and *C. latifolia.* The rhizome shape varied from large ovoid to ovate spheroidal shape and size of the rhizome from 8cm to 15 cm. Rhizome morphology varied not only among the species but also within the species as well as in cultivars according to their geographical location and cultivation. The study also highlighted the intraspecific variation for chromosome numbers. Chromosome numbers observed in *C. comosa*, were 2n = 62, 63 and 64; whereas in *C. latifolia*, 2n=63 and 84.

Paisooksantivatana *et al*. (2001a) evaluated genetic diversity in *C. alismatifolia* Gagnep. populations collected from cultivated and wild population in Thailand using allozyme polymorphism. High genetic diversity was observed in both cultivated and natural populations in which genetic diversity in cultivated population was comparatively lower than natural population. Among the natural populations, a high land population (H2) existed as an intermediate cluster between natural and cultivated population. Perusal of results showed a tendency of higher genetic diversity towards high altitude and origin of *C. alismatifolia* is found to be closely related to highland population.

Paisooksantivatana *et al*. (2001b) studied the genetic variation and genetic relationship between cultivated and wild population of *C. alismatifolia* collected from Thailand utilizing isozyme polymorphism. The study revealed low genetic diversity in cultivated population compared to wild population which was resulted from intensive selection and clonal

multiplication. Separation of H1 population collected from highest elevation into a distinct cluster is believed to be resulted from isolation and adaptation to new environment as well as gene transfer from a related species *C. aff. parviflora* Wall. The cultivated plants showed a closer association with wild plants from highland region than with lowland ones.

Islam *et al*. (2005) investigated genetic diversity among different populations of *C. zedoaria* collected from various geographical locations of Bangladesh using RAPD marker. The results indicated that population from hilly areas exhibited higher genetic diversity than plain and plateau land populations where the anthropogenic activities are prevalent. Moreover, the population from hilly areas showed a similar level of genetic diversity and thus they were difficult to discriminate from each other. Whereas plain and plateau populations showed a clear distinction in the 2D (dimensional) plot of Principal Coordinates Analysis (PCoA). The study revealed a presence of high intrapopulation genetic diversity than interpopulation diversity.

Islam *et al*. (2007) investigated intra and interpopulation genome size variation, chromosome number variation as well as genetic variation using RAPD markers in *C. zedoaria* collected from various parts of Bangladesh. The study showed that all the populations contained 2n=63 chromosomes with 2-3 satellite chromosomes with elongated secondary constriction. Significant variation was observed for 2C nuclear DNA content among the individual populations. Genetic diversity analysis using RAPD markers revealed that hilly populations possessed higher genetic diversity than plain and plateau land populations.

Skornickova *et al*. (2007) investigated chromosome number and genome size variation in *Curcuma* species collected from India. Most of the species showed low intraspecific genome size variation (3.4% on average). However, five *Curcuma* species *viz., C. caesia, C. longa, C. montana, C. prakasha* and *C. raktakanta* showed intraspecific variation for nuclear DNA content, maximum being in cultivated *C. longa* (15.1%).

Komatsu *et al*. (2008) investigated the relationship between genotype and phenotype of *C. kwangsiensis,* using morphological, genetic and chemical polymorphism in main cultivation areas of Guangxi Zhuangzu (Autonomous region) and Guangdong Province, China. *C. kwangsiensis* in the cultivation fields showed morphological variations including the season of flowering. *C. kwangsiensis* in Guangxi A. R. showed different morphology not only among different cultivation fields but also in the same field.

Hussain *et al*. (2008) studied the genetic diversity among the accessions of *Curcuma* species (*C. latifolia, C. malabarica, C. raktakanta* and *C. longa*) including the morphotypes of *C.*

longa collected from different habitat and conserved in the *in vitro* gene bank at NBPGR, New Delhi using RAPD markers. Cluster analysis separated 30 accessions in to two main clusters; cluster 1 comprised of two accessions of *C. longa* with unknown location and remaining accessions formed the cluster 2. Accessions of *C. longa* clustered according to their place of collection. Individual dendrogram constructed using 14 accessions collected from South India (34-69%), 7 accessions from North-East India (32-69%) and 13 morphotypes of *C. longa* showed considerable diversity (18-68%). Accessions of *C. longa* showed higher diversity which may be attributed to natural or conscious selection of genotypes by farmers/breeders over time which helped clones to evolve and adapt to local geographical location.

Zou *et al*. (2011) studied genetic diversity of six *Curcuma* species (*C. longa, C. phaeocaulis, C. sichuanensis, C. kwangsiensis, C. wenyujin* and *C. chuanhuangjiang*) using RAPD markers. The study showed close genetic relationship between *C. longa* and *C. sichuanensis* which was further supported by their similar floral, vegetative and rhizome characters. Two accessions of C. *kwangsiensis* showed morphological variation for stem and leaf midrib colour as one was blue and other was mauve. However, both the accession showed a low genetic variability between them and grouped under the same cluster. The study concluded that the morphological variation observed might be due to the growing environment.

Sigrist *et al*. (2011) employed 17 microsatellites to study the genetic diversity in *C. longa* collected from various states of Brazil. In addition to this, samples from India and Puerto Rico were also used for comparison. Most of the genetic variability in Brazil was found due to within the states. Genotypes from São Paulo State were found to be most divergent whereas genotypes from Minas Gerais State were found least divergent. When samples from India and Puerto Rico were included in the analysis, AMOVA study revealed major variation among the countries than between states of the countries.

Zheng *et al*. (2015) evaluated genetic diversity in five populations of *C. wenyujin* collected from various parts of China using RAPD and ISSR markers. Genetic diversity at the species level was higher than that those at the population level. The genetic differentiation among the population was relatively low indicating the occurrence of genetic variability mainly within population. The correlation study between geographical and genetic distance showed that genetic distance was independent of geographical distance.

Corcolon *et al*. (2015) studied genetic diversity in 22 turmeric rhizomes collected from seven provinces (Davao del Sur, Davao del Norte, South Cotabato, North Cotabato, Lanao del Sur, Sultan Kudarat and Maguindanao) of Mindanao, Philippines using RAPD markers. High

percentage of polymorphism obtained from the analysis indicated existence of genetic diversity in the turmeric accessions. In general, the clustering pattern did not give any location specificity. However, certain accessions from north cotabato, south cotabato and Maguindanao showed location specificity in their clustering. The probable reason behind the lack of location specificity and observed variation may have arisen from exchange and introduction of genotypes from one place to another in the form of planting material.

2.1.6. Species under study

2.1.6.1. *Curcuma amada* Roxb.

Curcuma amada Roxb. well known as mango ginger, is a perennial, rhizomatous aromatic herb. The rhizomes are morphologically similar to ginger (*Zingiber officinale* Rosc.) but impart a distinct raw mango flavour (Policegoudra *et al*., 2011; George and Britto, 2016). *C. amada* is found as wild in parts of West Bengal, and cultivated in Kerala, Tamil Nadu, Karnataka, Gujarat and Uttar Pradesh (Policegoudra *et al*., 2011).

2.1.6.1.1. Uses

Mango ginger is mainly used in culinary preparations *i.e*. they are used for making pickles. In addition to their use in pickles, they also find a pivotal role in candies, salads, sauces, chutneys and other medicines (Mridula and Jayachandran, 2001). Rhizomes find application in Ayurvedic medicines as appetiser, antipyretic, aphrodisiac, etc. It is also used as diuretic, expectorant, emollient, antipyretic and appetiser in the Unani system of medicine (Hussain *et al*., 1992; Warrier *et al*., 1994; Policegoudra *et al*., 2008). Rhizomes are also used for sprains (Chopra *et al*., 1980). Extensive studies on *C. amada* revealed that they have many bioactive components that can be exploited medicinally for various ailments. Essential oil reported the presence of α–pinene, car-3-ene and cis–ocimene as responsible for the characteristic mango flavor (Chopra *et al*., 1980; Choudhury *et al*., 1996; Srivastava *et al*., 2001; Singh *et al*., 2003; Mustafa *et al*., 2005; Policegoudra *et al*., 2011). *C. amada* can be used as a natural source of phenolic and terpenoid compounds, as well as a source of starch for developing products with health benefits (Policegoudra and Aradhya, 2008; Policegoudra *et al*., 2011). Crude rhizome extracts of mango ginger displayed potent antifungal activity against dermatophytic fungus *Trichophyton rubrum*, which causes skin infections in humans and also against the fungus *Aspergillus niger* (Gupta and Bernerjee, 1972). They are used externally in the form of a paste or combined with other medicinally important plants for bruises and skin diseases (Nadkarni and Nadkarni, 1982).

Ethnomedicinal studies have suggested that *C. amada* is one of the medicinally important plants, among the tribes of India. Tribes of Similipal bio-reserve, Orrisa used whole plant paste with crushed long peppers (*Piper longum* L.) for the treatment of piles (Kambaska, 2006). Rhizomes are used for improving blood quality. It is also used therapeutically as a carminative, stomachic and also for sprains (Rao *et al*., 1989; Kapoor, 1990).

Mango ginger is reported to have anti-inflammatory, antibacterial, antifungal, anti-tubercular, anticancer potential (Ghosh *et al*., 1980; Bhat *et al*., 1981; Majumdar *et al*., 2000; Chandarana *et al*., 2005; Jatoi *et al*., 2007; Krishnaraj *et al*., 2012; Angel *et al*., 2013; Ramachandran *et al*., 2015).

The extracts of mango ginger can also be used as a natural food preservative to inhibit the growth of foodborne pathogens, and the natural mango flavour will give an added advantage in culinary preparation (Policegoudra *et al*., 2006).

Leaf extracts and crude methanol, ethanol and aqueous extracts of mango ginger rhizomes can be used as a natural source of antioxidants for the pharmaceutical industry (Srinivasan and Chandrasekhar, 1992; Chirangini *et al*., 2004; Kumar *et al*., 2013; Angel *et al*., 2013; George and Britto, 2016).

C. amada is also an important source of biopesticide. Rhizome extract and essential oils from *C. amada* showed repellent activity against housefly (Singh and Singh, 1991) pulse beetle and weevils (Ahmed and Ahmed, 1991; Ahmad and Ahmad, 1992).

2.1.6.1.2. Morphology and variability

C. amada usually grows to a height of 60 – 90 cm in length with a long petiolate, oblong–lanceolate leaves tapering at both ends. Each plant bears 6 – 7 number of leaves that are glabrous with hairy tip (Warrier *et al*., 1994). Lateral inflorescence (rarely central) is produced earlier in the season (Syamkumar, 2008). Flowers are white or pale yellow (Rao *et al*., 2008). Rhizomes of the plants are fleshy and pale yellow to brown colour or buff coloured outside and creamy – yellow inside, and they are cylindrical or ellipsoidal branched (Policegoudra *et al*., 2011). Branching is sympodial. Fully matured plants yield 1.5 – 2 kg of rhizomes (Policegoudra *et al*., 2011). According to Srivastava *et al*. (2006), it is rather difficult to distinguish herbarium specimens of *C. amada* from *C. longa*.

Sabu (2006) and Syamkumar (2008) reported a different range of values for a number morphological characters in *C. amada* like plant height, leaf length, leaf width and number of leaves per tiller. Similarly, petiole length also found to vary from 5-10cm (Ravindran *et al*., 2007) to 14-26cm (Jatoi *et al*., 2015) in *Curcuma*.

Rao *et al*. (2008) investigated the natural variation of morphological traits and its correlation in a population of *C. amada* collected from random places (Andhra Pradesh, Maharashtra, Orissa private nurseries, wild habitats and organizations) and maintained under uniform conditions at the farm of Indian Institute of Integrative Medicine, Jammu. The highest variation was recorded for plant yield, rhizome weight and finger weight while the lowest was for finger length, leaf length and sheath length. β-myrcene, the major component of essential oil was found to vary from 7.2-92.5%. Finger weight had significant positive correlation with rest of the characters except sheath length. Study indicated that plant height, leaf length and herb yield enhanced the finger weight and corm weight.

Variability studies on *C. amada* accessions collected from various parts of Kerala reported presence of significant variation for plant height, number of leaves, number of tillers, leaf length, leaf breadth, leaf area, number and length of primary and secondary fingers, length of mother rhizome and yield (Jayasree, 2009; Jayasree *et al*., 2014).

Jatoi *et al*. (2015) evaluated morpho-agronomic characterization and genetic variability pattern in *C. amada* collected from Myanmar. Out of the nine accessions acquired, four accessions were from gene bank, three from rural farmers and one from local market (ZO102) from Myanmar. One accession (ZO89) was collected from local market in Thailand located near to Myanmar. Plant height, sheath length, leaf length, finger rhizome thickness and rhizome weight showed high to moderate variation during two years (2005 & 2006). Principal Component Analysis showed variation in distribution pattern of the nine accessions. for the two years. During 2005, the gene bank accessions existed as a distinct entity in the scatter plot whereas rest of the accessions remained undistinguishable from each other. Whereas in the second year, *C. amada* collected from three sources remained scattered and mixed on the plot. Study revealed that market accessions displayed a higher variability than farm and gene bank accessions which emphasised importance of inclusion of local market samples in diversity studies.

2.1.6.1.3. Variability for quality traits

The rhizomes of *C. amada* have characteristic raw mango flavour and pungent taste. Various studies have attempted to identify the components responsible for the mango aroma of *C. amada*. Gholap and Bandyopadhyay (1984) identified three terpene hydrocarbons, *i.e*.α-pinene, car-3-ene and cis-ocimene, and proposed that car-3-ene and cis-ocimene are the two compounds responsible for the characteristic mango flavour, whereas α-pinene gives the aroma. However, in another study, myrcene, cis- and trans- hydroocimene and ocimene were

found to be the primary character-affecting compounds of *C. amada* volatile oils, indicating that the aroma of mango ginger is influenced by variety of compounds (Rao *et al*., 1989).

Various studies have been conducted to investigate the chemical composition of volatile oils of *C. amada* (Bandyopadhay, 1993). More than 130 chemical compounds have been reported so far in *C. amada* of which 121 have been identified. Most of the studies used rhizome for analysis. Various studies revealed that constituents of essential oil show quantitative and qualitative variation for both major and minor chemical constituents in both dry and fresh rhizome and thus this will bring difference in the aroma too (Rao *et al*.,1989; Bandyopadhay, 1993; Mustafa *et al*., 2005). Major constituents identified are myrcene, ocimene, ar-turmerone, (z)-β-farnasene, guaia–6, 9–diene, cis-β-ocimene, trans-hydroocimene, α-longiopinene, α-guaiene, linalool, β-curcumene and turmerone (Rao *et al*., 1989; Choudhary *et al*., 1996; Gupta *et al*., 1999; Srivastava *et al*., 2001; Singh *et al*., 2003; Mustafa *et al*., 2005).

Considerable variation was observed for the curcumin content of leaf and rhizome in *C. longa* and *C. amada* at different stages of growth (Mehta *et al*.,1980). Comparison of essential oil from leaves and rhizomes of *C. amada* grown in foot hills of Uttarakhand, India, showed quantitative and qualitative variation for essential oil constituents (Padalia *et al*., 2013). According to Mridula and Jayachandran (2001) varying level of mineral nutrients plays an important role in the quality of volatile oil, non-volatile oil, fibre and starch of mango ginger. Study revealed that nitrogen and phosphorous application increased the volatile oil and fibre content. NPK supplied plants showed a higher starch content. Varying nutrients in the soil might be one of the reasons behind variation in quality traits in *C. amada* population collected from different location.

Policegoudra and Aradhya (2008) conducted a detailed study on structure and biochemical properties of starch from *C. amada*. Starch from *C. amada* exhibited peculiar structural and biochemical properties of its own. Scanning Electron Microscopy (SEM) revealed significant variation in shape and size of the starch granules, as they varied from oval, elliptic, irregular or cuboidal and polygonal. The starch from *C. amada* occupied in a position between turmeric and ginger starch.

Sajitha and Sasikumar (2015) reported qualitative variation in starch content among the four *Curcuma* species namely, *C. amada, C. caesia, C. aromatica* and *C. xanthorrhiza*. Scanning electron micrographs revealed variation in the shape and size of starch granules of *C. amada* as they varied from oval to elliptical shape with a smooth surface. Among the four species,

C. amada yielded the good amount of starch and topped in most of the physiochemical properties and thus it can be used as a suitable alternative starch source in food industries.

Jamir and Seshagirirao (2017) reported variation for size and shape of the starch granules isolated from four starchy *Curcuma* species including *C. amada*. The shape of the starch granules varied from oval to elliptical with size ranging from 10-30μm.

2.1.6.1.4. Molecular variability

Most of the studies on *C. amada* have focused on its phytochemical and pharmacological properties. However, very few studies have been done on its variation for morphological traits and molecular variations (Rao *et al*., 2008; Gilani *et al*., 2015).

Jatoi *et al*. (2006) used Rice SSR markers as RAPD markers to study the genetic diversity in three genera (*Zingiber*, *Alpinia* and *Curcuma*) of family Zingiberaceae. Three *C. amada* genotypes (ZO 18-1, ZO 23-1 and ZO 49) collected from Myanmar and Malaysia were considered under genus *Curcuma*. The number of amplified fragments ranged from 1.0 – 8.0 in *C. amada.* Three genera were clustered into four groups in which *Zingiber barbatum* clustered separately. *C. amada* formed a separate cluster which was close to *Alpinia officinarum*. In the cluster containing *C. amada*, Zo 18-1 and Zo 23-1 collected from Myanmar found closer to each other than ZO 49 from Malaysia. Results from cluster analysis was comparable with Principal Component Analysis (PCA). Although all the genera under study were distantly related, some of the sequenced fragments showed sequence homology of more than 95% among the genera.

Angel *et al*. (2008) studied genetic diversity in 11 starchy *Curcuma* species including *C. amada* collected from National Bureau of Plant Genetic Resources (NBPGR) Thrissur, Kerala. The genetic variability among the species was assessed by the level of polymorphism and degree of genetic relationship based on the cluster analysis. The results showed high level of genetic variability among the species, and there was no location specificity observed in the study. *C. amada* formed a subgroup with *C. caesia* and *C. malabarica.*

Ahmad *et al*. (2009) studied intergeneric and intrageneric variation of chloroplast DNA present in 11 species in four genera of Zingiberaceae acquired mainly from Myanmar using cpDNA primers and restriction analysis. *Curcuma* species showed interspecific variation in the restriction profiles of five chloroplast DNA, *trns-trnfM* being the most informative gene region. Cluster analysis discriminated various accessions at intergeneric and intrageneric level. Cluster I, II and III were formed by *Zingiber* species, V and VI by *Kaempferia* and *Alpinia*. Cluster IV, being the major cluster was formed by *Curcuma* species which was

further divided in to sub clusters. Three accessions of *C. amada* were grouped into two different subclusters; when ZO 43-1 and ZO 45-1 grouped together, accession ZO 23-1 grouped with *C. longa* showing intraspecific variation. The results evidenced interspecific and intergeneric variation across the tested samples.

Neutral (rice SSR-based RAPDs) and functional genomic (P450 based analogue) markers were utilised to study the genetic variation in mango ginger (*C. amada*) procured from farmers and Ex situ gene bank in Myanmar. The accessions acquired from gene bank represented central and Eastern part of the Shan state in Myanmar whereas those collected from farmers were landraces from Mandalay division. The high polymorphism, *i.e*. greater than 91% exhibited the existence of high genetic diversity among the germplasm studied. The gene bank accessions showed comparatively higher genetic diversity than farmers' accessions. The major source of molecular variance (85%, 93%) was explained within farmers and gene bank accessions whereas only 15% and 7% variation was accounted for between collection source as assessed using PBA and RSB-RAPD markers. The study also proposed intraspecific variation in the neutral regions more than the functional regions which resulted in the amplification of a higher number of alleles. The study also highlighted the importance of using two marker systems for genetic variability study as they cover different part of the genome (Jatoi *et al*., 2010).

Genetic diversity and proximity of nine *Curcuma* species from North-East India were assessed using PCR-based markers (RAPD, ISSR and AFLP). *C. amada* (Amingaon, Assam) and *C. zedoaria* (Darrang, Assam) clustered as two extremes in the dendrogram constructed using RAPD profile. In ISSR analysis, *C. amada, C. angustifolia* and *C. zedoaria* were found to be genetically closer to each other, whereas AFLP analysis grouped *C. amada* along with other multivariate species like *C. zedoaria* and *Curcuma* spp. in the same subset. The observed variation in the clustering pattern generated using different markers is because different markers scanned different segments of genome (Das *et al*., 2011).

Mohanty *et al*. (2014) studied the genetic diversity among and within two populations of Zingiberaceae belonging to two genus *Curcuma* and *Zingiber* using RAPD, ISSR and SSR markers. Among the ten species evaluated, *Zingiber officinale* and *C. longa* were the cultivated varieties whereas rest of the species were from wild habitats. At genomic level, cultivated and wild species separated from each other. Moreover, all the species grouped exclusively under their own respective genera. Jaccard's Similarity coefficients showed a closer association between *C. aromatica* and *C. amada*.

Akkara and Thaliyangal (2014) studied genetic stability in fifteen micropropagated *C. longa* morphotypes and ten wild *Curcuma* species including *C. amada* using RAPD markers. In-vitro cultures of *C. amada* were able to show stability in genetic makeup until fifth or sixth stage of subculture and after that genetic variability was observed in RAPD banding pattern of OPA05, OPC 05, OPD 07 and OPH 01. The study presumed influence of DNA methylation and increased subculture interval for the stability/instability of In-vitro cultures. The study also highlighted the importance of periodical monitoring of in-vitro cultures for their genetic stability.

Gilani *et al*. (2015) studied the molecular genetic diversity of curcuminoids genes in *C. amada* using 8 curcumin containing accessions and 6 curcumin free accessions. Flow cytometry study revealed that the ploidy level was higher in curcumin-containing accessions than in curcumin-free accessions of *C. amada*. Curcumin containing accessions showed a higher level gene expression (*CURS* and *DCS* genes) than rest of the accessions with an exception of one curcumin free accession (Z129) showing higher level of gene expression.

Saha *et al*. (2016) used 20 ISSR markers to study the genetic diversity at interspecific and intraspecific level among four *Curcuma* species (*C. caesia, C. amada*, *C. longa* and *C. zedoaria*) collected from Suryamaninagar, Jampui Hill, Madhupur and Baramura Hill of Tripura. Cluster analysis showed the presence of two clusters; *C. amada* and *C. longa* grouped under same cluster and *C. caesia* and *C. zedoaria* in another cluster. *C. amada* displayed affinity with cultivated population of *C. longa*. Moreover, they were similar for several morphological characters although they differed from each other for aroma and rhizome colour. Due to the difference in ploidy level, the somatic chromosome number varied in *C. amada* (42) and *C. longa* (63), however, it did not affect the similarity indices between them. Out of the two cultivated population of *C. longa*, cultivated *C. longa*3 and *C. longa* 1 from wild habits were found genetically closer to each other.

2.1.6.2. *Curcuma aromatica* Salisb.

Curcuma aromatica Salisb, widely known as ‘wild turmeric’ (Anoop, 2015), or ‘Kasturi Manjal’ in Malayalam is an aromatic medicinal plant, well known for its multifaceted uses. *C. aromatica* is being used in traditional systems of medicine like Ayurveda and Unani. *C. aromatica* is known as ‘Vanaharidra’ in Ayurveda (Joy *et al*., 1998; Anoop, 2015). Other than India, south-east Asian countries also explored the various uses of *C. aromatica*. Recently *C. aromatica* have become a novel plant material in pharmacological research (Sikha and Harini, 2015). *C. aromatica* is distributed in China, South Sri Lanka (Ravindran

et al., 2007) and South Asian region. In India it is cultivated in Kerala and Bengal (Ahmed *et al*., 2008).

2.1.6.2.1. Uses

C. aromatica is one of the most important plants from the Zingiberaceae family with highly potent pharmacological activities. The plant consists of many medicinally important chemical constituents in its essential oils. Rhizomes of *C. aromatica* are used as an appetiser and are useful in leucoderma and diseases of the blood. The plant has also been widely studied for various pharmacological activities such as antimicrobial, antidiabetic, antifungal, antibiotic, anti-inflammatory, antiangiogenic, anti-tumour, anti-melanogenic, anti-fibrosis, antioxidant, wound healing etc. (Husain *et al*., 1992; Kim *et al*., 1997; Jiang *et al*., 2005; Thippeswamy and Salimath, 2006; Ahmed *et al*., 2008; Marina *et al*., 2008; Jantan *et al*., 2008; Al Reza *et al*., 2010; Hu *et al*., 2011; Al-reza *et al*., 2011; Rajamma *et al*., 2012; Shahwar *et al*., 2012; Srividya *et al*., 2012; Rajiv *et al*., 2013; Zhao *et al*., 2014; Sikha and Harini, 2015) along with many miscellaneous activities, which are yet to be explored. They are also used to treat gastrointestinal ailments, insect bites, skin infection and arthritic pain (Santhanam and Nagarajan, 1990; Bakkali *et al*., 2008; Sharmin *et al*., 2013). The rhizome of *C. aromatica* has cosmetic application; its paste is used for enhancement of skin tone and complexion, to cure acne and reduce excessive facial hair growth (Chetana *et al*., 2012; Sikha and Harini, 2015).

Essential oil and various organic extracts (hexane, chloroform, ethyl acetate, dichloromethane, aqueous and methanol) from leaves and rhizomes of *C. aromatica* exhibited remarkable antibacterial activity against gram-positive, gram-negative bacteria (Singh *et al*., 2004; Ahmed *et al*., 2008; Sharma *et al*., 2010; Al-reza *et al*., 2011; Rajamma *et al*., 2012; Sharmin *et al*., 2013; Revathi and Malathy, 2013; Rachana and Venugopalan, 2014; Anjusha and Gangaprasad, 2014). *C. aromatica* is an effective personal protection measure against mosquito bites (Kojima *et al*., 1998; Pitasawat *et al*., 2003; Choochote *et al*., 2005) and has larvicidal activity (Madhu *et al*., 2010).

Recent studies showed that *C. aromatica* oil consist of many types of anti-tumour ingredients such as germacrone, β-elemene, curcumol, curdione and curcumin, which showed anti-tumorous properties in wide spectrum of cell lines (Shi *et al*., 1981; Jee *et al*., 1998; Wu *et al*., 2000; Deng *et al*., 2004; Hou *et al*., 2011; Yu *et al*., 2011; Zhi-jun *et al*., 2013; Liu *et al*., 2014).

2.1.6.2.2. Morphology and variability

C. aromatica is an erect, perennial herb. Rhizomes are large, tuberous and creamy coloured inside. They have unique aromatic taste and camphoraceous smell. The plant is morphologically more similar to *C. longa* (Das *et al*., 2011). They have large green leaves, which are oblong – lanceolate or oblong-epileptic with acuminate apex. The leaves are pubescent below, base deltoid with long petioles. Flowers are fragrant, white and pink toned with an orange lip and are borne on peduncles with the crown of bracts. Flowers are shorter than bracts, spikes 15-30cm long; ovate, recurved, rounded at the tip, pale green, connate below forming pouches for the flower. Flowering bracts are 3.8-5.0 cm and coma bracts are 5.0-7.5cm long, with a pink tinge (Warrier *et al*., 1994; Ahmed *et al*., 2008). Leaf length, leaf width, leaf texture and number of leaves per tiller varied within the species (Sabu, 2006; Syamkumar, 2008).

2.1.6.2.3. Variability for quality traits

Medicinal properties of *C. aromatica* is mainly due to its essential oil, which is an excellent source of mono-sesquiterpenes. Marked variations have been observed in the chemical constituents of *C. aromatica*, particularly essential oils (Tsai *et al*., 2011).

Choudhury *et al*. (1996) reported marked variation in essential oil content extracted from leaves, petioles and rhizomes collected from Assam. The percentage of essential oil constituents varied from 86.3% (petiole and rhizome) to 96.8% (leaf). The major components like 1,8-cineole (20.0%, 8.8% and 9.3%,), camphor (18.0%, 16.8% and 25.6%), isoborneol (6.4%, 6.8% and 8.2%), germacrone (11.8%, 0.2% and 10.6%), and camphene (9.4%, 1.2% and 7.4%) were found to be varying in leaf, petiole and rhizome sources.

GC-MS analysis of the essential oil isolated from *C. aromatica* collected from Japan and India showed marked variation for its constituents. Oil from Japanese rhizome mainly contained curdione, germacrone, 1,8-cineole, (45,55)-germacrone–4, 5-epoxide, β-elemene and linalool, whereas the oil from Indian rhizome was comprised of β-curcumene, ar-curcumene, xanthorrhizol, germacrone, camphor and curzereonone (Kojima *et al*., 1998). It may be due to the difference in geographical and climatic conditions (Behura *et al*., 2002).

Essential oil constituents from leaves of North India comprised of major components such as camphor, curzerenone, ar-turmerone, 1,8-cineole and α-turmerone, whereas rhizome oil consisted mainly camphor, curzerene, α-turmerine, ar-turmerine and 1,8-cineole (Bordoloi *et al*., 1999). Various other studies reported compounds like curzerenone, isoborneol and camphene in the rhizome and limonene in leaf oil; caryophyllene oxide, patchouli alcohol

and elsholtzia ketone in the petiole oil (Choudhary *et al*., 1996;). Twenty-three compounds representing 94.29% of the total hydro distilled essential oil were identified using GC-MS analysis. Major components were camphor (26.32%), borneol (16.45%), vinyl dimethyl carbinol (12.21%), caryophyllene oxide (6.33%), cubenol (5.59%), cucumber alcohol (5.19%), ledol (3.84%) and germacrene D (3.45%) (Al-reza *et al*., 2010).

Major components like α and β pinene, camphene, 1,8-cineole, isofuranogermacrene, camphor, isoborneol, borneol, germacrone and tetra methyl pyrazine were obtained from Chinese *C. aromatica* (Guo *et al*., 1980). Essential oils isolated from rhizomes collected from India and Indonesia contained about 19% ar-curcumene, 26% β-curcumene and 26% xanthorrhizol (Zwaving and Bos, 1992). Kuroyanagi *et al*. (1987) isolated three new sesquiterpenes, isozedoaronchiol, and methyl zedoarondiol and neocurdione from *C. aromatica* collected from Japan. Whereas essential oil from *C. aromatica* obtained from Thailand showed camphor (26.94%) ar-curcumene (23.18%) and xanthorrhizol (18.70%) as the main components of essential oils (Jarikasem *et al*., 2005).

C. aromatica Salisb var 'Bataguda' which was collected from high altitude research station in Orissa consisted of major compounds 1-8 cineole (28.01%) and linalool (7.67%) followed by α-pinene (4.74%) β-pinene (3.70%) and C8-aldehyde (2.62%). *C. aromatica* var 'Bataguda' showed a significant difference in the constituents when compared with other varieties from different location; e.g. Assam as germacrone, camphor and curdione are found to be absent in *C. aromatica* var 'Bataguda' (Behura *et al*., 2002).

Fattepurkar *et al*. (2009) detected variation for biochemical constituents in two collections of *C. aromatica* (CA 62/1, CA 62/2) grown in black cotton soil at Turmeric Research Centre, Sangali. The contents of moisture (7.01% and 7.52%) ash (4.72% and 4.84%) crude protein (7.09% and 4.05%) crude fat (2.87% and 2.66%), crude fiber (2.96% and 2.16%), total carbohydrate (76.35% and 78.31%) and acid value (4.66% and 4.73%) have been reported.

A sesquiterpene ketoxide, namely zederone and other compounds like β-sitosterol-3-0-β-d-gluco pyranoside were isolated for the first time from the ethyl acetate extracts of rhizomes from *C. aromatica* (Pant *et al*., 2001; Pant *et al*., 2013). Essential oil from the dried rhizome of *C. aromatica* using simultaneous steam distillation and solvent extraction apparatus detected two major compounds, curcumol (35.77%) and 1-8 cineole (12.22%) (Tsai *et al*., 2011).

Rajamma *et al*. (2012) reported variation in oleoresins and total phenol content in nine starchy *Curcuma* species including *C. aromatica*, *C. amada* and *C. caesia*. Antioxidant and anti-bacterial activities also varied from species to species. When *C. amada* showed

maximum antibacterial activity, *C. zedoaria, C. aromatica* and *C. caesia* showed highest antioxidant activities.

Angel *et al*. (2014) studied essential oil yield and composition in eight starchy *Curcuma* species including *C. aromatica*. Interspecific variation was observed for oil yield and compositions among the *Curcuma* species. Camphor, camphene and 1,8 cineole are the major three components detected in *C. aromatica*. The essential oil data was then subjected to cluster analysis to find out the relation between the species. Eight *Curcuma* species were grouped in to four clusters. *C. aromatica, C. brog* and *C. caesia* formed the fourth cluster. The classification of *Curcuma* species using essential oil data showed similarity with the classification made using RAPD markers on starchy *Curcuma* species (Angel *et al*., 2008).

Lee *et al*. (2014) studied variation in metabolic profiling of terpenoids and curcuminoids in *C. aromatica* and *C. longa* collected from two locations (Jeju-do and Jin-do) in South Korea. The study revealed that significant variation for curcuminoids and terpenoids within the plants were influenced by species and geographical locations.

The scanning electron microscopic study revealed variation in shape and size of starch granules in *C. aromatica* as the size varied from 9-60 μm in length and 6-24μm in width and oval to elliptical in shape. Starch granules of *C. aromatica* were large, flat and with surface ornamentation of concentric rings (Sajitha and Sasikumar, 2015). Jamir and Seshagirirao (2017) reported shape of starch granules in *C. aromatica* varying from large, flat, elongated to polygonal with size ranging from 5-28 μm.

2.1.6.2.4. Molecular variability

Syamkumar and Sasikumar (2007) used RAPD and ISSR markers to study the genetic diversity/relatedness of 15 *Curcuma* species including *C. aromatica*. In the UPGMA based cluster analysis, 15 *Curcuma* species were categorised into seven groups. The results were almost in congruence with the classification based on morphological characters. *C. aromatica* collected from two different places of Kerala (NBPGR Thrissur and Wayanad) formed a single group along with *C. haritha*. Two *C. aromatica* species also resembled closely for their morphological characters. As *C. aromatica* is a seed setting species, the observed variation for morphological traits is believed to be due to the seedling variation.

Islam (2004) conducted genetic diversity study on 16 *Curcuma* species collected from Bangladesh using RAPD markers. The main goal of the study was to find out the inter and intraspecific diversity level among the species as well as to testify whether wild/cultivated and rare/dominant species shows significant differentiation at genetic level. Results

evidenced that cultivated species like *C. aromatica, C. longa* and *C. amada* were separated from wild species. Among the three species, *C. longa* (Gazipur) and *C. aromatica* (Chittagong) were close to each other than *C. amada* (Gazipur). The AMOVA analysis revealed high level of genetic variability present within the species as the variation for individual plants within the species were 58.90% and 79.55% for wild/cultivated and rare/dominant species respectively.

Ahmad *et al*. (2009) studied genetic variation of chloroplast DNA present in 11 species including *C. aromatica* in four genera of Zingiberaceae mainly from Myanmar using Restriction Fragment Length Polymorphism (RFLP). The study showed significant intergeneric and intrageneric variation among the species. *Curcuma* species displayed interspecific variation for the five chloroplast DNA regions. The region *trns-trnsfM* was found to be the most informative part of *Curcuma* species. In the dendrogram, two accessions of *C. aromatica* were closely related, and they showed affinity with *C. xanthorrhiza* and *C. zedoaria*.

The interspecific genetic diversity of 7 *Curcuma* species using AFLP markers revealed that *C. angustifolia* was placed in a separate cluster inferring to its wild nature. But *C. caesia*, *C. aromatica* and *C. domestica* II (with dark yellow rhizome with strong odour collected from Nagaland) formed the same subgroup. The reason may be because both *C. domestica* II (Nagaland) and *C. aromatica* (Nagaon) are highland species which possess geographic similarity and have a peculiarly strong aroma and are thus found to be related. *C. aromatica* was found to be closely associated with *C. longa* for ISSR markers whereas, in case of RAPD markers, *C. domestica* I, II and *C. aromatica* clustered together owing to its physiological similarity of strong aroma (Das *et al*., 2011).

Mohanty *et al*. (2014) studied the genetic diversity among and within two populations of Zingiberaceae belonging to two genus *Curcuma* and *Zingiber* collected from Eastern India (Odisha and West Bengal) using RAPD, ISSR and SSR markers. Among the 10 species, *C. longa* and *Z. officinale* were the cultivated varieties and rest of the species were from wild populations. In cluster analysis all the species grouped under their own respective genera and showed a clear distinction between cultivated and wild species. Jaccard's Similarity coefficients of RAPD data showed a closer association between *C. aromatica* and *C. amada.* Similarly, combined markers (RAPD, ISSR & SSR) showed a closer association between *C. aromatica* and *C. amada.* Whereas in ISSR profile, *C. caesia* and *C. aromatica* were found to be closely associated.

Genetic diversity/relatedness at interspecific level in *Curcuma* species (*C. longa, C. aromatica, C. aeruginosa, C. zedoaria, C. caesia, C. haritha* and *C. ecalcarata*) collected from Kerala (Kannur and Thiruvananthapuram districts) were assessed using ISSR markers. Nine *Curcuma* species were grouped in to two major clusters. The similarity values ranged from 0.42 to 0.96 suggesting a high level of genetic diversity among the species. *C. aromatica* grouped with *C. ecalcarata;* they shared similar aerial morphology and rhizome characters. *C aeruginosa* and *C. zedoaria* clustered with *C. longa*, whereas *C. caesia, C. haritha* and *C. amada* formed outliers (Seema, 2015).

2.1.6.3. *Curcuma caesia* Roxb.

Curcuma caesia Roxb., commonly known as 'black turmeric' is a perennial herb with bluish-black rhizomes. Black turmeric is native to Northeast and Central India. The plant is also found in Papi hills of East Godavari, the foothills of the Himalayas and North Hill Forest of Sikkim (Baghel *et al*., 2013). The species is found in India, Java and Myanmar (Sharma *et al*., 2011).

2.1.6.3.1. Uses

The inner part of the rhizomes possesses characteristic sweet smell, due to the presence of essential oil and thus 'Turkomans' used these tubers as a rubefacient to rub their bodies after Turkish bath (Kirtikar and Basu, 1987). Northern tribes use black turmeric as a talisman to keep the evil spirits away, while in west Bengal, it plays an important place in the traditional system of medicine whereas in Madhya Pradesh the plant is regarded as very auspicious and it is believed that a person who possesses it will never experience a shortage of cereal and food (Pandey and Chowdhary, 2003). In Chattisgarh and Madhya Pradesh, tribes used rhizome as a folk medicine for the treatment of wounds, cold, cough, inflammation, piles, bronchitis, leucoderma, pneumonia, asthma, rheumatic pains, toothaches, infertility, etc. (Paliwal *et al*., 2011; Behar *et al*., 2014). Tribes of Assamese, Bodo, Mishing, Nepali and Santhal communities use fresh rhizome paste (50g) mixed with *Musa balbisiana* fruit 'barkash' and apply it once daily for curing gout, sprains and bruises. Likewise, Khampti, a major tribe of Arunachal Pradesh use a crushed paste of *C. caesia* to heal severe wounds and injuries (Saikia, 2006). Ethnobotanical survey on tribal healers inhabiting the Madhpur region in Bangladesh revealed that they used *C. caesia* to treat inflammation of tonsils (Mia *et al*., 2009).

Rhizomes of *C. caesia* are of high economic value owing to their alleged medicinal properties. The rhizomes are widely used in the treatment of epilepsy, asthma, leprosy, cancer, wound, haemorrhoids, fever, vomiting, menstrual disorder, smooth muscle relaxant (Karmakar *et al*., 2011).

The presence of various bioactive compounds is responsible for the anti-oxidative, anti-inflammatory, wound healing, hypoglycaemic, antibacterial, anticoagulant, antioxidant, anti-inflammatory, anti-ulcer and anticonvulsant, anti-asthmatic, anti-microbial, antifungal, analgesic, anthelmintic, anti-mutagenic, antipyretic and anti-ulcer activity of the *C. caesia* (Banerjee and Nigam, 1976; Rahman and Yusuf, 1996; Chirangini, 2004; Arulmozhi *et al*., 2006; Mannangatti and Narayanasamy, 2008; Mangla *et al*., 2010; Randeep *et al*., 2011; Krishnaraj *et al*., 2012; Das *et al*., 2012; Dhal *et al*., 2012; Jose and Thomas, 2014; Behar *et al*., 2014; Devi *et al*., 2015; Reenu *et al*., 2015; Shakya *et al*., 2015).

2.1.6.3.2. Morphology and variability

The plant is erect, ranging from 0.5 to 1 m in height which are differentiated into underground ovoid tuberous rhizome and upright shoot with leaves and flowers (Paliwal *et al*., 2011).

Rhizomes are cylindrical, finger shaped, bluish-black characterised with camphoraceous odour, bitter taste, about 2-8cm in diameter (Verma *et al*., 2010). The shape and size along with the intensity of the rhizome blue colour are often variable according to the nature of the soil and age of the rhizome (Seema, 2015). Rhizomes of *C. caesia* are laterally flattened, covered with adventitious roots, root scars and warts. Rhizomes are sessile and exhibits longitudinal circular wrinkles on the surface exhibiting the look of nodal and internodal zones to the rhizome (Behar *et al*., 2014). The surface of the rhizome is brown in colour with circular arrangements of remnants of scaly leaves, which gives a false impression of growth rings. The branching of the rhizome is sympodial. They have dark green leaves about 7 – 10 in numbers. Leaves are oblong-lanceolate and glabrous. Leaves are characterised with purple coloured to reddish brown leaf midrib (Seema, 2015). Petioles encircle each other forming a pseudo-axis, a typical characteristic of monocots (Sharma *et al*., 2011). Inflorescence of the plant is long dense which is 15-20 cm in length, which arises much before opening the leaf, the bracts are tinged with pink, and the bracts of coma are dark purple, which becomes crimson when old. Flowers are smaller than bracts, pale yellow with reddish border (Kritikar and Basu, 1965; Sharma *et al*., 2011). Calyx is 10-15 cm long, obtuse, three toothed and long tubular corollas, pale yellow, lip 3 – lobbed and semi-elliptic (Paliwal *et al*., 2011).

Spikes are positioned centrally or laterally with purple coloured calyx and corolla (Velayudhan *et al*., 1999).

Susngi and Laskar (2015) observed variation in characteristic ring formation in the rhizomes of *C. caesia* collected from various sites of Meghalaya. Samples acquired from Bhoirymbong, Pynursla and Shella had circular shapes whereas for rest of the samples, the shape varied from oval, elliptical to crescent shapes.

2.1.6.3.2. Variability for quality traits

A wide range of variation for essential oil content and its chemical components were reported in *C. caesia*. Various authors have reported different essential oil profile of *C. caesia*, which may be either because of false taxonomic identification of the specimen or due to environmental effects, maturity variations of the various varieties of rhizome, or different analytical techniques used for the extraction of oil (Behar *et al*., 2014).

Studies on essential oil contents of *C. caesia* reported various constituents such as 1,8-cineole (9.06%), ocimene (15.66%), δ-camphor (18.88%), 1-ar-curcumene (14.84%), δ-linalool (20.42%), δ-borneol (7%) and zingberol (12.60%) (Banerjee *et al*., 1984).

Behura (2000) reported chemical composition of essential oil in rhizome as α-pinene (0.60%), β-ocimene (E and Z) (2.1%), camphor (7.73%), linalool (0.99%), caryophyllene (3.15%), borneol (4.3%), camphene (1.67%), anethole (1.79%) and cis-b-ocimene (14.54%). Chattopadhyay *et al*. (2004) reported 30 components representing 97.48% of the oil with camphor (28.3%), ar-turmerone (12.3%), (z)-ocimene (8.2%), ar-curcumene (6.8%), 1-8, cineole (5.3%), elemene (4.8%), borneol (4.4%) bornyl acetate (3.3%) and curcumene (2.82%) as the major constituents. Behura and Srivastava (2004) reported that the volatile oil from *C. caesia* ranged from 1.5% to 1.8%. Essential oil content in the leaves contains α-pinene (1.5%), β-pinene (6.3%), myrcene (0.5%), limonene (2.1%), 1, 8-cineole (27%), camphor (1.68%), linalool (2.8%), β-elemene (2.4%), borneol (8.7%), α-terpenol (5.2%) and eugenol (2%).

Essential oil from *C. caesia* rhizome from Thailand was characterised by a high content of 1,8-cineole (30.4%) and a good amount of camphor (10.8%), curzerene (8.8%) and curzerenone (5.8%) (Pandey and Choudhary, 2003). This variation in camphor content motivated Pandey and Choudhary (2003) to conduct a detailed GC-MS investigation of *C. caesia.* They found that volatile constituents of the rhizome from central India has camphor (28.3%), ar-turmerone (12.3%), (z)-β-ocimene (8.2%), ar-curcumene (6.9%), 1, 8-cineole (5.3%), β-elemene (4.8%), borneol (4.4%), bornyl acetate (3.3%) and endo-fenchol (2.3%).

The study concluded that essential oil obtained from Indian collections were rather different from that of essential oil produced from Thailand, as it does not contain curzerene and curzerenone. Moreover, oil from the Thailand specimens displayed predominance of 1,8-cineole than camphor. Paliwal *et al*. (2011) based on GC-MS analysis of the volatile oil of *C. caesia* rhizomes from Madhya Pradesh reported 1, 8-cineole (27-48%) and camphor (14-28.3%) and ar-turmerone (12.3%) as the major constituents.

Rajamma *et al*. (2012) reported interspecific variation for oleoresins and total phenol content in nine starchy *Curcuma* species including *C. aromatica, C. amada* and *C. caesia*. Oleoresin content varied from 4% to 15%. High phenol content was detected in *C. caesia* than in *C. aromatica.* Antioxidant and antibacterial activities were also varied from species to species. When *C. amada* showed maximum antibacterial activity, *C. aromatica* and *C. caesia* showed high antioxidant activities.

A comparative study of phenol content and antioxidant activity of *C. caesia* and *C. amada* by Krishnaraj *et al*. (2012), reported interspecific variation for antioxidant activity and total phenol content in *C. caesia* (44.33 mg TAE $^{g-1}$) and *C. amada* 37.64mgTAE $^{g-1}$). Phenol content and antioxidant activities were significantly high in *C. caesia* rhizome extracts than the *C. amada*.

Reenu (2017) also reported varying values in *C. caesia* for starch (542.8-618mgg^{-1}), protein (82.1-121.9mgg^{-1}), soluble sugars (18.7-36.8mgg^{-1}), essential oil (2.2-3%), oleoresin (6.1-7.3%) and curcumin (0.022-0.032%) in six different accessions collected from various parts of India. Epicurzereonone and 1,8 cineole, the main essential oil constituents of *C. caesia* varied from 23.5 to 27.9% and 13.1 to 14.9% respectively among the accessions.

Scanning electron micrographs of starch granules revealed variation for size and shape of starch granules in *C. caesia* with shape varying from round to oval with smooth surface. The length of the starch granules varied from 10-39μm and width from 9μm to 23μm (Sajitha and Sasikumar, 2015). Similarly, Jamir and Seshagirirao (2017) also reported the variation in size (8-30μm) and shape (round to oval) of starch granules in *C. caesia.*

2.1.6.3.3. Molecular variability

Molecular studies in *C. caesia* is still in a primitive stage, and only a few studies are reported so far in *C. caesia* (Syamkumar and Sasikumar, 2007; Das *et al*., 2011).

Islam (2004) conducted genetic diversity study on 16 *Curcuma* species collected from Bangladesh using RAPD markers. The main goal of the study was to find out whether inter and intraspecific diversity exists among the species and to testify whether wild/cultivated

and rare/dominant species shows significant differentiation at genetic level. *C. caesia* and *Curcuma* sp. collected from wild regions of Chittagong separated from other wild species. The study revealed higher level of genetic variation among and within the species as the variation for individual plants within the species was 58.90% and 79.55% for wild/cultivated and rare/dominant species, respectively in AMOVA.

Angel *et al*. (2008) studied genetic diversity of 11 starchy *Curcuma* species *viz. C. caesia, C. aromatica, C. amada, C. zedoaria, C. aeruginosa, C. haritha, C. brog, C. leucorrhiza, C. rakthakanta C. malabarica* and *C. sylvatica* maintained at NBPGR, Thrissur, Kerala. The entities of the same plants collected from different places displayed similar features at morphological, biochemical and molecular level. In the UPGMA dendrogram, 11 species were divided in to three main clusters. *C. caesia* and *C. malabarica* grouped together and sub grouped with *C. amada* in cluster II. The results showed a high level of genetic variability among the starchy *Curcuma* species, and there was no geographical distinction among the species.

Genetic fingerprinting of nine *Curcuma* species including *C. caesia* from North East India using 12 RAPD, 19 ISSR and 4 AFLP to study their intra and interspecific genetic diversity confirmed maximum polymorphism in ISSR. The dendrogram based on these three marker data were similar with slight changes showing that interspecific differences were more significant compared to intra-varietal ones. In RAPD cluster analysis, *C. caesia* and *C. zedoaria* grouped in the same cluster. The majority of the morphological characters of the two species were more or less similar although flower colour and the internal anatomy of the rhizomes varied. All other species were grouped together in the same cluster along with cultivated species. In ISSR cluster analysis *C. caesia* was separately placed whereas rest of the species clustered together. For AFLP markers, *C. caesia, C. domestica* II (with dark yellow tuber) and *C. aromatica* were grouped separately. Dendrogram showed that the species that are the derivatives of genetically similar type grouped together (Das *et al*., 2011).

A study on genetic stability using RAPD markers in fifteen micropropagated *C. longa* morphotypes and ten wild *Curcuma* species including *C. caesia* revealed variation in genetic makeup of In-vitro cultures after fifth or sixth stage of subculture. Number of banding pattern of OPA05, OPC 05, OPD 07 and OPH 01 showed variation in fifth or sixth stage of subculture (Akkara and Thaliyangal, 2014). The study presumed influence of DNA methylation and increased subculture interval for the stability/instability of In-vitro cultures and highlighted the importance of periodical monitoring of in-vitro cultures for their genetic stability.

Susngi and Lasker (2015) assessed genetic diversity in *C. caesia* collected from nine different sites (Bhoirymbong, Pynursla, Umiam, Umden, Wahlong, Tangmang, Shella, Jowai and Pairong) of Meghalaya belonging to four districts using ISSR markers. Principal Component Analysis (PCA) plot revealed that Bhoirymbong, Umiam and Umden samples were genetically closer to one another than with other samples. Similarly, Tangmang, Wahlong and Pariong samples also displayed similarity with each other. Jowai and Shella samples were found related to each other than rest of the samples. Among the nine samples, the Pynursla sample was found genetically distinct from rest of the samples.

2.1.6.4. *Curcuma xanthorrhiza* Roxb.

Curcuma xanthorrhiza Roxb. is a medicinal plant also known as Java turmeric (Kim *et al*., 2014) indigenous to and widely used in East Asia (Suksamrarn *et al*., 1994; Jantan, 2012; Salea *et al*., 2014). Although it originates from Indonesia, it has been grown wild and cultivated in Thailand, the Philippines, Sri Lanka and Malaysia (Handayani *et al*., 2007; Devaraj *et al*., 2010). It has a round tuber with a yellow outer skin and orange-yellow flesh. The rhizome smells balmy and taste bitter. The plant is also known as 'Manja koova (Mal.)' and 'Temu lawak'(malay) (Devaraj *et al*., 2010). In Thailand, the plant is called 'Wan Chak Modlook' (Hwang and Rukayadi, 2006) and is used to treat various skin inflammations (Claeson *et al*., 1993), constipation, fever, arthritis and used as tonic after child birth (Aminah, 2007).

2.1.6.4.1. Uses

C. xanthorrhiza assumes significance in medicinal applications. *C. xanthorrhiza* is widely used in Indonesian folk medicine to treat liver disorders, stomachic, analgesic, rheumatic remedy etc. Essential oil, curcumin, methanolic and ethanolic extracts of *C. xanthorrhiza* were reported of having hepatoprotective, cholagogic effect, antioxidant, antibacterial, anti-metastatic, anti-microbial, anti-inflammatory, estrogenic, antimycotic, anticandidal and antitumour activity (Ozaki and Liang,1988; Osaki, 1990; Claeson *et al*.,1993; Lin *et al*., 1995; Claeson *et al*., 1996; Nurfina *et al*., 1997; Vimala *et al*., 1999; Hwang *et al*., 2000; Ireson *et al*., 2002; Choi *et al*., 2004; Rukayadi *et al*., 2006; Rukayadi and Hwang, 2007; Lee *et al*., 2008; Anggakusuma *et al*., 2009; Devaraj *et al*., 2010; Mary *et al*., 2012; Mangunwardoyo *et al*., 2012; Lew *et al*., 2015; Sylvester *et al*., 2015).

Yasni *et al*. (1993) reported an inhibitive effect on liver fatty acid synthase when rats were fed on a diet containing 4% powdered *C. xanthorrhiza* rhizome resulting in a decrease of the

triglyceride content of the liver. In an extended study, it was observed that the essential oil from *C. xanthorrhiza* has a lowering effect on the hepatic triglyceride content. This activity was attributed to the curcumin content in *C. xanthorrhiza* (Yasni *et al*., 1994).

In nude mouse that had been injected subcutaneous with prostate cancer cells, a diet of 2% curcumin caused a marked decrease in the extent of cell proliferation, a significant increase of apoptosis and micro vessel density (Dorai *et al*., 2001).

Xanthorrhizol had an anti-metastatic effect and induced apoptosis (Lee *et al*., 2002; Choi *et al*., 2005; Kang *et al*., 2009). Cheah *et al*. (2009) reported that when xanthorrhizol and curcumin were added together to human breast cancer cells in vitro, there was an increase in growth inhibition via apoptosis as compared to xanthorrhizol alone, indicating a synergistic effect of these two substances.

The effects of cassava starch-based edible coating enriched with *Kaempferia rotunda* L. and *C. xanthorrhiza* essential oil on patin fillets quality were studied. The results indicated that essential oil enrichment was able to maintain the patin fillets quality. Therefore, edible coating can be used as an alternative for fish preservation (Utami *et al*., 2004).

Xanthorrhizol isolated from *C. xanthorrhiza* is active against a variety of pathogenic microorganisms. Antimicrobial effects of xanthorrhizol included antibacterial (Hwang *et al*., 2000; Rukayadi *et al*., 2005), anticandidal (Lim *et al*., 2005; Rukayadi *et al*., 2006; Mustafa *et al*., 2010; Rukayadi *et al*., 2013) antifungal activities (Rukayadi *et al*., 2007; Anjusha and Gangaprasad, 2014) and anti-acne activity (Batubara *et al*., 2016).

2.1.6.4.2. Morphology and variability

The plant grows to a height of 2m. Rhizomes are erect with few branches, ovate with both palmate and pendulous tubers deep dark orange yellow, orange or orange-red inside, paler in colour in the younger parts. Rhizome has a pungent smell and bitter taste. Leaves are semi-erect, sessile on their green sheaths, broad lanceolar and oblong sheaths of scapes. Leaf margins are medium wavy. Leaf blades are green with purple midvein, ovate with distinct purple strip beside main green midrib, 10-18 cm x 30-80 cm (Hwang and Rukayadi, 2006). Mateblowski (1991) reported leaf length varying from 31-84 cm and leaf width 10-18 cm with long petiole length of 43-80 cm. whereas, Skornickova and Sabu (2005) reported petiole length ranging from 5-20 cm in *C. xanthorrhiza* with leaf lamina 30-100 x 10-28 cm. Inflorescence grows up to 25 cm, on separate shoots arising from rhizomes. The length of the peduncle is around 15-25 cm, spike is 16-25 cm x 8-10 cm; bracts are fertile with pale green, pubescent, apex 3-toothed; corolla tube lobes are pale-purple and ovate. Comas are

large and have a deep purple or crimson colour. Coma bracts are dark pink; flowers are yellow with pinkish corolla lobes, outer border of the corolla is red, lateral staminodes yellowish tinged with purple colour; labellum is yellowish with deeply coloured anther base with spurs (Hwang and Rukayadi, 2006; Ravindran *et al*., 2007).

Roots are usually short with large tubers, rootstocks are oblong, short, with an orange-yellow colour. The colour of the rhizome varied from dark yellow to reddish brown at the exterior and orange to dark orange-red in the interior of the rhizome (Skornickova and Sabu, 2005; Hwang and Rukayadi, 2006). The rhizome morphological traits of 'wan-chak-motluk' were found to vary not only among the species but also within the species depending on geographical location and cultivation (Soontornchainaksaeng *et al*., 2010).

2.1.6.4.3. Variability for qualitative traits

Biochemical analysis showed variation for various chemical components in *C. xanthorrhiza* such as starch (48.18-59.64%), crude fiber (2.58-4.83%), volatile oil such as, phelandrene, camphor, tumerol, sineol, borneol, and xanthorrhizol (1.48- 1.63%), and also curcuminoids like, curcumin and demethoxycurcumin (1.6-2.2%) (Mangunwardoyo *et al*., 2012).

Essential oil of the *C. xanthorrhiza* rhizome contain varying content of constituents such as monoterpenes, curcuminoids (curcumin 1, bisdemethoxycurcumin and demethoxycurcumin), sesquiterpenes, sesquiterpenoids (xanthorrhizol, cubenol, α-eudesmol, α-cis-bergamotene) and monoterpenoids (1, 8-cineole, 6, 7-epoxy myrcene) (Yasni *et al*., 1994; Jantan *et al*., 1999; Jarikasem *et al*., 2005; Zwaving and Bos, 1992; Jantan *et al*., 2012; Mary *et al*., 2012).

Zwaving and Bos (1992) reported essential oil composition of *C. xanthorrhiza* from Indonesia and India as Ar-curcumene (41.4%) and xanthorrhizol (21.5%). Whereas, a study from Thailand, showed major volatile oil components in *C. xanthorrhiza* as 1,8-cineole (37.58%) and curzerenone (13.70%). p-cymene-8-ol (4.26%), humulene oxide (2.64%) (Jarikasem *et al*., 2005).

2.1.6.4.4. Molecular variability

Islam (2004) conducted genetic diversity study on 16 *Curcuma* species collected from Bangladesh using RAPD markers. The study was conducted to find out the inter and intraspecific diversity among the species as well as to evaluate whether wild/cultivated and rare/dominant species showed significant differentiation at genomic level. The geographical location of the collected samples was categorised in to three habitats; Hilly areas

(Chittagong, Sitakundu and Srimangal), Plain (Savar) and plateau lands (Birganj and Kapasia). Six accessions of *C. xanthorrhiza* were collected from wild habitats of Srimangal area. *C. xanthorrhiza* grouped in the second cluster which was the most divergent cluster comprising of wild species *viz.*, *C. amarissima*, *C. aeruginosa*, *C. petiolata*, *C. zedoaria, C. rubescens, C. latifolia, C. viridiflora, C. australasica, C. angustifolia* and *C. elata*. In the dendrogram, *C. elata, C. angustifolia, C. xanthorrhiza* and *C. zedoaria* were found to be closely associated with each other. The clustering was in accordance with their morphology having large clumps of leafy stiffs and large rhizomes. It is also evident from the study that higher level of genetic variability was attributed within the species *i.e*. 58.90 % of the variation among the individual plants within species and 79.55% accounted for cultivated/wild and rare/dominant groups. The cultivated species were found separated from the wild species in multivariate cluster analysis.

Ahmad *et al*. (2009) studied genetic variation at intergeneric and intrageneric level of chloroplast DNA present in 11 species in four genera of Zingiberaceae mainly from Myanmar using cpDNA primers and restriction analysis. *Curcuma* species showed interspecific variation in the restriction profiles of five chloroplast DNA, *trns-trnfM* being the most informative gene region. Data was subjected to cluster analysis to discriminate various accessions at intergeneric and intrageneric level. Cluster I, II and III were formed by *Zingiber* species, V and VI by *Kaempferia* and *Alpinia*. Cluster IV, being the major cluster was formed by *Curcuma* species which was further divided in to subclusters, within the cluster, *C. xanthorrhiza* and *C. zedoaria* formed a subgroup, and they were found identical at the molecular level. The study was in congruence with the previous studies on the morphological and molecular similarity between *C. xanthorrhiza* and *C. zedoaria*. (Liu and Wu,1999; Syamkumar and Sasikumar, 2007). AMOVA studies showed significant cpDNA variation at intergeneric (57.99%) and intrageneric (42.01%) level.

2.1.7. Phenology

C. amada, C. aromatica, C. caesia and *C. xanthorrhiza* are widely distributed in various parts of South Asian regions and India. Flowering of different *Curcuma* species depends on the species, cultivars and climatic conditions. Two types of flowering are seen in *Curcuma* species, the early flowering (April-May) species developed terminal flowers whereas late flowering species (August- September) developed central flowering (Sirirugsa, 1999). Flowers emerges between 95 and 155 days after planting. After the emergence of flowers,

the inflorescence takes about 7-11 days for blossoming with a mean number of 26-35 flower per inflorescence. Inflorescence lasts for 1 to 2 weeks (Ravindran *et al*., 2007).

Fast and vigorous growth is observed in *C. aromatica* during monsoon season. The plant attains maturity, and foliage dries up by late autumn and the rhizomes show dormancy during winter (Anoop, 2015). The flowering period of *C. aromatica* was from July to September and September to December in *C. longa*. Seed propagation was evidenced in *C. aromatica* and *C. longa* (George, 1981; Sasikumar *et al*., 1996). The seeds of *C. aromatica* mature within 23-29 days after the opening of the flower and will germinate within 8-10 days (Nambiar *et al*., 1982). The anthesis in *C. longa* was reported to be occurring from 7 to 9 a.m. and anther dehiscence between 7.15 - 7.45 a.m. Earlier studies indicated that in Kerala climatic conditions, the flower opening takes place from 6.00 a.m. to 6.30 a.m. In *C. aromatica*, the number of days taken for flowering was from 95-104 days whereas in *C. longa* it was 118-143 days (Nambiar *et al*., 1982; Ravindran etal., 2007).

C. aromatica, C. caesia and *C. xanthorrhiza* are early flowering species and produces a beautiful lateral inflorescence. *C. amada* is a late flowering species possessing central inflorescence with greenish–white inflorescence (Nirmal Babu *et al*., 2007). *C. caesia* blooms in May, the inflorescence appears as the first leaf emerges from the rhizome (Asiatick society,1836) or in some cases like in *C. xanthorrhiza* inflorescence spike is found to be appearing before leaves (Behar *et al*., 2014).

Earlier studies on Zingiberaceae plants displayed variation in phenology. In *C. alismatifolia*, the shoots emerge within 2-3 weeks after planting (WAP) and the flower initiation around 5-6 WAP. The flowering period of *C. alismatifolia*, an ornamental crop is from July to August, and plant shows dormancy during winter (Changjeraja *et al*., 2007), *Curcuma bhatii* (R.M.Sm.) Skornick. and M. Sabu flowers during July to August (Skornickova and Sabu, 2005), In *Curcuma woodii* N. H. Xia & J. Chen, flowering period is from July to September (Chen *et al*., 2015), *C. indora* is dormant from November to April and starts sprouting in the first week of May. The emergence of flowering is observed during May to August (Prabhu Kumar *et al*., 2014).

Considerable variation was observed for the curcumin content of leaves and rhizomes in three cultivars of *C. longa* and a single type of *C. amada* at different stages of growth. Curcumin content of leaf decreased, while that of rhizome increased with increasing maturity (Mehta *et al*.,1980). Similarly, in *C. longa* Hanashiro *et al*. (2003) reported increase in curcumin content with the maturation of the plant. Likewise, in *C. amada* and *C. aromatica*, phenological variation was observed at three growth stages (90,140 and 180 days

after planting), for morphological characters, yield, dry recovery, curcumin, essential oil, protein and crude fiber content (Sajitha *et al*., 2014).

Chapter 3

Materials and Methods

3.1. Plant material

The present study was conducted at the Experimental farm of the ICAR-Indian Institute of Spices Research, Kozhikode, Kerala during 2012-2015. Different accessions of *Curcuma* species *viz*., *Curcuma amada* Roxb., *Curcuma aromatica* Salisb., *Curcuma caesia* Roxb. and *Curcuma xanthorrhiza* Roxb., which were collected and conserved in the previous months, were planted at the Experimental farm and used in the study. The experiment was laid out in Completely Randomized Design (CRD). The details of the *Curcuma* species and their accessions used in the study are given in the Table 6 &Figure 1.

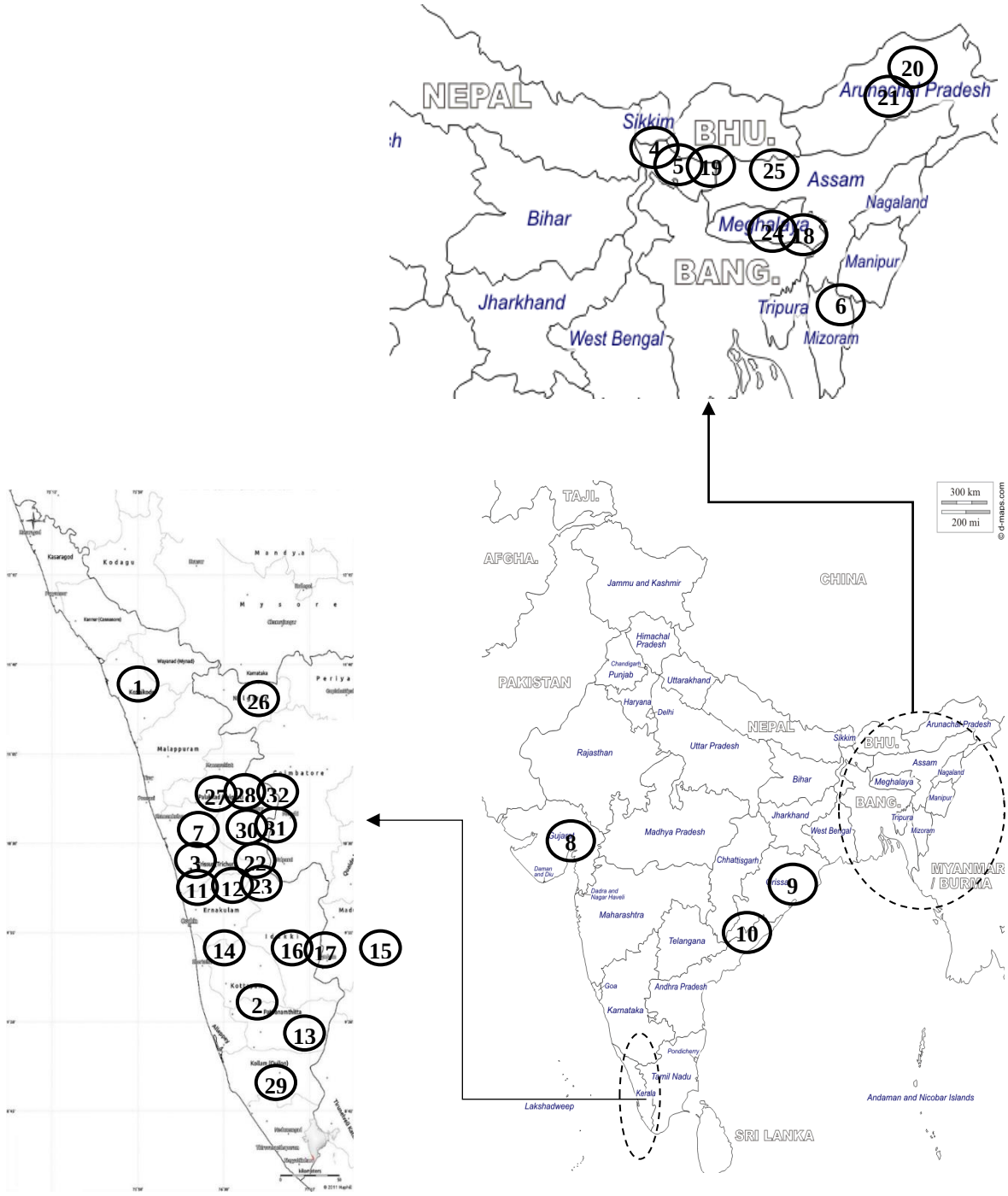


Figure 1. Places of collection of the *Curcuma* accessions

Table 6. Location details of *Curcuma* species and their accessions used in the study.

Sl No	Species	IC No	Acc. No.	Place of collection	Latitude (N)	Longitude (E)	Altitude (in M)
1	*C. amada* Roxb.		265	Arikulam, Kozhikode, Kerala	11° 28' 5"	75° 43' 21"	5
2	*C. amada*	IC 348623	347	Moovattupuzha, Kottayam, Kerala	9° 59' 21"	76° 34' 44"	20
3	*C. amada*	IC 348682	521	Thrissur, Kerala	10° 31' 39"	76° 12' 51"	21
4	*C. amada*	Coll No. 3933	752	Pundibari, Cooch Behar, West Bengal	26° 31' 27"	89° 6' 26"	44
5	*C. amada*	Coll No. 3934	753	Pundibari, Cooch Behar, West Bengal	26° 31' 27"	89° 6' 26"	44
6	*C. amada*	Coll No. 6130	848	Tuidam, Mamit, Mizoram	23° 55' 35"	92° 22' 8"	688
7	*C. amada*	IC 548485	1119	Thalappilly, Thrissur, Kerala	10° 39' 42"	76° 14' 10"	17
8	*C. amada*	Coll No.6369	1503	Anand, Gujarat	22° 33' 52"	72° 55' 43"	34
9	*C. amada*	Coll No. 6377	1511	Pottangi, Koraput, Odhisa	18° 34' 6"	82° 58' 32"	934
10	*C. amada*	Coll No. 6390	6390	Gundimeda, Vijayawada, Andhra Pradesh	16° 26' 41"	80° 38' 0"	22
11	*C. aromatica* Salisb.	IC 348719	711	Thrissur, Kerala	10° 31' 39"	76° 12' 51"	21
12	*C. aromatica*	IC 265019	1025	Thrissur, Kerala	10° 31' 39"	76° 12' 51"	21
13	*C. aromatica*	IC 266590	1113	Kakkad, Pathanamthitta, Kerala	9° 19' 38"	76° 58' 16"	77
14	*C. aromatica*	IC 266577	1124	Kunnathunadu, Ernakulam, Kerala	10° 0' 55"	76° 24' 18"	16
15	*C. aromatica*	IC 72760	1132	Ambasamudram, Tirunelveli, Tamil Nadu	8° 42' 33"	77° 27' 10"	49
16	*C. aromatica*	Coll No. 7901	1518	Anachal, Idukki, Kerala	10° 1' 22"	77° 2' 10"	901
17	*C. aromatica*	Coll No. 7907	1520	Thekkadi, Idukki, Kerala	9° 36' 11"	77° 9' 41"	902
18	*C. caesia* Roxb.	IC 349014	292	Shilong, Meghalaya	25° 34' 43"	91° 53' 35"	1416
19	*C. caesia*	-	751	Pundibari, Cooch Behar, West Bengal	26° 31' 27"	89° 6' 26"	44
20	*C. caesia*	-	1001	Tezu, Lohit, Arunachal Pradesh	27° 56' 1"	96° 9' 28"	217
21	*C. caesia*	-	1006	Hayuliang, Anjaw, Arunachal Pradesh	28° 4' 35"	96° 32' 17"	567
22	*C. caesia*	IC 360924	1135	Thrissur, Kerala	10° 31' 39"	76° 12' 51"	21
23	*C. caesia*	IC 266608	1154	Olakkara, Thrissur, Kerala	10° 31' 39"	76° 12' 51"	21
24	*C .caesia*	IC 348821	1171	Shilong, Meghalaya	25° 34' 43"	91° 53' 35"	1416
25	*C. xanthorrhiza* Roxb.	IC 348632	465	Jorhat, Assam	26° 44' 47"	94° 12' 9"	84
26	*C. xanthorrhiza*	Coll No. 3941	760	Kalpetta, Wayanad, Kerala	11° 37' 11"	76° 5' 3"	748
27	*C. xanthorrhiza*	IC 88830	1108	Parambikulam, Palakkad, Kerala	10° 23' 34"	76° 46' 32"	582
28	*C .xanthorrhiza*	IC 266539	1122	Parambikulam, Palakkad, Kerala	10° 23' 34"	76° 46' 32"	582
29	*C. xanthorrhiza*	IC 266521	1163	Kizhakkekara, Kollam, Kerala	9° 58' 40"	76° 35' 31"	43
30	*C. xanthorrhiza*	IC 88840	1164	Nilambur, Malappuram, Kerala	11° 16' 45"	76° 14' 23"	26
31	*C. xanthorrhiza*	IC 266548	1167	Nilambur, Malappuram, Kerala	11° 16' 45"	76° 14' 23"	26
32	*C. xanthorrhiza*	IC 88904	1168	Nilambur, Malappuram, Kerala	11° 16' 45"	76° 14' 23"	26

3.2 Characterization of *Curcuma* species

3.2.1 Morphological characterization

The plants were grown in beds (3m x 1m) as per the standard package of practices. Details of the quantitative and qualitative characters studied are given in the Table 7 and representative figures of four *Curcuma* species and their accessions are given in the Figures 3-6. All the observations are recorded from 3 random plants per plot.

Table 7. Morphological characters studied.

Sl.No	Character	Unit	Abbreviation
1	Plant height	cm	PH
2	Number of tillers per clump	number	NTC
3	Number of leaves per tiller	number	NLT
4	Petiole length	cm	PL
5	Leaf length	cm	LL
6	Leaf width	cm	LW
7	Colour of leaf sheath	colour	CLS
8	Leaf midrib colour	colour	LMC
9	Leaf texture	texture	LT
10	Pseudo stem colour	colour	PC
11	Rhizome habit	visual	RH
12	Weight of rhizome	g	WR
13	Rhizome length (primary)	cm	RL
14	Rhizome internode length	cm	RIL
15	Rhizome inner core colour	colour	RIC
16	Number of mother rhizome	number	NM
17	Number of primary rhizome	number	NP
18	Number of secondary rhizome	number	NS
19	weight of mother rhizome	g	WM
20	weight of primary rhizome	g	WP
21	Weight of secondary rhizome	g	WS
22	Dry recovery	g	DR
23	Aroma of rhizome	odour	AR
24	Flavour/ Taste of rhizome	taste	F/T

Fig 2A. Representative photographs of different accessions of *C.amada* - a). Acc. 265, b). Acc. 347, c). Acc. 521, d). Acc. 752, e). Acc. 753

Fig 2B. Representative photographs of different accessions of *C.amada* - f). Acc. 848, g). Acc. 1119, h). Acc. 1503, i). Acc. 1511, j). Acc. 6390

Fig 3A. Representative photographs of different accessions of *C.aromatica* - a). Acc. 711, b). Acc. 1025, c). Acc. 1113, d). Acc. 1124

Fig 3B. Representative photographs of different accessions of *C.aromatica* - e). Acc. 1132, f). Acc. 1518, g). Acc. 1520

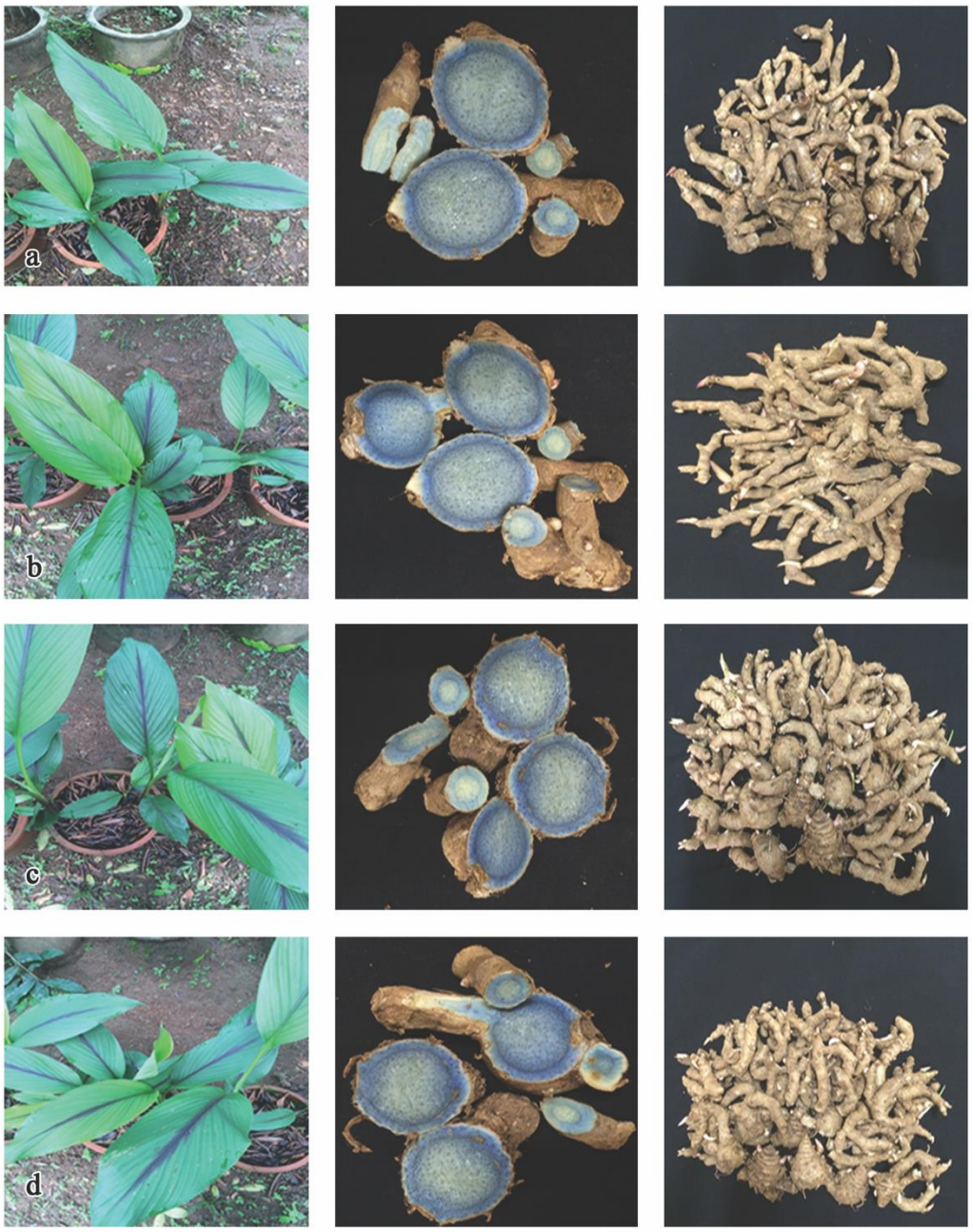


Fig 4A. Representative photographs of different accessions of *C.caesia* - a). Acc. 292, b). Acc. 751, c). Acc. 1001, d). Acc. 1006

Fig 4B. Representative photographs of different accessions of *C.caesia*- e). Acc. 1135 f). Acc. 1154, g). Acc. 1171

Fig 5A. Representative photographs of different accessions of *C.xanthorrhiza* - a). Acc. 465, b). Acc. 760, c). Acc. 1108, d). Acc. 1122

Fig 5B. Representative photographs of different accessions of *C.xanthorrhiza* - e). Acc. 1163, f). Acc.1164, g). Acc.1167, h). Acc.1168

3.2.1.1. Aerial morphological characters

Aerial morphological characterization was carried out on fully grown plants (180-200 days after planting).

3.2.1.1.1 Plant height (PH)

Height of the main tiller was considered as plant height, which was measured from the soil level to the tip of the leaf of the main shoot.

3.2.1.1.2 Number of tillers per clump (NTC)

Total number of tillers per clump was noted and the mean worked out.

3.2.1.1.3 Number of leaves per tiller (NLT)

Total number of leaves present on the main shoot counted and the mean worked out.

3.2.1.1.4 Petiole length (PL)

Petiole length was measured from the pseudostem to the base of the leaf blade; of the middle three leaves of the main tiller and mean was calculated.

3.2.1.1.5 Leaf length (LL)

Leaf length was measured from the tip of the petiole to the tip of leaf blade; of the middle three leaves of the main tiller and average of the three was calculated.

3.2.1.1.6 Leaf width (LW)

Leaf width was recorded at the maximum width of middle three leaves of the main shoot and the mean worked out.

3.2.1.1.7 Colour of leaf sheath (CLS)

By visual observation, colour of the sheath was recorded

3.2.1.1.8 Leaf midrib colour (LMC)

Plant’s leaf midrib colour was visually observed and recorded.

3.2.1.1.9 Leaf texture (LT)

Nature of leaf texture was recorded as glabrous or pubescent based on physical feeling and visual observation.

3.2.1.1.10 Pseudo stem colour (PC)

Colour of pseudo stem was visually recorded.

3.2.1.2 Rhizome Characters

The rhizome characters such as clump weight, rhizome length, rhizome number and weight of mother, primary and secondary rhizomes etc. were recorded after the harvest.

3.2.1.2.1 Rhizome habit (RH)

Rhizome habit was scored as compact, intermediate or loose.

3.2.1.2.2 Weight of rhizome (WR)

The whole weight of fresh clump was recorded as weight of rhizome and average computed.

3.2.1.2.3 Rhizome length (primary) (RL)

The length of the primary rhizomes was measured from tip to the origin.

3.2.1.2.4 Rhizome internode length (RIL)

The distance between two adjacent internodes was measured and categorized accordingly.

3.2.1.2.5 Rhizome inner core colour (RIC)

Primary rhizomes were sliced to visually record the inner core colour of the rhizomes.

3.2.1.2.6 Number of mother rhizome (NM)

Number of mother rhizomes per clump was counted and the mean worked out.

3.2.1.2.7 Number of primary rhizome (NP)

Primary rhizomes per clump was counted and mean worked out.

3.2.1.2.8 Number of secondary rhizome (NS)

Number of secondary rhizomes per clump was recorded and mean worked out.

3.2.1.2.9 Weight of mother rhizome (WM)

Weight of mother rhizomes per clump was recorded and mean computed.

3.2.1.2.10 Weight of primary rhizome (WP)

Gross weight of primary rhizomes per clump was recorded and the mean worked out.

3.2.1.2.11 Weight of secondary rhizome (WS)

Entire weight of secondary rhizomes per clump was taken to record the weight of secondary rhizomes and the average calculated.

3.2.1.2.12 Dry recovery (DR)

Dry recovery was recorded from the sun dried rhizome having a moisture range of 11-12%.

3.2.1.2.13. Aroma of rhizome (AR)

Aroma of the rhizomes was evaluated by smelling the rhizomes.

3.2.1.2.14. Flavour / taste of rhizome (F/T)

The taste of the fresh matured rhizomes was tested by conducting sensory testing (organoleptic analysis).

3.2.2. Biochemical characterization

3.2.2.1. Extraction of oleoresin

Dried and powdered rhizomes were used for the extraction of oleoresin from the four *Curcuma* species using Acetone (ASTA, 1968). Ten gram of sample was weighed and transferred to a glass column (18 x 450 mm) with stopcock. Fifty milliliter of acetone was added to the sample in the column and kept undisturbed overnight at room temperature. The extract was drained in to a pre-weighed 100ml beaker. Column was washed twice with 15ml of acetone. The extracts were pooled and evaporated to dryness. The amount of oleoresin was estimated gravimetrically.

$$\underset{\text{(Oleoresin)}}{\text{Acetone extract \%}}\left(\frac{v}{w}\right) = \frac{\text{Wt. of Residues(g)} \times 100}{\text{Weight of Samples (g)}}$$

3.2.2.2. Hydro distillation of essential oil

Essential oil was estimated as per the method described by AOAC (1975) using modified Clevenger apparatus. The rhizomes were harvested at full maturity and stored at ambient temperature. Prior to extraction of oils, the fresh rhizomes were cleaned and cut in to pieces and kept for drying at 80 °C for three days. Dried rhizomes were used for hydro-distillation. 25g of dried rhizome was accurately weighed and transferred to a short neck one litre round bottom flask with 500ml water. The trap and condenser tube were placed in position and boiled for 2½ hours. Oil collected in the trap was checked for two consecutive readings taken at one-hour interval until no change was observed. Then they were allowed to cool to room temperature and the reading was taken. Extracted oil was drained in to a 2ml Eppendorf tube sealed with parafilm and then stored in refrigerator for further analysis. The percentage of the oil was calculated as below:

$$\text{Volatile Oil, \%}\left(\frac{v}{w}\right) = \frac{\text{Weight. of Oil} \times 100}{\text{Weight of Samples (g)}}$$

3.2.2.3. GC-MS analysis of essential oil

The volatile oil constituents of samples were collected and analyzed using a gas chromatograph (Shimadzu GC 2010) equipped with mass spectroscope (Shimadzu QP-2010) and capillary column (RTX-Wax, 30mm × 0.25 mm id× 0.25µm). The column temperature was programmed as follows: -

- Injection port temperature: 250^0C.
- Flow rate; 1ml/min.
- Carrier gas: helium with linear velocity of 48.1 cm/s.
- Split ratio: 50.
- Ionization energy: 70 eV.
- Mass range: 40-650 amu.

Essential oil (0.1 µl) was injected into the equipment through the injection port. The sample run was set to 54 minutes and peaks were obtained. The peaks on the chromatogram were analyzed by the inbuilt library of the gas chromatogram unit. The constituents present in the oil were quantified by the mass spectrogram and the quantity of constituents was estimated. The compounds were identified using authentic standards (Sigma, USA) and library matching (Willey 275 & NBS 75 databases).

The constituents of the oil were correctly identified by comparing the retention indices with those of library and literature. Compound identifications showed by the database search with > 90 % hits were taken.

The formula used for calculating the retention indices (RI) is as below

$$RI_t = RT_x + \left[\left[\frac{RT_t - RT_x}{RT_y - RT_x}\right] \times 100\right]$$

Where, RIt = Retention Indices of test

RTt = Retention Time of test

RTx = Retention Time for first alkane standard

RTy = Retention Time of second alkane standard

3.2.2.4. Extraction of curcumin and quantification using spectrophotometer

Curcumin content was estimated colorimetrically using ASTA analytical method (ASTA, 1968).

- 100 mg of ground samples were weighed and then transferred in to a 100ml round bottom flask which was connected with a condenser and water cooled drip tip.
- Added 30 ml of ethanol (95%) to the sample in round bottom flask and refluxed for 2½ hours on a boiling water bath.
- The extract was cooled to room temperature and filtered quantitatively in to a 100 ml volumetric flask and made up with ethanol.
- 2ml of the filtered curcumin extract was pipetted in to a 25ml volumetric flask and diluted it to 25ml using ethanol.
- Absorbance of the curcumin extract and standard solution were measured at 425nm against an ethanol blank.
- The percentage curcumin content in the extracted samples were calculated using the following formula.

$$\text{Absorptivity of curcumin, a} = \frac{\text{Absorption of standard solution at 425nm}}{\text{Cell length(cm)} \times \text{concentration } (gl^{-1})}$$

$$\text{Curcuminin samples}(\%) = \frac{\text{Absorbance of the extract at 425nm}}{\text{Cell length(cm)} \times \text{a} \times \text{sample wt (g)}}$$

3.2.2.5. Separation and quantification of curcuminoids using HPLC

Curcuminoids (curcumin1, demethoxycurcumin and bisdemethoxycurcumin) were quantified by HPLC (High Pressure Liquid Chromatography) in a shimadzu HPLC model. The elution was carried out in porous silica pre packed Luna 5µ, RP- C18e 100A° column with dimensions of 250 x 4.60mm. A rheodyne injector with a 20µl loop was used for injecting the sample. The analyses were carried out in ambient temperature using Photodiode Array detector (PDA λ=425).

The HPLC method for isocratic elution and separation of curcuminoids are validated by altering the mobile phase, changing the proportions of the mobile phase (Acetonitrile: 0.1% orthophosphoric acid in HPLC grade water) used and by changing the flow rate. Acetonitrile: 0.1% orthophosphoric acid in varying proportions (50:50-90:10) was used with a flow rate of 0.60-2ml minute^{-1}. The proportion of the mobile phase and flow rate was optimized to 60:40 and 1ml minute^{1}.

The curcuminoids percentage in the samples was calculated by comparing with the peak area of the three curcuminoids in the standard curcumin using the following formula:

$$Curcumin\ 1(\%) = \frac{Sample\ area\ of\ curcumin \times standard\ weight \times standard\ purity}{standard\ area\ of\ curcumin \times sample\ weight}$$

$$Demethoxycurcumin(DMC)\ (\%) = \frac{Sample\ area\ of\ demethoxycurcumin \times standard\ weight \times standard\ purity}{standard\ area\ of\ curcumin \times sample\ weight}$$

$$Bisdemethoxycurcumin\ (BDMC)\ (\%) = \frac{Sample\ area\ of\ bisdemethoxycurcumin \times standard\ weight \times standard\ purity}{standard\ area\ of\ curcumin \times sample\ weight}$$

3.2.2.5.1. Preparation of stock solution

Standard stock solution was prepared using the standard curcumin (Sigma Aldrich, USA) in different concentrations and then stored in a light resistant container at 4°C before HPLC analysis.

3.2.2.5.2. Preparation of sample solution

Sample (0.1ml) from 100 ml stock solution was diluted to 1ml (1:10) using methanol (Merck) in an 10 ml volumetric flask just before loading the sample in the HPLC.

3.2.2.6. Protein estimation by Lowry's method (1951)

3.2.2.6.1. Principle

The blue colour developed as a result of the reduction of phosphomolybdic-phosphotungstic components in the Folin-ciocalteau reagents by the action of amino acids tyrosine and tryptophan present in the protein. In addition to that, the colour formed by the diuret reaction of the protein with the alkaline cupric tartarate are measured in the Lowry's method of protein estimation

3.2.2.6.2. Materials

- 2% sodium carbonate in 0.1 N NaOH (Reagent A).
- 0.5% copper sulphate ($CuSO_4.5H_2O$) in 1% potassium sodium tartarate (Reagent B).
- Alkaline copper solution: - Mix 50 ml of Reagent A and 1ml of Reagent B prior to use and mark it as Reagent C.
- Folin-ciocalteau reagent (Merck) as Reagent D.

3.2.2.6.3. Preparation of protein standard solution

Accurately weighed 50 mg of Bovine Serum Albumin (BSA) and dissolved it in distilled water and made up to 50 ml using a volumetric flask (concentration: 1mg ml^{-1})

working standard of the stock solution was prepared by diluting 10 ml of stock solution to a 50 ml with distilled water in a volumetric flask (concentration: 200 µg ml^{-1}).

For analysis of total protein, samples were dried in a hot air oven at 60-80°C. They were ground to a fine powder. 100 mg of powdered sample was extracted in hot 80 % ethanol and centrifuged at 2000g for 20 min. The pellet obtained from the above extraction was suspended in 10 ml Trichloroacetic acid and allowed to stand in an ice box for 15 min to precipitate the proteins. This was later centrifuged at 1000g for 30 min and the supernatant discarded. The pellet was re-extracted with absolute ethanol once and with hot ethanol twice, every time discarding the supernatant. The pellet which contains proteins and nucleic acids was dissolved in 5 ml of 1N NaOH and boiled at 100°C for 4-5min. This protein extract was made up to 50 ml volume and 1ml aliquots taken for protein assay.

3.2.2.6.4. Estimation of protein

- Pipetted out 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard solution in to a series of test tubes and marked.

- Pipetted out 0.2 ml of the sample extract in a test tube and marked.
- Made up the volume to 1ml in all the test tubes. A tube with 1ml of distilled water served as the blank.
- Then added 5 ml of the reagent C to each tube including the blank, mixed well and allowed it to stand for 10 minutes.
- Then added 0.5 ml of reagent D, mixed well and incubated at room temperature in the dark for 30 minutes until the blue colour developed.
- Read the absorbance at 660 nm.
- Plotted a standard curve using the standard protein absorbance versus concentration. Calculated the protein in the sample using the standard curve.

3.2.2.7. Qualitative analysis of starch

3.2.2.7.1. Estimation of starch by Anthrone reagent (Hodge and Hofreiter, 1962)

Starch is hydrolyzed in to simple sugars with the help of dilute acids and then measured colorimetrically.

3.2.2.7.1.1. Principle

In hot acidic medium starch is hydrolysed to glucose and dehydrated to hydroxymethyl furfural. This compound forms a green colored product with anthrone.

3.2.2.7.1.2. Materials

- Anthrone (MERCK): dissolved 200mg of anthrone in 100 ml of ice cold 95% Sulphuric acid.
- 80% ethanol (MERCK).
- 52% Perchloric acid (MERCK).
- Standard glucose: Prepared by dissolving 100 mg of glucose in 100 ml of water in a volumetric flask.
- Working standard: working standard of the stock solution was prepared by diluting 10 ml of stock solution to a 100 ml with distilled water in a volumetric flask.

3.2.2.7.1.3. Procedure

- Dried rhizomes of four *Curcuma* species were grounded in to powder.

- Homogenized 100 mg of the sample in hot 80% ethanol to remove sugars. The homogenized sample was centrifuged and residue was retained. The residue was then washed several times with hot 80% ethanol till the washings did not give any colour with anthrone reagent. The whole procedure was done in a 2 ml Eppendorf tubes at 37°C, 9167 g for 30 minutes. The residue was then dried.
- Transferred the residue to a screw cap bottle. 5 ml of water and 6.5 ml of 52% perchloric acid was added.
- Extracted the residue at 0°C for 20 minutes at 9167 g. Centrifuged and collected the supernatant.
- Extraction was repeated using fresh Perchloric acid. Centrifuged and collected the supernatant again and then made up to 100 ml using a volumetric flask.
- Pipetted out 0.1ml of the supernatant and made up the volume to 1 ml using distilled water.
- Standard solutions were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1ml of working standard and made up the volume to 1ml in each test tubes with distilled water.
- To each test tubes added 4 ml of anthrone reagent and heated it in a boiling water bath for 8 minutes.
- Test tubes were rapidly cooled by keeping it inside an ice bucket and intensity of green to dark green colour was measured at 630 nm.
- Glucose content of the sample was estimated by using the standard graph and then multiplied the values by a factor 0.9 to find out the starch content.

3.2.2.7.2. Extraction of starch for scanning electron microscopy study

Starch was extracted by combining the methods of Rani and Chawhaan (2012) and Zhou *et al*. (2013) with slight modifications and estimated using the protocol of Hodge and Hofreiter (1962).

- Fresh but mature rhizomes were washed, peeled, and immediately diced into 2–3 cm cubes.
- The cubes were suspended overnight in 0.1% solution of Sodium bisulphite in water. The samples were homogenized in 1% solution of Ammonium oxalate in water.
- Cell debris was removed from the homogenate by filtering through two layers of muslin cloth, and the filtrate was kept aside for the starch to settle to the bottom as sediment.

- The extraction process was repeated 3–4 times until all the starch was extracted from the material.
- The sediment was suspended in saline (0.9 %) solution at room temperature and shaken after adding toluene (0.1% by volume) to denature any residual cytoplasmic proteins.
- The starch settled to the bottom, and the layer consisting of proteins and toluene was discarded.
- Any brown layer that formed at the top was also discarded, the white layer re-suspended in water, and centrifuged several times (3000 g, 10 min) until the supernatant was free of colour and no browner layers were formed.
- The starch sediment was rinsed with 70% ethanol followed by 80% acetone and ether. Finally, the samples were dried at room temperature.

3.2.2.7.2.1. Scanning electron microscopy of starch granules

- The extracted starch was serially dehydrated in a series of progressively stronger solutions of ethanol (30%, 50%, 70%, and 90%).
- Gold coating tape was pasted on the circular stub of a scanning electron microscope (SEM) and the starch granules to be examined were sprinkled on the stub.
- The stub with sample was then coated with gold for 1 min by using a gold-coating machine and viewed under a scanning electron microscope (Hitachi SU 6600 FE).
- The size of starch granules was measured and the granules were photographed using a camera attached to the SEM.

3.2.2.7.2.2. Moisture content and ash content

Moisture content of *Curcuma* starch samples was analyzed in a fully automatic moisture meter (Shimadzu MOC- 120H). The heating unit consisted of a ceramic infrared heating element. About 4 g of powdered sample was taken in the sample pan and the temperature was set to 110°C. Once the cycle of instructions programmed for a particular product was completed, the moisture content of the product was obtained from the instrument and ash content of the starch was determined using the standard AOAC official procedures (AOAC, 1990).

- Crucibles were placed inside the muffle furnace at 550°C overnight to ensure that crucibles were devoid of all the impurities.

- Then the crucibles were cooled to room temperature and weighed to three decimal places.
- Weighed 5 g of the sample and added it into the pre-weighed crucibles.
- This was heated at 550°C overnight for three days until the samples turned to ash
- Ash with crucibles were weighed and recorded.

  Calculation

$$\text{Ash}(\%) = \frac{\text{Weight of ash}}{\text{Weight of Sample}} \times 100$$

3.2.2.7.2.3. Solubility and swelling power

Solubility and swelling power of the starch was estimated by the method of Leach *et al*. (1959).

- Aqueous suspension of 2% starch (w/v) were heated at constant temperature of 85 °C in a water bath for 30 minutes with intermittent shaking.
- This suspension was then cooled down and centrifuged at 3000 g for 15 minutes.
- Precipitated paste was separated from supernatant and weighed (Wp).
- Both the residues were then dried at 105 °C for 24 hours.
- Dry solids in precipitated paste (Wps) and supernatant (Ws) were calculated.
- Swelling power is the ratio of the weight of swollen starch granules after centrifugation (g) to their dry mass (g).
- The data obtained were used to calculate the swelling power and solubility using the following formula:

$$\text{SwellingPower} = \frac{\text{Wp}\big(\text{Hydrated starch granules (g)}\big)}{\text{Wps (Dry granules in precipitated paste(g))}}$$

Solubility is the percentage of dry mass of soluble in supernatant to the dry mass of whole starch sample (Wo).

$$\%\ \text{Solubility} = \frac{\text{Ws}}{\text{Wps}} \times 100$$

3.2.2.7.2.4. Water-holding capacity

Water holding capacity (WHC) of the samples was determined by using the method of Ju and Mittal (1995).

- The aqueous samples were prepared by adding 75ml of distilled water to 5g of starch in a centrifuge tube.
- The solution was agitated for 1h and then centrifuged at 3000g for 10 minutes.
- The free water from the centrifuge tubes were removed and weighed.
- The samples were drained for 10 minutes and starch samples were weighed.

$$\text{Water holding capacity \%} = \frac{\text{Mass of water added to the sample} - \text{Mass of water removed}}{\text{Mass of sample}} \times 100$$

3.2.2.8. Total Carbohydrates (Dubois *et al.*, 1956)

Phenol- Sulphuric acid method was used for the estimation of total carbohydrates.

3.2.2.8.1. Principle

Glucose obtained from the acid hydrolysis is dehydrated in hot acidic medium to hydroxymethyl furfural, which forms a yellow-orange product with phenol which has an absorption maximum at 490 nm.

3.2.2.8.2. Materials

- 5 % Phenol: redistilled (reagent grade-MERCK) phenol (50g) which was dissolved in water and diluted to one litre.
- 96% reagent grade Sulphuric acid (MERCK).
- Glucose standard stock: 10 mg in 100 ml of distilled water.
- Working standard: 10 ml of standard stock made up to 100 ml standard flask using distilled water.

3.2.2.8.3. Carbohydrate Extraction

Weighed 100 mg of the powdered sample and hydrolyzed in 5 ml of 2.5N HCl in a boiling water bath for 3h. It was cooled to room temperature, neutralized with solid Sodium carbonate (Na_2CO_3), until the effervescence ceased and made up to 100 ml in a standard flask.

3.2.2.8.4. Procedure

- Pipetted out 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard solution in to five test tubes.
- Pipetted out 0.1 ml of the extracted sample and then added 1 ml of 5% redistilled phenol and 5ml of 96% Sulphuric acid (H_2SO_4) and mixed well.
- The mixture was allowed to stand at room temperature for 10 min and then placed in a water bath at 25-30 °C for 20 min.
- The color was read at 490 nm against a glucose standard (25-100 µg ml^{-1}).
- The results were expressed as mg glucose 100 mg^{-1} dry weight of sample.

3.2.2.9. Estimation of total phenols

Estimation of total phenols was carried out using the Folin-Ciocalteau reagent, described by Malick and Singh (1980).

3.2.2.9.1. Principle

Phenols react with Phosphomolybdic acid in Folin-ciocalteau reagent in alkaline medium and produce blue colored complex (molybdenum blue).

3.2.2.9.2. Materials

- 80% ethanol
- Folin-ciocalteau reagent
- 20% sodium carbonate
- Stock standard solution: 100mg of Catechol was dissolved in 100ml of distilled water.
- Working standard solution: 10 ml of the stock solution was made up to 100ml with distilled water.

3.2.2.9.3. Total phenol extraction

- 1g of the sample was ground with a pestle and mortar in 10 ml of 80% ethanol.
- The homogenate was centrifuged for 20 minutes at 10000 rpm and saved the supernatant.
- The residue was re-extracted with 5ml of 80% ethanol.

- Evaporated the supernatant to dryness and then dissolved in a known volume of distilled water.

3.2.2.9.4. Procedure

- A 0.5ml of the supernatant was pipetted out in to a test tube and made up the volume to 3ml with distilled water.
- A blank was kept with 3ml distilled water.
- To this 0.5ml of Folin-Ciocalteau reagent was added.
- After 3 min, 2ml of 20% Sodium carbonate solution was added to it. It was mixed thoroughly and placed the tube in a boiling water bath for exactly 1 min. It was cooled and the absorbance was measured at 650 nm.
- A standard curve was obtained by treating different aliquots of working standard solution in the same manner.
- The concentration of phenols in the sample was found out using the standard curve and expressed as milligram phenols/gram material (mg/g).

3.2.2.10. Crude fiber

The crude fiber was determined by ASTA analytical method (1968).

- Weighed 1g of the sample and transferred it to crucibles. Placed the crucibles in to the Fibra plus.
- Acid wash:
  - Added 1.25% H_2SO_4 to the extracts from top.
  - Set initial temperature 500°C.
  - After boiling, decreased the temperature to 400°C and kept it for 45minutes.
  - Drained the alkali and washed the sample thrice with distilled water.
- Alkali wash:
  - Added 1.25% NaOH to the extracts from top.
  - Set initial temperature 500°C
  - After boiling decreased the temperature to 400°C and kept for 45 minutes.
  - Drained the alkali and washed the sample thrice with distilled water.
  - After alkali wash, crucibles were taken out and dried in hot air oven until the crucibles were free from moisture.
  - Cooled down the hot crucibles to room temperature using a desiccator.

- Weighed the crucibles and recorded the reading till it attained a constant value.
- Placed all the crucibles in the muffle furnace at 500°C for ashing.
- After ashing, crucibles were cooled down to room temperature and its weights were recorded.
    - Calculation: % of crude fiber = $(W_3/W) \times 100$.
    - $W_3 = W_1 - W_2$
    - W = Sample weight.

3.3. Molecular characterization

3.3.1. Isolation of DNA from young leaves

Fresh and young leaves of the four *Curcuma* species and their accessions were selected for DNA isolation. The leaves were washed with distilled water and wiped dry with tissue paper. Three grams of the leaves were weighed in a weighing balance and used for isolating DNA. The DNA was isolated using the modified CTAB method of Doyle & Doyle, (1987). The method used is described below:

- Ground three grams of leaf sample in to a fine powder using liquid nitrogen in a pre-chilled mortar and pestle.
- Transferred the powdered leaf sample to a 50 ml Oakridge tube containing 10 ml of freshly preheated CTAB extraction buffer.
- Incubated sample at 65°C for 60 minutes with occasional gentle spinning and mixing.
- Then added equal volume of Chloroform: Isoamyl alcohol (24:1) and mixed by gentle swirling. Centrifugation was done at 12000g for 10 min at 4°C.
- Transferred the aqueous phase to fresh tubes with cut tips.
- Added 2/3 volume of Isopropanol and mixed by gentle inversions and kept at room temperature for 30min.
- Scooped out the DNA strands in to Eppendorf tubes containing 1ml of 70% ethanol. Then, centrifuged it at 8000g for 2-5 min, discarded the supernatant and vacuum dried for 20min and dissolved in nuclease free water.
- Then added 10µg/ ml of RNase A and incubated it at 37°C for 30min in a water bath.
- Added equal volume of phenol: chloroform: isoamyl alcohol (25:24:1), mixed well and centrifuged at 14,000g for 15min.
- Collected the aqueous phase and extracted it with equal volume of chloroform: isoamyl alcohol (24:1) and centrifuged at 17500 g for 15min.

- To the aqueous phase added equal volume of chilled 100% ethanol and incubated at 4°C for 1h and then centrifuged at 5700 g for 5min.
- Decanted the supernatant carefully and then washed the pellet with 70% ethanol.
- Air dried the pellet and dissolved it in TE buffer.

3.3.1.1. Quantification of DNA

3.3.1.1.1. Agarose gel analysis

The quantity of the total DNA isolated from fresh young leaves was checked by agarose gel electrophoresis. Quantity of the isolated DNA was estimated by comparing it with standard DNA marker (Genei, Bengaluru, India). Agarose gel electrophoresis was carried out in a SUB20- maxi standard submarine electrophoresis system (Hoefer, USA) using a programmable electrophoresis PS300B 300-volt power supply (Hoefer, USA).

- Sealed the ends of the casting tray with two layers of tape. Placed the combs in gel casting tray.
- Prepared 0.8% agarose in 1× TBE (0.8g agarose in 100ml 1× TBE). Boiled the solution using a microwave oven until the solution became clear. Allowed it to cool (55-60°C) by swirling the flask occasionally to cool evenly.
- And then added 2µl of ethidium bromide (10mg ml^{-1}) and poured it to gel tray.
- After 20-25 minutes removed the tape and comb once the gel became solid and placed the gel in the tank and poured 1× TBE until the gel is fully immersed.
- Loaded the DNA samples in the well with 6× loading dye and standard DNA marker.
- Run the gel at 60-65 volts.
- Finally visualized the bands on a UV trans illuminator and documented the gel using a Gensys gel documentation system.

3.3.1.1.2. Spectrophotometric analysis

Spectrophotometer based DNA concentration and quality were measured using Biophotometer Plus (Eppendorf, Germany) at 260 nm and 280 nm.

3.3.1.2. Quality analysis of DNA

Quality of the isolated DNA was estimated using agarose gel electrophoresis and spectrophotometric analysis.

3.3.1.2.1. Agarose gel based analysis

The quality of the isolated DNA from fresh leaf samples were visualized using a 0.8% agarose gel.

3.3.2. DNA amplification

The isolated DNA from leaves were amplified using RAPD, ISSR and SSR primers.

3.3.2.1. RAPD analysis

RAPD analysis was carried out as per the method suggested by Williams *et al*. (1990) with slight modifications.

3.3.2.1.1. Optimization of PCR components for RAPD analysis

RAPD reaction was performed in a volume of 25µl total reaction volume by keeping the assay buffer concentration constant (1X), concentrations of all other PCR components for RAPD reactions such as template DNA, dNTP'S, *Taq* DNA polymerase, $MgCl_2$ and primer were tested in different concentrations.

- Template DNA concentration- 20-50ng DNA was used.
- *Taq* DNA polymerase- 0.5U-2U (Genei, Bengaluru,India) of enzyme concentration was taken for the assay.
- Concentration of dNTPs- final concentrations of 0.1mM-0.5mM were used (Genei, Bengaluru, India).
- $MgCl_2$- A final concentration of 1.5-3mM $MgCl_2$ per reaction.
- Primers- A concentration of 5-20 Pico moles per reaction of primer is used (Operon Technologies, USA).

3.3.2.1.2. RAPD-PCR amplification protocol

The reaction was performed in a 0.2ml microfuge tubes (Eppendorf). PCR amplification was carried out in an Eppendorf Vapo-protect thermal cycler. The reaction mixture of 25µl contained:

- Nuclease free water - 16.8 µl
- 10 X reaction buffer - 2.5 µl
- $MgCl_2$ (25mM μl^{-1}) - 1.5 µl

- dNTP mix (10mM $\mu l^{-1)}$ - 1 µl
- Primer (10 Pico moles μl^{-1}) - 2 µl
- *Taq* DNA polymerase (5U μl^{-1}) – 0.2 µl
- Template DNA (40ng μl^{-1}) - 1 µl

Various thermal cycling conditions and cycle repeats were performed for the optimization of RAPD-PCR. Among them the following temperature profile was found to be optimum.

- Pre-denaturation at 94°C for 3 minutes
- Denaturation at 94°C for 1 minute
- Annealing at 37°C for 1 minute
- Extension at 72°C for 1 minute

34 cycles

- Final extension at 72°C for 10 minutes
- Refrigerate at 4°C

RAPD-PCR amplification of genomic DNA was carried out using 26 arbitrary decamer oligonucleotide primers (Operon technologies, USA) (Table.8).

Table 8. Operon primers used for RAPD analysis

Sl. No	Primer	Sequence (5'- 3')
1	OPA 01	CAGGCCCTTC
2	OPA 04	AATCGGGCTG
3	OPA 05	AGGGGTCTTG
4	OPA07	GAAACGGGTG
5	OPA 08	GTGACGTAGG
6	OPA 17	GACCGCTTGT
7	OPA 19	CAAACGTCGG
8	OPB 10	CTGCTGGGAC
9	OPC 01	TTCGAGCCAG
10	OPC 03	GGGGGTCTTT
11	OPC 05	GATGACCGCC
12	OPC 20	ACTTCGCCAC
13	OPD 03	GTCGCCGTCA
14	OPD 04	TCTGGTGAGG
15	OPD 20	ACCCGGTCAC
16	OPL 03	CCAGCAGCTT
17	OPN 04	GACCGACCCA
18	OPN 16	AAGCGACCTG
19	OPN 18	GGTGAGGTCA
20	OPO 06	CCACGGGAAG
21	OPX 05	CCTTTCCCTC
22	OPX 08	CAGGGGTGGA
23	OPX 14	ACAGGTGCTG
24	OPAF 05	CCCGATCAGA
25	OPAF 14	GGTGCGCACT
26	OPAF 15	CACGAACCTC

3.3.2.2. ISSR analysis

ISSR reaction was carried out as per the method suggested by Syamkumar (2008) with slight modifications.

3.3.2.2.1. Optimization of PCR components for ISSR analysis

PCR amplification was carried out in a 25 µl reaction volume by keeping the assay buffer concentration constant (1X) and changing the rest of the components such as $MgCl_2$, template DNA, dNTP'S, *Taq* DNA polymerase and primer to check in different concentrations until the optimum was found.

- Template DNA concentration- 20-60ng DNA was used.
- *Taq* DNA polymerase- 0.5 U-2U (Genei, Bengaluru, India) of enzyme concentration was taken for the assay.
- Concentration of dNTPs- final concentrations of 0.1mM-0.5mM were used (Genei, Bengaluru, India).
- $MgCl_2$- A final concentration of 1.5-3mM $MgCl_2$ per reaction.
- Primers- A concentration of 10-60 Pico moles per reaction of primer is used (Sigma, USA).

3.3.2.2.2. ISSR-PCR amplification protocol

To a 0.2 µl thin microfuge tubes (Eppendorf) the following mixture was added in the order:

- Nuclease free water - 16.3 µl
- 10 X reaction buffer - 2.5 µl
- $MgCl_2$ (25mM μl^{-1}) - 2.0 µl
- dNTP mix (10mM $\mu l^{-1)}$ - 1.0 µl
- Primer (10 Pico moles μl^{-1}) - 2.0 µl
- *Taq* DNA polymerase (5U μl^{-1}) - 0.2 µl
- Template DNA (40ng μl^{-1}) - 1.0 µl

3.3.2.2.3. Optimization of ISSR-PCR temperature profile

Different temperature profiles were tested for optimizing PCR keeping the cycle repeats constant. The following temperature profile was found to be optimum.

- Pre-denaturation at 94°C for 3 minutes
- Denaturation at 94°C for 1 minute
- Annealing at 52°C for 1 minute
- Extension at 72°C for 1 minute

31 cycles

- Final extension at 72°C for 10 minutes
- Refrigerate at 4°C

ISSR –PCR amplification was performed using 21 ISSR primers (Table 9) from Sigma (USA).

Table 9. Primers used for ISSR analysis

Sl.No	Primer	Sequence (5'- 3')
1	ISSR 4	(AGC)4GT
2	ISSR 5	(CAC)3GC
3	ISSR 6	(CTC)3GC
4	ISSR 8	(GACA)3GC
5	ISSR 9	(TC)7G
6	ISSR 11	(AGTG)3TT
7	ISSR 12	(CCCT)4
8	ISSR 13	(AGTG)3
9	ISSR 14	(AG)8T
10	ISSR 16	(AC)7T
11	UBC 880	GGAGAGGAGAGGAGA
12	UBC 855	ACACACACACACACACCTT
13	UBC 850	(GT)8C
14	UBC 842	GAGAGAGAGAGAGAGACTG
15	UBC 841	(GA)8T
16	UBC 835	(AG)8TC
17	UBC 834	(AG)8CT
18	UBC 826	(AC)8C
19	UBC 818	(CA)8G
20	UBC 812	(GA)8A
21	UBC 811	(GA)8C

3.3.2.3. SSR analysis CuMiSat (*Curcuma* MicroSatellite) SSR markers developed by Siju *et al*. (2010) and Senan *et al*. (2013) were used for performing genomic SSR analysis in different accessions of *Curcuma* species. The primers used for the genomic SSR analysis are given in Table 10.

Table 10. Primers used for Genomic SSR analysis

Sl No	Marker	Sequence forward primer (5'-3') Reverse primer (3'-5')	Repeat motif	Ta (°C)
1	CuMiSat 1	AAACCGCAAGAAAACTGAAG CTCTTCCCTGAACGATTCC	(AG)6	62
2	CuMiSat 2	TATGTGATGGTTGGGACG GTAGTGGAGGAAGACGCC	(AG)16	62
3	CuMiSat 3	GCACTACTTCCTICTCGTTCAA CGTCGTAAAGATTAGCGTGTG	(AG)19	65
4	CuMiSat 5	AGCAGTGCGTCTTTCATC CTCTTGTCACGGAACCTC	(AG)13	63
5	CuMiSat 6	AAGAAACTCCAACCACAATCC CTTGTCTTCCTCCTCCATTG	(AG)12	62
6	CuMiSat 7	AGCATGTGTCTAGCTCTTTGC AAGCAGTCGTTCCTCTACTGAC	(AG)19	64
7	CuMiSat 8	CATTGCGTGCCCACTTCC CCTCCCTGTCGCTCTCCTC	(AG)17	65
8	CuMiSat 9	AGTTGTGAAAGGGATAGAGTAGTTG AAGAAAGCAAATGCCAAGG	(AG)21	62
9	CuMiSat 10	CACCCTATGAGTGCTAACTGAAG ACCTGCACCACGATCAAC	(AG)9	65
10	CuMiSat 11	ACAGTCCCCTTCCCACTC TCTTGTTCCTATGCTCTACGC	(AG)15	65
11	CuMiSat 12	AAGGTTGCTGCTTGTTGAGAA GCATATTGCCTTACATGCCTAA	(AG)7	62
12	CuMiSat 13	CCCGAAGCCATTTCTCAG TCGTCTCTCCTCTGCCAAC	(AG)8	65
13	CuMiSat 14	GCTGACTGTGGCAAAAGAGTC GCTGCGC'TTCTTCTTAATGAC	(AG)7	64
14	CuMiSat 16	CATTTGTTCTGCTCGCTTCTAC CTGCTCCGCTGTCTCTCAC	(AG)19	63
15	CuMiSat 17	ATGTGGTTGAGGAATGATGAGAC CTATTTCCCATAGCCCTTGTAGC	(AG)18	65
16	CuMiSat 18	GTTCACAGCTTTAGCAGGGACAA CTCCTCTCCATATTCTCCATCTCG	(CT)14	65
17	CuMiSat 19	CATGCAAATGGAAATTGACAC TGATAAATTGACACATGGCAGTC	(AC)16(AT)6	65
18	CuMiSat 20	CGATACGAGTCCATCTCTTCG CC'TTGCTTTGGTGGCTAGAG	(AC)6	65
19	CuMiSat 21	TCATTCAAAGTCCGATGGAA TTCGAGTGCAGAAGGAGAATTA	(AAG)9	62
20	CuMiSat 22	AATTTATTAGCCCGGACCAC AAGAAAGTGAGTAGAAACCAAAGC	(CTT)10	64
21	CuMiSat 28	TTCAACTTCTCCTCGCTCAG GCAAGGTCTGCATCTATTTCTC	(AAG)7 (GAT)5	65
22	CuMiSat 29	GTGGTATCCCCATGAAGAGC ATGACCAAGCCCTTTCACC	(AAG)10	65
23	CuMiSat 35	GGTTCGTCGCTGGAAAGTAAT GCATCTCAACAGGGGCTG	(CTT)10	60

3.3.2.3.1. Optimization of PCR components for genomic SSR analysis

Concentrations of all PCR components for genomic SSR reactions such as template DNA, dNTP'S, *Taq* DNA polymerase, forward primer, reverse primer and $MgCl_2$ were tested in different concentrations keeping the assay buffer concentrations constant (1X). The

amplification was carried out in 25µl reaction volume with the following components in order:

- Nuclease free water - 15.4 µl
- 10 X reaction buffer - 2.5 µl
- $MgCl_2$ (25mM μl^{-1}) - 1.5 µl
- dNTP mix (10mM $\mu l^{-1)}$ - 0.5 µl
- forward primer (2.5 Pico moles μl^{-1}) - 1.5 µl
- Reverse primer (2.5 Pico moles μl^{-1}) - 1.5 µl
- *Taq* DNA polymerase (5U μl^{-1}) - 0.1 µl
- Template DNA (10-50ng μl^{-1}) - 2 µl

Different temperature profiles and cycle repeats were tested for optimizing the PCR. For genomic SSR PCR amplification the following temperature profile was found to be optimum. The temperature profile used for genomic SSR-PCR amplification was as follows:

- Pre-denaturation at 94°C for 4 minutes
- Denaturation at 94°C for 30 seconds
- Annealing at T_a°C* for 45 seconds — 35 cycles
- Extension at 72°C for 1 minute
- Final extension at 72°C for 10 minutes
- Refrigerate at 4°C

(*-annealing temperature varied for different primers)

Amplified products were pre-checked in 2% agarose gel for successful amplification and then resolved in 10-15% denaturing polyacrylamide gels.

3.3.2.3.2 Denaturing PAGE

- Both the glass plates were thoroughly washed with warm water and liquid detergent.
- Then they were rinsed in deionized water and wiped using tissue paper soaked in 70% alcohol. Air dried glass plates were then assembled by placing the spacers and sealed off.
- Separation of alleles were performed in a 15% denaturing PAGE gels by mixing 60 ml urea: acrylamide mixture (appendix) with 300 µl Ammonium persulfate (10%) and 75 µl of TEMED (Sigma Aldrich, USA).

- Then this mix was poured in to the assembled gel plates and kept it for about 40-45 minutes for polymerization.
- After the acrylamide has polymerized, the combs were removed and all the wells were flushed out using a syringe needle filled with 1X TBE buffer. The tank was filled with 1X TBE buffer and the gel was subjected to pre-run at 200V for 45minutes.
- 4 µl of the amplified products were mixed with an equal volume of denaturing buffer (98% formamide, 10mM EDTA (pH 8.0), 0.05% xylene cyanol, and 0.05% bromophenol blue), denatured at 94°C for 5 minutes and immediately transferred to ice to prevent annealing.
- Wells were washed again with 1 X TBE buffer to remove the urea from the wells that would have diffused in to the bottom of the wells from the gel.
- Then the denatured samples were loaded.
- The gel was then allowed to run at constant power of 220V for 4-5 hours.

3.3.2.3.3 Silver staining of PAGE gels

- Once the electrophoresis was completed the polyacrylamide gels were subjected to silver staining using the method described by Benbouza *et al*. (2006) as mentioned below. The composition of the solutions used for silver staining is listed in Appendix. All the steps were performed with mild shaking.
- Once the electrophoresis was finished the glass plates were disassembled carefully and the gels were transferred to a tray containing 1500 ml of cold (10-12 °C) fixing solution for 5-10 minutes.
- After decanting the fixing solution, gels were washed with distilled water for 30 seconds.
- Washed gels were then placed in sufficient amount of impregnating solution for 5-10 minutes.
- Gels were then washed quickly with distilled water for 10-15 seconds.
- Then gels were developed in a 1500 ml developing solution at room temperature for 3-6 minutes, until bands with desirable intensity was achieved.
- When adequate intensity of bands was achieved, further development was stopped by impregnating the gel in 1500 ml of stop solution for 3-5 minutes.

- Washed the gels in distilled water for 1 minutes and then transferred to transparent OHP sheets for further use.

3.4. Phenological variation in two species of *Curcuma*

A field experiment was also laid out in Completely Randomized Design (CRD) at the ICAR-Indian Institute of Spices Research, Peruvannamuzhi farm, Kozhikode, Kerala, India during 2012-2013. Plant height, leaf number, tiller number, yield and dry recovery were recorded from 90, 140 and 180 days after planting (DAP) of the two *Curcuma* species *viz*., *C. amada* and *C. aromatica*. The biochemical parameters such as oil, curcumin, fiber, starch and protein were estimated at these growth stages, using the standard protocols as described in sections 3.2.2.2, 3.2.2.4, 3.2.2.10, 3.2.2.7.1, 3.2.2.6 (AOAC, 1995; ASTA, 1968; Hodge and Hofreiter, 1962; Lowry *et al*., 1951). Data was analysed statistically as per the standard procedure.

3.5. Population diversity study of four *Curcuma* species

To understand the population differentiation, accessions of *C. amada, C. aromatica, C. caesia* and *C. xanthorrhiza* collected from different regions of India were grouped and studied based on the locations from which they were collected and then analysed using POPGENE, version 1.31.

3.6. Statistical analysis

Morphological and biochemical data were analysed using one-way ANOVA (Panse and Sukhatme, 1985) using SPSS and XLSTAT software. Post Hoc text DMRT was carried out to establish the groupings among the accessions and among the species. Two-way ANOVA was used to analyse the significance of phenological variation at three growth stages using SPSS software.

On the basis of mean square values obtained, percentage of variations within and among species as well as within and among accessions were also determined (Sokal and Rohlf, 1973). Associations among accessions and species were investigated by the Principal Component Analysis (PCA) method using XLSTAT software.

The genotypic and phenotypic correlations were calculated by Kwon and Torrie (1964) technique. Falconer (1989) formula was used to calculate the genetic advance in percentage of mean.

$$\text{Genetic Varance}\left(\sigma_g^2\right) = \frac{(MSg - MSe)}{r}$$

$$\text{Environmental Variance}(\sigma_e^2) = MSe$$

$$\text{Phenotypic Variance}\left(\sigma_p^2\right) = \text{Genetic Variance}\left(\sigma_g^2\right) + \text{Environmental Variance}(\sigma_e^2)$$

Genotypic and Phenotypic coefficient of variation is calculated as

$$GCV\ \% = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \times 100; \quad PCV\ \% = \frac{\sqrt{\sigma_p^2}}{\bar{x}} \times 100; \quad ECV\ \% = \frac{\sqrt{\sigma_e^2}}{\bar{x}} \times 100$$

$$\text{Heritability } (H^2) \text{ is calculated as } H^2 = \frac{\sigma_p^2}{\sigma_g^2}$$

$$\text{The expected genetic advance}(GA) = K \times H^2 \times \sqrt{\sigma_p^2}$$

$$\text{Genetic advance as percentage of mean}(GA\%) == \frac{K \times H^2 \times \sqrt{\sigma_p^2}}{\bar{x}} \times 100$$

where σ_g^2 is the genetic variance; $\sigma_g^2$is the environmental variance;

σ_g^2 is the phenotypic variance;

$GCV\ \% =$ genotypic coefficient of variation;

$PCV\ \% =$ phenotypic coefficient of variation;

$ECV\% =$ environmental coefficient of variation.

$H^2$is broad sense heritability of the trait;

$K = 2.06$ for a selection instensity of 5%;

GA is the genetic advance and GA% is genetic advance as a percentage of mean.

Inter and intraspecific variation of quantitative morphological characters among four species of *Curcuma* were assessed by converting the data in to a binary format and then similarity matrices were prepared using NTSYS pc version 2.02i. The character state with values <= 0 was represented with the binary code '0' whereas those with >0 were represented with '1' (Sokal and Sneath, 1963).

Standardized character states are computed as

$$X'ij = \frac{xij - \bar{x}i}{Si}$$

X'ij = standard character state code for 'i' and Operational Taxonomic Unit 'j'

xij = row score

$\bar{x}$ and Si = mean and standard deviation for the character respectively.

For assessing genetic variation among the species only clear and prominent bands were scored. RAPD, ISSR and SSR products were recorded for the presence (1) and absence (0) of bands and the data were entered in a binary matrix. For RAPD and ISSR markers a range of bands between 250-2000 bp were considered but for SSR markers it was between 90-350 bp.

Jaccard's, Sorensen's Dice and Simple Matching similarity coefficients via SIMQUAL of the NTSYS pc ver 2.02i package (Rohlf, 1993) were used for analyzing molecular data. Unweighted Pair Group Method of Arithmetic mean (UPGMA) dendrogram was constructed using SAHN clustering programme of the NTSYSpc version 2.02i.

Polymorphic Information Content (PIC) was used to compare the efficiency of each primer, computed using the formula $PIC=1-\Sigma pi^2$ where pi is the frequency of i^{th} allele at a given locus (Anderson *et al*.,1993) and Marker Index was calculated (Powell *et al*.,1996).

Genetic diversity was assessed using POPGENE version 1.31 (Yeh *et al*.,1997). The levels of genetic diversity present within in the four *Curcuma* species were assessed using variable such as, observed number of alleles per locus (Na), effective number of alleles per locus (Ne), percentage of polymorphic loci (P), Shannon's Information Index (I), Nei's gene diversity (h).

AMOVA (Analysis of Molecular Variance) was evaluated using the software -GenAlex (version 6) in order to examine the interspecific and intraspecific genetic variability (Peakall and Smouse, 2006).

Correlations of various similarity matrices, genetic and geographic distances were examined using Mantel test to determine the level of correlation between various matrices.

Chapter 4

Results

4.1. Morphological characterization

4.1.1. Aerial morphological characters

4.1.1.1. Plant Height (PH)

Significant variation for plant height was observed among and within the species. Among the four species of *Curcuma*, minimum plant height was recorded in *C. aromatica* (110.74 ± 7.49cm) and maximum in *C. xanthorrhiza* (161.47 ± 5.50 cm). In *C. amada*, the height of the main tiller ranged from 114.80 ± 8.55 cm (Acc. 347) to 169.33 ± 2.33cm (Acc. 752) while in *C. aromatica*, the height of the main tiller ranged from 63.67 ± 1.45cm (Acc. 1520) to 148.33 ± 4.41cm (Acc. 1132). In *C. caesia*, maximum plant height was recorded in Acc. 292 (131.00 ± 6.27cm) and minimum plant height in Acc. 1154 (81.00 ± 4.93cm). In *C. xanthorrhiza*, plant height ranged from 137.67 ± 17.90cm (Acc. 1122) to 193.33 ± 14.24cm (Acc. 760) (Table 11).

The percentage of variation calculated using mean square values revealed that plant height displayed higher variation within the species to the tune of 60% as compared to variation among the species (40%). Within the four *Curcuma* species, majority of the variation was observed among the accessions (*C. aromatica*-88%; *C. amada*-70%; *C. caesia*-57%) except in *C. xanthorrhiza,* wherein most of the variation was found within the accessions (54%) (Table 12).

Table 11. Mean plant height of four *Curcuma* species

Sl. No	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	Plant height(cm)	Accession	Plant height(cm)	Accession	Plant height(cm)	Accession	Plant height(cm)
1	265	159.67 ± 8.37 ^{ab}	711	146.67 ± 14.11 ^{a}	292	131.00 ± 6.27 ^{a}	465	184.33 ± 7.84 ^{ab}
2	347	114.80 ± 8.55 ^{c}	1025	76.00 ± 8.33 ^{cd}	751	105.33 ± 10.73 ^{b}	760	193.33 ± 14.24 ^{a}
3	521	144.33 ± 8.41 ^{b}	1113	137.67 ± 3.18 ^{a}	1001	115.67 ± 4.81 ^{ab}	1108	152.33 ± 8.99 ^{bc}
4	752	169.33 ± 2.33 ^{a}	1124	111.17 ± 1.92 ^{b}	1006	114.67 ± 9.70 ^{ab}	1122	137.67 ± 17.90 ^{c}
5	753	156.67 ± 3.33 ^{b}	1132	148.33 ± 4.41 ^{a}	1135	112.67 ± 1.45 ^{ab}	1163	155.00 ± 13.05 ^{bc}
6	848	155.27 ± 1.40 ^{ab}	1518	91.67 ± 4.41 ^{bc}	1154	81.00 ± 4.93 ^{c}	1164	138.00 ± 4.04 ^{c}
7	1119	141.33 ± 8.11 ^{b}	1520	63.67 ± 1.45 ^{d}	1171	122.00 ± 2.52 ^{ab}	1167	147.00 ± 6.81 ^{bc}
8	1511	121.67 ± 4.10 ^{c}	-	-	-	-	1168	184.10 ± 11.44 ^{ab}
9	1503	148.40 ± 7.85 ^{b}	-	-	-	-	-	-
10	6390	121.57 ± 2.14 ^{c}	-	-	-	-	-	-
Mean ± SE		143.30 ± 3.63 ^{b}		110.74 ± 7.49 ^{c}		111.76 ± 3.87 ^{c}		161.47 ± 5.50 ^{a}
Accessions		P< 0.05		P< 0.05		P< 0.05		P< 0.05
Species		P< 0.05						

Values with the different superscript are significantly different (P<0.05), SE- Standard error

Table 12. F-value and percentage of variation for plant height in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	60.00	210.00	133.82	3.31	22.45**	40%	60%
C. amada	100.40	174.00	143.30	3.63	8.89**	70%	30%
C. aromatica	60.00	168.00	110.74	7.49	26.04**	88%	12%
C. caesia	73.00	142.00	111.76	3.87	5.70**	57%	43%
C. xanthorrhiza	115.00	210.0	161.47	5.50	3.89*	46%	54%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.1.1.2. Number of Tillers per Clump (NTC)

Number of tillers per clump showed significant variation among species. The number of tillers per clump ranged from 2.57±0.15 (*C. aromatica*) to 3.81±0.25 (*C. caesia*). However, within the species only *C. aromatica* and *C. xanthorrhiza* showed significant variation.

In *C. aromatica*, tiller number was maximum in Acc. 711 (3.67 ± 0.33) and minimum in Acc. 1520 (2.00 ± 0.00). Among the eight accessions of *C. xanthorrhiza*, maximum tiller number was observed in Acc. 1163 (3.67 ± 0.67) and minimum in Acc. 1167 (2.00 ± 0.00) (Table 13).

The percentage of variation obtained using mean square values showed that variation of NTC within the species was as high as 81% compared to variation present among the species which displayed a meagre variation of 19%. The same scenario was observed within the accessions of the four species where variation within the accessions was higher (*C. xanthorrhiza*-59%; *C. aromatica*-53%) as compared to variation between the accessions (Table 14).

Table 13. Mean number of tillers per clump in four *Curcuma* species

Sl. No	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	No. of tillers per clump	Accession	No. of tillers per clump	Accession	No. of tillers per clump	Accession	No. of tillers per clump
1	265	3.33 ± 0.33 ^{a}	711	3.67 ± 0.33 ^{a}	292	4.33 ± 0.67 ^{abc}	465	2.33 ± 0.33 ^{b}
2	347	3.33 ± 0.33 ^{a}	1025	2.33 ± 0.33 ^{bc}	751	3.00 ± 0.58 ^{bc}	760	2.67 ± 0.33 ^{ab}
3	521	3.00 ± 0.00 ^{a}	1113	3.00 ± 0.00 ^{ab}	1001	2.67 ± 0.33 ^{c}	1108	3.00 ± 0.00 ^{ab}
4	752	3.00 ± 0.58 ^{a}	1124	2.33 ± 0.33 ^{bc}	1006	3.67 ± 0.33 ^{abc}	1122	3.67 ± 0.33 ^{a}
5	753	3.33 ± 0.67 ^{a}	1132	2.33 ± 0.33 ^{bc}	1135	3.33 ± 0.33 ^{abc}	1163	3.67 ± 0.67 ^{a}
6	848	3.67 ± 0.33 ^{a}	1518	2.33 ± 0.33 ^{bc}	1154	4.67 ± 0.88 ^{ab}	1164	3.00 ± 0.00 ^{ab}
7	1119	2.67 ± 0.33 ^{a}	1520	2.00 ± 0.00 ^{c}	1171	5.00 ± 0.58 ^{a}	1167	2.00 ± 0.00 ^{b}
8	1511	3.67 ± 0.33 ^{a}	-	-	-	-	1168	2.33 ± 0.33 ^{b}
9	1503	3.00 ± 0.58 ^{a}	-	-	-	-	-	-
10	6390	2.33 ± 0.33 ^{a}	-	-	-	-	-	-
Mean ± SE		3.13 ± 0.13 ^{b}		2.57 ± 0.15 ^{c}		3.81 ± 0.25 ^{a}		2.83 ± 0.16 ^{bc}
Accessions		NS		P< 0.05		NS		P< 0.05
Species		P< 0.05						

NS = Not Significant (P>0.05), Values with the different superscript are significantly different (P<0.05), SE-Standard error

Table 14. F-value and percentage of variation for number of tillers per clump in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	2.00	6.00	3.08	0.10	8.54**	19%	81%
C. amada	2.00	4.00	3.13	0.13	1.00	30%	70%
C. aromatica	2.00	4.00	2.57	0.15	4.07*	47%	53%
C. caesia	2.00	6.00	3.81	0.26	2.43	29%	71%
C. xanthorrhiza	2.00	5.00	2.83	0.16	3.43*	41%	59%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.1.1.3. Number of Leaves per Tiller (NLT)

Among the four species of *Curcuma*, the number of leaves per tiller ranged from 6.48 ± 0.24 (*C. caesia*) to 8.43 ± 0.34 (*C. aromatica*). Analysis of variance revealed significant variation among the four *Curcuma* species. Within the species, only *C. aromatica* and *C. xanthorrhiza* showed significant variation.

In *C. aromatica*, the maximum NLT was observed in Acc. 1113 (11.67 ± 0.33) and minimum in Acc. 1520 and Acc.1025 (7.33 ± 0.33). Whereas in accessions of *C. xanthorrhiza*, the total number of leaves per tiller (NLT) ranged from 5.67 ± 0.33(Acc. 1122) to 9.33 ± 0.33 (Acc. 1167) (Table 15).

The percentage of variation obtained from the mean square values indicated high value for within the species variation (79%) than among the species (21%). Accessions of *C. amada* and *C. caesia* did not show significant variation whereas *C. aromatica* (80%) and *C. xanthorrhiza* (70%) showed significant variation among the accessions than within the accessions (Table 16).

Table 15. Mean number of leaves per tiller in four *Curcuma* species

Sl. No	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	No. of leaves per tiller	Accession	No. of leaves per tiller	Accession	No. of leaves per tiller	Accession	No. of leaves per tiller
1	265	7.33 ± 1.33 ^{ab}	711	8.00 ± 0.58 ^{bc}	292	5.33 ± 0.67 ^{b}	465	7.33 ± 0.33 ^{bc}
2	347	8.00 ± 0.58 ^{a}	1025	7.33 ± 0.33 ^{c}	751	5.67 ± 0.33 ^{ab}	760	6.67 ± 0.67 ^{cd}
3	521	6.00 ± 0.58 ^{ab}	1113	11.67 ± 0.33 ^{a}	1001	7.33 ± 0.33 ^{a}	1108	6.00 ± 0.58 ^{cd}
4	752	6.67 ± 0.33 ^{ab}	1124	7.67 ± 0.33 ^{c}	1006	6.67 ± 0.33 ^{ab}	1122	5.67 ± 0.33 ^{d}
5	753	6.67 ± 0.33 ^{ab}	1132	8.00 ± 0.58 ^{bc}	1135	6.33 ± 0.88 ^{ab}	1163	7.33 ± 0.33 ^{bc}
6	848	7.00 ± 1.00 ^{ab}	1518	9.00 ± 0.00 ^{b}	1154	7.00 ± 0.58 ^{ab}	1164	8.67 ± 0.33 ^{ab}
7	1119	6.00 ± 0.58 ^{ab}	1520	7.33 ± 0.33 ^{c}	1171	7.00 ± 0.58 ^{ab}	1167	9.33 ± 0.33 ^{a}
8	1511	7.33 ± 0.33 ^{ab}	-	-	-	-	1168	6.33 ± 0.33 ^{cd}
9	1503	5.67 ± 0.33 ^{b}	-	-	-	-	-	-
10	6390	6.67 ± 0.67 ^{ab}	-	-	-	-	-	-
Mean ± SE		6.73 ± 0.22 ^{b}		8.43 ± 0.34 ^{a}		6.48 ± 0.24 ^{b}		7.17 ± 0.28 ^{b}
Accessions		NS		P< 0.05		NS		P< 0.05
Species		P< 0.05						

NS = Not Significant (P>0.05), Values with the different superscript are significantly different (P<0.05), SE-Standard error

Table 16. F-value and percentage of variation for number of leaves per tiller in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	4.00	12.00	7.15	0.15	9.55**	21%	79%
C. amada	5.00	10.00	6.73	0.21	1.10	3%	97%
C. aromatica	7.00	12.00	8.43	0.34	14.87**	80%	20%
C. caesia	4.00	8.00	6.48	0.24	1.73	17%	83%
C. xanthorrhiza	5.00	10.00	7.17	0.28	9.14**	70%	30%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.1.1.4. Petiole Length (PL)

Petiole length varied from 21.43 ± 1.57 cm (*C. aromatica)* to 27.92 ± 1.41cm (*C. xanthorrhiza)* and analysis of variance showed significant variation for petiole length among the species whereas within the species only *C. amada* and *C. aromatica* showed significant variation. Within the accessions of *C. amada*, petiole length varied from 18.33 ± 0.33cm (Acc. 1511) to 28.33 ± 0.88 cm (Acc. 753 and Acc.265). In *C. aromatica*, Acc. 1520 recorded the lowest petiole length of 12.33 ± 0.33 cm and Acc. 1113 recorded the highest petiole length of 31.33 ± 1.86 cm (Table 17).

Percentage of variation for this trait was maximum within the species (88%) than among species (12%). Among the accessions, *C. caesia* and *C. xanthorrhiza* did not show any significant variation for petiole length whereas in *C. amada* (57%) and *C. aromatica* (73%) majority of the variation was present among the accessions (Table 18).

Table 17. Mean petiole length in four *Curcuma* species

Sl. No	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	Petiole length (cm)	Accession	Petiole length (cm)	Accession	Petiole length (cm)	Accession	Petiole length (cm)
1	265	28.33 ± 0.88 ^{a}	711	25.00 ± 3.06 ^{ab}	292	23.90 ± 1.72 ^{ab}	465	26.00 ± 1.53 ^{b}
2	347	21.33 ± 0.33 ^{bc}	1025	16.00 ± 1.53 ^{cd}	751	23.67 ± 3.38 ^{ab}	760	39.00 ± 5.77 ^{a}
3	521	25.33 ± 1.20 ^{ab}	1113	31.33 ± 1.86 ^{a}	1001	28.67 ± 2.03 ^{a}	1108	24.33 ± 1.45 ^{b}
4	752	26.00 ± 3.06 ^{a}	1124	16.33 ± 1.20 ^{cd}	1006	24.33 ± 1.76 ^{ab}	1122	27.33 ± 5.90 ^{b}
5	753	28.33 ± 0.88 ^{a}	1132	27.00 ± 1.53 ^{ab}	1135	19.00 ± 3.06 ^{b}	1163	26.33 ± 3.18 ^{b}
6	848	27.07 ± 1.16 ^{a}	1518	22.00 ± 3.51 ^{bc}	1154	19.00 ± 1.73 ^{b}	1164	23.67 ± 1.76 ^{b}
7	1119	24.33 ± 1.33 ^{ab}	1520	12.33 ± 0.33 ^{d}	1171	24.40 ± 0.87 ^{ab}	1167	26.67 ± 3.53 ^{b}
8	1511	18.33 ± 0.33 ^{c}	-	-	-	-	1168	30.00 ± 1.15 ^{ab}
9	1503	26.43 ± 2.02 ^{a}	-	-	-	-	-	-
10	6390	20.90 ± 1.24 ^{bc}	-	-	-	-	-	-
Mean ± SE		24.64 ± 0.71 ^{b}		21.43 ± 1.57 ^{b}		23.28 ± 0.99 ^{b}		27.92 ± 1.41 ^{a}
Accessions		P< 0.05		P< 0.05		NS		NS
Species		P< 0.05						

NS = Not Significant (P>0.05), Values with the different superscript are significantly different (P<0.05), SE- Standard error

Table 18. F-value and percentage of variation for Petiole Length in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	12.00	39.00	24.46	0.62	5.25**	12%	88%
C. amada	18.00	30.00	24.64	0.71	5.40**	57%	43%
C. aromatica	12.00	32.00	21.43	1.57	10.44**	73%	27%
C. caesia	15.00	32.00	23.28	0.99	2.31	27%	73%
C. xanthorrhiza	20.00	39.00	27.91	1.41	1.91	21%	79%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.1.1.5. Leaf Length (LL)

Among the four species of *Curcuma*, maximum leaf length was observed in *C. xanthorrhiza* (66.58 ± 2.02 cm) and minimum in *C. caesia* (46.20 ± 1.81 cm). Analysis of variance for leaf length revealed significant variation among the four species. Within the accessions of individual species only *C. amada* and *C. aromatica* showed significant variation.

In *C. amada*, leaf length ranged from 50.33 ± 3.18 cm (Acc.1511) to 67.83 ± 2.42 cm (Acc. 848). Among the accessions of *C. aromatica*, leaf length was minimum for Acc.1520 (30.33 ± 5.17 cm) and maximum for Acc. 711 (70.33 ± 5.55 cm) (Table 19).

The percentage of variation obtained from the mean squares values revealed that in four species of *Curcuma* 66% of the variation was contributed by variation present within the species than among the species (34%). In *C. amada*, the major source variation was from within the accessions (63%) whereas in *C. aromatica*, the variability within the accessions was just 24% (Table 20).

Table 19. Mean leaf length in four *Curcuma* species

Sl. No	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	Leaf length (cm)	Accession	Leaf length (cm)	Accession	Leaf length (cm)	Accession	Leaf length (cm)
1	265	62.33 ± 2.33 ^{ab}	711	70.33 ± 5.55 ^{a}	292	55.07 ± 5.45 ^{a}	465	66.33 ± 5.93 ^{a}
2	347	50.67 ± 4.48 ^{b}	1025	36.33 ± 2.60 ^{de}	751	46.67 ± 5.24 ^{a}	760	73.33 ± 4.10 ^{a}
3	521	57.33 ± 5.90 ^{ab}	1113	59.67 ± 0.33 ^{ab}	1001	49.00 ± 4.16 ^{a}	1108	63.33 ± 3.76 ^{a}
4	752	67.67 ± 1.20 ^{a}	1124	51.67 ± 2.33 ^{bc}	1006	47.33 ± 2.03 ^{a}	1122	60.33 ± 9.40 ^{a}
5	753	60.67 ± 4.63 ^{ab}	1132	60.00 ± 6.81 ^{ab}	1135	44.33 ± 1.20 ^{ab}	1163	65.33 ± 3.18 ^{a}
6	848	67.83 ± 2.42 ^{a}	1518	43.67 ± 1.86 ^{cd}	1154	34.00 ± 2.08 ^{b}	1164	59.67 ± 0.33 ^{a}
7	1119	61.33 ± 4.63 ^{ab}	1520	30.33 ± 5.17 ^{e}	1171	47.00 ± 5.00 ^{a}	1167	67.67 ± 7.17 ^{a}
8	1511	50.33 ± 3.18 ^{b}	-	-	-	-	1168	76.67 ± 6.01 ^{a}
9	1503	60.33 ± 0.20 ^{ab}	-	-	-	-	-	-
10	6390	52.73 ± 4.03 ^{b}	-	-	-	-	-	-
Mean ± SE		59.12 ± 1.48 ^{b}		50.29 ± 3.23 ^{c}		46.20 ± 1.81 ^{c}		66.58 ± 2.02 ^{a}
Accessions		P< 0.05		P< 0.05		NS		NS
Species		P< 0.05						

NS = Not Significant (P>0.05), Values with the different superscript are significantly different (P<0.05), SE-Standard error

Table 20. F-value and percentage of variation of leaf length in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	20.00	85.00	56.23	1.31	17.55**	34%	66%
C. amada	42.00	70.40	59.12	1.48	2.93*	37%	63%
C. aromatica	20.00	79.00	50.29	3.23	11.92**	76%	24%
C. caesia	30.00	64.00	46.20	1.81	2.57	31%	69%
C. xanthorrhiza	49.00	85.00	66.58	2.02	1.12	3%	97%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.1.1.6. Leaf Width (LW)

Within the four species of *Curcuma*, maximum leaf width was observed in *C. xanthorrhiza* (15.83 ± 0.76 cm) and minimum in *C. caesia* (11.55 ± 0.29cm). Analysis of variance indicated significant differences among species for leaf width. However, within the species, none of the species except *C. xanthorrhiza*, showed significant variation for the trait. In *C. xanthorrhiza*, the Acc.1163 recorded minimum leaf width of 13.17 ± 0.44 cm and the Acc.1164 recorded maximum leaf width of 23.67 ± 1.76 cm (Table 21).

The percentage of variation calculations revealed that in four species of *Curcuma* 71% of the variation was contributed by variation present within the species than among the species (29%). In *C. xanthorrhiza*, majority of the variation for leaf width was due to among the accessions source (63%) (Table 22).

Table 21. Mean leaf width in four *Curcuma* species

Sl. No	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	Leaf width (cm)	Accession	Leaf width (cm)	Accession	Leaf width (cm)	Accession	Leaf width (cm)
1	265	13.67 ± 0.33 [a]	711	15.50 ± 1.76 [a]	292	11.80 ± 0.42 [ab]	465	14.90 ± 1.82 [b]
2	347	11.67 ± 0.33 [a]	1025	13.13 ± 0.69 [a]	751	10.63 ± 0.49 [b]	760	14.63 ± 1.13 [b]
3	521	12.67 ± 0.88 [a]	1113	14.87 ± 1.07 [a]	1001	12.20 ± 0.65 [ab]	1108	13.73 ± 0.87 [b]
4	752	14.00 ± 0.58 [a]	1124	13.52 ± 1.10 [a]	1006	13.13 ± 0.96 [a]	1122	14.67 ± 1.06 [b]
5	753	14.23 ± 1.39 [a]	1132	15.33 ± 1.20 [a]	1135	11.40 ± 0.92 [ab]	1163	13.17 ± 0.44 [b]
6	848	13.77 ± 1.18 [a]	1518	13.67 ± 0.67 [a]	1154	10.33 ± 0.35 [b]	1164	23.67 ± 1.76 [a]
7	1119	12.13 ± 0.94 [a]	1520	13.77 ± 1.57 [a]	1171	11.33 ± 0.67 [ab]	1167	17.20 ± 1.07 [b]
8	1511	13.20 ± 0.12 [a]	-	-	-	-	1168	14.70 ± 1.61 [b]
9	1503	12.27 ± 0.43 [a]	-	-	-	-	-	-
10	6390	11.63 ± 0.50 [a]	-	-	-	-	-	-
Mean ± SE		12.92 ± 0.27 [c]		14.26 ± 0.43 [b]		11.55 ± 0.29 [d]		15.83 ± 0.76 [a]
Accessions		NS		NS		NS		P< 0.05
Species		P< 0.05						

NS = Not Significant (P>0.05), Values with the different superscript are significantly different (P<0.05), SE-Standard error

Table 22. F-value and percentage of variation of leaf width in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	9.80	27.00	13.64	0.28	14.29**	29%	71%
C. amada	10.90	17.00	12.92	0.27	1.60	15%	85%
C. aromatica	11.36	19.00	14.26	0.43	0.62	21%	79%
C. caesia	9.80	15.00	11.55	0.29	1.98	22%	78%
C. xanthorrhiza	11.60	27.00	15.83	0.76	6.75**	63%	37%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.1.1.7. Colour of Leaf Sheath (CLS)

Colour of leaf sheath (CLS) varied from green (Hex code #008000) to dark green (Hex code #006400) among the four *Curcuma* species. *C. amada* and *C. aromatica* exhibited dark green colour, while *C. caesia* and *C. xanthorrhiza* exhibited green colour (Figure 6).

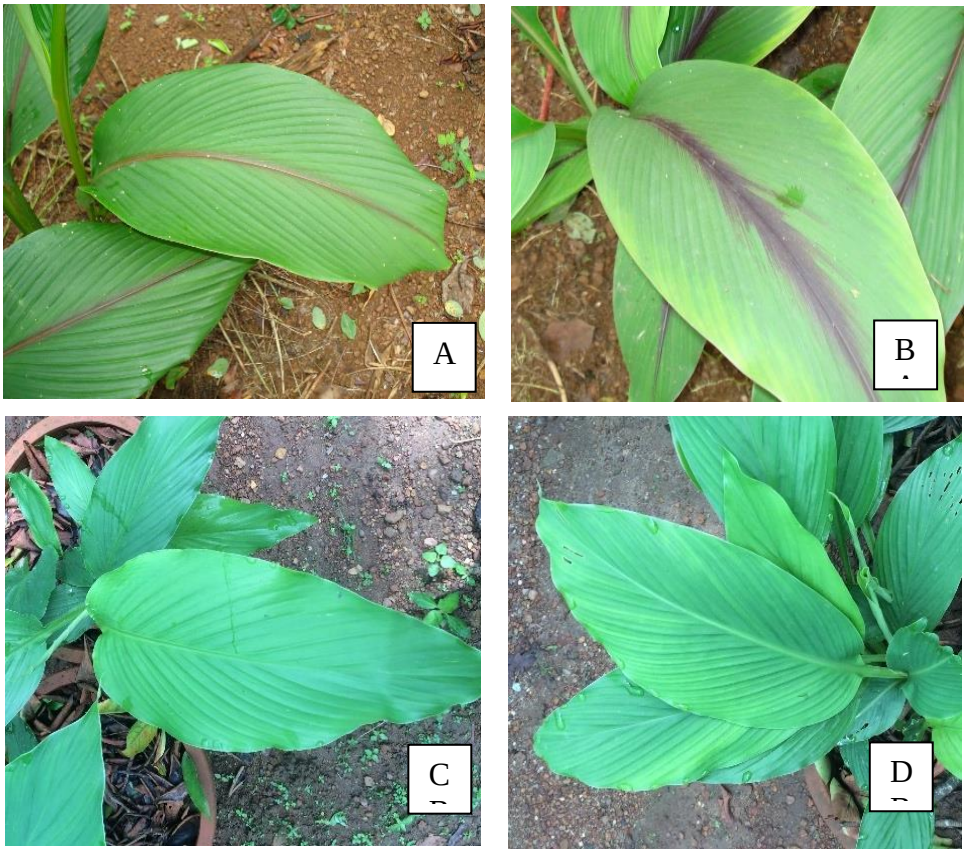


Fig. 6. Colour of leaf sheath and leaf mid rib colour in four *Curcuma* species A). *C. xanthorrhiza,* B). *C. caesia,* C). *C. amada,* D). *C. aromatica.*

4.1.1.8. Leaf Midrib Colour (LMC)

Leaf midrib colour of the four species varied from green (Hex code #008000) to purple (Hex code # 800080) and dark purple (Hex code # 660066). *C. amada* and *C. aromatica* had a green coloured leaf mid rib whereas *C. caesia* had a dark purple coloured leaf midrib. *C. xanthorrhiza* had a purple coloured leaf midrib (Figure 6).

4.1.1.9. Leaf Texture (LT)

Three species *viz*., *C. amada, C. caesia* and *C. xanthorrhiza* had a glabrous leaf lamina whereas *C. aromatica* had a pubescent leaf lamina (abaxial side).

4.1.1.10. Pseudo stem colour (PC)

Among the four *Curcuma* species studied, accessions of *C. caesia* showed considerable variation for the colour of pseudo stem. Acc. 751 and Acc. 1001 showed a purple tinge on their pseudo stem whereas rest of the accessions of *C. caesia* as well as other three species had a green pseudo stem colour (Figure 7).

4.1.2. Rhizome morphological characters

4.1.2.1. Rhizome Habit (RH)

Rhizome habit of accessions of *C. amada, C. aromatica* and *C. caesia* are intermediate in nature, whereas accessions of *C. xanthorrhiza* showed a loose rhizome habit. Primary fingers were very less in *C. xanthorrhiza* (Figure 8).

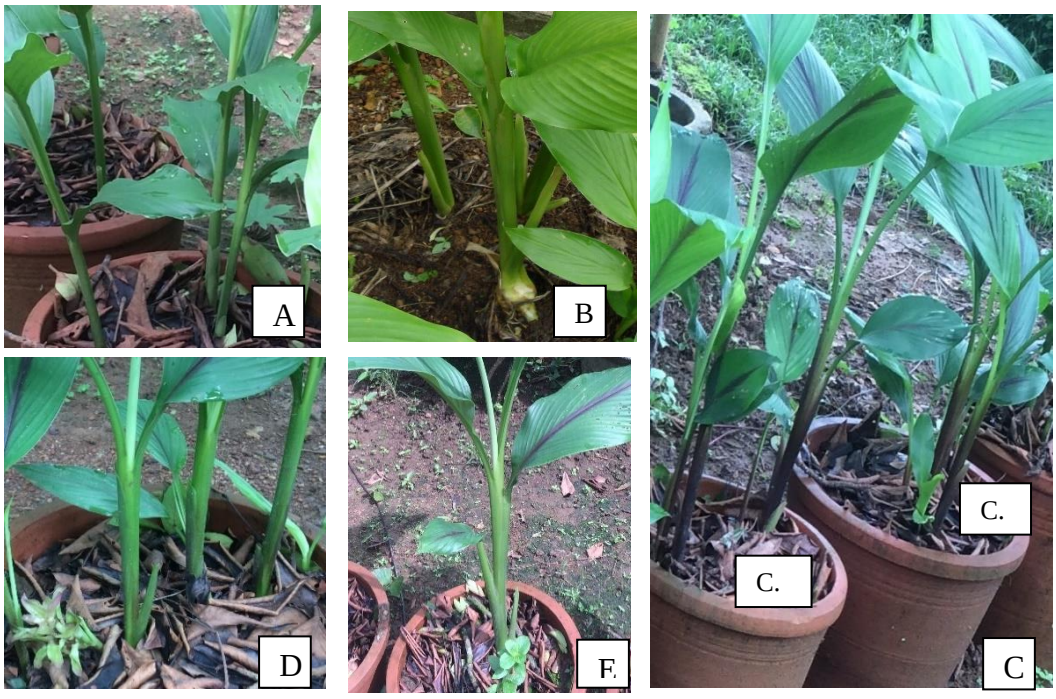


Fig. 7. Pseudo stem colour of four *Curcuma* species A). *C. amada* B). *C. aromatica* C). *C. caesia* [C.a). – Acc. 751 and C.b). – Acc.1001] D). *C. caesia* E). *C. xanthorrhiza*

Fig. 8. Rhizome habit of four *Curcuma* species a). *C. amada,* b). *C. aromatica,* c). *C. caesia,* d). *C. xanthorrhiza.*

4.1.2.2. Rhizome Weight (RW)

Rhizome weight showed significant variation among the four species. Minimum rhizome weight was recorded in *C. amada* (229.30±2.48g) and maximum in *C. xanthorrhiza* (716.09±46.44g) (Table 23). The percentage of variation observed among the species was 71% while within the species it was 29%. Within the species, the rhizome weight varied significantly in all the three species, except in *C. amada.* Among the accessions of *C. aromatica*, the weight of rhizome ranged from 224.20±4.52g (Acc.1518) to 277.23±2.85g (Acc.1124) with 80% of the variation observed among the accessions and 20% of the variation within the accessions. In *C. caesia*, maximum rhizome weight was in the Acc.1001 (648.39±6.85g) and minimum in Acc.751 (251.25±5.27g) with 98% of the variation present among accessions and a meagre 2% within accessions. *C. xanthorrhiza* showed 68% of the variation for rhizome weight among the accessions and 32% within accessions with rhizome weight ranging from 361.09±1.55g (Acc. 1167) to 1048.25±24.29g (Acc. 1168) (Table 24).

Table 23. Mean rhizome weight in four *Curcuma* species

Sl. No	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	Rhizome weight (g)	Accession	Rhizome weight (g)	Accession	Rhizome weight (g)	Accession	Rhizome weight (g)
1	265	217.60 ± 7.41 ^{a}	711	250.23 ± 8.26 ^{bc}	292	416.67 ± 1.70 ^{c}	465	670.36 ± 18.36 ^{bc}
2	347	231.69 ± 8.27 ^{a}	1025	265.65 ± 5.73 ^{ab}	751	251.25 ± 5.27 ^{d}	760	707.89 ± 167.77 ^{bc}
3	521	235.67 ± 13.08 ^{a}	1113	250.06 ± 1.85 ^{bc}	1001	648.39 ± 6.85 ^{a}	1108	720.56 ± 8.98 ^{bc}
4	752	237.01 ± 13.85 ^{a}	1124	277.23 ± 2.85 ^{a}	1006	428.32 ± 4.80 ^{c}	1122	831.54 ± 114.54 ^{ab}
5	753	230.79 ± 8.56 ^{a}	1132	275.90 ± 6.97 ^{a}	1135	405.91 ± 21.52 ^{c}	1163	866.77 ± 3.97 ^{ab}
6	848	233.11 ± 5.36 ^{a}	1518	224.20 ± 4.52 ^{d}	1154	533.43 ± 6.64 ^{b}	1164	522.24 ± 8.33 ^{cd}
7	1119	224.09 ± 7.59 ^{a}	1520	236.87 ± 1.96 ^{cd}	1171	409.52 ± 5.23 ^{c}	1167	361.09 ± 1.55 ^{d}
8	1511	226.88 ± 7.17 ^{a}	-	-	-	-	1168	1048.25 ± 24.29 ^{a}
9	1503	225.69 ± 6.61 ^{a}	-	-	-	-	-	-
10	6390	230.51 ± 0.10 ^{a}	-	-	-	-	-	-
Mean ± SE		229.30 ± 2.48 ^{c}		254.31 ± 4.42 ^{c}		441.93 ± 25.61 ^{b}		716.09 ± 46.44 ^{a}
Accessions		NS		P< 0.05		P< 0.05		P< 0.05
Species		P< 0.05						

NS = Not Significant (P>0.05), Values with the different superscript are significantly different (P<0.05), SE-Standard error

Table 24. F-value and percentage of variation of rhizome weight in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	206.88	1090.32	402.98	23.96	77.80**	71%	29%
C. amada	206.88	261.87	229.31	2.48	0.46	16%	84%
C. aromatica	217.92	284.70	254.31	4.42	14.89**	80%	20%
C. caesia	242.11	660.26	441.93	25.61	166.23**	98%	2%
C. xanthorrhiza	358.40	1090.32	716.09	46.44	8.42**	68%	32%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.1.2.3. Rhizome Length (RL)

Analysis of variance showed no significant variation for rhizome length among the species. The average rizhome length among all the four species was observed to be 8.66 ± 0.15 cm. Within the species, only *C. amada* and *C. xanthorrhiza exhibited* significant variation. In *C. amada*, maximum rhizome length was observed in Acc.1119 (9.83 ± 0.34 cm) and minimum in Acc. 848 (6.90 ± 0.26 cm) whereas in *C. xanthorrhiza*, rhizome length varied from 7.00 ± 0.50 cm (Acc. 1163) to 10.75 ± 0.14 cm (Acc.1168) (Table 25).

The percentage of variation obtained from mean square values displayed lack of significant variation among the species (2%) for rhizome length. Within the species, *C. amada* and *C. xanthorrhiza* showed 77% and 63% variation among the accessions and 23% and 37% variation within in the accessions, respectively (Table 26).

Table 25. Mean rhizome length in four *Curcuma* species

Sl. No	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	Rhizome length (cm)	Accession	Rhizome length (cm)	Accession	Rhizome length (cm)	Accession	Rhizome length (cm)
1	265	8.37 ± 0.09 ^{bc}	711	8.13 ± 1.37 ^{a}	292	8.63 ± 1.59 ^{ab}	465	8.63 ± 0.73 ^{bc}
2	347	9.03 ± 0.54 ^{ab}	1025	7.80 ± 0.50 ^{a}	751	10.77 ± 0.99 ^{a}	760	7.57 ± 0.92 ^{c}
3	521	9.20 ± 0.66 ^{ab}	1113	9.83 ± 0.90 ^{a}	1001	6.53 ± 0.35 ^{b}	1108	7.97 ± 0.67 ^{bc}
4	752	6.93 ± 0.37 ^{d}	1124	7.63 ± 0.26 ^{a}	1006	8.65 ± 0.84 ^{ab}	1122	8.33 ± 0.44 ^{bc}
5	753	7.57 ± 0.56 ^{cd}	1132	9.77 ± 0.20 ^{a}	1135	10.30 ± 0.68 ^{a}	1163	7.00 ± 0.50 ^{c}
6	848	6.90 ± 0.26 ^{d}	1518	9.43 ± 0.37 ^{a}	1154	8.87 ± 0.82 ^{ab}	1164	7.35 ± 0.66 ^{c}
7	1119	9.83 ± 0.34 ^{a}	1520	9.03 ± 0.38 ^{a}	1171	8.70 ± 1.42 ^{ab}	1167	9.90 ± 0.81 ^{ab}
8	1511	8.37 ± 0.29 ^{bc}	-	-	-	-	1168	10.75 ± 0.14 ^{a}
9	1503	9.80 ± 0.25 ^{a}	-	-	-	-	-	-
10	6390	8.37 ± 0.09 ^{bc}	-	-	-	-	-	-
Mean ± SE		8.52 ± 0.22 ^{a}		8.80 ± 0.29 ^{a}		8.92 ± 0.43 ^{a}		8.44 ± 0.32 ^{a}
Accessions		P< 0.05		NS		NS		P< 0.05
Species		NS						

NS = Not Significant (P>0.05), Values with the different superscript are significantly different (P<0.05), SE- Standard error

Table 26. F-value and percentage of variation of rhizome length in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	5.60	12.70	8.66	0.15	0.52	2%	98%
C. amada	6.20	10.50	8.52	0.22	7.51**	77%	23%
C. aromatica	5.60	11.30	8.81	0.29	1.85	44%	56%
C. caesia	6.00	12.70	8.92	0.43	1.75	43%	57%
C. xanthorrhiza	6.00	11.30	8.44	0.32	3.96*	63%	37%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.1.2.4. Rhizome Internode Length (RIL)

Significant variation was observed among the four species for rhizome internode length, which ranged from 1.17±0.05 cm (*C. aromatica*) to 1.43±0.07 cm (*C. caesia*). The percentage of variation observed among and within the species was 9% and 91%, respectively. While among the accessions of four species, only *C. amada* showed significant variation. The percentage of variation estimated using mean square values showed major variation (51%) from within the accessions than among the accessions of *C. amada* (49%). Accessions of rest of the three species did not show significant variation among themselves (Table 27).

Among the accessions of *C. amada*, rhizome internode length (RIL) varied from 1.03 ± 0.09cm (Acc. 752) to 1.47 ± 0.09 cm (Acc. 1119) (Table 28).

Table 27. Mean rhizome internode length in four *Curcuma* species

Sl. No	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	Rhizome internode length (cm)	Accession	Rhizome internode length (cm)	Accession	Rhizome internode length (cm)	Accession	Rhizome internode length (cm)
1	265	1.30 ± 0.10 ^{bc}	711	1.00 ± 0.15 ^{a}	292	1.23 ± 0.19 ^{a}	465	1.37 ± 0.09 ^{ab}
2	347	1.37 ± 0.09 ^{bc}	1025	0.97 ± 0.23 ^{a}	751	1.60 ± 0.21 ^{a}	760	1.40 ± 0.06 ^{ab}
3	521	1.43 ± 0.03 ^{abc}	1113	1.13 ± 0.03 ^{a}	1001	1.23 ± 0.15 ^{a}	1108	1.37 ± 0.23 ^{ab}
4	752	1.03 ± 0.09 ^{d}	1124	1.30 ± 0.15 ^{a}	1006	1.20 ± 0.06 ^{a}	1122	1.33 ± 0.18 ^{ab}
5	753	1.23 ± 0.03 ^{bcd}	1132	1.30 ± 0.06 ^{a}	1135	1.57 ± 0.32 ^{a}	1163	1.23 ± 0.03 ^{b}
6	848	1.20 ± 0.06 ^{cd}	1518	1.13 ± 0.09 ^{a}	1154	1.73 ± 0.17 ^{a}	1164	1.20 ± 0.00 ^{b}
7	1119	1.47 ± 0.09 ^{ab}	1520	1.37 ± 0.15 ^{a}	1171	1.47 ± 0.09 ^{a}	1167	1.70 ± 0.12 ^{a}
8	1511	1.33 ± 0.03 ^{bc}	-	-	-	-	1168	1.35 ± 0.09 ^{ab}
9	1503	1.37 ± 0.09 ^{bc}	-	-	-	-	-	-
10	6390	1.30 ± 0.10 ^{bc}	-	-	-	-	-	-
Mean ± SE		1.34 ± 0.03 ^{a}		1.17 ± 0.05 ^{b}		1.43 ± 0.07 ^{a}		1.37 ± 0.05 ^{a}
Accessions		P< 0.05		NS		NS		NS
Species		P< 0.05						

NS = Not Significant (P>0.05), Values with the different superscript are significantly different (P<0.05), SE-Standard error

Table 28. F-value and percentage of variation of rhizome internode length in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	0.60	2.10	1.33	0.03	4.22**	9%	91%
C. amada	0.90	1.76	1.34	0.04	4.27**	49%	51%
C. aromatica	0.60	1.60	1.17	0.05	1.26	7%	93%
C. caesia	1.00	2.10	1.43	0.07	1.32	8%	92%
C. xanthorrhiza	1.00	1.90	1.37	0.05	1.53	13%	87%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance.

4.1.2.5. Rhizome Inner Core Colour (RIC)

Although rhizome inner core colour showed interspecific variation there was no intraspecific variation for the rhizome inner core colour in all the four species. All the ten accessions of *C. amada* showed creamy white (Hex code #FFFFE0) inner core colour. Likewise, seven accessions of *C. aromatica* were uniformly pale yellow (Hex code #FFFFE0) coloured. Accessions of *C. caesia* possessed peculiar bluish black (Hex code #00008B) colour and eight accessions of *C. xanthorrhiza* were orange yellow (Hex code #FF8C00) in colour. Rhizome inner core colour variation is shown in the Figure 9.

Fig 9. Representative photographs of mother rhizome and rhizome inner core colour of different species of *Curcuma* A). *C. xanthorrhiza,* B). *C. amada,* C). *C. aromatica,* D). *C. caesia.*

4.1.2.6. Number of Mother Rhizome (NM)

Among the four species, number of mother rhizome was highest in *C. caesia* (4.86 ± 0.24) and lowest in *C. xanthorrhiza* (2.29 ± 0.09). The observed values of the number of mother rhizome are given in Table 29. Variance analysis revealed significant variation among the four species of *Curcuma*. Percentage of variation using mean square values revealed observed variation among the species was more (60%) than within the species (40%) variation. Within the species, only *C. amada* showed significant variation with a percentage of variation of 81% within and 19% among the accessions (Table 30).

The number of mother rhizome in accessions of *C. amada* ranged from 3.00 ± 0.00 (Acc.752) to 4.00 ± 0.00 (Acc. 347).

Table 29. Mean number of mother rhizomes in four *Curcuma* species

Sl. No	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	No. of mother rhizomes	Accession	No. of mother rhizomes	Accession	No. of mother rhizomes	Accession	No. of mother rhizomes
1	265	3.67 ± 0.33 ^{ab}	711	2.67 ± 0.33 ^{a}	292	5.33 ± 0.33 ^{a}	465	2.67 ± 0.33 ^{a}
2	347	4.00 ± 0.00 ^{a}	1025	3.00 ± 0.58 ^{a}	751	4.67 ± 0.88 ^{a}	760	2.00 ± 0.00 ^{a}
3	521	3.67 ± 0.33 ^{ab}	1113	2.33 ± 0.33 ^{a}	1001	4.33 ± 0.33 ^{a}	1108	2.33 ± 0.33 ^{a}
4	752	3.00 ± 0.00 ^{b}	1124	3.00 ± 0.58 ^{a}	1006	5.00 ± 0.58 ^{a}	1122	2.33 ± 0.33 ^{a}
5	753	3.33 ± 0.33 ^{ab}	1132	3.33 ± 0.33 ^{a}	1135	4.00 ± 0.58 ^{a}	1163	2.00 ± 0.00 ^{a}
6	848	3.67 ± 0.33 ^{ab}	1518	2.00 ± 0.00 ^{a}	1154	5.33 ± 0.33 ^{a}	1164	2.33 ± 0.33 ^{a}
7	1119	3.67 ± 0.33 ^{ab}	1520	2.33 ± 0.33 ^{a}	1171	5.33 ± 1.20 ^{a}	1167	2.33 ± 0.33 ^{a}
8	1511	3.67 ± 0.33 ^{ab}		-	-	-	1168	2.33 ± 0.33 ^{a}
9	1503	3.67 ± 0.33 ^{ab}		-	-	-	-	-
10	6390	3.67 ± 0.33 ^{ab}		-	-	-	-	-
Mean ± SE		3.43 ± 0.12 ^{b}		2.67 ± 0.16 ^{c}		4.86 ± 0.24 ^{a}		2.29 ± 0.09 ^{c}
Accessions		P< 0.05		NS		NS		NS
Species		P< 0.05						

NS = Not Significant (P>0.05), Values with the different superscript are significantly different (P<0.05), SE-Standard error

Table 30. F-value and percentage of variation of number of mother rhizomes in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	2.00	7.00	3.29	0.12	48.25**	60%	40%
C. amada	2.00	4.00	3.43	0.12	4.14**	49%	51%
C. aromatica	2.00	4.00	2.67	0.16	1.40	10%	90%
C. caesia	3.00	7.00	4.86	0.24	0.63	21%	79%
C. xanthorrhiza	2.00	3.00	2.29	0.09	0.55	19%	81%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.1.2.7. Number of Primary Rhizomes (NP)

There was significant variation among and within the four species for the number of primary rhizomes which ranged from 10.17 ± 0.44 (*C. amada*) to 20.43 ± 1.47 (*C. caesia*). The observed values for the number of primary rhizomes are given in Table 31. Percentage of variation obtained from the mean square values revealed that 45% of the variation was contributed by the variation existing among the species whereas 55% of the variation was contributed by variation present within the species (Table 32).

The number of primary rhizome in the accessions of *C. amada* varied from 8.00 ± 0.58 (Acc.752, Acc. 1503) to 13.67 ± 0.88 (Acc.848). In *C. amada*, the percentage of variation estimated using mean square values showed 52% variation among the accessions and 48% within the accessions (Table 22). In *C. aromatica*, the number of primary rhizomes varied from 8.33 ± 0.67 (Acc.1518) to 13.67 ± 0.67 (Acc.1025) and the variation was significant with a percentage of variation of 54% among accessions and 46% within the accessions. *C. caesia* showed a range from 13.00 ± 0.58 (Acc. 1171) to 33.00 ± 0.58 (Acc.1001), and the variation among the accessions of *C. caesia* had a percentage variation of 95%. In *C. xanthorrhiza*, Acc.1167 showed least number of primary rhizomes (5.33 ± 0.33) and Acc.1163 showed the highest number of primary rhizomes (19.67 ± 0.88). The variation present among the accessions of *C. xanthorrhiza* was significant with percentage variation of 76% among the accessions.

Table 31. Mean number of primary rhizomes in four *Curcuma* species

Sl. No	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	No. of primary rhizomes	Accession	No. of primary rhizomes	Accession	No. of primary rhizomes	Accession	No. of primary rhizomes
1	265	12.33 ± 0.88 ^{ab}	711	9.67 ± 0.33 ^{bc}	292	19.50 ± 0.87 ^{c}	465	17.33 ± 1.20 ^{ab}
2	347	10.33 ± 0.67 ^{bc}	1025	13.67 ± 0.67 ^{a}	751	20.50 ± 0.87 ^{c}	760	11.33 ± 2.91 ^{cd}
3	521	11.00 ± 2.08 ^{abc}	1113	8.67 ± 0.88 ^{c}	1001	33.00 ± 0.58 ^{a}	1108	13.67 ± 0.88 ^{bc}
4	752	8.00 ± 0.58 ^{cd}	1124	12.00 ± 1.15 ^{ab}	1006	26.00 ± 1.15 ^{b}	1122	15.00 ± 1.15 ^{bc}
5	753	10.67 ± 0.88 ^{abc}	1132	11.67 ± 0.88 ^{ab}	1135	16.00 ± 1.15 ^{d}	1163	19.67 ± 0.88 ^{a}
6	848	13.67 ± 0.88 ^{a}	1518	8.33 ± 0.67 ^{c}	1154	15.00 ± 0.58 ^{de}	1164	8.67 ± 0.33 ^{de}
7	1119	9.67 ± 0.88 ^{bcd}	1520	11.67 ± 1.20 ^{ab}	1171	13.00 ± 0.58 ^{e}	1167	5.33 ± 0.33 ^{e}
8	1511	11.00 ± 0.58 ^{abc}	-	-	-	-	1168	12.00 ± 1.15 ^{cd}
9	1503	8.00 ± 0.58 ^{cd}	-	-	-	-	-	-
10	6390	12.33 ± 0.88 ^{ab}	-	-	-	-	-	-
Mean ± SE		10.17 ± 0.44 ^{c}		10.81 ± 0.49 ^{bc}		20.43 ± 1.47 ^{a}		12.88 ± 0.98 ^{b}
Accessions		P< 0.05		P< 0.05		P< 0.05		P< 0.05
Species		P< 0.05						

Values with the different superscript are significantly different (P<0.05), SE-Standard error

Table 32. F-value and percentage of variation of number of primary rhizomes in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	5.00	34.00	13.23	0.59	26.71**	45%	55%
C. amada	6.00	15.00	10.17	0.44	4.61**	52%	48%
C. aromatica	7.00	15.00	10.81	0.49	5.05**	54%	46%
C. caesia	12.00	34.00	20.43	1.48	66.43**	95%	5%
C. xanthorrhiza	5.00	21.00	12.88	0.98	11.87**	76%	24%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.1.2.8. Number of Secondary rhizomes (NS)

Number of secondary rhizomes among the four species under study varied from 10.08 ± 1.11 to 40.79 ± 2.26; highest in *C. caesia* and lowest in *C. xanthorrhiza* (Table 33). Analysis of variance showed significant variations among the species under study for the number of secondary rhizome. Seventy-two percent of the variation was accounted by the variation among the species and remaining 28% was contributed by variation within the species. Within the species, except *C. amada,* all the species showed significant variation for number of secondary rhizomes. The percentage of variation observed among the accessions was 95% (*C. caesia*), 77% *(C. aromatica*), and 76% (*C. xanthorrhiza*), respectively (Table 34).

In *C. aromatica,* the number of secondary rhizomes varied from 19.33 ± 0.33(Acc. 1518) to 34.67 ± 0.67 (Acc. 1025). Among the accessions of *C. caesia,* number of secondary rhizome varied from 29.00 ± 0.58 to 60.50 ± 0.29; highest being in Acc. 1001 and lowest in Acc. 1171. Number of secondary rhizome in the accessions of *C. xanthorrhiza* varied from 5.33 ± 0.33 (Acc. 1167) to 21.00 ± 0.58 (Acc. 1168) (Table 33).

Table 33. Mean number of secondary rhizomes in four *Curcuma* species

Sl. No	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	No. of secondary rhizomes	Accession	No. of secondary rhizomes	Accession	No. of secondary rhizomes	Accession	No. of secondary rhizomes
1	265	23.00 ± 0.58 ^{a}	711	23.00 ± 0.58 ^{cd}	292	35.50 ± 0.87 ^{de}	465	9.67 ± 0.88 ^{bc}
2	347	24.33 ± 2.19 ^{a}	1025	34.67 ± 0.67 ^{a}	751	32.00 ± 2.31 ^{ef}	760	7.00 ± 0.58 ^{c}
3	521	27.33 ± 4.26 ^{a}	1113	22.00 ± 1.53 ^{cd}	1001	60.50 ± 0.29 ^{a}	1108	10.33 ± 0.67 ^{bc}
4	752	24.00 ± 0.58 ^{a}	1124	27.67 ± 0.88 ^{b}	1006	39.00 ± 1.15 ^{cd}	1122	14.00 ± 4.00 ^{b}
5	753	25.33 ± 4.84 ^{a}	1132	26.00 ± 1.15 ^{bc}	1135	48.50 ± 1.44 ^{b}	1163	6.33 ± 0.67 ^{c}
6	848	27.00 ± 0.58 ^{a}	1518	19.33 ± 0.33 ^{d}	1154	41.00 ± 1.15 ^{c}	1164	7.00 ± 0.58 ^{c}
7	1119	23.33 ± 4.84 ^{a}	1520	25.67 ± 2.85 ^{bc}	1171	29.00 ± 0.58 ^{f}	1167	5.33 ± 0.33 ^{c}
8	1511	20.33 ± 1.86 ^{a}	-	-	-	-	1168	21.00 ± 0.58 ^{a}
9	1503	25.00 ± 0.58 ^{a}	-	-	-	-	-	-
10	6390	24.73 ± 0.37 ^{a}	-	-	-	-	-	-
Mean ± SE		24.44 ± 0.80 ^{b}		25.48 ± 1.11 ^{b}		40.79 ± 2.26 ^{a}		10.08 ± 1.11 ^{c}
Accessions		NS		P< 0.05		P< 0.05		P< 0.05
Species		P< 0.05						

NS = Not Significant (P>0.05), Values with the different superscript are significantly different (P<0.05), SE-Standard error

Table 34. F-value and percentage of variation of number of secondary rhizomes in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	5.00	61.00	24.65	1.26	82.89**	72%	28%
C. amada	18.00	35.00	24.44	0.80	0.54	19%	81%
C. aromatica	19.00	36.00	25.48	1.11	12.63**	77%	23%
C. caesia	28.00	61.00	40.79	2.27	72.12**	95%	5%
C. xanthorrhiza	5.00	22.00	10.08	1.11	11.59**	76%	24%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.1.2.10. Weight of Mother Rhizome (WM)

Analysis of variance revealed significant variation for the weight of mother among the accessions rhizome, which varied from 84.41 ± 2.11g to 328.72 ± 21.54g; highest being in *C. xanthorrhiza* and lowest in *C. aromatica.* In *C. aromatica* weight of mother rhizome ranged from 76.23 ± 6.94g (Acc. 1518) to 99.05 ± 1.27g (Acc. 1025). Among accessions of *C. caesia,* the values ranged from 87.09 ± 1.03g (Acc. 751) to 199.87 ± 2.41g (Acc. 1154). In *C. xanthorrhiza*, maximum weight of mother rhizome was recorded for Acc.1163 (460.16 ± 25.39 g) and minimum in Acc. 1167 (193.90 ± 2.51 g) (Table 35).

The percentage of variation for the weight of mother rhizomes was 76% among the species and remaining 24% was within the species variation (Table 36). Within the four species, all the species except *C. amada* showed significant variation for weight of mother rhizome. Percentage of variation was found higher among the accessions of *C. aromatica* (56%) and *C. caesia* (89%) than within the accessions. *C. xanthorrhiza* exhibited a reverse trend (Table 36).

Table 35. Mean weight of mother rhizomes in four *Curcuma* species

Sl. No	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	Weight of mother rhizomes(g)	Accession	Weight of mother rhizomes(g)	Accession	Weight of mother rhizomes(g)	Accession	Weight of mother rhizomes(g)
1	265	80.64 ± 6.30 ^{a}	711	80.35 ± 1.19 ^{c}	292	153.31 ± 2.94 ^{b}	465	296.71 ± 3.58 ^{bcd}
2	347	82.49 ± 1.78 ^{a}	1025	99.05 ± 1.27 ^{a}	751	87.09 ± 1.03 ^{c}	760	409.54 ± 105.86 ^{ab}
3	521	84.49 ± 8.37 ^{a}	1113	81.90 ± 3.41 ^{bc}	1001	183.89 ± 3.10 ^{a}	1108	350.44 ± 4.26 ^{abc}
4	752	88.13 ± 4.51 ^{a}	1124	83.03 ± 4.36 ^{bc}	1006	142.44 ± 3.39 ^{b}	1122	381.81 ± 61.34 ^{abc}
5	753	81.67 ± 8.29 ^{a}	1132	93.27 ± 2.63 ^{ab}	1135	136.54 ± 17.05 ^{b}	1163	460.16 ± 25.39 ^{a}
6	848	91.41 ± 2.55 ^{a}	1518	76.23 ± 6.94 ^{c}	1154	199.87 ± 2.41 ^{a}	1164	281.89 ± 4.19 ^{bcd}
7	1119	86.87 ± 2.28 ^{a}	1520	77.01 ± 2.13 ^{c}	1171	134.63 ± 1.95 ^{b}	1167	193.90 ± 2.51 ^{d}
8	1511	82.33 ± 5.76 ^{a}	-	-	-	-	1168	255.34 ± 7.62 ^{cd}
9	1503	87.78 ± 1.06 ^{a}	-	-	-	-	-	-
10	6390	86.68 ± 0.33 ^{a}	-	-	-	-	-	-
Mean ± SE		85.25 ± 1.44 ^{c}		84.41 ± 2.11 ^{c}		148.25 ± 7.89 ^{b}		328.72 ± 21.54 ^{a}
Accessions		NS		P< 0.05		P< 0.05		P< 0.05
Species		P< 0.05						

NS = Not Significant (P>0.05), Values with the different superscript are significantly different (P<0.05), SE-Standard error

Table 36. F-value and percentage of variation of weight of mother rhizomes in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	65.34	519.48	159.71	11.75	104.47**	76%	24%
C. amada	65.34	94.62	85.25	1.44	0.48	17%	83%
C. aromatica	69.24	101.10	84.41	2.11	5.49**	56%	44%
C. caesia	85.30	204.04	148.25	7.89	28.39**	89%	11%
C. xanthorrhiza	189.56	519.48	328.72	21.54	3.92*	46%	54%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.1.2.11. Weight of Primary rhizome (WP)

The weight of primary rhizome varied from 101.40 ± 2.14g to 302.28 ± 24.83g. Maximum weight was recorded in *C. xanthorrhiza* and minimum in *C. amada* (Table 37). Significant variation was observed among the four species. Percentage of variation obtained from mean square values exhibited 59% variation among the species and the rest within the species (Table 38). Among the accessions of *C. amada*, minimum weight was recorded in Acc. 265 (88.36 ± 9.51g) and maximum weight in Acc.752 (114.77 ± 4.87g). The weight of primary rhizome among the accessions of *C. aromatica* ranged from 93.04 ± 2.44g (Acc. 1518) to 127.30 ± 3.56g (Acc. 1124). In *C. caesia*, maximum weight was recorded for Acc.1001 (280.00 ± 0.58g) and minimum in Acc.751 (81.50 ± 3.38). In *C. xanthorrhiza*, Acc. 1167 marked the lowest weight for primary rhizome (143.27 ± 0.58g) and Acc. 1168 recorded maximum weight for primary rhizome (521.36 ± 18.04g). Within the species, major source of variation was present among the accessions except for *C. amada*.

C. aromatica (67%), *C. caesia* (99%), and *C. xanthorrhiza* (67%), showed major source of variation among the accession than within the accession source. Whereas in *C. amada*, the major source of variation was present within the accessions (65%) (Table 38).

Table 37. Mean weight of primary rhizomes in four *Curcuma* species

Sl. No	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	Weight of primary rhizomes (g)	Accession	Weight of primary rhizomes (g)	Accession	Weight of primary rhizomes (g)	Accession	Weight of primary rhizomes (g)
1	265	88.36 ± 9.51 ^{c}	711	112.88 ± 7.76 ^{bc}	292	152.00 ± 3.34 ^{c}	465	287.00 ± 10.76 ^{b}
2	347	100.16 ± 5.55 ^{abc}	1025	113.33 ± 3.82 ^{bc}	751	81.50 ± 3.38 ^{e}	760	245.56 ± 49.33 ^{bc}
3	521	111.83 ± 4.19 ^{a}	1113	111.05 ± 1.91 ^{bc}	1001	280.00 ± 0.58 ^{a}	1108	334.67 ± 6.04 ^{b}
4	752	114.77 ± 4.87 ^{a}	1124	127.30 ± 3.56 ^{a}	1006	152.68 ± 2.02 ^{c}	1122	336.38 ± 94.51 ^{b}
5	753	110.14 ± 3.30 ^{ab}	1132	118.29 ± 1.12 ^{ab}	1135	141.95 ± 2.07 ^{d}	1163	338.45 ± 21.39 ^{b}
6	848	100.56 ± 3.16 ^{abc}	1518	93.04 ± 2.44 ^{d}	1154	183.78 ± 3.11 ^{b}	1164	211.51 ± 13.83 ^{bc}
7	1119	90.39 ± 2.75 ^{c}	1520	102.58 ± 2.27 ^{cd}	1171	140.05 ± 1.91 ^{d}	1167	143.27 ± 0.58 ^{c}
8	1511	104.27 ± 9.61 ^{abc}	-	-	-	-	1168	521.36 ± 18.04 ^{a}
9	1503	92.76 ± 3.12 ^{bc}	-	-	-	-	-	-
10	6390	100.72 ± 0.34 ^{abc}	-	-	-	-	-	-
Mean ± SE		101.40 ± 2.14 ^{c}		111.21 ± 2.57 ^{c}		161.71 ± 12.55 ^{b}		302.28 ± 24.83 ^{a}
Accessions		P< 0.05		P< 0.05		P< 0.05		P< 0.05
Species		P< 0.05						

Values with the different superscript are significantly different (P<0.05), SE-Standard error

Table 38. F-value and percentage of variation of weight of primary rhizomes in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	69.35	552.61	166.95	10.72	47.06**	59%	41%
C. amada	69.35	124.38	101.39	2.14	2.81*	35%	65%
C. aromatica	89.34	134.39	111.21	2.57	8.12**	67%	33%
C. caesia	75.64	281.00	161.71	12.55	573.89**	99%	1%
C. xanthorrhiza	142.27	552.61	302.28	24.83	8.10**	67%	33%

*All values are significant at 0.05 level of significance, **All values are significant at 0.01 level of significance.

4.1.2.12. Weight of Secondary Rhizomes (WS)

The weight of secondary rhizomes (WS) varied from 42.68 ± 1.29 g to 131.97 ± 6.55 g, highest being in *C. caesia* and lowest in *C. amada*. Significant variation was observed among the species for weight of secondary rhizome with a percentage of variation of 32% among and 68% within the species. Within the species, only *C. caesia* and *C. xanthorrhiza* showed significant variation for weight of secondary rhizome. Among the accessions of *C. caesia*, highest weight was recorded in Acc.1001 (184.50 ± 3.18 g) and lowest in Acc. 751 (82.67 ± 0.86 g). The weight of secondary rhizome in the accessions of *C. xanthorrhiza* ranged from 23.92 ± 0.38 (Acc.1167) to 271.55 ± 13.87 g (Acc. 1168) (Table 39).

The study revealed that in *C. caesia* and *C. xanthorrhiza*, major source of variation was among the accessions (99% and 66%, respectively) and rest of the variation was within the accessions (Table 40).

Table 39. Mean weight of secondary rhizomes of four *Curcuma* species

Sl. No	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	Weight of secondary rhizomes (g)	Accession	Weight of secondary rhizomes (g)	Accession	Weight of secondary rhizomes (g)	Accession	Weight of secondary rhizomes (g)
1	265	48.61 ± 5.48 ^{a}	711	57.00 ± 2.91 ^{ab}	292	111.37 ± 1.30 ^{e}	465	86.65 ± 4.13 ^{b}
2	347	49.04 ± 1.94 ^{a}	1025	53.26 ± 4.93 ^{b}	751	82.67 ± 0.86 ^{f}	760	52.78 ± 13.08 ^{b}
3	521	39.35 ± 3.16 ^{ab}	1113	57.12 ± 2.14 ^{ab}	1001	184.50 ± 3.18 ^{a}	1108	35.45 ± 6.34 ^{b}
4	752	34.11 ± 6.88 ^{b}	1124	66.90 ± 2.35 ^{a}	1006	133.20 ± 0.61 ^{c}	1122	113.36 ± 81.09 ^{b}
5	753	38.98 ± 3.15 ^{ab}	1132	64.34 ± 3.71 ^{ab}	1135	127.42 ± 2.41 ^{d}	1163	68.16 ± 4.25 ^{b}
6	848	41.14 ± 0.72 ^{ab}	1518	54.93 ± 4.85 ^{b}	1154	149.79 ± 1.12 ^{b}	1164	28.84 ± 1.31 ^{b}
7	1119	46.84 ± 4.54 ^{a}	1520	57.28 ± 2.07 ^{ab}	1171	134.84 ± 1.37 ^{c}	1167	23.92 ± 0.38 ^{b}
8	1511	40.28 ± 2.89 ^{ab}	-	-	-	-	1168	271.55 ± 13.87 ^{a}
9	1503	45.15 ± 2.83 ^{ab}	-	-	-	-	-	-
10	6390	43.32 ± 0.64 ^{ab}	-	-	-	-	-	-
Mean ± SE		42.68 ± 1.29 ^{c}		58.69 ± 1.51 ^{bc}		131.97 ± 6.55 ^{a}		85.09 ± 18.10 ^{b}
Accessions		NS		NS		P< 0.05		P<0.05
Species		P< 0.05						

NS = Not Significant (P>0.05), Values with the different superscript are significantly different (P<0.05), SE- Standard error

Table 40. F-value and percentage of variation of weight of secondary rhizomes in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	23.24	295.57	76.32	5.82	16.35**	32%	68%
C. amada	23.24	59.34	42.68	1.29	1.65	16%	84%
C. aromatica	44.27	71.30	58.69	1.51	2.08	24%	76%
C. caesia	81.17	190.00	131.97	6.55	318.87**	99%	1%
C. xanthorrhiza	23.27	295.57	85.09	18.10	7.54**	66%	34%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.1.2.13. Dry Recovery (DR)

Significant variation was observed among the four species for dry recovery. Percentage of dry recovery ranged from 21.75 ± 0.73% to 24.61 ± 0.23%. The highest percentage of dry recovery was recorded in *C. amada* and lowest in *C. xanthorrhiza*. Within the species, except *C. amada* rest of the species showed significant variations for this trait (Table 41). The percentage of variation obtained from the mean square values revealed that the majority of the variation is due to within the species source (82%) (Table 42). The dry recovery in *C. aromatica* varied from 19.74±0.37% (Acc.1124) to 25.12±0.55% (Acc.711) with major source of variation present among the source (74%). In *C. caesia*, dry recovery varied from 19.25 ± 0.49% (Acc. 1154) to 25.81 ± 0.33% (Acc.1171). The major source of variation was present among the accessions (90%) for *C. caesia*. In *C. xanthorrhiza* the values varied from 15.86 ±0.15% to 26.35 ± 0.29%, the lowest being in Acc. 1164 and highest in Acc.1108. The major source of variation was distributed among (98%) the accessions for *C. xanthorrhiza* (Table 42).

Table 41. Mean dry recovery in four *Curcuma* species

Sl. No	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	Dry recovery(%)	Accession	Dry recovery(%)	Accession	Dry recovery(%)	Accession	Dry recovery(%)
1	265	25.19±0.50 ^{ab}	711	25.12±0.55 ^{a}	292	24.33±0.81 ^{ab}	465	25.72±0.18^{a}
2	347	25.51±0.64 ^{ab}	1025	24.93±0.33 ^{a}	751	23.89±0.47 ^{b}	760	23.89±0.51^{b}
3	521	23.48±0.34^{b}	1113	23.35±0.66 ^{a}	1001	23.35±0.37^{bc}	1108	26.35±0.29^{a}
4	752	23.82±1.21 ^{ab}	1124	19.74±0.37 ^{c}	1006	24.47±0.35^{ab}	1122	20.62±0.42^{c}
5	753	23.89±0.79 ^{ab}	1132	20.66±1.30 ^{bc}	1135	22.04±0.28^{c}	1163	20.43±0.33^{c}
6	848	23.79±0.89 ^{ab}	1518	23.07±0.88 ^{ab}	1154	19.25±0.49^{d}	1164	15.86±0.15^{e}
7	1119	24.41±0.43 ^{ab}	1520	22.68±0.91 ^{ab}	1171	25.81±0.33^{a}	1167	23.11±0.59^{b}
8	1511	25.66±0.36 ^{ab}	-	-	-	-	1168	18.06±0.02^{d}
9	1503	24.56±0.32 ^{ab}	-	-	-	-	-	-
10	6390	25.76±0.33^{a}	-	-	-	-	-	-
Mean ± SE		24.61 ± 0.23^{a}		22.79 ± 0.48^{bc}		23.30 ± 0.46^{ab}		21.75 ± 0.73^{c}
Accessions		NS		P< 0.05		P<0.05		P< 0.05
Species		P< 0.05						

NS = Not Significant (P>0.05), Values with the different superscript are significantly different (P<0.05).

Table 42. F-value and percentage of variation of dry recovery in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	15.62	26.69	23.11	0.26	6.62**	18%	82%
C. amada	22.08	26.42	24.61	0.23	1.78	44%	56%
C. aromatica	18.82	26.22	23.79	0.48	6.59**	74%	26%
C. caesia	18.34	26.45	23.30	0.46	20.14**	90%	10%
C. xanthorrhiza	15.62	26.69	21.75	0.73	104.83**	98%	2%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance.

4.1.2.14. Aroma of rhizome (AR)

Among the four species, *C. amada* possessed characteristic raw mango aroma. Whereas, rest of the species had a camphoraceous aroma, among which *C. aromatica* possessed a strong intensity for the aroma.

4.1.2.15. Flavour/ taste of rhizome (F/T)

Among the four *Curcuma* species, all the species had a bitter taste except *C. amada*. *C. amada* had a characteristic mango flavour with a gingery taste.

4.1.3. Variability, heritability and genetic advance

Phenotypic and genotypic coefficients of variation (PCV and GCV), heritability (h^2) and genetic advance (GA) were calculated for those morphological characters which showed significant variations.

In *C. amada*, the highest values for PCV and GCV were observed for number of primary rhizomes (24%,18%) followed by number of mother rhizomes (20%,14%). Whereas the lowest value for PCV and GCV were recorded for weight of primary rhizomes (12%,7%) (Table 43). PCV and GCV values greater than 20% are regarded as high, whereas values between 10%-20% are considered medium and values less than 10% to be low (Deshmukh *et al*., 1986). Results showed a narrow difference between PCV and GCV for most of the characters including plant height, petiole length, rhizome length, rhizome internode length etc. Phenotypic Coefficient of Variation was more or less same or a little bit higher than genotypic coefficient of variation. Variation for all these characters indicated that environment played a very little role in the expression of these characters.

The heritability (h^2) ranged from 38% (weight of primary rhizomes) to 72% (plant height). Heritability values greater than 80% are very high, values from 60-79% are moderately high, 40-59% are medium and values less than 40% are low (Singh, 2001). Accordingly, among the traits, plant height (72%) and rhizome length (68%) showed moderately high values for heritability. Petiole length (59%), rhizome internode length (52%), number of primary rhizomes (55%) and number of mother rhizomes (51%) showed a moderate heritability. Low heritability (<40%) was recorded for weight of primary rhizome (38%). High heritability values indicate

that the characters are least influenced by environment and selection for improvement of such characters may be useful.

Genetic advance as percentage of mean (GAM) ranged from 9% (weight of primary rhizome) to 27% (number of primary rhizome). Values from 0-10% are low,10-20% are moderate and 20% and above are high (Johnson *et al*., 1955). Based on this delineation, characters like number of primary rhizomes (27%), number of mother rhizomes (21%), plant height (21%), rhizome length (20%), and petiole length (20%) recorded high genetic advance, rhizome internode length (16%) showed moderate value and weight of primary rhizome (9%) showed low value for genetic advance.

Table 43. Estimates of genetic parameters for aerial morphological and rhizome characters in *C. amada*

Character	Mean	Phenotypic Variance	Genotypic Variance	GCV %	PCV %	h^2	GA	GA as % of Mean	F Value
PH	143.30	415.25	300.87	12%	14%	72%	30.42	21%	8.89**
PL	24.64	15.76	9.37	12%	16%	59%	4.86	20%	5.40**
RL	8.52	1.51	1.03	12%	14%	68%	1.73	20%	7.51**
RIL	1.34	0.04	0.02	11%	15%	52%	0.21	16%	4.27**
NM	3.43	0.48	0.24	14%	20%	51%	0.73	21%	4.14**
NP	10.17	6.10	3.33	18%	24%	55%	2.78	27%	4.61**
WP	101.40	140.97	53.01	7%	12%	38%	9.20	9%	2.81*

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

GCV-Genotypic coefficients of variation, PCV- Phenotypic coefficients of variation, h^2 - heritability, GA-Genetic Advance.

PH-Plant height, PL-Petiole length, RL-Rhizome length, RIL-Rhizome internode length, NM-Number of mother rhizome, NP-Number of primary rhizome, WP- Weight of primary rhizome.

Estimates of phenotypic, genotypic variances, Phenotypic coefficients of variation (PCV), Genotypic coefficients of variation (GCV), heritability and genetic advance as percentage of the mean of *C. aromatica* are given in Table 44. The genotypic coefficients of variance (GCV) ranged from 8% (Rhizome weight & Dry recovery) to 31% (Plant height). Similarly, phenotypic coefficients of variation ranged from 8% (rhizome weight) to 35% (petiole length).

The current study showed a narrow difference between PCV and GCV for most of the characters including plant height, number of leaves per tiller, petiole length, leaf length, number of secondary rhizomes etc. The phenotypic coefficient of variation was almost same or little bit greater than genotypic coefficient of variation for all these characters.

Heritability ranged from 51% (number of tillers per clump) to 89% (plant height). Characters like plant height (89%), number of leaves per tiller (82%) and rhizome weight (82%) recorded very high values for heritability. Moderately high heritability was recorded for characters like number of secondary rhizomes (79%), leaf length (78%), petiole length (76%), weight of primary rhizomes (70%), dry recovery (65%) and weight of mother rhizomes (60%). The number of tillers per clump (51%) and number of primary rhizomes (57%) showed medium to low heritability.

Table 44. Estimates of genetic parameters for aerial morphological and rhizome characters in *C. aromatica*

Character	Mean	Phenotypic Variance	Genotypic Variance	GCV %	PCV %	h^2	GA	GA as % of Mean	F Value
PH	110.74	1,294.98	1,156.45	31%	32%	89%	66.20	60%	26.04**
NTC	2.57	0.48	0.24	19%	27%	51%	0.72	28%	4.07*
NLT	8.43	2.68	2.20	18%	19%	82%	2.77	33%	14.87**
PL	21.43	55.68	42.25	30%	35%	76%	11.66	54%	10.44**
LL	50.29	237.98	186.70	27%	31%	78%	24.93	50%	11.92**
RW	254.31	447.27	367.81	8%	8%	82%	35.83	14%	14.89**
NP	10.81	5.37	3.08	16%	21%	57%	2.74	25%	5.05**
NS	25.48	28.10	22.33	19%	21%	79%	8.68	34%	12.63**
WM	84.41	99.86	59.89	9%	12%	60%	12.35	15%	5.49**
WP	111.21	149.73	105.36	9%	11%	70%	17.74	16%	8.13**
DR	22.79	5.26	3.42	8%	10%	65%	3.07	13%	6.59**

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

GCV-Genotypic coefficients of variation, PCV- Phenotypic coefficients of variation, h^2 -heritability, GA-Genetic advance.

PH-Plant height, NTC- Number of tillers per clump, NLT-Number of leaf per tiller, PL-petiole length, LL-Leaf length, RW- Rhizome weight, NP-Number of primary rhizome, NS-Number of secondary rhizome, WM-weight of mother rhizome, WP- Weight of primary rhizome, DR-Dry recovery.

Genetic advance as percentage of mean (GAM) ranged from 13% (dry recovery) to 60% (Plant height). Based on the delineation proposed by Johnson *et al*. (1995), high GAM was recorded for the majority of the characters under study. Characters like plant height (60%), number of tiller per clump (28%), number of leaves per tiller (33%), petiole length (54%), leaf length (50%), number of secondary rhizomes (34%) and number of primary rhizomes (25%) recorded high genetic advance whereas weight of primary rhizome (16%), weight of mother rhizomes (15%), rhizome weight (14%) and dry recovery (13%) showed moderate values for genetic advance.

In case of *C. caesia*, highest PCV and GCV are observed for the weight of primary rhizome (37% each) and lowest GCV and PCV for dry recovery (9%, 10%) (Table 45).

Table 45. Estimates of genetic parameters for aerial morphological and rhizome characters in *C. caesia*

Character	Mean	Phenotypic Variance	Genotypic Variance	GCV %	PCV %	h^2	GA	GA as % of Mean	F value
PH	111.76	334.81	204.36	13%	16%	61%	23.01	21%	5.70**
RW	441.93	15,269.72	14,997.42	28%	28%	98%	250.02	57%	166.23**
NP	20.43	50.51	48.30	34%	35%	96%	14.00	69%	66.44**
NS	40.79	119.12	114.30	26%	27%	96%	21.57	53%	72.12**
WM	148.25	1,437.50	1,295.60	24%	26%	90%	70.39	47%	28.39**
WP	161.71	3,670.98	3,651.85	37%	37%	99%	124.16	77%	573.89**
WS	131.97	1,000.03	990.68	24%	24%	99%	64.53	49%	318.87**
DR	23.30	4.96	4.29	9%	10%	86%	3.97	17%	20.14**

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance.

GCV- Genotypic coefficients of variation, PCV- Phenotypic coefficients of variation, h^2 -heritability, GA-Genetic advance.

PH-Plant height, RW-Rhizome weight, NP-Number of primary rhizome, NS-Number of secondary rhizome. WM-Weight of mother rhizome, WP- Weight of primary rhizome, WS-Weight of secondary rhizome, DR- Dry recovery.

Like in *C. amada* and *C. aromatica*, the data showed a narrow difference between PCV and GCV for all the characters. Values of PCV and GCV were more or less same, indicating a very little role of environment in the expression of traits. All the characters exhibited high heritability which ranged from 61% (plant height) to 99% (weight of primary rhizome and weight of secondary rhizome). Majority of the characters showed a very high heritability as per the range of values suggested by Singh (2001). Genetic advance as percentage of mean (GAM) ranged from 17% (dry recovery) to 77% (weight of primary rhizome). All the traits, except dry recovery (17%) exhibited high genetic advance as percentage of mean. High values for genetic advance indicates that the character is governed by additive genes.

The variance components and coefficients of variation for *C. xanthorrhiza* are given in the Table 46. Highest genotypic coefficients of variation (GCV) and phenotypic coefficients of variation (PCV) were observed for weight of secondary rhizomes (89%, 107%) and lowest for plant height (12%, 17%) (Table 46). The GCV and PCV for the number of secondary rhizomes (49%,

56%), weight of primary rhizomes (35%, 42%), number of primary rhizomes (34%, 39%), rhizome weight (28%, 33%) were higher than rest of the characters. Lower values for GCV and PCV are recorded for characters like plant height (12%, 17%), rhizome length (13%, 19%) and dry recovery (17%, 17%). The magnitude of PCV was higher than the corresponding GCV for all the traits which reflects the influence of environment on the expression of these traits.

Table 46. Estimates of genetic parameters for aerial morphological and rhizome characters in *C. xanthorrhiza*

Character	Mean	Phenotypic Variance	Genotypic Variance	GCV %	PCV %	h^2	GA	GA as % of Mean	F Value
PH	161.47	757.67	371.65	12%	17%	49%	27.81	17%	3.89*
NTC	2.83	0.60	0.27	18%	27%	45%	0.72	25%	3.43*
NLT	7.17	2.01	1.47	17%	20%	73%	2.14	30%	9.14**
LW	15.83	14.76	9.70	20%	24%	66%	5.20	33%	6.75**
RL	8.44	2.53	1.26	13%	19%	50%	1.63	19%	3.96*
RW	716.09	55,182.35	39,296.92	28%	33%	71%	344.61	48%	8.42**
NP	12.88	24.85	19.48	34%	39%	78%	8.05	63%	11.87**
NS	10.08	31.89	24.85	49%	56%	78%	9.06	90%	11.59**
WM	328.72	11,628.72	5,730.86	23%	33%	49%	109.48	33%	3.92*
WP	302.28	15,764.39	11,079.90	35%	42%	70%	181.79	60%	8.09**
WS	85.09	8,363.90	5,732.55	89%	107%	69%	129.13	152%	7.54**
DR	21.75	13.78	13.39	17%	17%	97%	7.43	34%	104.83**

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

GCV- Genotypic coefficients of variation, PCV- Phenotypic coefficients of variation, h^2 -heritability, GA-Genetic advance.

PH-Plant height, NTC- Number of tillers per clump, NLT-Number of leaf per tiller, LW-Leaf width, RL-Rhizome length, RW-Rhizome weight, NP-Number of primary rhizome, NS-Number of secondary rhizome, WM- Weight of mother rhizome, WP- Weight of primary rhizome, WS-Weight of secondary rhizome, DR- Dry recovery.

The magnitude of heritability varied from 45% (number of tillers per clump) to 97% (dry recovery). Moderately high heritability was observed for traits like number of primary rhizomes (78%), number of secondary rhizome (78%), number of leaves per tiller (73%), rhizome weight (71%), weight of primary rhizome (70%), weight of secondary rhizome (69%), leaf width (66%). In *C. xanthorrhiza* majority of the characters under study showed moderately high heritability except dry recovery which showed very high heritability (97%). Genetic advance as

percentage of mean (GAM) ranged from 17% (plant height) to 152% (weight of secondary rhizomes). Very high GAM was recorded for rhizome weight (48%), weight of primary rhizome (60%), number of primary rhizomes (63%), number of secondary rhizomes (90%), and weight of secondary rhizome (152%). The majority of the characters showed higher values for GAM. Rhizome length (19%) and plant height (17%) showed lowest values for GAM. Traits like dry recovery, number of primary and secondary rhizome, weight of primary and secondary rhizomewith moderate to high GCV, heritability and genetic advance are most amenable to selection.

4.1.4. Principal Component Analysis (PCA) of Morphological Data

Principal component analysis was carried out to ascertain the relationship among and within the species. Sixteen morphological traits revealed a total of sixteen principal components for the entire 32 accessions under study. The contribution of Principal Component (PC) 1, 2 and 3 was 30.48%, 22.14% and 11.99%, respectively. The first two principal components explained 52.62% of the variation and first three principal components explained 64.61% of the variation. The results of the principal component analysis for the entire accessions are depicted in Table 47. The principal component 1 showed high loading factor for leaf length, plant height, weight of mother rhizome, weight of primary rhizome, rhizome weight, petiole length and leaf width. Principal component 2 showed high loading factor for weight of secondary rhizome, number of primary rhizomes, rhizome weight, weight of primary rhizome, number of mother rhizome, number of secondary rhizomes, number of tillers per clump and weight of mother rhizome. Principal component 3 showed high loading factor for dry recovery, plant height, petiole length, leaf length and number of tillers per clump.

Table 47. Principal Components (PC) for quantitative characters in thirty-two accessions.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Eigenvalue	4.88	3.54	1.92	1.69	1.06	0.96	0.62	0.49	0.29
Variability (%)	30.48	22.14	11.99	10.56	6.65	6.00	3.88	3.08	1.84
Cumulative %	30.48	52.62	64.61	75.17	81.82	87.82	91.70	94.78	96.62
Plant Height	0.78	-0.04	0.54	0.15	0.16	0.02	-0.13	0.05	-0.03
No. of Tillers per Clump	-0.23	0.44	0.39	-0.20	-0.17	0.66	0.11	-0.26	0.00
No. of Leaves per Tiller	0.11	-0.54	-0.35	-0.24	0.41	0.18	0.55	-0.03	-0.09
Petiole Length	0.66	0.09	0.51	0.08	0.37	0.05	0.19	0.17	-0.07
Leaf Length	0.80	-0.14	0.46	0.12	0.20	0.02	-0.15	-0.01	-0.04
Leaf Width	0.62	-0.43	-0.31	-0.27	0.12	0.26	-0.14	0.18	0.29
Rhizome Length	-0.23	-0.03	-0.21	0.82	0.33	0.02	0.05	-0.17	0.26
Rhizome Internode Length	-0.15	0.24	-0.12	0.79	-0.23	0.21	0.15	0.35	-0.13
Rhizome Weight	0.70	0.66	-0.21	0.02	-0.10	-0.05	0.09	-0.09	0.01
No. of Mother Rhizome	-0.65	0.51	0.32	-0.02	0.13	0.33	-0.04	0.12	0.16
No. of Primary Rhizome	-0.14	0.78	0.04	-0.36	0.08	-0.24	0.16	0.28	0.20
No. of Secondary Rhizome	-0.71	0.48	0.01	-0.19	0.35	-0.13	-0.10	0.13	-0.15
Weight of Mother Rhizome	0.76	0.42	-0.12	-0.03	-0.38	0.03	0.21	0.07	0.05
Weight of Primary Rhizome	0.72	0.59	-0.23	0.04	-0.03	-0.14	0.00	-0.16	-0.01
Weight of Secondary Rhizome	0.04	0.84	-0.22	0.10	0.40	-0.03	-0.04	-0.20	-0.05
Dry Recovery	-0.35	-0.18	0.68	0.09	-0.17	-0.41	0.32	-0.14	0.14

C. amada and *C. aromatica* interleaved in the 2-dimensional phase of principal component analysis. However, almost all accessions of *C. xanthorrhiza* clustered closer depicting similar morphological traits among the accessions. However, Acc.1167 and Acc.1164 (*C. xanthorrhiza*) were isolated from their counterparts and plotted in a different quadrant along with *C. amada* and *C. aromatica*. Similarly, accessions of *C. caesia* clustered together as a separate group although Acc.1001 was remotely placed with respect to rest of the accessions. Figure 10. shows the 2-dimensional plot of the Principal Component Analysis.

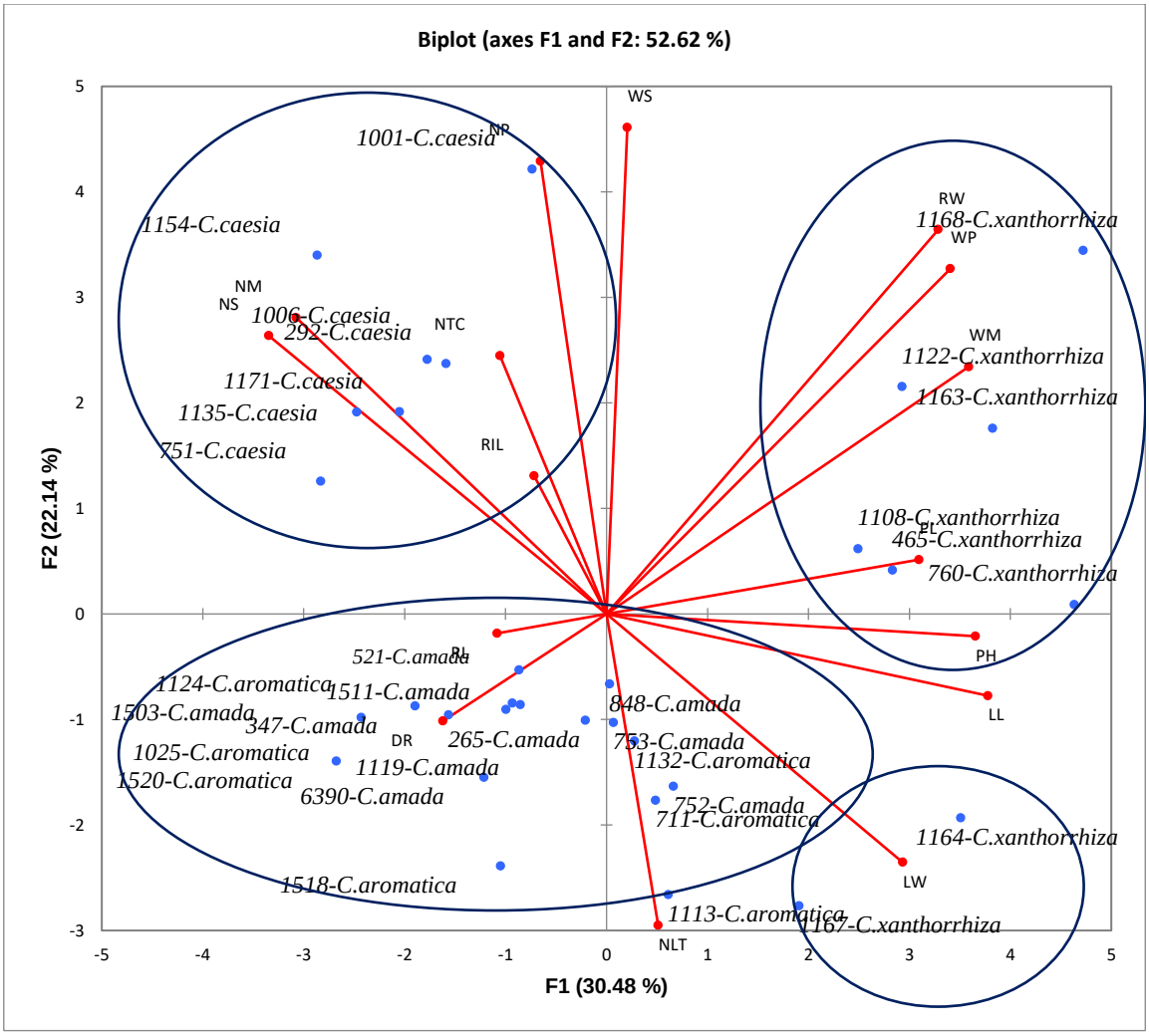


Fig. 10. Two dimensional loading plot of Principal Component Analysis (PCA) based on morphological data in four *Curcuma species.*

In case of *C. amada*, 16 morphological traits revealed a total of nine principal components. The contribution of principal Component 1, 2 and 3 was 37.55%, 26.32% and 13.56%, respectively. The first two principal components explained 63.88% of the variation and first three principal components explained 77.44% of the variation. The results of the principal component analysis are depicted in Table 48. The principal component 1 showed high loading factor for number of mother rhizome, weight of secondary rhizome, rhizome internode length, number of leaves per tiller, rhizome length, number of primary rhizome and dry recovery. Principal component 2 showed high loading factor for petiole length, number of tillers from clump, number of primary rhizome, dry recovery, leaf length, plant height, leaf width and number of leaves per tiller.

Principal Component 3 showed high loading factor for weight of mother rhizome and leaf length.

Table 48. Principal Components (PC) of morphological characters in *C. amada*.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Eigenvalue	6.01	4.21	2.17	1.65	0.96	0.59	0.20	0.15	0.06
Variability (%)	37.55	26.33	13.56	10.30	6.00	3.67	1.26	0.94	0.40
Cumulative %	37.55	63.88	77.44	87.74	93.74	97.41	98.67	99.60	100.00
Plant Height	-0.78	0.41	0.39	-0.16	-0.10	0.10	0.15	-0.04	-0.11
No. of Tillers per Clump	0.05	0.78	-0.44	-0.33	0.23	-0.19	-0.04	0.02	0.06
No. of Leaves per Tiller	0.59	0.40	-0.28	-0.46	-0.28	-0.29	0.19	-0.05	0.01
Petiole Length	-0.34	0.77	0.23	-0.14	0.12	0.39	0.18	-0.05	0.12
Leaf Length	-0.59	0.55	0.50	0.00	0.17	-0.27	0.04	0.04	0.00
Leaf Width	-0.74	0.43	-0.34	0.28	-0.12	0.17	-0.01	0.19	-0.04
Rhizome Length	0.70	-0.57	0.28	0.23	-0.04	0.06	0.18	-0.13	-0.05
Rhizome Internode Length	0.79	-0.20	0.18	0.21	0.47	0.16	0.02	0.06	0.07
Rhizome Weight	-0.60	-0.66	-0.36	0.10	0.16	-0.12	0.15	0.06	0.03
No. of Mother Rhizome	0.85	0.01	0.12	-0.34	0.35	-0.10	0.03	0.09	-0.04
No. of Primary Rhizome	0.48	0.75	-0.27	-0.02	0.28	0.17	-0.09	-0.10	-0.12
No. of Secondary Rhizome	-0.50	0.20	-0.41	0.55	0.42	-0.18	0.10	-0.11	-0.05
Weight of Mother Rhizome	-0.56	-0.22	0.72	-0.11	0.25	-0.20	-0.02	0.05	-0.01
Weight of Primary Rhizome	-0.64	-0.56	-0.49	-0.14	0.09	0.01	0.03	-0.08	0.04
Weight of Secondary Rhizome	0.78	0.31	-0.10	0.46	-0.14	-0.05	0.16	0.19	-0.02
Dry Recovery	0.20	0.59	0.26	0.66	-0.20	-0.22	-0.08	-0.11	0.08

The principle component analysis showed very high dispersion among the accessions of *C. amada*. Acc.1503 and Acc.1119 plotted together under same quadrant. Acc.752, Acc. 848 and Acc. 753 fell in the same quadrant and latter two showed a likely grouping. Likewise, Acc.347, Acc.265 and Acc.1511 plotted under the same quadrant and latter two showed closer association. Acc.6390 and Acc.521 formed their own separate quadrant. Acc.347 and Acc. 752 stood out separately in their group (Figure 11).

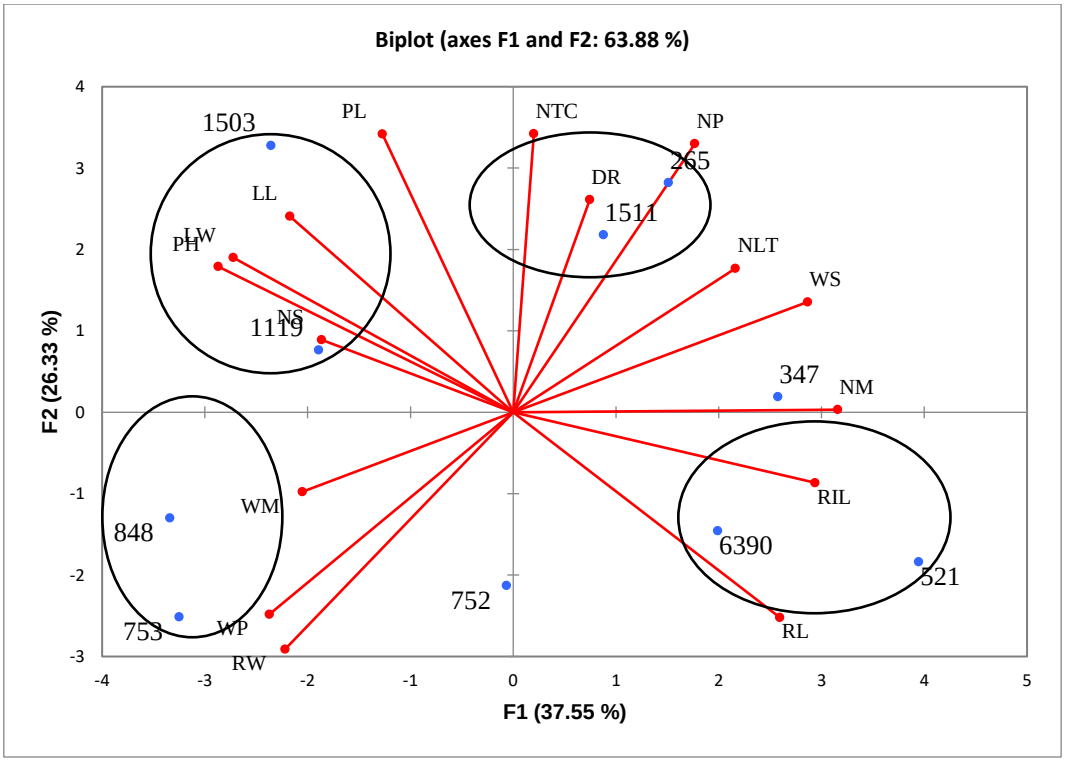


Fig. 11.Two dimensional loading plot of Principal Component Analysis (PCA) based on morphological data in *C. amada.*

In case of *C. aromatica*, 16 morphological traits revealed a total of six principal components. The contribution of Principal Components 1, 2 and 3 was 40.31%, 24.78% and 16.07%, respectively. The first two principal components explained 65.09% of the variation and first three principal components explained 81.16% of the variation. The results of the Principal component analysis in *C. aromatica* are depicted in Table 49. The principal component 1 showed high loading factor for number of primary rhizomes, number of secondary rhizomes, rhizome weight, number of mother rhizome, dry recovery and weight of primary rhizome. Principal component 2 showed high loading factor for rhizome internode length, weight of secondary rhizome, rhizome weight, plant height and weight of primary rhizome. PCA 3 showed high loading factor for leaf width, dry recovery, number of mother rhizome and leaf length.

Table 49. Principal Components (PC) of morphological characters in *C. aromatica*.

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	6.45	3.97	2.57	2.32	0.40	0.29
Variability (%)	40.31	24.78	16.07	14.49	2.53	1.83
Cumulative %	40.31	65.09	81.16	95.64	98.17	100.00
Plant Height	-0.70	0.46	0.47	-0.17	0.21	-0.04
No. of Tillers per Clump	-0.51	-0.56	0.33	-0.50	-0.08	0.27
No. of Leaves per Tiller	-0.71	0.09	-0.53	0.36	-0.14	0.26
Petiole Length	-0.88	0.12	0.05	0.34	0.31	-0.02
Leaf Length	-0.72	0.16	0.54	-0.40	0.05	-0.01
Leaf Width	-0.44	0.13	0.79	0.35	-0.16	-0.10
Rhizome Length	-0.42	0.49	0.15	0.74	0.08	0.06
Rhizome Internode Length	0.03	0.97	-0.01	0.06	-0.24	-0.03
Rhizome Weight	0.66	0.74	0.02	-0.10	0.06	0.03
No. of Mother Rhizome	0.58	0.50	0.56	-0.01	-0.03	0.32
No. of Primary Rhizome	0.95	-0.05	0.18	0.13	0.20	-0.03
No. of Secondary Rhizome	0.92	-0.27	0.02	0.30	-0.04	0.00
Weight of Mother Rhizome	0.75	0.02	0.31	0.58	0.08	0.08
Weight of Primary Rhizome	0.47	0.52	-0.26	-0.61	0.24	0.10
Weight of Secondary Rhizome	0.04	0.92	-0.08	-0.31	-0.17	-0.11
Dry Recovery	0.49	-0.46	0.71	-0.19	-0.09	-0.06

The Principle Component Analysis showed very high dispersion among the accessions of *C. aromatica*. Acc. 1518 and Acc.1520 fell in the same quadrant and showed a likely grouping. Similar is the case with Acc. 1025 and Acc.1113. Acc.1132, Acc.1124 and Acc.711 distantly plotted in the loading plot. (Figure 12).

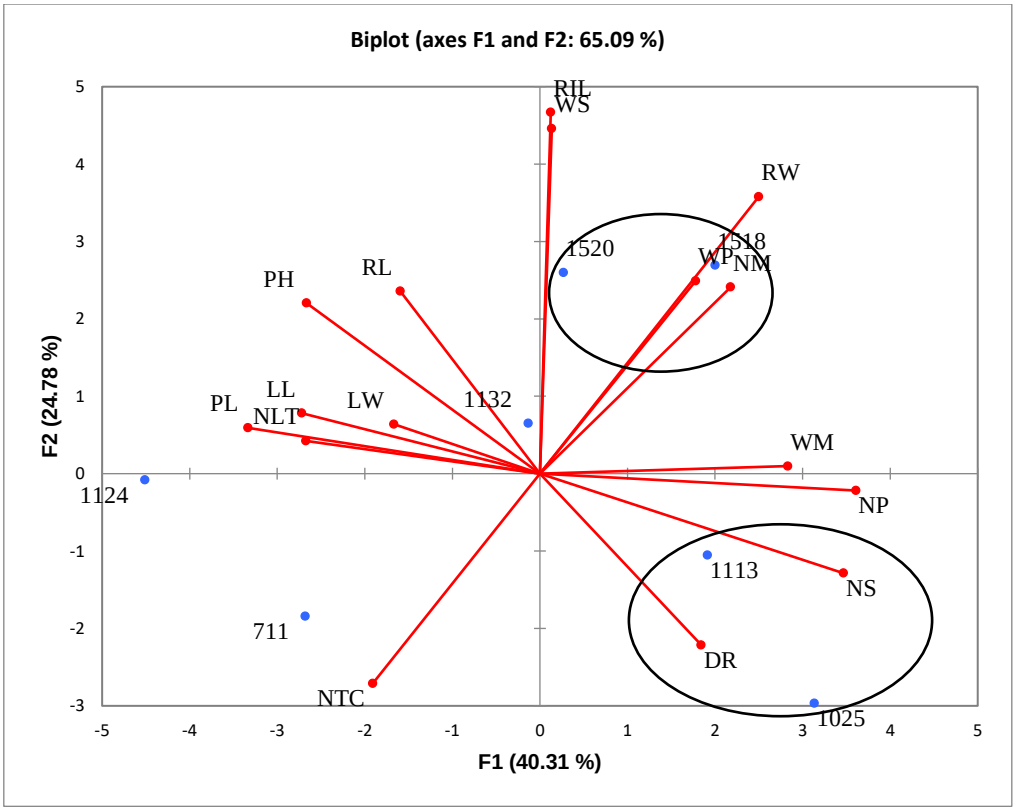


Fig. 12. Two dimensional loading plot of Principal Component Analysis (PCA) based on morphological data in *C. aromatica.*

In case of *C. caesia*, 16 morphological traits revealed a total of six principal components. The contribution of Principal Component 1, 2 and 3 was 49.15%, 23.65% and 14.88%, respectively. The first two principal components explained 72.80% of the variation and first three principal components explained 87.68% of the variation (Table 50). The principal component 1 showed high loading factor for the weight of primary rhizome, weight of secondary rhizome, number of primary rhizomes, weight of mother rhizome, number of secondary rhizomes, number of leaves per tiller, petiole length and leaf width. Principal component 2 showed high loading factor for leaf length, plant height and number of tillers per clump. Principal component 3 showed high loading factor for leaf width, a number of tillers per clump and rhizome internode length.

Table 50. Principal Components (PC) of morphological characters in *C. caesia.*

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	7.87	3.78	2.38	1.61	0.25	0.11
Variability (%)	49.15	23.65	14.88	10.05	1.57	0.70
Cumulative %	49.15	72.80	87.68	97.73	99.30	100.00
Plant Height	0.16	0.85	0.18	0.40	-0.24	-0.08
No. of Tillers per Clump	-0.38	0.66	0.25	0.59	0.08	0.08
No. of Leaves per Tiller	0.79	-0.42	0.41	-0.09	0.03	-0.14
Petiole Length	0.70	0.30	-0.45	-0.41	-0.14	0.17
Leaf Length	0.10	0.99	-0.08	-0.02	-0.09	-0.02
Leaf Width	0.48	0.12	0.87	0.00	-0.01	-0.02
Rhizome Length	-0.89	-0.37	0.07	0.13	-0.22	0.03
Rhizome Internode Length	-0.40	-0.72	0.48	0.19	-0.12	0.17
Rhizome Weight	0.99	0.01	0.02	0.16	0.02	0.05
No. of Mother Rhizome	-0.41	0.67	0.34	-0.45	0.24	0.08
No. of Primary Rhizome	0.89	0.06	-0.15	-0.40	-0.13	-0.02
No. of Secondary Rhizome	0.86	-0.42	-0.05	0.27	0.07	0.02
of Mother Rhizome	0.89	0.22	0.19	0.34	0.07	0.07
Weight of Primary Rhizome	0.99	0.01	-0.11	0.09	0.02	0.07
Weight of Secondary Rhizome	0.97	-0.19	0.10	0.13	-0.02	0.01
Dry Recovery	-0.06	-0.09	-0.85	0.51	0.10	-0.02

The Principle Component Analysis showed very high dispersion among the accessions of *C. caesia*. Acc.1171, Acc.1154 and Acc.1001 fell in the same quadrant and latter two showed a likely grouping. Acc.292 and Acc.751 also plotted together in same quadrant. However, rest of the two accessions (Acc.1135 and Acc. 1006) dispersed into two different quadrants (Figure 13).

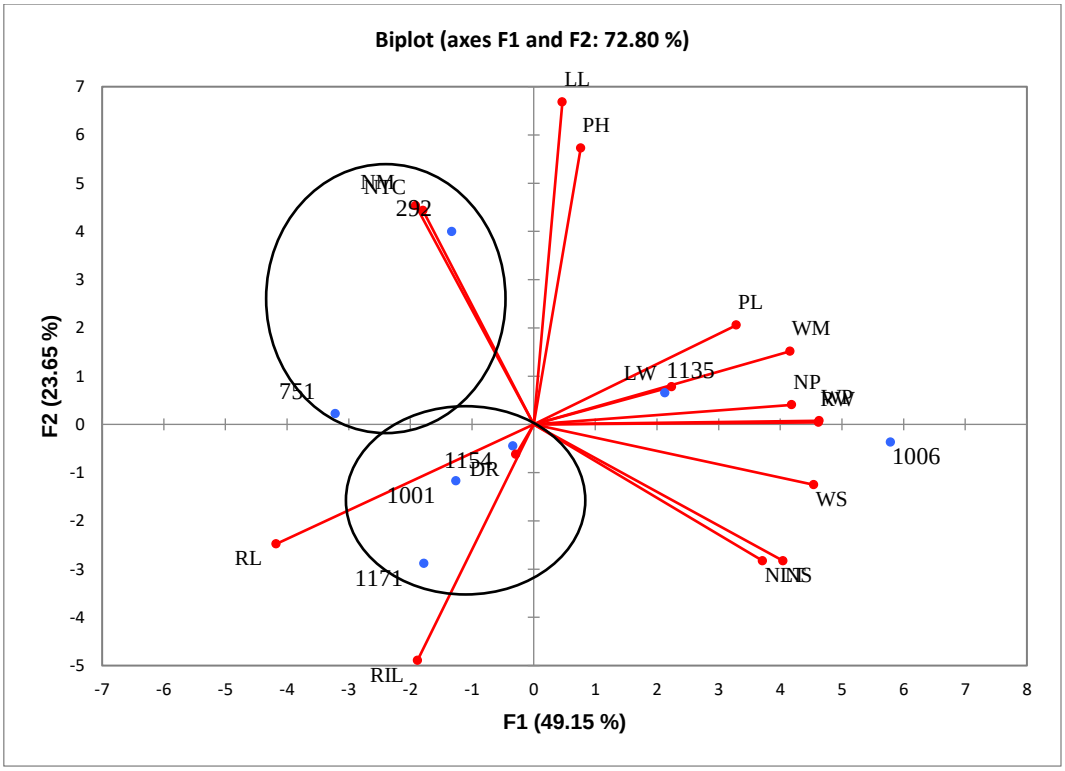


Fig. 13.Two dimensional loading plot of Principal Component Analysis (PCA) based on morphological data in *C. caesia.*

In case of *C. xanthorrhiza*, 16 morphological traits revealed a total of seven principal components. The contribution of Principal Component 1, 2 and 3 was 31.61%, 22.19% and 21.43%, respectively. The first two principal components explained 53.80% of the variation and first three principal components explained 75.23% of the variation (Table 51). The principal component 1 showed high loading factor for the number of tillers per clump, weight of primary rhizome, number of secondary rhizome, rhizome weight, rhizome internode length, weight of secondary rhizome and rhizome length. Principal component 2 showed high loading factor for rhizome internode length, rhizome length, dry recovery, number of secondary rhizome, plant height and weight of secondary rhizome. Principal component 3 showed high loading factor for leaf width, number of leaf per tiller and rhizome length.

Table 51. Principal Components (PC) of morphological characters in *C. xanthorrhiza*

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalue	5.37	3.77	3.64	1.69	1.31	0.72	0.49
Variability (%)	31.61	22.19	21.43	9.95	7.73	4.21	2.88
Cumulative %	31.61	53.80	75.23	85.18	92.91	97.12	100.00
Plant Height	-0.82	0.35	-0.43	-0.01	-0.12	0.00	-0.01
No. of Tillers per Clump	0.88	-0.44	-0.06	0.16	-0.05	0.00	0.05
No. of Leaves per Tiller	-0.47	-0.44	0.48	0.54	-0.19	0.05	0.14
Petiole Length	-0.64	0.01	-0.62	-0.39	-0.11	0.04	0.21
Leaf Length	-0.79	-0.03	-0.58	-0.05	0.08	0.18	0.00
Leaf Width	-0.30	-0.23	0.78	-0.02	-0.28	0.31	0.27
Rhizome Length	0.33	0.80	0.33	0.10	0.33	0.12	0.11
Rhizome Internode Length	0.40	0.76	-0.22	-0.40	0.16	-0.12	0.17
Rhizome Weight	0.62	-0.18	-0.70	0.16	-0.02	0.22	0.15
No. of Mother Rhizome	-0.40	0.31	0.33	0.20	0.63	0.44	0.04
No. of Primary Rhizome	0.14	-0.11	-0.46	0.74	0.39	-0.24	0.05
No. of Secondary Rhizome	0.68	0.69	0.15	-0.16	-0.08	0.10	0.04
of Mother Rhizome	0.22	-0.60	-0.64	-0.10	0.31	0.07	0.27
Weight of Primary Rhizome	0.71	0.19	-0.38	0.22	-0.22	0.36	-0.30
Weight of Secondary Rhizome	0.36	0.59	-0.14	0.40	-0.48	-0.06	0.32
Dry Recovery	-0.51	0.43	-0.58	0.28	-0.30	0.21	-0.11

The Principle Component Analysis showed very high dispersion among the accessions of *C. xanthorrhiza*. Acc. 465 and Acc.760 fell in the same quadrant. Similarly, Acc.1122 and Acc.1163 as well as Acc.1164 and Acc.1167 also grouped under the same quadrant. However, Acc.1108 and Acc.1168 showed a distict status in the loading plot (Figure 14).

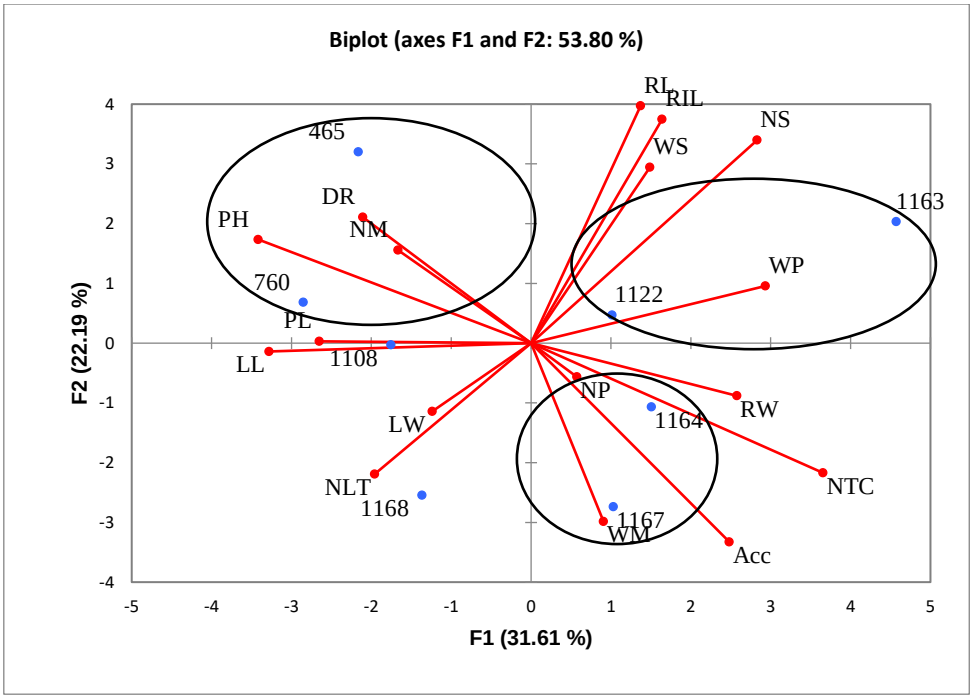


Fig. 14. Two dimensional loading plot of Principal Component Analysis (PCA) based on morphological data in *C. xanthorrhiza.*

4.1.5. Cluster analysis

Morphological characters including aerial and rhizome characters were used for the cluster analysis. The UPGMA dendrogram was constructed for individual species based on the data obtained from the similarity coefficients. Simple Matching (SM) coefficient matrix was considered for the cluster analysis of individual species. Ten accessions of *C. amada* clustered into three main groups splitting at coefficient 0.52. The groupings were as follows.

Group I – Acc.265, Acc.848, Acc.752, Acc.753

Group II – Acc.347, Acc.1503

Group III- Acc.521, Acc.1119, Acc.1511, Acc.6390

The similarity matrix based on Simple Matching coefficient is denoted in Table 42. The dendrogram constructed using SAHN clustering algorithm is shown in Figure 15.

Table 52. Similarity matrix of *C. amada* generated using Simple Matching similarity coefficients

Accession	265	347	521	752	753	848	1119	1503	1511	6390
265	1.00									
347	0.56	1.00								
521	0.31	0.50	1.00							
752	0.44	0.13	0.50	1.00						
753	0.56	0.25	0.63	0.75	1.00					
848	0.69	0.38	0.50	0.63	0.75	1.00				
1119	0.38	0.56	0.44	0.44	0.19	0.31	1.00			
1503	0.69	0.63	0.38	0.38	0.50	0.50	0.31	1.00		
1511	0.44	0.38	0.63	0.50	0.38	0.50	0.81	0.13	1.00	
6390	0.19	0.63	0.50	0.38	0.25	0.25	0.69	0.25	0.63	1.00

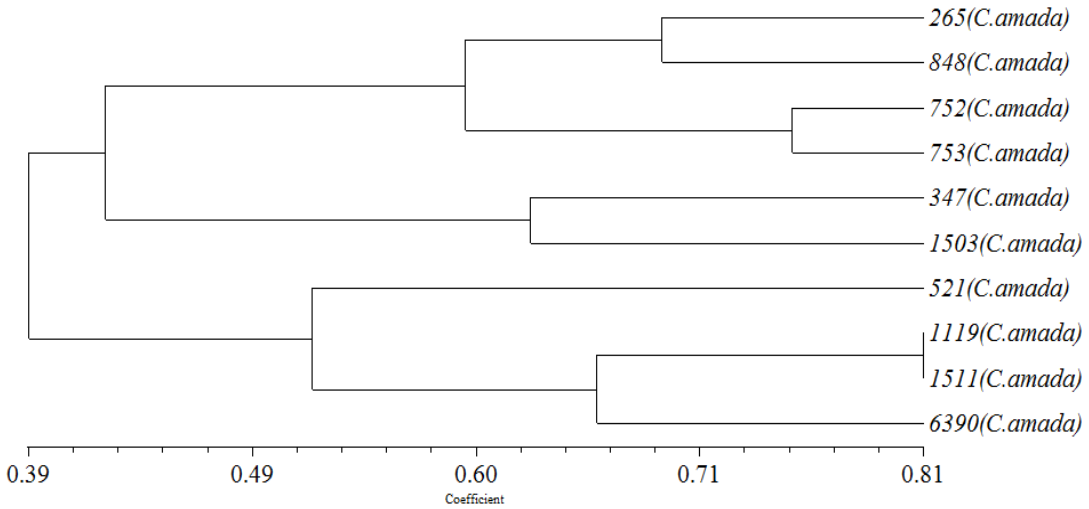


Fig. 15. UPGMA dendrogram of *C. amada* constructed using Simple Matching similarity coefficients.

First group was formed by four accessions; Acc.265, Acc.848, Acc.752 and Acc.753. Among which Acc.753 and Acc.752 which were collected from Cooch Behar (West Bengal) showed closer association with each other. Acc.347 and Acc.1503 formed the second group followed by four accessions, *viz*., Acc.521, Acc.1119, Acc.1511 and Acc.6390 forming the third group; Acc. 1119 (Thrissur, Kerala) and Acc.1511 (Koraput, Odisha) showed maximum proximity with each other among the ten accessions.

In case of *C. aromatica*, the seven accessions were clustered into two groups by splitting at coefficient 0.50. The groupings obtained were as follows:

Group I- Acc.711, Acc.1113, Acc.1518, Acc.1520

Group II – Acc.1025, Acc.1124, Acc.1132

The similarity matrix and dendrogram of seven accessions of *C. aromatica* based on Simple Matching coefficients is denoted in Table 53& Figure 16.

Table 53. The similarity matrix of *C. aromatica* generated using Simple Matching similarity coefficients

Accession	711	1025	1113	1124	1132	1518	1520
711	1.00						
1025	0.38	1.00					
1113	0.75	0.13	1.00				
1124	0.38	0.63	0.25	1.00			
1132	0.38	0.50	0.38	0.75	1.00		
1518	0.56	0.44	0.69	0.19	0.19	1.00	
1520	0.38	0.63	0.38	0.50	0.38	0.69	1.00

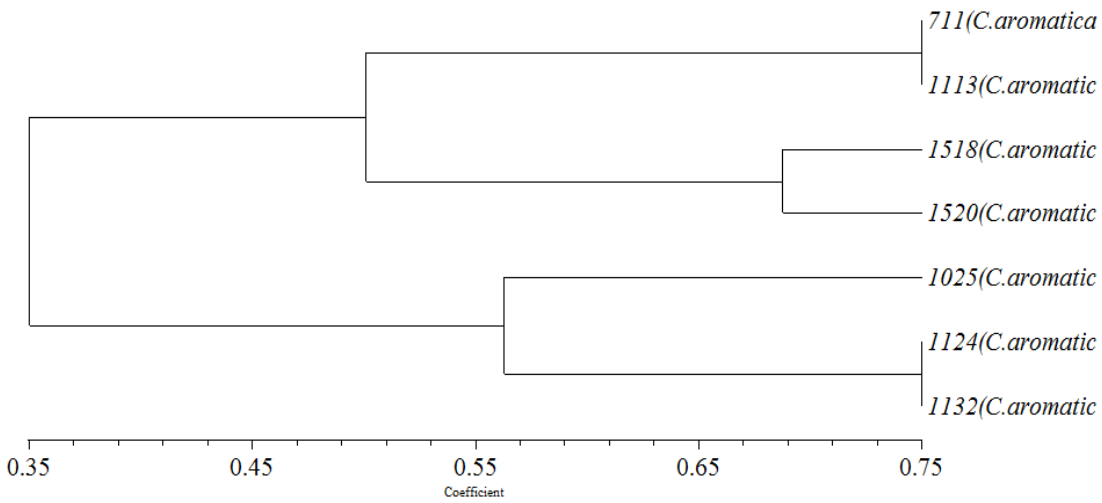


Fig. 16. UPGMA dendrogram of *C. aromatica* constructed using Simple Matching similarity coefficients.

Seven accessions of *C. aromatica* were clustered into two main groups. First group was formed by Acc.711, Acc.1113, Acc.1518 and Acc.1520. Acc.711 and Acc.1113 which was collected from Thrissur and Pathanamthitta districts of Kerala showed maximum affinity with each other

with a coefficient value of 0.75. Acc.1518 and Acc.1520 which were collected from Idukki (Kerala) grouped together. Likewise, in second group Acc.1124 (Ernakulam, Kerala) and Acc.1132 (Tirunelveli, Tamil Nadu) showed closer affinity with each other with a coefficient value of 0.75.

Seven accessions of *C. caesia* were clustered into four groups by splitting at simple matching (SM) similarity coefficient of 0.541. The grouping pattern obtained was as follows.

Group I - Acc.292, Acc.1006, Acc.1171,

Group II - Acc.1001

Group III - Acc.751, Acc.1135

Group IV - Acc.1154

The similarity matrix of the seven accessions of *C. caesia* based on Simple Matching coefficient is given in Table 54. The dendrogram of the seven accessions constructed using SAHN clustering algorithm is shown in Figure 17.

Table 54. The similarity matrix generated using Simple Matching similarity coefficients

Accession	292	751	1001	1006	1135	1154	1171
292	1.00						
751	0.50	1.00					
1001	0.50	0.38	1.00				
1006	0.69	0.56	0.69	1.00			
1135	0.38	0.63	0.25	0.31	1.00		
1154	0.31	0.19	0.44	0.25	0.44	1.00	
1171	0.69	0.56	0.44	0.75	0.44	0.50	1.00

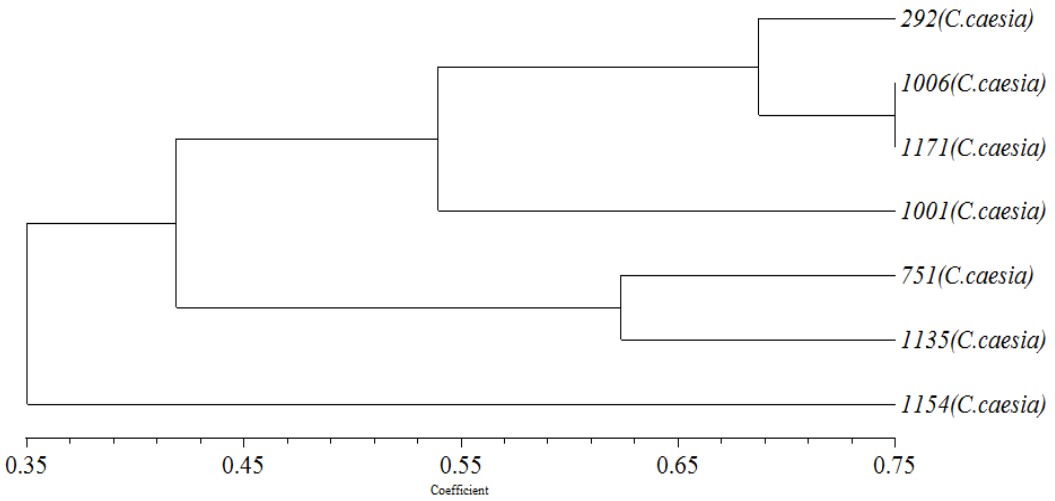


Fig. 17. UPGMA dendrogram of *C. caesia* constructed using Simple Matching similarity coefficients

First group was formed by Acc.292, Acc.1006, Acc.1171; among which Acc.1006 (Anjaw, Arunachal Pradesh) and Acc.1171 (Shilong, Meghalaya) showed closer association with a similarity coefficient of 0.75.Acc. 1001 formed the second group. Acc.751 (Cooch Behar, West Bengal) and Acc.1135 (Thrissur, Kerala) which were collected from two different location of India grouped together. Acc.1154 (Thrissur, Kerala) formed the fourth group. Grouping was independent of geographical origin.

In case of *C. xanthorrhiza*, eight accessions were clustered into four groups by splitting at coefficients 0. 590.The groupings obtained was as follows.

Group I – Acc.465, Acc.1164, Acc.1167

Group II – Acc.760

Group III- Acc.1108, Acc.1122, Acc.1163

Group IV – Acc.1168

The similarity matrix of the eight accessions of *C. xanthorrhiza* based on Simple Matching coefficient was denoted in Table 55. The dendrogram of the eight accessions constructed using SAHN clustering algorithm is shown in Figure 18.

Table 55. The similarity matrix generated using Simple Matching similarity coefficients

Accession	465	760	1108	1122	1163	1164	1167	1168
465	1.00							
760	0.44	1.00						
1108	0.44	0.38	1.00					
1122	0.44	0.25	0.88	1.00				
1163	0.44	0.38	0.75	0.75	1.00			
1164	0.56	0.38	0.50	0.50	0.63	1.00		
1167	0.63	0.56	0.31	0.19	0.31	0.69	1.00	
1168	0.50	0.44	0.44	0.56	0.31	0.31	0.38	1.00

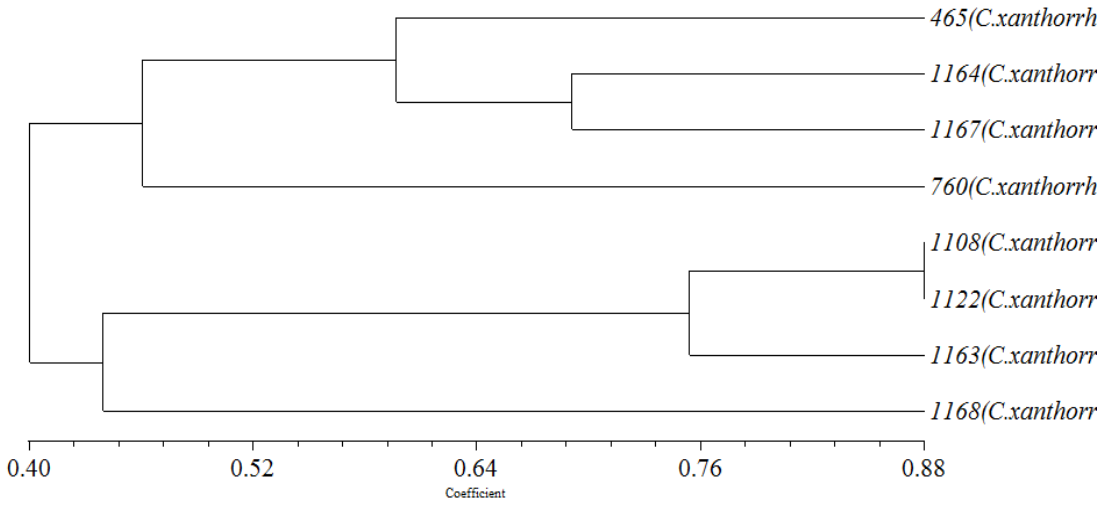


Fig. 18. UPGMA dendrogram of *C. xanthorrhiza* constructed using Simple Matching similarity coefficients.

First group was formed by Acc.465, Acc.1164 and Acc.1167. Acc.760 and Acc. 1168 formed the second and fourth group respectively. Third group was formed by Acc.1108, Acc.1122 and Acc.1163. Acc.1108 and Acc.1122 which were collected from Palakkad (Kerala) found to be closely related with each other with a coefficient value of 0.88.

A UPGMA based dendrogram (Figure 19) was constructed for four *Curcuma* species using Jaccard's similarity coefficients. The dendrogram formed eight groups splitting at Jaccard's similarity coefficient of 0.438. The eight groups formed were as follows:

Group I	-	Acc. 265, 1132, 1113, 1167, 521, 1511, 1119, 752, 753, 848 and 711
Group II	-	Acc. 465, 1168, 760, 1122, 1163, 1108, 1164
Group III	-	Acc. 292, Acc.1006, Acc.751, Acc.1171, Acc.1135, Acc.1154, Acc.1001
Group IV	-	Acc. 347, Acc.1503
Group V	-	Acc. 1025
Group VI	-	Acc.1124
Group VII	-	Acc. 1518, Acc.1520
Group VIII	-	Acc. 6390

The similarity matrix of thirty-two accessions under study based on Jaccard's coefficient is presented in Table 56.

Table 56. Jaccard's similarity coefficients based on morphological data from four *Curcuma* species

		C. amada										*C. aromatica*							*C. caesia*							*C. xanthorrhiza*							
		256	347	521	752	753	848	1119	1503	1511	6390	711	1025	1113	1124	1132	1518	1520	292	751	1001	1006	1135	1154	1171	465	760	1108	1122	1163	1164	1167	1168
C. amada	**256**	1.00																															
	347	0.27	1.00																														
	521	0.55	0.45	1.00																													
	752	0.50	0.43	0.20	1.00																												
	753	0.73	0.38	0.60	0.43	1.00																											
	848	0.60	0.15	0.30	0.31	0.36	1.00																										
	1119	0.31	0.60	0.56	0.38	0.45	0.08	1.00																									
	1503	0.60	0.15	0.18	0.42	0.50	0.50	0.18	1.00																								
	1511	0.64	0.31	0.36	0.36	0.70	0.56	0.25	0.75	1.00																							
	6390	0.38	0.21	0.07	0.73	0.31	0.27	0.15	0.40	0.33	1.00																						
C. aromatica	711	0.58	0.38	0.33	0.43	0.64	0.50	0.33	0.67	0.89	0.42	1.00																					
	1025	0.20	0.55	0.25	0.36	0.21	0.08	0.36	0.17	0.23	0.33	0.31	1.00																				
	1113	0.20	0.89	0.36	0.36	0.31	0.17	0.50	0.17	0.33	0.23	0.42	0.60	1.00																			
	1124	0.17	0.30	0.38	0.15	0.18	0.25	0.38	0.11	0.20	0.09	0.30	0.33	0.33	1.00																		
	1132	0.31	0.33	0.08	0.64	0.23	0.44	0.17	0.44	0.36	0.67	0.45	0.25	0.36	0.22	1.00																	
	1518	0.60	0.07	0.18	0.42	0.36	0.50	0.08	0.50	0.40	0.40	0.36	0.17	0.00	0.11	0.30	1.00																
	1520	0.60	0.25	0.44	0.42	0.67	0.33	0.44	0.50	0.40	0.27	0.36	0.08	0.17	0.11	0.30	0.33	1.00															
C. caesia	292	0.46	0.29	0.14	0.67	0.38	0.36	0.14	0.36	0.42	0.89	0.50	0.31	0.31	0.08	0.60	0.36	0.25	1.00														
	751	0.08	0.20	0.25	0.08	0.09	0.13	0.25	0.00	0.10	0.00	0.09	0.38	0.22	0.40	0.00	0.13	0.00	0.00	1.00													
	1001	0.73	0.20	0.45	0.43	0.64	0.36	0.33	0.50	0.55	0.42	0.50	0.31	0.13	0.18	0.23	0.67	0.50	0.38	0.20	1.00												
	1006	0.31	0.60	0.56	0.29	0.45	0.08	0.75	0.08	0.25	0.15	0.33	0.36	0.50	0.22	0.08	0.08	0.30	0.23	0.25	0.33	1.00											
	1135	0.27	0.80	0.45	0.43	0.38	0.07	0.60	0.07	0.21	0.31	0.29	0.55	0.70	0.18	0.23	0.07	0.25	0.38	0.20	0.29	0.78	1.00										
	1154	0.36	0.38	0.14	0.54	0.29	0.36	0.14	0.25	0.31	0.55	0.38	0.55	0.42	0.18	0.45	0.36	0.15	0.64	0.20	0.29	0.23	0.38	1.00									
	1171	0.33	0.15	0.08	0.42	0.15	0.50	0.00	0.33	0.27	0.56	0.25	0.27	0.17	0.11	0.63	0.50	0.20	0.50	0.13	0.36	0.00	0.15	0.50	1.00								
C. xanthorrhiza	465	0.64	0.13	0.25	0.58	0.42	0.40	0.15	0.40	0.33	0.60	0.31	0.23	0.07	0.09	0.36	0.75	0.40	0.55	0.10	0.70	0.15	0.21	0.42	0.56	1.00							
	760	0.46	0.29	0.14	0.82	0.38	0.25	0.23	0.36	0.31	0.70	0.29	0.31	0.21	0.00	0.45	0.50	0.36	0.64	0.09	0.50	0.23	0.38	0.50	0.50	0.70	1.00						
	1108	0.46	0.64	0.60	0.43	0.64	0.15	0.78	0.25	0.42	0.21	0.50	0.42	0.55	0.30	0.14	0.15	0.36	0.29	0.20	0.38	0.78	0.64	0.29	0.00	0.21	0.29	1.00					
	1122	0.40	0.30	0.57	0.07	0.30	0.43	0.22	0.25	0.33	0.00	0.30	0.20	0.33	0.33	0.10	0.11	0.25	0.08	0.17	0.18	0.22	0.18	0.18	0.11	0.09	0.00	0.30	1.00				
	1163	0.64	0.31	0.50	0.46	0.89	0.27	0.50	0.56	0.60	0.33	0.55	0.23	0.23	0.20	0.25	0.40	0.75	0.31	0.10	0.70	0.36	0.31	0.21	0.17	0.45	0.42	0.55	0.20	1.00			
	1164	0.20	0.10	0.29	0.08	0.10	0.14	0.13	0.00	0.00	0.00	0.00	0.11	0.00	0.20	0.00	0.33	0.14	0.00	0.25	0.22	0.13	0.10	0.10	0.14	0.25	0.10	0.10	0.20	0.11	1.00		
	1167	0.30	0.09	0.25	0.17	0.09	0.29	0.11	0.13	0.10	0.10	0.09	0.10	0.00	0.17	0.11	0.50	0.13	0.09	0.20	0.33	0.11	0.09	0.09	0.29	0.38	0.20	0.09	0.17	0.10	0.67	1.00	
	1168	0.30	0.20	0.43	0.27	0.33	0.13	0.43	0.13	0.10	0.10	0.09	0.00	0.10	0.17	0.11	0.13	0.50	0.09	0.00	0.20	0.25	0.20	0.00	0.00	0.22	0.20	0.33	0.17	0.38	0.25	0.20	1.00

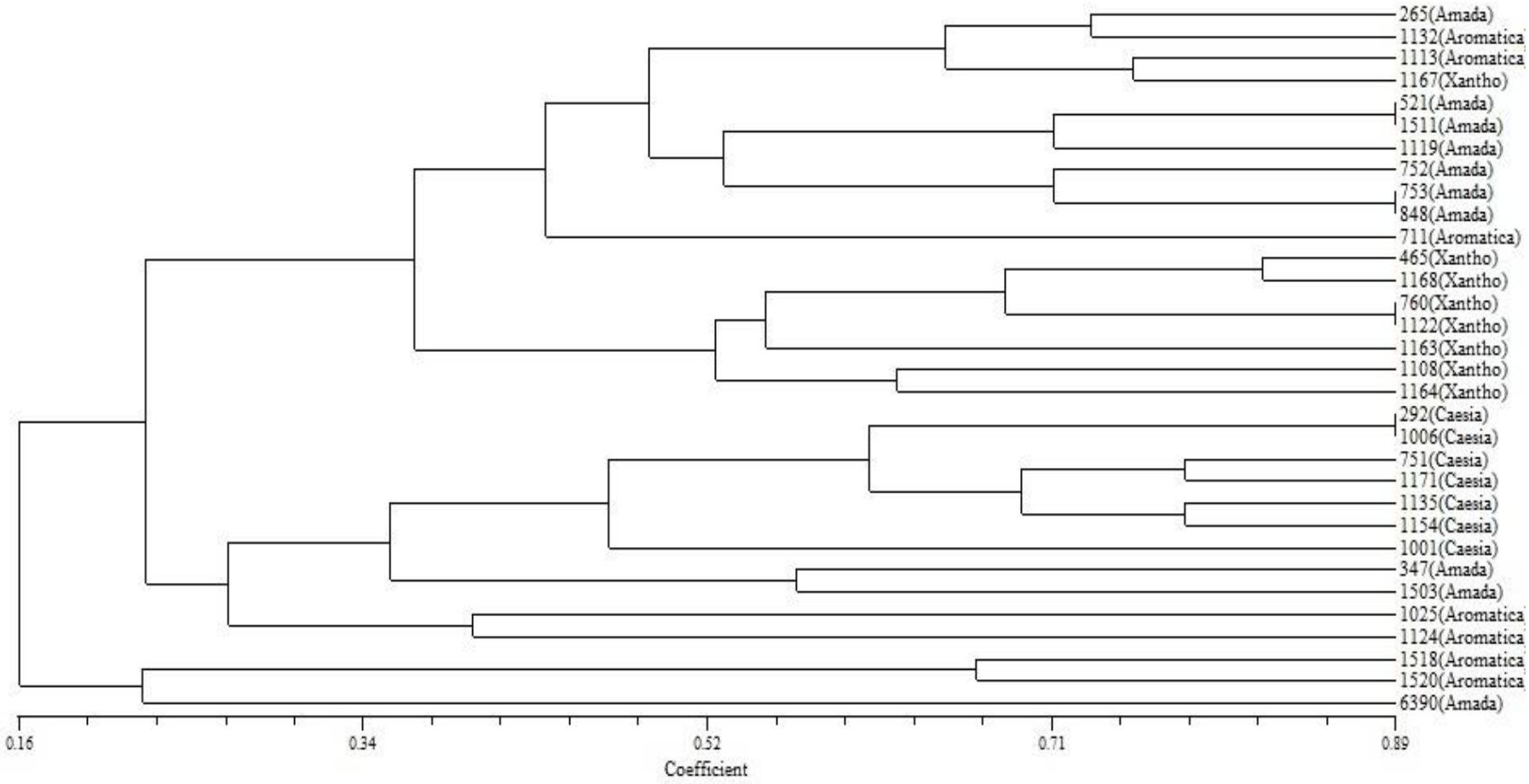


Fig. 19. UPGMA dendrogram of four *Curcuma* species constructed using Jaccard's similarity coefficients.

First group was formed by three accessions from *C. aromatica* (Acc. 1132, Acc.1113, Acc.711) seven accessions from *C. amada* (Acc. 265, Acc.521, Acc.1511, Acc.1119, Acc. 752, Acc.753, Acc. 848) and a single accession from *C. xanthorrhiza* (Acc. 1167). In the first group maximum similarity was observed between Acc.521 and Acc.1511 as well as Acc. 753 and Acc. 848 (0.89) of *C. amada*. Second group was formed by the accessions of *C. xanthorrhiza*–Acc.465, Acc.1168, Acc. 760, Acc.1122, Acc.1163, Acc.1108 and Acc.1164. Maximum similarity was observed between Acc. 760 and Acc. 1122 (0.89). Accessions from *C. caesia* formed the third group, in which Acc. 292 and Acc. 1006 showed maximum similarity of 0.89. Two accessions from *C. amada* (Acc. 347 & Acc.1503) formed the fourth group. Fifth, sixth and eight groups were formed by Acc. 1025 (*C. aromatica*), Acc.1124 (*C. aromatica*) and Acc. 6390 (*C. amada*), respectively. Acc.1518 and Acc. 1520 (*C. aromatica*) formed the seventh group.

4.2. Biochemical Characterization of four *Curcuma* species

4.2.1. Oleoresin

Significant variation was observed for oleoresin content among and within the four *Curcuma* species. Least oleoresin content was recorded in *C. caesia* (6.92%) and maximum in *C. aromatica* (10.68%) (Table 57). Variance analysis showed that 63% of the variation was contributed by within the species whereas the remaining 37% of variation was accounted by among the species (Table 58).

Among the accessions of *C. amada*, the oleoresin content ranged from 4.38% (Acc.753) to 11.37% (Acc.521), with an average value of 7.76%. In case of *C. aromatica*, the oleoresin content ranged from 9.63% (Acc. 1124) to 12.45% (Acc.1520) with an average value of 10.68%. Among the accessions of *C. caesia*, highest oleoresin content was recorded in Acc. 1006 (7.51%) followed by Acc. 1135 (7.31%) and Acc.1154 (7.28%).

In *C. xanthorrhiza*, the oleoresin content among accessions ranged from 7.23% (Acc. 1167) to 11.71% (Acc.1164), with an average oleoresin content of 9.29%. The percentage of variation obtained from the mean square values showed that maximum variation was contributed by variations present among accessions of *C. amada, C. xanthorrhiza, C. aromatica* and *C. caesia* (97%, 96%, 85% and 52%, respectively) (Table 58).

Table 57. Mean oleoresin content in four *Curcuma* species

Sl. No	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	Oleoresin (%)	Accession	Oleoresin (%)	Accession	Oleoresin (%)	Accession	Oleoresin (%)
1	265	5.04 ± 0.04^{ab}	711	11.67 ± 0.28^{b}	292	6.71 ± 0.18^{abc}	465	10.61 ± 0.24^{d}
2	347	9.95 ± 0.05^{e}	1025	9.94 ± 0.16^{a}	751	6.91 ± 0.04^{bcd}	760	9.67 ± 0.12^{c}
3	521	11.37 ± 0.33^{f}	1113	9.83 ± 0.08^{a}	1001	6.53 ± 0.19^{ab}	1108	8.92 ± 0.14^{b}
4	752	4.43 ± 0.33^{a}	1124	9.63 ± 0.11^{a}	1006	7.51 ± 0.31^{d}	1122	7.43 ± 0.21^{a}
5	753	4.38 ± 0.26^{a}	1132	9.73 ± 0.42^{a}	1135	7.31 ± 0.17^{cd}	1163	8.76 ± 0.14^{b}
6	848	5.36 ± 0.31^{bc}	1518	11.51 ± 0.30^{b}	1154	7.28 ± 0.19^{cd}	1164	11.71 ± 0.25^{e}
7	1119	7.74 ± 0.20^{c}	1520	12.45 ± 0.26^{c}	1171	6.18 ± 0.31^{a}	1167	7.23 ± 0.10^{a}
8	1511	10.52 ± 0.34^{e}	-	-	-	-	1168	9.99 ± 0.11^{c}
9	1503	9.89 ± 0.09^{e}	-	-	-	-	-	-
10	6390	8.87 ± 0.28^{d}	-	-	-	-	-	-
Mean ± SE		7.76 ± 0.48^{a}		10.68 ± 0.25^{c}		6.92 ± 0.12^{a}		9.29 ± 0.30^{b}
Accessions		P< 0.05		P< 0.05		P<0.05		P< 0.05
Species		P< 0.05						

Values with the different superscript are significantly different (P<0.05), SE-Standard error

Table 58. F-value and percentage of variation for oleoresin content in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	4.08	12.94	8.60	0.23	19.47**	37%	63%
C. amada	4.08	12.04	7.76	0.49	117.95**	97%	3%
C. aromatica	9.01	12.94	10.68	0.25	20.75**	85%	15%
C. caesia	5.68	8.14	6.92	0.12	4.86**	52%	48%
C. xanthorrhiza	7.09	12.06	9.29	0.30	79.93**	96%	4%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.2.2. Essential Oil

Essential oil yield from dried rhizomes ranged from 2.39% to 5.06%. Among the four *Curcuma* species, *C. aromatica* recorded with highest percentage of essential oil (5.06%), followed by *C. xanthorrhiza* (3.17%), *C. caesia* (2.79%) and lowest being in *C. amada* (2.39%) (Table 59). Significant variation was observed among the four species as well as within the species. Among the four species, *C. aromatica* stood apart in terms of essential oil content as it recorded the highest essential oil content. Among the species variation (71%) was more than within the species variation (29%).

Among the accessions of *C. amada*, essential oil yield ranged from 3.17% (Acc. 1503) to 1.17% (Acc. 265). Ninety-six percentage of the variation for essential oil content was contributed by the differences present among the accessions. In *C. aromatica*, a similar trend was observed with 94% of the variation among the accessions source. Acc.1025 yielded the maximum essential oil content of 6.03% followed by Acc. 1520 (5.60%), least being in Acc. 1124 (4.27%). Accessions of *C. caesia* yielded an average essential oil content of 2.79%, ranging from 2.30% (Acc. 292) to 3.13% (Acc. 1001). Among eight accessions of *C. xanthorrhiza*, Acc. 1168 recorded the highest amount of essential oil (4.80%) and lowest in the Acc. 1167 (2.43%), with an average essential oil content of 3.17%. Maximum percentage of variation was contributed by variation present among the accessions (85%, 94%) for *C. caesia* and *C. xanthorrhiza* respectively (Table 60).

Table 59. Mean essential oil content in four *Curcuma* species

Sl. No	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	Essential Oil (%)	Accession	Essential Oil (%)	Accession	Essential Oil (%)	Accession	Essential Oil (%)
1	265	1.17 ± 0.09^{e}	711	4.77 ± 0.15^{bc}	292	2.30 ± 0.06^{b}	465	3.63 ± 0.09^{b}
2	347	2.77 ± 0.15^{b}	1025	6.03 ± 0.26^{a}	751	2.97 ± 0.12^{a}	760	2.73 ± 0.15^{d}
3	521	2.23 ± 0.15^{d}	1113	4.83 ± 0.33^{bc}	1001	3.13 ± 0.19^{a}	1108	2.72 ± 0.22^{d}
4	752	2.37 ± 0.09^{cd}	1124	4.27 ± 0.15^{c}	1006	2.75 ± 0.20^{ab}	1122	3.15 ± 0.08^{c}
5	753	2.67 ± 0.17^{bc}	1132	5.23 ± 0.09^{ab}	1135	2.70 ± 0.06^{ab}	1163	2.70 ± 0.12^{d}
6	848	2.77 ± 0.15^{b}	1518	4.70 ± 0.15^{bc}	1154	2.73 ± 0.12^{ab}	1164	3.23 ± 0.12^{c}
7	1119	1.37 ± 0.09^{e}	1520	$5.60 \pm 0.12s^{b}$	1171	3.00 ± 0.12^{a}	1167	2.43 ± 0.12^{d}
8	1511	2.70 ± 0.15^{a}			-	-	1168	4.80 ± 0.12^{a}
9	1503	3.17 ± 0.20^{bc}	-	-	-	-	-	-
10	6390	2.67 ± 0.07^{bc}	-	-	-	-	-	-
Mean ± SE		2.39 ± 0.12^{c}		5.06 ± 0.14^{a}		2.79 ± 0.07^{bc}		3.17 ± 0.15^{b}
Accessions		P< 0.05	P< 0.05		P< 0.05		P< 0.05	
Species		P<0.05						

Values with the different superscript are significantly different (P<0.05), SE-Standard error

Table 60. F-value and percentage of variation for essential oil characters in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	1.20	6.40	3.28	0.12	78.97**	71%	29%
C. amada	1.20	3.60	2.41	0.12	90.07**	96%	4%
C. aromatica	4.11	6.40	5.03	0.15	52.41**	94%	6%
C. caesia	2.20	3.52	2.83	0.08	21.41**	85%	15%
C. xanthorrhiza	2.35	4.80	3.23	0.15	52.78**	94%	6%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.2.2.1. GC-MS analysis of Essential Oil

GC-MS analysis of the essential oil from four *Curcuma* species yielded a total of 30 major compounds, constituting an average of 87.84% of the oil. Composition of the essential oil yield from four species under study is presented in Table 61-65.

Among the four species the lowest number of identified compounds were detected in *C. amada* and highest number in *C. caesia* and *C. xanthorrhiza*. Maximum amount of total volatile components was observed in *C. xanthorrhiza* (89.91%) followed by *C. amada* (89.88%), *C. aromatica* (86.32%) and *C. caesia* (84.87%).

The major constituents of essential oil from the accessions of *C. amada* were β-myrcene (64.44%), followed by β-pinene (10.94%), perillene (3.61%) and β-ocimene (3.13%). A total of nine compounds amounts to 89.88% of the total oil content in the species. Acc. 848 recorded the highest amount of β-myrcene (75.43%), followed by Acc. 753 (74.96%) and Acc. 521 (70.34%). Lowest amount was recorded in Acc.1503 (54.12%), with an average value of 64.44% among the accessions (Table 61). Figure 15 shows the major three oil components in *C. amada*.

Table 61. GC-MS analysis of essential oil from the accessions of *C. amada*

Compound	RI	**265**	**347**	**521**	**752**	**753**	**848**	**1119**	**1503**	**1511**	**6390**	Avg
Alpha Pinene	938	2.98	2.97	2.46	0.96	1.24	0.89	1.68	2.84	2.89	0.97	1.99
Beta Pinene	975	14.92	11.08	12.31	9.36	9.45	8.78	8.86	12.35	12.76	9.57	10.94
Beta Myrcene	991	56.23	68.52	70.34	63.72	74.96	75.43	67.56	54.12	58.13	55.37	64.44
D-Limonene	1030	0.90	0.67	0.65	0.36	0.27	0.35	0.33	0.53	0.73	0.42	0.52
Eucalyptol (1,8-Cineole)	1033	0.76	0.84	0.96	0.38	0.48	0.74	0.44	0.47	0.81	0.64	0.65
Beta Ocimene	1046	2.43	2.89	2.17	2.13	3.49	5.41	2.56	3.89	1.23	5.12	3.13
Perillene	1102	5.34	4.28	4.38	1.36	1.83	0.93	1.68	4.10	6.94	5.21	3.61
trans β-caryophyllene	1417	1.53	1.68	1.74	2.45	2.53	2.42	1.87	1.88	1.36	1.43	1.89
Beta Farnesene	1464	1.42	2.59	2.56	1.49	1.42	3.98	3.52	2.54	4.21	3.42	2.72
Grand Total		86.51	95.52	97.57	82.21	95.67	98.93	88.50	82.72	89.06	82.15	89.88

*RI- Retention Index

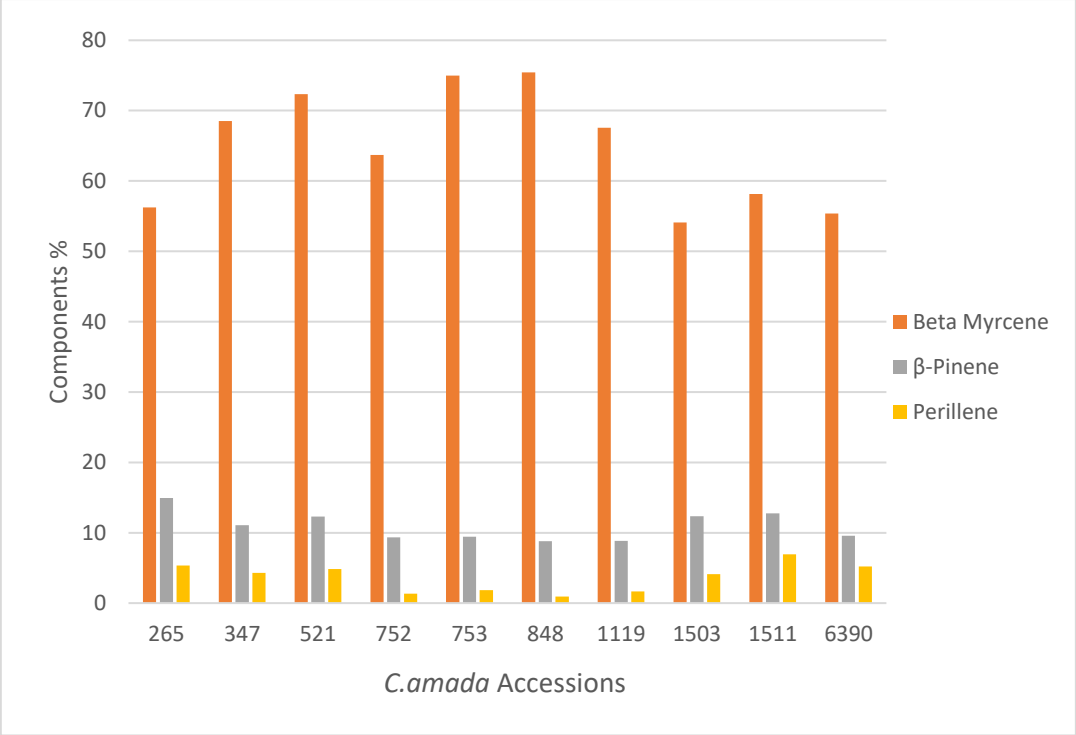


Fig. 20. Histogram showing the three major oil components in *C. amada*

In *C. aromatica*, 14 constituents accounted for 86.32% of total essential oil. The major components were camphor (27.82%), curdione (18.26%), 1, 8 cineole (9.11%), borneol (8.63%), camphene (4.60%), germacrone (3.59%), curzerene (3.12%) and germacrene D (2.73%). Camphor was found maximum in Acc.1518 (32.27%) and minimum in Acc.1124 (24.18%). Second major compound curdione was found maximum in Acc.1124 (20.19%), followed by Acc. 1132 (19.29%). The identified components are shown in Table 52. The three major identified compounds are depicted in Figure 21.

Table 62. GC-MS analysis of essential oil from the accessions of *C. aromatica*

Compound	RI	**711**	**1025**	**1113**	**1124**	**1132**	**1518**	**1520**	Avg
α-Pinene	938	1.50	1.04	1.08	0.89	1.06	1.19	1.20	1.14
Camphene	953	4.94	4.36	4.72	3.78	4.09	5.48	4.82	4.60
β-Pinene	975	0.97	0.48	0.58	0.49	0.53	0.54	0.92	0.64
Beta myrcene	991	1.06	1.01	1.05	0.78	0.82	1.18	1.28	1.03
D-Limonene	1030	2.11	1.97	2.47	1.96	1.98	1.78	1.63	1.99
Eucalyptol(1,8-Cineole)	1033	9.35	8.98	8.67	8.04	7.78	11.61	9.32	9.11
Camphor	1145	27.63	27.34	27.91	24.18	27.26	32.27	28.18	27.82
Borneol	1165	8.22	8.83	8.42	7.39	8.29	9.24	10.02	8.63
α-terpineol	1186	2.13	1.39	1.66	1.14	1.16	2.63	1.22	1.62
β-elemene	1394	2.13	1.61	2.12	2.02	1.91	2.39	2.16	2.05
Germacrene D	1481	2.66	2.92	2.84	2.69	2.65	2.43	2.89	2.73
Curzerene	1499	3.18	4.30	3.55	2.76	2.96	2.15	2.96	3.12
Germacrone	1694	3.92	5.95	3.82	2.52	2.52	3.22	3.18	3.59
Curdione	1698	17.45	17.61	17.15	20.19	19.29	17.99	18.17	18.26
Grand Total		87.25	87.79	86.04	78.83	82.30	94.10	87.95	86.32

*RI- Retention Index

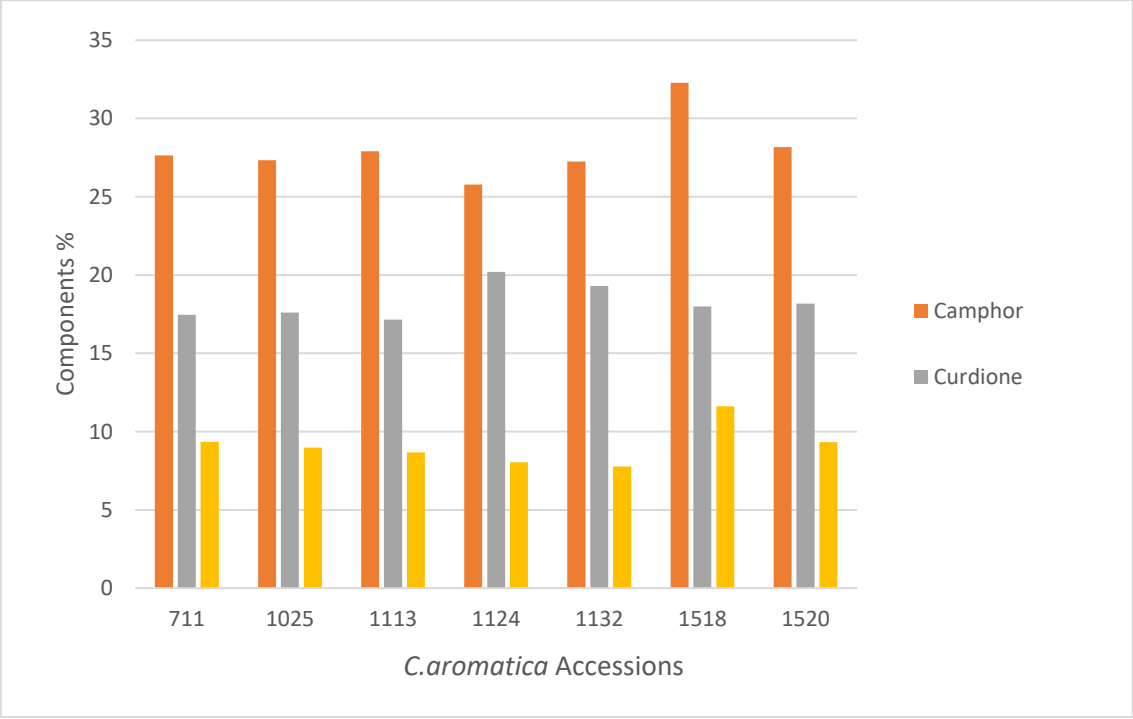


Fig. 21. Histogram showing the three major oil components in *C. aromatica*

GC-MS analysis of the essential oil from *C. caesia* revealed twenty components comprising 84.87% of the total volatile oils. The predominant components found in the accessions of *C. caesia* were epicurzerenone (22.88%), 1, 8 cineole (22.43%), camphor (8.95%), curzerene (3.90%), borneol (3.27%), isocurcumenol (3.05%), β-elemene (2.75%), isobornyl acetate (2.48%), and germacrone (2.77%).

Out of the seven accessions, Acc. 1006 accounted for maximum amount of volatile oil (88.92%) components and minimum in Acc. 292 (82.06%). Major component epicurzerenone was found maximum (24.86%) in the Acc. 1171, followed by Acc. 1006 (24.15%) and minimum in Acc. 1135 (20.83%). Acc. 292 recorded minimum amount of camphor (7.05%) and 1, 8 cineole (19.72%). Although major two components were lowest in Acc. 292, components like isocurcumenol, β-elemene and germacrone were found highest in Acc. 292 (Table 63). Figure 22 shows the three major components of the essential oil in *C. caesia* accessions.

Table 63. GC-MS analysis of essential oil from the accessions of *C. caesia*

Compound	RI	**292**	**751**	**1001**	**1006**	**1135**	**1154**	**1171**	Avg
α-Pinene	938	0.23	0.72	0.68	0.99	0.89	0.72	0.72	0.71
Camphene	953	2.16	2.43	2.57	2.97	2.72	2.57	2.59	2.57
β-Pinene	975	1.89	1.74	2.31	2.48	2.18	1.64	1.48	1.96
Beta Myrcene	991	0.31	0.42	0.45	0.49	0.51	0.44	0.47	0.44
D-Limonene	1030	0.69	0.54	0.49	0.57	0.52	0.59	0.61	0.57
Eucalyptol (1,8-Cineole)	1033	19.72	22.14	22.67	23.97	23.89	22.08	22.51	22.43
2-nonanol	1097	0.41	0.54	0.85	0.74	0.86	0.73	0.74	0.70
Camphor	1145	7.05	8.59	8.79	9.38	9.82	9.31	9.73	8.95
Isoborneol	1155	1.01	1.28	2.13	1.89	3.11	1.55	1.89	1.84
Borneol	1165	2.44	2.69	2.98	4.08	2.96	3.74	4.03	3.27
α-terpineol	1191	0.95	0.63	0.89	0.94	2.18	1.99	1.97	1.36
Isobornyl Acetate	1283	2.36	1.92	2.37	2.48	2.70	2.39	3.11	2.48
β-elemene	1394	3.94	2.14	2.49	2.64	2.33	2.94	2.79	2.75
β-caryophyllene	1419	0.58	0.37	0.51	0.53	0.54	0.58	0.47	0.51
Germacrene D	1481	0.56	0.98	1.11	1.07	1.04	1.27	1.10	1.02
Curzerene	1499	3.97	3.81	3.82	3.94	3.57	4.11	4.05	3.90
Germacrene B	1557	1.14	0.58	0.84	0.56	0.59	0.67	0.59	0.71
EpiCurzerenone	1605	23.18	23.67	22.31	24.15	20.83	21.19	24.86	22.88
Isocurcumenol	1615	5.71	4.89	3.14	2.67	1.14	1.87	1.94	3.05
Germacrone	1694	3.76	2.28	2.96	2.38	3.02	2.64	2.32	2.77
Grand Total		82.06	82.36	84.36	88.92	85.40	83.02	87.97	84.87

*RI- Retention Index

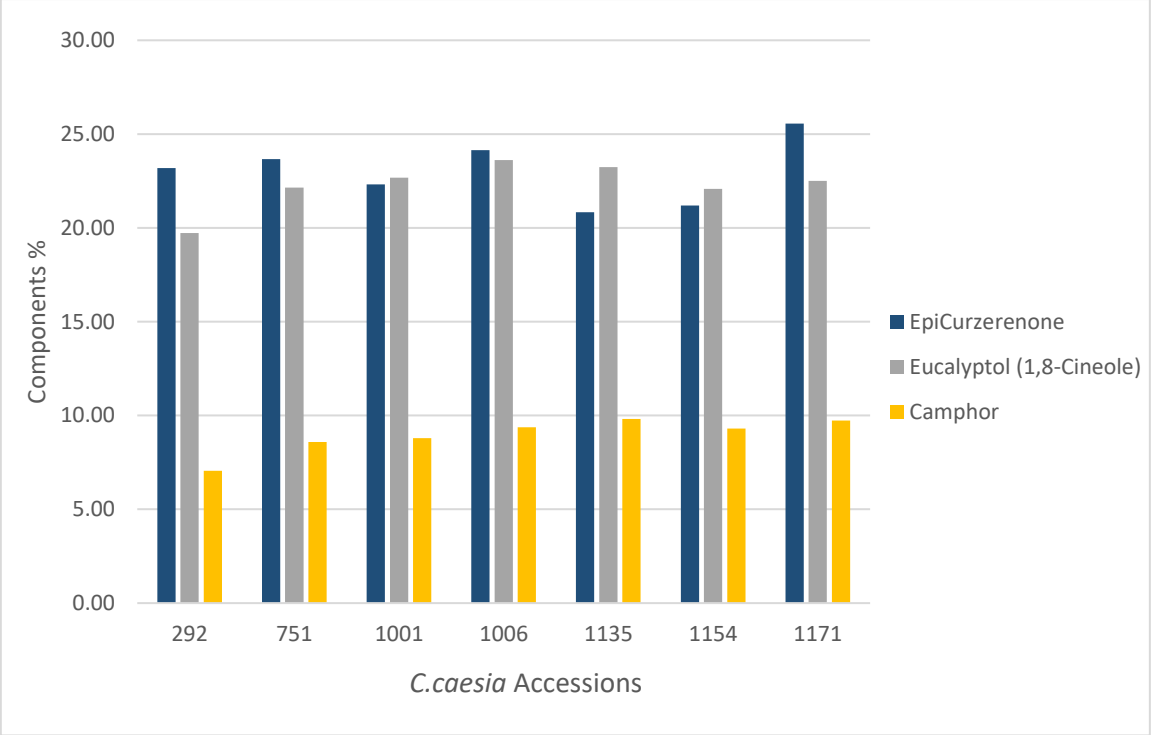


Fig. 22. Histogram showing the three major oil components in *C. caesia*

In *C. xanthorrhiza*, the two major components found were alpha cedrene (23.69%) and xanthorrhizol (23.66%), constituted 89.90% of the total essential oil. Ar-curcumene (18.69%), camphor (8.63%), germacrone (3.82%), curzerene (2.61%), and β-farnesene (2.55%) were the other major volatile oil components present in *C. xanthorrhiza*.

Acc. 465 had the highest amount of total volatile oil constituents (97%) and lowest in the Acc. 1167 (84.22%). Major component alpha cedrene was found maximum in Acc. 1168 (26.26%) and minimum in Acc. 1167 (22.22%) (Table 64). Figure 23 shows the three major components of the essential oil in *C. xanthorrhiza* accessions.

Table 64. GC-MS analysis of essential oil from the accessions of *C. xanthorrhiza*

Compound	RI	**465**	**760**	**108**	**1122**	**1163**	**1164**	**1167**	**1168**	Avg
α-Pinene	938	0.48	0.25	0.41	0.30	0.36	0.34	0.29	0.39	0.35
Camphene	953	0.76	0.75	0.80	0.74	0.86	0.90	0.57	0.95	0.77
β-Pinene	975	0.13	0.12	0.13	0.10	0.12	0.15	0.10	0.12	0.12
Beta myrcene	991	0.23	0.21	0.22	0.20	0.22	0.24	0.17	0.24	0.21
D-Limonene	1030	0.21	0.22	0.23	0.21	0.23	0.25	0.17	0.26	0.22
Eucalyptol (1,8-Cineole)	1033	0.32	0.20	0.12	0.21	0.11	0.12	0.10	0.11	0.17
Camphor	1145	9.55	8.92	8.24	8.04	8.52	8.78	8.34	8.29	8.63
Camphene hydrate	1150	0.97	0.35	0.42	0.39	0.40	0.38	0.31	0.46	0.46
Isoborneol	1155	0.87	0.32	0.32	0.29	0.34	0.38	0.26	0.41	0.40
Borneol	1165	0.62	0.25	0.23	0.19	0.24	0.26	0.18	0.27	0.28
α-terpineol	1191	0.38	0.10	0.11	0.11	0.14	0.12	0.10	0.14	0.15
β-elemene	1394	0.33	0.51	0.30	0.25	0.31	0.28	0.24	0.35	0.32
Alpha Cedrene	1412	24.65	24.52	25.78	22.50	23.44	22.71	22.22	26.26	23.69
Beta farnesene	1464	3.06	2.66	2.50	2.31	2.17	2.84	2.30	2.84	2.55
Ar-Curcumene	1483	23.03	17.53	17.76	18.10	16.84	20.07	17.51	20.48	18.69
Zingiberene	1495	2.12	0.50	0.50	0.45	0.46	0.28	0.64	0.28	0.71
Curzerene	1499	2.38	3.19	2.78	2.42	2.77	2.41	2.34	2.57	2.61
Germacrene B	1557	0.38	3.69	2.84	2.47	2.67	0.34	2.36	0.42	2.11
Germacrone	1694	1.78	5.12	3.97	4.25	4.38	3.26	3.99	3.29	3.82
Xanthorrhizol	1751	24.75	24.61	25.97	22.64	23.52	22.07	22.03	26.41	23.66
Grand Total		97.00	94.02	93.63	86.17	88.10	86.18	84.22	94.54	89.90

*RI- Retention Index

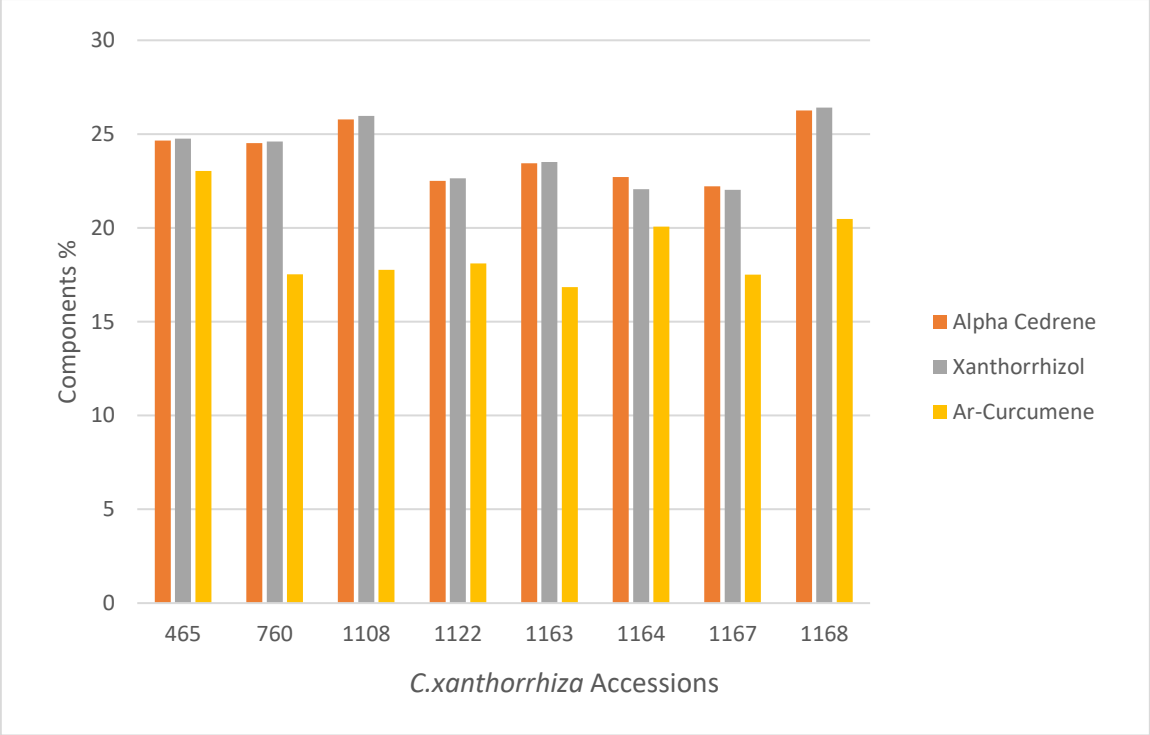


Fig. 23. Histogram showing the three major oil components in *C. xanthorrhiza*

Out of the total 30 compounds identified from four *Curcuma* species, some of the volatile oil constituents were unique to certain *Curcuma* species studied. Beta-ocimene was specific to *C. amada* while highest amount of curdione was present in the species *C. aromatica*. 2-nonanol, β-caryophyllene, epicurzerenone, isocurcumenol were found in *C. caesia*, Xanthorrhizol was recorded in *C. xanthorrhiza*. The consolidated list of compounds identified from all the four species is given in Table 65.

Table 65. Composition of the essential oil from four *Curcuma* species

Compound	RI	*C. amada*	*C. aromatica*	*C. caesia*	*C. xanthorrhiza*
α-Pinene	938	1.99	1.14	0.71	0.35
Camphene	953	-	4.60	2.57	0.77
β-Pinene	975	10.94	0.64	1.96	0.12
Beta Myrcene	991	64.44	1.03	0.44	0.21
D-Limonene	1030	0.52	1.99	0.57	0.22
Eucalyptol (1,8-Cineole)	1033	0.65	9.11	22.43	0.17
Beta Ocimene	1046	3.13	-	-	-
2-nonanol	1097	-	-	0.70	-
Perillene	1102	3.61	-	-	-
Camphor	1145	-	27.82	8.95	8.63
Camphene hydrate	1150	-	-	-	0.46
Isoborneol	1155	-	-	1.84	0.40
Borneol	1165	-	8.63	3.27	0.28
α-terpineol	1191	-	1.62	1.36	0.15
Isobornyl Acetate	1283	-	-	2.48	-
β-elemene	1394	-	2.05	2.75	0.32
Alpha Cedrene	1412	-	-	-	23.69
Trans β-caryophyllene	1417	1.89	-	-	-
β-caryophyllene	1419	-	-	0.51	-
Beta Farnesene	1464	2.72	-	-	2.55
Germacrene D	1481	-	2.73	1.02	-
Ar-Curcumene	1483	-	-	-	18.69
Zingiberene	1495	-	-	-	0.71
Curzerene	1499	-	3.12	3.90	2.61
Germacrene B	1557	-	-	0.71	2.11
EpiCurzerenone	1605	-	-	22.88	-
Isocurcumenol	1615	-	-	3.05	-
Germacrone	1694	-	3.59	2.77	3.82
Curdione	1698	-	18.26	-	-
Xanthorrhizol	1751	-	-	-	23.66
Total		89.88%	86.32%	84.87%	89.91%

4.2.3. Extraction of curcumin and quantification using spectrophotometer

Total curcumin extracted using the hydro-distillation method from four *Curcuma* species is given in the Table 66.

Among the four species, *C. xanthorrhiza* yielded maximum total curcumin (1.54%) followed by *C. amada* (0.06%), *C. aromatica* (0.05%) and minimum in *C. caesia* (0.03%). Analysis of variance showed that the curcumin content among the accessions varied significantly except in *C. caesia*. Among the species variation (92%) was higher than within the species variation (8%). In case of *C. amada*, Acc. 521 (0.102%) yielded maximum amount of total curcumin and minimum by Acc. 753 (0. 016%). In case of *C. amada,* 45% of the variation was accounted by variation present among the accessions and 55% within the accessions. But in *C. aromatica,* all the accessions recorded more or less similar values for total curcumin content, except Acc. 711 (0.075%) being the highest and the variation was mainly contributed by differences present among the accessions (83%) rather than within accessions as in *C. amada.* Acc.1163 of *C. xanthorrhiza* recorded maximum amount of total curcumin (2.03%) followed by Acc. 760 (1.84%) and Acc. 1167 (1.84%). Minimum amount of total curcumin was recorded in Acc. 1122 (0.96%). The percentage of variation was maximum (88%) among the accessions than within the accessions (12%) (Table 67).

Table 66. Percentage of curcumin in four *Curcuma* species

	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	Curcumin (%)	Accession	Curcumin (%)	Accession	Curcumin (%)	Accession	Curcumin (%)
1	265	0.043 ± 0.002^{abc}	711	0.075 ± 0.002^{d}	292	0.024 ± 0.002^{a}	465	1.48 ± 0.08^{c}
2	347	0.067 ± 0.002^{bcd}	1025	0.036 ± 0.003^{a}	751	0.025 ± 0.010^{a}	760	1.84 ± 0.03^{de}
3	521	0.102 ± 0.046^{d}	1113	0.037 ± 0.003^{ab}	1001	0.034 ± 0.017^{a}	1108	1.22 ± 0.17^{b}
4	752	0.045 ± 0.002^{abc}	1124	0.045 ± 0.003^{bc}	1006	0.039 ± 0.001^{a}	1122	0.96 ± 0.04^{a}
5	753	0.016 ± 0.001^{ab}	1132	0.046 ± 0.003^{c}	1135	0.026 ± 0.001^{a}	1163	2.03 ± 0.02^{e}
6	848	0.055 ± 0.001^{abcd}	1518	0.048 ± 0.001^{c}	1154	0.024 ± 0.000^{a}	1164	1.75 ± 0.07^{d}
7	1119	0.047 ± 0.000^{abc}	1520	0.037 ± 0.002^{ab}	1171	0.031 ± 0.004^{a}	1167	1.84 ± 0.06^{de}
8	1511	0.098 ± 0.001^{d}	-	-	-	-	1168	1.17 ± 0.04^{ab}
9	1503	0.072 ± 0.000^{cd}	-	-	-	-	-	-
10	6390	0.023 ± 0.001^{ab}	-	-	-	-	-	-
Mean ± SE		0.057 ± 0.006^{a}		0.05 ± 0.003^{a}		0.03 ± 0.003^{a}		1.54 ± 0.079^{b}
Accessions		P< 0.05		P< 0.05		NS		P< 0.05
Species		P< 0.05						

NS = Not Significant (P>0.05), Values with the different superscript are significantly different (P<0.05), SE-Standard error

Table 67. F-value and percentage of variation for curcumin in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	0.012	2.073	0.418	0.069	354.178**	92%	8%
C. amada	0.012	0.164	0.057	0.006	3.762**	45%	55%
C. aromatica	0.032	0.080	0.046	0.003	17.889**	83%	17%
C. caesia	0.015	0.067	0.029	0.003	0.744	24%	76%
C. xanthorrhiza	0.875	2.073	1.535	0.079	25.167**	88%	12%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.2.3.1. Separation and quantification of curcuminoids using HPLC

The amount of curcuminoids in four *Curcuma* species and their accessions was estimated. (Tables 68 – 71). As species like *C. amada, C. caesia, C. aromatica* are low curcumin yielding species, the total curcuminoids values were very low in these species. In all the three species, Curcumin 1 was the major curcuminoids, followed by Demethoxycurcumin (DMC) and Bisdemethoxycurcumin (BDMC). Significant variation was observed among the four species (86%) for total curcuminoids, whereas within the species, only the accessions of *C. xanthorrhiza* showed significant variation. Ninety-nine percentage of the variation was accounted by the variation present among the accessions of *C. xanthorrhiza* (Table 72).

In *C. xanthorrhiza,* the mean value of total curcuminoids was 0.131% (Table 72), highest being in the Acc. 1163 (0.213%) followed by Acc. 1164 (0.175%) and Acc. 760 (0.161%) in that order (Table 71). In all the accessions studied, Curcumin1 was the major curcuminoids followed by DMC and BDMC.

Variance analysis revealed that major source of variation (86%) was contributed by among the species. However, in case of *C. xanthorrhiza,* 99% of the total variation for this trait was contributed by among the accessions source, remaining only 1% of variation accounted by within the accessions source (Table 72). Figures 24 and 25 show the curcuminoids profile of all the thirty-two accessions under study.

Table 68. Percentage of curcumin1, demethoxycurcumin, bisdemethoxycurcumin and total curcuminoids in the accessions of *C. amada*

Sl. No	Accession	Bisdemethoxycurcumin ($x10^{-2}$ %)	Demethoxycurcumin ($x10^{-2}$ %)	Curcumin1 ($x10^{-2}$ %)	Total curcuminoids ($x10^{-2}$ %)
1	265	0.016 ± 0.003[a]	0.076 ± 0.009 [a]	0.255 ± 0.028 [a]	0.347 ± 0.039 [a]
2	347	0.019 ± 0.001 [a]	0.076 ± 0.003 [a]	0.248 ± 0.012 [a]	0.344 ± 0.016 [a]
3	521	0.020 ± 0.002 [a]	0.087 ± 0.008 [a]	0.296 ± 0.027 [a]	0.403 ± 0.037 [a]
4	752	0.016 ± 0.003 [a]	0.070 ± 0.009 [a]	0.245 ± 0.032 [a]	0.330 ± 0.043 [a]
5	753	0.020 ± 0.003 [a]	0.087 ± 0.011 [a]	0.304 ± 0.038 [a]	0.410 ± 0.052 [a]
6	848	0.016 ± 0.002 [a]	0.075 ± 0.005 [a]	0.252 ± 0.015 [a]	0.343 ± 0.022 [a]
7	1119	0.023 ± 0.009 [ab]	0.147 ± 0.087 [a]	0.422 ± 0.217 [a]	0.592 ± 0.313 [a]
8	1503	0.016 ± 0.002 [a]	0.077 ± 0.005 [a]	0.261 ± 0.015 [a]	0.354 ± 0.022 [a]
9	1511	0.026 ± 0.009 [ab]	0.160 ± 0.089 [a]	0.436 ± 0.203 [a]	0.622 ± 0.302 [a]
10	6390	0.033 ± 0.001 [b]	0.162 ± 0.025 [a]	0.519 ± 0.042 [a]	0.714 ± 0.069 [a]
		P<0.05	NS	NS	NS

NS = Not Significant (P>0.05), Values with the different superscript are significantly different (P<0.05)

Table 69. Percentage of curcumin 1, demethoxycurcumin, bisdemethoxycurcumin and total curcuminoids in the accessions of *C. aromatica.*

Sl. No	Accession	Bisdemethoxycurcumin ($x10^{-2}$ %)	Demethoxycurcumin ($x10^{-2}$ %)	Curcumin1 ($x10^{-2}$ %)	Total curcuminoids ($x10^{-2}$ %)
1	711	0.003 ± 0.001[a]	0.030 ± 0.009 [a]	0.087 ± 0.022 [a]	0.121 ± 0.030 [a]
2	1025	0.005 ± 0.002 [a]	0.032 ± 0.019 [a]	0.067 ± 0.046 [a]	0.104 ± 0.067 [a]
3	1113	0.003 ± 0.001 [a]	0.021 ± 0.009 [a]	0.052 ± 0.020 [a]	0.077 ± 0.030 [a]
4	1124	0.004 ± 0.002 [a]	0.033 ± 0.025 [a]	0.070 ± 0.052 [a]	0.107 ± 0.079 [a]
5	1132	0.003 ± 0.001 [a]	0.032 ± 0.015 [a]	0.072 ± 0.033 [a]	0.106 ± 0.049 [a]
6	1518	0.004 ± 0.001 [a]	0.026 ± 0.005 [a]	0.084 ± 0.011 [a]	0.115 ± 0.014 [a]
7	1520	0.006 ± 0.001 [a]	0.035 ± 0.009 [a]	0.075 ± 0.019 [a]	0.115 ± 0.030 [a]
		NS	NS	NS	NS

NS = Not Significant (P>0.05), Values with the different superscript are significantly different (P<0.05)

Table 70. Percentage of curcumin1, demethoxycurcumin, bisdemethoxycurcumin and total curcuminoids in the accessions of *C. caesia.*

Sl. No	Accession	Bisdemethoxycurcumin ($x10^{-2}$ %)	Demethoxycurcumin ($x10^{-2}$ %)	Curcumin1 ($x10^{-2}$ %)	Total curcuminoids ($x10^{-2}$ %)
1	292	0.008 ± 0.002 [a]	0.053 ± 0.014 [a]	0.150 ± 0.045 [a]	0.211 ± 0.060 [a]
2	751	0.006 ± 0.004 [a]	0.051 ± 0.032 [a]	0.126 ± 0.079 [a]	0.182 ± 0.114 [a]
3	1001	0.005 ± 0.003 [a]	0.040 ± 0.020 [a]	0.116 ± 0.050 [a]	0.161 ± 0.072 [a]
4	1006	0.005 ± 0.003 [a]	0.042 ± 0.022 [a]	0.113 ± 0.053 [a]	0.161 ± 0.077 [a]
5	1135	0.005 ± 0.002 [a]	0.033 ± 0.014 [a]	0.095 ± 0.040 [a]	0.133 ± 0.055 [a]
6	1154	0.009 ± 0.004 [a]	0.070 ± 0.037 [a]	0.163 ± 0.087 [a]	0.243 ± 0.128 [a]
7	1171	0.006 ± 0.004 [a]	0.051 ± 0.036 [a]	0.122 ± 0.087 [a]	0.179 ± 0.127 [a]
		NS	NS	NS	NS

Table 71. Percentage of curcumin 1, demethoxycurcumin, bisdemethoxycurcumin and total curcuminoids in the accessions of *C. xanthorrhiza*

Sl. No	Accession	Bisdemethoxycurcumin ($x10^{-2}$ %)	Demethoxycurcumin ($x10^{-2}$ %)	Curcumin1 ($x10^{-2}$ %)	Total curcuminoids ($x10^{-2}$ %)
1	465	0.203 ± 0.017 [a]	2.697 ± 0.135 [b]	8.307 ± 0.300 [d]	11.203 ± 0.452 [c]
2	760	0.480 ± 0.007 [d]	4.943 ± 0.035 [d]	10.690 ± 0.067 [e]	16.115 ± 0.091 [d]
3	1108	0.237 ± 0.003 [b]	2.810 ± 0.045 [b]	6.867 ± 0.203 [b]	9.910 ± 0.245 [b]
4	1122	0.237 ± 0.007 [b]	2.753 ± 0.028 [b]	5.740 ± 0.040 [a]	8.723 ± 0.074 [a]
5	1163	0.603 ± 0.003 [e]	6.403 ± 0.034 [f]	14.323 ± 0.044 [f]	21.333 ± 0.074 [f]
6	1164	0.577 ± 0.009 [e]	5.453 ± 0.084 [e]	11.463 ± 0.197 [e]	17.493 ± 0.289 [e]
7	1167	0.180 ± 0.000 [a]	2.380 ± 0.000 [a]	6.460 ± 0.010 [b]	9.020 ± 0.010 [a]
8	1168	0.310 ± 0.010 [c]	3.400 ± 0.076 [c]	7.397 ± 0.142 [c]	11.103 ± 0.224 [c]
		P< 0.05	P< 0.05	P< 0.05	P< 0.05

Values with the different superscript are significantly different (P<0.05)

Table 72. F-value and percentage of variation for total curcuminoids in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	0.0001	0.200	0.071	0.000	200.845**	86%	14%
C. amada	0.0002	0.003	0.004	0.000	1.621	16%	84%
C. aromatica	0.0001	0.001	0.002	0.000	0.773	25%	75%
C. caesia	0.0001	0.001	0.002	0.000	0.256	10%	90%
C. xanthorrhiza	0.0100	0.200	0.131	0.000	404.074**	99%	1%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

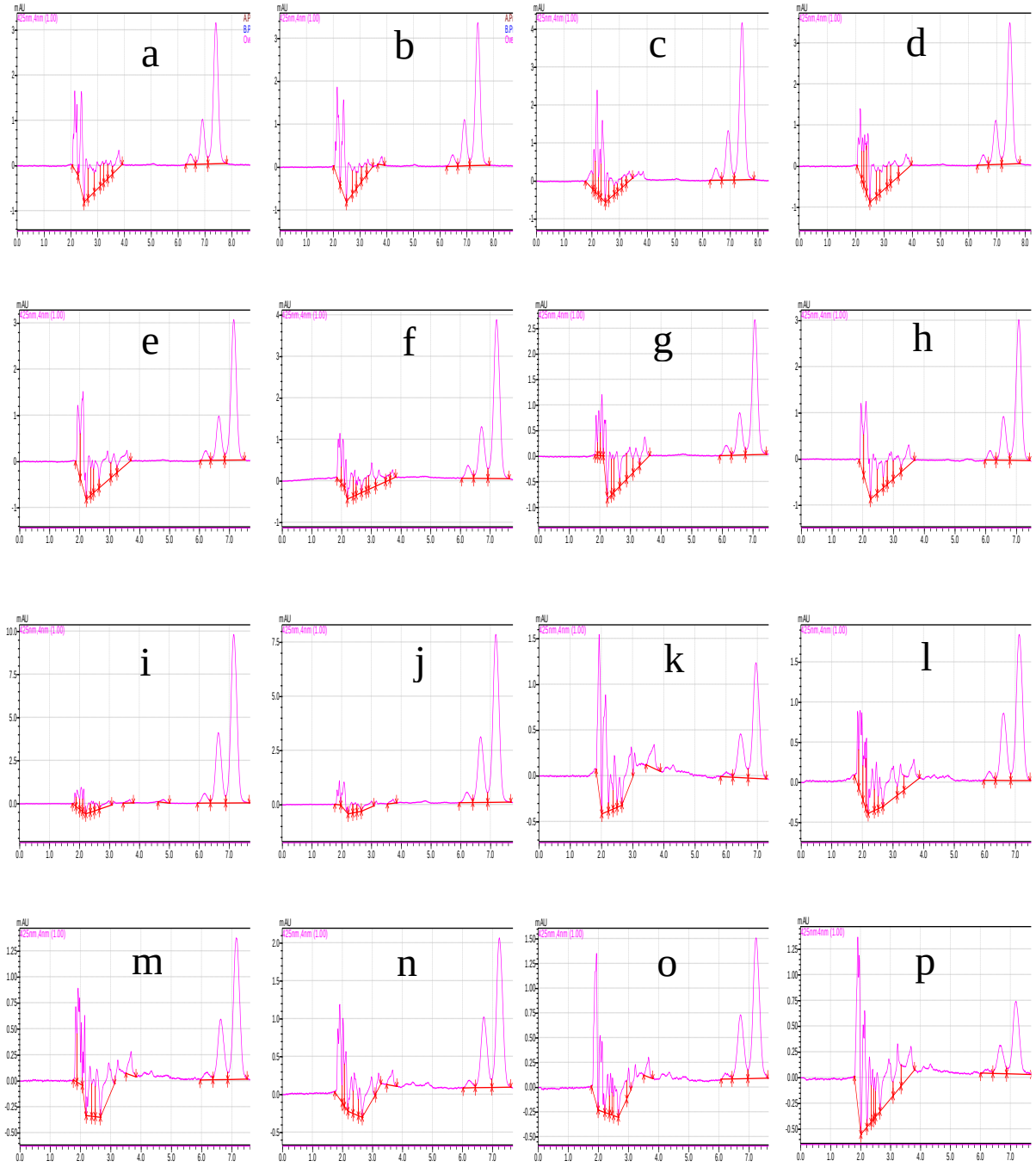


Fig. 24. Total curcuminoids profiles of *C. amada* (a – j) and *C. aromatica* (k – p) a – p is Acc. 265, Acc. 347, Acc. 521, Acc. 752, Acc. 753, Acc. 848, Acc. 1119, Acc. 1503, Acc. 1511, Acc. 6390, Acc. 711, Acc. 1025, Acc. 1113, Acc. 1124, Acc. 1132 and Acc. 1518.

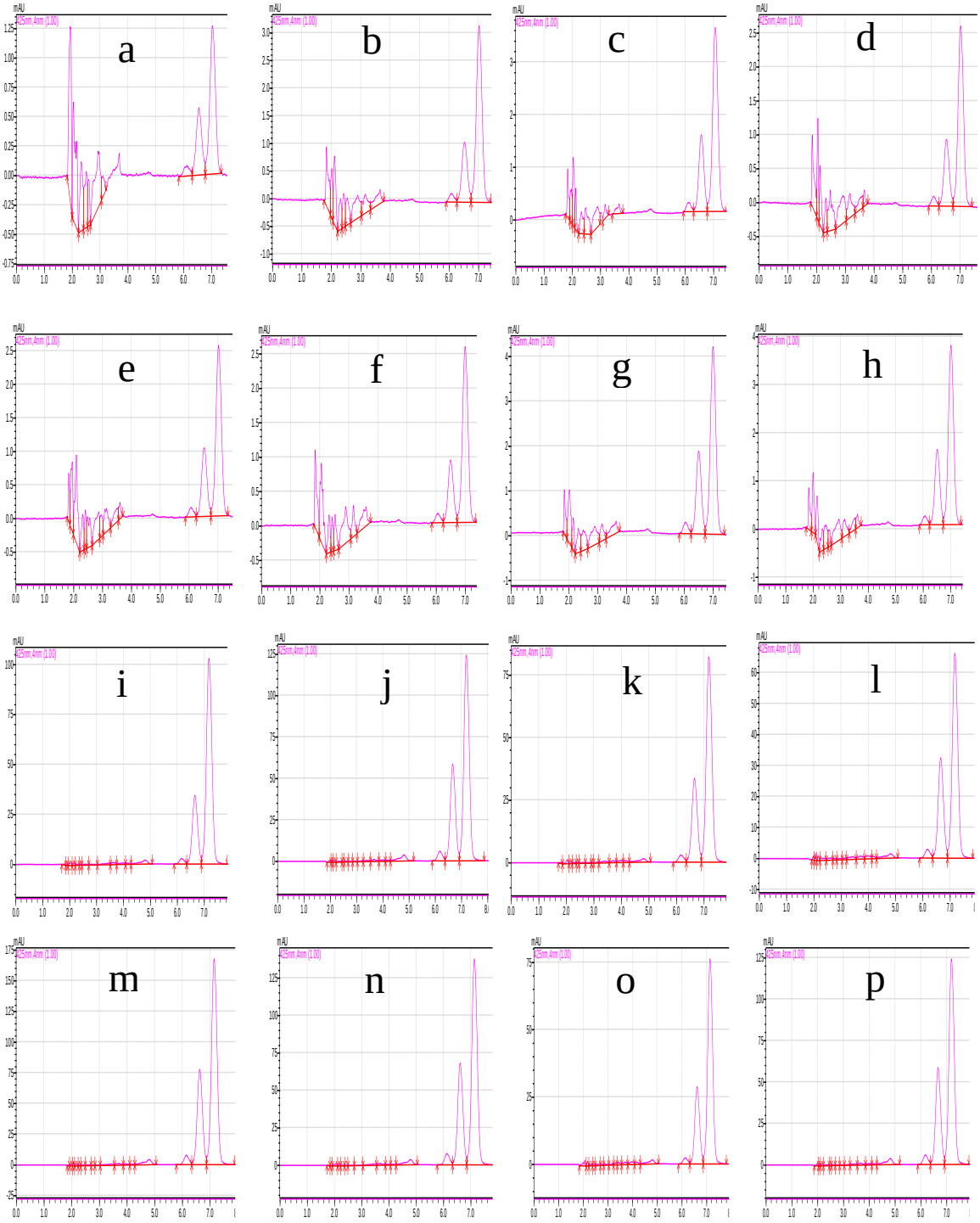


Fig. 25. Total curcuminoids profiles of *C. aromatica* (a), *C. caesia* (b-h), *C. xanthorrhiza* (i-p) a – p is Acc. 1520, Acc. 292, Acc. 751, Acc. 1001, Acc. 1006, Acc. 1135, Acc. 1154, Acc. 1171, Acc. 465, Acc. 760, Acc. 1108, Acc. 1122, Acc. 1163, Acc. 1164, Acc. 1167 and Acc. 1168.

4.2.4. Protein estimation by Lowry's method (1951)

Total protein was estimated from the accessions of four *Curcuma* species using Lowry's method (Table 73). No significant variation was observed among the species for protein content whereas among the accessions there existed significant variation for the trait. Among the accession source contributed maximum variation in all the species (76%-*C. xanthorrhiza* , 74%-*C. amada*, 73%-*C. caesia* and 68%-*C. aromatica*) (Table 74).

In *C. amada*, the protein content ranged from 7.91% (Acc. 1119) to 10.90% (Acc. 347), with an average value of 9.34%. In case of *C. aromatica*, Acc. 1520 accounted for the highest amount of total protein (10.47%) followed by Acc. 1025 (9.50%), while lowest protein content was recorded in Acc. 1124 (6.83%) with an average protein content of 8.85%. Protein content in the accessions of *C. caesia* ranged from 8.50% (Acc.1006) to 12.47% (Acc.1171) with an average value of 9.75%. In *C. xanthorrhiza*, Acc.1122 showed maximum amount of protein content (11.08%) and minimum in Acc.1167 (7.21%) accounting for an average protein content of 8.90%.

Table 73. Mean protein content in four *Curcuma* species.

	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	Protein (%)	Accession	Protein (%)	Accession	Protein (%)	Accession	Protein (%)
1	265	7.97 ± 0.29^{a}	711	9.36 ± 0.60^{cd}	292	9.26 ± 0.20^{ab}	465	10.30 ± 0.26^{d}
2	347	10.90 ± 0.06^{d}	1025	9.50 ± 0.61^{cd}	751	8.53 ± 0.33^{a}	760	9.84 ± 0.36^{cd}
3	521	9.21 ± 0.31^{b}	1113	7.87 ± 0.20^{ab}	1001	9.47 ± 0.48^{ab}	1108	8.43 ± 0.49^{ab}
4	752	9.16 ± 0.23^{b}	1124	6.83 ± 0.20^{a}	1006	8.50 ± 0.38^{a}	1122	11.08 ± 0.60^{d}
5	753	9.12 ± 0.33^{b}	1132	9.11 ± 0.36^{bc}	1135	9.48 ± 0.56^{ab}	1163	8.73 ± 0.46^{bc}
6	848	10.17 ± 0.20^{cd}	1518	8.83 ± 0.19^{bc}	1154	10.53 ± 0.41^{b}	1164	8.03 ± 0.45^{ab}
7	1119	7.91 ± 0.17^{a}	1520	10.47 ± 0.41^{d}	1171	12.47 ± 0.54^{c}	1167	7.21 ± 0.18^{a}
8	1511	10.39 ± 0.43^{d}	-	-	-	-	1168	7.58 ± 0.20^{ab}
9	1503	9.13 ± 0.20^{b}	-	-	-	-	-	-
10	6390	9.44 ± 0.48^{bc}	-	-	-	-	-	-
Mean ± SE		9.34 ± 0.19^{ab}		8.85 ± 0.28^{a}		9.75 ± 0.32^{b}		8.90 ± 0.29^{a}
Accessions		P< 0.05		P< 0.05		P< 0.05		P< 0.05
Species		NS						

NS = Not Significant (P>0.05), values with the different superscript are significantly different (P<0.05), SE-Standard error

Table 74. F-value and percentage of variation of protein content in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	6.50	13.41	9.21	0.13	2.34	4%	96%
C. amada	7.50	11.20	9.34	0.19	10.58**	74%	26%
C. aromatica	6.50	11.20	8.85	0.28	8.60**	68%	32%
C. caesia	7.90	13.41	9.75	0.32	10.29**	73%	27%
C. xanthorrhiza	6.98	12.03	8.90	0.29	11.68**	76%	24%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.2.5. Total starch

Starch yield of different species is presented in Table 75. Significant variation was observed for total starch content among and within the four species. The percentage of variation obtained from the mean square values showed that variation was mainly contributed by the differences present within the species (86%) than among the species (14%).Variance analysis revealed that majority of the variation was contributed by variations existing among the accessions in *C. caesia* (90%), *C. xanthorrhiza* (90%), *C. aromatica* (87%) *and C. amada* (79%) (Table 76).

Starch content of the four *Curcuma* species ranged from 43.82% to 48.23%. Highest amount of starch was recorded in *C. amada* and lowest in *C. aromatica*. Among the accessions of *C. amada*, maximum starch content was recorded in Acc. 1511 (51.30%) and minimum in Acc. 521 (40.34%) with an average value of 48.23%. In case of *C. aromatica*, Acc. 1113, yielded highest amount of starch (52.37%) followed by Acc. 711 (46.62%), lowest percentage of starch was recorded in Acc. 1124 (35.28%), with a mean starch value of 43.82%.

In case of *C. caesia*, the percentage of starch ranged from 39.61% (Acc. 1171) to 51.65% (Acc. 1006), with an average value of 44.28%. Percentage of starch ranged from 41.43% (Acc.1108) to 51.67% (Acc.760) among the accessions of *C. xanthorrhiza*. *C. xanthorrhiza* displayed a mean starch content of 45.09%.

Table 75. Mean starch content in four *Curcuma* species.

	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	Starch (%)	Accession	Starch (%)	Accession	Starch (%)	Accession	Starch (%)
1	265	49.10 ± 0.86^{bc}	711	46.62 ± 0.64^{c}	292	45.75 ± 0.89^{b}	465	42.59 ± 0.76^{a}
2	347	46.98 ± 0.73^{b}	1025	41.57 ± 0.71^{b}	751	41.39 ± 0.69^{a}	760	51.67 ± 0.34^{c}
3	521	40.34 ± 0.46^{a}	1113	52.37 ± 0.57^{d}	1001	42.01 ± 0.25^{a}	1108	41.43 ± 0.83^{a}
4	752	49.33 ± 0.62^{bc}	1124	35.28 ± 1.91^{a}	1006	51.65 ± 0.55^{c}	1122	49.91 ± 0.05^{c}
5	753	48.78 ± 0.59^{bc}	1132	44.55 ± 0.96^{bc}	1135	47.67 ± 1.17^{b}	1163	45.89 ± 0.69^{b}
6	848	49.53 ± 1.09^{bc}	1518	42.50 ± 1.07^{b}	1154	41.91 ± 0.36^{a}	1164	45.52 ± 1.02^{b}
7	1119	50.55 ± 0.48^{c}	1520	43.84 ± 0.98^{bc}	1171	39.61 ± 0.80^{a}	1167	42.04 ± 0.54^{a}
8	1511	51.30 ± 0.81^{c}	-	-	-	-	1168	41.63 ± 0.89^{a}
9	1503	48.81 ± 1.36^{bc}	-	-	-	-	-	-
10	6390	47.61 ± 0.89^{b}	-	-	-	-	-	-
Mean ± SE		48.23 ± 0.58^{b}		43.82 ± 1.12^{a}		44.28 ± 0.91^{a}		45.09 ± 0.79^{a}
Accessions		P< 0.05		P< 0.05		P< 0.05		P< 0.05
Species		P< 0.05						

Values with the different superscript are significantly different (P<0.05), SE-Standard error

Table 76. F-value and percentage of variation of starch content in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	32.01	53.31	45.62	0.45	6.40**	14%	86%
C. amada	39.64	52.68	48.23	0.58	13.32**	79%	21%
C. aromatica	32.01	53.31	43.82	1.13	23.85**	87%	13%
C. caesia	38.69	52.74	44.28	0.91	33.65**	90%	10%
C. xanthorrhiza	39.98	52.34	45.09	0.80	31.02**	90%	10%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.2.6. Total phenol

Total phenol content (TPC) of the four *Curcuma* species in the dried rhizome samples ranged from 2.25 mg/g (*C. amada*) to 6.01 mg/g (*C. xanthorrhiza*) (Table 77). Significant variation was observed for total phenol content among the four species. Variance analysis revealed that among the species variation was more (73%) than within the species source (27%). Within the accessions of the four species, the percentage of variation was significant in all the species except in *C. xanthorrhiza*. The percentage of variation among the accessions of *C. amada*, *C. caesia* and *C. aromatica* were 49%, 39% and 36%, respectively. In all the three species the percentage of variation within the accessions (*C. aromatica*-64%, *C. caesia*-61% and *C. amada*-51%, respectively) was higher than among the accessions (Table 78).

The total phenol content in the accessions of *C. amada* ranged from 1.43 mg/g (Acc. 1119) to 3.36 mg/g (Acc. 6390) with an average value of 2.25 mg/g. Accessions of *C. aromatica* yielded an average total phenol content of 4.94 mg/g. Acc. 711 displayed maximum amount of total phenol content (6.63 mg/g) and minimum being in the Acc. 1124 (3.05 mg/g). In *C. caesia*, mean value of total phenol content was observed to be 3.22 mg/g, highest amount being present in the Acc. 292 (3.99 mg/g) followed by Acc. 1001 (3.89 mg/g) and Acc. 751 (3.36 mg/g). Lowest TPC was observed to be present in Acc. 1006 (2.20 mg/g).

Table 77. Mean total phenol content in four *Curcuma* species

	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	Phenol (mg/g)	Accession	Phenol (mg/g)	Accession	Phenol (mg/g)	Accession	Phenol (mg/g)
1	265	2.70 ± 0.29^{bc}	711	6.63 ± 0.37^{c}	292	3.99 ± 0.55^{c}	465	5.98 ± 0.30^{a}
2	347	2.69 ± 0.36^{bc}	1025	4.86 ± 0.36^{abc}	751	3.36 ± 0.30^{bc}	760	5.94 ± 0.29^{a}
3	521	2.59 ± 0.32^{bc}	1113	5.36 ± 0.29^{bc}	1001	3.89 ± 0.26^{c}	1108	5.87 ± 0.30^{a}
4	752	1.50 ± 0.39^{a}	1124	3.05 ± 1.13^{a}	1006	2.20 ± 0.22^{a}	1122	6.11 ± 0.29^{a}
5	753	1.89 ± 0.29^{ab}	1132	5.40 ± 0.27^{bc}	1135	3.08 ± 0.36^{abc}	1163	6.13 ± 0.29^{a}
6	848	2.59 ± 0.33^{bc}	1518	5.17 ± 0.42^{abc}	1154	3.31 ± 0.22^{abc}	1164	5.78 ± 0.02^{a}
7	1119	1.43 ± 0.29^{a}	1520	4.08 ± 1.05^{ab}	1171	2.72 ± 0.41^{ab}	1167	6.18 ± 0.30^{a}
8	1511	1.44 ± 0.31^{a}	-	-	-	-	1168	6.13 ± 0.33^{a}
9	1503	2.35 ± 0.32^{abc}	-	-	-	-	-	-
10	6390	3.36 ± 0.32^{c}	-	-	-	-	-	-
Mean ± SE		2.25 ± 0.14^{a}		4.94 ± 0.31^{c}		3.22 ± 0.17^{b}		6.01 ± 0.09^{d}
Accessions		P< 0.05		P< 0.05		P< 0.05		NS
Species		P< 0.05						

NS = Not Significant (P>0.05), values with the different superscript are significantly different (P<0.05), SE-Standard error

Table 78. F-value and percentage of variation of phenol content in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	0.74	7.02	3.99	0.18	89.63**	73%	27%
C. amada	0.74	3.97	2.25	0.14	4.16**	49%	51%
C. aromatic	1.44	7.02	4.94	0.31	2.98*	36%	64%
C. caesia	1.78	4.93	3.22	0.17	3.26*	39%	61%
C. xanthorrhiza	5.42	6.79	6.01	0.09	0.27	10%	90%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.2.7. Total carbohydrates

C. amada showed highest amount of total carbohydrates (67.30%) followed by *C. xanthorrhiza* (63.93%), *C. caesia* (58.79%) and *C. aromatica* (58.78%). The total carbohydrate content of the four *Curcuma* species is given in Table 79.

Significant variation was observed for total carbohydrates among the four species. Variance analysis revealed that among the species, variation was slightly more (52%) than within the species source (48%). Within the accessions of the four species, the percentage of variation was significant in all the species except in *C. xanthorrhiza*. The percentage of variation among the accessions of *C. amada*, *C. aromatica* and *C. caesia* was 95%, 94% and 82%, respectively (Table 80).

In *C. amada*, Acc.1511 (74.70%) recorded maximum amount of total carbohydrates followed by Acc. 521 (73.52%), Acc.1503 (71.96%) and Acc. 753 (61.00%). In *C. aromatica*, total carbohydrates among the accessions ranged from 56.24% (Acc. 1518) to 61.36% (Acc.1113) with an average value of 58.78%. Total carbohydrates in *C. caesia* ranged from 55.20% (Acc.1135) to 62.17% (Acc. 1171), with a mean value of 58.79%.

Table 79. Mean total carbohydrates content in four *Curcuma* species

	Species							
	C. amada		*C. aromatica*		*C. caesia*		*C. xanthorrhiza*	
	Accession	Carbohydrates (%)	Accession	Carbohydrates (%)	Accession	Carbohydrates (%)	Accession	Carbohydrates (%)
1	265	66.24 ± 0.79^{de}	711	57.90 ± 0.16^{b}	292	61.79 ± 0.58^{d}	465	65.63 ± 0.13^{e}
2	347	66.81 ± 0.07^{e}	1025	59.61 ± 0.15^{c}	751	59.58 ± 0.28^{c}	760	68.00 ± 0.47^{g}
3	521	73.52 ± 0.35^{gh}	1113	61.36 ± 0.37^{d}	1001	57.64 ± 0.37^{bc}	1108	66.02 ± 0.17^{e}
4	752	63.39 ± 1.00^{bc}	1124	56.95 ± 0.27^{a}	1006	55.92 ± 0.25^{ab}	1122	63.95 ± 0.36^{d}
5	753	61.00 ± 0.23^{a}	1132	58.48 ± 0.09^{b}	1135	55.20 ± 1.44^{a}	1163	59.87 ± 0.12^{b}
6	848	61.80 ± 0.55^{ab}	1518	56.24 ± 0.37^{a}	1154	59.27 ± 0.36^{c}	1164	62.81 ± 0.26^{c}
7	1119	68.78 ± 0.50^{f}	1520	60.89 ± 0.30^{d}	1171	62.17 ± 0.50^{d}	1167	58.17 ± 0.12^{a}
8	1511	74.70 ± 0.50^{h}	-	-	-	-	1168	66.97 ± 0.53^{f}
9	1503	71.96 ± 0.37^{g}	-	-	-	-	-	-
10	6390	64.81 ± 0.97^{cd}	-	-	-	-	-	-
Mean ± SE		67.30 ± 0.87^{c}		58.78 ± 0.41^{a}		58.79 ± 0.60^{a}		63.93 ± 0.68^{b}
Accessions		P< 0.05		P< 0.05		P< 0.05		NS
Species		P< 0.05						

NS = Not Significant (P>0.05), Values with the different superscript are significantly different (P<0.05), SE-Standard error

Table 80. F-value and percentage of variation of carbohydrate content in four *Curcuma* species

Description	Minimum	Maximum	Mean	Std Err	F (28,92)	Among	Within
Species	52.35	75.71	62.73	0.52	35.72**	52%	48%
C. amada	60.76	75.71	67.30	0.87	63.54**	95%	5%
C. aromatica	55.77	62.01	58.78	0.41	53.11**	94%	6%
C. caesia	52.35	62.94	58.80	0.60	16.69**	82%	18%
C. xanthorrhiza	57.94	68.74	63.93	0.68	125.61	97%	3%

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

4.2.8. Variability, heritability and genetic advance

Phenotypic and genotypic coefficients of variation (PCV and GCV), heritability and genetic advance were calculated for those characters which showed significant variation.

In case of *C. amada*, all the characters except total curcuminoids showed significant variation. Estimates of PCV were higher than GCV for majority of the traits although for some traits like oleoresin, total carbohydrates and essential oil, the values were at par. The genotypic coefficients of variation ranged from 6% (total starch) to 43% (curcumin) whereas the phenotypic coefficients of variation ranged from 7% (total starch and total carbohydrates) to 62% (curcumin). The GCV and PCV estimates of total phenol content (25%,36%), oleoresin (35% each), curcumin (43%,62%) and essential oil (27% each) were higher compared to total protein (10%,11%), total starch (6%,7%) and total carbohydrates (7% each). All the characters showed high heritability which ranged from 48 (curcumin) to 97% (oleoresin and essential oil). Very high estimates of heritability were observed for the other traits like starch (80%), oleoresin (97%), total carbohydrates (95%) and essential oil (97%). High heritability indicates that selection will be effective and characters are least influenced by environment (Table 81).

Table 81. Estimates of genetic parameters for biochemical characters in *C. amada*

Character	Mean	Phenotypic Variance	Genotypic Variance	GCV %	PCV %	h^2 (%)	GA	GA as % of Mean	F
Total Protein (%)	9.34	1.10	0.84	10	11	76	1.64	18	10.58**
Total Starch (%)	48.23	10.65	8.56	6	7	80	5.40	11	13.32**
Total Phenol (mg/g)	2.25	0.64	0.33	25	36	51	0.85	38	4.16**
Oleoresin (%)	7.76	7.56	7.37	35	35	97	5.52	71	117.94**
Total Carbohydrate (%)	67.30	24.09	22.98	7	7	95	9.65	14	63.54**
Curcumin (%)	0.06	0.00	0.00	43	62	48	0.03	62	3.76**
Essential oil (%)	2.41	0.44	0.42	27	27	97	1.32	55	90.07**

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

GCV- Genotypic coefficients of variation, PCV- Phenotypic coefficients of variation, h^2 -heritability, GA-Genetic advance.

Genetic advance as percentage of mean (GAM) ranged from 11% (total starch) to 71% (oleoresin). Among the traits oleoresin (71%), curcumin (62%), essential oil (55%) and total phenol (38%) exhibited higher percentage of genetic advance whereas rest of the traits showed a moderately high values for GAM.

Estimates of phenotypic coefficients of variation (PCV), genotypic coefficients of variation (GCV), heritability and genetic advance of *C. aromatica* are given in the Table 82. The genotypic coefficients of variance (GCV) ranged from 3% (total carbohydrates) to 29% (curcumin). Similarly, phenotypic coefficients of variation ranged from 3% (total carbohydrates) to 30% (total phenol content and curcumin).

Table 82. Estimates of genetic parameters for biochemical characters in *C. aromatica*

Character	Mean	Phenotypic Variance	Genotypic Variance	GCV %	PCV %	h^2(%)	GA	GA as % of Mean	F
Protein (%)	8.85	1.73	1.24	13	15	72	1.94	22	8.60**
Starch (%)	43.82	29.14	25.76	12	12	88	9.83	22	23.85**
Total phenol content(mg/g)	4.94	2.12	0.84	19	30	40	1.19	24	2.98*
Oleoresin (%)	10.68	1.48	1.28	11	11	87	2.17	20	20.75**
Total Carbohydrates (%)	58.78	3.88	3.67	3	3	95	3.84	7	53.11**
Curcumin (%)	0.05	0.00	0.00	29	30	91	0.03	57	30.25**
Essential oil (%)	5.03	0.49	0.47	14	14	94	1.37	27	52.41**

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance

GCV- Genotypic coefficients of variation, PCV- Phenotypic coefficients of variation, h^2 -heritability, GA-Genetic advance.

Heritability ranged from 40% (total phenol content) to 95% (total carbohydrates). Characters like oleoresin (87%), total starch (88%), curcumin (91%), essential oil (94%) and total carbohydrates (95%) recorded very high values for heritability. Moderately high heritability was recorded for total protein (72%). Genetic advance as percentage of mean (GAM) ranged from 7% (total carbohydrates) to 57% (curcumin). All the traits except total carbohydrates (7%) exhibited higher percentage for genetic advance.

In case of *C. caesia*, the highest PCV and GCV estimates were observed for total phenol content (16%, 25%) and lowest GCV and PCV were observed for total carbohydrates (4%, 5%) (Table 83).

Table 83. Estimates of genetic parameters for biochemical characters in *C. caesia*

Character	Mean	Phenotypic Variance	Genotypic Variance	GCV %	PCV %	h^2(%)	GA	GA as % of Mean	F
Total protein (%)	9.75	2.29	1.73	13	16	76	2.35	24	10.29**
Total starch (%)	44.28	19.23	17.61	9	10	92	8.27	19	33.65**
Total phenol content (mg/g)	3.22	0.64	0.28	16	25	43	0.71	22	3.26*
Oleoresin (%)	6.92	0.33	0.18	6	8	56	0.66	10	4.86**
Total carbohydrates (%)	58.79	8.16	6.85	4	5	84	4.94	8	16.69**
Essential oil (%)	2.83	0.13	0.11	12	13	87	0.64	23	21.41**

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance.

GCV- Genotypic coefficients of variation, PCV- Phenotypic coefficients of variation, h^2 -heritability, GA-Genetic advance.

The difference between the PCV and GCV estimates was very narrow in *C. amada* and *C. aromatica*, indicating very little role of environment for the expression of biochemical traits. All the characters exhibited high heritability which ranged from 43% (total phenol content) to 92% (total starch). Genetic advance as percentage of mean (GAM) ranged from 8% (total carbohydrates) to 24% (total protein). High values for genetic advance indicates that the character is governed by additive genes.

In *C. xanthorrhiza*, the highest genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were observed for total curcuminoids (35% each) and the lowest was recorded for total carbohydrates (5% each). The GCV and PCV values for total curcuminoids (35% each), curcumin (25%, 26%) and essential oil (23%, 24%) were more or less equal suggesting environmental influence in the expression of these traits (Table 84).

Table 84. Estimates of genetic parameters for biochemical characters in *C. xanthorrhiza*

Character	Mean	Phenotypic Variance	Genotypic Variance	GCV %	PCV %	h^2(%)	GA	GA as % of Mean	F
Total protein (%)	8.90	2.21	1.72	15	17	78	2.39	27	11.68**
Total starch (%)	45.09	16.47	14.97	9	9	91	7.60	17	31.02**
Oleoresin (%)	9.29	2.40	2.31	16	17	96	3.07	33	79.93**
Carbohydrates (%)	63.93	12.19	11.90	5	5	98	7.02	11	125.61**
Curcumin (%)	1.53	0.16	0.14	25	26	89	0.74	48	25.17**
Essential oil (%)	3.23	0.58	0.55	23	24	95	1.49	46	52.78**
Curcuminoids (%)	0.13	2.15×10^{-3}	2.13×10^{-3}	35	35	99	0.09	72	404.07**

* All values are significant at 0.05 level of significance, ** All values are significant at 0.01 level of significance
GCV- Genotypic coefficients of variation, PCV- Phenotypic coefficients of variation, h^2 -heritability, GA-Genetic advance.

All the characters showed high heritability which ranged from 78% (total protein) to 99% (total curcuminoids). Genetic advance as percentage of mean (GAM) ranged from 11 -72%. Among the traits highest genetic advance was exhibited by total curcuminoids (72%) followed by curcumin (48%), essential oil (46%), oleoresin (33%) and total protein (27%) whereas total starch (17%) and total carbohydrates (11%) recorded moderately high values for GAM.

4.2.9. Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was carried out using the eight biochemical characters namely total protein, total starch, total phenol, oleoresin, total carbohydrate, curcumin, essential oil and total curcuminoids contents. The resulting 2D (2 dimensional) PCA graph is presented in Figure 26.

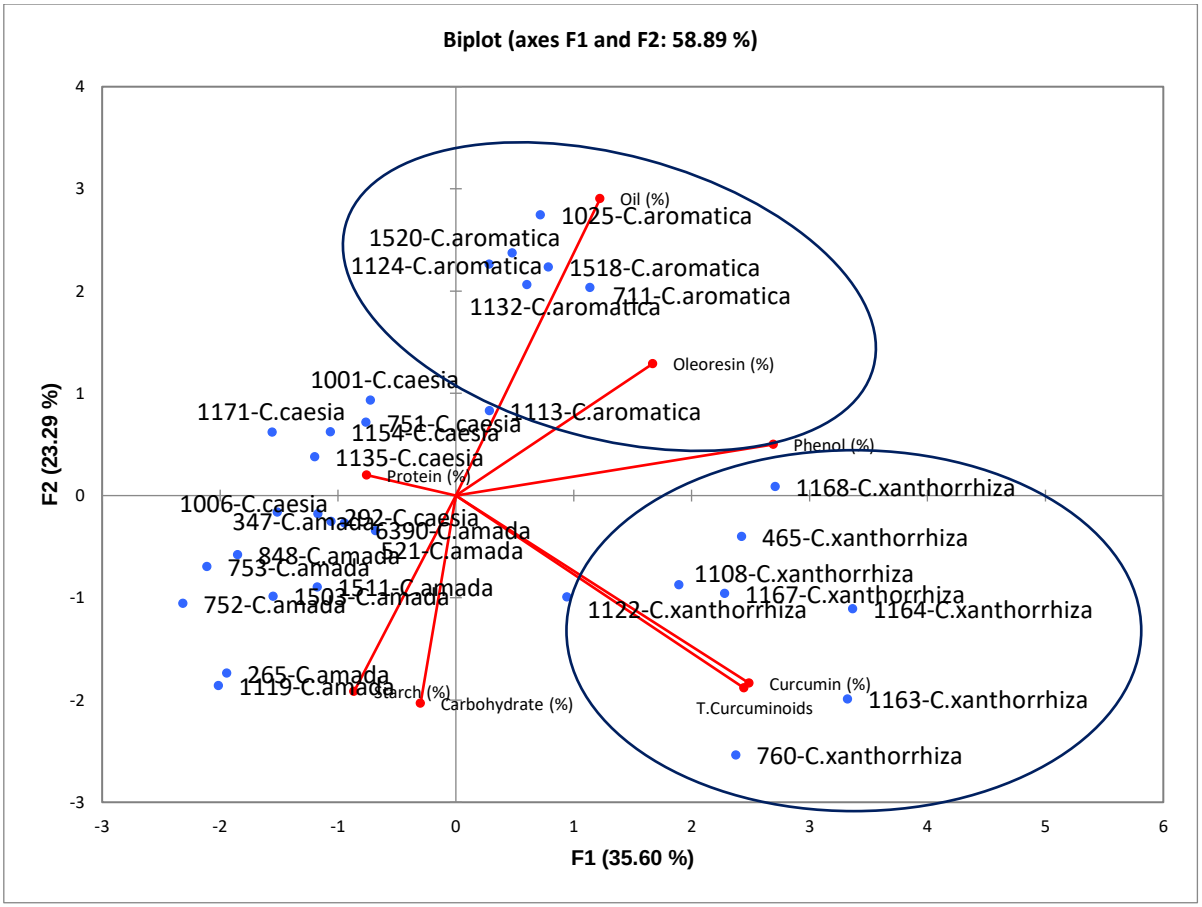


Fig. 26. Two dimensional loading plot of Principal Component Analysis based on biochemical characters.

Eight biochemical traits revealed a total of eight principal components. The contribution of the principal components 1, 2 and 3 was 35.60%, 23.29% and 15.68%, respectively. The first two principal components explained 58.89% of the variation while the first three principal components explained 74.57% of the variation (Table 85). The principal component 1 showed high loading factor for total phenol, curcumin, total curcuminoids, oleoresin and essential oil content. Principal component 2 showed high loading factor for essential oil, oleoresin, phenol and protein content. Principal component 3 showed high loading factor for total carbohydrates, oleoresin, total protein, essential oil and total starch content.

Table 85. Principal Components (PC) for qualitative characters in four *Curcuma* species.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Eigenvalue	2.85	1.86	1.25	0.88	0.76	0.21	0.16	0.03
Variability (%)	35.60	23.29	15.68	10.96	9.50	2.65	1.98	0.34
Cumulative %	35.60	58.89	74.57	85.53	95.03	97.68	99.66	100.00
Total protein (%)	-0.10	-0.55	0.68	-0.34	-0.21	-0.24	0.04	-0.01
Total starch (%)	0.56	0.35	0.60	-0.34	0.06	0.29	0.03	0.00
Total phenol (mg/g)	-0.26	0.06	0.56	0.76	-0.20	0.06	0.01	0.00
Oleoresin (%)	0.41	0.79	0.24	0.05	0.23	-0.23	-0.20	0.01
Total carbohydrates (%)	-0.29	-0.52	0.21	0.10	0.77	0.02	0.00	0.01
Curcumin (%)	0.82	-0.51	-0.05	0.13	-0.05	0.04	-0.16	-0.11
Essential oil (%)	0.91	0.14	-0.06	0.18	0.15	-0.13	0.29	-0.02
Total Curcuminoids	0.84	-0.50	-0.09	0.11	-0.08	0.01	-0.08	0.12

The Principle Component Analysis revealed that accessions of *C. aromatica* and *C. xanthorrhiza* grouped separately from other accessions in two different quadrants and showed clear distinction. However, Acc.1168 of *C. xanthorrhiza* fell in different quadrant. Accessions of *C. caesia* and *C. amada* which showed closer association for biochemical characters.

In case of *C. amada*, 8 biochemical traits revealed a total of eight principal components. The contributions of the principal component 1, 2 and 3 were 51.50%, 26.13% and 9.88%, respectively. The first two principal components generated 77.62% of the variation and first three principal components accounted for 87.50% of the variation. The results of principal component analysis in *C. amada* is depicted in Table 86. The principal component 1 showed high loading factor for curcumin, oleoresin, total carbohydrates, total phenol and total protein content. Principal component 2 showed high loading factor for oil and protein content. Principal component 3 showed high loading factor for total curcumin content, phenol and protein content.

Table 86. Principal Components (PC) for qualitative characters in the accessions of *C. amada*.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Eigenvalue	4.12	2.09	0.79	0.71	0.21	0.07	0.01	0.00
Variability (%)	51.50	26.13	9.88	8.86	2.65	0.90	0.08	0.00
Cumulative %	51.50	77.62	87.50	96.36	99.01	99.92	100.00	100.00
Total protein (%)	0.44	0.79	0.35	-0.17	0.13	-0.09	-0.04	0.00
Total starch (%)	-0.90	0.00	0.30	0.02	0.32	0.03	0.03	0.00
Total phenol (mg/g)	0.60	0.20	0.42	0.65	-0.09	0.03	0.01	0.00
Oleoresin (%)	0.89	-0.26	0.24	-0.27	0.01	-0.11	0.05	0.00
Total carbohydrates (%)	0.80	-0.59	0.01	-0.11	0.02	-0.02	-0.01	0.01
Curcumin (%)	0.95	-0.12	0.03	-0.13	0.15	0.20	-0.01	0.00
Essential oil (%)	0.14	0.94	-0.09	-0.26	-0.14	0.08	0.03	0.00
Total curcuminoids(%)	-0.64	-0.34	0.58	-0.30	-0.21	0.06	-0.01	0.00

The principle component analysis showed very high dispersion among the accessions of *C. amada*. Acc. 848, Acc.1119 and Acc. 1503 fell in the same quadrant and showed a likely grouping. Similarly, Acc.347 and Acc. 753 as well as Acc.6390 and Acc.265 displayed closer association with each other. Likewise, Acc. 347 and Acc.753, Acc. 265 and Acc.6390 showed closer association. Acc. 521 and Acc.752 stood separately in their respective quadrant (Figure 27).

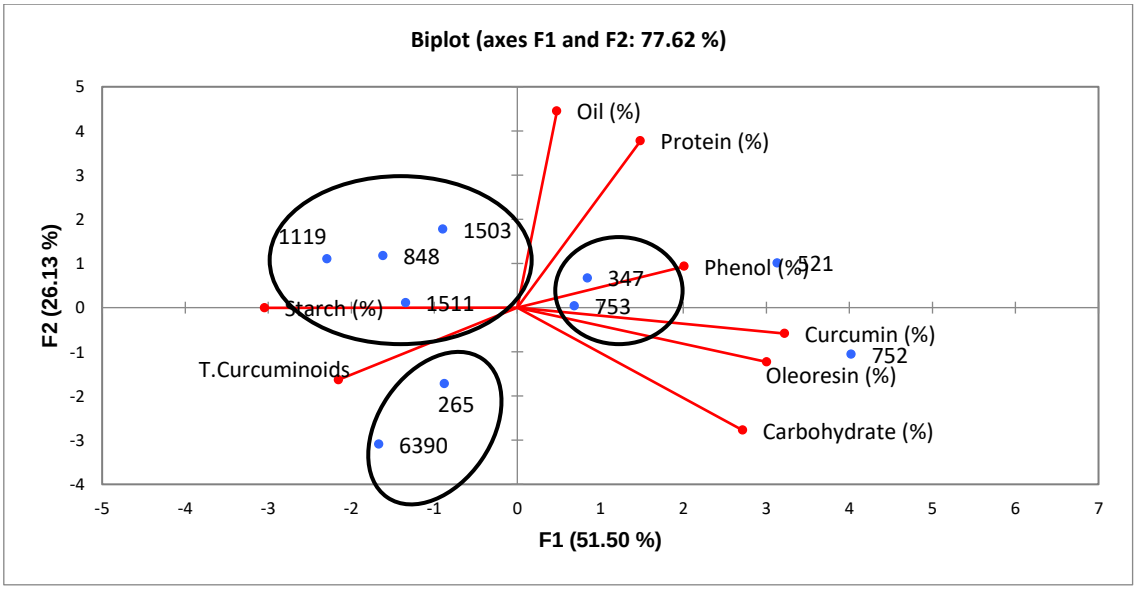


Fig. 27. Two dimensional loading plot of Principal Component Analysis (PCA) based on biochemical data from *C. amada*.

In case of *C. aromatica*, 8 biochemical traits revealed a total of six principal components. The contributions of principal components 1, 2 and 3 were 46.15%, 35.15% and 14.44%, respectively. The first two principal components explained 81.30% of the variation while the first three principal components explained 95.74% of the variation. Among the four species, variation was highest in *C. aromatica* (Table 87 and Figure 28). The principal component 1 showed high loading factor for curcumin, oleoresin, total curcumin, phenol and protein content. Principal component 2 showed high loading factor for total carbohydrates, starch and oil content. PCA 3 showed high loading factor for essential oil content and total protein content.

Table 87. Principal Components (PC) for qualitative characters in the accessions of *C. aromatica.*

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	3.69	2.81	1.16	0.17	0.16	0.02
Variability (%)	46.15	35.15	14.44	2.06	1.96	0.24
Cumulative %	46.15	81.30	95.74	97.80	99.76	100.00
Total protein (%)	0.85	0.27	0.42	-0.13	0.06	0.08
Total starch (%)	0.17	0.82	-0.52	-0.04	0.18	-0.05
Total phenol (mg/g)	0.83	0.50	-0.12	-0.21	-0.09	-0.02
Oleoresin (%)	0.92	0.05	-0.27	0.25	-0.12	0.01
Total carbohydrates (%)	-0.28	0.94	-0.09	0.15	0.08	0.07
Curcumin (%)	0.89	-0.35	-0.28	0.02	-0.08	0.00
Essential oil (%)	0.36	0.56	0.73	0.13	-0.01	-0.07
Total Curcuminoids	0.63	-0.71	0.06	0.06	0.30	-0.01

The principle component analysis showed very high dispersion among the accessions of *C. aromatica*. Acc.1518 and Acc.1132 fell in the same quadrant and showed a very high similarity. Similar was the case of Acc. 1113 and Acc.1124. However, Acc. 711, Acc. 1520 and Acc.1025 stood separately in the group.

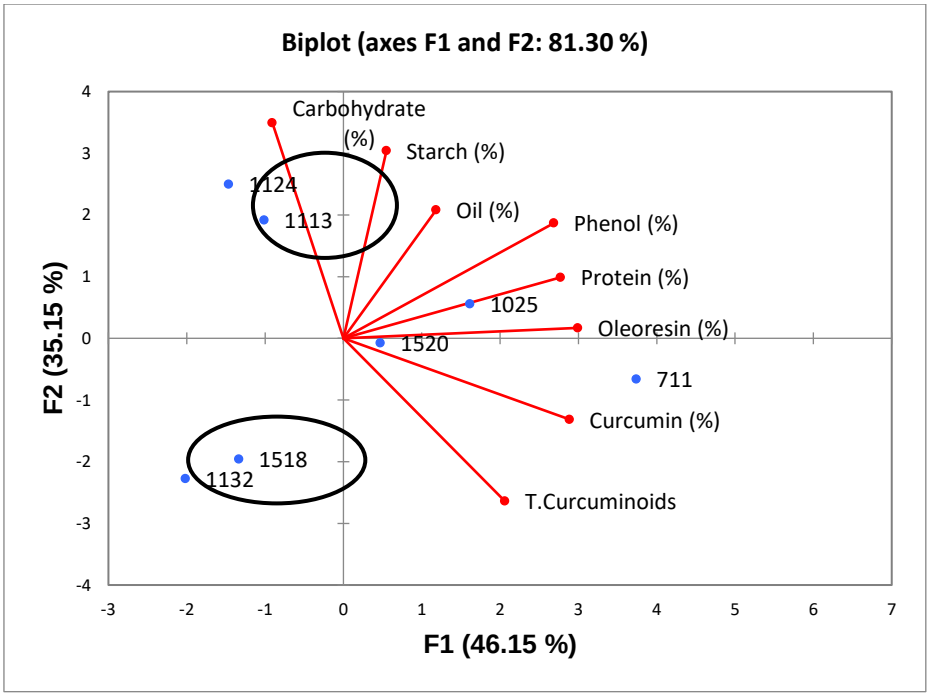


Fig. 28. Two dimensional loading plot of Principal Component Analysis (PCA) based on biochemical data from *C. aromatica*.

In case of *C. caesia*, 8 biochemical traits revealed a total of six principal components. The contributions of principal components 1, 2 and 3 were 47.82%, 29.06% and 14.03%, respectively. The first two principal components explained 76.88% of the variation and first three principal components explained 90.91% of the variation (Table 88 and Figure 29). The principal component 1 showed high loading factor for total curcumin content, phenol and carbohydrate content. Principal component 2 showed high loading factor for essential oil and curcumin content. PCA 3 showed high loading factor for total carbohydrates and curcumin content.

Table 88. Principal Components (PC) for qualitative characters in the accessions of *C. caesia*.

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	3.83	2.33	1.12	0.65	0.06	0.02
Variability (%)	47.82	29.06	14.03	8.13	0.75	0.21
Cumulative %	47.82	76.88	90.91	99.05	99.79	100.00
Total protein (%)	0.14	0.15	-0.95	0.21	0.06	-0.02
Total starch (%)	-0.70	-0.67	-0.08	0.24	-0.04	-0.03
Total phenol (mg/g)	0.95	0.24	-0.16	-0.01	-0.12	0.02
Oleoresin (%)	-0.82	-0.43	-0.14	-0.33	0.09	0.04
Total carbohydrates (%)	0.91	-0.22	0.30	0.05	0.14	-0.07
Curcumin (%)	-0.59	0.46	0.26	0.60	0.02	0.02
Essential oil (%)	-0.13	0.98	0.01	-0.13	0.09	0.03
Total Curcuminoids	0.73	-0.63	0.00	0.23	0.07	0.09

The principle component analysis showed very high dispersion among the accessions of *C. caesia*. Acc.1154, Acc.1135 and Acc.1171 fell in the same quadrant and showed high similarity. Similarly, Acc. 1006 and Acc.1001 fell in the same quadrant. However, Acc. 751 and Acc. 292 stood separately in the group.

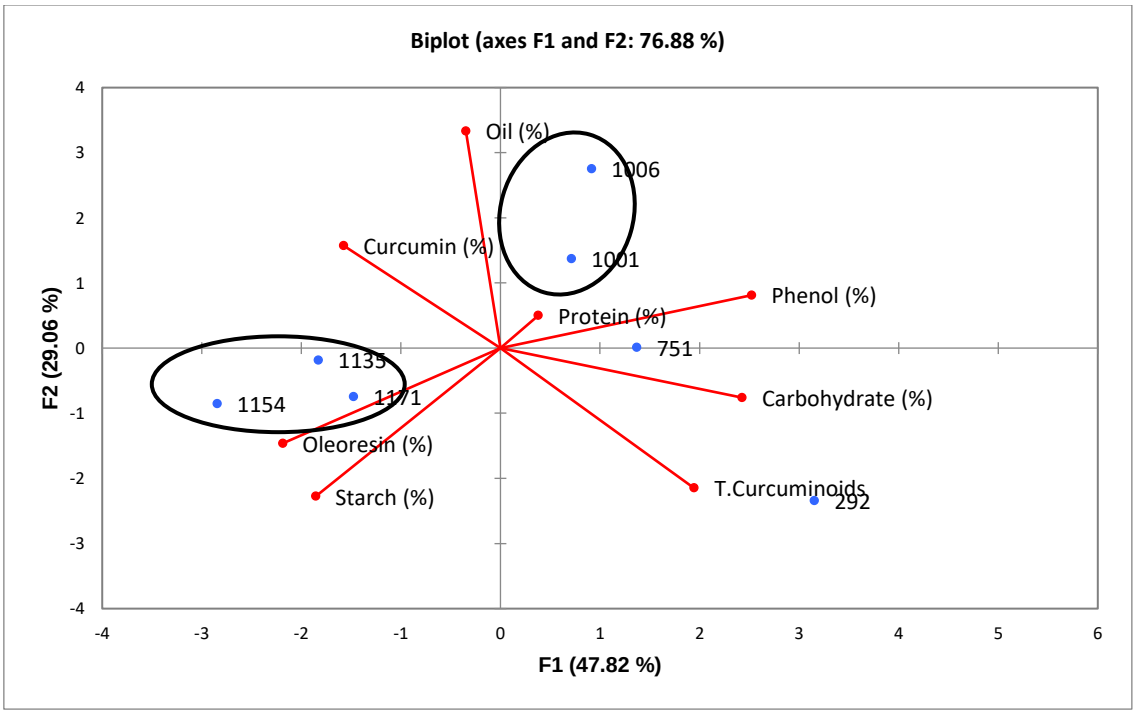


Fig. 29. Two dimensional loading plot of Principal Component Analysis (PCA) based on biochemical data from *C. caesia*.

In *C. xanthorrhiza*, 8 biochemical traits revealed a total of six principal components. The contributions of principal component 1, 2 and 3 were 38.71%, 27.60% and 17.12%, respectively. The first two principal components revealed 66.32% of the variation and first three principal components revealed 83.43% of the variation (Table 89 and Figure 30). The principal component 1 showed high loading factor for total curcuminoids and curcumin contents. Principal component 2 showed high loading factor for total phenol, total starch and total protein content. Principal Component 3 showed high loading factor for essential oil, oleoresin and starch content.

Table 89. Principal Components (PC) for quantitative characters in the accessions of *C. xanthorrhiza.*

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	3.10	2.21	1.37	0.85	0.39	0.08
Variability (%)	38.71	27.60	17.12	10.66	4.87	1.01
Cumulative %	38.71	66.32	83.43	94.09	98.96	99.96
Total protein (%)	-0.75	0.49	0.38	0.00	0.11	-0.19
Total Starch (%)	0.05	0.69	0.39	0.50	-0.35	0.07
Total Phenol (mg/g)	0.18	0.83	0.28	-0.26	0.35	0.14
Oleoresin (%)	0.25	-0.78	0.57	0.03	0.04	0.06
Total carbohydrates (%)	-0.68	-0.35	0.13	0.57	0.28	0.06
Curcumin (%)	0.88	-0.10	0.36	0.20	0.17	-0.11
Essential oil (%)	-0.50	-0.26	0.70	-0.42	-0.17	0.02
Total curcuminoids	0.97	0.11	0.20	0.02	-0.02	-0.03

The Principle Component Analysis showed very high dispersion among the accessions of *C. xanthorrhiza*. A closer association and high similarity were observed between Acc. 465 and Acc.1122. Acc.1164, Acc.1163 and Acc.760 plotted in same quadrant but they were distantly related with each other. Acc.1168, Acc.1167, Acc.1108 seen scattered in the loading plot separated from each other.

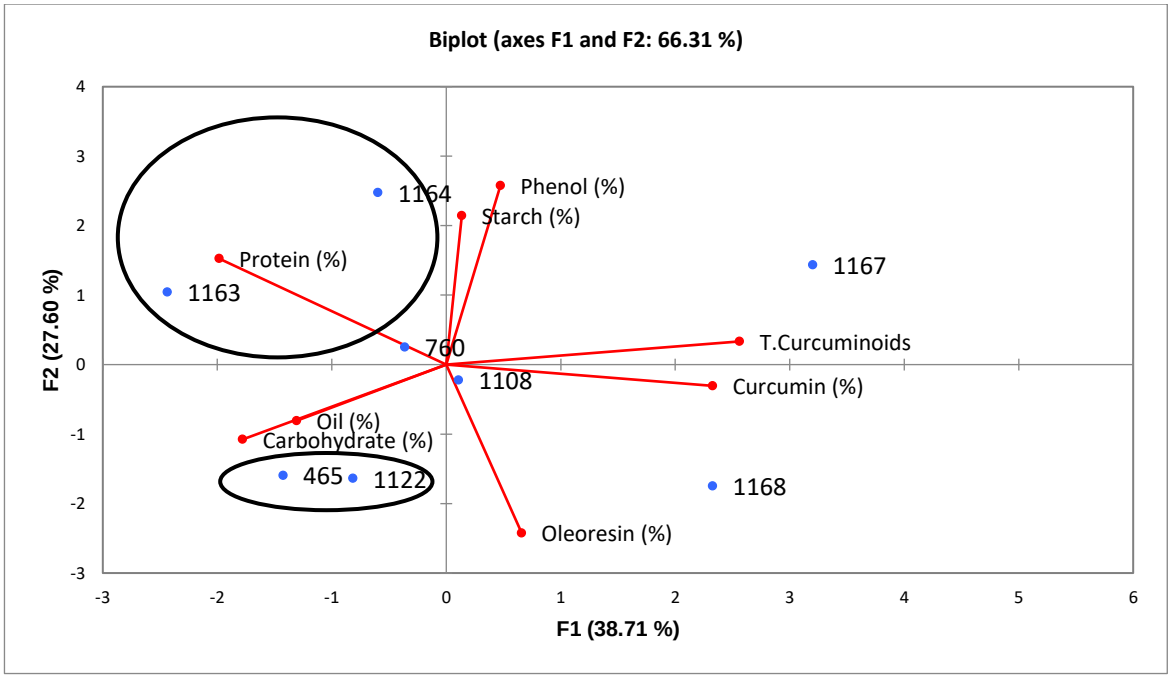


Fig. 30. Two dimensional loading plot of Principal Component Analysis (PCA) based on biochemical data from *C. xanthorrhiza*.

4.2.10. Cluster analysis

A UPGMA based dendrogram was constructed using the biochemical data obtained from four *Curcuma* species using Simple Matching similarity coefficients.

In *C. amada*, the dendrogram clustered ten accessions of *C. amada* into three groups splitting at similarity coefficient of 0.562. The three groups were as follows:

Group I – Acc.265, Acc.848, Acc.752, Acc.753, Acc.1119

Group II – Acc.347, Acc. 6390

Group III – Acc.521, Acc.1511, Acc.1503

The similarity matrix obtained using Simple Matching coefficients is depicted in Table 90 and the dendrogram constructed using UPGMA SAHN clustering programin Figure 31.

Table 90. Similarity matrix of *C. amada* obtained using Simple Matching similarity coefficients

Accession	265	347	521	752	753	848	1119	1503	1511	6390
265	1.00									
347	0.38	1.00								
521	0.50	0.63	1.00							
752	0.75	0.38	0.25	1.00						
753	0.63	0.25	0.13	0.88	1.00					
848	0.75	0.63	0.25	0.75	0.63	1.00				
1119	0.63	0.00	0.38	0.63	0.75	0.38	1.00			
1503	0.25	0.63	0.50	0.50	0.38	0.50	0.38	1.00		
1511	0.38	0.50	0.63	0.38	0.50	0.38	0.50	0.63	1.00	
6390	0.38	0.75	0.38	0.38	0.50	0.63	0.25	0.38	0.50	1.00

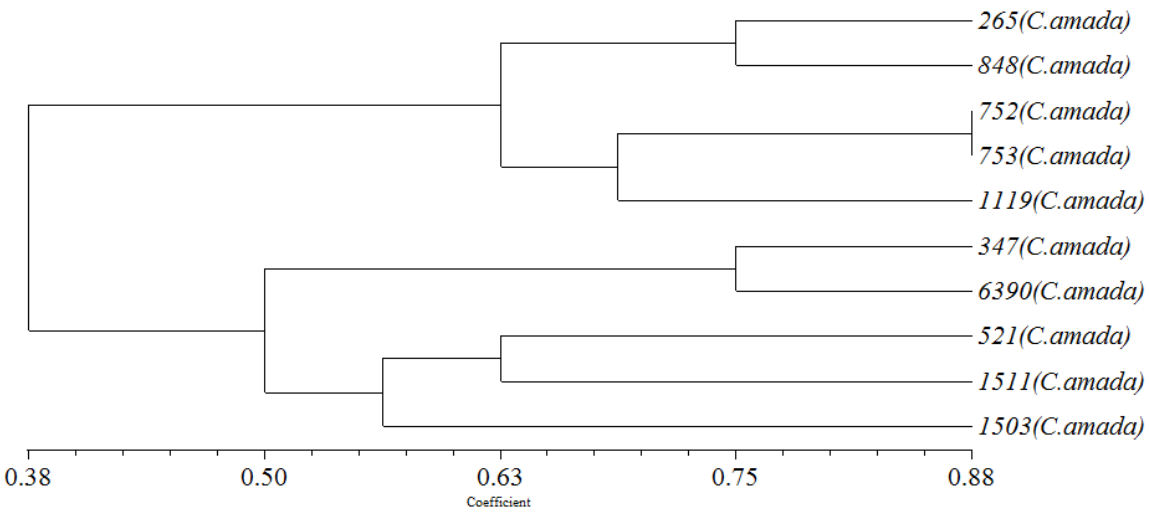


Fig. 31. UPGMA dendrogram of *C. amada* constructed using the Simple Matching similarity coefficients

First group was formed by five accessions (Acc.265, Acc.848, Acc.752, Acc.753 and Acc.1119), of which Acc.752 and Acc.753, showed closer proximity with each other as they both were collected from Cooch Behar, (West Bengal). Rest of the accessions did not show any geographical influence in the clustering pattern. Second group was formed by Acc.347 and Acc.6390 and the last group was formed by Acc. 521, Acc.1511 and Acc.1503.

In case of *C. aromatica*, the UPGMA dendrogram clustered the seven accessions into three groups by splitting at similarity coefficient of 0.50. The similarity matrix obtained by using

Simple Matching coefficients is depicted in Table 91 and the dendrogram constructed using UPGMA SAHN clustering algorithm is given in Figure 32.

The three groups were as follows:

Group I - Acc.711, Acc.1518, Acc.1132

Group II - Acc.1025, Acc.1124, Acc.1113

Group III - Acc.1520

Table 91. Similarity matrix of *C. aromatica* obtained using Simple Matching similarity coefficients

Accession	711	1025	113	1124	1132	1518	1520
711	1.00						
1025	0.13	1.00					
113	0.38	0.50	1.00				
1124	0.25	0.63	0.63	1.00			
1132	0.63	0.50	0.50	0.38	1.00		
1518	0.75	0.13	0.38	0.50	0.38	1.00	
1520	0.50	0.63	0.38	0.25	0.38	0.25	1.00

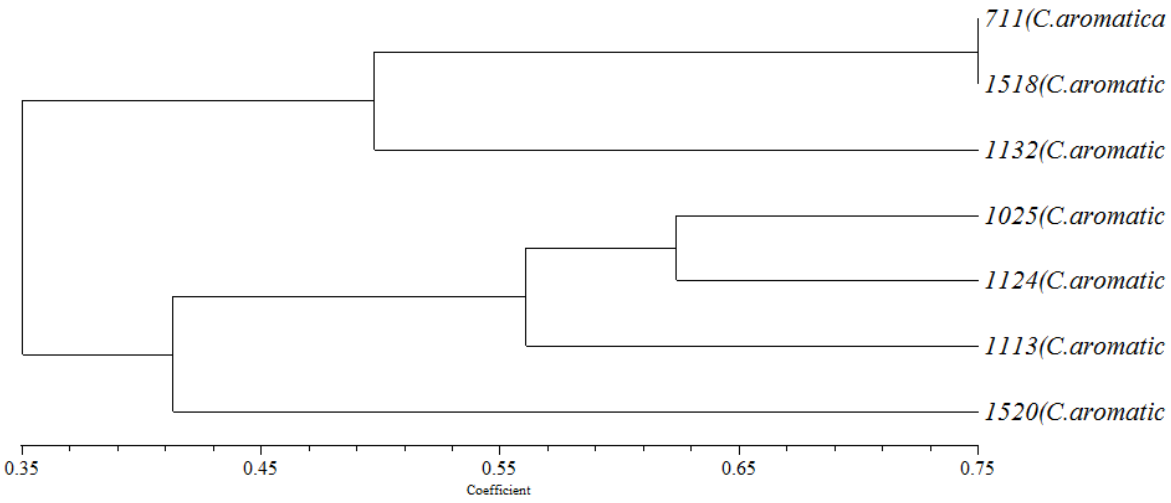


Fig. 32. UPGMA dendrogram of *C. aromatica* constructed using the Simple Matching similarity coefficients.

Acc. 711, Acc.1518 and Acc.1132 formed the first group, while the Acc.1025, Acc.1124 and Acc.1113 formed the second group and the last group was formed by a single accession, Acc.1520.

In case of *C. caesia*, the UPGMA dendrogram clustered seven accessions of *C. caesia* into three groups splitting at coefficient of 0.62. The similarity matrix obtained by using Simple Matching coefficients is depicted in Table 92 and the dendrogram constructed using UPGMA SAHN clustering algorithm is given in Figure 33. The groups formed were as follows.

Group I – Acc.292, Acc.1154

Group II – Acc. 751, Acc. 1001, Acc. 1171

Group III – Acc.1006, Acc.1135

Table 92. Similarity matrix of *C. caesia* obtained using Simple Matching similarity coefficients

Accession	292	751	1001	1006	1135	1154	1171
292	1.00						
751	0.63	1.00					
1001	0.38	0.75	1.00				
1006	0.38	0.25	0.50	1.00			
1135	0.50	0.38	0.38	0.88	1.00		
1154	0.63	0.50	0.25	0.25	0.38	1.00	
1171	0.25	0.63	0.63	0.38	0.25	0.38	1.00

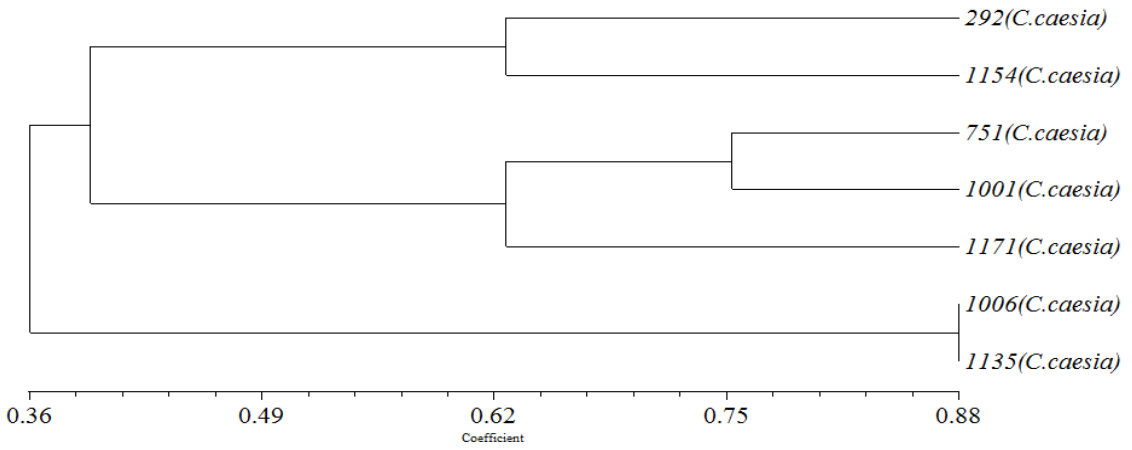


Fig. 33. UPGMA dendrogram of *C. caesia* constructed using Simple Matching similarity coefficients

Acc. 292 and Acc.1154 formed first group while the second group was formed by Acc. 751, Acc.1001 and Acc.1171. Acc.1006 and Acc.1135 constituted the third group and interestingly they were closely associated with each other although they were collected from different geographical regions such as Eastern Arunachal Pradesh (Anjaw, Arunachal Pradesh) and central Kerala (Thrissur), respectively.

In case of *C. xanthorrhiza*, the UPGMA dendrogram clustered the eight accessions into three groups by splitting at Simple Matching similarity coefficient of 0.58. The similarity matrix obtained using Simple Matching coefficients is depicted in Table 93 and the dendrogram constructed using UPGMA SAHN clustering algorithm in Figure 34. The groups formed were as follows:

Group I – Acc.465, Acc.1168, Acc.1108, Acc.1122

Group II – Acc.760, Acc.1164

Group III – Acc.1163, Acc.1167

Table 93. Similarity matrix of *C. xanthorrhiza* obtained using Simple Matching similarity coefficients

Accession	465	760	1108	1122	1163	1164	1167	1168
465	1.00							
760	0.50	1.00						
1108	0.63	0.38	1.00					
1122	0.63	0.38	0.50	1.00				
1163	0.00	0.50	0.38	0.38	1.00			
1164	0.38	0.63	0.25	0.25	0.63	1.00		
1167	0.25	0.25	0.63	0.38	0.75	0.38	1.00	
1168	0.75	0.25	0.63	0.63	0.25	0.38	0.50	1.00

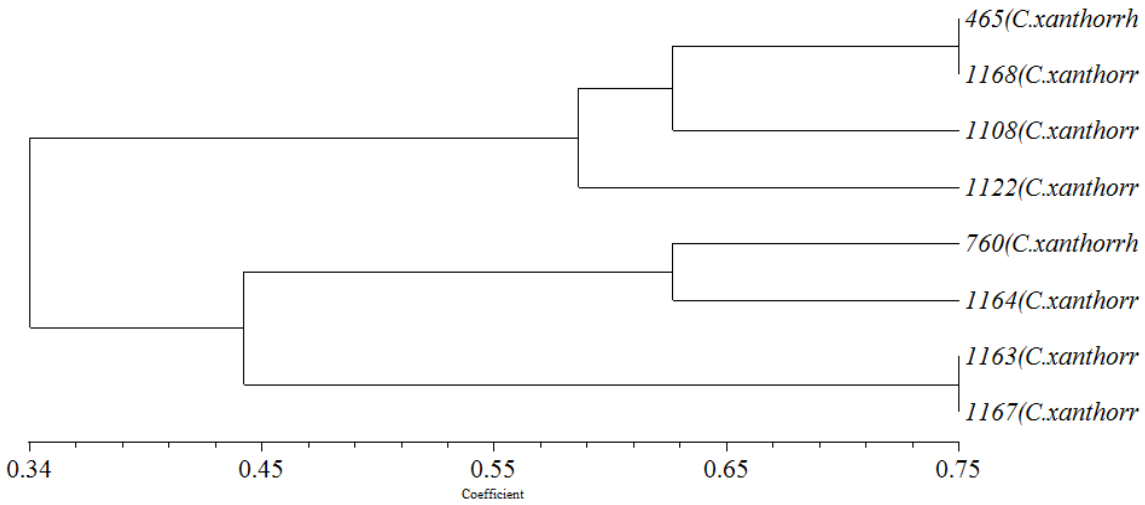


Fig. 34. UPGMA dendrogram of *C. xanthorrhiza* constructed using the Simple Matching similarity coefficients.

First group was formed by Acc. 465, Acc.1168, Acc.1108 and Acc.1122 of which Acc.465 and Acc.1168 showed a closer association with each other although they were collected from two different geographical regions *viz*., Jorhat (Assam) and Malappuram (Kerala), respectively. The second group was formed by Acc.760 and Acc.1164 and the last group was formed by two accessions *viz*., Acc.1163 and Acc.1167 and they showed a closer association with each other.

4.2.11. Qualitative analysis of starch

Total starch yield and physiochemical properties of starch such as moisture content, ash content, solubility, water holding capacity, swelling power and qualitative characters such as size and shape of the starch granules (Figure 35) are given in the Tables 94 and 95, respectively.

Table 94. Yield and physicochemical properties of starch from four *Curcuma* species.

Species	Starch (%)	Moisture (%)	Ash (%)	Solubility (%)	Swelling power (g/g)	Water holding capacity (%)
C. amada	48.48 ± 0.31	9.22 ± 0.08	4.58 ± 0.01	1.21 ± 0.02	4.48 ± 0.04	157.72 ± 0.85
C. aromatica	45.90 ± 0.10	9.26 ± 0.08	11.45 ± 0.01	1.09 ± 0.02	3.96 ± 0.05	133.33 ± 0.51
C. caesia	45.24 ± 0.25	8.94 ± 0.09	3.55 ± 0.02	0.47 ± 0.01	3.74 ± 0.04	121.62 ± 0.79
C. xanthorrhiza	46.11 ± 0.18	9.60 ± 0.12	3.83 ± 0.03	1.07 ± 0.02	4.07 ± 0.01	142.50 ± 0.42

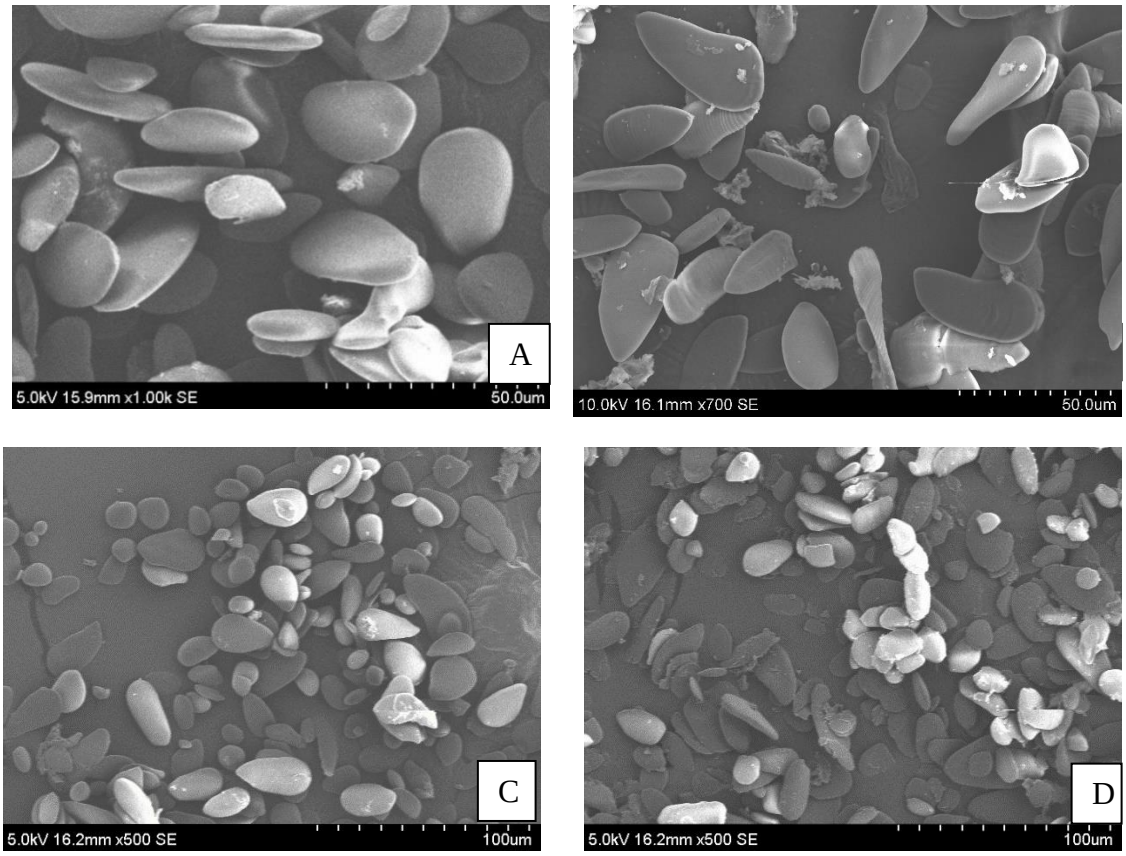


Fig. 35. Scanning electron micrographs of starch granules from four *Curcuma* species. A). *C. amada*, B). *C. aromatica*, C). *C. caesia* and D). *C. xanthorrhiza*.

Table 95. Mean size and shape of starch granules of four *Curcuma* species.

Species	Parameter		
	Size		Shape
	Length(µm)	Width(µm)	
C. amada	16-48	11-26	Oval to elliptical, smooth surface
C. aromatica	9-60	6-24	Oval to elliptical, large, flat with concentric rings
C. caesia	10-39	9-23	Round to oval, small, smooth surface
C. xanthorrhiza	9-47	8-23	Oval to elliptical, some were rounded, with smooth surface

Starch content (dry weight basis) of the four *Curcuma* species under study ranged from 45.24% (*C. caesia*) to 48.48% (*C. amada*). *C. amada* recorded maximum swelling power, solubility and

water holding capacity (4.48 g/g, 1.21%, 157.72%) whereas *C. caesia* recorded minimum values for these parameters (3.74 g/g, 0.47%, 121.62%).

Scanning electron micrographs (Figure 35) of the four *Curcuma* species revealed variation for the size and shape of the starch granules. Starch granules of *C. amada* were oval to elliptical with smooth surface, 16-48μm long and 11-26 μm wide while in *C. aromatica* they were oval to elliptical, flat with concentric rings on the surface, 9-60 μm long and 6-24 μm wide (Figure 36). In *C. caesia* round to oval with a smooth surface, 10-39 μm long and 9-23 μm wide starch granules were observed and in *C. xanthorrhiza* elongated (9-47 μm) and broad (8-23 μm) granules were seen.

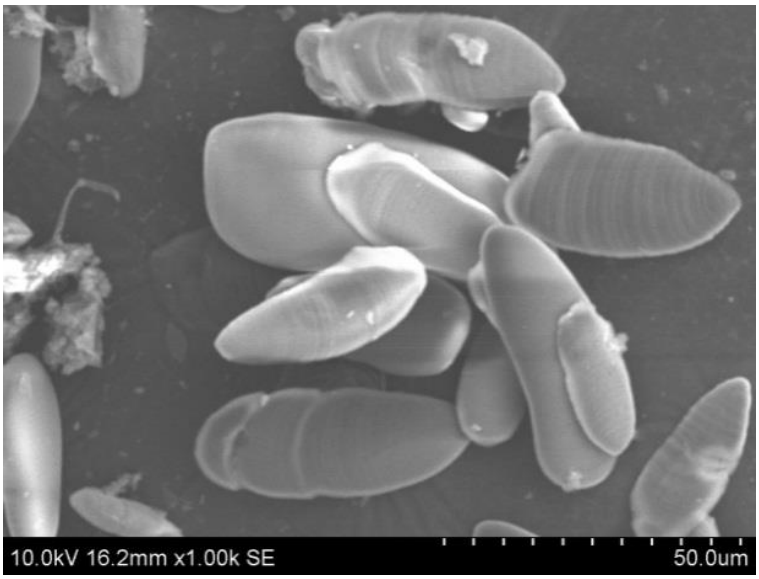


Fig. 36. Scanning electron micrograph of Starch granules of *C aromatica* showing characteristic ring formation

4.3. Phenological variation in two species of *Curcuma* at three growth stages

The results of field experiments conducted on two *Curcuma* species *viz*. *C. amada* and *C. aromatica* on aerial morphological traits, yield and dry recovery revealed that highest plant height was recorded at 180 days after planting (DAP) in both the species; 83.25 cm for *C. aromatica* and 78.75 cm for *C. amada*. Leaf number was highest at 90DAP followed by 140 DAP and least in 180DAP in case of *C. amada.* Similar trend was observed in *C. aromatica*. Tiller number did not vary with the different growth stages of the plants. Details of the aerial morphological characters are given in Table 96.

Table 96. Mean aerial morphological characters of two *Curcuma* species at three growth stages.

Species	Plant height (cm)				Leaf number				Tiller number			
	Days after planting				Days after planting				Days after planting			
	90	140	180	Mean	90	140	180	Mean	90	140	180	Mean
C. amada	64.72^{bc}	76.00^{ab}	78.75^{ab}	73.16	9.00^{a}	8.50^{ab}	8.00^{ab}	8.50	2.25^{a}	2.25^{a}	2.00^{a}	2.20
C. aromatica	54.33^{c}	73.25^{ab}	83.25^{a}	70.28	9.25^{a}	8.25^{ab}	7.25^{b}	8.25	1.75^{a}	1.75^{a}	1.75^{a}	1.75
Mean	59.53	74.63	81.00		9.13	8.40	7.63		2.00	2.00	1.88	
LSD (P<0.05) Species x Growth stage	15.85				1.17				*NS			
CV (%)	14.87				9.44							

* NS = Not Significant (P>0.05), CV- Coefficient of Variation, LSD- Least Significant Difference, Values with the different superscript are significantly different (P<0.05)

Mean yield and dry recovery of *Curcuma* species were maximum in 180DAP in *C. amada* (398.50 g, 14.93%). Dry recovery at 140DAP and 180DAP did not vary much (Table 97).

Table 97. Mean yield and dry recovery of two *Curcuma* species at three growth stages.

Species	Yield (g)				Dry recovery (%)			
	Days after planting				Days after planting			
	90	140	180	Mean	90	140	180	Mean
C. amada	99.00^{c}	184.00^{bc}	398.50^{a}	227.17	10.51^{d}	13.30^{cd}	14.93^{bc}	12.91
C. aromatica	62.50^{c}	133.00^{bc}	274.00^{a}	156.50	13.73^{c}	17.49^{ab}	18.81^{a}	16.68
Mean	80.75	158.50	336.25		12.12	15.40	16.87	
LSD (P< 0.05) Species x Growth stage	15.76				2.95			
CV (%)	19.28				13.41			

CV- Coefficient of Variation, LSD- Least Significant Difference

In case of biochemical parameters, maximum essential oil yield was recovered at 90 DAP in both the species of *Curcuma*. Maximum essential oil yield was recovered at 90 DAP in both the species of *Curcuma*, as the age of the plant increased, the oil yield decreased gradually from 4.42 to 2.10 % in *C. amada*, and from 6.98 to 5.20 % in *C. aromatica* (Table 98). Percentage of curcumin in *C. aromatica* slightly increased from 0.036 (90 DAP) to 0.047% (180 DAP), though there was not much difference between 140 DAP and 180 DAP. In *C. amada* the curcumin

content decreased slightly from 0.06 % (90 DAP) to 0.055 % (140 DAP) and then it registered an increase (0.09%) at 180 DAP.

Table 98. Mean oil and curcumin content of two *Curcuma* species at three growth stages

Species	Essential oil (%)				Curcumin (%)			
	Days after planting				Days after planting			
	90	140	180	Mean	90	140	180	Mean
C. amada	4.43^{c}	2.90^{d}	2.10^{e}	3.142	0.06^{ab}	0.06^{ab}	0.09^{a}	0.07
C. aromatica	6.98^{a}	5.90^{b}	5.20^{bc}	6.025	0.04^{b}	0.05^{ab}	0.05^{ab}	0.04
Mean	5.70	4.40	3.65		0.05	0.05	0.07	
LSD (P<0.05) Species x Growth stage	0.78				0.07			
CV (%)	11.47				6.70			

CV- Coefficient of Variation, LSD- Least Significant Difference, Values with the different superscript are significantly different (P<0.05)

In *C. aromatica*, starch content ranged from 37.80% (90 DAP) to 45.85% (180 DAP), whereas in *C. amada*, the starch content ranged from 18.02% (90 DAP) to 48.75% (180 DAP) (Table 99).

Total protein content in *C. aromatica* ranged from 8.25% to 9.98%, maximum at 180 DAP and minimum at 140DAP. But in case of *C. amada*, the scenario was different. Protein content was maximum at 90 DAP (5.90%) and minimum at 180 DAP (3.25%). Protein content in this species decreased with the increase of age. However, there was no significant variation between the two species for protein content across the three stages of growth. Crude fiber content too showed similar trend in the two species albeit statistically significant.

Table 99. Mean starch, protein and crude fiber content of two *Curcuma* species at three growth stages.

Species	Starch (%)				Protein (%)				Crude fiber (%)			
	Days after planting				Days after planting				Days after planting			
	90	140	180	Mean	90	140	180	Mean	90	140	180	Mean
C. amada	18.01^{c}	35.10^{b}	48.75^{a}	33.95	10.56^{a}	9.36^{a}	9.08^{a}	9.66	2.80^{b}	2.83^{b}	3.03^{b}	2.88
C. aromatica	37.80^{b}	37.21^{b}	46.85^{ab}	40.62	8.53^{a}	8.25^{a}	9.98^{a}	8.92	5.90^{a}	3.28^{b}	3.25^{b}	4.14
Mean	27.91	36.16	47.80		9.54	8.80	9.53		4.35	3.05	3.14	
LSD (P<0.05) Species x Growth stage	10.23				*NS				0.64			
CV (%)	18.55								12.18			

*NS–Not Significant, CV- Coefficient of Variation, LSD- Least Significant Difference, Values with the different superscript are significantly different (P<0.05)

4.4. Molecular characterization of four *Curcuma* species

4.4.1. DNA Isolation

Both the qualitative and quantitative estimation of the DNA extracted from leaves of four *Curcuma* species and their accessions yielded good quality of DNA with no secondary metabolites, in the range of 108 – 210 μg g^{-1} (Table 100). The ratio of absorbance at 260nm and 280nm (A260|280) ranged from 1.74 – 2.08. The isolated DNA showed conspicuous bands in 0.80% Agarose gel electrophoresis (Figure 37).

Table 100. DNA yield from accessions of four *Curcuma* species

Sl. No.	*C. amada*	DNA Yield (µg/g)	Sl. No.	*C. caesia*	DNA Yield (µg/g)
1	Acc. 265	108	1	Acc. 292	201
2	Acc. 347	188	2	Acc. 751	191
3	Acc. 521	167	3	Acc. 1001	187
3	Acc. 752	172	4	Acc.1006	185
4	Acc. 753	200	5	Acc. 1135	178
5	Acc. 848	185	6	Acc. 1154	169
6	Acc. 1119	170	7	Acc. 1171	160
8	Acc. 1503	167			
9	Acc. 1511	169			
10	Acc. 6390	172			
Sl. No.	*C. aromatica*	DNA Yield (µg/g)	Sl. No.	*C. xanthorrhiza*	DNA Yield (µg/g)
1	Acc.711	192	1	Acc. 465	174
2	Acc. 1025	174	2	Acc. 760	169
3	Acc. 1113	182	3	Acc. 1108	158
4	Acc. 1124	180	4	Acc. 1122	173
5	Acc. 1132	169	5	Acc.1163	157
6	Acc. 1518	184	6	Acc. 1164	176
7	Acc. 1520	180	7	Acc. 1167	208
			8	Acc. 1168	210

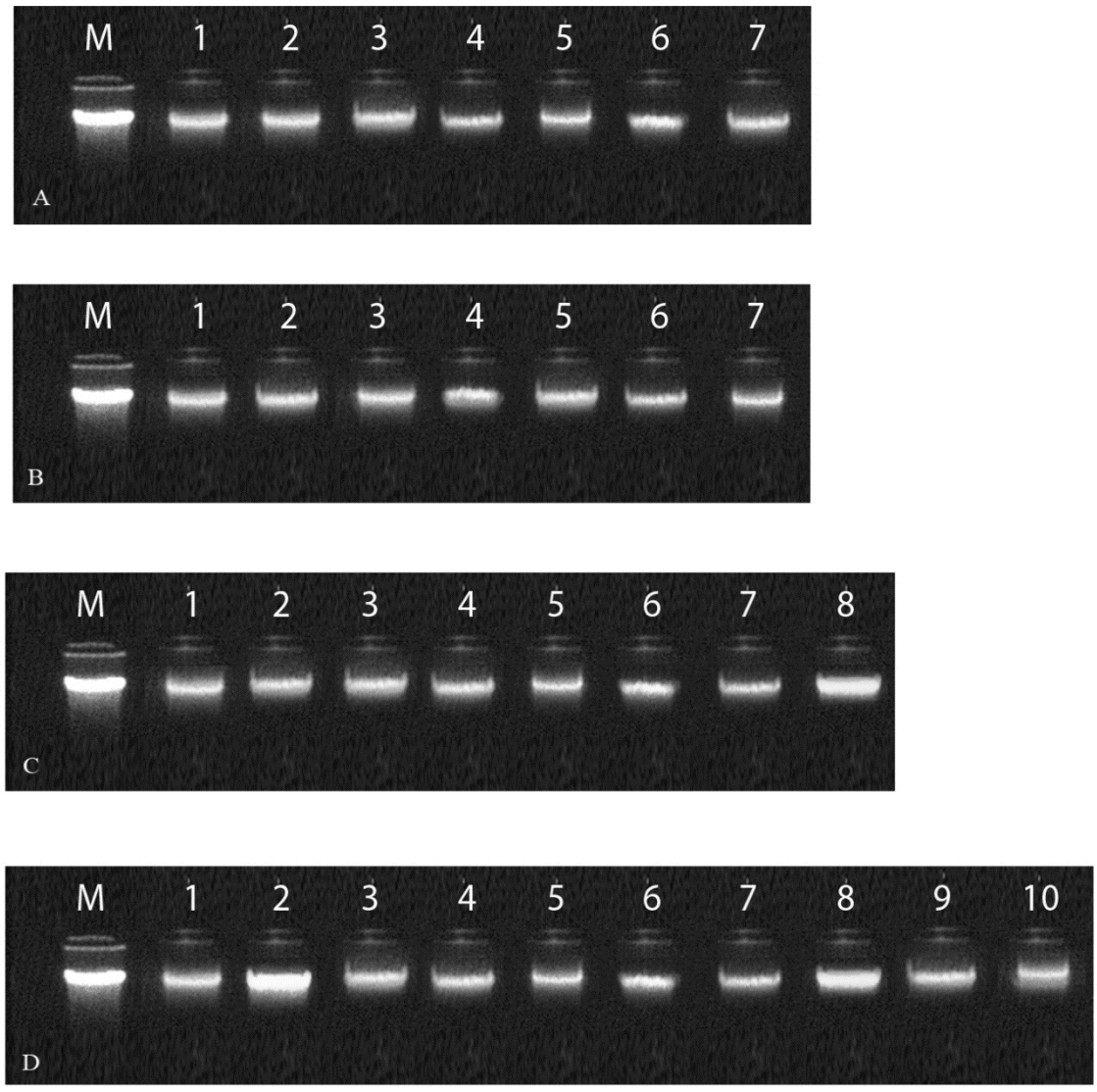


Fig. 37. Genomic DNA isolated from different accessions of four *Curcum*a species.
A). *C. aromatica*. M-marker, Lane 1-7 are Acc. 711, Acc. 1025, Acc. 1113, Acc. 1124, Acc. 1132, Acc. 1518 and Acc. 1520.
B). *C. caesia*. M-marker, Lane 1-7 are Acc. 292, Acc. 751, Acc. 1001, Acc. 1006, Acc. 1135, Acc. 1154 and Acc. 1171.
C). *C. xanthorrhiza*. M-marker,Lane 1-8 are Acc. 465, Acc. 760, Acc. 1108, Acc. 1122, Acc. 1163, Acc. 1164, Acc. 1167 and Acc. 1168.
D). *C. amada*. M-marker (Human genomic DNA), Lane 1-10 are Acc. 265, Acc. 347, Acc.521, Acc. 752, Acc. 753, Acc. 848, Acc. 1119, Acc. 1503, Acc. 1511 and Acc. 6390, respectively.

4.4.2. RAPD Analysis

Twenty-six RAPD Primers yielded a total 994 bands, out of which 648 are polymorphic. The amplification product was 38.23 per primer and the products ranged from 200bp to 2000bp. Some of the representative agarose gels are presented in Figures 38-41. The details of the RAPD primers, mean number of bands amplified per primer and number of polymorphic bands, the percentage of polymorphism and PIC (Polymorphism Information Content) and MI (marker index) are given in Table 101.

The number of scored bands per primer ranged from 27 (OPD 3, OPA 19) to 53 (OPA 17). The percentage of polymorphic bands ranged from 37% (OPA 19) to 86% (OPA5) with an average percentage of polymorphism of 65%. The PIC value for RAPD loci ranged from 0.15 (OPX5, OPC20) to 0.34 (OPC 5, OPA 1). The average PIC and MI values are 0.26 and 17.46, respectively.

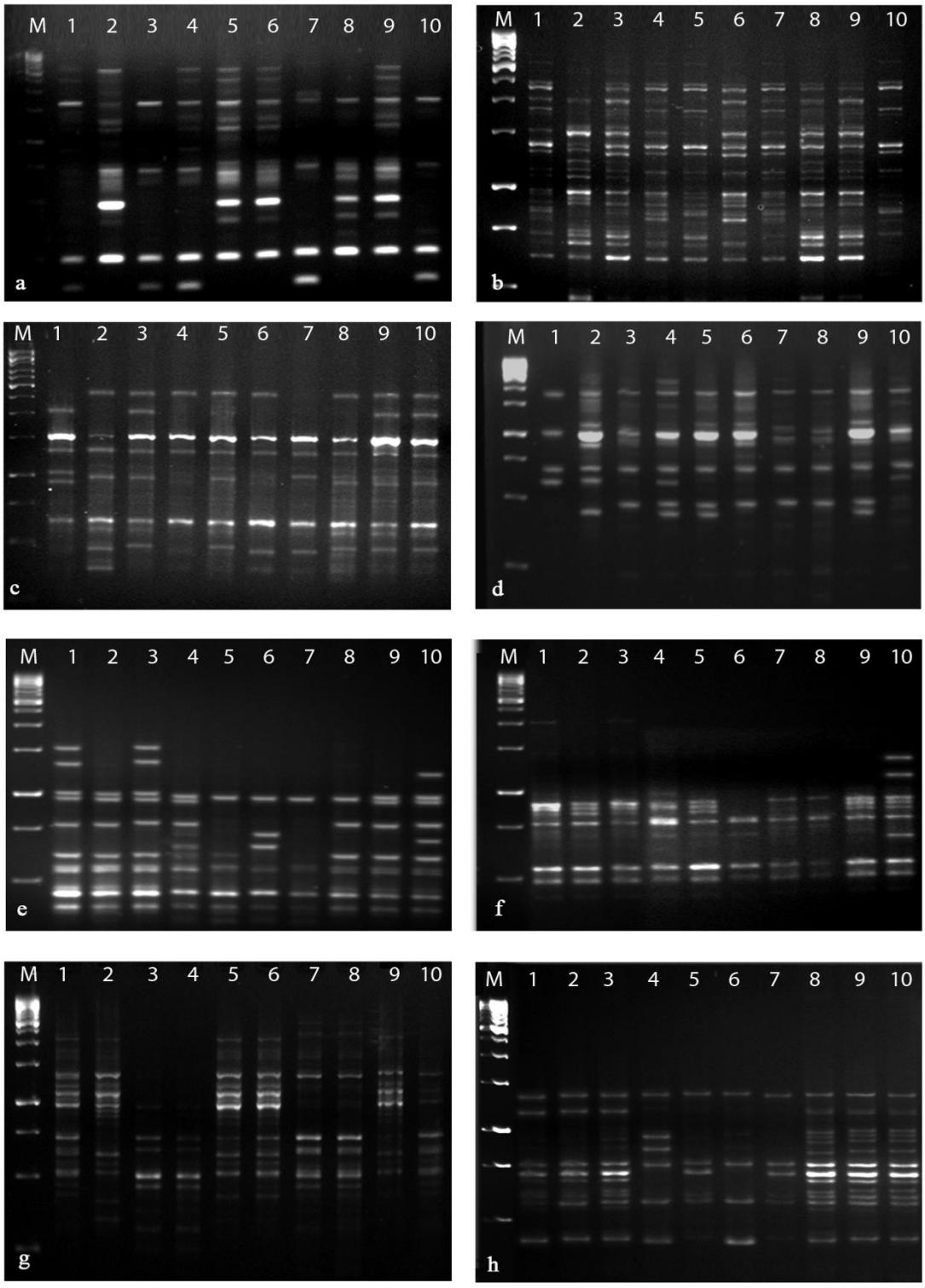


Fig. 38. a-h are the RAPD profiles of *C. amada* using primers OPA 05, OPC 05, OPN 04, OPA 07, OPC 20, OPD 03, OPN 16 and OPAF 14. M-marker (1kb ladder), Lane 1-10 are Acc. 265, Acc. 347, Acc.521, Acc. 752, Acc. 753, Acc. 848, Acc. 1119, Acc. 1503, Acc. 1511 and Acc. 6390, respectively.

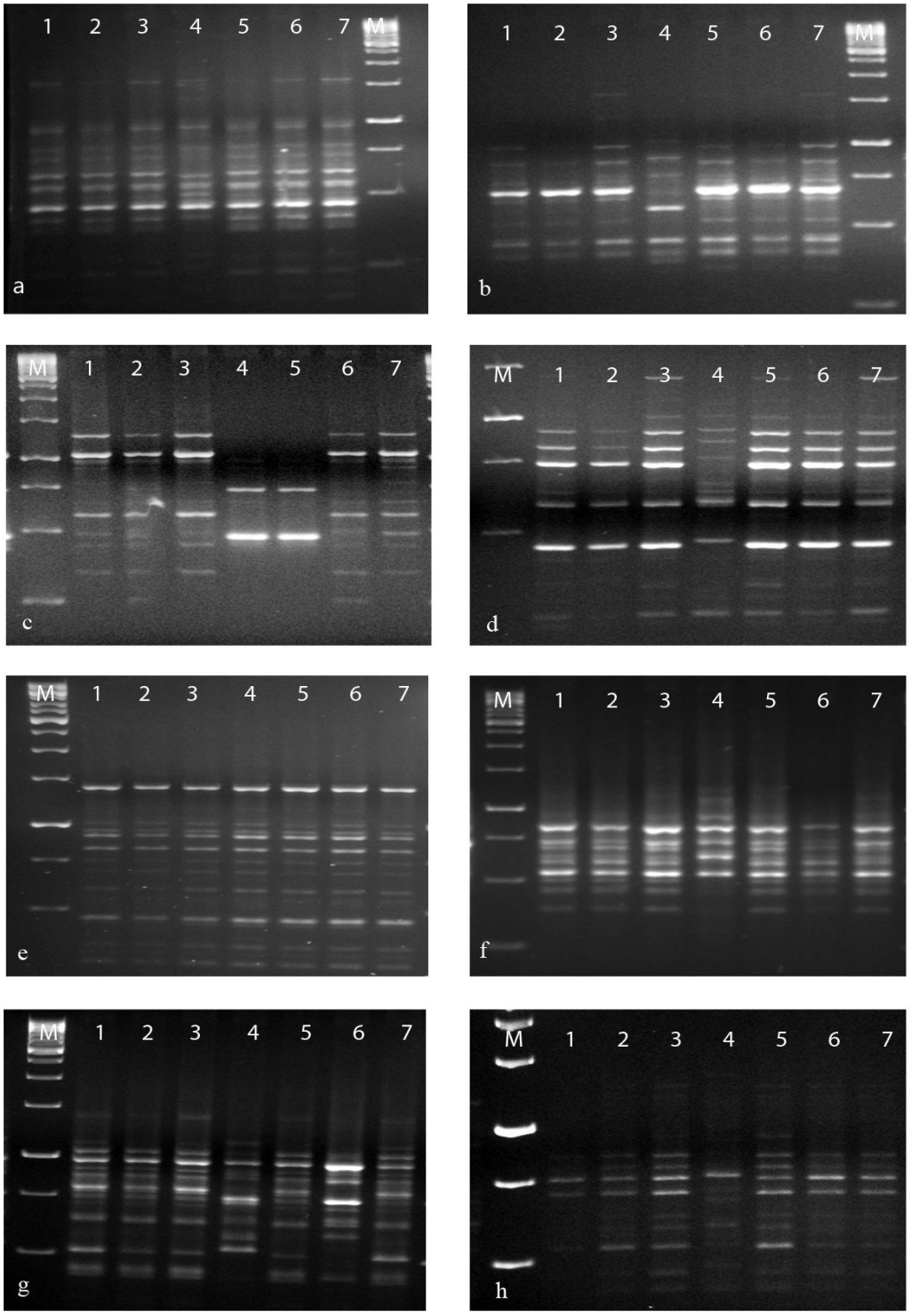


Fig. 39. a-h are the RAPD profiles of *C. aromatica* using primers OPC 20, OPD 20, OPA 08, OPA 04, OPC 05, OPB 10, OPAF 15 and OPAF 14. M-marker (1kb ladder), Lane 1-7 are Acc. 711, Acc. 1025, Acc. 1113, Acc. 1124, Acc. 1132, Acc. 1518 and Acc. 1520, respectively.

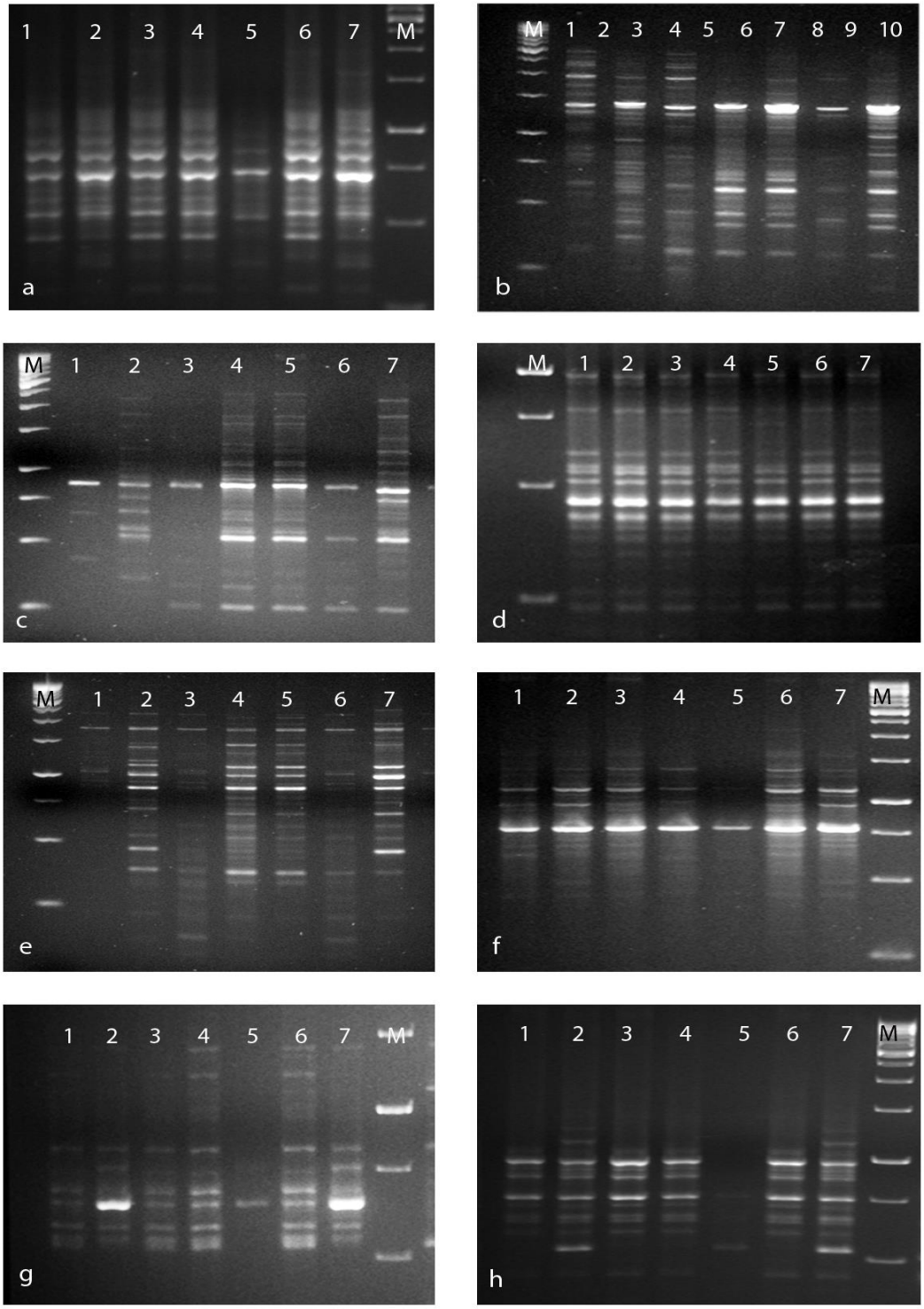


Fig. 40. a-h are the RAPD profiles of *C. caesia* using primersOPN 16, OPA 04, OPC 03, OPC 05, OPA 07, OPA 19, OPL 03 and OPAF 15. M-marker (1kb ladder), Lane 1-7 are Acc. 292, Acc. 751, Acc. 1001, Acc. 1006, Acc. 1135, Acc. 1154 and Acc. 1171, respectively.

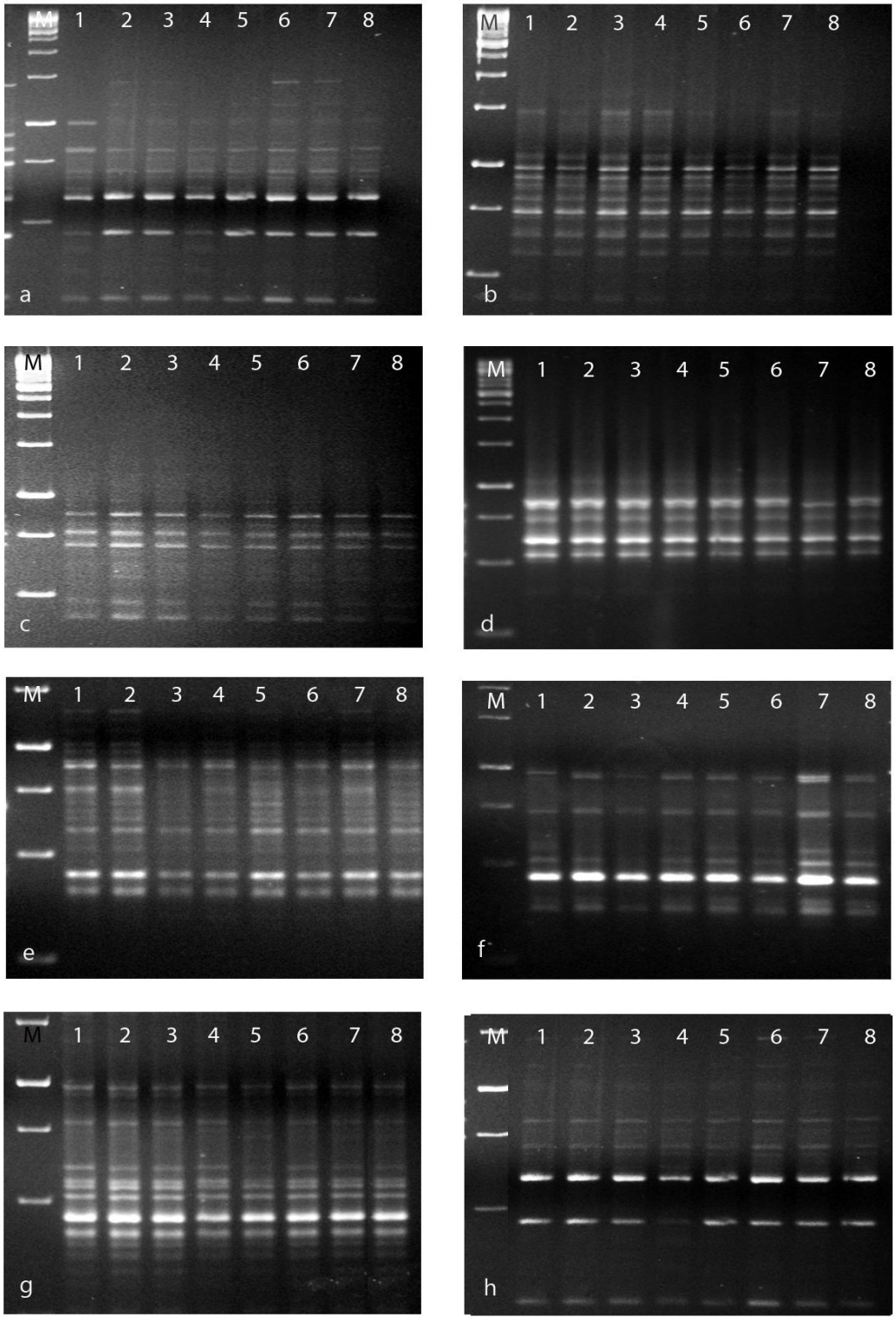


Fig. 41. a-h are the RAPD profiles of *C. xanthorrhiza* using primers OPA 04, OPAF 15, OPA 07, OPB 10, OPD 20, OPX 15, OPAF 14 and OPC 20. M-marker (1 kb ladder), Lane 1-8 are Acc. 465, Acc. 760, Acc. 1108, Acc. 1122, Acc. 1163, Acc. 1164, Acc. 1167 and Acc. 1168, respectively.

Table 101. Sequence information and amplification details of RAPD primers used in four *Curcuma* species.

Primer	Sequence (5'-3')	Total no. of bands	Total no of polymorphic bands	POL (%)	PIC	MI
OPA1	CAGGCCCTTC	28	19	68	0.34	22.77
OPA4	AATCGGGCTG	47	32	68	0.27	18.14
OPA5	AGGGGTCTTG	36	31	86	0.32	27.23
OPA7	GAAACGGGTG	40	30	75	0.25	18.42
OPA8	GTGACGTAGG	31	25	81	0.30	24.26
OPA17	GACCGCTTGT	53	30	57	0.24	13.86
OPA19	CAAACGTCGG	27	10	37	0.32	12.04
OPB10	CTGCTGGGAC	40	22	55	0.19	10.50
OPC1	TTCGAGCCAG	32	23	72	0.26	18.69
OPC3	GGGGGTCTTT	42	27	64	0.28	18.30
OPC5	GATGACCGCC	51	39	76	0.34	26.13
OPC20	ACTTCGCCAC	41	16	39	0.15	5.73
OPD3	GTCGCCGTCA	27	22	81	0.33	27.04
OPD4	TCTGGTGAGG	42	22	52	0.19	9.78
OPD20	ACCCGGTCAC	37	26	70	0.25	17.49
OPL3	CCAGCAGCTT	34	26	76	0.30	22.63
OPN4	GACCGACCCA	40	29	73	0.31	22.44
OPN16	AAGCGACCTG	52	32	62	0.23	14.28
OPN18	GGTGAGGTCA	28	16	57	0.18	10.01
OPO6	CCACGGGAAG	35	26	74	0.30	21.93
OPX5	CCTTTCCCTC	36	14	39	0.15	5.89
OPX8	CAGGGGTGGA	30	19	63	0.25	15.96
OPX14	ACAGGTGCTG	42	31	74	0.30	22.09
OPAF5	CCCGATCAGA	45	33	73	0.28	20.63
OPAF14	GGTGCGCACT	37	27	73	0.25	17.92
OPAF15	CACGAACCTC	41	21	51	0.19	9.91
Mean		38	25	65	0.26	17.46

PIC-Polymorphism Information Content, MI-Marker Index, POL-Polymorphism

4.4.2.1. Genetic diversity analysis using RAPD data

The average values of observed alleles (Na), effective alleles (Ne), Nei's genetic diversity index (h) and Shannon's information Index (I) among the four species are presented in Table 102.

Table 102. Estimates of genetic variance using RAPD in four *Curcuma* species

Species	n	(Na)	(Ne)	(h)	(I)	% P	Gst
C. amada	10	1.23 ± 0.13	1.14 ± 0.09	0.08 ± 0.05	0.13 ± 0.08	66.02	
C. aromatica	7	1.31 ± 0.17	1.17 ± 0.11	0.11 ± 0.06	0.16 ± 0.09	67.37	
C. caesia	7	1.25 ± 0.16	1.15 ± 0.11	0.09 ± 0.06	0.13 ± 0.09	75.07	
C. xanthorrhiza	8	1.22 ± 0.14	1.11 ± 0.09	0.07 ± 0.05	0.11 ± 0.07	54.65	
Inter species	32	2.00 ± 0.00	1.56 ± 0.05	0.34 ± 0.02	0.51 ± 0.03	65.29	0.74

Na - total number of alleles; Ne - number of effective alleles; h - Nei's (1973) gene diversity index; I - Shannon's information index; %P - percentage of polymorphism; genetic differentiation Gst = (Ht-Hs)/Ht; Nm = 0.5*(1-Gst)/Gst

The observed number of alleles ranged from 1.22 ± 0.14 (*C. xanthorrhiza*) to 1.31 ± 0.17 (*C. aromatica*). Nei's gene diversity index (h) and Shannon's information index (I) were highest for *C. aromatica* (h = 0.11 ± 0.06; I = 0.16 ± 0.09), whereas they were lowest in *C. xanthorrhiza* (h = 0.07 ± 0.05; I = 0.11 ± 0.07). Highest percentage of polymorphism was displayed by *C. caesia* (%P = 75.07%) and the lowest by *C. xanthorrhiza* (%P = 54.65%), with an average value of 65.29%. High Gst value (0.74) shows that species are well differentiated from each other.

4.4.2.2. Cluster analysis

4.4.2.2.1. Cluster analysis of individual species.

The genetic similarity was calculated using Simple Matching similarity coefficients for individual species. In *C. amada*, the similarity matrices showed the maximum similarity between Acc. 521 and Acc. 265, with a value of 0.89. The least similar accessions were Acc.848 and Acc.521 with a value of 0.58 in the Simple Matching similarity matrix (Table 103).

Table 103. RAPD similarity matrix of different accessions of *C. amada*

Accession	265	347	521	752	753	848	1119	1503	1511	6390
265	1.00									
347	0.81	1.00								
521	0.89	0.79	1.00							
752	0.66	0.63	0.67	1.00						
753	0.74	0.80	0.69	0.71	1.00					
848	0.60	0.67	0.58	0.79	0.74	1.00				
1119	0.71	0.74	0.70	0.69	0.81	0.71	1.00			
1503	0.81	0.81	0.84	0.61	0.72	0.64	0.71	1.00		
1511	0.81	0.81	0.80	0.69	0.75	0.66	0.66	0.83	1.00	
6390	0.77	0.74	0.75	0.65	0.69	0.62	0.70	0.79	0.77	1.00

The UPGMA dendrogram constructed using the Simple Matching similarity matrix clustered the ten accessions of *C. amada* into four groups (Figure 42) splitting at coefficient value of 0.765. The groups formed were as follows:

Group I - Acc. 265, Acc.521, Acc. 1503, Acc.1511 and Acc. 347

Group II - Acc.6390

Group III - Acc. 753 and Acc. 1119

Group IV - Acc.752 and Acc. 848

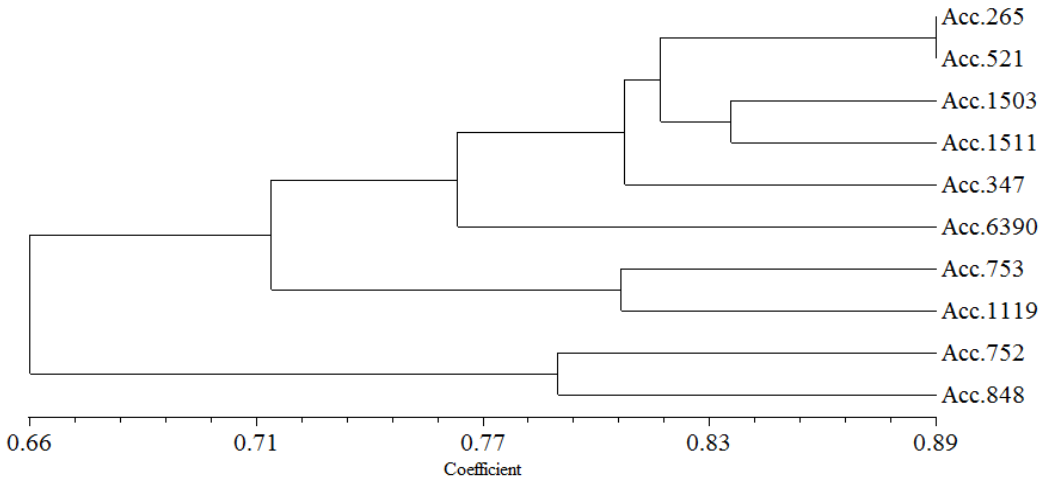


Fig. 42. RAPD based UPGMA-Dendrogram of genetic similarity among ten accessions of *C. amada.*

The first group, which comprised of five accessions, formed the major group, with a maximum similarity between Acc.265 and Acc.521 which were collected from two different locations, *i.e*. Kozhikode (Kerala) and Thrissur (Kerala). The second group was formed by a single accession Acc.6390, which was collected from Gundimeda (Vijayawada, Andhra Pradesh). The third group was formed Acc. 753 (Cooch Behar, West Bengal) and Acc. 1119 (Thrissur, Kerala) and the last group was formed by Acc. 752 (Cooch Behar, West Bengal) and Acc. 848 (Mamit, Mizoram).

RAPD similarity matrix of *C. aromatica* showed maximum similarity between Acc. 1132 and Acc. 1518 (0.84). The minimum similarity was between Acc. 1025 and Acc. 1124 (0.56) (Table 104).

Table 104. RAPD similarity matrix of different accessions of *C. aromatica*

Accession	711	1025	1113	1124	1132	1518	1520
711	1.00						
1025	0.72	1.00					
1113	0.78	0.77	1.00				
1124	0.58	0.56	0.58	1.00			
1132	0.74	0.81	0.82	0.71	1.00		
1518	0.82	0.78	0.84	0.62	0.84	1.00	
1520	0.73	0.78	0.76	0.59	0.82	0.79	1.00

The UPGMA dendrogram constructed using the Simple Matching similarity matrix clustered the seven accessions of *C. aromatica* into two groups (Figure 43) splitting at coefficient value of 0.768. The groups formed were as follows:

Group I - Acc. 711, Acc.1025, Acc.1113, Acc.1132, Acc.1518, Acc.1520

Group II - Acc.1124

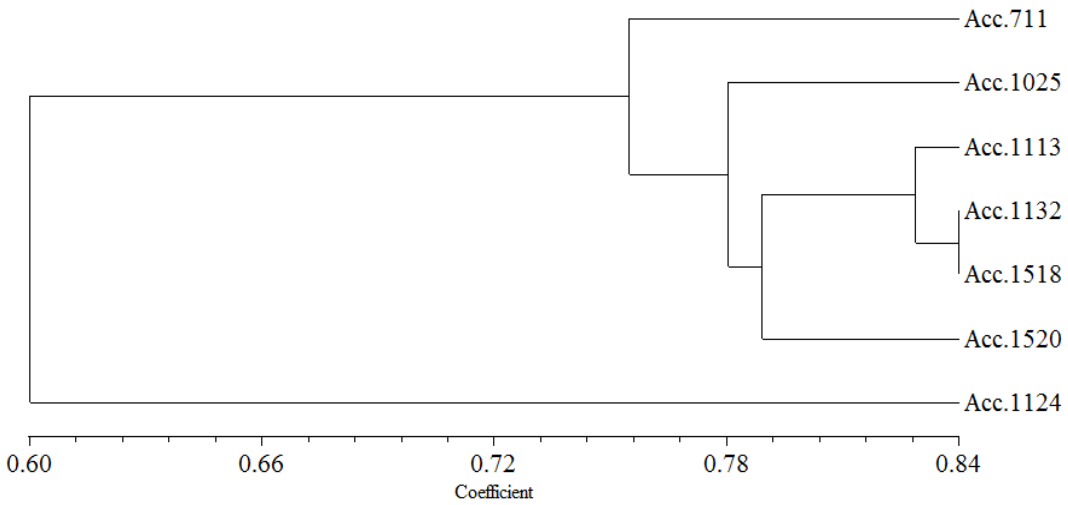


Fig. 43. RAPD based UPGMA-Dendrogram of genetic similarity among seven accessions of *C. aromatica.*

The first group was formed by six accessions, in which Acc.1132 (Tirunelveli, Tamil Nadu) and Acc.1518 (Idukki, Kerala) showed the highest similarity with each other. The second group was formed by Acc.1124 (Ernakulam, Kerala) which was distinct from the rest of the accessions. Among the seven accessions of *C. caesia*, maximum similarity was found between Acc. 1001 and Acc. 1154 with a coefficient value of 0.82 and the minimum similarity was between Acc. 292 and Acc. 1135 (0.48) (Table 105). The dendrogram obtained from the data is given in Figure 44.

Table 105. RAPD similarity matrix of different accessions of *C. caesia*

Accession	292	751	1001	1006	1135	1154	1171
292	1.00						
751	0.67	1.00					
1001	0.81	0.68	1.00				
1006	0.73	0.77	0.80	1.00			
1135	0.48	0.57	0.55	0.59	1.00		
1154	0.75	0.69	0.82	0.77	0.55	1.00	
1171	0.62	0.78	0.67	0.80	0.53	0.73	1.00

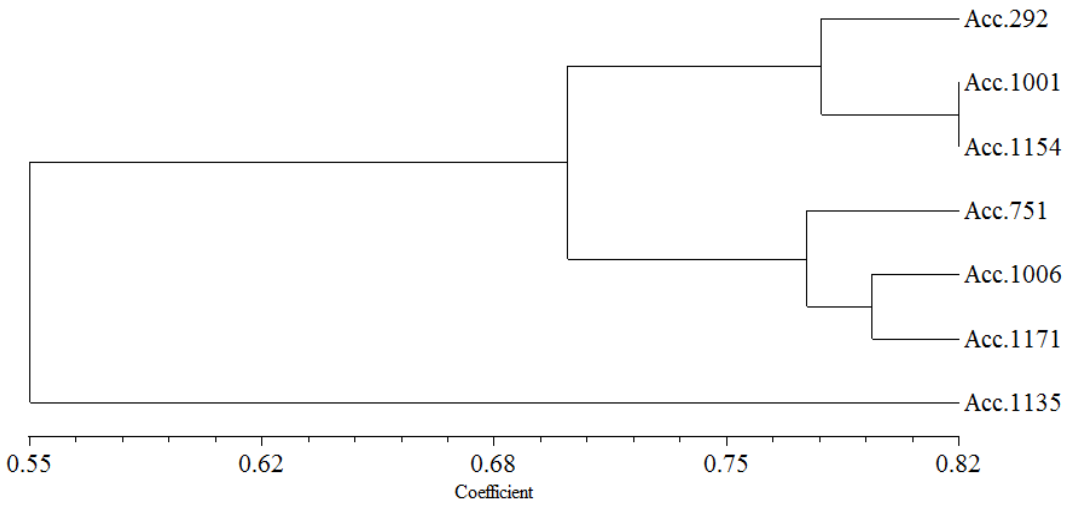


Fig. 44. RAPD based UPGMA-Dendrogram of genetic similarity among seven accessions of *C. caesia*

The UPGMA dendrogram clustered the seven accessions into three groups splitting at a coefficient value of 0.777. The groupings were as below:

Group I	-	Acc. 292, Acc. 1001 and Acc. 1154
Group II	-	Acc. 751, Acc. 1006 and Acc. 1171
Group III	-	Acc. 1135

The first and second group contains three accessions each, whereas the third group contains only one accession. In the first group, the maximum similarity (0.82) was between Acc. 1001(Lohit, Arunachal Pradesh) and Acc. 1154 (Thrissur, Kerala), although they were collected from two different geographical regions of India. Acc. 751, Acc.1006 and Acc.1171 formed the second group. Acc.1006 and Acc.1171 exhibited maximum similarity within the subgroup with a similarity value of 0.80. The third group consisting of the lone accession, Acc.1135 which was collected from Thrissur (Kerala) showed least genetic similarity with rest of the accessions of *C. caesia.*

In *C. xanthhorrhiza*, the similarity matrix using Simple Matching similarity coefficients obtained from the RAPD data showed the highest similarity between Acc. 1122 and Acc. 1163 with a coefficient value of 0.88. The lowest similarity was between Acc. 465 and Acc.1167 with a coefficient value of 0.69 (Table 106).

Table 106. RAPD similarity matrix of different accessions of *C. xanthorrhiza*

Accession	465	760	1108	1122	1163	1164	1167	1168
465	1.00							
760	0.78	1.00						
1108	0.78	0.82	1.00					
1122	0.78	0.86	0.84	1.00				
1163	0.73	0.81	0.79	0.88	1.00			
1164	0.70	0.79	0.81	0.83	0.86	1.00		
1167	0.69	0.84	0.74	0.84	0.86	0.85	1.00	
1168	0.80	0.78	0.77	0.80	0.77	0.76	0.73	1.00

The dendrogram (UPGMA) constructed using Simple Matching similarity coefficients is depicted in Figure 45.

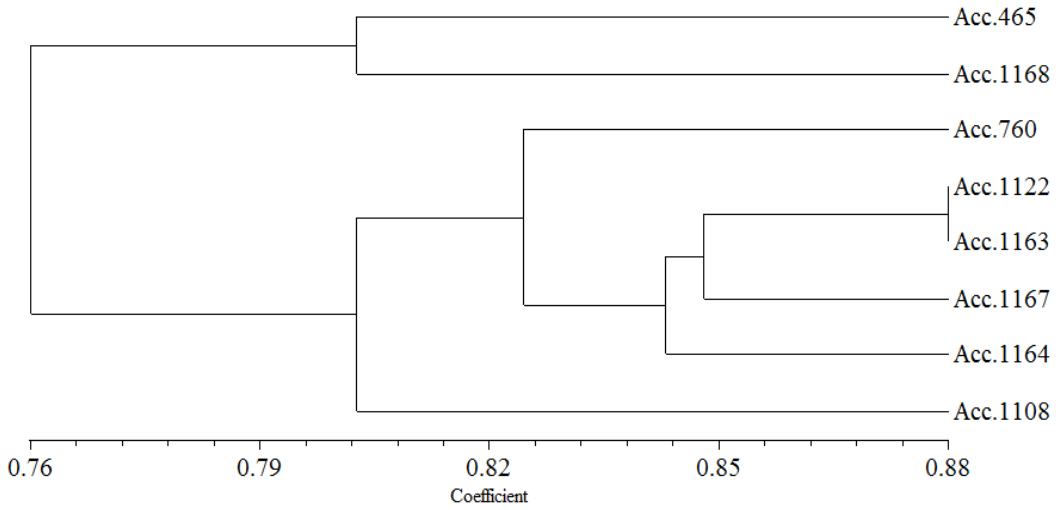


Fig. 45. RAPD based UPGMA-Dendrogram of genetic similarity among eight accessions of *C. xanthorrhiza*

The UPGMA dendrogram constructed using the Simple Matching similarity coefficients clustered the eight accessions into four groups splitting at coefficient value of 0.819. The groups formed were as below:

Group I - Acc. 465

Group II - Acc. 1168

Group III	-	Acc. 760, Acc. 1122, Acc. 1163, Acc. 1167 and Acc. 1164
Group IV	-	Acc. 1108

First, second and fourth group have only one accession each namely Acc. 465, Acc. 1168 and Acc. 1108. In third group, which comprised of five accessions, Acc. 1122 and 1163 were closely related to each other with coefficient value 0.88, which were collected from Palakkad and Kollam districts of Kerala.

4.4.2.2.2. Cluster analysis of Four Species

The similarity matrices for the thirty-two accessions from the four species were constructed using Simple Matching, Jaccard's and Sorensen-Dice algorithms. (Tables 107, 108 &109). The dendrogram (UPGMA) constructed using the three similarity coefficients are depicted in Figures 46, 47 and 48.

The UPGMA dendrogram based on the Simple Matching, Jaccard's and Sorensen-Dice similarity coefficients clustered thirty-two accessions into four groups splitting at coefficients 0.822, 0.470 and 0.638, respectively in Simple matching, Jaccard's and Sorensen-Dice UPGMAs.

The four groupings were:

Group I	-	*C. amada* Acc. 265, 521, 1503, 1511, 347. 6390, 753, 1119, 752 and 848
Group II	-	*C. caesia* Acc. 292, 1001, 1154, 751, 1006, 1171 and 1135
Group III	-	*C. xanthorrhiza* Acc. 465, 1168, 760, 1122, 1163, 1167, 1164 and 1108
Group IV	-	*C. aromatica* Acc. 711, 1025, 1113, 1132, 1518, 1520 and 1124

The UPGMA based SAHN clustering program has clustered the 32 accessions into 4 different groups, each group containing accessions of their respective species. Group 1 contained all the ten accessions of *C. amada*, group 2 was formed by seven accessions of *C. caesia*. Likewise, group 3 and 4 were formed by eight accessions of C. *xanthorrhiza* and seven accessions of *C. aromatica*, respectively. The only differences between the clusters obtained through Simple matching coefficients and Jaccard's / Sorensen-Dice was that Simple Matching showed closer association between *C. amada* and *C. caesia* as obtained by the Nei's genetic distance algorithm,

whereas Jaccard's and Sorensen dice showed a closer association between *C. aromatica* and *C. xanthorrhiza*.

Among the accessions, the maximum similarity was observed between Acc. 265 and Acc.521 of *C. amada* (SM coefficient = 0.96, Jaccard's = 0.87 and Dice = 0.93) followed by Acc.1122 and Acc.1163 as well as Acc. 1122 and Acc.760 of *C. xanthorrhiza*. Genetically least similar were Acc. 1511 (*C. amada*) and Acc. 711 (*C. aromatica)* with a similarity coefficient of 0.52 for Simple Matching similarity coefficient. Besides Acc. 848 (*C. amada*) and Acc. 1001 (*C. caesia*) with coefficient values 0.08 and 0.15 for Jaccard's and Sorensen-Dice, respectively. Acc. 752 and Acc. 1001, Acc. 711 and Acc. 6390 also exhibited the least similarity in Jaccard's and Sorensen-Dice similarity coefficients.

Table 107. RAPD similarity matrix of four *Curcuma* species using Simple Matching similarity coefficients

		C.amada										*C.aromatica*							*C.caesia*							*C.xanthorrhiza*							
		265	347	521	752	753	848	1119	1503	1511	6390	711	1025	1113	1124	1132	1518	1520	292	751	1001	1006	1135	1154	1171	465	760	1108	1122	1163	1164	1167	1168
C.amada	265	1.00																															
	347	0.94	1.00																														
	521	0.96	0.93	1.00																													
	752	0.88	0.87	0.88	1.00																												
	753	0.91	0.93	0.89	0.90	1.00																											
	848	0.86	0.89	0.85	0.93	0.91	1.00																										
	1119	0.90	0.91	0.89	0.89	0.93	0.90	1.00																									
	1503	0.93	0.93	0.95	0.87	0.90	0.88	0.90	1.00																								
	1511	0.94	0.93	0.93	0.89	0.91	0.88	0.88	0.94	1.00																							
	6390	0.92	0.91	0.91	0.88	0.89	0.87	0.90	0.93	0.92	1.00																						
C.aromatica	711	0.53	0.54	0.52	0.54	0.56	0.56	0.57	0.53	0.52	0.53	1.00																					
	1025	0.53	0.55	0.54	0.57	0.56	0.58	0.58	0.54	0.53	0.54	0.87	1.00																				
	1113	0.55	0.56	0.54	0.57	0.58	0.58	0.59	0.55	0.54	0.56	0.90	0.89	1.00																			
	1124	0.53	0.54	0.53	0.55	0.55	0.56	0.57	0.54	0.52	0.53	0.80	0.80	0.80	1.00																		
	1132	0.53	0.54	0.53	0.55	0.55	0.56	0.56	0.53	0.52	0.53	0.88	0.91	0.91	0.88	1.00																	
	1518	0.54	0.55	0.54	0.55	0.57	0.57	0.58	0.54	0.53	0.55	0.91	0.89	0.92	0.83	0.92	1.00																
	1520	0.53	0.54	0.52	0.55	0.55	0.56	0.57	0.53	0.52	0.53	0.87	0.90	0.88	0.82	0.92	0.90	1.00															
C.caesia	292	0.60	0.61	0.62	0.63	0.63	0.64	0.66	0.61	0.59	0.63	0.60	0.59	0.62	0.58	0.57	0.61	0.58	1.00														
	751	0.60	0.61	0.62	0.63	0.63	0.64	0.66	0.61	0.59	0.62	0.58	0.59	0.61	0.58	0.57	0.60	0.59	0.89	1.00													
	1001	0.59	0.60	0.60	0.61	0.61	0.62	0.64	0.60	0.58	0.62	0.58	0.57	0.62	0.58	0.57	0.60	0.58	0.94	0.90	1.00												
	1006	0.58	0.59	0.60	0.61	0.60	0.62	0.64	0.59	0.57	0.60	0.57	0.56	0.59	0.57	0.56	0.58	0.57	0.91	0.93	0.94	1.00											
	1135	0.64	0.65	0.65	0.68	0.66	0.69	0.70	0.65	0.63	0.67	0.62	0.61	0.65	0.63	0.61	0.63	0.62	0.83	0.86	0.86	0.87	1.00										
	1154	0.59	0.61	0.61	0.63	0.62	0.63	0.65	0.61	0.59	0.62	0.58	0.56	0.61	0.57	0.56	0.60	0.57	0.92	0.90	0.94	0.93	0.86	1.00									
	1171	0.58	0.59	0.60	0.61	0.61	0.62	0.64	0.59	0.58	0.60	0.55	0.56	0.59	0.56	0.55	0.57	0.56	0.88	0.93	0.90	0.94	0.85	0.91	1.00								
C.xanthorrhiza	465	0.56	0.59	0.57	0.57	0.58	0.58	0.59	0.57	0.56	0.58	0.54	0.56	0.56	0.54	0.53	0.55	0.55	0.60	0.60	0.59	0.58	0.61	0.57	0.57	1.00							
	760	0.54	0.56	0.55	0.55	0.56	0.57	0.57	0.55	0.54	0.55	0.53	0.54	0.54	0.52	0.52	0.54	0.53	0.59	0.60	0.57	0.57	0.60	0.57	0.57	0.91	1.00						
	1108	0.56	0.59	0.57	0.57	0.59	0.59	0.59	0.58	0.56	0.58	0.54	0.56	0.57	0.54	0.53	0.56	0.55	0.62	0.62	0.60	0.59	0.62	0.59	0.58	0.91	0.93	1.00					
	1122	0.56	0.57	0.57	0.56	0.57	0.57	0.58	0.57	0.55	0.57	0.53	0.54	0.55	0.53	0.52	0.54	0.54	0.59	0.60	0.57	0.57	0.61	0.56	0.57	0.91	0.95	0.93	1.00				
	1163	0.56	0.57	0.57	0.58	0.57	0.58	0.59	0.57	0.55	0.57	0.55	0.56	0.56	0.55	0.54	0.55	0.56	0.60	0.61	0.59	0.58	0.63	0.58	0.58	0.89	0.93	0.91	0.95	1.00			
	1164	0.57	0.59	0.58	0.58	0.58	0.59	0.59	0.58	0.56	0.58	0.55	0.55	0.57	0.54	0.54	0.56	0.55	0.63	0.62	0.61	0.59	0.63	0.59	0.59	0.88	0.92	0.92	0.93	0.95	1.00		
	1167	0.56	0.57	0.57	0.58	0.57	0.58	0.59	0.58	0.56	0.57	0.54	0.55	0.55	0.54	0.53	0.54	0.55	0.60	0.61	0.59	0.58	0.62	0.57	0.58	0.88	0.94	0.90	0.94	0.95	0.94	1.00	
	1168	0.57	0.59	0.58	0.58	0.59	0.60	0.60	0.59	0.57	0.59	0.57	0.58	0.58	0.55	0.56	0.58	0.57	0.62	0.63	0.61	0.60	0.64	0.60	0.59	0.92	0.91	0.90	0.92	0.91	0.91	0.89	1.00

Table 108. RAPD similarity matrix of four *Curcuma* species using Jaccard's similarity coefficients

		C.amada										*C.aromatica*							*C.caesia*							*C.xanthorrhiza*							
		265	347	521	752	753	848	1119	1503	1511	6390	711	1025	1113	1124	1132	1518	1520	292	751	1001	1006	1135	1154	1171	465	760	1108	1122	1163	1164	1167	1168
C.amada	265	1.00																															
	347	0.80	1.00																														
	521	0.87	0.77	1.00																													
	752	0.63	0.61	0.64	1.00																												
	753	0.72	0.77	0.67	0.66	1.00																											
	848	0.58	0.63	0.55	0.73	0.68	1.00																										
	1119	0.67	0.69	0.65	0.62	0.75	0.63	1.00																									
	1503	0.79	0.79	0.82	0.59	0.69	0.60	0.67	1.00																								
	1511	0.80	0.79	0.79	0.67	0.73	0.63	0.63	0.82	1.00																							
	6390	0.74	0.71	0.72	0.60	0.64	0.57	0.64	0.75	0.74	1.00																						
C.aromatica	711	0.13	0.13	0.12	0.11	0.13	0.11	0.12	0.12	0.12	0.10	1.00																					
	1025	0.14	0.15	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.11	0.66	1.00																				
	1113	0.13	0.13	0.11	0.11	0.13	0.11	0.12	0.12	0.13	0.10	0.71	0.69	1.00																			
	1124	0.15	0.15	0.14	0.13	0.14	0.12	0.14	0.14	0.15	0.11	0.55	0.55	0.53	1.00																		
	1132	0.15	0.16	0.15	0.13	0.14	0.13	0.14	0.14	0.15	0.12	0.70	0.77	0.76	0.69	1.00																	
	1518	0.13	0.13	0.12	0.11	0.13	0.11	0.12	0.13	0.13	0.10	0.76	0.71	0.77	0.59	0.80	1.00																
	1520	0.14	0.15	0.13	0.12	0.14	0.12	0.13	0.13	0.14	0.10	0.67	0.74	0.69	0.57	0.78	0.73	1.00															
C.caesia	292	0.12	0.12	0.13	0.11	0.11	0.10	0.12	0.12	0.11	0.11	0.15	0.14	0.15	0.15	0.14	0.15	0.14	1.00														
	751	0.13	0.13	0.14	0.13	0.13	0.12	0.14	0.13	0.13	0.11	0.13	0.16	0.14	0.15	0.15	0.15	0.16	0.61	1.00													
	1001	0.11	0.11	0.12	0.09	0.10	0.08	0.11	0.10	0.10	0.10	0.13	0.12	0.15	0.14	0.14	0.14	0.14	0.75	0.63	1.00												
	1006	0.14	0.14	0.15	0.13	0.13	0.12	0.14	0.13	0.13	0.12	0.15	0.15	0.15	0.17	0.16	0.15	0.16	0.68	0.74	0.76	1.00											
	1135	0.11	0.12	0.13	0.12	0.11	0.11	0.14	0.11	0.11	0.11	0.12	0.11	0.14	0.16	0.14	0.12	0.14	0.39	0.48	0.46	0.53	1.00										
	1154	0.12	0.13	0.13	0.11	0.11	0.10	0.12	0.12	0.12	0.11	0.13	0.12	0.14	0.14	0.13	0.15	0.13	0.68	0.64	0.78	0.74	0.46	1.00									
	1171	0.14	0.15	0.16	0.14	0.14	0.13	0.15	0.14	0.14	0.12	0.14	0.15	0.15	0.17	0.16	0.15	0.16	0.59	0.75	0.65	0.78	0.49	0.70	1.00								
C.xanthorrhiza	465	0.17	0.19	0.18	0.14	0.15	0.14	0.14	0.17	0.17	0.15	0.17	0.20	0.17	0.18	0.18	0.17	0.19	0.15	0.16	0.14	0.17	0.12	0.13	0.16	1.00							
	760	0.16	0.17	0.17	0.14	0.15	0.14	0.14	0.16	0.16	0.14	0.17	0.19	0.16	0.18	0.18	0.17	0.18	0.16	0.17	0.14	0.17	0.12	0.13	0.18	0.77	1.00						
	1108	0.14	0.16	0.15	0.12	0.14	0.12	0.12	0.15	0.15	0.13	0.15	0.18	0.15	0.16	0.16	0.16	0.18	0.15	0.16	0.13	0.15	0.10	0.12	0.14	0.75	0.79	1.00					
	1122	0.17	0.18	0.18	0.14	0.15	0.14	0.14	0.17	0.17	0.14	0.16	0.19	0.16	0.18	0.18	0.17	0.19	0.15	0.17	0.13	0.17	0.12	0.12	0.16	0.77	0.85	0.80	1.00				
	1163	0.16	0.16	0.16	0.14	0.14	0.13	0.13	0.16	0.16	0.13	0.17	0.19	0.16	0.19	0.18	0.17	0.20	0.14	0.17	0.13	0.16	0.12	0.12	0.16	0.71	0.80	0.75	0.87	1.00			
	1164	0.15	0.17	0.16	0.13	0.14	0.12	0.12	0.16	0.15	0.13	0.16	0.17	0.15	0.17	0.17	0.16	0.17	0.16	0.17	0.14	0.16	0.12	0.13	0.16	0.69	0.78	0.77	0.81	0.84	1.00		
	1167	0.17	0.17	0.17	0.15	0.15	0.14	0.14	0.17	0.17	0.14	0.17	0.18	0.16	0.19	0.18	0.17	0.19	0.15	0.17	0.14	0.17	0.13	0.13	0.17	0.69	0.83	0.71	0.83	0.85	0.83	1.00	
	1168	0.15	0.17	0.15	0.12	0.14	0.13	0.13	0.15	0.15	0.14	0.18	0.19	0.17	0.18	0.18	0.18	0.18	0.14	0.17	0.14	0.17	0.12	0.12	0.16	0.76	0.76	0.71	0.77	0.74	0.72	0.70	1.00

Table 109. RAPD similarity matrix of four *Curcuma* species using Sorensen-Dice similarity coefficients

		C.amada										*C.aromatica*							*C.caesia*							*C.xanthorrhiza*							
		265	347	521	752	753	848	1119	1503	1511	6390	711	1025	1113	1124	1132	1518	1520	292	751	1001	1006	1135	1154	1171	465	760	1108	1122	1163	1164	1167	1168
C.amada	265	1.00																															
	347	0.89	1.00																														
	521	0.93	0.87	1.00																													
	752	0.78	0.75	0.78	1.00																												
	753	0.83	0.87	0.80	0.79	1.00																											
	848	0.73	0.78	0.71	0.85	0.81	1.00																										
	1119	0.80	0.82	0.79	0.77	0.86	0.77	1.00																									
	1503	0.88	0.88	0.90	0.74	0.82	0.75	0.80	1.00																								
	1511	0.89	0.89	0.88	0.80	0.84	0.77	0.78	0.90	1.00																							
	6390	0.85	0.83	0.84	0.75	0.78	0.72	0.78	0.86	0.85	1.00																						
C.aromatica	711	0.23	0.23	0.21	0.19	0.23	0.20	0.21	0.22	0.22	0.17	1.00																					
	1025	0.25	0.26	0.24	0.24	0.25	0.25	0.24	0.24	0.25	0.19	0.80	1.00																				
	1113	0.23	0.23	0.21	0.19	0.22	0.20	0.21	0.21	0.22	0.18	0.83	0.82	1.00																			
	1124	0.26	0.26	0.25	0.23	0.25	0.22	0.25	0.25	0.26	0.20	0.71	0.71	0.69	1.00																		
	1132	0.27	0.27	0.26	0.24	0.25	0.23	0.24	0.25	0.26	0.21	0.82	0.87	0.86	0.82	1.00																	
	1518	0.23	0.24	0.22	0.20	0.23	0.20	0.21	0.22	0.23	0.19	0.86	0.83	0.87	0.74	0.89	1.00																
	1520	0.25	0.25	0.23	0.22	0.24	0.21	0.23	0.23	0.25	0.19	0.80	0.85	0.81	0.73	0.88	0.84	1.00															
C.caesia	292	0.22	0.22	0.23	0.20	0.19	0.18	0.22	0.21	0.20	0.19	0.26	0.24	0.26	0.26	0.25	0.26	0.25	1.00														
	751	0.23	0.23	0.25	0.23	0.22	0.22	0.25	0.23	0.22	0.20	0.23	0.27	0.25	0.27	0.26	0.26	0.27	0.76	1.00													
	1001	0.20	0.20	0.21	0.17	0.18	0.15	0.20	0.19	0.18	0.18	0.23	0.21	0.26	0.25	0.25	0.25	0.24	0.86	0.77	1.00												
	1006	0.24	0.25	0.26	0.23	0.23	0.22	0.25	0.24	0.23	0.21	0.25	0.26	0.27	0.29	0.28	0.27	0.28	0.81	0.85	0.87	1.00											
	1135	0.21	0.21	0.23	0.21	0.19	0.20	0.24	0.21	0.20	0.20	0.21	0.21	0.24	0.27	0.25	0.22	0.25	0.56	0.65	0.63	0.70	1.00										
	1154	0.21	0.22	0.24	0.20	0.19	0.18	0.22	0.21	0.21	0.19	0.23	0.21	0.24	0.25	0.23	0.26	0.23	0.81	0.78	0.87	0.85	0.63	1.00									
	1171	0.25	0.26	0.27	0.25	0.24	0.23	0.26	0.25	0.25	0.21	0.24	0.26	0.26	0.29	0.27	0.26	0.28	0.74	0.86	0.79	0.88	0.65	0.82	1.00								
C.xanthorrhiza	465	0.29	0.32	0.30	0.24	0.27	0.24	0.25	0.29	0.30	0.26	0.30	0.33	0.29	0.31	0.31	0.30	0.32	0.26	0.28	0.25	0.29	0.21	0.23	0.28	1.00							
	760	0.28	0.30	0.29	0.24	0.26	0.24	0.24	0.28	0.28	0.24	0.29	0.32	0.28	0.31	0.30	0.29	0.31	0.27	0.30	0.24	0.30	0.22	0.24	0.30	0.87	1.00						
	1108	0.25	0.28	0.26	0.21	0.24	0.21	0.22	0.26	0.25	0.22	0.26	0.30	0.27	0.27	0.28	0.27	0.30	0.26	0.27	0.23	0.26	0.18	0.21	0.25	0.86	0.88	1.00					
	1122	0.29	0.31	0.30	0.25	0.26	0.24	0.25	0.29	0.29	0.25	0.28	0.31	0.28	0.30	0.30	0.28	0.32	0.26	0.29	0.23	0.29	0.21	0.22	0.28	0.87	0.92	0.89	1.00				
	1163	0.27	0.28	0.28	0.24	0.24	0.23	0.23	0.27	0.27	0.23	0.29	0.32	0.27	0.31	0.31	0.29	0.33	0.25	0.29	0.23	0.27	0.22	0.22	0.28	0.83	0.89	0.86	0.93	1.00			
	1164	0.27	0.29	0.27	0.23	0.24	0.22	0.22	0.27	0.26	0.23	0.27	0.29	0.27	0.29	0.29	0.28	0.29	0.28	0.29	0.25	0.28	0.21	0.23	0.27	0.81	0.87	0.87	0.89	0.91	1.00		
	1167	0.29	0.30	0.29	0.26	0.26	0.24	0.25	0.29	0.29	0.25	0.29	0.31	0.27	0.32	0.31	0.28	0.32	0.26	0.29	0.24	0.29	0.22	0.23	0.29	0.81	0.91	0.83	0.90	0.92	0.91	1.00	
	1168	0.26	0.28	0.27	0.22	0.25	0.23	0.22	0.27	0.26	0.24	0.30	0.32	0.29	0.30	0.31	0.30	0.31	0.25	0.29	0.24	0.28	0.21	0.22	0.27	0.87	0.86	0.83	0.87	0.85	0.84	0.83	1.00

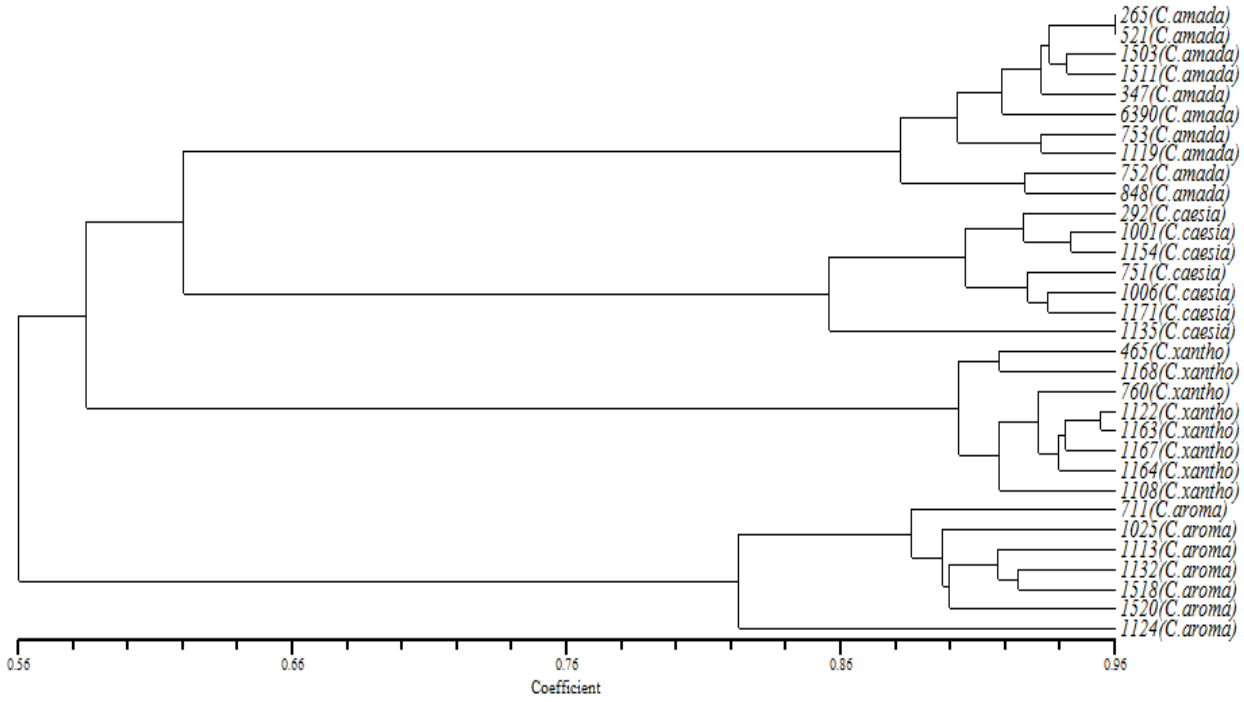


Fig. 46. UPGMA dendrogram of genetic similarity among four *Curcuma* species based on RAPD markers using Simple Matching similarity coefficients

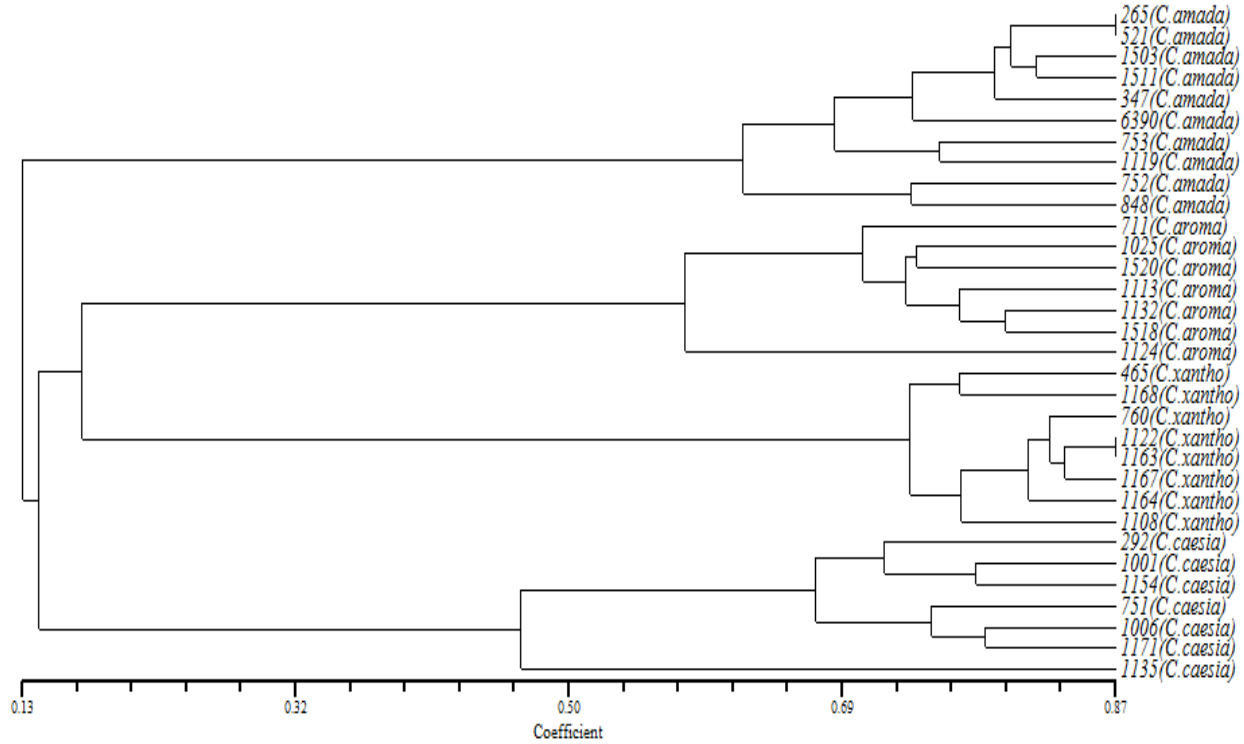


Fig. 47. UPGMA dendrogram of genetic similarity among four *Curcuma* species based on RAPD markers using Jaccard's similarity coefficients

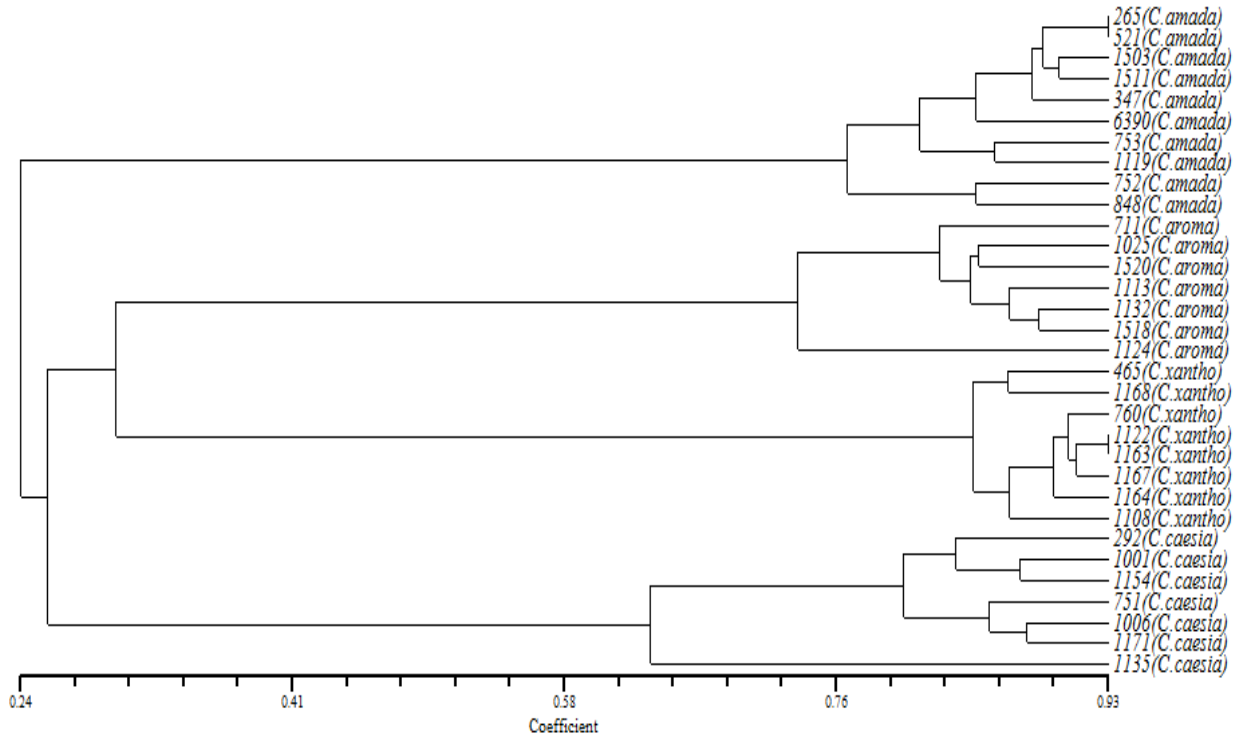


Fig.48. UPGMA dendrogram of genetic similarity among four *Curcuma* species based on RAPD markers using Sorensen-Dice similarity coefficients

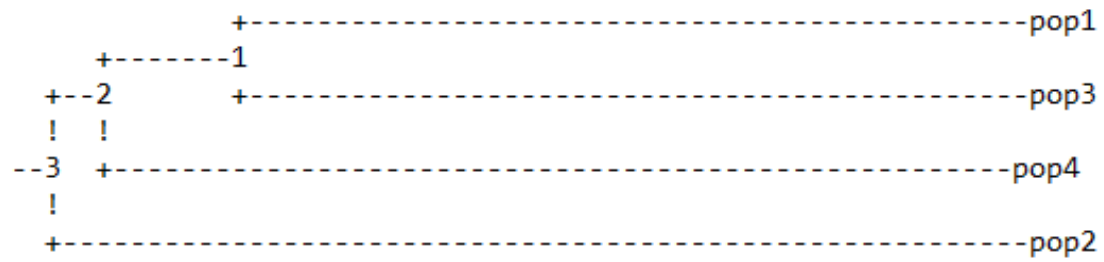
4.4.2.3. Genetic distance among the species

The Nei's genetic distance (1972) among the species was estimated using the POPGENE. Table 110 shows the Nei's genetic distance among the species. The upper diagonal matrix shows the Nei's genetic identity and the lower diagonal matrix shows the Nei's genetic distance.

Table 110. Nei's genetic distance and genetic identity of four *Curcuma* species using RAPD data

Species	*C. amada*	*C. aromatica*	*C. caesia*	*C. xanthorrhiza*
C. amada	****	0.604	0.677	0.622
C. aromatica	0.504	****	0.649	0.601
C. caesia	0.391	0.433	****	0.647
C. xanthorrhiza	0.475	0.509	0.435	****

C. amada and *C. caesia* showed closer association (0.677) with lowest genetic distance value of 0.391 followed by *C. xanthorrhiza* and *C. aromatica.* The dendrogram constructed using Nei's genetic distance and genetic identity is given in Figure 49.



pop1 – C. amada; pop2 – C. aromatica; pop3 – C. caesia; pop4 – C. xanthorrhiza

Fig. 49. Nei's genetic distance based dendrogram of genetic similarity among four *Curcuma* species based on RAPD markers.

4.4.3. ISSR Analysis

Twenty-one ISSR primers were used to characterize the inter and intraspecific genetic diversity among the four *Curcuma* species, including thirty-two individual accessions which resulted in the amplification of 784 bands of which 440 were polymorphic bands. The bands amplified were in the range of 200 bp to 2000 bp. The average number of amplified bands per primer was 37 and an average number of polymorphic bands per primer was 21. Some of the representative agarose gels profile are presented in Figures 50-53. The details of the ISSR primers, mean number of bands amplified per primer and number of polymorphic bands, the percentage of polymorphism, PIC (Polymorphism information content) and MI (Marker index) are given in Table 111.

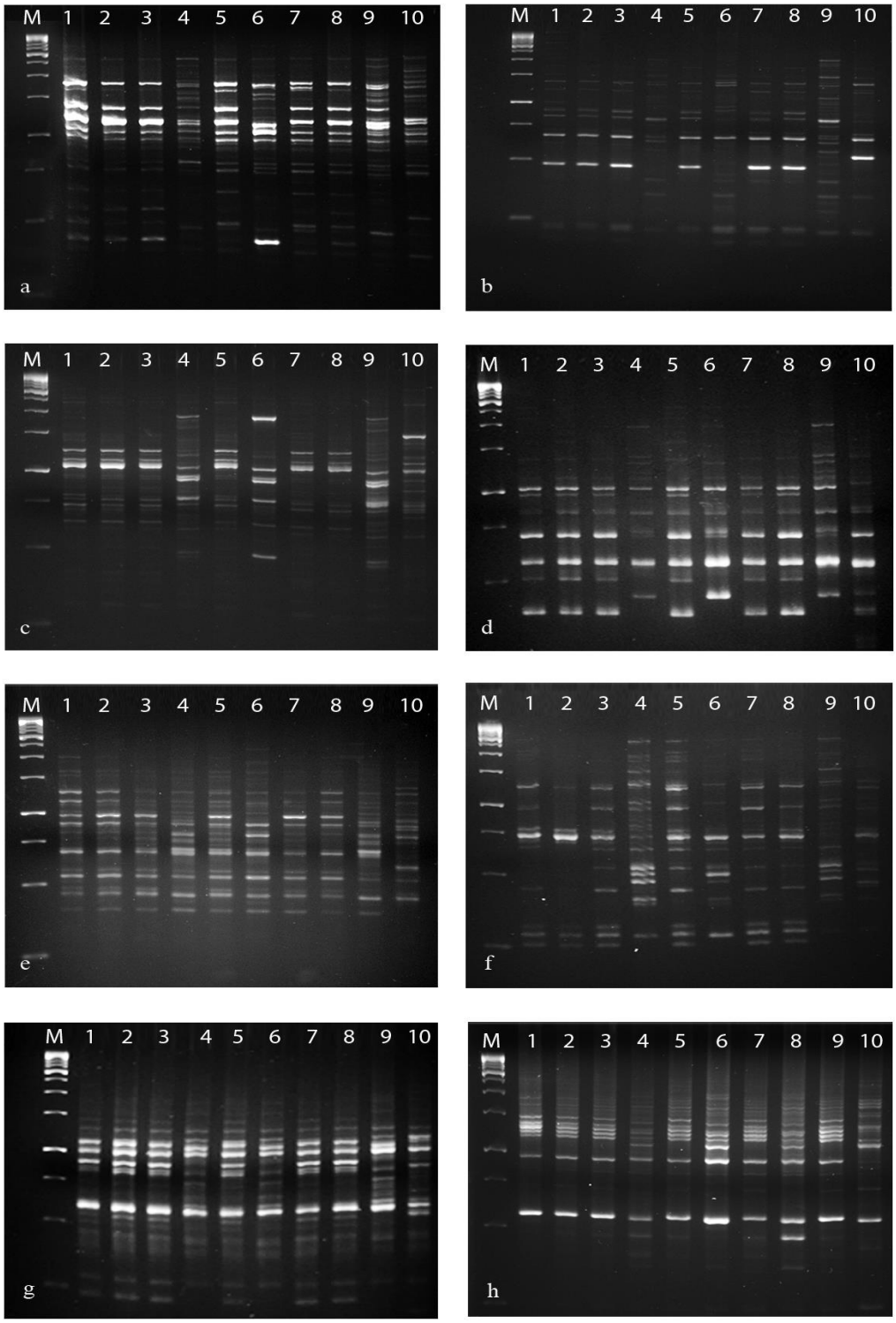


Fig. 50. a-h are the ISSR profiles of *C. amada* using primers ISSR 04, ISSR 06, ISSR 08, ISSR 16, ISSR 13, UBC 880, UBC 811 and UBC 835. M-marker (1kb ladder), Lane 1-10 are Acc. 265, Acc. 347, Acc.521, Acc. 752, Acc. 753, Acc. 848, Acc. 1119, Acc. 1503, Acc. 1511 and Acc. 6390, respectively

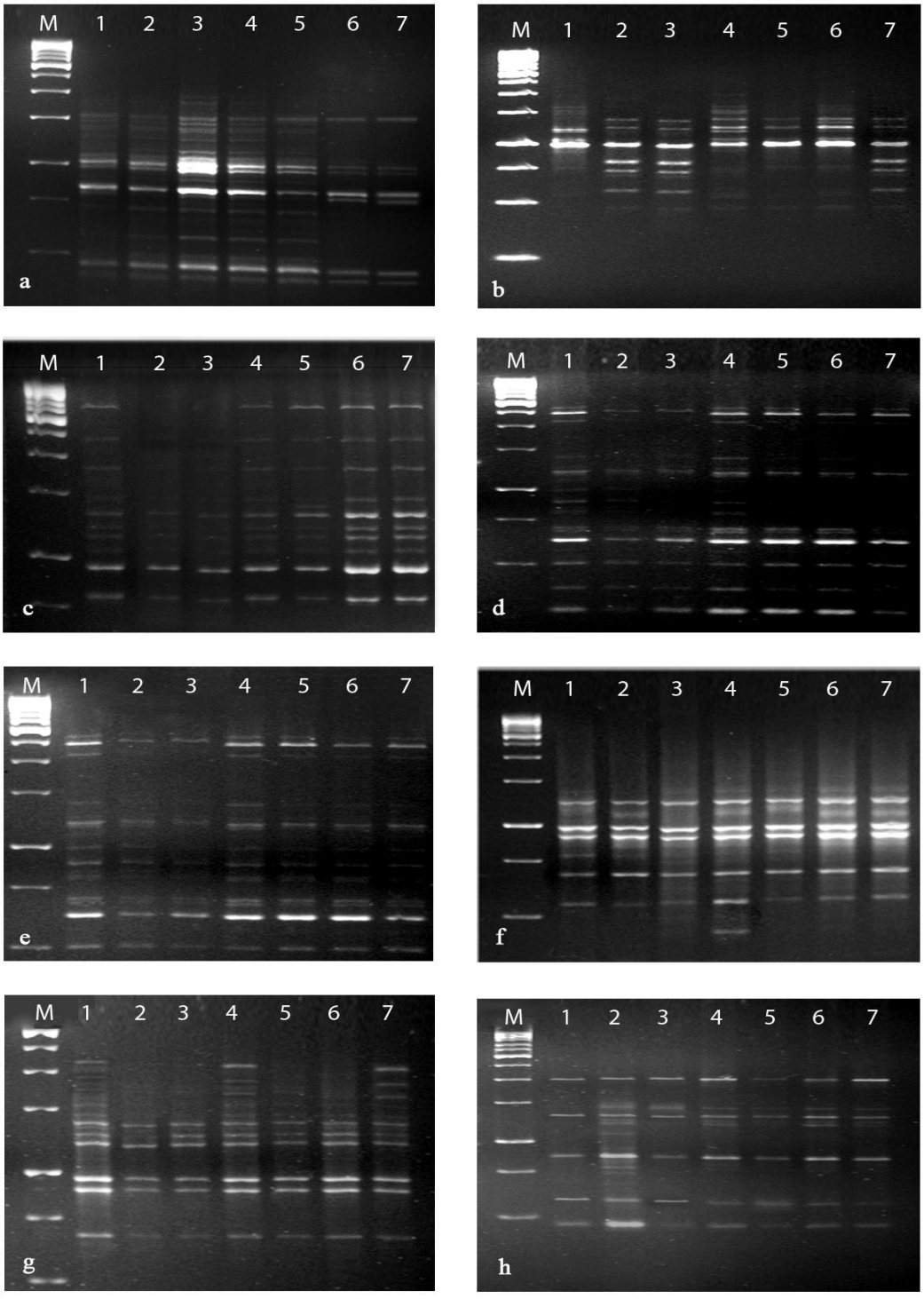


Fig. 51. a-h are the ISSR profiles of *C. aromatica* using primers ISSR 05, ISSR 08, ISSR 14, ISSR 13, UBC 880, UBC, 811, UBC 850 and UBC 835. M-marker (1kb ladder), Lane 1-7 are Acc. 711, Acc. 1025, Acc. 1113, Acc. 1124, Acc. 1132, Acc. 1518 and Acc. 1520, respectively.

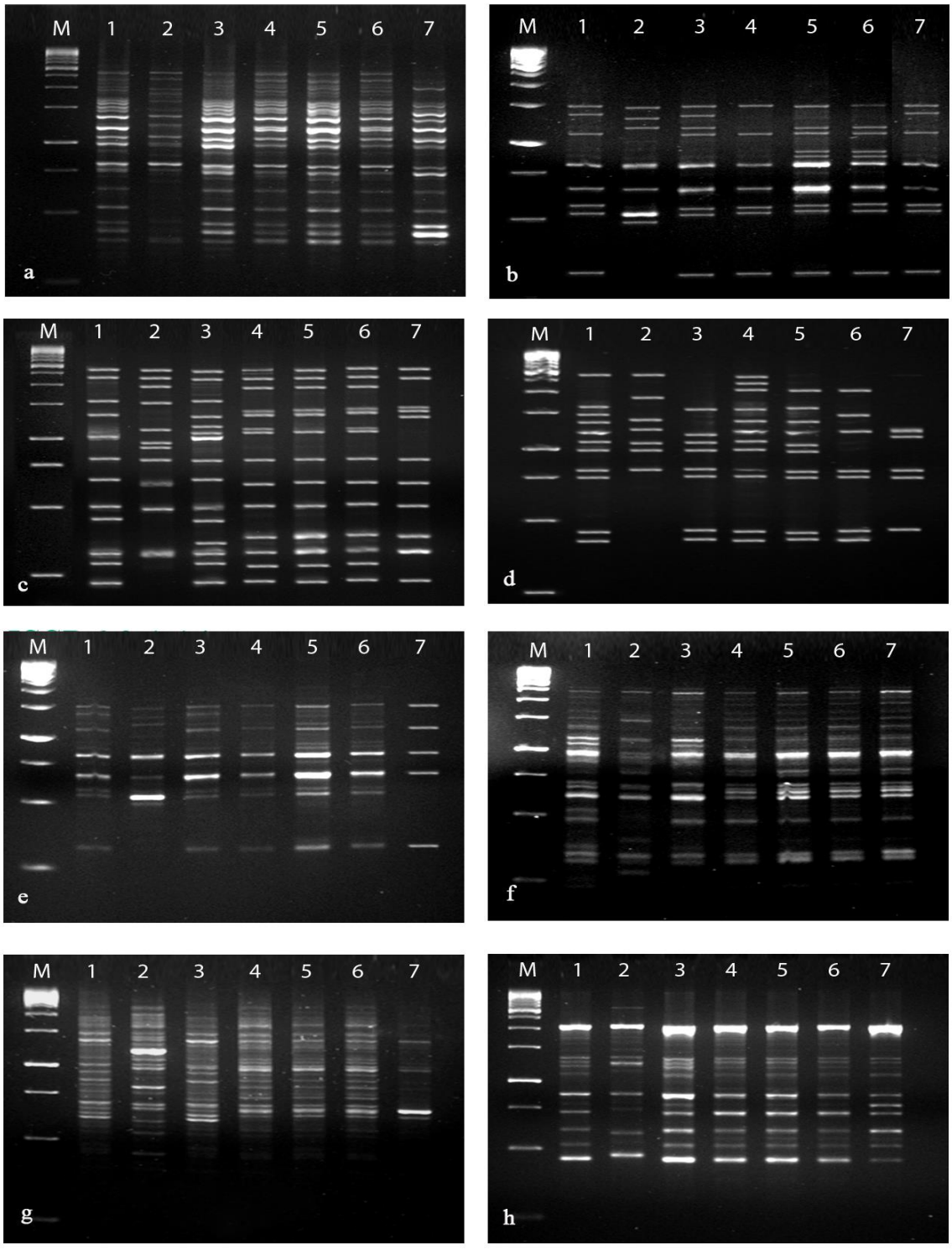


Fig. 52. a-h are the ISSR profiles of *C. caesia* using primers ISSR 05, ISSR 06, ISSR 16, ISSR 04, ISSR 14, UBC 850, UBC 811 and UBC 835. M-marker (1kb ladder), Lane 1-7 are Acc. 292, Acc. 751, Acc. 1001, Acc. 1006, Acc. 1135, Acc. 1154 and Acc. 1171, respectively.

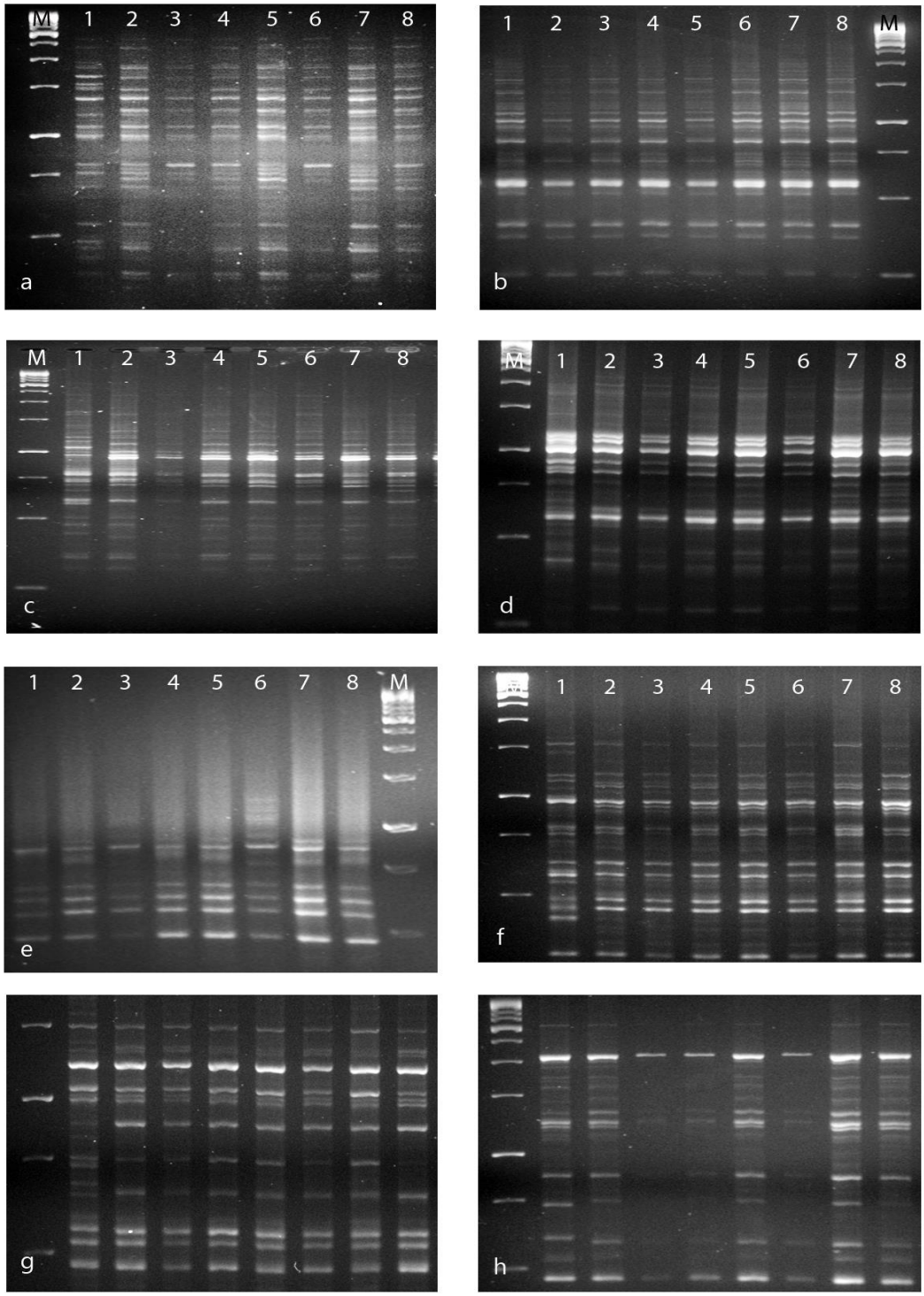


Fig. 53. a-h are the ISSR profiles of *C. xanthorrhiza* using primers ISSR 06, ISSR 12, ISSR 08, ISSF 16, UBC 826, UBC 812, UBC 880 and UBC 835. M-marker (1 kb ladder), Lane 1-8 are Acc. 465 Acc. 760, Acc. 1108, Acc. 1122, Acc. 1163, Acc. 1164, Acc. 1167 and Acc. 1168, respectively

The number of scored bands per primer ranged from 15 (UBC 818) to 51 (ISSR 14). The percentage of polymorphism ranged from 26% (ISSR 12) to 72% (ISSR 8) with an average value of 55%. The PIC value for ISSR loci ranged from 0.11 (ISSR 12) to 0.33 (UBC 842), with an average PIC and MI (Marker index) as 0.22 and 21.76, respectively.

Table 111. Sequence information and amplification details of ISSR primers used in four *Curcuma* species.

Primer	Sequence (5'- 3')	Total no of bands	No of Polymorphic bands	POL (%)	PIC	MI
ISSR4	(AGC)4GT	43	26	60%	0.24	14.38
ISSR5	(CAC)3GC	45	24	53%	0.18	9.76
ISSR6	(CTC)3GC	47	31	66%	0.27	18.02
ISSR8	(GACA)3GC	47	34	72%	0.26	19.03
ISSR9	(TC)7G	23	10	43%	0.19	8.32
ISSR11	(AGTG)3TT	26	17	65%	0.19	12.45
ISSR12	(CCCT)4	38	10	26%	0.11	2.92
ISSR13	(AGTG)3	36	18	50%	0.20	10.23
ISSR14	(AG)8T	51	28	55%	0.22	11.89
ISSR16	(AC)7T	38	21	55%	0.23	12.51
UBC880	GGAGAGGAGAGGAGA	46	27	59%	0.24	14.17
UBC855	ACACACACACACACACCTT	40	15	38%	0.15	5.54
UBC850	$(GT)^8C$	36	25	69%	0.26	18.12
UBC842	GAGAGAGAGAGAGAGACTG	32	22	69%	0.33	22.79
UBC841	$(GA)^8T$	36	20	56%	0.20	11.27
UBC835	$(AG)^8TC$	41	26	63%	0.23	14.57
UBC834	$(AG)^8CT$	34	20	59%	0.18	10.51
UBC826	$(AC)^8C$	38	16	42%	0.19	8.17
UBC818	$(CA)^8G$	15	6	40%	0.21	8.21
UBC812	$(GA)^8A$	33	18	55%	0.17	9.40
UBC811	$(GA)^8C$	39	26	67%	0.31	20.52
Mean		37	21	55%	0.22	12.51

POL% - % Polymorphism; PIC-Polymorphism Information Content, MI-Marker Index

4.4.3.1. Genetic diversity analysis using ISSR data

The genetic variance observed among four species using ISSR markers are presented in Table 112.

Table 112. Estimates of genetic variance using ISSR in four *Curcuma* species

	n	(Na)	(Ne)	(h)	(I)	% P	Gst
C. amada	10	1.27 ± 0.14	1.16 ± 0.09	0.10 ± 0.05	0.14 ± 0.08	64.50%	
C. aromatica	7	1.14 ± 0.13	1.09 ± 0.09	0.05 ± 0.05	0.08 ± 0.07	44.50%	
C. caesia	7	1.25 ± 0.17	1.13 ± 0.09	0.08 ± 0.06	0.13 ± 0.08	60.46%	
C. xanthorrhiza	8	1.17 ± 0.13	1.08 ± 0.07	0.05 ± 0.04	0.08 ± 0.07	50.69%	
Inter species	32	2.00 ± 0.01	1.54 ± 0.05	0.33 ± 0.02	0.50 ± 0.03	55.38%	0.79

Na - total number of alleles' Ne - number of effective alleles; h - Nei's (1973) gene diversity index; I - Shannon's information index; %P - percentage of polymorphism; genetic differentiation Gst = (Ht-Hs)/Ht;

The observed number of alleles ranged from 1.14 ± 0.13 (*C. aromatica*) to 1.27 ± 0.14 (*C. amada*). Nei's gene diversity index (h) and Shannon's index (I) were highest in *C. amada* (h = 0.10 ± 0.05; I = 0.14 ± 0.08), whereas Nei's gene diversity index (h) was lowest in *C. xanthorrhiza* (h = 0.05 ± 0.04). Shannon's Index (I) was lowest in *C. aromatica* and *C. xanthorrhiza* with I = 0.08 ± 0.07. Highest percentage of polymorphism was observed in *C. amada* (% P = 64.50%) and the lowest in *C. aromatica* (%P = 44.50%), with an average value of 55.38%. A high Gst value of 0.79 suggested that species are well differentiated from each other.

4.4.3.2. Cluster Analysis

4.4.3.2.1. Cluster analysis of Individual Species.

The genetic similarity was calculated using Simple Matching similarity coefficients for individual species based on ISSR profile. In *C. amada*, the similarity matrices showed maximum similarity between Acc. 521 and Acc. 1503, (0.93) and the least similar accessions were Acc.265 and Acc.752 (0.60) (Table 113).

Table 113. ISSR similarity matrix of different accessions of *C. amada*

Accession	265	347	521	752	753	848	1119	1503	1511	6390
265	1.00									
347	0.93	1.00								
521	0.91	0.93	1.00							
752	0.60	0.63	0.60	1.00						
753	0.88	0.91	0.91	0.69	1.00					
848	0.60	0.65	0.61	0.83	0.64	1.00				
1119	0.88	0.90	0.90	0.65	0.92	0.68	1.00			
1503	0.89	0.91	0.93	0.64	0.89	0.66	0.92	1.00		
1511	0.63	0.64	0.64	0.86	0.68	0.80	0.66	0.62	1.00	
6390	0.64	0.66	0.70	0.68	0.68	0.68	0.68	0.68	0.69	1.00

The UPGMA dendrogram constructed using the similarity matrix of genetic distances is represented in the Figure 54.

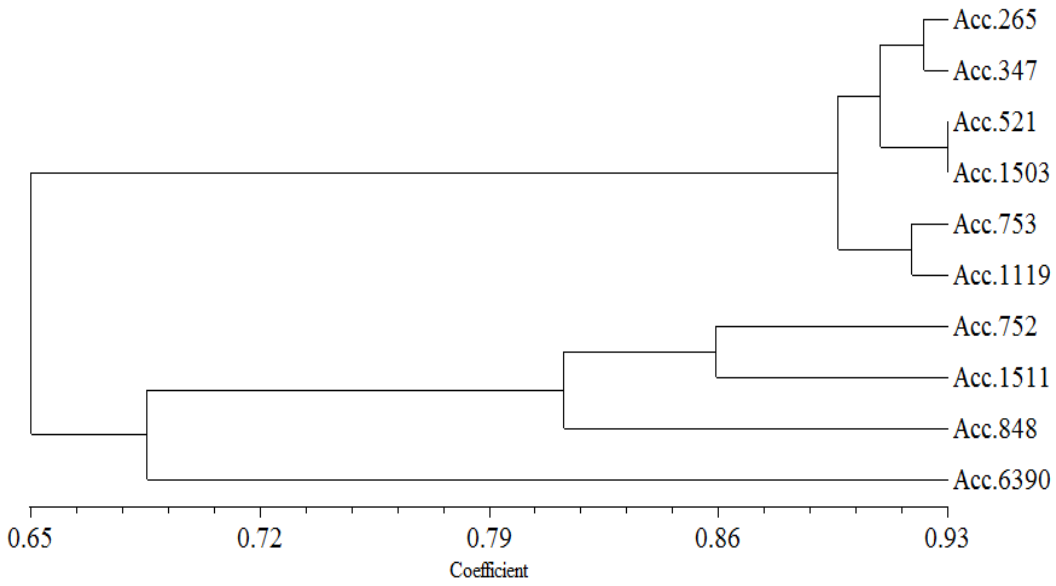


Fig. 54. ISSR based UPGMA dendrogram of genetic similarity among ten accessions of *C. amada.*

The UPGMA dendrogram constructed using Simple Matching similarity coefficients clustered the ten accessions into three groups splitting at a coefficient value of 0.813. The groups formed were as follows:

Group I - Acc. 265, Acc. 347, Acc. 521, Acc. 1503, Acc. 753 and Acc. 1119

Group II - Acc. 752, Acc. 1511 and Acc. 848

Group III - Acc. 6390

The first group consisted of six accessions, with a maximum similarity between Acc. 521 and Acc. 1503(0.93) although they were collected from Thrissur (Kerala) and Anand (Gujarat), respectively. The second group has three accessions (Acc.752, Acc.1511 and Acc.848) and the third group has only one member, Acc.6390.

In case of *C. aromatica*, the similarity matrix constructed using the ISSR data showed the maximum similarity between Acc. 1132 and Acc. 1124 with a similarity coefficient value of 0.95. The minimum similarity was between Acc. 1113 and Acc. 1158 with a value of 0.65. Table 114 shows the similarity matrix of accessions constructed using the Simple Matching algorithm.

Table 114. ISSR similarity matrix of different accessions of *C. aromatica*

Accession	711	1025	1113	1124	1132	1518	1520
711	1.00						
1025	0.84	1.00					
1113	0.82	0.88	1.00				
1124	0.86	0.87	0.85	1.00			
1132	0.88	0.86	0.86	0.95	1.00		
1518	0.72	0.68	0.65	0.75	0.76	1.00	
1520	0.76	0.71	0.68	0.77	0.77	0.91	1.00

The UPGMA dendrogram constructed using Simple Matching similarity coefficients is given in Figure 55.

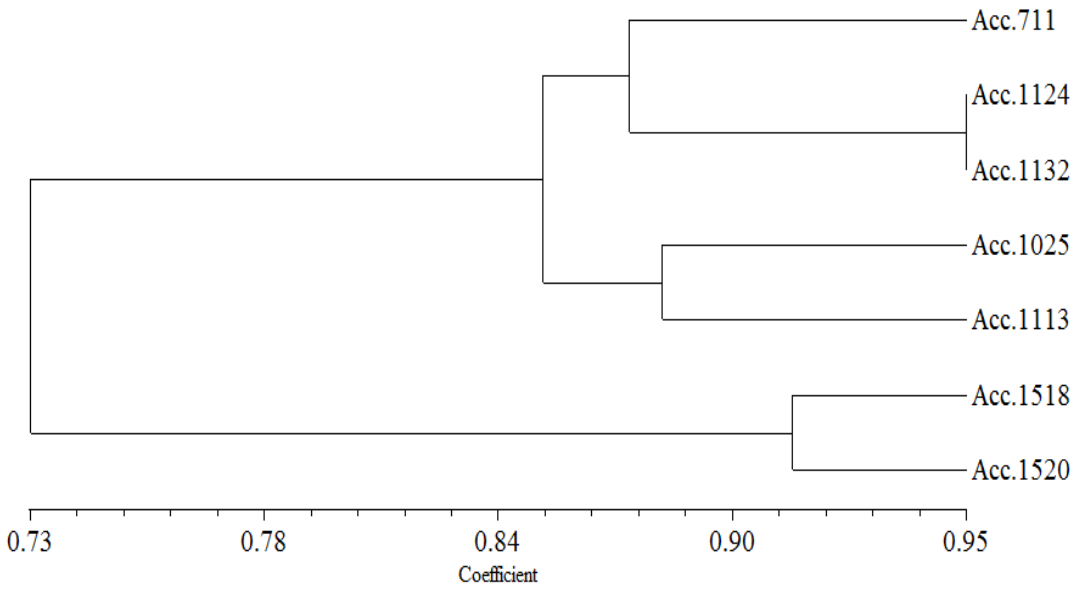


Fig. 55. ISSR based UPGMA-Dendrogram of seven accessions of *C. aromatica.*

The seven accessions of *C. aromatica* clustered into three groups in the dendrogram, splitting at coefficient value of 0. 850. The groups were as follows:

Group I	-	Acc. 711, Acc. 1124, and Acc.1132
Group II	-	Acc. 1025 and Acc. 1113
Group III	-	Acc. 1518 and Acc. 1520

The first group was formed by Acc. 711, Acc.1124 and Acc.1132. Acc. 1132 and Acc.1124 were closely related to each other with a coefficient value of 0.95. The second and third groups consisted of two accessions each *viz*., Acc. 1025 and Acc. 1113 in the second group and Acc. 1518 and Acc. 1520 in the third group, respectively. Acc.1518 and Acc.1520 which formed a separate group, was collected from Idukki district of Kerala.

In case of *C. caesia*, the cluster analysis carried out using Simple Matching similarity coefficients showed the maximum similarity between Acc. 1135 and Acc. 1154, with a coefficient value of 0.96. The least similarity was between Acc. 1171 and Acc. 751 with a coefficient value of 0.52. Table 115 shows the similarity matrix of *C. caesia* accessions using simple matching similarity coefficients. Dendrogram constructed based on Simple Matching similarity coefficients is represented in Figure 56.

Table 115. ISSR similarity matrix of different accessions of *C. caesia*

Accession	292	751	1001	1006	1135	1154	1171
292	1.00						
751	0.63	1.00					
1001	0.89	0.62	1.00				
1006	0.83	0.65	0.81	1.00			
1135	0.83	0.67	0.86	0.92	1.00		
1154	0.83	0.65	0.82	0.93	0.96	1.00	
1171	0.69	0.52	0.70	0.78	0.77	0.78	1.00

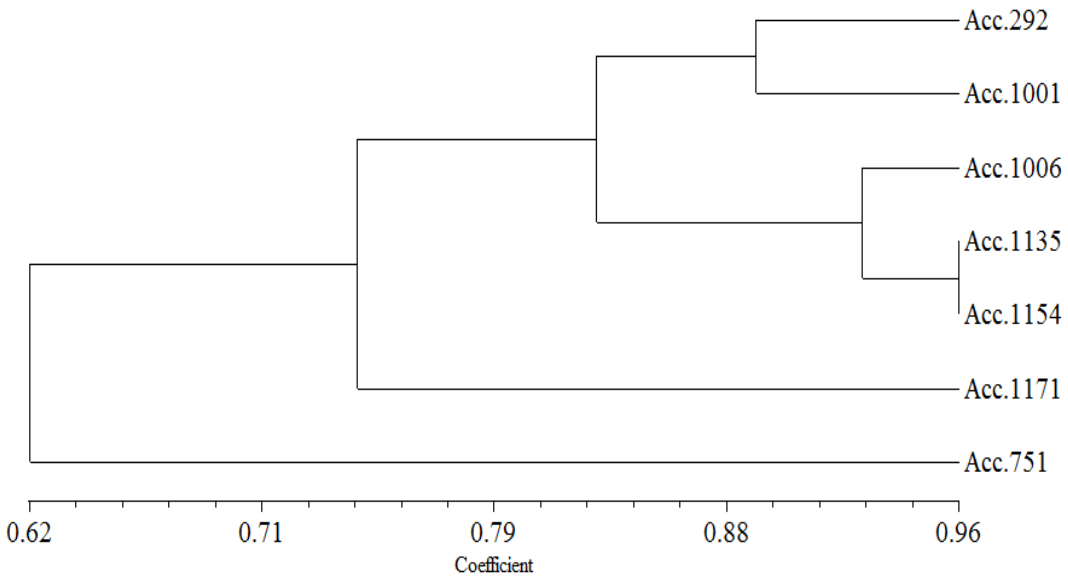


Fig. 56. ISSR based UPGMA-Dendrogram of seven accessions of *C. caesia*

The UPGMA dendrogram clustered the seven accessions of *C. caesia* into four groups splitting at a coefficient value of 0. 830. The groups were as follows:

Group I - Acc. 292 and Acc. 1001

Group II - Acc. 1006, Acc. 1135 and Acc. 1154

Group III - Acc. 1171

Group IV - Acc. 751

The first group was formed by Acc. 292 and Acc.1001 with a similarity coefficient of 0.875. The second group consisted of three accessions; Acc. 1006, Acc. 1135 and Acc. 1154 whereas the third and fourth group had only one accession each; Acc.1171 and Acc.751, respectively. In the second group, Acc. 1135 and Acc. 1154 showed maximum similarity with a coefficient value of 0.96, and they were collected from Thrissur, Kerala.

In case of *C. xanthorrhiza*, the similarity matrix constructed using Simple Matching similarity coefficients showed the maximum similarity between Acc. 1163 and Acc. 1167 with a coefficient value of 0.97. The minimum similarity was between Acc. 465 and Acc. 1108 with similarity coefficient value of 0.57 (Table 116).

Table 116. ISSR similarity matrix of different accessions of *C. xanthorrhiza*

Accessions	465	760	1108	1122	1163	1164	1167	1168
465	1.00							
760	0.77	1.00						
1108	0.57	0.66	1.00					
1122	0.73	0.89	0.67	1.00				
1163	0.75	0.95	0.67	0.92	1.00			
1164	0.70	0.82	0.79	0.83	0.84	1.00		
1167	0.76	0.95	0.65	0.91	0.97	0.83	1.00	
1168	0.74	0.94	0.69	0.89	0.96	0.86	0.96	1.00

Dendrogram constructed based on Simple Matching similarity coefficients is represented in Figure 57.

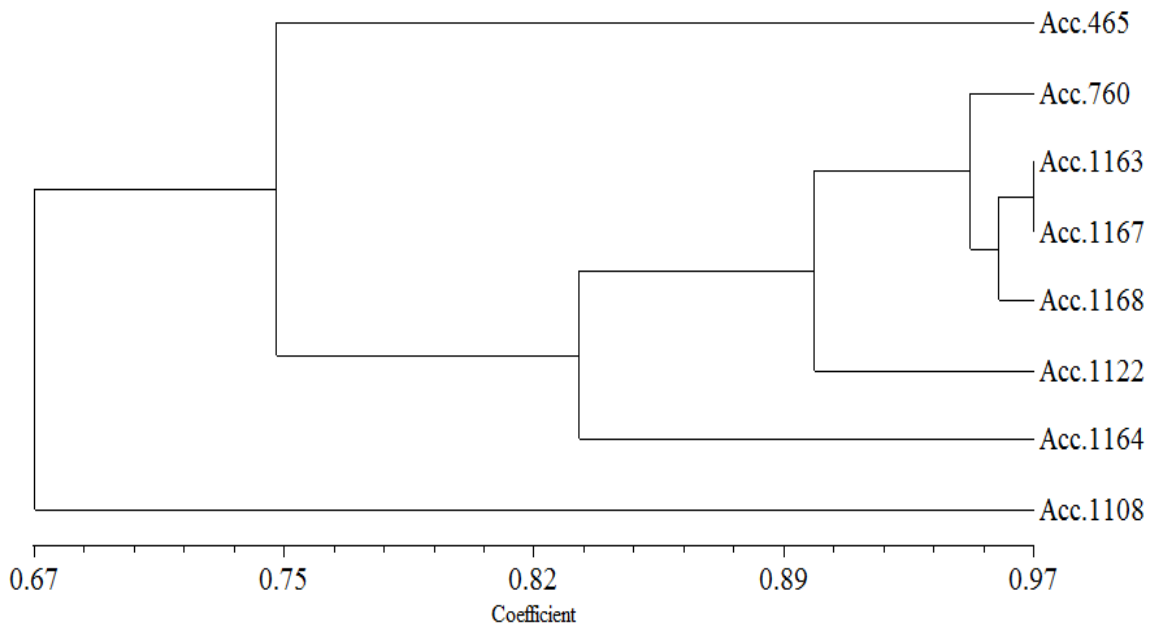


Fig. 57. ISSR based UPGMA-Dendrogram of eight accessions of *C. xanthorrhiza*

The UPGMA dendrogram constructed using the Simple Matching similarity matrix clustered the eight accessions of *C. xanthorrhiza* into three groups splitting at coefficient value of 0.817. The groups formed were as follows:

Group I	-	Acc. 465
Group II	-	Acc. 760, Acc. 1163, Acc. 1167, Acc. 1168, Acc. 1122. and Acc.1164
Group III	-	Acc. 1108

First and third group were formed by one accession each; Acc. 465 and Acc. 1108, respectively. Second group, which was the major group, comprised of six accessions; Acc. 760, Acc. 1163, Acc. 1167, Acc. 1168, Acc. 1122 and Acc. 1164. Acc.1164 and Acc.1167 were closely related to each other with a coefficient value of 0.97 followed by Acc. 1168.

4.4.3.2.2. Cluster Analysis of four species

The similarity matrices for the thirty-two accessions from four species using Simple Matching, Jaccard's and Sorensen-Dice algorithms are given in Tables 117-119. The UPGMA dendrogram constructed using the three similarity coefficients is depicted in Figures 58, 59 and 60.

The UPGMA dendrogram based on the Simple Matching, Jaccard's and Sorensen-Dice similarity coefficients clustered the thirty-two accessions into four groups splitting at a coefficient 0.845, 0.591 and 0.741, respectively.

The four groupings were:

Group I	*C. amada* Acc. 265, 521, 1503, 1511, 347. 6390, 753, 1119, 752 and 848
Group II	*C. xanthorrhiza* Acc. 465, 1168, 760, 1122, 1163, 1167, 1164 and 1108
Group III	*C. aromatica* Acc. 711, 1025, 1113, 1132, 1518, 1520 and 1124
Group IV	*C. caesia* Acc. 292, 1001, 1154, 751, 1006, 1171 and 1135

The UPGMA based SAHN clustering program has clustered 32 accessions into four different groups, each group containing accessions of respective species. Group I was formed by the ten accessions of *C. amada*, group II by eight accessions of *C. xanthorrhiza*, similarly group III and IV were formed by seven accessions eachof *C. aromatica* and *C. caesia*, respectively. The only difference between the clusters obtained from Simple Matching coefficients and Jaccard's / Sorensen-Dice is that in simple matching *C. amada* and *C. xanthorrhiza* were closely associated with *C. aromatica* confirming the observation in the Nei's genetic distance algorithm, whereas in Jaccard's and Sorensen dice, *C. amada* and *C. xanthorrhiza* were closely associated with *C. caesia.*

Among the accessions, maximum similarity was observed between the accessions of *C. xanthorrhiza*, Acc.1163 and Acc.1167 (Simple Matching coefficient = 0.99, Jaccard's = 0.97 and Dice = 0.98) followed by Acc. 1168 and Acc.1163 as well as Acc. 1167 and Acc.1168 of *C. xanthorrhiza*. Genetically least similar was Acc. 711 (*C. aromatica*) and Acc. 1108 (*C. xanthorrhiza*) with a similarity coefficient 0.09 and 0.16 for Jaccard's and Sorensen-Dice, respectively. Acc. 1025 (*C. aromatica)* and Acc. 1108 (*C. xanthorrhiza*), Acc. 1518 (*C. aromatica*) and Acc. 1108 (*C. xanthorrhiza*) also exhibited the least similarity in Jaccard's and Sorensen-Dice similarity coefficients.

Table 117. ISSR similarity matrix of four *Curcuma* species using Simple Matching similarity coefficients

		C.amada										*C.aromatica*							*C.caesia*							*C.xanthorrhiza*							
		265	347	521	752	753	848	1119	1503	1511	6390	711	1025	1113	1124	1132	1518	1520	292	751	1001	1006	1135	1154	1171	465	760	1108	1122	1163	1164	1167	1168
C.amada	265	1.00																															
	347	0.97	1.00																														
	521	0.96	0.97	1.00																													
	752	0.83	0.84	0.83	1.00																												
	753	0.95	0.96	0.96	0.87	1.00																											
	848	0.83	0.85	0.83	0.93	0.85	1.00																										
	1119	0.95	0.96	0.96	0.85	0.97	0.86	1.00																									
	1503	0.95	0.96	0.97	0.85	0.96	0.86	0.97	1.00																								
	1511	0.84	0.85	0.85	0.94	0.87	0.91	0.86	0.84	1.00																							
	6390	0.85	0.86	0.87	0.86	0.86	0.87	0.86	0.86	0.87	1.00																						
C.aromatica	711	0.60	0.60	0.58	0.58	0.57	0.57	0.59	0.59	0.56	0.59	1.00																					
	1025	0.59	0.59	0.58	0.57	0.57	0.56	0.58	0.58	0.56	0.58	0.95	1.00																				
	1113	0.59	0.59	0.57	0.57	0.57	0.57	0.58	0.58	0.56	0.58	0.95	0.96	1.00																			
	1124	0.59	0.59	0.58	0.55	0.57	0.56	0.58	0.58	0.55	0.58	0.96	0.96	0.96	1.00																		
	1132	0.59	0.58	0.57	0.55	0.56	0.56	0.57	0.58	0.54	0.58	0.96	0.96	0.96	0.99	1.00																	
	1518	0.61	0.61	0.62	0.58	0.59	0.58	0.60	0.61	0.58	0.62	0.92	0.90	0.89	0.93	0.93	1.00																
	1520	0.61	0.61	0.62	0.58	0.59	0.57	0.60	0.60	0.58	0.61	0.93	0.91	0.90	0.93	0.93	0.97	1.00															
C.caesia	292	0.54	0.54	0.55	0.52	0.53	0.52	0.53	0.53	0.53	0.55	0.56	0.57	0.56	0.55	0.54	0.59	0.59	1.00														
	751	0.57	0.58	0.58	0.55	0.57	0.55	0.57	0.56	0.55	0.58	0.61	0.60	0.59	0.59	0.58	0.62	0.62	0.85	1.00													
	1001	0.55	0.55	0.55	0.52	0.54	0.52	0.54	0.54	0.54	0.54	0.56	0.57	0.56	0.55	0.54	0.59	0.58	0.95	0.84	1.00												
	1006	0.54	0.55	0.54	0.52	0.54	0.52	0.53	0.53	0.52	0.54	0.57	0.57	0.56	0.57	0.55	0.60	0.60	0.93	0.86	0.92	1.00											
	1135	0.55	0.56	0.55	0.53	0.55	0.53	0.54	0.54	0.54	0.55	0.57	0.57	0.56	0.56	0.55	0.59	0.59	0.93	0.87	0.94	0.97	1.00										
	1154	0.55	0.55	0.55	0.52	0.55	0.53	0.54	0.54	0.53	0.55	0.57	0.56	0.56	0.56	0.55	0.59	0.59	0.93	0.86	0.93	0.97	0.98	1.00									
	1171	0.57	0.58	0.58	0.55	0.57	0.55	0.57	0.56	0.55	0.57	0.61	0.60	0.60	0.59	0.58	0.63	0.63	0.87	0.80	0.88	0.91	0.90	0.91	1.00								
C.xanthorrhiza	465	0.61	0.61	0.60	0.59	0.58	0.57	0.58	0.59	0.59	0.58	0.60	0.59	0.60	0.58	0.58	0.61	0.61	0.56	0.59	0.56	0.57	0.56	0.55	0.60	1.00							
	760	0.62	0.61	0.61	0.58	0.59	0.57	0.59	0.60	0.59	0.59	0.59	0.58	0.59	0.58	0.57	0.61	0.61	0.56	0.59	0.56	0.57	0.56	0.56	0.61	0.93	1.00						
	1108	0.65	0.65	0.65	0.61	0.63	0.62	0.64	0.64	0.62	0.64	0.64	0.64	0.64	0.63	0.62	0.67	0.67	0.62	0.65	0.61	0.62	0.61	0.61	0.65	0.87	0.89	1.00					
	1122	0.62	0.62	0.61	0.59	0.59	0.58	0.60	0.60	0.60	0.60	0.60	0.59	0.60	0.59	0.58	0.62	0.62	0.57	0.61	0.57	0.58	0.57	0.57	0.61	0.92	0.96	0.90	1.00				
	1163	0.61	0.61	0.60	0.58	0.59	0.57	0.59	0.60	0.59	0.58	0.59	0.58	0.59	0.58	0.57	0.61	0.61	0.56	0.59	0.56	0.56	0.56	0.55	0.59	0.92	0.99	0.90	0.98	1.00			
	1164	0.64	0.63	0.63	0.61	0.61	0.61	0.62	0.63	0.61	0.62	0.62	0.61	0.62	0.61	0.60	0.64	0.64	0.59	0.62	0.58	0.59	0.58	0.58	0.63	0.91	0.94	0.94	0.95	0.95	1.00		
	1167	0.62	0.62	0.61	0.59	0.59	0.58	0.60	0.60	0.60	0.59	0.59	0.59	0.60	0.58	0.57	0.61	0.61	0.56	0.59	0.56	0.57	0.56	0.56	0.60	0.93	0.99	0.89	0.97	0.99	0.95	1.00	
	1168	0.62	0.61	0.61	0.58	0.59	0.57	0.60	0.60	0.59	0.59	0.60	0.59	0.60	0.59	0.58	0.61	0.61	0.56	0.59	0.56	0.57	0.56	0.55	0.60	0.92	0.98	0.90	0.97	0.99	0.96	0.99	1.00

Table118. ISSR similarity matrix of four *Curcuma* species using Jaccard's similarity coefficients

		C.amada										*C.aromatica*							*C.caesia*							*C.xanthorrhiza*							
		265	347	521	752	753	848	1119	1503	1511	6390	711	1025	1113	1124	1132	1518	1520	292	751	1001	1006	1135	1154	1171	465	760	1108	1122	1163	1164	1167	1168
C.amada	265	1.00																															
	347	0.90	1.00																														
	521	0.88	0.90	1.00																													
	752	0.56	0.58	0.56	1.00																												
	753	0.84	0.88	0.88	0.66	1.00																											
	848	0.56	0.60	0.56	0.79	0.60	1.00																										
	1119	0.84	0.87	0.87	0.61	0.90	0.63	1.00																									
	1503	0.86	0.89	0.91	0.60	0.87	0.62	0.90	1.00																								
	1511	0.59	0.60	0.60	0.83	0.65	0.75	0.62	0.59	1.00																							
	6390	0.59	0.61	0.64	0.63	0.63	0.63	0.63	0.62	0.64	1.00																						
C.aromatica	711	0.15	0.15	0.13	0.13	0.14	0.13	0.14	0.15	0.12	0.13	1.00																					
	1025	0.16	0.15	0.14	0.13	0.15	0.12	0.15	0.15	0.13	0.14	0.82	1.00																				
	1113	0.16	0.15	0.13	0.13	0.14	0.13	0.14	0.15	0.13	0.13	0.81	0.87	1.00																			
	1124	0.17	0.16	0.15	0.13	0.15	0.13	0.16	0.16	0.13	0.14	0.85	0.86	0.85	1.00																		
	1132	0.17	0.16	0.15	0.13	0.15	0.13	0.16	0.16	0.13	0.14	0.87	0.86	0.85	0.95	1.00																	
	1518	0.14	0.14	0.15	0.10	0.13	0.11	0.14	0.15	0.12	0.14	0.70	0.67	0.64	0.74	0.74	1.00																
	1520	0.16	0.15	0.16	0.12	0.15	0.11	0.14	0.15	0.13	0.15	0.74	0.70	0.68	0.76	0.77	0.89	1.00															
C.caesia	292	0.15	0.15	0.15	0.12	0.16	0.12	0.14	0.14	0.15	0.14	0.13	0.15	0.14	0.14	0.14	0.14	0.14	1.00														
	751	0.16	0.16	0.16	0.14	0.17	0.13	0.16	0.15	0.15	0.15	0.16	0.15	0.15	0.16	0.15	0.15	0.16	0.60	1.00													
	1001	0.15	0.16	0.15	0.13	0.17	0.13	0.15	0.15	0.16	0.14	0.13	0.15	0.14	0.14	0.14	0.14	0.14	0.87	0.59	1.00												
	1006	0.14	0.14	0.14	0.12	0.16	0.12	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.15	0.14	0.14	0.15	0.80	0.61	0.78	1.00											
	1135	0.17	0.17	0.16	0.15	0.18	0.14	0.16	0.16	0.17	0.16	0.15	0.16	0.15	0.16	0.16	0.15	0.16	0.80	0.65	0.84	0.91	1.00										
	1154	0.16	0.16	0.16	0.14	0.18	0.14	0.15	0.15	0.16	0.15	0.14	0.15	0.14	0.16	0.15	0.15	0.16	0.81	0.62	0.80	0.91	0.95	1.00									
	1171	0.13	0.15	0.14	0.11	0.15	0.12	0.14	0.14	0.13	0.13	0.14	0.14	0.14	0.14	0.14	0.14	0.15	0.64	0.47	0.65	0.73	0.73	0.74	1.00								
C.xanthorrhiza	465	0.18	0.17	0.16	0.16	0.17	0.14	0.15	0.16	0.18	0.14	0.13	0.12	0.13	0.13	0.13	0.11	0.13	0.14	0.15	0.15	0.15	0.16	0.14	0.14	1.00							
	760	0.20	0.19	0.18	0.16	0.18	0.15	0.17	0.18	0.18	0.15	0.12	0.12	0.13	0.13	0.13	0.11	0.13	0.15	0.15	0.15	0.15	0.16	0.15	0.16	0.77	1.00						
	1108	0.16	0.15	0.15	0.11	0.14	0.12	0.14	0.14	0.13	0.13	0.09	0.09	0.10	0.10	0.10	0.09	0.11	0.13	0.14	0.12	0.12	0.13	0.13	0.13	0.54	0.62	1.00					
	1122	0.18	0.18	0.17	0.15	0.17	0.14	0.16	0.17	0.17	0.14	0.12	0.11	0.13	0.13	0.12	0.11	0.12	0.15	0.16	0.15	0.15	0.16	0.15	0.15	0.72	0.88	0.61	1.00				
	1163	0.20	0.19	0.18	0.16	0.18	0.15	0.17	0.18	0.18	0.15	0.13	0.13	0.14	0.14	0.13	0.12	0.13	0.15	0.16	0.15	0.15	0.16	0.15	0.15	0.75	0.95	0.63	0.91	1.00			
	1164	0.18	0.18	0.17	0.15	0.17	0.15	0.16	0.18	0.17	0.15	0.12	0.11	0.13	0.12	0.12	0.11	0.12	0.14	0.15	0.13	0.14	0.15	0.14	0.14	0.68	0.80	0.73	0.80	0.82	1.00		
	1167	0.20	0.19	0.18	0.16	0.19	0.15	0.18	0.18	0.19	0.15	0.12	0.13	0.14	0.14	0.13	0.12	0.13	0.15	0.16	0.15	0.15	0.16	0.15	0.15	0.76	0.95	0.61	0.90	0.97	0.80	1.00	
	1168	0.19	0.19	0.18	0.15	0.18	0.14	0.17	0.18	0.18	0.15	0.12	0.13	0.14	0.14	0.13	0.12	0.13	0.15	0.15	0.15	0.14	0.16	0.14	0.14	0.74	0.94	0.65	0.88	0.95	0.84	0.95	1.00

Table 119. ISSR similarity matrix of four *Curcuma* species using Sorensen-Dice similarity coefficients

		C.amada										*C.aromatica*							*C.caesia*							*C.xanthorrhiza*							
		265	347	521	752	753	848	1119	1503	1511	6390	711	1025	1113	1124	1132	1518	1520	292	751	1001	1006	1135	1154	1171	465	760	1108	1122	1163	1164	1167	1168
C.amada	265	1.00																															
	347	0.95	1.00																														
	521	0.94	0.95	1.00																													
	752	0.72	0.74	0.72	1.00																												
	753	0.91	0.94	0.94	0.79	1.00																											
	848	0.72	0.75	0.72	0.88	0.75	1.00																										
	1119	0.91	0.93	0.93	0.76	0.95	0.77	1.00																									
	1503	0.92	0.94	0.95	0.75	0.93	0.76	0.94	1.00																								
	1511	0.74	0.75	0.75	0.90	0.79	0.86	0.76	0.74	1.00																							
	6390	0.74	0.76	0.78	0.77	0.77	0.77	0.77	0.77	0.78	1.00																						
C.aromatica	711	0.27	0.26	0.23	0.23	0.25	0.23	0.25	0.26	0.22	0.23	1.00																					
	1025	0.27	0.26	0.25	0.23	0.26	0.22	0.25	0.26	0.23	0.24	0.90	1.00																				
	1113	0.27	0.26	0.23	0.23	0.25	0.23	0.25	0.26	0.23	0.23	0.89	0.93	1.00																			
	1124	0.29	0.27	0.26	0.23	0.27	0.23	0.27	0.28	0.23	0.25	0.92	0.93	0.92	1.00																		
	1132	0.29	0.27	0.26	0.22	0.27	0.24	0.27	0.28	0.24	0.25	0.93	0.92	0.92	0.97	1.00																	
	1518	0.25	0.25	0.25	0.19	0.24	0.20	0.24	0.25	0.21	0.25	0.82	0.80	0.78	0.85	0.85	1.00																
	1520	0.27	0.27	0.27	0.21	0.26	0.20	0.25	0.27	0.23	0.26	0.85	0.82	0.81	0.86	0.87	0.94	1.00															
C.caesia	292	0.26	0.26	0.26	0.22	0.27	0.22	0.25	0.25	0.26	0.25	0.23	0.26	0.24	0.25	0.24	0.25	0.25	1.00														
	751	0.27	0.27	0.27	0.24	0.29	0.23	0.27	0.26	0.25	0.26	0.27	0.27	0.26	0.27	0.26	0.26	0.27	0.75	1.00													
	1001	0.27	0.27	0.26	0.24	0.29	0.23	0.26	0.26	0.27	0.25	0.23	0.26	0.25	0.25	0.24	0.25	0.25	0.93	0.74	1.00												
	1006	0.25	0.25	0.25	0.22	0.27	0.21	0.24	0.24	0.24	0.24	0.24	0.25	0.24	0.27	0.25	0.25	0.26	0.89	0.76	0.88	1.00											
	1135	0.29	0.29	0.28	0.26	0.31	0.25	0.28	0.28	0.28	0.27	0.26	0.27	0.26	0.28	0.27	0.26	0.27	0.89	0.78	0.91	0.95	1.00										
	1154	0.27	0.28	0.27	0.24	0.30	0.24	0.27	0.27	0.27	0.26	0.25	0.26	0.25	0.27	0.26	0.26	0.28	0.89	0.77	0.89	0.96	0.98	1.00									
	1171	0.24	0.25	0.24	0.20	0.26	0.21	0.24	0.24	0.23	0.22	0.24	0.25	0.24	0.25	0.24	0.24	0.27	0.78	0.64	0.79	0.85	0.84	0.85	1.00								
C.xanthorrhiza	465	0.31	0.30	0.28	0.28	0.28	0.25	0.26	0.28	0.30	0.24	0.22	0.22	0.23	0.23	0.23	0.20	0.22	0.25	0.25	0.25	0.25	0.27	0.25	0.25	1.00							
	760	0.33	0.32	0.30	0.27	0.31	0.26	0.29	0.30	0.31	0.26	0.21	0.22	0.24	0.23	0.22	0.20	0.23	0.26	0.27	0.26	0.26	0.28	0.26	0.27	0.87	1.00						
	1108	0.27	0.26	0.26	0.19	0.24	0.21	0.24	0.25	0.23	0.23	0.16	0.17	0.19	0.18	0.17	0.17	0.19	0.23	0.24	0.22	0.22	0.23	0.23	0.22	0.70	0.76	1.00					
	1122	0.31	0.30	0.29	0.26	0.29	0.24	0.28	0.29	0.29	0.25	0.21	0.20	0.23	0.22	0.21	0.20	0.22	0.26	0.28	0.25	0.26	0.27	0.26	0.26	0.84	0.93	0.76	1.00				
	1163	0.33	0.32	0.31	0.27	0.31	0.26	0.30	0.31	0.31	0.26	0.22	0.22	0.25	0.24	0.23	0.21	0.23	0.26	0.27	0.26	0.26	0.28	0.26	0.26	0.86	0.97	0.78	0.96	1.00			
	1164	0.31	0.30	0.29	0.26	0.29	0.25	0.28	0.30	0.29	0.25	0.21	0.20	0.23	0.22	0.21	0.20	0.22	0.24	0.26	0.24	0.25	0.26	0.25	0.25	0.81	0.89	0.84	0.89	0.90	1.00		
	1167	0.34	0.33	0.31	0.28	0.31	0.26	0.30	0.31	0.31	0.27	0.22	0.23	0.24	0.24	0.23	0.21	0.23	0.26	0.27	0.26	0.26	0.28	0.26	0.26	0.86	0.97	0.76	0.95	0.98	0.89	1.00	
	1168	0.33	0.32	0.30	0.27	0.30	0.25	0.29	0.30	0.30	0.26	0.22	0.23	0.25	0.24	0.23	0.21	0.23	0.25	0.26	0.25	0.25	0.27	0.25	0.25	0.85	0.97	0.79	0.94	0.98	0.91	0.98	1.00

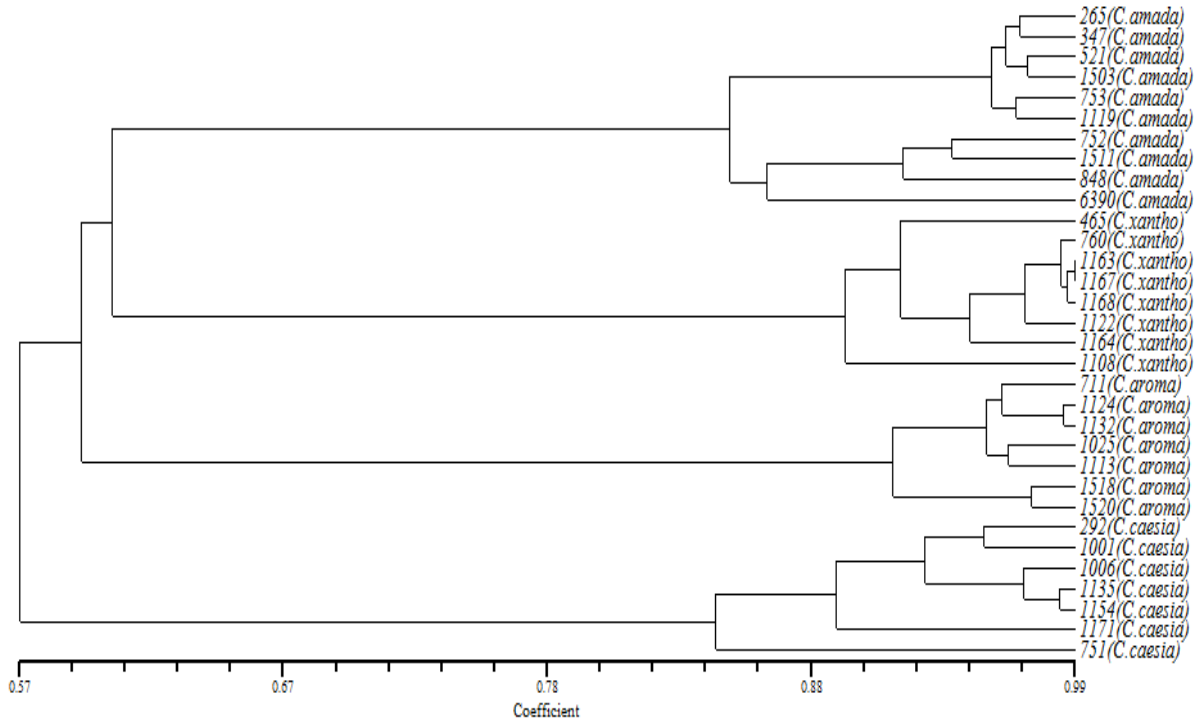


Fig. 58. UPGMA-Dendrogram of four *Curcuma* species based on ISSR markers using Simple Matching similarity coefficients

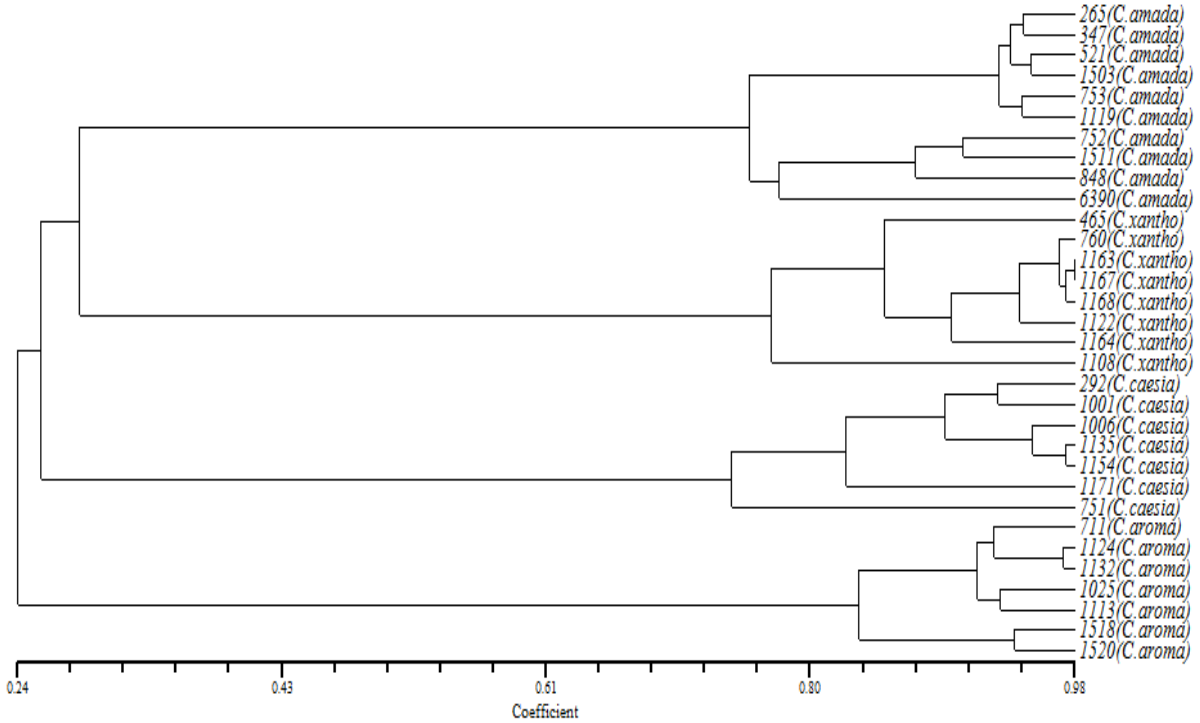


Fig. 59. UPGMA-Dendrogram of four *Curcuma* species based on ISSR markers using Jaccard's similarity coefficients

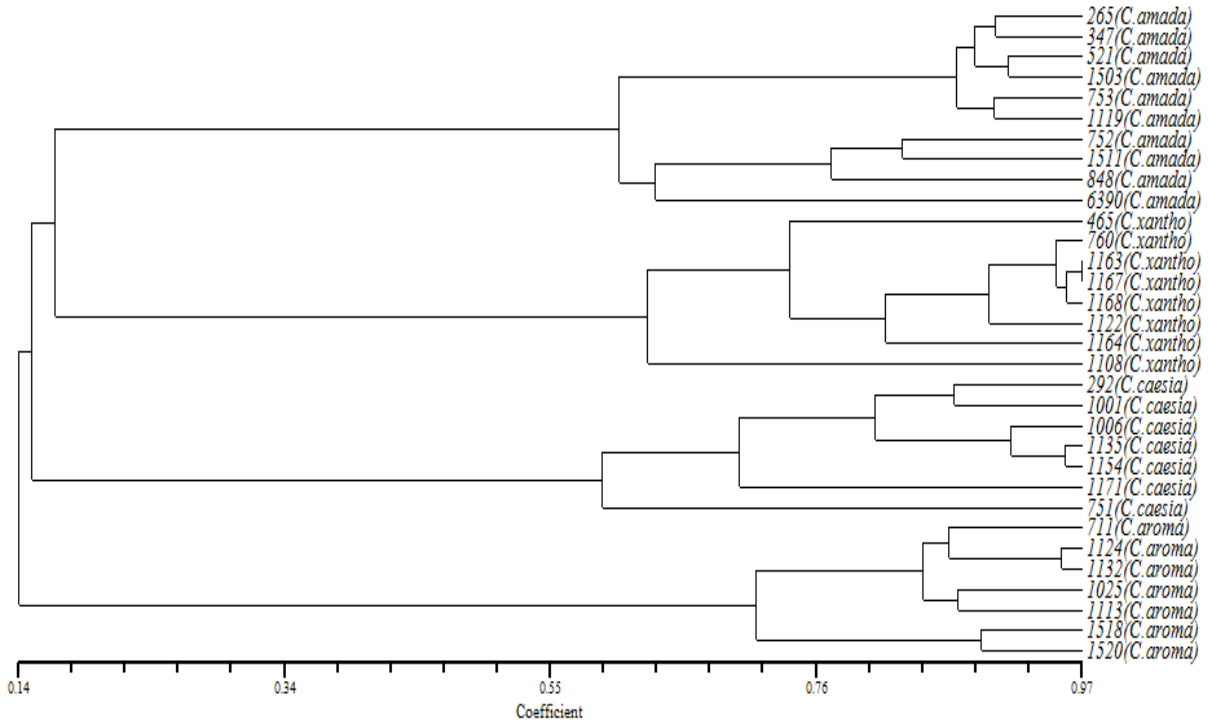


Fig. 60. UPGMA-Dendrogram of four *Curcuma* species based on ISSR markers using Sorensen-Dice similarity coefficients

4.4.3.3. Genetic distance among the species

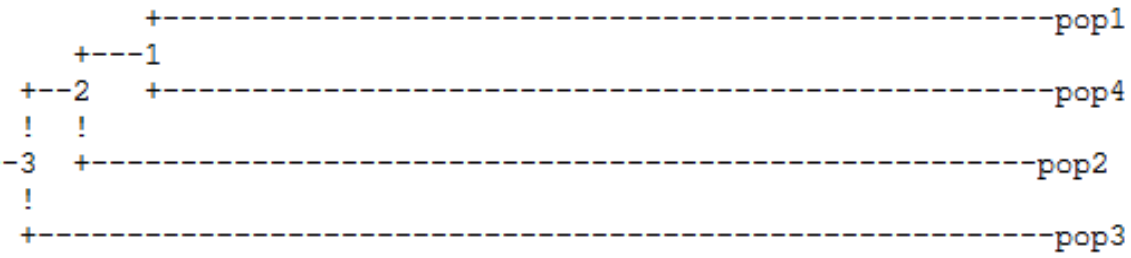
The Nei's genetic distance among the species was estimated using the POPGENE. Table 120 shows the Nei's genetic distance among the species. The upper diagonal matrix shows the Nei's genetic identity and the lower diagonal matrix shows the Nei's genetic distance.

Table 120. Nei's genetic distance and genetic identity of four *Curcuma* species using ISSR data

Species	*C. amada*	*C. aromatica*	*C. caesia*	*C. xanthorrhiza*
C. amada	****	0.628	0.599	0.652
C. aromatica	0.465	****	0.620	0.636
C. caesia	0.513	0.478	****	0.623
C. xanthorrhiza	0.428	0.452	0.473	****

The species *C. amada* and *C. xanthorrhiza* showed closer association (0.652) followed by *C. aromatica* and *C. xanthorrhiza* (0.636). The genetic distance was found to be highest between

C. caesia and *C. amada* (0.513) followed by *C. caesia* and *C. aromatica* (0.478). The dendrogram constructed using Nei's genetic distance and genetic identity is given in Figure 61.



Pop1- *C. amada*; pop2 – *C. aromatica;* pop3 – *C. caesia;* pop4 – *C. xanthorrhiza*

Fig. 61. Nei's genetic distance based dendrogram of genetic similarity among four *Curcuma* species based on ISSR markers.

4.4.4. SSR Analysis

Twenty-three SSR markers resulted in the amplification of 484 bands of which 362 were polymorphic bands. The bands amplified werein the range of 90 bp to 300 bp. The average number of amplified bands per primer was 21.04 and the average number of polymorphic bands per primer was 15.74. Some of the representative agarose gels are presented in the Figures 62 and 63. Details of the SSR primers, mean number of bands amplified per primer and number of polymorphic bands, the percentage of polymorphism, polymorphism information content (PIC) and marker index (MI) are given in Table 121.

The number of scored bands per primer ranged from 6 (CuMiSat 20) to 23 (CuMiSat 18). The percentage of polymorphic bands ranged from 48% (CuMiSat 16) to 100% (CuMiSat 17, CuMiSat 19) with an average percentage of polymorphism of 75%. The PIC (Polymorphism Information Content) value for SSR loci ranged from 0.14 (CuMiSat 20) to 0.46 (CuMiSat 17), with an average PIC and MI of 0.30 and 23.57, respectively.

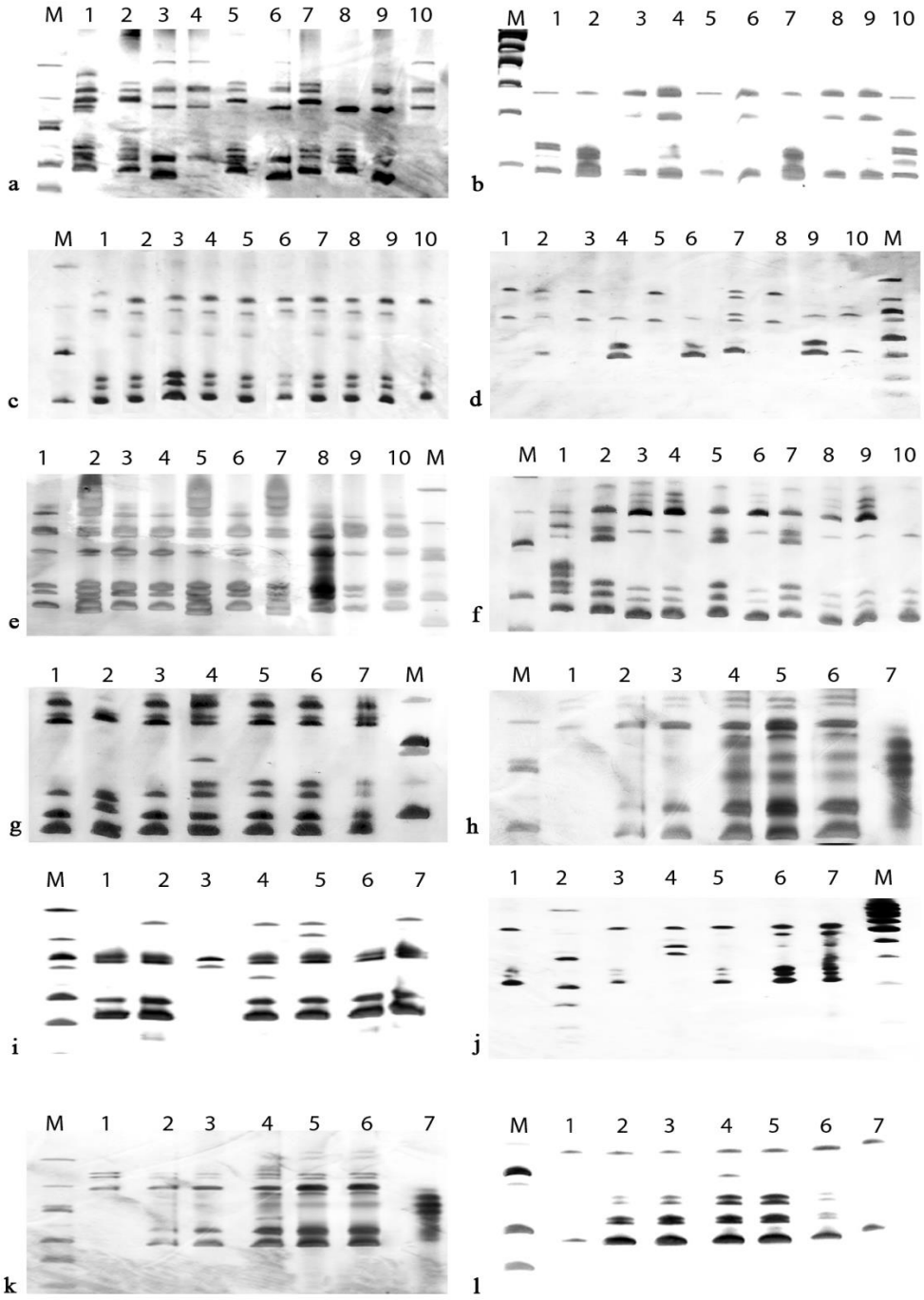


Fig. 62. SSR profiles of *C. amada*(a-f)and *C. aromatica*(h-l)using primers CuMiSat 18, CuMiSat 11, CuMiSat 17, CuMiSat 10, CuMiSat 14, CuMiSat 3, CuMiSat 20, CuMiSat 7,CuMiSat 29, CuMiSat 9, CuMiSat 18 and CuMiSat 10. *C. amada*, M-marker (Ultra-low range DNA ladder), Lane 1-10 are Acc. 265, Acc. 347, Acc.521, Acc. 752, Acc. 753, Acc. 848, Acc. 1119, Acc. 1503, Acc. 1511 and Acc. 6390. *C. aromatica*, M- marker (Ultra-low range DNA ladder), Lane 1-7 are Acc. 711, Acc. 1025, Acc. 1113, Acc. 1124, Acc. 1132, Acc. 1518 and Acc. 1520 respectively.

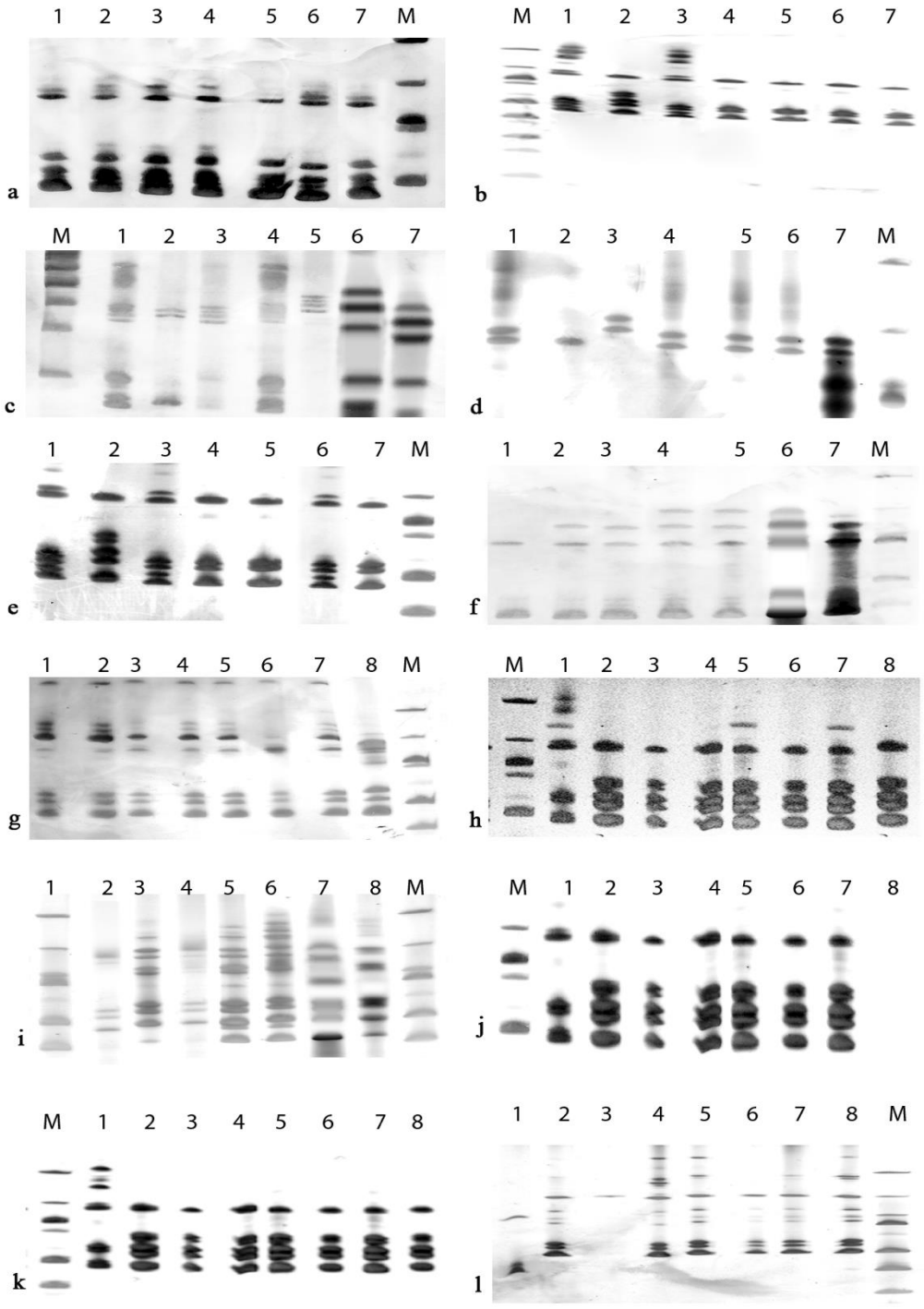


Fig. 63. SSR profiles of *C. caesia* (a-f) and *C. xanthorrhiza* (h-l) using primers CuMiSat 10, CuMiSat 11, CuMiSat 16, CuMiSat 19, CuMiSat 20, CuMiSat 3, CuMiSat 7, CuMiSat 35, CuMiSat 28, CuMiSat 3, CuMiSat 10 and CuMiSat 11.
C. caesia. M-marker (Ultra-low range DNA ladder), Lane 1-7 are Acc. 292, Acc. 751, Acc. 1001, Acc. 1006, Acc. 1135, Acc. 1154 and Acc. 1171.
C. xanthorrhiza. (Ultra-low range DNA ladder),Lane 1-8 are Acc. 465, Acc. 760, Acc. 1108, Acc. 1122, Acc. 1163, Acc. 1164, Acc. 1167 and Acc. 1168, respectively.

Table 121. Sequence information and amplification details of SSR primers used in four *Curcuma* species.

Primer	Forward primer (5'-3') Reverse primer (3'-5')	Total no of bands	No of Polymorphic bands	POL (%)	PIC	MI
CuMiSat1	AAACCGCAAGAAAACTGAAG CTCTTCCCTGAACGATTCC	27	20	74	0.39	28.54
CuMiSat2	TATGTGATGGTTGGGACG GTAGTGGAGGAAGACGCC	13	8	62	0.28	17.15
CuMiSat3	GCACTACTTCCTICTCGTTCAA CGTCGTAAAGATTAGCGTGTG	23	18	78	0.32	24.91
CuMiSat5	AGCAGTGCGTCTTTCATC CTCTTGTCACGGAACCTC	27	18	67	0.26	17.29
CuMiSat6	AAGAAACTCCAACCACAATCC CTTGTCTTCCTCCTCCATTG	20	10	50	0.15	7.36
CuMiSat7	AGCATGTGTCTAGCTCTTTGC AAGCAGTCGTTCCTCTACTGAC	16	14	88	0.30	26.61
CuMiSat8	CATTGCGTGCCCACTTCC CCTCCCTGTCGCTCTCCTC	15	12	80	0.32	25.65
CuMiSat9	AGTTGTGAAAGGGATAGAGTAGTTG AAGAAAGCAAATGCCAAGG	26	21	81	0.39	31.57
CuMiSat10	CACCCTATGAGTGCTAACTGAAG ACCTGCACCACGATCAAC	19	18	95	0.33	31.12
CuMiSat11	ACAGTCCCCTTCCCACTC TCTTGTTCCTATGCTCTACGC	18	13	72	0.26	18.56
CuMiSat12	AAGGTTGCTGCTTGTTGAGAA GCATATTGCCTTACATGCCTAA	19	14	74	0.25	18.52
CuMiSat13	CCCGAAGCCATTTCTCAG TCGTCTCTCCTCTGCCAAC	19	19	100	0.45	44.68
CuMiSat14	GCTGACTGTGGCAAAAGAGTC GCTGCGC'TTCTTCTTAATGAC	25	19	76	0.24	18.18
CuMiSat16	CATTTGTTCTGCTCGCTTCTAC CTGCTCCGCTGTCTCTCAC	33	16	48	0.23	11.33
CuMiSat17	ATGTGGTTGAGGAATGATGAGAC CTATTTCCCATAGCCCTTGTAGC	16	16	100	0.46	45.59
CuMiSat18	GTTCACAGCTTTAGCAGGGACAA CTCCTCTCCATATTCTCCATCTCG	31	23	74	0.30	22.18
CuMiSat19	CATGCAAATGGAAATTGACAC TGATAAATTGACACATGGCAGTC	22	22	100	0.41	41.16
CuMiSat20	CGATACGAGTCCATCTCTTCG CC'TTGCTTTGGTGGCTAGAG	12	6	50	0.14	7.23
CuMiSat21	TCATTCAAAGTCCGATGGAA TTCGAGTGCAGAAGGAGAATTA	21	18	86	0.29	25.09
CuMiSat22	AATTTATTAGCCCGGACCAC AAGAAAGTGAGTAGAAACCAAAG C	15	8	53	0.20	10.90
CuMiSat28	TTCAACTTCTCCTCGCTCAG GCAAGGTCTGCATCTATTTCTC	17	9	53	0.24	12.94
CuMiSat29	GTGGTATCCCCATGAAGAGC ATGACCAAGCCCTTTCACC	24	18	75	0.34	25.45
CuMiSat35	GGTTCGTCGCTGGAAAGTAAT GCATCTCAACAGGGGCTG	26	22	85	0.36	30.07
Mean		21.04	15.74	75	0.30	23.57

POL% - % Polymorphism; PIC-Polymorphism Information Content, MI-Marker Index

4.4.4.1. Genetic diversity analysis using SSR data

The genetic variance observed among four species using SSR markers are presented in Table 122.

Table 122. Estimates of genetic variance using SSR in four *Curcuma* species

Species	N	(Na)	(Ne)	(h)	(I)	% P	Gst
C. amada	10	1.30 ± 0.15	1.20 ± 0.11	0.12 ± 0.06	0.17 ± 0.09	82.39%	
C. aromatica	7	1.30 ± 0.17	1.17 ± 0.11	0.10 ± 0.06	0.16 ± 0.09	78.98%	
C. caesia	7	1.26 ± 0.17	1.17 ± 0.12	0.10 ± 0.06	0.15 ± 0.09	72.31%	
C. xanthorrhiza	8	1.22 ± 0.15	1.13 ± 0.10	0.08 ± 0.05	0.12 ± 0.08	62.91%	
Inter species	32	2.00 ± 0.01	1.53 ± 0.05	0.33 ± 0.02	0.50 ± 0.02	74.77%	0.70

Na - total number of alleles; Ne - number of effective alleles; h - Nei's (1973) gene diversity index; I - Shannon's information index; %P - percentage of polymorphism; Genetic differentiation Gst = (Ht-Hs)/Ht; Nm = 0.5*(1-Gst)/Gst

The observed number of alleles ranged from 1.22 ± 0.15 (*C. xanthorrhiza*) to 1.30 ± 0.17 (*C. aromatica*) & 1.30 ± 0.15 (*C. amada*). The effective number of alleles ranged from 1.13 ± 0.10 (*C. xanthorrhiza*) to 1.20 ± 0.11 (*C. amada*). Nei's gene diversity index (h) and Shannon's index (I) were highest in *C. amada* (h = 0.12 ± 0.06; I = 0.17 ± 0.09) while these estimates were lowest in *C. xanthorrhiza* (h = 0.08 ± 0.05 &I = 0.12 ± 0.08). Highest percentage of polymorphic bands was observed in *C. amada* (%P = 82.39%) and the lowest in *C. xanthorrhiza* (%P = 62.91%), with an average value of 74.77% among the species. Results showed a high value for Gst (0.70), which indicated that species are well differentiated from each other.

4.4.4.2. Cluster Analysis

4.4.4.2.1. Cluster Analysis of Individual Species.

The genetic similarity was calculated using Simple Matching similarity coefficients for individual species. In *C. amada*, the similarity matrix showed maximum similarity between Acc.753 and Acc.1119, with a similarity coefficient value of 0.84 and the least similar accessions were Acc.521 and Acc.753 with a value of 0.49 (Table 123).

Table 123. SSR similarity matrix of different accessions of *C. amada*

Accession	265	347	521	752	753	848	1119	1503	1511	6390
265	1.00									
347	0.73	1.00								
521	0.55	0.57	1.00							
752	0.59	0.60	0.73	1.00						
753	0.69	0.74	0.49	0.58	1.00					
848	0.55	0.52	0.76	0.82	0.55	1.00				
1119	0.62	0.77	0.53	0.58	0.84	0.58	1.00			
1503	0.64	0.66	0.65	0.80	0.64	0.74	0.70	1.00		
1511	0.57	0.58	0.77	0.72	0.50	0.80	0.57	0.75	1.00	
6390	0.61	0.58	0.68	0.71	0.54	0.74	0.59	0.74	0.75	1.00

The UPGMA dendrogram constructed using the similarity matrix of genetic distance is represented in Figure 64.

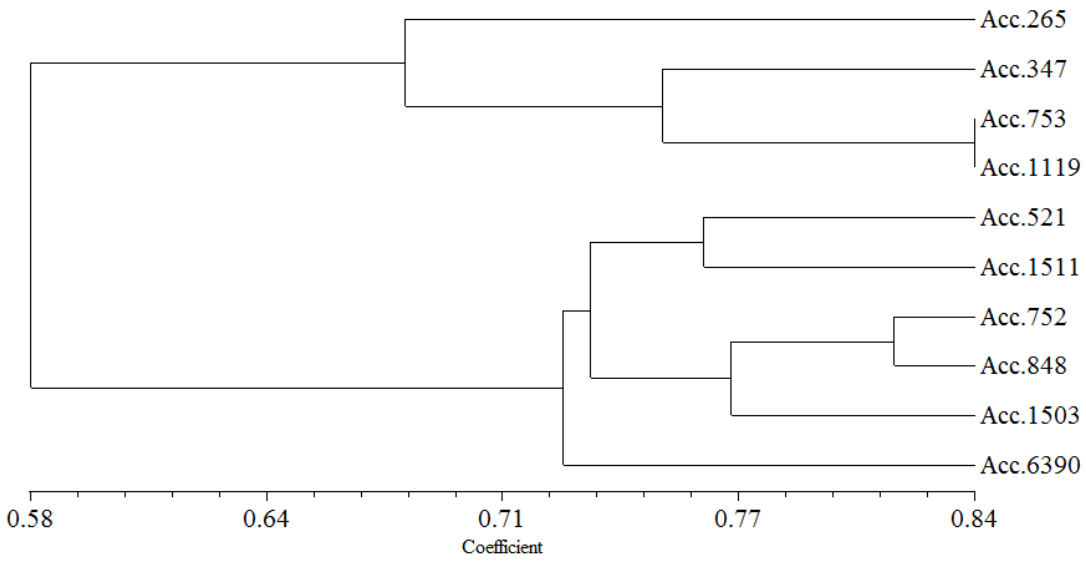


Fig. 64. SSR based UPGMA-Dendrogram of ten accessions of *C. amada.*

The dendrogram constructed clustered the ten accessions into four groups splitting at a coefficient value of 0.814. The groups formed are as follows:

Group I	-	Acc. 265
Group II	-	Acc. 347, Acc. 753 and Acc. 1119
Group III	-	Acc. 521, Acc. 1511, Acc. 752, Acc. 848, and Acc. 1503
Group IV	-	Acc. 6390

The first and fourth groups were formed by single accessions – Acc. 265 and Acc.6390, respectively. The second group was formed by three accessions *viz*., Acc. 347, Acc.753 and Acc.1119. The third group had the maximum number of accessions – Acc. 521, Acc.1511, Acc.752, Acc.848 and Acc.1503 with a maximum similarity between Acc.752 and Acc.848 (0.82).

Genetic similarity among the seven accessions of *C. aromatica* was estimated using Simple Matching similarity coefficients. The similarity matrix generated with SSR profile showed maximum similarity between Acc. 1132 and Acc. 1518 with a similarity coefficient value of 0.85. Minimum similarity was seen between Acc. 711 and Acc. 1520 with a value of 0.46 (Table 124).

Table 124. SSR similarity matrix of different accessions of *C. aromatica*

Accession	711	1025	1113	1124	1132	1518	1520
711	1.00						
1025	0.72	1.00					
1113	0.69	0.79	1.00				
1124	0.51	0.61	0.73	1.00			
1132	0.58	0.76	0.78	0.69	1.00		
1518	0.64	0.79	0.78	0.69	0.85	1.00	
1520	0.46	0.68	0.61	0.52	0.68	0.76	1.00

The dendrogram (UPGMA) constructed using Simple Matching similarity coefficients is depicted in Figure 65.

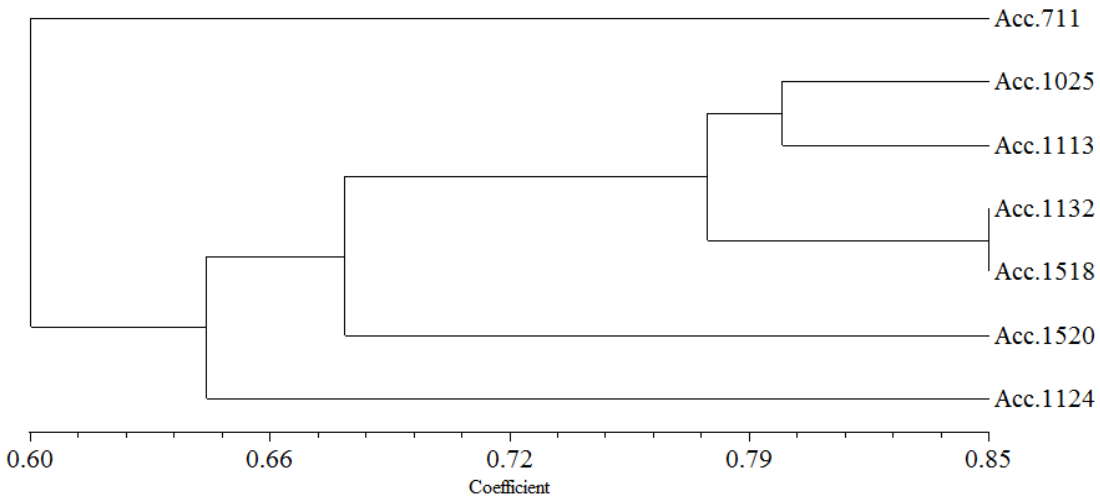


Fig. 65. SSR based UPGMA-Dendrogram of seven accessions of *C. aromatica.*

The UPGMA dendrogram constructed using the Simple Matching similarity matrix clustered the seven accessions of *C. aromatica* into four groups splitting at coefficient value of 0.777. The groups formed were as follows:

Group I	-	Acc. 711
Group II	-	Acc. 1025, Acc. 1113, Acc.1132 and Acc. 1518
Group III	-	Acc. 1520
Group IV	-	Acc. 1124

First group was formed by a single accession, Acc. 711. Second group had the highest number of accessions namely Acc.1025, Acc.1113, Acc.1132 and Acc.1518. Acc. 1132 and 1518 shared maximum similarity with each other. Third and fourth groups were formed by single accession each *viz*., Acc.1520 and Acc.1124, respectively.

In case of *C. caesia*, the similarity matrix using the Simple Matching similarity coefficients showed maximum similarity between Acc.1135 and Acc.1154 with coefficient value of 0.76. Minimum similarity was exhibited byAcc. 292 and Acc.751 with similarity coefficient value of 0.61 (Table 125).

Table 125. SSR similarity matrix of different accessions of *C. caesia*

Accession	292	751	1001	1006	1135	1154	1171
292	1.00						
751	0.61	1.00					
1001	0.73	0.76	1.00				
1006	0.70	0.66	0.68	1.00			
1135	0.67	0.73	0.71	0.72	1.00		
1154	0.75	0.69	0.71	0.70	0.76	1.00	
1171	0.65	0.70	0.70	0.70	0.74	0.68	1.00

The UPGMA dendrogram constructed based on Simple Matching similarity coefficients of *C. caesia* is given in Figure 66.

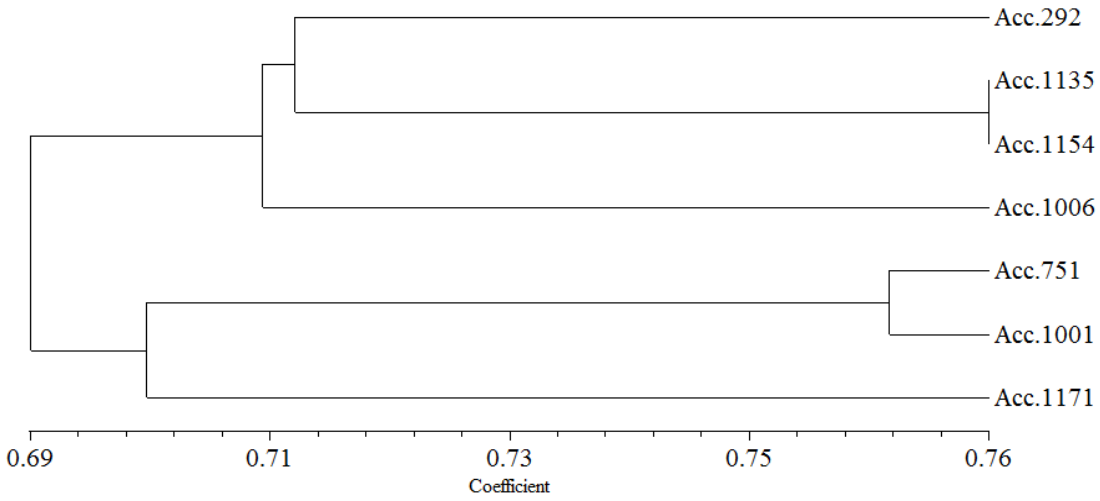


Fig. 66. SSR based UPGMA-Dendrogram of seven accessions of *C. caesia*.

The UPGMA dendrogram constructed using the Simple Matching similarity matrix clustered the seven accessions of *C. caesia* into four groups splitting at coefficient value of 0.711. The groups formed were as follows:

Group I - Acc. 292, Acc. 1135and 1154

Group II - Acc. 1006

Group III - Acc. 751 and 1001

Group IV - Acc. 1171

The first group was formed by three accessions *viz*., Acc. 292, Acc. 1154 and Acc.1135. Maximum similarity was found between Acc.1135 and Acc.1154, which were collected from Thrissur, Kerala.The second and fourth groups were formed by Acc. 1006 and Acc.1171, respectively. Acc.751 and Acc.1001 formed the third group.

Among the *C. xanthorrhiza* accessions, similarity matrix generated using Simple Matching similarity coefficients showed highest similarity between the Acc.1122 and Acc.760 besides Acc.1167 and Acc.1164 with a coefficient value of 0.87. Acc.1122 and Acc.465 showed the least similarity (0.63) (Table 126).

Table 126. SSR Similarity matrix of different accessions of *C. xanthorrhiza*

Accessions	465	760	1108	1122	1163	1164	1167	1168
465	1.00							
760	0.70	1.00						
1108	0.67	0.69	1.00					
1122	0.63	0.87	0.71	1.00				
1163	0.65	0.80	0.76	0.82	1.00			
1164	0.74	0.73	0.65	0.73	0.78	1.00		
1167	0.76	0.80	0.73	0.78	0.85	0.87	1.00	
1168	0.72	0.74	0.69	0.69	0.76	0.73	0.76	1.00

The UPGMA dendrogram (Figure 67) constructed using Simple Matching similarity matrix clustered the eight accessions into four groups splitting at a coefficient value of 0.77. The groupings were as follows:

Group I - Acc. 465

Group II - Acc. 760 and Acc. 1122, Acc.1163, Acc. 1164 and Acc.1167.

Group III - Acc.1168

Group IV - Acc. 1108

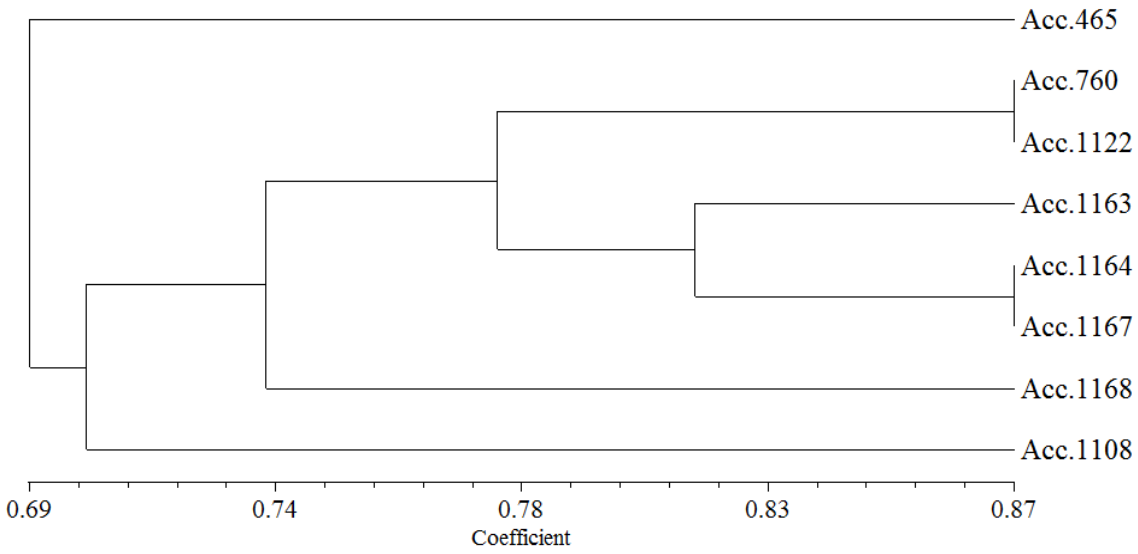


Fig. 67. SSR based UPGMA-Dendrogram of eight accessions of *C. xanthorrhiza.*

First, third and fourth groups were formed by Acc. 465, Acc.1168 and Acc. 1108, respectively. Second group, was comprised of five accessions *viz*., Acc.760, Acc.1122, Acc. 1163, Acc. 1164 and Acc.1167. Acc.1164 and Acc.1167 as well as Acc.760 and Acc.1122 were closely related to each other with coefficient value of 0.87.

4.4.4.2.2. Cluster analysis of four species

The similarity matrices obtained from four *Curcuma*species using Simple Matching, Jaccard's and Sorensen-Dice similarity coefficients are depicted in Tables 127-129. Figures 68- 70 shows the UPGMA dendrogram.

Among the accessions, maximum similarity was observed between Acc.1164 and Acc.1167 of *C. xanthorrhiza* (Simple Matching coefficient = 0.95, Jaccard's = 0.86 and Dice = 0.92) besides Acc. 760 and Acc.1122 (SM coefficient = 0.95, Jaccard's = 0.84 and Dice = 0.91). Genetically least similar accessions were Acc. 1171 (*C. caesia*) and Acc. 1167 (*C. xanthorrhiza*) with a similarity coefficient of 0.54 in case of Simple matching; Acc. 753 (*C. amada*) and Acc. 1164 (*C. xanthorrhiza*) with coefficient as 0.08 and 0.15 in case of Jaccard's and Sorensen-Dice, respectively. Acc. 753 (*C. amada*) and Acc. 1520 (*C. aromatica*); Acc. 753 (*C. amada*) and Acc. 1168 (*C. xanthorrhiza*) also exhibited the least similarity in Jaccard's and Sorensen-Dice similarity coefficients.

The UPGMA dendrogram based on the Simple Matching, Jaccard's and Sorensen-Dice similarity coefficients separated thirty-two accessions into four groups splitting at coefficients of 0.841, 0.524 and 0.686, respectively in Simple matching, Jaccard's and Sorensen-Dice.

The four groupings obtained based on Simple Matching, Jaccard's and Sorensen-Dice matrices were given below:

Group I	*C. amada*-Acc. 265, 521, 1503, 1511, 347. 6390, 753, 1119, 752 and 848
Group II	*C. caesia*-Acc. 292, 1001, 1154, 751, 1006, 1171 and 1135
Group III	*C. aromatica*-Acc. 711, 1025, 1113, 1132, 1518, 1520 and 1124
Group IV	*C. xanthorrhiza*-Acc. 465, 1168, 760, 1122, 1163, 1167, 1164 and 1108

The UPGMA based SAHN clustering program has clustered 32 accessions into four different groups using Simple Matching, Jaccard's and Sorensen-Dice similarity coefficients, with each group consisting of accessions from their own respective species. Group 1, 2, 3 and 4 were formed by ten accessions of *C. amada*, seven accessions of *C. caesia*, seven accessions of *C. aromatica* and eight accessions of *C. xanthorrhiza*, respectively.

Table 127. SSR similarity matrix of four *Curcuma* species using Simple Matching similarity coefficients

		C.amada										*C.aromatica*							*C.caesia*							*C.xanthorrhiza*							
		265	347	521	752	753	848	1119	1503	1511	6390	711	1025	1113	1124	1132	1518	1520	292	751	1001	1006	1135	1154	1171	465	760	1108	1122	1163	1164	1167	1168
C.amada	265	1.00																															
	347	0.90	1.00																														
	521	0.83	0.84	1.00																													
	752	0.85	0.85	0.90	1.00																												
	753	0.88	0.90	0.81	0.84	1.00																											
	848	0.83	0.82	0.91	0.93	0.83	1.00																										
	1119	0.86	0.91	0.82	0.84	0.94	0.84	1.00																									
	1503	0.86	0.87	0.87	0.93	0.87	0.90	0.89	1.00																								
	1511	0.84	0.84	0.91	0.90	0.82	0.92	0.84	0.90	1.00																							
	6390	0.85	0.84	0.88	0.89	0.83	0.90	0.85	0.90	0.90	1.00																						
C.aromatica	711	0.65	0.61	0.63	0.65	0.65	0.64	0.63	0.62	0.63	0.64	1.00																					
	1025	0.60	0.55	0.59	0.60	0.59	0.60	0.57	0.57	0.57	0.60	0.89	1.00																				
	1113	0.59	0.55	0.58	0.59	0.59	0.59	0.57	0.57	0.57	0.59	0.89	0.92	1.00																			
	1124	0.63	0.58	0.62	0.64	0.60	0.63	0.58	0.60	0.59	0.62	0.82	0.86	0.90	1.00																		
	1132	0.59	0.55	0.57	0.60	0.59	0.60	0.57	0.57	0.56	0.60	0.84	0.91	0.92	0.88	1.00																	
	1518	0.60	0.56	0.59	0.60	0.60	0.61	0.58	0.59	0.57	0.61	0.87	0.92	0.92	0.88	0.94	1.00																
	1520	0.62	0.60	0.61	0.63	0.62	0.62	0.61	0.60	0.59	0.61	0.80	0.88	0.86	0.82	0.88	0.91	1.00															
C.caesia	292	0.60	0.58	0.59	0.60	0.61	0.60	0.59	0.57	0.57	0.57	0.62	0.57	0.56	0.58	0.55	0.56	0.59	1.00														
	751	0.62	0.62	0.61	0.64	0.66	0.63	0.63	0.61	0.60	0.61	0.64	0.59	0.59	0.60	0.57	0.57	0.61	0.85	1.00													
	1001	0.63	0.61	0.61	0.63	0.65	0.63	0.62	0.61	0.59	0.62	0.65	0.59	0.60	0.60	0.58	0.59	0.63	0.89	0.91	1.00												
	1006	0.64	0.62	0.62	0.65	0.66	0.64	0.63	0.61	0.61	0.63	0.62	0.57	0.57	0.59	0.57	0.58	0.63	0.89	0.87	0.88	1.00											
	1135	0.64	0.62	0.63	0.66	0.66	0.65	0.63	0.63	0.61	0.63	0.65	0.60	0.61	0.62	0.59	0.59	0.64	0.88	0.90	0.89	0.89	1.00										
	1154	0.60	0.58	0.59	0.60	0.61	0.60	0.59	0.57	0.57	0.58	0.61	0.56	0.56	0.58	0.55	0.55	0.60	0.90	0.88	0.89	0.89	0.91	1.00									
	1171	0.61	0.59	0.59	0.62	0.63	0.61	0.59	0.59	0.58	0.59	0.60	0.55	0.56	0.57	0.54	0.55	0.59	0.87	0.88	0.89	0.88	0.90	0.88	1.00								
C.xanthorrhiza	465	0.64	0.61	0.61	0.64	0.61	0.62	0.60	0.60	0.59	0.60	0.64	0.59	0.59	0.62	0.59	0.60	0.64	0.59	0.60	0.61	0.60	0.60	0.59	0.58	1.00							
	760	0.61	0.59	0.59	0.62	0.60	0.63	0.59	0.59	0.58	0.59	0.63	0.58	0.58	0.62	0.59	0.59	0.62	0.57	0.60	0.60	0.59	0.60	0.57	0.57	0.89	1.00						
	1108	0.64	0.62	0.61	0.64	0.63	0.63	0.61	0.61	0.58	0.62	0.66	0.60	0.61	0.64	0.60	0.61	0.62	0.59	0.63	0.63	0.62	0.62	0.59	0.59	0.88	0.89	1.00					
	1122	0.62	0.59	0.60	0.63	0.61	0.63	0.59	0.60	0.59	0.59	0.65	0.61	0.60	0.65	0.59	0.61	0.63	0.57	0.61	0.59	0.59	0.60	0.57	0.57	0.87	0.95	0.90	1.00				
	1163	0.62	0.58	0.58	0.60	0.60	0.61	0.58	0.58	0.58	0.58	0.63	0.58	0.60	0.62	0.58	0.59	0.61	0.57	0.60	0.59	0.58	0.59	0.56	0.56	0.88	0.93	0.91	0.94	1.00			
	1164	0.59	0.55	0.57	0.57	0.55	0.59	0.55	0.55	0.56	0.56	0.62	0.59	0.58	0.61	0.57	0.59	0.61	0.56	0.57	0.57	0.56	0.56	0.56	0.54	0.91	0.91	0.88	0.91	0.92	1.00		
	1167	0.59	0.57	0.56	0.59	0.57	0.59	0.57	0.57	0.55	0.56	0.62	0.57	0.57	0.61	0.56	0.58	0.60	0.55	0.58	0.57	0.57	0.57	0.55	0.54	0.91	0.93	0.91	0.92	0.95	0.95	1.00	
	1168	0.61	0.59	0.59	0.60	0.59	0.62	0.58	0.58	0.58	0.60	0.61	0.56	0.57	0.60	0.57	0.57	0.61	0.57	0.61	0.60	0.59	0.60	0.57	0.56	0.90	0.91	0.89	0.89	0.91	0.91	0.92	1.00

Table 128. SSR similarity matrix of four *Curcuma* species using Jaccard's similarity coefficients

		C.amada										*C.aromatica*							*C.caesia*							*C.xanthorrhiza*							
		265	347	521	752	753	848	1119	1503	1511	6390	711	1025	1113	1124	1132	1518	1520	292	751	1001	1006	1135	1154	1171	465	760	1108	1122	1163	1164	1167	1168
C.amada	265	1.00																															
	347	0.66	1.00																														
	521	0.50	0.54	1.00																													
	752	0.50	0.54	0.67	1.00																												
	753	0.59	0.67	0.45	0.50	1.00																											
	848	0.47	0.48	0.71	0.76	0.48	1.00																										
	1119	0.54	0.71	0.50	0.51	0.78	0.52	1.00																									
	1503	0.58	0.62	0.63	0.75	0.58	0.70	0.65	1.00																								
	1511	0.53	0.56	0.74	0.68	0.47	0.74	0.53	0.71	1.00																							
	6390	0.53	0.53	0.63	0.64	0.46	0.68	0.53	0.70	0.70	1.00																						
C.aromatica	711	0.12	0.11	0.15	0.13	0.12	0.14	0.11	0.14	0.15	0.14	1.00																					
	1025	0.12	0.11	0.16	0.14	0.12	0.15	0.12	0.15	0.15	0.16	0.66	1.00																				
	1113	0.11	0.10	0.16	0.13	0.11	0.14	0.11	0.14	0.15	0.14	0.63	0.77	1.00																			
	1124	0.14	0.11	0.18	0.17	0.10	0.17	0.10	0.16	0.15	0.16	0.45	0.59	0.69	1.00																		
	1132	0.12	0.11	0.15	0.14	0.12	0.15	0.12	0.15	0.14	0.15	0.53	0.73	0.75	0.66	1.00																	
	1518	0.13	0.11	0.16	0.14	0.12	0.16	0.12	0.16	0.15	0.16	0.58	0.76	0.75	0.65	0.82	1.00																
	1520	0.09	0.11	0.14	0.12	0.09	0.12	0.12	0.14	0.12	0.12	0.38	0.63	0.57	0.48	0.63	0.70	1.00															
C.caesia	292	0.13	0.13	0.16	0.14	0.14	0.15	0.14	0.15	0.15	0.12	0.15	0.15	0.14	0.14	0.14	0.14	0.13	1.00														
	751	0.11	0.14	0.16	0.15	0.15	0.15	0.14	0.15	0.14	0.12	0.13	0.13	0.14	0.12	0.12	0.11	0.11	0.57	1.00													
	1001	0.11	0.13	0.15	0.14	0.14	0.14	0.13	0.15	0.13	0.13	0.13	0.14	0.14	0.13	0.13	0.13	0.13	0.67	0.68	1.00												
	1006	0.15	0.16	0.18	0.18	0.17	0.18	0.16	0.18	0.17	0.16	0.11	0.13	0.12	0.13	0.13	0.13	0.14	0.66	0.59	0.61	1.00											
	1135	0.13	0.15	0.18	0.18	0.16	0.17	0.14	0.18	0.16	0.15	0.14	0.15	0.15	0.15	0.14	0.13	0.14	0.62	0.66	0.63	0.65	1.00										
	1154	0.13	0.15	0.17	0.15	0.14	0.16	0.14	0.16	0.16	0.13	0.13	0.15	0.14	0.14	0.14	0.13	0.14	0.72	0.64	0.66	0.66	0.71	1.00									
	1171	0.13	0.14	0.16	0.16	0.16	0.15	0.14	0.17	0.15	0.14	0.11	0.13	0.13	0.12	0.12	0.12	0.12	0.62	0.64	0.65	0.65	0.68	0.65	1.00								
C.xanthorrhiza	465	0.14	0.14	0.16	0.16	0.10	0.15	0.12	0.16	0.14	0.13	0.14	0.15	0.14	0.16	0.15	0.16	0.15	0.15	0.12	0.12	0.14	0.12	0.15	0.12	1.00							
	760	0.11	0.12	0.14	0.14	0.10	0.16	0.11	0.14	0.14	0.12	0.12	0.13	0.13	0.16	0.15	0.14	0.13	0.13	0.12	0.11	0.13	0.12	0.13	0.12	0.66	1.00						
	1108	0.11	0.12	0.12	0.12	0.09	0.12	0.10	0.13	0.10	0.11	0.13	0.12	0.14	0.15	0.13	0.13	0.10	0.12	0.12	0.12	0.12	0.11	0.12	0.10	0.61	0.63	1.00					
	1122	0.11	0.11	0.14	0.15	0.09	0.16	0.11	0.15	0.13	0.11	0.15	0.16	0.15	0.19	0.15	0.16	0.14	0.11	0.11	0.09	0.11	0.11	0.12	0.11	0.60	0.84	0.64	1.00				
	1163	0.12	0.11	0.12	0.11	0.09	0.13	0.10	0.13	0.13	0.10	0.13	0.14	0.15	0.16	0.15	0.14	0.12	0.12	0.12	0.11	0.11	0.10	0.12	0.10	0.62	0.77	0.70	0.78	1.00			
	1164	0.12	0.12	0.15	0.12	0.08	0.15	0.11	0.13	0.14	0.12	0.15	0.18	0.17	0.19	0.16	0.18	0.17	0.15	0.12	0.12	0.13	0.11	0.15	0.13	0.72	0.72	0.62	0.71	0.76	1.00		
	1167	0.12	0.13	0.13	0.14	0.10	0.14	0.12	0.15	0.13	0.11	0.15	0.16	0.15	0.18	0.15	0.16	0.14	0.14	0.13	0.12	0.13	0.12	0.14	0.11	0.73	0.77	0.69	0.75	0.82	0.86	1.00	
	1168	0.11	0.12	0.15	0.12	0.09	0.15	0.11	0.13	0.13	0.13	0.10	0.12	0.13	0.14	0.13	0.13	0.12	0.13	0.13	0.12	0.13	0.12	0.14	0.11	0.68	0.71	0.64	0.65	0.72	0.72	0.75	1.00

Table 129. SSR similarity matrix of four *Curcuma* species using Sorensen-Dice similarity coefficients

		C.amada										*C.aromatica*							*C.caesia*							*C.xanthorrhiza*							
		265	347	521	752	753	848	1119	1503	1511	6390	711	1025	1113	1124	1132	1518	1520	292	751	1001	1006	1135	1154	1171	465	760	1108	1122	1163	1164	1167	1168
C.amada	265	1.00																															
	347	0.80	1.00																														
	521	0.66	0.70	1.00																													
	752	0.67	0.70	0.80	1.00																												
	753	0.74	0.80	0.62	0.66	1.00																											
	848	0.64	0.65	0.83	0.86	0.65	1.00																										
	1119	0.70	0.83	0.67	0.68	0.87	0.69	1.00																									
	1503	0.73	0.77	0.77	0.86	0.74	0.82	0.79	1.00																								
	1511	0.69	0.72	0.85	0.81	0.64	0.85	0.69	0.83	1.00																							
	6390	0.69	0.70	0.78	0.78	0.63	0.81	0.70	0.82	0.82	1.00																						
C.aromatica	711	0.21	0.19	0.26	0.24	0.21	0.24	0.21	0.25	0.26	0.24	1.00																					
	1025	0.22	0.19	0.28	0.24	0.21	0.26	0.22	0.26	0.26	0.27	0.79	1.00																				
	1113	0.20	0.19	0.27	0.24	0.20	0.25	0.20	0.25	0.26	0.25	0.78	0.87	1.00																			
	1124	0.24	0.20	0.30	0.29	0.19	0.30	0.19	0.28	0.27	0.27	0.62	0.74	0.82	1.00																		
	1132	0.22	0.20	0.26	0.25	0.21	0.27	0.22	0.26	0.25	0.27	0.70	0.85	0.86	0.79	1.00																	
	1518	0.22	0.20	0.27	0.25	0.21	0.27	0.22	0.28	0.26	0.27	0.73	0.86	0.86	0.79	0.90	1.00																
	1520	0.17	0.19	0.24	0.21	0.16	0.22	0.21	0.24	0.22	0.21	0.55	0.77	0.72	0.64	0.78	0.83	1.00															
C.caesia	292	0.23	0.24	0.28	0.24	0.24	0.26	0.24	0.25	0.26	0.22	0.26	0.26	0.24	0.24	0.24	0.25	0.23	1.00														
	751	0.19	0.25	0.27	0.26	0.27	0.26	0.25	0.27	0.24	0.22	0.23	0.23	0.24	0.22	0.22	0.20	0.20	0.73	1.00													
	1001	0.21	0.22	0.26	0.25	0.24	0.25	0.23	0.26	0.24	0.23	0.23	0.24	0.24	0.22	0.23	0.22	0.22	0.80	0.81	1.00												
	1006	0.26	0.27	0.30	0.30	0.29	0.31	0.27	0.30	0.29	0.27	0.20	0.23	0.22	0.22	0.23	0.24	0.25	0.80	0.74	0.76	1.00											
	1135	0.23	0.26	0.30	0.30	0.28	0.30	0.25	0.31	0.28	0.26	0.25	0.26	0.26	0.25	0.25	0.23	0.25	0.77	0.79	0.77	0.79	1.00										
	1154	0.23	0.25	0.29	0.26	0.25	0.28	0.25	0.27	0.27	0.24	0.23	0.26	0.25	0.25	0.25	0.23	0.24	0.84	0.78	0.80	0.80	0.83	1.00									
	1171	0.23	0.24	0.28	0.28	0.27	0.26	0.24	0.29	0.26	0.24	0.20	0.23	0.23	0.22	0.21	0.21	0.22	0.77	0.78	0.78	0.79	0.81	0.79	1.00								
C.xanthorrhiza	465	0.25	0.25	0.27	0.27	0.19	0.26	0.21	0.27	0.24	0.22	0.25	0.26	0.25	0.28	0.27	0.27	0.26	0.26	0.21	0.21	0.24	0.21	0.26	0.22	1.00							
	760	0.20	0.22	0.25	0.25	0.18	0.28	0.20	0.25	0.24	0.21	0.21	0.24	0.24	0.28	0.26	0.25	0.23	0.23	0.21	0.20	0.23	0.21	0.23	0.21	0.80	1.00						
	1108	0.19	0.21	0.21	0.22	0.17	0.22	0.18	0.22	0.18	0.19	0.23	0.22	0.24	0.26	0.23	0.22	0.17	0.21	0.22	0.21	0.21	0.19	0.21	0.18	0.76	0.78	1.00					
	1122	0.19	0.20	0.25	0.25	0.17	0.27	0.19	0.25	0.23	0.19	0.26	0.27	0.26	0.32	0.26	0.28	0.25	0.20	0.21	0.17	0.20	0.19	0.21	0.19	0.75	0.91	0.78	1.00				
	1163	0.21	0.20	0.22	0.20	0.16	0.23	0.18	0.23	0.23	0.18	0.23	0.25	0.27	0.28	0.25	0.25	0.22	0.22	0.21	0.19	0.21	0.19	0.21	0.19	0.77	0.87	0.82	0.88	1.00			
	1164	0.22	0.21	0.26	0.22	0.15	0.26	0.19	0.23	0.25	0.21	0.26	0.30	0.29	0.32	0.28	0.31	0.28	0.26	0.22	0.21	0.23	0.21	0.26	0.22	0.84	0.83	0.77	0.83	0.86	1.00		
	1167	0.22	0.22	0.24	0.24	0.17	0.25	0.22	0.26	0.22	0.21	0.25	0.27	0.26	0.30	0.27	0.28	0.24	0.24	0.23	0.21	0.23	0.22	0.25	0.20	0.85	0.87	0.82	0.86	0.90	0.92	1.00	
	1168	0.20	0.22	0.26	0.21	0.16	0.26	0.20	0.24	0.24	0.23	0.19	0.21	0.23	0.24	0.23	0.23	0.21	0.22	0.23	0.21	0.24	0.22	0.24	0.20	0.81	0.83	0.78	0.79	0.84	0.84	0.85	1.00

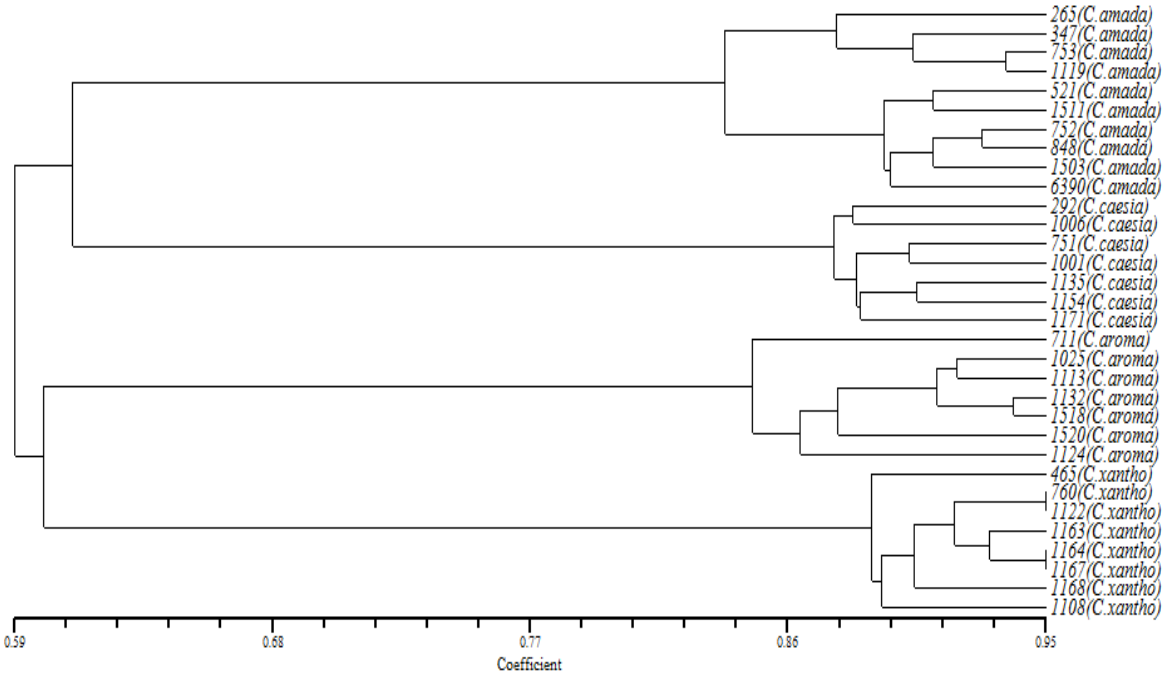


Fig. 68. UPGMA-Dendrogram of four *Curcuma* species based on SSR markers using Simple Matching similarity coefficients.

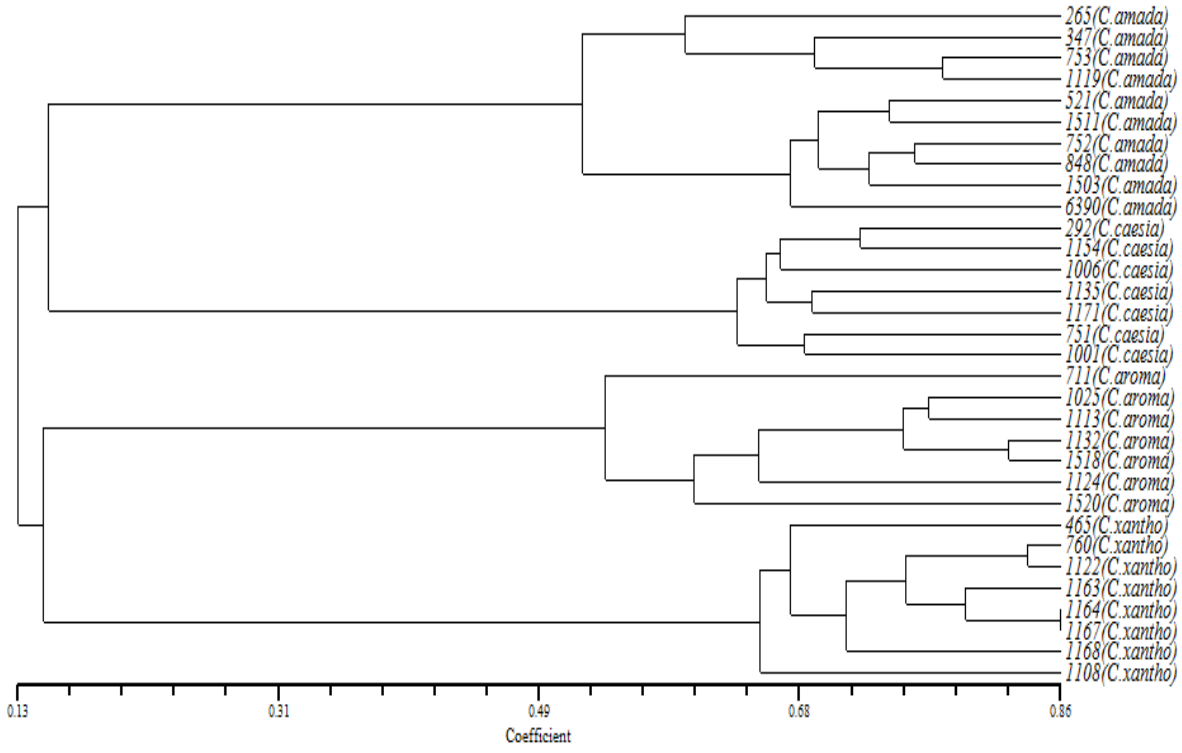


Fig. 69. UPGMA-Dendrogram of four *Curcuma* species based on SSR markers using Jaccard's similarity coefficients.

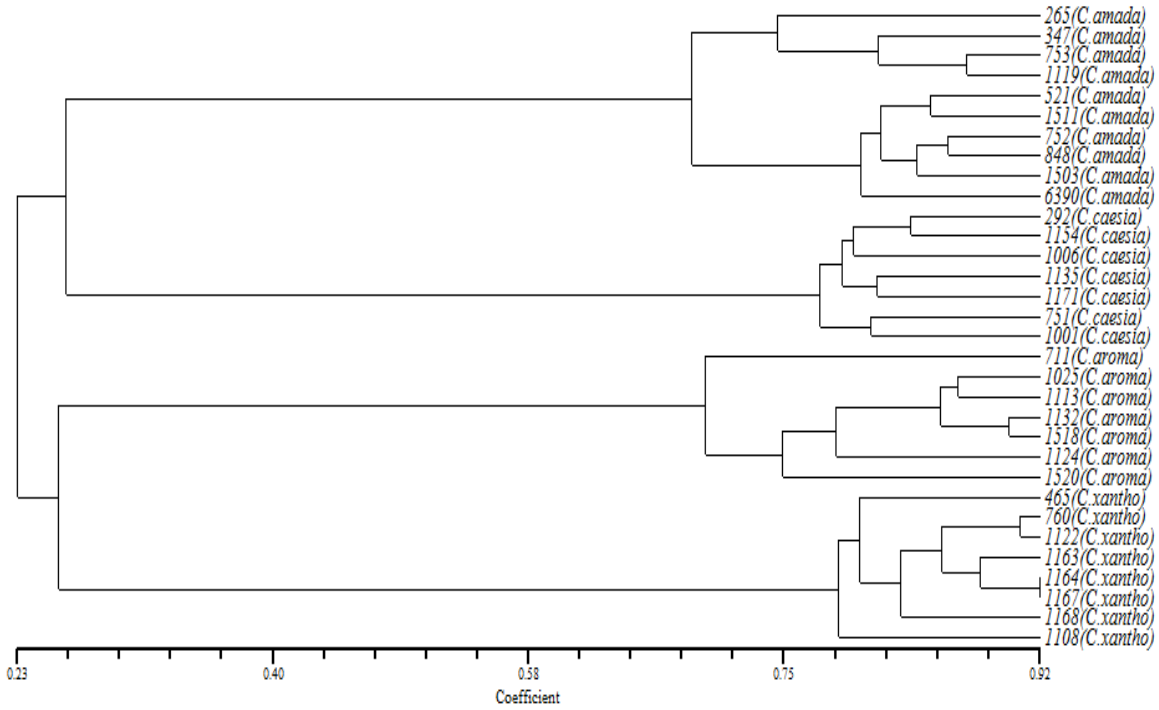


Fig. 70. UPGMA-Dendrogram of four *Curcuma* species based on SSR markers using Sorensen-Dice similarity coefficients.

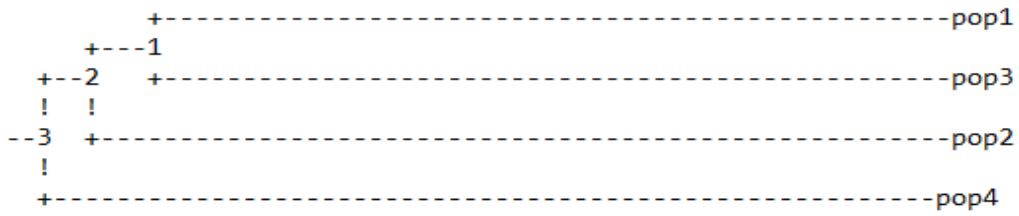
4.4.4.3. Genetic distance among theSpecies

The Nei's genetic distance (1972) among the species was estimated using SSR marker profile. Table 130 shows the Nei's genetic distance among the species. The upper diagonal matrix shows the Nei's genetic identity and the lower diagonal matrix shows the Nei's genetic distance.

Table 130. Nei's genetic distance and genetic identity of four *Curcuma* species based on SSR markers

Species	*C. amada*	*C. aromatica*	*C. caesia*	*C. xanthorrhiza*
C. amada	****	0.672	0.685	0.658
C. aromatica	0.397	****	0.654	0.662
C. caesia	0.378	0.425	****	0.638
C. xanthorrhiza	0.418	0.413	0.449	****

C. amada and *C. caesia* showed closer association (0.378) followed by *C. amada* and *C. aromatica.* The dendrogram constructed using Nei's genetic distance and genetic identity is given in Figure 71.



Pop1 – *C. amada;* pop2 – *C. aromatica;* pop3 – *C. caesia;* pop4 – *C. xanthorrhiza*

Fig. 71. Dendrogram of genetic similarity among four *Curcuma* species based on SSR markers using Nei's genetic distance.

4.4.5. Characterization of four *Curcuma* species using combined data of RAPD, ISSR and SSR markers

Thirty-two accessions belonging to four *Curcuma* species were characterized using combined data of RAPD, ISSR and SSR markers which generated a total of 2262 bands, out of which 1450 bands were polymorphic in nature with an average polymorphic percentage of 65%.

4.4.5.1. Cluster analysis of individual species

Accessions of *C. amada*, *C. aromatica* and *C. xanthorrhiza* showed similar grouping patterns in the dendrogram constructed using Simple Matching, Jaccard's and Sorensen-Dice similarity coefficients. However, in *C. caesia*, the dendrogram constructed using the Simple Matching algorithm slightly varied from Jaccard's and Sorensen-Dice. Handel *et al*. (2004) stated that it is preferable to use Simple Matching function to analyse diversity within species.

The similarity matrix constructed based on the combined RAPD, ISSR and SSR data on accessions of *C. amada* showed maximum similarity between Acc. 265 and Acc. 347 with similarity coefficient value of 0.84. The minimum similarity was between Acc. 265 and Acc. 848 with a value of 0.59 in Simple Matching similarity matrix (Table 131).

Table 131. Similarity matrix of different accessions of *C. amada* obtained from the combined RAPD, ISSR and SSR data

Accession	265	347	521	752	753	848	1119	1503	1511	6390
265	1.00									
347	0.84	1.00								
521	0.83	0.80	1.00							
752	0.62	0.62	0.65	1.00						
753	0.79	0.83	0.74	0.68	1.00					
848	0.59	0.63	0.63	0.81	0.66	1.00				
1119	0.76	0.81	0.75	0.65	0.86	0.67	1.00			
1503	0.81	0.82	0.84	0.66	0.78	0.67	0.79	1.00		
1511	0.69	0.70	0.73	0.77	0.68	0.74	0.64	0.73	1.00	
6390	0.69	0.68	0.72	0.68	0.65	0.67	0.67	0.73	0.73	1.00

The UPGMA dendrogram constructed using the Simple Matching similarity coefficients of *C. amada* accessions is given in Figure 72. The UPGMA dendrogram constructed using the Simple Matching similarity coefficients clustered the ten accessions of *C. amada* into three groups splitting at coefficient value of 0.780. The groups formed are as follows:

Group I - Acc. 265, Acc. 347, Acc. 521, Acc. 1503, Acc. 753 and Acc. 1119

Group II - Acc. 752 and Acc. 848, Acc. 1511

Group III - Acc. 6390

First group being the major one consisted of six accessions, among which Acc.753 and Acc.1119 showed maximum similarity (0.86). Second group was formed by three accessions, Acc.752, Acc.848 and Acc.1511. Acc. 6390 formed the third group. Similar to the dendrogram constructed using individual marker system, Acc. 6390 maintained a separate status for combined marker system as well.

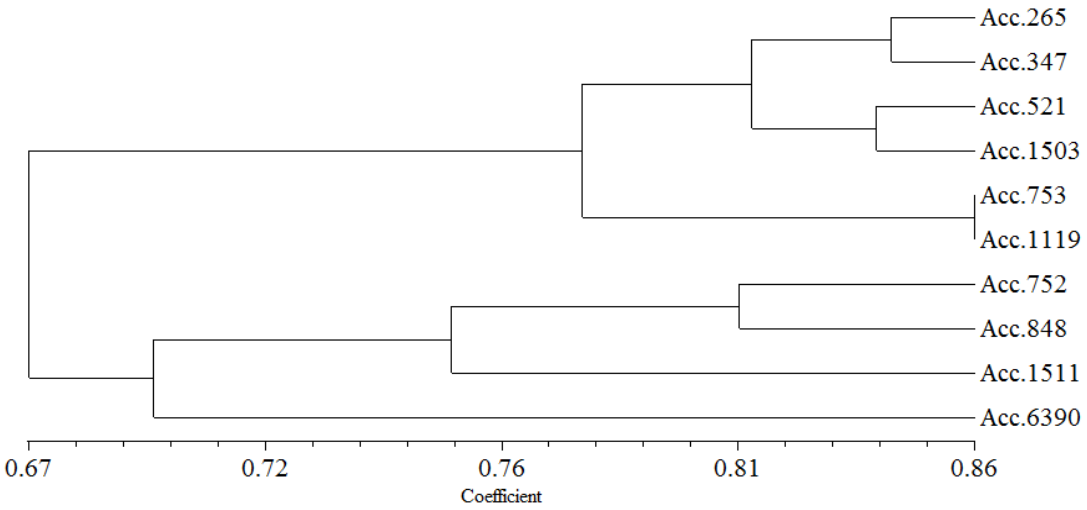


Fig. 72. UPGMA-Dendrogram of ten accessions of *C. amada* based on combined RAPD, ISSR and SSR data.

The similarity matrix obtained from the Simple Matching coefficients in *C. aromatica* was given in Table 132. Acc. 1132 and Acc. 1113 showed maximum similarity with a coefficient value of 0.82. Minimum similarity was between Acc. 1124 and Acc. 1520 with a similarity value of 0.64.

Table 132. Similarity matrix of different accessions of *C. aromatica* generated from the combined RAPD, ISSR and SSR data

Accession	711	1025	1113	1124	1132	1518	1520
711	1.00						
1025	0.75	1.00					
1113	0.78	0.80	1.00				
1124	0.65	0.67	0.69	1.00			
1132	0.75	0.81	0.82	0.79	1.00		
1518	0.75	0.75	0.77	0.68	0.82	1.00	
1520	0.68	0.74	0.70	0.64	0.78	0.81	1.00

The UPGMA dendrogram constructed using the Simple Matching similarity coefficients is given in Figure 73. The UPGMA dendrogram constructed using the Simple Matching similarity coefficients clustered the seven accessions of *C. aromatica* into three groups splitting at coefficient value of 0.760. The groups formed are as follows:

Group I	-	Acc. 711
Group II	-	Acc. 1025, Acc. 1113, Acc. 1132, Acc. 1518 and Acc. 1520
Group III	-	Acc. 1124

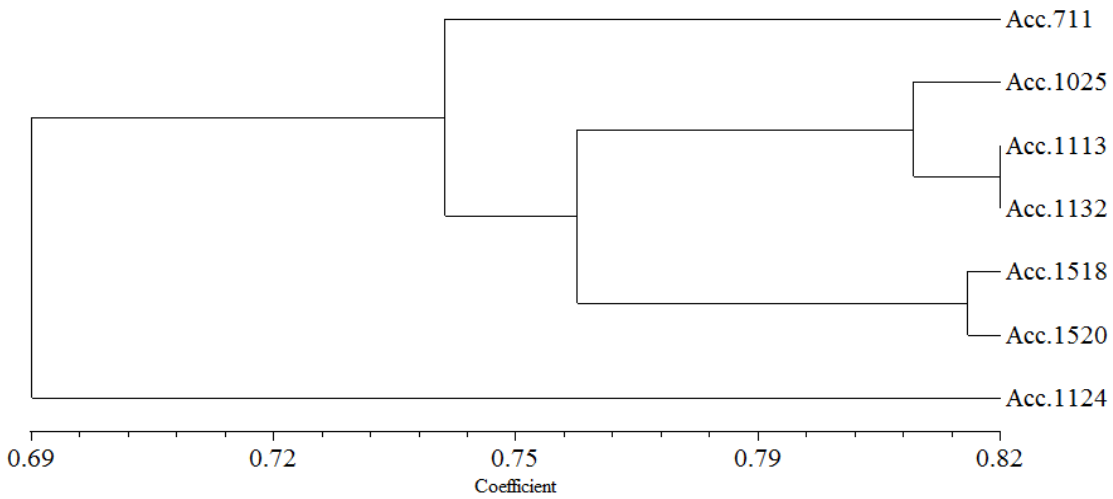


Fig. 73. Dendrogram of genetic similarity among seven accessions of *C. aromatica* based on combined RAPD, ISSR and SSR data.

First and third groups were formed by Acc.711 and Acc.1124, respectively. Remaining five accessions formed the second group. Acc. 1113 and Acc.1132 showed maximum similarity among the accessions while Acc.1124 showed least genetic similarity with rest of the accessions of *C. aromatica*.

The similarity matrix obtained from Simple Matching coefficients in *C. caesia* is presented in Table 133. Acc.292 and Acc.1001 as well as Acc.1006 and Acc.1154 showed maximum similarity among the accessions (0.82). Minimum similarity was between Acc. 292 and Acc. 751 (0.64).

Table 133. Similarity matrix of different accessions of *C. caesia* obtained from the combined RAPD, ISSR and SSR data

Accession	292	751	1001	1006	1135	1154	1171
292	1.00						
751	0.64	1.00					
1001	0.82	0.67	1.00				
1006	0.76	0.70	0.78	1.00			
1135	0.66	0.65	0.72	0.76	1.00		
1154	0.78	0.68	0.80	0.82	0.77	1.00	
1171	0.65	0.66	0.69	0.77	0.68	0.74	1.00

The UPGMA dendrogram constructed using the Simple Matching similarity coefficients of *C. caesia* accessions is given in Figure 74. The UPGMA dendrogram constructed using the Simple Matching similarity coefficients clustered the seven accessions of *C. caesia* into three groups splitting at coefficient value of 0.726.

The clustering pattern obtained is as follows:

Group I - Acc. 292, Acc.1001, Acc.1006, Acc.1154 and Acc.1135

Group II - Acc. 1171

Group III - Acc. 751

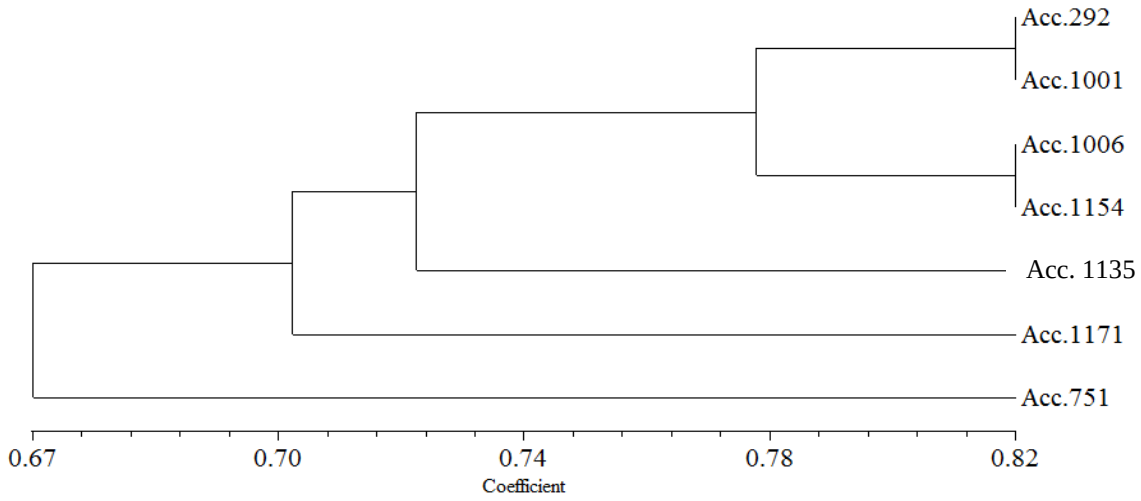


Fig. 74. Dendrogram of genetic similarity among seven accessions of *C. caesia* based on combined RAPD, ISSR and SSR data.

First group was formed by five accessions whereas second and third groups were formed by the single accessions, Acc.1171 and Acc.751, respectively.

The similarity matrix obtained from Simple Matching coefficients in *C. xanthorrhiza* accessions is shown in Table 134. Acc. 1163 and Acc. 1167 showed maximum similarity with a coefficient value of 0.89. Minimum similarity was between Acc. 465 and Acc. 1108 with a value of 0.69.

Table 134. Similarity matrix of different accessions of *C. xanthorrhiza* generated from the combined RAPD, ISSR and SSR data

Accession	465	760	1108	1122	1163	1164	1167	1168
465	1.00							
760	0.76	1.00						
1108	0.69	0.74	1.00					
1122	0.73	0.87	0.75	1.00				
1163	0.72	0.86	0.74	0.88	1.00			
1164	0.71	0.79	0.77	0.81	0.84	1.00		
1167	0.73	0.87	0.71	0.85	0.89	0.85	1.00	
1168	0.76	0.82	0.72	0.81	0.83	0.79	0.81	1.00

The UPGMA dendrogram constructed using the Simple Matching similarity coefficients of *C. xanthorrhiza* accessions is given in Figure 75. The UPGMA dendrogram constructed using the Simple Matching similarity coefficients clustered the eight accessions of *C. xanthorrhiza* into four groups splitting at coefficient value of 0.815.

The clustering pattern was as follows:

Group I - Acc. 465

Group II - Acc. 760, Acc.1122, Acc.1163, Acc.1167 and Acc.1164

Group III - Acc.1168

Group IV - Acc. 1108

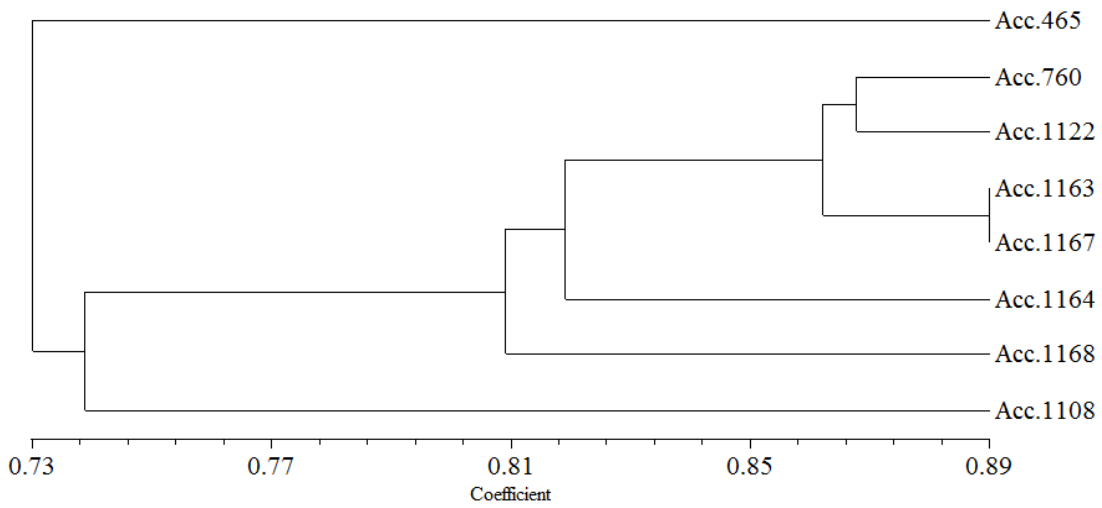


Fig. 75. Dendrogram of genetic similarity among eight accessions of *C. xanthorrhiza* based on combined RAPD, ISSR and SSR data.

First, third and fourth groups were formed by Acc.465, Acc.1168 and Acc.1108 respectively, whereas the second group was formed by the remaining five accessions, among which Acc.1163 and Acc.1167 exhibited maximum similarity with each other. Acc. 465 and Acc. 1108 showed least genetic similarity with rest of the accessions. A similar pattern of clustering was observed with individual marker system as well.

4.4.5.2. Cluster analysis of four *Curcuma* Species

The similarity matrices for the thirty-two accessions from four species using Simple Matching, Jaccard's and Sorensen-Dice coefficients are presented in Tables 135-137. The dendrogram (UPGMA) constructed using the three similarity coefficients are depicted in Figures 76, 77 and 78.

The UPGMA dendrogram based on the Simple matching, Jaccard's and Sorensen-Dice similarity coefficients clustered the thirty-two accessions into four major groups splitting at coefficients 0.874, 0.612 and 0.762 for Simple matching, Jaccard's and Sorensen-Dice, respectively.

The four groupings formed were:

Group I	*C. amada* Acc. 265, 521, 1503, 1511, 347. 6390, 753, 1119, 752 and 848
Group II	*C. caesia* Acc. 292, 1001, 1154, 751, 1006, 1171 and 1135

Group III *C. xanthorrhiza* Acc. 465, 1168, 760, 1122, 1163, 1167, 1164 and 1108

Group IV *C. aromatica* Acc. 711, 1025, 1113, 1132, 1518, 1520 and 1124

The UPGMA based SAHN clustering has clustered the 32 accessions into four different groups. Group I was formed by ten accessions of *C. amada*. Group II, III and IV were formed by accessions of *C. caesia*, *C. xanthorrhiza* and *C. aromatica,* respectively. The only difference in dendrogram, constructed using Simple Matching, Jaccard's and Sorensen-Dice coefficient was that in case of Simple Matching coefficient, *C. caesia* and *C. amada* showed closer association followed by *C. xanthorrhiza* and *C. aromatica,* whereas in case of Jaccard's and Sorensen-Dice coefficients, *C. aromatica* and *C. xanthorrhiza* showed closer association followed by *C. amada* and *C. caesia*.

Among the accessions, maximum similarity was observed between Acc.1163 and Acc.1167 of *C. xanthorrhiza* (Simple Matching = 0.96, Jaccard's = 0.88 and Dice = 0.94) followed by Acc. 1122 and Acc.1163 as well as Acc. 760 and Acc.1122 of *C. xanthorrhiza.* Genetically least similar accessions were Acc. 1511 (*C. amada*) and Acc. 1132 (*C. aromatica*) with similarity coefficient of 0.54 for Simple matching; Acc. 1135 (*C. caesia*) and Acc. 1108 (*C. xanthorrhiza*) with a coefficient of 0.11 and 0.20 in case of Jaccard's and Sorensen-Dice, respectively. Acc. 752 (*C. amada*) and Acc. 1518 (*C. aromatica*), Acc. 752 (*C. amada*) and Acc. 1108 (*C. xanthorrhiza*) also exhibited least similarity in Jaccard's and Sorensen-Dice similarity coefficients.

Table 135. Similarity matrix of four *Curcuma* species obtained from the combined RAPD, ISSR and SSR data using Simple matching similarity coefficients.

		C.amada										*C.aromatica*							*C.caesia*							*C.xanthorrhiza*							
		265	347	521	752	753	848	1119	1503	1511	6390	711	1025	1113	1124	1132	1518	1520	292	751	1001	1006	1135	1154	1171	465	760	1108	1122	1163	1164	1167	1168
C.amada	265	1.00																															
	347	0.94	1.00																														
	521	0.93	0.93	1.00																													
	752	0.86	0.86	0.87	1.00																												
	753	0.92	0.94	0.90	0.88	1.00																											
	848	0.84	0.86	0.86	0.93	0.87	1.00																										
	1119	0.91	0.93	0.90	0.87	0.95	0.87	1.00																									
	1503	0.93	0.93	0.94	0.87	0.92	0.87	0.92	1.00																								
	1511	0.88	0.88	0.90	0.91	0.88	0.90	0.86	0.90	1.00																							
	6390	0.88	0.88	0.89	0.88	0.87	0.88	0.87	0.90	0.90	1.00																						
C.aromatica	711	0.58	0.57	0.57	0.58	0.58	0.58	0.59	0.57	0.56	0.58	1.00																					
	1025	0.57	0.56	0.56	0.57	0.57	0.58	0.58	0.56	0.55	0.57	0.90	1.00																				
	1113	0.57	0.57	0.56	0.57	0.58	0.58	0.58	0.56	0.55	0.57	0.91	0.92	1.00																			
	1124	0.57	0.57	0.57	0.57	0.57	0.57	0.58	0.57	0.55	0.57	0.86	0.87	0.88	1.00																		
	1132	0.56	0.56	0.55	0.56	0.56	0.57	0.57	0.56	0.54	0.56	0.90	0.93	0.93	0.92	1.00																	
	1518	0.58	0.57	0.58	0.57	0.58	0.58	0.59	0.58	0.56	0.59	0.90	0.90	0.91	0.88	0.93	1.00																
	1520	0.58	0.58	0.57	0.57	0.58	0.58	0.59	0.57	0.56	0.58	0.88	0.90	0.89	0.86	0.91	0.93	1.00															
C.caesia	292	0.58	0.58	0.58	0.58	0.59	0.58	0.60	0.57	0.56	0.59	0.59	0.58	0.59	0.57	0.56	0.59	0.59	1.00														
	751	0.59	0.60	0.60	0.60	0.61	0.60	0.62	0.59	0.58	0.60	0.60	0.59	0.60	0.59	0.58	0.60	0.60	0.87	1.00													
	1001	0.58	0.58	0.58	0.58	0.60	0.59	0.60	0.58	0.56	0.59	0.59	0.57	0.59	0.57	0.56	0.59	0.59	0.93	0.88	1.00												
	1006	0.58	0.58	0.58	0.58	0.59	0.59	0.60	0.57	0.56	0.58	0.58	0.57	0.58	0.57	0.56	0.59	0.59	0.91	0.89	0.92	1.00											
	1135	0.61	0.61	0.61	0.62	0.62	0.62	0.63	0.61	0.59	0.62	0.61	0.59	0.61	0.60	0.58	0.61	0.61	0.88	0.87	0.90	0.91	1.00										
	1154	0.58	0.58	0.58	0.58	0.59	0.59	0.60	0.57	0.56	0.59	0.58	0.56	0.58	0.57	0.55	0.59	0.58	0.92	0.88	0.93	0.93	0.91	1.00									
	1171	0.58	0.59	0.59	0.59	0.60	0.59	0.60	0.58	0.57	0.59	0.58	0.57	0.58	0.58	0.56	0.59	0.59	0.87	0.87	0.89	0.92	0.88	0.91	1.00								
C.xanthorrhiza	465	0.59	0.60	0.59	0.59	0.59	0.59	0.59	0.58	0.58	0.58	0.58	0.58	0.58	0.57	0.56	0.58	0.59	0.58	0.60	0.58	0.58	0.59	0.57	0.58	1.00							
	760	0.58	0.59	0.58	0.58	0.58	0.58	0.58	0.58	0.57	0.57	0.57	0.56	0.57	0.56	0.55	0.57	0.58	0.58	0.59	0.57	0.58	0.59	0.56	0.58	0.91	1.00						
	1108	0.61	0.62	0.61	0.60	0.61	0.61	0.61	0.61	0.59	0.61	0.60	0.60	0.60	0.59	0.58	0.61	0.61	0.61	0.63	0.61	0.60	0.62	0.60	0.61	0.89	0.91	1.00					
	1122	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.58	0.58	0.58	0.57	0.58	0.57	0.56	0.58	0.59	0.58	0.60	0.58	0.58	0.59	0.56	0.58	0.91	0.95	0.91	1.00				
	1163	0.59	0.59	0.58	0.58	0.58	0.58	0.59	0.58	0.57	0.58	0.58	0.57	0.58	0.57	0.56	0.58	0.59	0.58	0.60	0.58	0.57	0.59	0.56	0.58	0.90	0.95	0.91	0.96	1.00			
	1164	0.60	0.60	0.60	0.59	0.59	0.60	0.59	0.59	0.58	0.59	0.59	0.58	0.59	0.58	0.57	0.60	0.60	0.60	0.61	0.59	0.59	0.60	0.58	0.59	0.90	0.93	0.92	0.93	0.94	1.00		
	1167	0.59	0.59	0.58	0.58	0.58	0.58	0.59	0.58	0.57	0.58	0.58	0.57	0.57	0.57	0.56	0.58	0.58	0.58	0.60	0.57	0.57	0.59	0.56	0.58	0.90	0.95	0.90	0.95	0.96	0.95	1.00	
	1168	0.59	0.60	0.59	0.58	0.59	0.59	0.59	0.59	0.58	0.59	0.59	0.58	0.59	0.57	0.57	0.59	0.59	0.59	0.61	0.59	0.59	0.60	0.57	0.59	0.92	0.94	0.90	0.93	0.94	0.92	0.93	1.00

Table 136. Similarity matrix of four *Curcuma* species obtained from the combined RAPD, ISSR and SSR data using Jaccard's similarity coefficients

		C.amada										*C.aromatica*							*C.caesia*							*C.xanthorrhiza*							
		265	347	521	752	753	848	1119	1503	1511	6390	711	1025	1113	1124	1132	1518	1520	292	751	1001	1006	1135	1154	1171	465	760	1108	1122	1163	1164	1167	1168
C.amada	265	1.00																															
	347	0.81	1.00																														
	521	0.79	0.77	1.00																													
	752	0.58	0.58	0.61	1.00																												
	753	0.74	0.79	0.70	0.63	1.00																											
	848	0.55	0.59	0.59	0.76	0.61	1.00																										
	1119	0.71	0.76	0.70	0.59	0.82	0.61	1.00																									
	1503	0.77	0.79	0.81	0.62	0.74	0.63	0.75	1.00																								
	1511	0.66	0.67	0.70	0.73	0.64	0.70	0.61	0.70	1.00																							
	6390	0.64	0.63	0.67	0.62	0.60	0.61	0.61	0.69	0.69	1.00																						
C.aromatica	711	0.14	0.13	0.13	0.12	0.13	0.12	0.13	0.13	0.13	0.12	1.00																					
	1025	0.14	0.14	0.14	0.13	0.14	0.14	0.14	0.15	0.14	0.13	0.71	1.00																				
	1113	0.14	0.13	0.13	0.12	0.13	0.12	0.13	0.13	0.13	0.12	0.73	0.76	1.00																			
	1124	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.15	0.14	0.13	0.62	0.65	0.66	1.00																		
	1132	0.15	0.15	0.15	0.13	0.14	0.14	0.14	0.15	0.14	0.13	0.72	0.79	0.79	0.76	1.00																		
	1518	0.13	0.13	0.14	0.11	0.13	0.12	0.13	0.14	0.13	0.13	0.70	0.71	0.72	0.65	0.78	1.00																
	1520	0.14	0.14	0.14	0.12	0.13	0.12	0.13	0.14	0.13	0.12	0.63	0.70	0.66	0.61	0.75	0.77	1.00															
C.caesia	292	0.13	0.14	0.14	0.12	0.13	0.12	0.13	0.13	0.13	0.13	0.14	0.14	0.14	0.14	0.14	0.14	0.14	1.00														
	751	0.14	0.14	0.15	0.13	0.15	0.13	0.15	0.14	0.14	0.13	0.14	0.15	0.14	0.15	0.15	0.14	0.15	0.60	1.00													
	1001	0.13	0.13	0.14	0.12	0.14	0.12	0.13	0.13	0.13	0.12	0.13	0.14	0.14	0.14	0.14	0.14	0.14	0.78	0.62	1.00												
	1006	0.14	0.15	0.15	0.14	0.15	0.13	0.14	0.14	0.14	0.13	0.14	0.14	0.14	0.16	0.15	0.15	0.15	0.72	0.66	0.74	1.00											
	1135	0.14	0.15	0.15	0.14	0.15	0.14	0.15	0.15	0.14	0.14	0.13	0.14	0.15	0.16	0.15	0.13	0.15	0.61	0.59	0.66	0.71	1.00										
	1154	0.14	0.14	0.15	0.13	0.14	0.13	0.14	0.14	0.14	0.13	0.13	0.14	0.14	0.15	0.14	0.14	0.14	0.74	0.63	0.76	0.79	0.72	1.00									
	1171	0.14	0.14	0.15	0.13	0.15	0.13	0.14	0.14	0.14	0.13	0.13	0.14	0.14	0.15	0.14	0.14	0.15	0.62	0.61	0.65	0.73	0.62	0.71	1.00								
C.xanthorrhiza	465	0.17	0.18	0.17	0.15	0.15	0.14	0.14	0.16	0.17	0.14	0.15	0.16	0.15	0.16	0.16	0.15	0.16	0.15	0.15	0.14	0.15	0.13	0.14	0.15	1.00							
	760	0.16	0.17	0.17	0.15	0.15	0.14	0.15	0.16	0.16	0.14	0.14	0.16	0.15	0.16	0.16	0.15	0.16	0.15	0.16	0.14	0.16	0.14	0.14	0.16	0.75	1.00						
	1108	0.14	0.15	0.14	0.11	0.13	0.12	0.12	0.14	0.13	0.12	0.13	0.14	0.13	0.14	0.14	0.13	0.14	0.13	0.14	0.13	0.13	0.11	0.12	0.13	0.65	0.71	1.00					
	1122	0.16	0.17	0.17	0.14	0.15	0.14	0.14	0.17	0.16	0.14	0.14	0.16	0.15	0.16	0.15	0.15	0.16	0.14	0.16	0.13	0.15	0.13	0.13	0.15	0.72	0.86	0.71	1.00				
	1163	0.17	0.16	0.16	0.14	0.15	0.14	0.14	0.16	0.16	0.13	0.15	0.16	0.15	0.16	0.16	0.15	0.16	0.14	0.16	0.13	0.15	0.13	0.13	0.14	0.71	0.84	0.70	0.87	1.00			
	1164	0.16	0.16	0.16	0.13	0.14	0.14	0.13	0.16	0.16	0.13	0.14	0.15	0.15	0.16	0.15	0.15	0.16	0.15	0.15	0.13	0.15	0.13	0.14	0.15	0.69	0.77	0.72	0.79	0.81	1.00		
	1167	0.17	0.17	0.17	0.15	0.15	0.14	0.15	0.17	0.17	0.14	0.15	0.16	0.15	0.17	0.16	0.15	0.16	0.15	0.16	0.14	0.15	0.14	0.14	0.15	0.72	0.86	0.68	0.84	0.88	0.83	1.00	
	1168	0.16	0.16	0.16	0.13	0.15	0.14	0.14	0.16	0.16	0.14	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.16	0.14	0.15	0.13	0.13	0.14	0.74	0.80	0.68	0.78	0.80	0.76	0.79	1.00

Table 137. Similarity matrix of four *Curcuma* species obtained from the combined RAPD, ISSR and SSR data using Sorensen-Dice similarity coefficients

		C.amada										*C.aromatica*							*C.caesia*							*C.xanthorrhiza*							
		265	347	521	752	753	848	1119	1503	1511	6390	711	1025	1113	1124	1132	1518	1520	292	751	1001	1006	1135	1154	1171	465	760	1108	1122	1163	1164	1167	1168
C.amada	265	1.00																															
	347	0.89	1.00																														
	521	0.88	0.87	1.00																													
	752	0.73	0.74	0.76	1.00																												
	753	0.85	0.88	0.82	0.77	1.00																											
	848	0.71	0.74	0.74	0.86	0.76	1.00																										
	1119	0.83	0.87	0.82	0.75	0.90	0.76	1.00																									
	1503	0.87	0.88	0.89	0.77	0.85	0.77	0.86	1.00																								
	1511	0.80	0.80	0.82	0.84	0.78	0.82	0.76	0.83	1.00																							
	6390	0.78	0.77	0.80	0.77	0.75	0.76	0.76	0.81	0.82	1.00																						
C.aromatica	711	0.24	0.23	0.23	0.22	0.24	0.22	0.22	0.24	0.23	0.21	1.00																					
	1025	0.25	0.25	0.25	0.24	0.25	0.24	0.24	0.25	0.24	0.22	0.83	1.00																				
	1113	0.24	0.23	0.23	0.22	0.23	0.22	0.22	0.24	0.23	0.21	0.84	0.87	1.00																			
	1124	0.27	0.26	0.26	0.24	0.24	0.24	0.24	0.27	0.25	0.23	0.76	0.79	0.79	1.00																		
	1132	0.26	0.26	0.26	0.23	0.25	0.24	0.25	0.26	0.25	0.24	0.84	0.88	0.88	0.87	1.00																	
	1518	0.24	0.23	0.24	0.20	0.23	0.21	0.22	0.25	0.23	0.23	0.82	0.83	0.84	0.78	0.88	1.00																
	1520	0.24	0.25	0.25	0.22	0.23	0.21	0.23	0.25	0.24	0.22	0.77	0.83	0.79	0.76	0.86	0.87	1.00															
C.caesia	292	0.24	0.24	0.25	0.22	0.24	0.21	0.24	0.23	0.24	0.22	0.25	0.25	0.25	0.25	0.24	0.25	0.25	1.00														
	751	0.24	0.25	0.26	0.24	0.26	0.23	0.26	0.25	0.24	0.23	0.25	0.26	0.25	0.26	0.25	0.25	0.26	0.75	1.00													
	1001	0.23	0.24	0.24	0.21	0.24	0.21	0.23	0.23	0.23	0.22	0.23	0.24	0.25	0.24	0.24	0.24	0.24	0.88	0.77	1.00												
	1006	0.25	0.25	0.26	0.24	0.26	0.23	0.25	0.25	0.25	0.23	0.24	0.25	0.25	0.27	0.26	0.25	0.27	0.84	0.79	0.85	1.00											
	1135	0.25	0.26	0.26	0.25	0.26	0.24	0.26	0.26	0.25	0.25	0.24	0.24	0.25	0.27	0.26	0.24	0.26	0.76	0.74	0.79	0.83	1.00										
	1154	0.24	0.25	0.26	0.23	0.25	0.23	0.25	0.25	0.25	0.23	0.24	0.24	0.25	0.26	0.24	0.25	0.25	0.85	0.78	0.86	0.88	0.84	1.00									
	1171	0.24	0.25	0.26	0.24	0.25	0.23	0.25	0.25	0.25	0.22	0.23	0.25	0.25	0.26	0.25	0.25	0.26	0.76	0.76	0.79	0.85	0.77	0.83	1.00								
C.xanthorrhiza	465	0.29	0.30	0.29	0.26	0.26	0.25	0.25	0.28	0.29	0.25	0.26	0.28	0.26	0.28	0.27	0.26	0.28	0.26	0.26	0.25	0.26	0.24	0.24	0.26	1.00							
	760	0.28	0.29	0.29	0.25	0.26	0.25	0.25	0.28	0.28	0.24	0.25	0.27	0.26	0.28	0.27	0.25	0.27	0.26	0.27	0.24	0.27	0.24	0.25	0.27	0.86	1.00						
	1108	0.24	0.26	0.25	0.21	0.23	0.21	0.22	0.25	0.23	0.22	0.22	0.24	0.24	0.24	0.24	0.23	0.25	0.24	0.25	0.22	0.24	0.20	0.22	0.23	0.79	0.83	1.00					
	1122	0.28	0.29	0.29	0.25	0.26	0.25	0.25	0.28	0.28	0.24	0.25	0.27	0.26	0.28	0.27	0.26	0.28	0.25	0.27	0.23	0.26	0.23	0.23	0.26	0.84	0.92	0.83	1.00				
	1163	0.28	0.28	0.28	0.25	0.26	0.24	0.25	0.28	0.27	0.23	0.26	0.27	0.26	0.28	0.27	0.26	0.28	0.25	0.27	0.23	0.25	0.24	0.23	0.25	0.83	0.91	0.83	0.93	1.00			
	1164	0.27	0.27	0.28	0.24	0.24	0.24	0.24	0.27	0.27	0.23	0.25	0.26	0.26	0.27	0.26	0.26	0.27	0.26	0.26	0.24	0.26	0.23	0.24	0.25	0.82	0.87	0.84	0.88	0.90	1.00		
	1167	0.29	0.29	0.29	0.26	0.26	0.25	0.26	0.29	0.28	0.25	0.26	0.27	0.26	0.29	0.27	0.26	0.28	0.26	0.27	0.24	0.27	0.25	0.24	0.26	0.84	0.92	0.81	0.91	0.94	0.91	1.00	
	1168	0.27	0.28	0.28	0.24	0.25	0.24	0.24	0.27	0.27	0.25	0.25	0.27	0.26	0.27	0.27	0.25	0.27	0.25	0.27	0.24	0.26	0.24	0.24	0.25	0.85	0.89	0.81	0.88	0.89	0.86	0.88	1.00

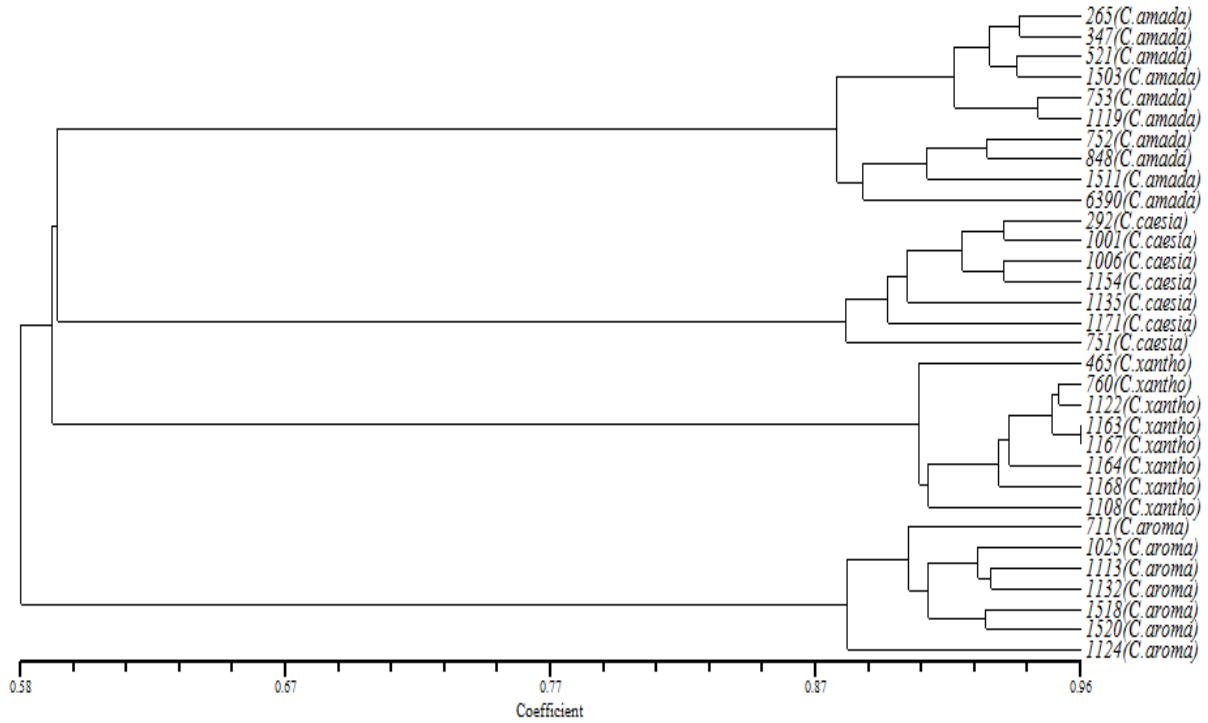


Fig. 76. Dendrogram of genetic similarity among four *Curcuma* species based on combined RAPD, ISSR and SSR data using Simple-matching similarity coefficients

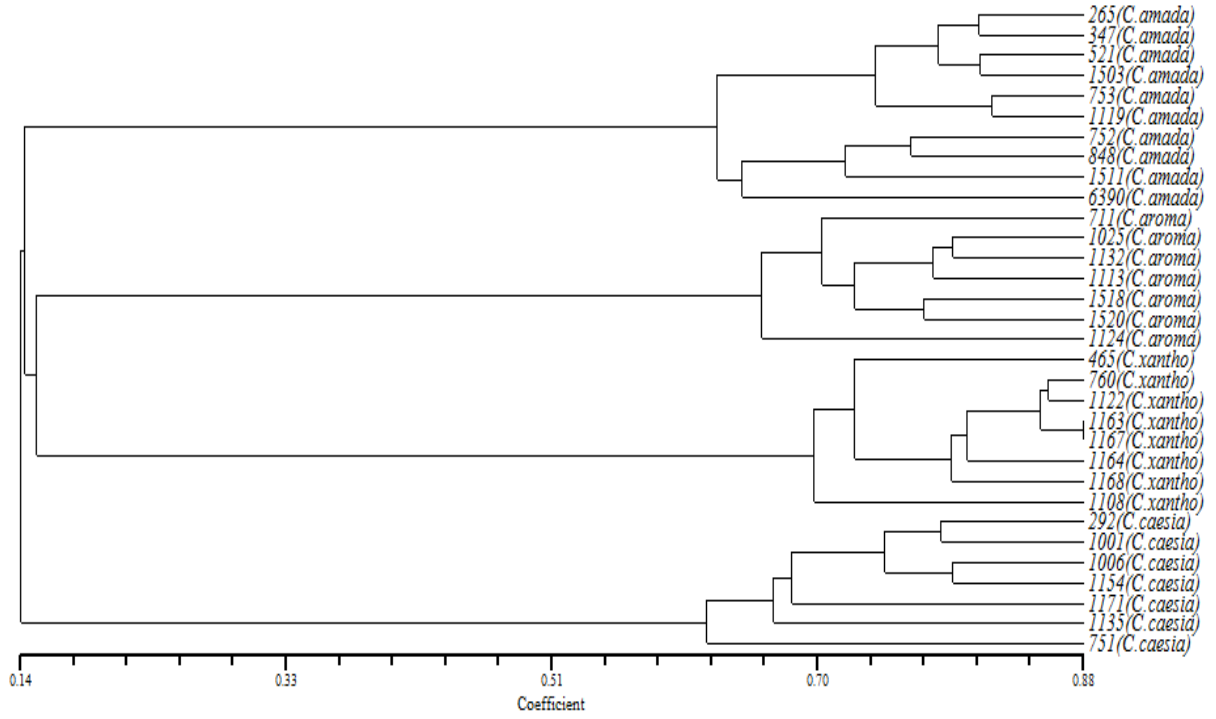


Fig. 77. Dendrogram of genetic similarity among four *Curcuma* species based on combined RAPD, ISSR and SSR data using Jaccard's similarity coefficients

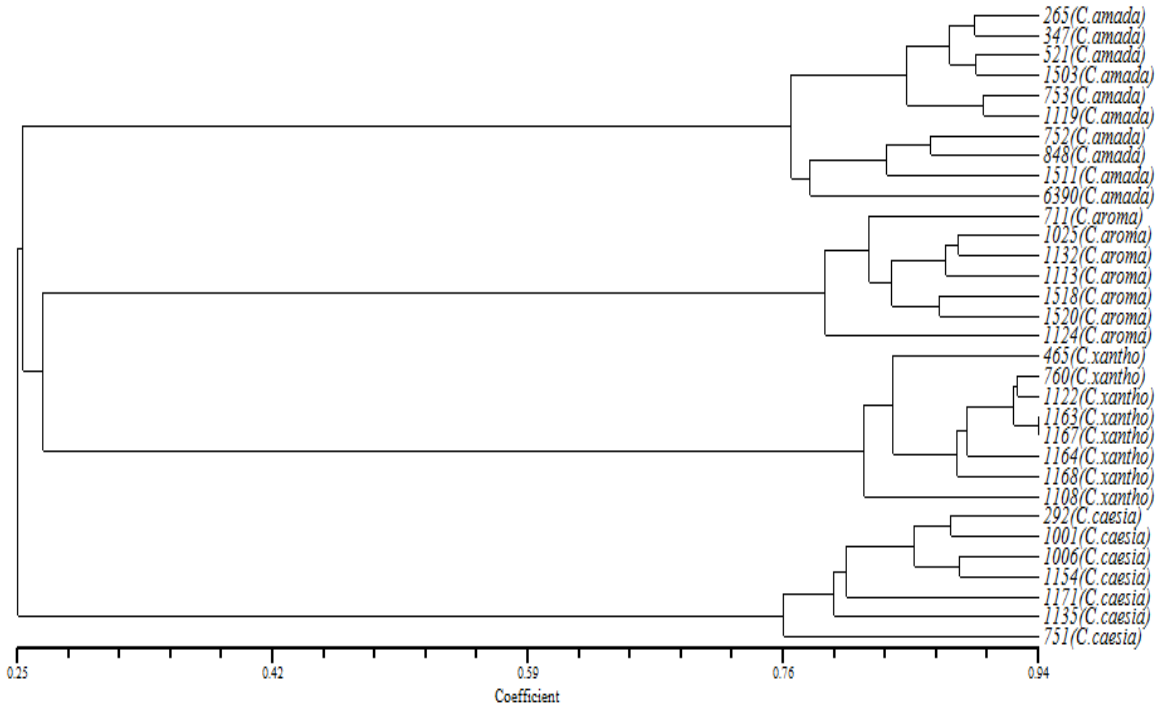


Fig. 78. Dendrogram of genetic similarity among four *Curcuma* species based on combined RAPD, ISSR and SSR data using Sorensen-Dice similarity coefficients.

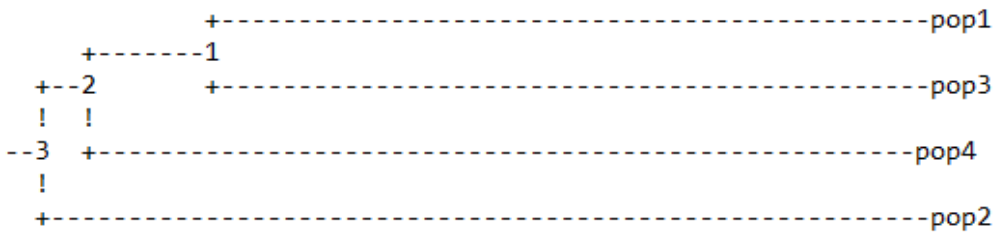
4.4.5.3. Genetic distance between species

The Nei's genetic distance (1972) between the four species was estimated using combined RAPD, ISSR and SSR data. Table 138 shows the Nei's genetic distance among the species. The upper diagonal matrix shows the Nei's genetic identity and the lower diagonal matrix shows the Nei's genetic distance.

Table 138. Nei's genetic distance and genetic identity of four *Curcuma* species obtained from the combined RAPD, ISSR and SSR data.

Species	*C. amada*	*C. aromatica*	*C. caesia*	*C. xanthorrhiza*
C. amada	****	0.627	0.650	0.640
C. aromatica	0.467	****	0.639	0.627
C. caesia	0.431	0.448	****	0.636
C. xanthorrhiza	0.446	0.467	0.452	****

C. amada and *C. caesia* (0.431) showed closer association followed by *C. xanthorrhiza* and *C. aromatica.* The dendrogram constructed using Nei's genetic distance and genetic identity is given in Figure 79.



Pop1 – *C. amada;* pop2 – *C. aromatica;* pop3 – *C. caesia;* pop4 – *C. xanthorrhiza*

Fig. 79. Dendrogram of genetic similarity between four species based on combined RAPD, ISSR and SSR data.

4.4.6. Genetic distance Vs geographic distance within individual species.

Nei's genetic distance derived from the combined RAPD, ISSR and SSR markers of four *Curcuma* species were used to analyse the relationship between the geographical location and genetic distance.

Ten accessions of *C. amada* collected from Kerala clustered together, majority of the accessions did not show any geographical location based clustering. Acc.6390, which was collected from Vijayawada (Andhra Pradesh) stood out from rest of the accessions of *C. amada*. This was further supported by its separate status in dendrogram constructed using morphological and molecular data as well as for several qualitative characters like total phenol, total curcuminoids and composition of essential oil. The Nei's genetic distance among the ten accessions is given in Table 139. The UPGMA dendrogram constructed using Nei's genetic distance is depicted in Figure 80.

Table 139. Nei's genetic distance matrix of *C. amada* accessions

Accession	265	347	521	752	753	848	1119	1503	1511	6390
265	0.00									
347	0.17	0.00								
521	0.19	0.22	0.00							
752	0.48	0.47	0.42	0.00						
753	0.24	0.18	0.30	0.39	0.00					
848	0.52	0.46	0.47	0.21	0.41	0.00				
1119	0.27	0.21	0.29	0.43	0.15	0.40	0.00			
1503	0.22	0.20	0.17	0.41	0.25	0.40	0.23	0.00		
1511	0.37	0.36	0.32	0.26	0.39	0.30	0.45	0.32	0.00	
6390	0.38	0.39	0.33	0.39	0.42	0.40	0.40	0.31	0.31	0.00

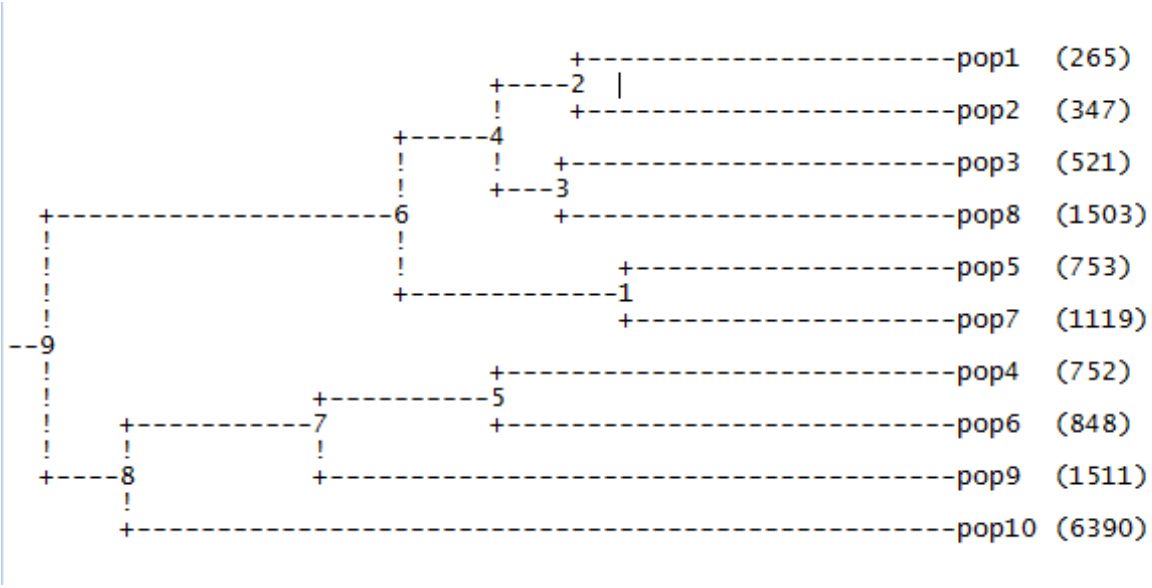


Fig. 80. Nei's genetic distance based UPGMA dendrogram of *C. amada* using combined markers.

Mantel test between genetic and geographic distance yielded a variation value of 0.13 at a significance level of 0.05 indicating that genetic distance was independent of geographical distance.

In case of *C. aromatica*, Acc.1518 and Acc.1520 which were collected from Idukki district of Kerala clustered together. Acc.1124 collected from Ernakulam showed least similarity with rest of the accessions which was supported by dendrogram constructed using RAPD and SSR markers. Acc.1124 recorded higher values for several morphological traits (weight of mother and secondary rhizome, number of secondary rhizome etc.) and lower values for majority of the

qualitative characters such as total phenol, total starch, total protein, oleoresin etc. The Nei's genetic distance among the accessions is given in Table 140. The UPGMA dendrogram constructed using Nei's genetic distance is depicted in Figure 81.

Table 140. Nei's genetic distance matrix of *C. aromatica* accessions

Accession	711	1025	1113	1124	1132	1518	1520
711	0.00						
1025	2.15	0.00					
1113	2.37	2.37	0.00				
1124	1.92	1.92	2.50	0.00			
1132	2.51	2.51	2.74	2.38	0.00		
1518	2.15	2.15	2.56	1.94	2.31	0.00	
1520	2.29	2.29	2.63	2.09	2.14	1.81	0.00

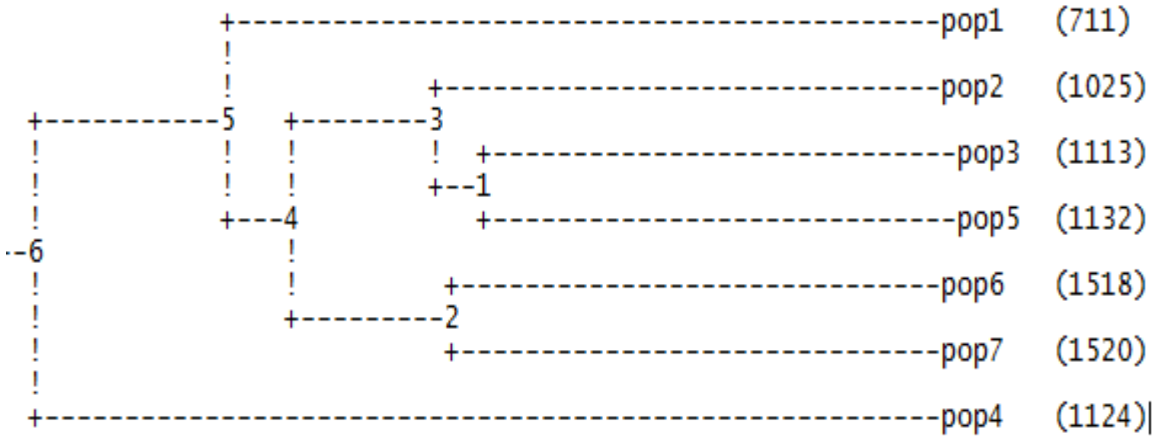


Fig. 81. Nei's genetic distance based UPGMA dendrogram of *C. aromatica* using combined markers.

Mantel test between genetic and geographic distance yielded a variation value of 0.01 at a significance level of 0.32 indicating that genetic distance was independent of geographical distance.

Accessions of *C. caesia* also did not generate any positive correlation between genetic and geographic distance. Acc.1135 and Acc.1154 collected from Thrissur (Kerala) found to be closely related with accessions from the Northeastern part of India. Acc.751 which was collected

from Cooch Behar (West Bengal) found to be least similar with rest of the accessions. The Nei's genetic distance among the accessions is given in Table 141. The UPGMA dendrogram constructed using Nei's genetic distance is depicted in Figure 82.

Table 141. Nei's genetic distance matrix of *C. caesia* accessions

Accession	292	751	1001	1006	1135	1154	1171
292	0.00						
751	0.45	0.00					
1001	0.20	0.40	0.00				
1006	0.27	0.36	0.25	0.00			
1135	0.41	0.43	0.33	0.28	0.00		
1154	0.25	0.39	0.22	0.20	0.26	0.00	
1171	0.43	0.42	0.37	0.26	0.39	0.30	0.00

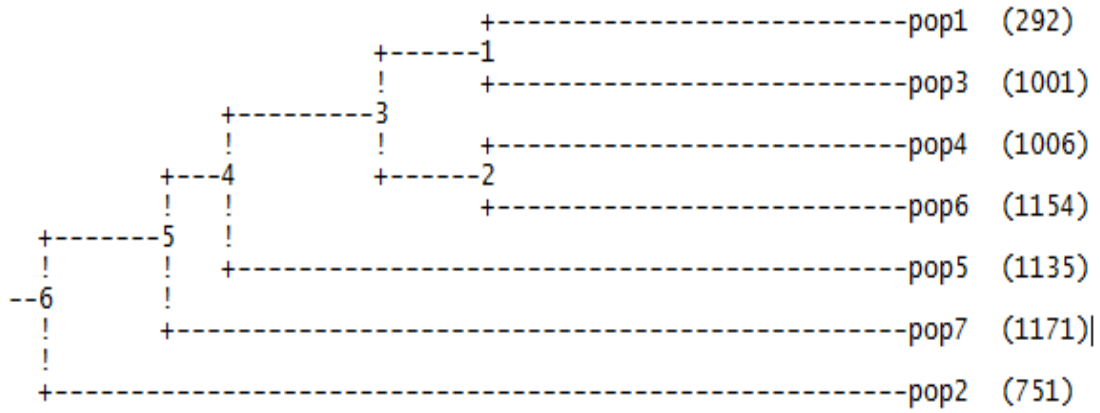


Fig. 82. Nei's genetic distance based UPGMA dendrogram of *C. caesia* using combined markers.

The correlation between genetic and geographic distance was as low as 0.02 at a significance level of 0.21 suggesting the independence of the two attributes.

Out of the eight accessions from *C. xanthorrhiza*, Acc.465 was collected from Jorhat (Assam) and the rest were from various parts of Kerala. In the dendrogram constructed using genetic distance, Acc.465 and Acc.1108 Palakkad (Kerala) were found to be separated from rest of the accessions. The result was further supported by Principal Component Analysis, where Acc.465 and Acc.1108 were seen in the same quadrant. Acc.465 exhibited higher values for essential oil

composition, oleoresin. The Nei's genetic distance among the ten accessions is given in Table 142. The UPGMA dendrogram constructed using Nei's genetic distance is depicted in Figure 83.

Table 142. Nei's genetic distance matrix of *C. xanthorrhiza* accessions

Accession	465	760	1108	1122	1163	1164	1167	1168
465	0.00							
760	0.27	0.00						
1108	0.37	0.30	0.00					
1122	0.31	0.14	0.29	0.00				
1163	0.33	0.15	0.30	0.12	0.00			
1164	0.34	0.23	0.26	0.21	0.18	0.00		
1167	0.31	0.14	0.34	0.16	0.11	0.17	0.00	
1168	0.27	0.19	0.33	0.21	0.19	0.24	0.21	0.00

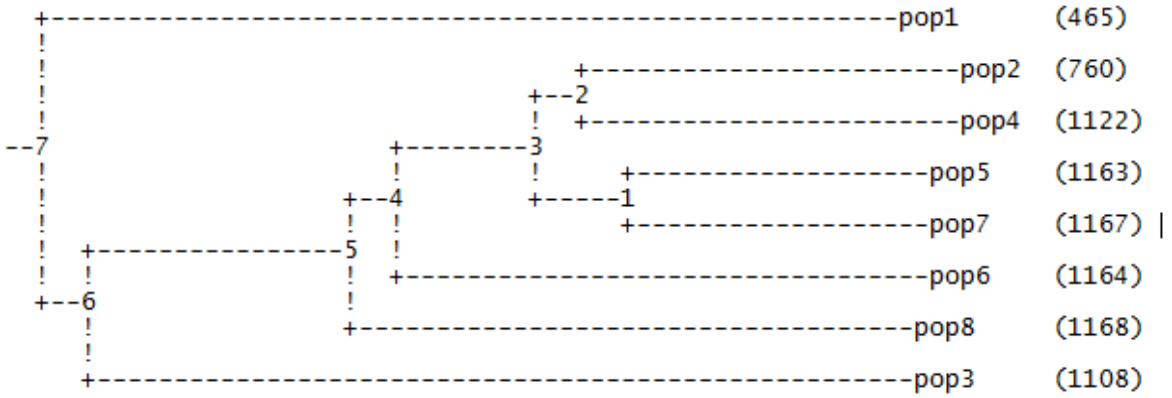


Fig. 83. Nei's genetic distance based UPGMA dendrogram of *C. xanthorrhiza* using combined markers.

Mantel test revealed that the correlation between genetic and geographic distance was as low as 0.34 at a significance level of 0.09 suggesting the independence of the two attributes.

4.4.7. Correlation among various markers

Correlation between various similarity matrices obtained were estimated using the Mantel test. (Table 143).

A very high correlation was observed among the different DNA markers, be it individual marker or combination of three markers. Further, the mantel test proved high similarity between three

similarity matrices (Simple Matching, Jaccard's and Sorensen-Dice) used (0.99) both in individual and combination of three markers.

Table 143. Correlation of various similarity matrices in four *Curcuma* species.

		RAPD			ISSR			SSR			RAPD+ISSR+SSR		
		SM	J	DICE	SM	J	DICE	SM	J	DICE	SM	J	DICE
RAPD	SM	1											
	J		1										
	DICE			1									
ISSR	SM	0.93			1								
	J		0.96			1							
	DICE			0.97			1						
SSR	SM	0.96			0.95			1					
	J		0.97			0.96			1				
	DICE			0.97			0.97			1			
RAPD+ISSR +SSR	SM	0.98			0.98			0.98			1		
	J		0.99			0.99			0.98			1	
	DICE			0.99			0.99			0.98			1

4.4.8. Analysis of molecular variance (AMOVA)

Analysis of molecular variance of four *Curcuma* species using three marker systems showed that variation was mainly contributed by the differences present among the species. Among individual and combined markers systems, ISSR marker produced maximum percentage of polymorphism (80%) followed by combined RAPD+ISSR+SSR markers (78%) (Table 144).

Table 144. Summary of AMOVA (Analysis of Molecular Variance) analysis of four *Curcuma* species using individual and combined marker systems

Markers	Source of Variations	df	Sum of Square	Estimated Variance	Variation %
RAPD	Among species	3	2906.87	117.54	77%
	within the species (Residual error)	28	1008.69	36.02	23%
	Total	31	3915.56	153.56	
	PhiPT (ΦPT)	0.76			
ISSR	Among species	3	2590.12	105.53	80%
	within the species (Residual error)	28	721.13	25.76	20%
	Total	31	3311.25	131.28	
	PhiPT (ΦPT)	0.80			
SSR	Among species	3	1273.80	50.96	72%
	within the species (Residual error)	28	562.14	20.08	28%
	Total	31	1835.94	71.04	
	PhiPT (ΦPT)	0.72			
RAPD + ISSR + SSR	Among species	3	6770.80	274.03	78%
	within the species (Residual error)	28	2291.95	81.86	22%
	Total	31	9062.75	355.88	
	PhiPT (ΦPT)	0.77			

d.f.- degrees of freedom. PhiPT (ΦPT), an analogue of F*st (Wright's fixation Index)* calculated to describe genetic differentiation between the populations. Probability (P) for Φ*pt* was based on 999 permutations across the full data set.

4.5. Population Diversity Study of Four *Curcuma* Species

4.5.1. *C. amada*

To understand the population diversity of the species, ten accessions of *C. amada*, collected from different regions of India, was grouped and studied based on the locations from which they were collected. The grouping of the various accessions and the location details are given in Table 145.

Table 145. Grouping of *C. amada* population

Population No.	Accession Number	Place of collection	Latitude (N)	Longitude (E)	Altitude (in M)
POP 1	265	Arikulam, Kozhikode, Kerala	11° 28' 5"	75° 43' 21"	5
	347	Moovattupuzha, Kottayam, Kerala	9° 59' 21"	76° 34' 44"	20
	521	Thrissur, Kerala	10° 31' 39"	76° 12' 51"	21
	1119	Thalappilly, Thrissur, Kerala	10° 39' 42"	76° 14' 10"	17
POP 2	752	Pundibari, Cooch Behar, West Bengal	26° 31' 27"	89° 6' 26"	44
	753	Pundibari, Cooch Behar, West Bengal	26° 31' 27"	89° 6' 26"	44
	848	Tuidam, Mamit, Mizoram	23° 55' 35"	92° 22' 8"	688
POP 3	1503	Anand, Gujarat	22° 33' 52"	72° 55' 43"	34
POP 4	1511	Pottangi, Koraput, Odhisa	18° 34' 6"	82° 58' 32"	934
	6390	Gundimeda, Vijayawada, Andhra Pradesh	16° 26' 41"	80° 38' 0"	22

*POP-Population

Accessions were grouped into various population based on the locations from which they were collected. Accessions collected from Southern India, namely Acc. 265, Acc.347, Acc.521 and Acc.1119 has been classified as POP 1. Accessions collected from the North-Eastern part of India, namely Acc. 752, Acc. 753 and Acc.848 have been classified as POP 2. Acc. 1503 collected from Gujarat has been classified as POP 3 while Acc.1511 and Acc. 6390 collected from East India – Vijayawada and Odisha were classified as POP 4.

In order to estimate the genetic distance among the population, combined use of RAPD, ISSR and SSR markers were employed. Genetic differences among the population have been highest in POP 2 which consists of the accessions collected from the North-East regions (42.46%) followed by POP 1 (36.92%) which consists of accessions from the Southern region. The lowest genetic diversity was among the POP 4 which had accessions from the Eastern part of India (Table 146).

Table 146. Nei's genetic diversity indices of *C. amada* population

	n	(Na)	(Ne)	(h)	(I)	%P	Gst	Nm
POP 1	4	1.37 ± 0.48	1.26 ± 0.36	0.15 ± 0.20	0.22 ± 0.29	36.92%		
POP 2	3	1.42 ± 0.49	1.34 ± 0.40	0.19 ± 0.22	0.27 ± 0.31	42.46%		
POP 3	1	1.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00%		
POP 4	2	1.27 ± 0.44	1.27 ± 0.44	0.13 ± 0.22	0.19 ± 0.31	26.77%		
C. amada	10	1.69 ± 0.46	1.43 ± 0.37	0.25 ± 0.19	0.37 ± 0.27	69.08%	0.504	0.492

Na - total number of alleles' Ne - number of effective alleles; h - Nei's (1973) gene diversity index; I - Shannon's information index; %P - percentage of polymorphism; Gst = (Ht-Hs)/Ht; Nm = 0.5*(1-Gst)/Gst, POP-Population.

The coefficient of genetic differentiation among population of *C. amada* (Gst) was 0.50, representing 50% genetic variability among population and remaining 50% within population. Comparatively medium genetic differentiation among population was further attributed by limited gene flow (Nm=0.492).

The Nei's genetic distance (1972) among the population was estimated using the POPGENE software. Table 147 shows the Nei's genetic distance among the population of *C. amada*. The upper diagonal matrix shows the Nei's genetic identity and the lower diagonal matrix shows the Nei's genetic distance.

The genetic distance among population of *C. amada* ranged from 0.122 to 0.248 (Table 147), the genetic distance between POP1 and POP3 being the minimum, indicating a close relationship between them, while the genetic distance between POP 3 and POP 2 was maximum, indicating a distant relationship between them. The genetic distance among population was consistent with their geographic distance. Genetic identity among population varied from 0.781 to 0.885.

Table 147. Nei's genetic distance and genetic identity of population of *C. amada* obtained from combined RAPD, ISSR and SSR data.

POP ID	POP 1	POP 2	POP 3	POP 4
POP 1	****	0.832	0.885	0.803
POP 2	0.184	****	0.781	0.832
POP 3	0.122	0.248	****	0.785
POP 4	0.220	0.184	0.242	****

*POP-Population, POP ID-Population identity

The dendrogram constructed using the genetic distances / genetic identity is depicted in Figure 84.

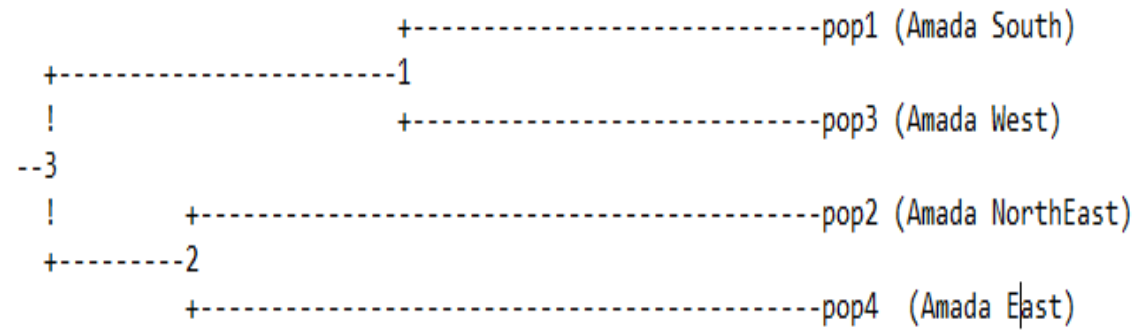


Fig. 84. UPGMA dendrogram based on Nei's genetic distance

The Mantel test of correlation between genetic and geographical diversity was not significant as the mantel test yielded r^2 value of 0.131 at a significance level of 3%.

Analysis of molecular variance present among and within the population is presented in Table 148. Due to data limitation from the western region, Acc. 1503 had to be discarded for carrying out the AMOVA analysis.

Table 148. AMOVA analysis of *C. amada* population

Source of variation	d.f.	SS	Est. Var.	Variation (%)	ϕ stat	P value
Among Population	2	220.69	7.79	8	0.08	NS*
Within Population (Residual error)	6	527.08	87.85	92		
Total	8	747.78	95.64			

d.f.-degrees of freedom. SS- sum of squares, Est. Var-estimated variation, *NS-Not significant (P>0.05).

No significant variation could be observed among or within population.

4.5.2. *C. aromatica*

To understand the population differentiation, seven accessions of *C. aromatica*, collected from different regions of Kerala, were grouped and studied based on the locations from which they were collected. The grouping of the various accessions and the location details are given in Table 149.

Table 149. Grouping details of *C. aromatica* population

Population No.	Accession Number	Place of collection	Latitude (N)	Longitude (E)	Altitude (in M)
POP 1	711	Thrissur, Kerala	10° 31' 39"	76° 12' 51"	21
	1025	Thrissur, Kerala	10° 31' 39"	76° 12' 51"	21
POP 2	1113	Kakkad, Pathanamthitta, Kerala	9° 19' 38"	76° 58' 16"	77
	1124	Kunnathunadu, Ernakulam, Kerala	10° 0' 55"	76° 24' 18"	16
	1132	Ambasamudram, Tirunelveli, Tamil Nadu	8° 42' 33"	77° 27' 10"	49
POP 3	1518	Anachal, Idukki, Kerala	10° 1' 22"	77° 2' 10"	901
	1520	Thekkadi, Idukki, Kerala	9° 36' 11"	77° 9' 41"	902

*POP- Population

Accessions collected from Thrissur (Kerala) namely Acc. 711 and Acc.1025 have been classified as POP 1. Accession collected from Pathanamthitta, Ernakulam and Tirunelveli, *viz*., Acc.1113, Acc.1124 and Acc.1132 have been classified as POP 2. Accessions 1518 and 1520 collected from Idukki were classified as POP 3.

In order to estimate the genetic distance among the population, combined use of RAPD, ISSR and SSR markers were employed. The combined marker system yielded total loci of 663 bands

with a total polymorphic level of 69.08%. Genetic diversity among the population was highest for POP 2 (35.29%) as the accessions were collected from diverse regions of Ernakulam, Pathanamthitta and Tirunelveli. Lowest genetic diversity was observed in population 3 (18. 55%, where accessions collected were very close geographically (Idukki region). Nei's coefficient of genetic differentiation (Gst) was 0. 429 which showed that 43% of the total genetic variation was attributed to inter-populations and remaining 57% intra-populations. The level of gene flow was found to be 0.664, which implied a limited gene flow would be the reason for the low level of genetic differentiation among the population. Genetic diversity calculated is given in Table 150.

Table 150. Nei's genetic diversity indices of *C. aromatica* population

	n	(Na)	(Ne)	(h)	(I)	%P	Gst	Nm
POP 1	2	1.25 ± 0.43	1.25 ± 0.43	0.12 ± 0.22	0.17 ± 0.30	24.74%		
POP 2	3	1.35 ± 0.48	1.28 ± 0.38	0.16 ± 0.21	0.22 ± 0.30	35.29%		
POP 3	2	1.19 ± 0.39	1.19 ± 0.39	0.09 ± 0.19	0.13 ± 0.27	18.55%		
C. aromatica	7	1.64 ± 0.48	1.36 ± 0.33	0.22 ± 0.18	0.33 ± 0.27	63.95%	0.429	0.664

Na - total number of alleles' Ne - number of effective alleles; h - Nei's (1973) gene diversity index; I - Shannon's information index; %P - percentage of polymorphism; Gst = (Ht-Hs)/Ht; Nm = 0.5*(1-Gst)/Gst, POP-Population

The Nei's genetic distance (1972) among the populations was estimated using combined marker data. Table 151 shows the Nei's genetic distance among the population of *C. aromatica*. The upper diagonal matrix shows the Nei's genetic identity and the lower diagonal matrix shows the genetic distance.

Table 151. Nei's genetic distance and genetic identity of population of *C. aromatica* obtained from the combined RAPD, ISSR and SSR data

POP ID	POP 1	POP 2	POP 3
POP 1	****	0.864	0.820
POP 2	0.146	****	0.836
POP 3	0.199	0.179	****

*POP-Population, POP ID- Population identity

The dendrogram constructed using the genetic distances / genetic identity is depicted in Figure 85.

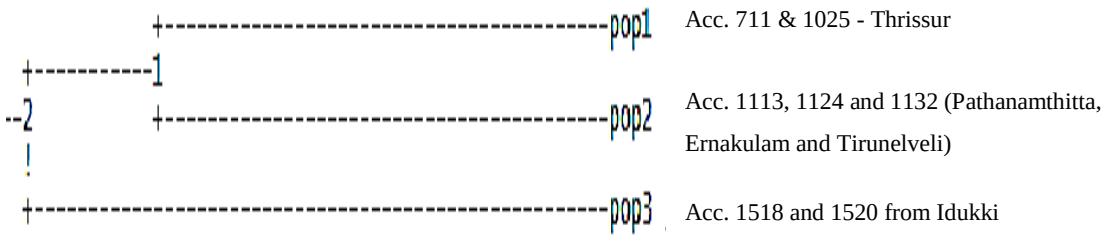


Fig. 85. UPGMA dendrogram based on Nei's genetic distance.

The UPGMA dendrogram showed closer association of population 1(Thrissur) and population 2 (Pathanamthitta, Ernakulam and Tirunelveli).

The mantel test of correlation between genetic and geographic distance was not significant. Analysis of molecular variance present among and within the population is given in Table 152.

Table 152. AMOVA analysis of *C. aromatica* population

Source of variation	d.f.	SS	Est. Var.	%	ϕ stat	P value
Among Population	2	209.07	12.98	15	0.15	0.017*
Within Population (Residual error)	4	299.50	74.88	85		
Total	6	508.57	87.85	100		

d.f.- degrees of freedom, SS- sum of squares, Est. Var-estimated variation, *P<0.05-significant.

A significant variation of 15% among the population and 85% within the population could be observed. The ϕ PT value was found to be 0.15 which showed medium genetic differentiation among the populations.

4.5.3. *C. caesia*

Seven accessions of *C. caesia*, collected from different regions of India, were grouped based on the locations from which they were collected. The grouping of the various accessions and the location details are given in Table 153.

Table 153. Grouping details of *C. caesia* population

Population No.	Accession Number	Place of collection	Latitude (N)	Longitude (E)	Altitude (in M)
POP 1	1001	Tezu, Lohit, Arunachal Pradesh	27° 56' 1"	96° 9' 28"	217
	1006	Hayuliang, Anjaw, Arunachal Pradesh	28° 4' 35"	96° 32' 17"	567
POP 2	1135	Thrissur, Kerala	10° 31' 39"	76° 12' 51"	21
	1154	Olakkara, Thrissur, Kerala	10° 31' 39"	76° 12' 51"	21
POP 3	292	Shilong, Meghalaya	25° 34' 43"	91° 53' 35"	1416
	1171	Shilong, Meghalaya	25° 34' 43"	91° 53' 35"	1416
	751	Pundibari, Cooch Behar, West Bengal	26° 31' 27"	89° 6' 26"	44

Two accessions collected from Arunachal Pradesh *viz*., Acc. 1001 and Acc.1006 were classified as POP 1. Acc.1135 and Acc.1154 collected from Thrissur were grouped as POP 2. Accessions collected from Meghalaya (Acc.292 and Acc.1171) and West Bengal (Acc.751) were grouped as POP 3.

The combined marker system yielded total loci of 628 bands with a polymorphic percentage of 68.63%. Genetic variability among the population was highest in POP 3 which consists of the accessions collected from the Meghalaya and West Bengal regions (52.55%) followed by POP 2 (23.25%) which consists of accessions from the Southern region. The lowest level of genetic variation was observed in the accessions collected from Arunachal Pradesh (21.97%) *i.e*. POP 1. The coefficient of genetic differentiation (Gst=0.326) was found to be low implying that, genetic variability was more in the intra-population level than in interpopulation level. The low genetic differentiation was further supported by a higher gene flow (Nm=1.034) (Table 154).

Table 154. Nei's genetic diversity indices of *C. caesia* population

	N	(Na)	(Ne)	(h)	(I)	%P	Gst	Nm
POP 1	2	1.22 ± 0.41	1.22 ± 0.41	0.11 ± 0.21	0.15 ± 0.29	21.97		
POP 2	2	1.23 ± 0.42	1.23 ± 0.42	0.12 ± 0.21	0.16 ± 0.29	23.25		
POP 3	3	1.53 ± 0.5	1.42 ± 0.4	0.23 ± 0.22	0.33 ± 0.32	52.55		
C. caesia	7	1.69 ± 0.46	1.39 ± 0.35	0.24 ± 0.18	0.36 ± 0.26	68.63	0.326	1.034

Na - total number of alleles' Ne - number of effective alleles; h - Nei's (1973) gene diversity index; I - Shannon's information index; %P -Percentage of polymorphism; Gst = (Ht-Hs)/Ht; Nm = 0.5*(1-Gst)/Gst, POP-Population.

The Nei's genetic distance (1972) among the populations was estimated using combined marker data (Table 155). The upper diagonal matrix shows the Nei's genetic identity and the lower diagonal matrix shows the Nei's genetic distance.

Table 155. Nei's genetic distance and genetic identity of populations of *C. caesia* obtained from the combined RAPD, ISSR and SSR data.

POP ID	POP 1	POP 2	POP 3
POP 1	****	0.873	0.891
POP 2	0.136	****	0.847
POP 3	0.116	0.166	****

*POP-Population, POP ID- Population identity

The dendrogram constructed using the genetic distances / genetic identity is depicted in Figure 86.

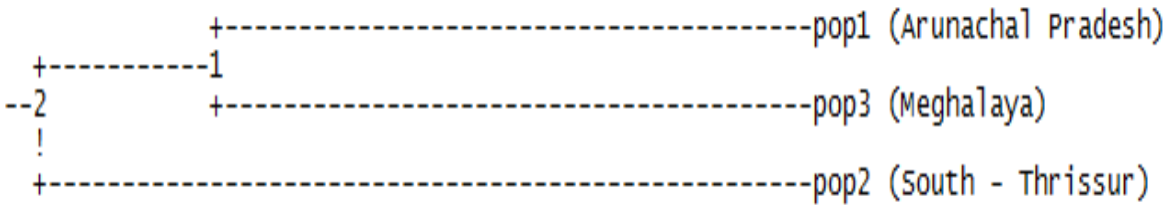


Fig. 86. UPGMA dendrogram based on Nei's genetic distance.

The dendrogram showed closer association among populations collected from North eastern region which were geographically closer than the accessions collected from South. The Mantel

test of correlation between genetic and geographical diversity was not significant ($r^2 = 0.414$, at a significance level of 32.3%).

AMOVA study was conducted to establish the variation among and within populations (Table 156).

Table 156. AMOVA analysis of *C. caesia* accessions

Source of variation	d.f.	SS	Est. Var.	Variation(%)	ϕstat	P value
Among Population	2	183.02	2.98	3	0.034	NS*
Within Population (Residual error)	4	338.83	84.71	97		
Total	6	521.86	87.68	100		

d.f.- degrees of freedom. SS -sum of squares, Est. Var-estimated variation, *NS-Not significant (P>0.05).

AMOVA was not significant. Hence no further variance analysis was attempted.

4.5.4. *C. xanthorrhiza*

Eight accessions of *C. xanthorrhiza*, collected from different regions of India were grouped based on the locations from which they were obtained. The grouping of the various accessions and the location details are given in Table 157.

Table 157. Grouping details of *C. xanthorrhiza* population

Population No.	Accession Number	Place of collection	Latitude (N)	Longitude (E)	Altitude (in M)
POP 1	465	Jorhat, Jorhat, Assam	26° 44' 47"	94° 12' 9"	84
POP 2	1108	Parambikulam, Palakkad, Kerala	10° 23' 34"	76° 46' 32"	582
	1122	Parambikulam, Palakkad, Kerala	10° 23' 34"	76° 46' 32"	582
	1163	Kizhakkekara, Kollam, Kerala	9° 58' 40"	76° 35' 31"	43
POP 3	1164	Nilambur, Malappuram, Kerala	11° 16' 45"	76° 14' 23"	26
	1167	Nilambur, Malappuram, Kerala	11° 16' 45"	76° 14' 23"	26
	1168	Nilambur, Malappuram, Kerala	11° 16' 45"	76° 14' 23"	26
	760	Kalpetta, Wayanad, Kerala	11° 37' 11"	76° 5' 3"	748

*POP-Population

Acc. 465, collected from Jorhat (Assam) was considered as POP 1. Accessions collected from Palakkad (Acc.1108, 1122) and Kollam (Acc. 1163) were considered as POP 2. Accessions

collected from Malappuram (Acc.1164, Acc. 1167 and Acc.1168) and from Wayanad (Acc.760) were grouped as POP 3.

In order to estimate the genetic distance among the population, combined use of RAPD, ISSR and SSR markers were employed. The combined marker system yielded a total of 608 bands with a total polymorphic level of 56.58%. Genetic diversity calculated is given in Table 158.

Table 158. Nei's genetic diversity indices of *C. xanthorrhiza*.

	n	(Na)	(Ne)	(h)	(I)	%P	Gst	Nm
POP 1	1	1 ± 0	1 ± 0	0 ± 0	0 ± 0	0.00		
POP 2	3	1.31 ± 0.46	1.25 ± 0.37	0.14 ± 0.21	0.2 ± 0.29	33.39		
POP 3	4	1.33 ± 0.47	1.23 ± 0.34	0.13 ± 0.19	0.2 ± 0.28	31.09		
C. xanthorrhiza	8	1.57 ± 0.5	1.3 ± 0.32	0.18 ± 0.18	0.28 ± 0.26	56.58	0.532	0.439

Na - total number of alleles' Ne - number of effective alleles; h - Nei's (1973) gene diversity index; I - Shannon's information index; %P - percentage of polymorphism; Gst = (Ht-Hs)/Ht; Nm = 0.5*(1-Gst)/Gst, POP-Population.

Genetic variation among the population was highest for population 2 (h = 0.14 ± 0.21; I = 0.20 ± 0.29) as the accessions were collected from the diverse regions of Palakkad and Kollam. This population also showed a higher level of polymorphism (33.39%). Lowest genetic variation was observed in POP 3, which were mainly collected from Malappuram region (h = 0.13 ± 0.19; I = 0.20 ± 0.28). They have also shown the lowest level of polymorphism (31.09%).

The Nei's genetic distance (1972) among the populations was estimated using combined RAPD, ISSR and SSR data (Table 159). The upper diagonal matrix shows the Nei's genetic identity and the lower diagonal matrix shows the Nei's genetic distance.

Table 159. Nei's genetic distance and genetic identity of populations of *C. xanthorrhiza* obtained from the combined RAPD, ISSR and SSR data.

POP ID	POP 1	POP 2	POP 3
POP 1	****	0.770	0.796
POP 2	0.262	****	0.935
POP 3	0.228	0.067	****

The dendrogram constructed using the genetic distances / genetic identity is depicted in Figure 87.

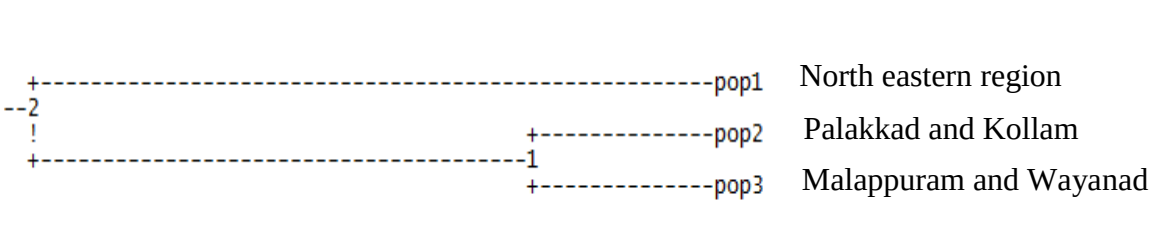


Fig. 87. UPGMA dendrogram based on Nei's genetic distance.

The dendrogram showed very close association with populations collected from Wayanad and Malappuram region. The Gst value obtained was 0.532, which shows a moderate genetic differentiation among the populations.

The Mantel test of correlation between genetic and geographical diversity was not significant (r^2 =0.989, at a significance level of 14.6%).

Due to data limitation, Acc. 465 from North East region had to be removed for carrying out the analysis. AMOVA was not significant. Hence no further analysis was done. The AMOVA results obtained is given in Table 160.

Table 160. AMOVA analysis of accessions of *C. xanthorrhiza*

Source of variation	d.f.	SS	Est. Var.	Variation(%)	ϕstat	P value
Among Population	1	59.23	0.48	1	0.008	NS*
Within Population (Residual error)	5	287.92	57.58	99		
Total	6	347.14	58.06	100		

d.f. -degrees of freedom. SS- sum of squares, Est. Var-estimated variation, *NS-Not significant (P>0.05).

Chapter 5

Discussion

5.1. General

Genus *Curcuma* is an important entity of family Zingiberaceae, composed of about 70-80 species, annual or perennial herbs (Purseglove, 1974; Sirirugsa, 1999) which consist of economically and medicinally important species. *Curcuma* species expanded themselves into a wide range of habitat ranging from sea level to hilly slopes (Sasikumar, 2005). A lot of disparity have been reported for the number of genera and species in the family and, the reason for this ambiguity is attributed to the active stage of evolution of the family (Larsen *et al*., 1999). The number of *Curcuma* species occurring in various countries are being revised from time to time as new species are being identified and some of the existing species are now treated as synonyms.

Although viable seed set is reported in some species like *C. longa* and *C. aromatica* (George, 1981; Sasikumar *et al*., 1996), majority of the *Curcuma* species are asexually reproduced, thus the chances of genetic variation in these species are believed to be very low (Widen *et al*., 1994). However, existence of genetic diversity in *Curcuma* species has been reported (Apavatjrut *et al*., 1999; Velayudhan *et al*., 1999; Chen *et al*.,1989; Sabu,1991; Symakumar and Sasikumar, 2007; Das *et al*., 2011; Zheng *et al*., 2015; Basak *et al*., 2017). Thus in the absence of sexual reproduction, the observed variation may be attributed to environmental effects, somatic mutation and/or numerical/structural chromosomal changes (Singh *et al*., 2014).

Curcuma species show intraspecific variation for various taxonomically important characters and thus making them taxonomically confusing genus for plant breeders and taxonomists (Mangaly and Sabu, 1993; Apavatjrut *et al*., 1999). Variations in characters like plant height, leaf sheath colour and texture, leaf mid rib colour, position and colour of the inflorescence, fertile bract colour, size and shape, rhizome inner core colour etc. have already been reported (Santapau, 1952; Sabu,1991; Velayudhan *et al*., 1999; Sabu, 2006).

Like morphological characters, biochemical traits were also reported to be varying with respect to species, explant, location and environment (Zwaving and Bos, 1992; Behura *et al*., 2002; Raina *et al*., 2005; Paliwal *et al*., 2011; Angel *et al*., 2014). The observed variation in morphological characters and biochemical traits could be emanating from intraspecific

genetic differentiation, geographical or environmental factors. Earlier works have established genetic basis for intraspecific variation in *Curcuma* species existing in wild and cultivated population (Paisooksantivatana, 2001a & 2001b; Islam *et al*., 2005; Nayak *et al*., 2006; Komatsu *et al*., 2008; Soontornchainaksaeng and Jenjittikul, 2010; Taheri *et al*., 2012; Taheri *et al*., 2014).

5.2. Morphological characterization of four *Curcuma* species

In the current study, four *Curcuma* species studied exhibited significant variation for morphological characters. While quantitative morphological characters showed both inter and intraspecific variation, the qualitative characters did not exhibit any intraspecific variations with a few exceptions. Acc.751 and Acc.1001 of *C. caesia* showed intraspecific variation for pseudostem colour. These two accessions showed a purple tinge on their pesudostem, whereas rest of the accessions of *C. caesia* were devoid of purple tinge. The purple tinge on pseudostem was not mentioned in earlier studies on *C. caesia* done by Paliwal *et al*. (2011) and Jose *et al*. (2014). However, variations in qualitative morphological characters were reported in other *Curcuma* species. Two accessions of *C. kwangsiensis* showed morphological variation for stem and leaf midrib colour as one was blue and other was mauve. However, both the accession showed very low genetic variability and were grouped under the same cluster. The study concluded that the morphological character variation observed might be due to the growing environment (Zou *et al*., 2011). Jan *et al*. (2012) also reported variations in qualitative morphological traits in *C. longa;* leaves of *C. longa* plants collected from Bannu and Haripur were light green in colour whereas leaves from Kasur area were dark green. Likewise, turmeric rhizomes from Bannu and Haripur were yellow in colour while rhizomes from Kasur had dark orange colour. The observed variation in pseudostem colour of Acc. 1001 could have been exuded from environmental factors as they showed genetic similarity with rest of the accessions. However, Acc. 751, maintained distinct status in molecular analysis as well. Previous works in *Curcuma* (Santapau, 1952; Sabu, 1991; Noli *et al*., 1999; Sabu, 2006; Syamkumar, 2008) have already reported the incongruence in the morphological characters of the same species.

Although, clonally propagated plants like *Curcuma* species have limited scope of variation, strikingly high intraspecific variation observed in the morphological characters can be attributed to both genetic and non-genetic factors. In such species, in the absence of sexual reproduction, the variation may have originated as a result of selection pressures in different

geographical location or due to somatic mutations or chromosomal changes or both which may get inherited to progenies and gradually get embedded in them. Abdullah *et al*, (2009) also reported similar possibilities in *C. alismatifolia*, where they suggested that variation observed in *C. alismatifolia* was not solely by genetic mutation but it can also due to somatic mutation that includes point mutations, karyotypic changes, somatic gene arrangements etc. Jayasree, (2009) suggested a possible role of mutations for the morphological variations observed in *C. amada* genotypes. A wide range of variations in morphological characters such as plant height, leaf length, leaf width, leaf texture, number of leaves per tiller, number of tillers per clump, number and weight of primary, secondary and mother rhizome, rhizome yield etc. were previously reported in *Curcuma* species such as *C. amada, C. caesia, C. aromatica, C. xanthorrhiza*, *C. zedoaria,* and *C. longa* (Pillai and Nambiar, 1974; Sabu, 1991;Vimala, 2002; Sabu, 2006; Syamkumar, 2008; Aminah, 2007; Kumar *et al*., 2013; Chatterjee *et al*., 2012; Bahl *et al*., 2014; Jatoi *et al*., 2015).

Among the accessions of four species, accessions of *C. xanthorrhiza* showed comparatively higher values for characters such as plant height, petiole length, leaf length and leaf width. Aminah (2008) also reported higher values for plant height, canopy width, leaf length and leaf width in *C. xanthorrhiza* when compared to *C. mangga, C. zedoaria, C. aeruginosa, C. heyneana* and *C. domestica*. High values observed for petiole length in the current study were justified by previous studies, which reported petiole length greater than 20cm in these *Curcuma* species (Vimala, 2002; Skornickova and Sabu, 2005; Jatoi *et al*., 2015). Olatunji, (1970) also commented about the characteristic long petioles seen in *Curcuma* and *Scaphochlamys* which reached 20cm or more.

All the four *Curcuma* species under study showed high value for dry recovery; highest in *C. amada*, followed by *C. caesia, C. aromatica* and *C. xanthorrhiza*. The probable reason behind the high dry recovery in these *Curcuma* species might be due to their starchy rhizomes (43.82-48.23% starch content). Earlier workers reported dry recovery ranging from 25-30% in these species (Ratnambal, 1986). Shankar *et al*. (2014) also reported dry recovery in the range of 15.21-30.67% in *C. angustifolia* which is a well-known starchy *Curcuma* species. Netaji *et al*. (2000) and Mahesh *et al*. (2014) reported a positive correlation between yield and starch content in crops like wheat and maize.

5.2.1. Multivariate analysis

Multivariate techniques such as Principal Component Analysis (PCA) and cluster analysis are efficient tools in evaluating diversity and for determining the relationship among and within the species (Lansari *et al*., 1994; Vanijajiva *et al*., 2005; Sorkheh *et al*., 2009). These techniques evaluate large data by reducing it into more interpretable and easily visualized groups (Colic *et al*., 2012). In Principal Component analysis (PCA), the geometrical distances among the individuals in loading plot have direct correspondence to its genetic distances (Placide *et al*., 2015). Roy *et al*. (2011) suggested that Principal Component Analysis and cluster analysis are effective for the grouping of turmeric accessions which will ultimately facilitate the characterization of accessions for crop improvement. Similarly, in other species like almond (Lansari *et al*., 1994), butterfly pea, (Morris, 2009), bread wheat (Beheshtizadeh *et al*., 2013), sweet potato (Placide *et al*., 2015) etc. authors established the usefulness of Principal Component Analysis and cluster analysis as an important tool for providing the information about variability, either intra-specific or inter-specific variations present in germplasm.

Morphological characters were subjected to Principal Component Analysis and cluster analysis. The study revealed that accessions of *C. amada* and *C. aromatica* showed very close resemblance to each other. All the accessions were interleaved and hardly possible to distinguish from each other. The close proximity of *C. amada* and *C. aromatica* in morphological characters has been reported earlier (Dixit *et al*., 2009; Mohanty *et al*., 2014). *C. amada* and *C. aromatica* were almost similar for visual aerial morphological characters, having leafy shoots reaching up to 1m or more, oblong-lanceolate green leaf sheaths, with green mid rib colour (Ravindran *et al*., 2007). However, *C. amada* possesses a unique raw mango flavour and aroma for its rhizomes (Policegoudra *et al*., 2011) with leaves hairy on ventral side (Velayudhan *et al*., 1999) whereas leaves of *C. aromatica* were densely pubescent on their abaxial side (Anand and Saxena, 2015) and its rhizome has camphoraceous aroma (Sikha and Harini, 2015). Acc. 1167 showed close proximity to the *C. amada* and *C. aromatica* group. This anomaly could be because of the similarity in unstable characters such as plant height, rhizome length, number of mother rhizomes, dry recovery etc.

Intraspecific variation in four species was further confirmed in Principal Component Analysis and cluster analysis as accessions from individual species formed two or more

groups in loading plot and dendrogram. In the cluster analysis, Acc. 6390 collected from Gundimeda, Vijayawada (Andhra Pradesh) maintained a unique status from rest of the accessions of *C. amada* as it formed a lone group. The lone status may be attributed to variation in characters like leaf width, number of tillers per clump, rhizome internode length, number of primary rhizomes and dry recovery. Accessions belonging to different regions were also seen clustering together showing similarity in the cluster analysis. Velayudhan *et al*. (1999) reported abnormality in the grouping of *Curcuma* species belonging to different taxonomic groups in the same cluster using morphological traits. Likewise, Zou *et al*. (2011) also reported the unlikely clustering of *Curcuma* genotypes collected from the same source into different clusters and whereas those from different places are grouped together. Probable reason may be the movement of rhizomes along with the migration of farmers from place to place (Singh *et al*., 2012).

Two accessions of *C aromatica*, Acc. 1124 and Acc. 711 stood apart from the rest of the accessions of *C. aromatica.* This unique status is also visible at the molecular level. The observed variation may be due to genetic factors rather than environmental conditions. A similar scenario was reported by Chaudhary *et al*. (2006) in *C. longa* varieties. Apart from vegetative reproduction, *C. aromatica* and *C. longa* are reported to produce open pollinated progenies (George,1981; Sasikumar *et al*., 1996) thus variation observed could also be attributed to its seed setting nature.

Acc.1001 and Acc. 1154 of *C. caesia* was found to have a very weak association to rest of the accessions with respect to morphological characters. However, in molecular analysis, the majority of the markers showed that Acc. 1171 was least similar with rest of the accessions whereas, Acc.1154 showed high genetic similarity with rest of the accessions. Thus the observed distinct status of Acc.1154 and Acc.1001 for morphology may be due to the growing environment. Zou *et al*. (2011) also reported similar scenario in *C. kwangsiensis*, as two accessions showed morphological variation for stem and leaf midrib colour but they showed low genetic variability and grouped together.

Acc.1168 of *C. xanthorrhiza* which showed least similarity with rest of the accessions for morphological traits also formed a lone group in majority of the molecular marker based dendrogram. The variation may be associated with genetic factors as reported in other *Curcuma* species (Chaudhary *et al*., 2006).

By and large none of the accessions from four *Curcuma* species showed any location specificity in the dendrogram except a few accessions. Accessions like Acc.752 & Acc.753 (Pundibari, West Bengal) of *C. amada*; Acc.1518 & Acc.1520 (Anachal & Thekkady of Idukki) of *C. aromatica*; Acc. 1108 & Acc. 1122 (Parambikulam, Kerala) and Acc.1164 and Acc.1167 (Nilambur, Malappuram, Kerala) of *C. xanthorrhiza* showed a closer association with each other for morphological characters based on their place of collection. Presence and absence of clustering of accessions based on their place of collection was supported by several studies in turmeric (Islam, 2004; Zou *et al*., 2011; Singh *et al*., 2012), in castor (Gajera *et al*., 2010), in Azuki bean (Yee *et al*.,1999) and in groundnut (Dwivedi *et al*., 2001). The first three component of principal analysis, which explained the variability among the species, revealed that *C. xanthorrhiza* and *C. amada* as the most variable species morphologically. The accessions were highly dispersed with one or two accessions standing out in both the species.

5.2.2. Variability, heritability and genetic advance

The estimate of genetic parameters among the accessions of the four species revealed that the Phenotypic Coefficient of Variation (PCV) was more or less same or a little bit higher than Genotypic Coefficient of Variation (GCV) for morphological characters. This indicated the possible role of environment in the expression of morphological characters to some extent. Such characters might be exhibiting an additive polygenic nature as suggested by Jayasree (2009) in *C. amada*. However, the combined role of gene(s) in controlling a trait and influence of environment for its expression can neither be ignored nor ruled out.

The value of GCV and PCV varied among the four species for different characters. The GCV and PCV values varied from species to species in previous studies too. Jayasree (2009) observed the highest GCV and PCV values for number of tillers, leaf area and plant height in *C. amada*. For *C. angustifolia*, total rhizome yield, number and weight of primary, secondary and mother rhizomes showed highest GCV and PCV values (Shankar *et al*., 2014). Petiole length, dry recovery, leaf length, number of leaves, leaf width, plant height and rhizome length showed highest GCV and PCV values in *C. longa* (Gupta *et al*., 2016).

Heritability is defined as the ability of a trait to get inherited to its progeny. The majority of the agronomic traits of crop plants are polygenic in nature and thus influenced by number of alleles and environment to some extent (Jayasree, 2009). According to Singh (2001), heritability values greater than 80% are very high, values from 60-79% are moderately high,

40-59% are medium and values less than 40% are low. Likewise, according to Johnson *et al*. (1955), genetic advance as percentage of mean (GAM) values from 0 - 10% is low, 10 - 20% are moderate and 20% and above are high. High heritability values were observed for characters like plant height, number of tillers per clump, number of leaves per tillers, rhizome weight, number and weight of mother, primary, secondary rhizomes etc. Jayasree *et al*. (2014) also reported moderate to high heritability for growth and yield characters like plant height, number of tillers, leaf area, yield etc. in *C. amada*. The characters which exhibit high heritability, as well as genetic advance, could be used as an important tool for the selection of characters as these characters are controlled by additive genes to a large extent, and improvement of these traits through phenotypic selection is fruitful (Panse and Sukhatme, 1995; Singh *et al*., 2012; Gupta *et al*., 2016).

Current results are supported by earlier findings of Philips and Nair (1986); Jayasree (2009); Jan *et al*. (2012); Singh *et al*. (2012); Rajyalakshmi *et al*. (2013), Shankar *et al*. (2014); Hanchinamani *et al*. (2016); Gupta *et al*. (2016) where high heritability coupled with genetic advance is reported for characters like plant height, number of tillers per clump, leaf length, leaf width, number and weight of mother, primary and secondary rhizomes etc. in various *Curcuma* species.

5.3. Biochemical Characterization of four *Curcuma* species

The medicinal and nutritive value of a plant lies in the variety of chemical substances (phenols, flavonoids, carbohydrates, starch, protein etc.) produced in them. Knowledge of these chemical substances are very crucial for the discovery of new therapeutic medicines, nutritive values as well as to understand the value of folk remedies (Mojab *et al*., 2003). Members of the genus *Curcuma* are well known for their use as spices, dyes, cosmetics, tonics, starch, perfumes, food and for aesthetic purposes. Bioactive components present in *Curcuma* species are responsible for their medicinal uses (Dutta, 2015). These bioactive components tend to vary within and among the species as well as from place to place owing to different agro climatic conditions and environment (Sandeep *et al*., 2015). Pioneer studies on various *Curcuma* species showed variation in their quality traits like curcumin, oleoresin, essential oil, curcuminoids, total protein, total starch, total phenol and total carbohydrates (Radhakrishnan *et al*., 1995; Jayaprakasha *et al*., 2002; Behura *et al*., 2002; Poilcegoudra and Aradhya, 2008; Li *et al*., 2011; Krishnaraj *et al*., 2012; Rajamma *et al*., 2012; Rani and Chawaan, 2012; Ashraf *et al*., 2012; Angel *et al*., 2013; Shamrao *et al*., 2013).

The study revealed that except for total protein content, there exist significant variation among the four species for the quality traits studied. Similarly, significant intraspecific variation was detected for most of the quality traits except one or two traits in each species.

5.3.1. Oleoresin

Oleoresins extracted from four *Curcuma* species were viscous in nature and brownish yellow in colour. Oleoresins from aromatic plants like *Curcuma* species is credited with volatile essential oil as well as the non-volatile fraction and is being used widely in food, cosmetic and pharmaceutical industries (Rajamma *et al*., 2012). Significant variation exists for oleoresin content among and within the four species with major variation from within the species source. Chatterjee *et al*. (2012) also reported variation for oleoresin content among the germplasm of mango ginger which varied from 4.21% to 6.57%. Syamkumar (2008) also emphasized the variation for oleoresin content in cultivars/varieties of *C. longa*. Earlier studies evidenced that variation in oleoresin content in *Curcuma* are influenced by factors like choice of species, post-harvesting techniques, type of extraction method used and varying environmental conditions (Green *et al*., 2008; Singh *et al*., 2012; Chatterjee *et al*., 2012). *Curcuma* species with high oleoresin content is vital owing to its natural antioxidant and antibacterial properties (Rajamma *et al*., 2012). In the current study, maximum oleoresin content was reported in *C. aromatica* (10.68%) and least in *C. caesia* (6.92%). Our results are almost in congruence with that reported by Rajamma *et al*. (2012), in which oleoresin yield was found maximum in *C. xanthorrhiza* (10%), *C. amada* (8.8%) and *C. aromatica* (7.8%) and least in *C. caesia* (4%). Reenu (2017) also reported a similar range of values (6.1-7.3%) for oleoresin content in *C. caesia* accessions.

5.3.2. Essential oil

Essential oils are natural, volatile complex compounds characterized with a strong aroma and are formed by aromatic plants as secondary metabolites. They are usually known for their antibacterial, antiseptic, antimicrobial, anti-inflammatory, fungicidal and various other medicinal properties. (Bakkali *et al*., 2008). Due to their aroma and antimicrobial activities, they are being frequently used in aromatherapy, pharmaceutical and food industries (Bakkali *et al*., 2008). Many *Curcuma* species are valued for its essential oil content as they are the indispensable ingredient of flavour, fragrance, pharmaceutical industries and ethnomedicinal use (Tripathi *et al*., 2013).

The present study showed the existence of significant variation in essential oil content among and within the four *Curcuma* species. A perusal of early works showed incongruence in the essential oil profile of same *Curcuma* species using same plant parts for the oil extraction. This anomaly may be attributed to several factors. Previous studies have revealed presence of significant variation for content and constituents of essential oils in *Curcuma* species with geographical locations (Li *et al*., 2011), plant source (Jantan, 1999; Garg *et al*., 1999; Rao *et al*., 2008) type of extraction chosen (Bakkali *et al*., 2008), soil, climatic conditions, age and organ of the plant (Massotti *et al*., 2003; Angioni *et al*., 2006).

Qualitative and quantitative variation for essential oil constituents were already reported in *C. amada* (Bandyopadhay, 1993; Mustafa *et al*., 2005; Padalia *et al*., 2013), *C. aromatica* (Choudhury *et al*., 1996; Behura *et al*., 2002; Tsai *et al*., 2011; Angel *et al*., 2014), *C. caesia* (Pandey and Choudhary, 2003; Paliwal *et al*., 2011) and *C. xanthorrhiza* (Zwaving and Bos, 1992; Jarikesem *et al*., 2005; Jantan *et al*., 2012).

Among the four species under study, *C. aromatica* recorded the highest percentage of essential oil followed by *C. xanthorrhiza, C. caesia* and least in *C. amada*. Syamkumar (2008) also reported *C. aromatica* as a better source of essential oil than *C. longa*. The intensive camphoraceous aroma of *C. aromatica* may be indicating high essential oil content it.

5.3.2.1. GC-MS analysis of essential oil

The essential oil is a complex mixture which contains around 20-60 components at different concentrations, among which 2-3 components are usually found in very high concentrations (20-70%) compared to rest of the components which are present in trace amounts (Tripathi *et al*., 2013). GC-MS analysis of essential oil from four *Curcuma* species identified a total of 30 major compounds. There was a wide range of variability for the type and quantity of essential oil components among and within the four species. The lowest number of identified compounds were present in *C. amada* and the highest number in *C. caesia* and *C. xanthorrhiza*. The essential oil constituents obtained in the current study is in accordance with previous works reported in these four *Curcuma* species. (Jantan *et al*., 1999; Pandey and Chowdhury, 2003; Syamkumar, 2008; Policegoudra *et al*., 2011). Various studies revealed that constituents of essential oil show quantitative and qualitative variation within the species for both major and minor chemical constituents in both dry and fresh rhizome (Rao *et al*., 1989; Garg *et al*., 1999; Mustafa *et al*., 2005; Rukayadi *et al*., 2006; Sarangthem

et al., 2010; Al-Reza *et al*., 2011). As different accessions within the same species show varying level of major essential oil components, there is an ample opportunity for bioprospecting the desirable oil constituents.

According to Li *et al*. (2011), the essential oils obtained from flowers and leaves usually contained monoterpenes while those from rhizomes and roots are dominated by sesquiterpenes; which was in accordance with our result, as sesquiterpenes dominated the rhizome oil. Similar results were reported by various authors on essential oil components of *Curcuma* species (Kuroyanagi *et al*.,1987; Bandyopadhay, 1993; Kojima *et al*., 1998; Behura *et al*., 2002; Pandey and Choudhary, 2003; Syamkumar, 2008).

Out of total 30 components identified from four *Curcuma* species, some of the volatile oil constituents were unique to certain *Curcuma* species studied. Beta-ocimene was specific to *C. amada*, the highest amount of curdione was present in the species *C. aromatica*. 2-nonanol, β-caryophyllene, epicurzerenone, isocurcumenol were found in *C. caesia*, xanthorrhizol was recorded in *C. xanthorrhiza*. A perusal of earlier literary works also highlighted the presence of unique essential oil compounds in these *Curcuma* species (Jantan, 1999; Choudhury *et al*.,1996; Singh *et al*., 2003; Pandey and Chowdhury, 2003; Syamkumar, 2008; Devaraj *et al*., 2010).

5.3.3. Curcumin

Curcumin (diferuloylmethane) which is responsible for the yellow colour pigment is a polyphenol compound derived from a number of *Curcuma* species and is one among the extensively studied naturally-derived therapeutic products in recent decades (Perrone *et al*., 2015). The study showed significant variation for curcumin content among the species. Except for *C. caesia*, rest of the species showed variation for curcumin content within the species. Variation in curcumin content is well documented in *Curcuma* species. Previous studies reported variation in curcumin content from species to species, cultivar to cultivar, and geographical regions (Radhakrishnan *et al*., 1995; Hegde *et al*., 1997; Anasuya, 2004; Hrideek, 2006; Rao *et al*., 2006; Kamble *et al*., 2011; Ashraf *et al*., 2012). According to Hayakawa *et al*. (2011) one possible reason for the intraspecific variation for curcumin content in *C. longa* may be caused by hybridization or introgression of *C. longa* with other *Curcuma* species like *C. aromatica*. As per their study homogeneous *C. longa* has high curcumin content whereas heterogeneous hybrid of *C. longa* has medium curcumin content.

Among the four species, maximum curcumin content was observed in *C. xanthorrhiza*. Curcumin content among the accessions of *C. xanthorrhiza* ranged from 0.96% to 2.03%. Rohman *et al*. (2015) reported curcumin content in *C. xanthorrhiza* in the range of 1.66 - 2.97%. Rest of the species showed lower concentrations for curcumin content. Earlier studies also reported lower concentrations of curcumin in species like *C. amada* (Gupta *et al*., 1999), *C. aromatica* (Nahar and Sarker, 2007), *C. aeruginosa*, *C. mangga*. (Bos *et al*., 2007) and *C. caesia* (Reenu, 2017).

5.3.3.1. HPLC analysis of curcuminoids

Curcuminoids, one of the major biologically active component of *Curcuma* species are mainly comprised of curcumin 1, demethoxycurcumin and bisdemethoxycurcumin. Among which curcumin 1 is the most explored component (Khanna,1999: Jayaprakasha *et al*., 2006). Several methods have been reported for the quantification of curcuminoids *viz*., GC (Gas Chromatography), fluorimetric, Spectrophotometric methods, TLC (Thin Layer Chromatography), HPLC (High Performance Liquid Chromatography) and HPTLC (High Performance Thin Layer Chromatography) (Tonnessen and Karlsen, 1983; Jasim and Ali,1992; Chauhan *et al*., 1999; Jayaprakasha *et al*., 2002; Panadda *et al*., 2009; Paramasivam *et al*., 2009). However, among the methods mentioned above HPLC methods are probably the most convenient one (Thomas *et al*., 2011). Various authors reported a range of mobile phase for the identification of curcuminoids *viz*., ethanol (Tonnesen and Karlsen,1983), methanol, acetic acid, acetonitrile (Jayaprakasha *et al*., 2002), chloroform (Syamkumar, 2008), formic acid (Avula *et al*., 2012) as curcuminoids separation in HPLC method is known to be influenced by the mobile phase used (Jayaprakasha *et al*., 2002). The variation in curcumin yield estimated using spectrophotometric and HPLC method is well documented. The probable reason behind this variation may be due to the presence of other similar compounds absorbing in the region of 420-430nm thus influencing the spectrophotometric results or it can be due to the difference in intensities of three curcuminoids in varying organic solvents used.

In the current study, different types and composition of mobile phase were tested and desired resolution of curcuminoids peaks was obtained by using Acetonitrile and 0.1% orthophosphoric acid (60:40 and 1ml minute^{1}). Among the four species, except *C. xanthorrhiza*, rest of the species yielded very low concentrations of curcuminoids. Similar results were reported in *C. amada*, *C. aromatica, C. aeruginosa* and *C. mangga* (Gupta *et*

al., 1999; Nahar and Sarker, 2007; Bos *et al*., 2007). Although significant variation was observed among the species, within the species only the accessions of *C. xanthorrhiza* displayed variation. In all the species, Curcumin 1 was the major curcuminoids detected. Curcuminoids contents often vary with location, maturity, varieties, cultivation condition and plant sources as reported in previous studies (Jayaprakasha *et al*., 2002; Pothitirat and Gritsanapan, 2005; Pothitirat and Gritsanapan, 2008; Li *et al*., 2011).

5.3.4. Total Protein

Nutritional components like total protein are generally high in *Curcuma* species, which add up to their use as spices and medicine (Behar, 2014). In the present study, among the four *Curcuma* species, protein content did not vary significantly. However, within the species protein content varied significantly. Pioneer study by Chatterjee *et al*. (2012) reported significant variation (5.37%-7.86%) for protein content among the germplasm of mango ginger acquired from different locations like West Bengal, Odisha and Kerala. Reenu (2017) reported protein content ranging from 8.21% to 12.19% in accessions of *C. caesia* collected from different locations in India.

Alikhan and Youngs (1973) reported intra and interspecific variation for protein content in homozygous pea cultivars. They suggested environmental, physiological and developmental factors as the probable reason for the intraspecific protein variation without ruling out the fact that both genetic and non-genetic factors can affect the protein content.

Previous studies reported significant variation for protein content in various *Curcuma* species including the species under study (*C. longa, C. aeruginosa, C. amada, C. aromatica, C. brog, C. caesia, C. malabarica, C. rakthakanta, C. sylvatica* and *C. zedoaria*) (Policegoudra and Aradhya, 2007; Fattepurkar *et al*., 2009; Angel *et al*., 2013).

An increase in protein content prior to sprouting was also reported in *Curcuma* rhizomes (Policegoudra and Aradhya, 2008) and potato (Macdonald and Osborne, 1988; Alam *et al*., 1994). In contrast to the early findings, our study reported a lack of variation in *C. amada* and *C. aromatica* for total protein content at different growth stages.

5.3.5. Total starch

Starch has wide applications in the food, cosmetic, textile, paper, pharmaceutical industries (Betancur and Chel, 1997; Santana and Meireles, 2014). With the increased demand from various starch based industries, studies have been extended to explore non-conventional starch sources with different properties as native starch sources are favoured over chemically

modified starches (Santana and Meireles, 2014; Sajitha and Sasikumar, 2015). Starch in tubers, cereals and roots are the predominant dietary source of energy for humans. Cassava (*Manihot esculenta* Crantz) and sweet potato (*Ipomoea batatas* Lam) are widely used in Asian countries as starch sources. Many *Curcuma* species including *C. caulina, C. angustifolia, C. montana, C. pseudomontana, C. zedoaria, C. malabarica, C. decipiens, C. rubescens,* and *C. haritha* are reported to be potential sources of starch (Velayudhan *et al*., 1999). However, starchy crops like *Curcuma* have not been exploited much for extraction of starches except a few species like *C. zedoaria, C. malabarica, C. longa, C. aromatica, C. amada, C. angustifolia*. (Jyothi *et al*., 2003; Braga *et al*., 2006; Al-Reza *et al*., 2010; Policegoudra *et al*., 2011; Rani and Chawhaan, 2012).

Starch yield results in four *Curcuma* species indicated significant variation among and within species as starch content among four species varied from 43.82% to 48.23%. Current results were in accordance with earlier findings that starch content and its characteristics in *Curcuma* species were found to vary with accessions, maturity of plant, location etc. from 9.20 % to 59.64 % (Srivastava *et al*., 2006; Angel *et al*., 2008; Policegoudra and Aradhya, 2008; Moorthy, 2001; Rani *et al*., 2010; Mangunwardoyo *et al*., 2012; Shamrao *et al*., 2013). Maximum starch content was recorded in *C. amada* and minimum in *C. aromatica*. In each *Curcuma* species, certain accessions showed high starch yield compared to rest of the accessions; Acc.1511 of *C. amada*, Acc.1113 of *C. aromatica*, Acc.1006 of *C. caesia* and Acc.760 of *C. xanthorrhiza*. Such high starch yielding accessions can be subjected to bioprospecting for starch sources with desirable starch properties.

5.3.5.1. Qualitative analysis of starch

The starch granules of the four *Curcuma* species varied greatly in shape and size. *C. aromatica* granules were the largest (9-60 µm long), showed surface ornamentation, and were different from the granules of the rest of the species in having concentric rings. Starch granules of *C. amada* were oval to elliptical in shape with a smooth surface (16-48 µm long), Round to oval, small with a smooth surface (10-39 µm long) in *C. caesia* and in *C. xanthorrhiza* the starch granules were oval to elliptical, some were rounded with smooth surface (9-47 µm long).

Earlier Scanning Electron Microscopic (SEM) studies in different *Curcuma* species also reported wide variation in the size and shape of starch granules: elliptical and 14–46 µm long in *C. zedoaria* and 16–42 µm in *C. malabarica* (Jyothi *et al*., 2003); oval, irregular or

cuboidal or elliptic and polygonal in *C. amada*, either small (3–20 μm long) or large (20–48 μm long) in *C. amada* (Policegoudra and Aradhya, 2008); ranging in length from 6 to 25 μm in *C. malabarica*, *C. longa*, C. *sylvatica*, *C. caesia*, *C. zedoaria*, *C. raktakanta*, *C. aeruginosa*, and *C. aromatica* (Vimala and Nambisan, 2005) and from 20 to 25 μm in *C. longa* and 20 to 30 μm in *C. zedoaria* (Leonel *et al*., 2003); small, rounded, oval to elliptical or spherical, 3.32–32.55 μm long and 2.29–8.47 μm wide in *C. angustifolia* (Rani and Chawhaan, 2012). The physiology of a plant and its chloroplasts and amyloplasts influence the morphology of starch granules (Singh *et al*., 2003). Earlier studies in potato, yam, ginger, cassava, and some other *Curcuma* species have shown that starch granules vary considerably not only with species but also with location (Braga *et al*., 2006, Zhou *et al*., 2013). Starch yield in the present experiment varied among and within the species. The four species did not differ a great deal in terms of their moisture content (8.94–9.60%). The moisture content of dry tuber starch is usually 6–16% (Moorthy, 2002). Policegoudra *et al*. (2011) reported 9.8% moisture in *C. amada* and Braga *et al*. (2006) reported a figure of 11.8% in *C. longa*.

The solubility and swelling power of starch granules are positively correlated, implying that solubilization increases with the extent of swelling (Srichuwong *et al*., 2005), which is borne out in the present experiment as well; *C. amada* topped in terms of both solubility and swelling power. The swelling power of starch granules is also reported to be influenced by hydrogen bonding and the structure of amylopectin molecules (Tester *et al*., 1993, Hoover, 2001). The solubility and swelling power of starch granules indicate the strength of the binding force between granules, which ultimately decides the suitability of starch from a given source for a specific purpose. Low solubility is attributed to the amylose–lipid complex in starch granules, which lowers their swelling power (Leach *et al*., 1959). The water-holding capacity of starch granules depends on the extent of swelling and is thus directly influenced by solubility and swelling power. A loose association between amylose and amylopectin molecules in starch granules is believed to increase their water-holding capacity (Soni *et al*., 1987). Wotton and Bamunuarachchi (1978) attributed the variation in water-holding capacity to the difference in available water-binding sites in starch granules. The morphological structure of granules also influences their swelling power, solubility, and water-holding capacity (Zhou *et al*., 1998; Singh and Singh, 2001; Kaur *et al*., 2002).

Although the four *Curcuma* species did not differ significantly in terms of moisture content, they did show qualitative differences in size, shape, solubility, swelling power, water-

holding capacity, and ash content. *C. amada* with the greater swelling power of its starch, can be used in the food industry whereas starch from *C. caesia*, given its low solubility, will be useful not only in metabolic products but also in textiles because its smaller granules can penetrate fabrics easily, imparting desirable stiffness to clothes (Sajitha and Sasikumar, 2015; Jamir and Seshagirirao, 2017).

5.3.6. Total phenol

Plant phenols are the structurally diverse group of secondary metabolites synthesized as byproducts from the condensation of acetate units (e.g. terpenoids), modification of aromatic amino acids (e.g. lignin precursors, catechols and coumarins), flavonoids, isoflavonoids and tannins (Bennette and Wallsgrove, 1994). Phenols are a major group of antioxidant phytochemicals which have an important role because of their potential biological and free radical scavenging activities (Prakash *et al*., 2007). The role of plant phenolics in various biochemical responses and its antimicrobial activities are well documented (Bennette and Wallsgrove, 1994). Spices and herbs are well known as an excellent source of phenolic compounds which show good antioxidant activities (Zheng and Wang, 2001).

The study showed significant variation for total phenol content among the four species. Within the species, all the species except *C. xanthorrhiza* showed significant variation for total phenol content. This variation in phenol content is expected to owe to their variation in constituents and type of phenols present (Krishnaraj *et al*., 2012) which can greatly differ within the genotypes of same species as well as from species to species (Jang *et al*., 2007). Similarly, previous studies evidenced a wide range of variation for total phenol content in various herbs, spices and vegetables (Tangkanakul *et al*., 2009).

In the present study, the total phenol content in four *Curcuma* species ranged from 2.25 mg g^{-1} to 6.01 mg g^{-1}. The highest amount of total phenol content was detected in *C. xanthorrhiza* and lowest in *C. amada*. Total phenol content in dried turmeric varied from 1.72 to 7.46g GAE/100g as reported in previous works (Wojdylo *et al*., 2007; Surveswaran *et al*., 2007). In previous studies, the phenol content and antioxidant activities of *C. caesia* were found higher than *C. amada* (Krishnaraj *et al*., 2012; Sahu and Saxena, 2013). A good yield of total phenol content observed in *C. xanthorrhiza* may be ascribed to the presence of curcumin content in them as curcumin is a naturally occurring polyphenol known for its antioxidant activities (Rao *et al*., 1995; Lim *et al*., 2011). Previous studies have highlighted the antioxidant activities of various *Curcuma* species owing to its phenol content (Kaur and

Kapoor, 2002; Rajamma *et al*., 2012; Reenu *et al*., 2015). Higher total phenolic content attributable to higher antioxidant activities in plant extracts is reported earlier (Wong *et al*., 2006). Maizura *et al*. (2011) also reported a similar positive linear relationship between antioxidant activity and total phenol content in turmeric, ginger and kesum (*Polygonum minus* Huds.).

5.3.7. Total carbohydrates

Carbohydrates are the main source of available energy for the plants as they are utilized as a substrate for growth and development. Major storage regions of carbohydrates are generally stem bases like stolons, rhizomes and corms (White, 1973; Hongpakdee and Ruamrungsri, 2012).

Total carbohydrates varied significantly among the four species under study (58.78% - 67.30%). Among the species, *C. amada* showed highest amount of total carbohydrates followed by *C. xanthorrhiza* and lowest in *C. aromatica*. However, unlike rest of the species, accessions of *C. xanthorrhiza* did not show any significant variation for total carbohydrates. Variation in carbohydrate content among and within the plant species was found to be influenced by temperature, irrigation, growth rate, developmental stage, seasonal change and also by the balance between photosynthesis and respiration (White, 1973: Hongpakdee and Ruamrungsri, 2012; Angel *et al*., 2013; Mohamed *et al*., 2014). The above-mentioned factors might have contributed to the interspecific and intraspecific variation of total carbohydrate content in *C. amada*, *C. caesia* and *C. aromatica.*

Maciel and Criley (2002) reported the presence of high amount of carbohydrates (>70%) in *C. zedoaria.* High carbohydrate content in *Curcuma* species was borne out in the current study as well.

Although the starch content and total carbohydrates were higher in *C. amada* and *C. xanthorrhiza*, total protein and essential oil content were not high among these species. This was also reported by Netaji *et al*. (2000) and Mahesh *et al*. (2014) in maize and wheat, where the significant positive correlation was observed between grain yield and starch content and negative correlation with protein and oil content at genotypic and phenotypic level.

5.3.8. Multivariate analysis

The data matrix of eight biochemical traits was subjected to multivariate analysis using Principal component analysis (PCA) and cluster analysis. The result of the PCA study proved the existence of similarity between the accessions of *C. amada* and *C. caesia* as loading plot showed an admixture of accessions from both the species and difficult to differentiate. Krishnaraj *et al*. (2012) reported a similar level of phenolics and superoxide radical scavenging activities in *C. caesia* and *C. amada*. Closer association of *C. amada* and *C. caesia* also evident at the genetic level using RAPD, SSR and combined marker analysis. The close association of *C. amada* and *C. caesia* was observed in a phylogenetic study done by Vinitha *et al*. (2014). Unlikely clustering of *C. caesia* with morphologically different species, like *C. zedoaria* was already reported (Saha *et al*., 2016).

The PCA analysis on the individual species showed widespread dispersion among the accessions proving the intraspecific variation for quality traits in these species. This is in line with the analysis of variance wherein almost all the biochemical traits under study showed significant variation within the species. There was only close association among 2 or 3 accessions and few accessions stood out within the four species; Acc. 521 and Acc. 752 of *C. amada*; Acc. 1025, Acc. 1520 and Acc. 711 of *C. aromatica*; Acc. 292 and Acc. 751 of *C. caesia* and Acc. 1167 and Acc. 1168 of *C. xanthorrhiza* were found distinct from their counterparts.

Principal Component Analysis could successfully distinguish the genotypes from one another for various quality traits. In earlier studies, the efficiency of using PCA was highlighted as it could assess the slightest variation for the quality characters and there by discriminate the genotypes from each other based on the traits. The 2D scatter plot of first three Principal Components clearly displayed high variability for the biochemical traits within each species. The cluster analysis based on biochemical traits were in accordance with the Principal Component Analysis. Each species has one or two accessions that showed a very different grouping with similarity coefficient value as low as 0.25. Both Principal Component Analysis and Cluster analysis could not draw a location specific grouping of the accessions.

Li *et al*. (2009) utilized PCA for the essential oil quality assessment from *C. longa* collected from different geographical locations in China. PCA could detect the slight variation in the chemical components in the *C. longa* samples and three groups were formed according to

the chemical components in essential oil. Similarly, Xiang *et al*. (2011) and Lee *et al*. (2014) used PCA to investigate the difference present in the quality of essential oil and secondary metabolites (curcuminoids and terpenoids) profiling of *C. phaeocaulis, C. kwangsiensis, C. wenyujin, C. aromatica* and *C. longa* obtained from different locations and PCA could successfully separate the samples from different species and ecotypes.

The first three component of principal analysis, which explained the variability among the species, revealed that *C. xanthorrhiza* and *C. amada* as the most variable species biochemically. This was in conjunction with the morphological results. The accessions were highly dispersed with one or two accessions standing out in both the species.

5.3.9. Variability, heritability and genetic advance

In plant breeding, success of any selection is dependent on the heritable variation, which gives information about the transmission of characters from parent to progeny. Thus evaluation of heritability and effect of environment on variation in quality traits will aid in its selection. Genetic parameter study was conducted in order to select the promising accessions with desirable quality traits for documentation and conservation. In the current study, significant variation was observed for quality traits among the four species. The narrow differences between genotypic and phenotypic variation for most of the traits suggest that the environment has little effect on the expression of these characters and a chance of high selection gain (Malek *et al*., 2014).

Traits such as total phenol content, essential oil content and total curcumin content recorded high phenotypic and genotypic coefficient of variation (PCV and GCV) across all the four species. High heritability coupled with genetic advance was observed for traits like total starch, total carbohydrates, essential oil, protein, curcumin and oleoresin among four *Curcuma* species. High heritability coupled with genetic advance observed for quality traits in the present study shows that effective selection can be made for these traits (Panse and Sukhatme, 1957; Ravishanker *et al*., 2013).

High phenotypic and genotypic variation accompanied with heritability for percentage of essential oil, oleoresin and curcumin content has been reported in *C. longa* (Singh *et al*. (2003), Sinkar *et al*. (2005), Prajapati *et al*. (2014), Singh and Ramakrishna (2014) and for oleoresin content in ginger (Ravishanker *et al*., 2013).

5.3.10. Phenological variation in two species of *Curcuma* at three growth stages

In order to assess the effect of maturity on growth, yield and quality traits, a study was conducted in two economically important *Curcuma* species *viz*., *C. amada* and *C. aromatica,* at three different stages of growth *i.e*. 90,140 and 180 days after planting. The study clearly indicated that growth stages affects yield and quality parameters as significant variations was observed in growth parameters and quality traits such as starch, curcumin, crude fibre and oil content. Species x growth stage interaction was also significant in these cases. However, protein and tiller number did not show any significant variation over three growth stages and remained almost same.

As the age of the plants increased, a decrease in the number of green leaves plant-^{1} was observed. A similar observation was reported in *C. longa* (Asghari *et al*., 2009). Percentage of curcumin in *C. aromatica* and *C. amada* slightly increased with the growth stage. Which was in accordance with the results of Hanashiro *et al*. (2003) in *C. longa* where curcumin content increased with maturation of plant. Starch content in *Curcuma* species is known to vary with the location, maturity, accession etc. (Srivastava *et al*., 2007; Angel *et al*., 2008; Policegoudra *et al*., 2008; Shamrao *et al*., 2013). The relatively high starch content in the present study may be due to the genotype and the stage of maturity. A comparative analysis of starch and sugar content in *C. longa* during dormant period till sprouting indicated a rapid starch to sugar conversion, as starch content decreased the total sugar content increased as sprouting started (Panneerselvam and Jaleel, 2008). Seasonal variation for growth and yield characters and for quality traits like chlorophyll (a and b), protein, carbohydrates, phenol content were stressed in *C. longa* (Sumathi *et al*., 2008).

Though the two species exhibited uniformity for all the aerial growth attributes, yield and starch, they exhibited varied expression for curcumin, crude fibre and protein content. The study indicated the possibility of significant accumulation of photosynthates even after 140 days in both the species as evidenced by the increase in fresh yield and dry recovery. This information would be useful in devising or rescheduling the fertilizer requirement for these species (Sajitha *et al*., 2014) besides assessing the genetic variation.

5.4. Molecular characterization of four *Curcuma* species

DNA based molecular markers play an important role in the evaluation of genetic diversity since they provide credible information about polymorphism, as the genetic composition is unique for each species and is not influenced by external factors like physiological and

environmental conditions. But morphological and biochemical characters are prone to environmental changes, and thus a combination of morphological, biochemical and molecular analysis will be preferable to study the diversity in plants.

Microsatellite markers (SSR) are ideal choice for genetic diversity studies owing to their locus specificity, high polymorphism, co-dominant segregation, multiallelic nature, reproducibility and they are easy to interpret (Sorkheh *et al*., 2009) and abundant in plant genomes (Powell *et al*., 1996) but time and high cost for identifying SSR motifs as well as primer designing limited its wide spread use in plants (Beckman and Soller, 1990; Akkaya *et al*., 1992; Roder *et al*., 1995). Dominant markers like RAPD and ISSR markers are useful in detecting genetic polymorphisms among accessions by covering a wide range of sequences in the genome. Moreover, they are cost effective, simpler to use and no prior knowledge of the target regions is required (Sharma, 2008). RAPD profile usually represent a widely distributed portion of the genome, on the other hand, Inter Simple Sequence Repeats (ISSR) profile are abundant throughout the genome and show a higher level of polymorphism since they are generated from micro satellite and minisatellite rich regions of the genomes (Kojima *et al*., 1998; Joshi *et al*., 2000: Semagn *et al*., 2006).

Genetic diversity based on combined use of molecular markers is believed to cover diverse regions of the genome, both neutral and functional regions, so combined use of more than one dominant marker will be more valuable to comprehend variability in the germplasm (Jatoi *et al*., 2010). In previous studies, molecular markers like RAPD, ISSR, SSR, AFLP etc. are utilised to study the genetic diversity present at the inter and intraspecific as well as at population level (Xiao *et al*., 2000; Sreeja *et al*., 2002; Nayak *et al*., 2006; Jatoi *et al*., 2006; Angel *et al*., 2008; Donipati and Sreeramaulu, 2015).

The three DNA fingerprinting methods used in this study were compared for their efficiency to generate polymorphism among the four *Curcuma* species. Each marker used was informative with regard to the amount of polymorphism generated. Molecular markers could able to show inter and intraspecific variation in four *Curcuma* species under study. All the three markers displayed a varying level of polymorphism. Russell *et al*. (1997) also reported wide variability for polymorphism developed using RAPD, RFLP, AFLP and SSR markers in Barley, Verma and Rana (2013) using RAPD, ISSR and DAMD (Directed Amplification of Minisatellite DNA) markers in Curry leaf plant and Basak *et al*. (2017) using RAPD and ISSR markers in turmeric. Among the three markers used in the present study, SSR marker

exhibited higher polymorphism followed by RAPD and then ISSR. Russell *et al*. (1997) and Sorkheh *et al*. (2009) also reported the efficiency of SSR marker when compared to other markers. Higher polymorphism for RAPD marker than ISSR was earlier reported in *Myristica* spp. (Sheeja *et al*., 2013) and rice bean (Muthusamy *et al*., 2008). The polymorphic efficiency of RAPD markers might be attributed to its polyallelic nature (Muthusamy *et al*., 2008). Mantel test revealed a high correlation among different similarity matrices generated from all the three different molecular markers used in this study. High correlation coefficient observed between SSR and RAPD indicated the similar discriminating power of the marker among the *Curcuma* species.

In the diversity studies, for ascertaining genetic distances and genetic similarity, various kinds of similarity matching indices are used. The available similarity matching indices are, Simple Matching (Sokal and Michener, 1958), Rogers and Tanimoto (Rogers and Tanimoto, 1960), Jaccard's (Jaccard, 1901), Sorensen-Dice (Dice, 1945; Sorensen, 1948), Ochiai (Ochiai, 1957), Ochiai II (Ochiai, 1957). Even though there are various algorithms and coefficients available for estimating the genetic similarity, there needs to be some justification for preferring one coefficient over the other. Duarte *et al*. (1999) consider the use of Distortion degree, the correlation between original and estimated distances and stress value to arrive at the most preferred coefficients. Ochiai and Sorensen-Dice coefficient showed the lowest levels of stress and distortion degree followed by Ochai II, Jaccard's and Simple Matching. Sokal and Sneath (1963) suggested that coefficients should be easier to interpret and hence suggested the use of Jaccard's for assessing genetic distances. Both Sorensen-Dice and Jaccard's considers only presence of bands, however ignores the absence of bands. Simple Matching algorithms consider absence of bands for estimating genetic similarity which is vital especially when genetic dissimilarity among closely related species are estimated (Hallden *et al*, 1994). The difference between Jaccard's and Sorensen-Dice is that Sorensen-Dice gives double weightage to the presence of band whereas Jaccard's provide single weightage. Hence, all three similarity coefficients – Simple Matching (SM), Jaccard's and Sorensen-Dice are considered for analysing the result in this study.

In all the species, the dendrogram obtained using various coefficient matrices showed very high similarity. However, the dendrogram constructed using Simple Matching similarity coefficients was slightly different from the dendrogram obtained using the Jaccard's and Sorensen-Dice similarity coefficients. Syamkumar (2008) reported a similar trend for

Simple Matching similarity coefficients when 15 *Curcuma* species were characterized using RAPD markers.

In RAPD, SSR and Combined markers (RAPD+ISSR+SSR) analysis, dendrogram based on Simple Matching similarity coefficients displayed closer relationship between *C. amada* and *C. caesia*. Principal Component Analysis based on quality traits also deciphered similar relationship between *C. amada* and *C. caesia*, where accessions of both the species were seen plotted so closely. However, in the dendrogram based on Jaccard's and Sorensen-Dice, *C. aromatica* and *C. xanthorrhiza* showed proximity.

Contrary to above-mentioned results, dendrogram based on ISSR markers showed a different association among the species, a closer relationship was evident between *C. amada* and *C. xanthorrhiza* for all the three similarity coefficients. These results were not in accordance with the clustering pattern obtained from the morphological study. The close similarity indices between morphologically diverse species like *C. amada* and *C. caesia* was further confirmed by the similar results from Nei's genetic distance based dendrogram. The close association of *C. amada* and *C. caesia* was observed in a phylogenetic study done by Vinitha *et al*. (2014) using plastid DNA and ITS sequences and natural hybridization was attributed to be the reason for their close proximity. In a study, addressing the phylogenetic relationship of *C. longa* and its allied species using *Cp*DNA sequences supported the hypothesis that the evolutionary history of the genus *Curcuma* underwent recent diversification (Hayakawa *et al*., 2011).

Earlier in barley (Hou *et al*., 2005), rice bean (Muthusamy *et al*., 2008), ginger and turmeric (Mohanty *et al*., 2014) a lack of congruence were reported among the clustering pattern of RAPD, ISSR and SSR markers. The evidenced variation in the clustering pattern with respect to molecular marker used is not uncommon as different marker is scanning different region in the genome and thus provide different genetic information (Souframanien and Gopalakrishna, 2004; Jatoi *et al*., 2010; Basak *et al*., 2017).

The dendrogram clustering revealed that majority of the accessions from individual species intermingled with each other in different subclusters irrespective of their geographical affiliations. Pioneers reported similar trend, where genetic distance was independent of geographical distance in population of *C. wenyujin* collected from various parts of China (Zheng *et al*., 2015), eleven starchy *Curcuma* species collected from NBPGR (Thrissur)

(Angel *et al*., 2008) and among 19 turmeric cultivars collected from Northeast India (Basak *et al*., 2017).

Molecular marker based study could successfully confirm the intraspecific variation among the four *Curcuma* species as cluster analysis based on accessions from individual species showed two or more groupings in all the dendrogram. In all the dendrogram constructed using RAPD, ISSR, SSR and combined markers, Acc. 6390 of *C. amada*, collected from Gundimeda, Vijayawada (Andhra Pradesh) maintained a unique status, by showing genetic divergence from rest of the accessions of *C. amada*. A similar scenario was also seen in the dendrogram generated using morphological characters. This variation observed in Acc. 6390 may be attributed to their place of collection as geographically separated plants tend to adapt to their growing environment by genetically modifying themselves (Souframanien and Gopalakrishna, 2004).

Acc. 752 (Pundibari, West Bengal) and Acc. 848 (Tuidam, Mizoram) as well as Acc.753 (Pundibari, West Bengal) and Acc. 1119 (Thalapilly, Kerala) of *C. amada* always grouped together in all the dendrogram irrespective of their place of collection as well as molecular marker and similarity coefficients used. This could be due to migration of same genotypes through farmers to different states as suggested by Basak *et al*. (2017) in turmeric cultivars of Northeast India. Clustering pattern irrespective of their geographical location was reported by previous authors in blackgram (Souframanien and Gopalakrishnan, 2004), sesame (Pandey *et al*., 2015) and turmeric (Basak *et al*., 2017).

In case of *C. aromatica*, the number of clusters in various dendrogram constructed using molecular markers varied from 2 to 4 showing intraspecific variation. In all the dendrogram generated using RAPD, SSR and combined markers Acc. 1124 (Kunnathunadu, Kerala) of *C. aromatica* showed a distinct status, as it separated into a lone group. Acc. 1124 stood apart from the rest of the accessions of *C. aromatica* for a number of morphological characters and quality traits as well. The dendrogram constructed using ISSR profile has given a different scenario where Acc. 1124 found very closely related to Acc.1132 and grouped under the same cluster. Likewise, Acc. 711(Thrissur, Kerala) also exhibited a distinct status among the accessions of *C. aromatica*, as it formed a lone group in the dendrogram constructed using SSR and combined molecular marker system. Moreover, the accession also maintained its distinct status in the dendrogram constructed using morphological traits. The intraspecific variation observed in *C. aromatica* may be associated

with its mode of reproduction. The predominant mode of reproduction in *C. aromatica* is asexual and propagation occurs mainly through underground rhizomes. However, viable seed set is also reported in *C. aromatica* like in *C. longa* and the observed variation might be attributed to its true seedling variation (George, 1981; Sasikumar *et al*.,1996). By and large, the distribution of accessions did not reflect their place of collection, however Acc. 1518 and Acc. 1520 collected from Anachal and Thekkady (Idukki, Kerala) grouped together in the dendrogram constructed using ISSR and combined markers.

Clustering pattern in seven accessions of *C. caesia* showed intraspecific variation as two or more groupings were observed in dendrogram. In RAPD marker, Acc. 1135 displayed the least similarity with rest of the accessions of *C. caesia* and separated into a lone group in the dendrogram. But ISSR, SSR and combined markers deciphered a different clustering pattern where Acc. 1171 formed a lone group. Acc. 751 showed a similar trend, except in the dendrogram generated using SSR profile. Although majority of the accessions were clustered irrespective of their place of collection, two accessions *viz*., Acc.1135 and Acc. 1154 collected from Thrissur, Kerala grouped together in the dendrogram generated using ISSR and SSR profiles. The distinct clustering of above-mentioned accessions was further supported by Principal Component Analysis of quality traits. The differences in clustering pattern may be due to the underlying fact that different marker amplifies different part of the genome or accessions show variation in its genetic makeup (Basak *et al*., 2017).
The observed intraspecific variation may be associated with cultivation and targeted genotype selection of desirable characters in *C. amada, C. caesia* and *C. aromatica* as these species are economically important.

Clustering pattern of accessions of *C. xanthorrhiza* was almost similar in all the dendrogram irrespective of the molecular markers used. Acc. 465 collected from Jorhat (Assam), Acc. 1108 collected from Parambikulam (Kerala) and Acc.1168 collected from Nilambur (Malappuram, Kerala) showed the least similarity with rest of the accessions of *C. xanthorrhiza*, and they formed a separate group of their own in almost all the dendrogram.

The accessions belonging to the same geographical location did not always occupy the same cluster. Lack of location specificity in the clustering was also reported earlier in *C. longa* (Gupta *et al*., 2016). Molecular marker based dendrogram was quite different from the one constructed using morphological and biochemical traits. Morphologically similar accessions like Acc.1108 and Acc. 1122 (Parambikulam, Palakkad, Kerala) were found genetically

distinct from each other. A similar incongruence between morphological and molecular marker based clustering pattern was earlier reported (Shegro *et al*., 2013; Biabani *et al*., 2013). Moreover, all genetic differentiation need not result in morphological differentiation (Siva and Krishnamurthy, 2005). The morphological similarity might be attributed to the environmental conditions of same geographical location from which accessions were collected.

In genetic diversity analysis based on Nei's genetic distance, majority of the marker deciphered a higher level of polymorphism in *C. amada*. This variation might have resulted from the cultivation and distribution of *C. amada* genotypes to a diverse geographical location through farmers which in turn compelled them to adapt to the new growing environment by making changes at the genetic level (Souframanien and Gopalakrishnan, 2004). Another probable reason for the higher polymorphism might be attributable to the diverse sampling location of *C. amada* compared to rest of the species. Contrast to this, species like *C. xanthorrhiza* growing in wild conditions are less prone to human interference thus giving a meagre scope of genetic variation among them. Similar results were reported in curry leaf plant, where cultivated accessions have a slightly higher variation than wild plants (Verma and Rana, 2013).

Analysis of Molecular Variance revealed that majority of the polymorphism was present among the species than within the species. SSR marker was able to reveal maximum variation present within the species than any other molecular marker. The efficiency of SSR marker to study diversity in closely related individuals and its high polymorphic nature is already reported by various authors (Sorkheh *et al*., 2009; Sigrist *et al*., 2011; Siju *et al*., 2010; Senan *et al*., 2013).

The present study revealed no significant correlation between genetic distance geographical distance, which was consistent with previous study reported in *C. wenyujin* (Zheng *et al*., 2015). The reason may be the rapid exchange of germplasm within the country. In general, the study indicated that morphological, biochemical and genetic diversity did not corroborate completely with each other in the cluster analysis. Zhang *et al*. (2012) revealed that molecular based genetic diversity may not able to fully explain the morphological and biochemical diversity observed in a plant, and thus a combination of these data matrices will be a preferable choice.

In light of the results from present and previous study, it can be concluded that the cluster analysis and Principal Component Analysis could successfully disclose inter and intraspecific diversity in four species with equal effectiveness. The observed intraspecific variation in four *Curcuma* species at morphological, biochemical and molecular level may be contributed by several factors; like genetic variation, including DNA mutation and chromosomal variation (particularly polyploidization), environmental factors, including phenotypic plasticity of the species and germplasm exchange, as geographically separated plants tend to adapt to their growing environment by genetically modifying themselves. The observed variability within the four *Curcuma* species can be utilized for selecting high yielding plants with desirable traits.

5.5. Population Diversity studies in four *Curcuma* species

A population diversity study was initiated with an aim to assess the level of genetic diversity and relationship among different population of four *Curcuma* species acquired from various parts of India. In order to achieve a comprehensive analysis of genetic diversity, RAPD, ISSR and SSR profiles were pooled together to generate a cumulative dendrogram based on Nei's genetic distance.

The dendrogram based on Nei's genetic distance separated four populations of *C. amada* into two clusters. Population from south and western part of India formed the first cluster and population from Northeast and east formed the second cluster. Nei's genetic diversity indices showed a higher polymorphism in the population 2 which was from Northeast part of India. This could be probably because of the high altitude combined with larger geographic distances between the two collection sites and highly varying climatic conditions among the north eastern regions. Islam *et al*. (2007) also reported higher polymorphism in *C. zedoaria* collected from hilly population than plain and plateau land population. The lowest genetic divergence was among the Population 4 which had accessions from East India which are geographically closer. Low genetic divergence may have ascribed to rapid germplasm movement across the states by the farmers/settlers which resulted in the wide spread distribution of same germplasm in various parts of India in course of time. Nm value of 0.49 further indicates the occurrence of gene flow between *C. amada* accessions albeit very limited. Basak *et al*. (2017) also reported similar gene flow among the populations of *C. longa* collected from four northeast states of India.

AMOVA couldn't generate a significant variation among and within the population, the reason may be the low sample size in each population.

Among the three population of *C. aromatica*, collected from various parts of Kerala, Population 2 showed highest genetic diversity. The probable reason may be the wide range of collection areas involved (Ernakulam, Pathanamthitta and Tirunelveli). Population 3, comprising closely located regions of Idukki showed lower genetic diversity. Islam *et al*. (2007) also stressed genetic similarity between closely located hilly land population of *C. zedoaria.* The possible reason for this phenomenon might be that hilly population are encompassing geographically similar and ecologically undisturbed conditions when compared to low land population which are constantly subjected to anthropogenic interference. The dendrogram showed closer association with population 1 collected from Thrissur region and population 2 collected from Pathanamthitta, Ernakulam and Tirunelveli. This could be because of the climatic/regional condition since these regions fall under mid land region compared to high altitude and dense forests of Idukki.

The moderate genetic differentiation and gene flow found in *C. aromatica* populations may be attributed to its both vegetative reproduction and open pollinating nature. In *C. aromatica*, the variation within the population was found to be higher (85%) than among population (15%). The observed variation might be attributed to its seed setting nature (George, 1981). Hamrick and Loveless (1989) also mentioned a common trend in tropical plants of maintaining high genetic diversity within the populations. Paisooksantivatana *et al*. (2001) reported high intrapopulation variation in *C. alismatifolia* collected from Thailand using allozyme polymorphism. Similarly, Islam *et al*. (2005) also revealed the presence of high intrapopulation genetic diversity in *C. zedoaria* than interpopulation diversity.

Among the three populations of *C. caesia*, higher genetic diversity was detected in population 3 collected from Meghalaya and West Bengal regions. The probable reason for the high genetic diversity may be the larger geographic distances between the two collection sites in population 3, *i.e*. Meghalaya and West Bengal. The north-eastern regions of India represent a geographically diverse area with varying altitude and temperature. A vast genetic diversity is believed to exist among the indigenous *Curcuma* species in these areas (Roy *et al*., 2011).

Moreover, these areas are coming under hilly regions and thus our results are in congruence with earlier findings which suggested that hilly population possess high genetic diversity than plain and plateau land population (Paisooksantivatana *et al*., 2001a; Islam *et al*., 2007). The lowest genetic variation was seen in Population 1, which was collected from Arunachal Pradesh. Basak *et al*. (2017) also reported low genetic diversity in the population of *C. longa* from Arunachal Pradesh compared to Assam, Meghalaya and Manipur. The low genetic differentiation and high gene flow observed in *C. caesia* population may be associated to closely placed collection sites as majority of the accessions were obtained from North eastern part of India which enabled the exchange or transfer of same plant material between the state/population. Although Nei's genetic diversity revealed higher intrapopulation variation than interpopulation variation, AMOVA studies couldn't support this significant variation which may be due to small population size.

Among the three population of *C. xanthorrhiza*, population collected from Palakkad and Kollam exhibited higher genetic diversity than rest of the population which may be due to their large geographical distance when compared to rest of the population which were collected from nearby places. The dendrogram showed very close association with population 2 (Palakkad and Kollam) and population 3 collected from Wayanad and Malappuram region owing to their low geographical distance. Paisooksantivatana *et al*. (2001a) also reported genetic similarity between closely placed high land population of *C. alismatifolia* from Thailand using allozymes. AMOVA analysis didn't show any significant variation among and within the population.

The lack of sufficient sample size in each population acted as a limiting factor for the proper evaluation of intra and interpopulation variation in all the four *Curcuma* species assessed. May be in future, a study involving more sample size in each population may shed some light towards the population diversity among these species.

A few important findings have been emerged from the present study. Despite being vegetatively propagated, four *Curcuma* species showed wide range of variation not only at the interspecific level but also at the intraspecific level for morphological, biochemical and molecular parameters as evidenced from various analysis. The accessions of different *Curcuma* species under study clustered into different groups, irrespective of their geographical affiliations except a few accessions, suggesting considerable intraspecific diversity amongst the genotypes. Some of the variation observed in morphological

characters were associated with its phenotypic plasticity as those variations were failed to express at the genetic level. Such phenotypic variation was already reported in other *Curcuma* species. The observed intraspecific variation in four *Curcuma* species at morphological, biochemical and molecular level may be contributed by genetic variation, environmental factors and germplasm exchange, as geographically separated plants tend to adapt to their growing environment by genetically modifying themselves.

Among the four species *C. amada* showed high genetic diversity compared to rest of the species as revealed from Nei's genetic diversity index (h), Shannon's information index (I) and percentage of polymorphism. *C. amada* also showed high intraspecific variability both in morphological traits and biochemical traits compared to *C. caesia* and *C. aromatica*. Unlike *C. longa* and *C. aromatica* no seed set was reported so far in *C. amada* (Sabu, 2006) and thus the intraspecific diversity observed in *C. amada* may be associated with genetic factors due to the rapid evolution of non-coding regions as suggested in previous studies (Nayak *et al*., 2006; Ahmad *et al*., 2009; Jatoi *et al*., 2010). Breeding and propagation system of *Curcuma* species is a major area of concern and need to be explored in depth as many species have undergone complex evolutionary and adaptive changes. According to early reports, higher polyploids reproduce exclusively vegetatively than lower polyploids (Zaveska *et al*., 2011) and higher genetic variation observed in the clonal species like *C. amada* with 2n=42, may have resulted from occasional sexual reproduction which need confirmation. It has been well established that, only by generating genetic variation any given plant species will be able to survive in a changing environment for a long term. Likewise, in *Curcuma* species, the long history of cultivation in wide range of geographical locations and climatic conditions might have expedited the micro-evolutionary process in these *Curcuma* species and resulted in the changes in the genetic level especially in *C. amada*, the main cultivated species among the four.

Some of the accessions in the present study were more diverse, may be due to differences in origin or ecotype. The divergent accessions such as Acc.6390 of *C. amada*, Acc.1124 and Acc.711 of *C. aromatica*, Acc.751 and Acc.1171 of *C. caesia* and Acc.1168, Acc.1108 and Acc.465 of *C. xanthorrhiza* should be further confirmed at the genomic sequence level and using intergenic spacers of chloroplast DNA as well as for any variation in the chromosomal level to get a clear picture. Though the number of populations used in the present study doesn't represent the total population variation in these species, we could provide an insight

on the genetic diversity on the population from the four species. Among the four species, *C. amada* populations found more diverse than rest of the species. Population study with more sample size might provide a better knowledge about the genetic diversity. The diversity observed in the *C. amada* populations may have attributed to its long term cultivation and distribution into diverse geographical places.

The biochemical characterization of four *Curcuma* species proved that these species can serve as promising alternative starch source for various industrial purpose not only because they offer many desirable physiochemical properties but also because they are easy to cultivate, widely adapted and resilient to adverse climatic conditions. *C. amada* with favourable starch properties like high solubility and swelling power, can find a place in food industries. Whereas starch from *C. caesia*, given its low solubility and small granule size, will be useful in metabolic products and textile industries. The species/accessions can also be utilized for bioprospecting economically/pharmacologically important phytoconstituents like essential oil, oleoresin, phenol, starch etc.

Phenological variation study in *C. amada* and *C. aromatica* at three growth stages showed significant variation in growth, yield and quality traits as the plant matured. The study indicated significant accumulation of photosynthates even after 140 days. The information would be useful for various crop improvement strategies such as devising or rescheduling the fertilizer requirement for these species besides assessing the genetic variation.
Estimates of genetic parameters in four *Curcuma* species showed possibility of effective selection for a few morphological characters and quality traits which showed high heritability and genetic advance.

The present study gives an insight about the broad genetic structure of four *Curcuma* species. The study also highlighted the feasibility of using molecular marker system along with morphological and biochemical parameters for assessing the genetic diversity. Among the three marker system tested, SSR marker proved to be more advantageous in predicting the intraspecific variation. The information generated has a pivotal role in the successful utilization and conservation of these economically important *Curcuma* genetic resources since each of them have significant role in traditional system of medicine, food, cosmetics and other uses.

Chapter 6

Summary and Conclusion

Zingiberaceae is an important family in the plant kingdom and many members of the family are used in fresh and processed form for medicinal, ornamental, aromatic and aesthetic purposes. *Curcuma* is an important genus of family *Zingiberaceae*, composed of 70-80 species of annual or perennial herbs. The genus consists of economically and medicinally important species, characterized by volatile oils and oleoresins, well known for their broad range of pharmacological and culinary uses.

Genus *Curcuma* is reported to display diversity in habitat, morphology, biochemical and ethno-medicinal use. Intraspecific variation for various taxonomically important characters and biochemical traits has already been reported. The intraspecific variation observed in the *Curcuma* species was believed to be influenced by both genetic and non-genetic factors. Diversity studies are often carried out in *Curcuma* species to assess relationship and genetic variability among germplasm in order to conserve the genetic resources and for crop improvement. The classical approaches for analyzing genetic diversity includes morphology, comparative anatomy, physiology, embryology etc. that have been often complemented with molecular marker based studies.

Curcuma species such as *C. amada*, *C. aromatica, C. caesia* and *C. xanthorrhiza* are reported to have multifaceted properties including ethno-botanical values in traditional systems of medicines, cosmetics and culinary uses. These species are rich source of bioactive secondary metabolites which are responsible for its pharmacological uses. Previous studies have revealed intraspecific variation in morphological characters and biochemical traits in these underutilized economically important species.

The present study was an attempt to evaluate the genetic diversity present among the four economically important *Curcuma* species (*C. amada, C. aromatica, C. caesia* and *C. xanthorrhiza*) using morphological, biochemical and molecular parameters with special reference to intraspecific diversity. To understand the diversity in different population, accessions from the four species were grouped into different population and studied.

In order to assess the effect of maturity on growth, yield and quality traits, a phenological study in *C. amada* and *C. aromatica* at three growth stages has also been carried out. Besides biochemical characterization of primary and secondary metabolites, qualitative analysis of starch from four *Curcuma* species has also been carried out with special reference to starch yield and their structural characteristics and some physicochemical properties to bring out the potential use of *Curcuma* species as an alternative starch source.

6.1. Morphological characterization of four *Curcuma* species

The experiments were conducted at the Experimental farm of the ICAR-Indian Institute of Spices Research, Peruvanamuzhi, Kozhikode, Kerala during 2012-2015. Thirty-two accessions belonging to four *Curcuma* species *viz*., *C. amada*, *C. aromatica*, *C. caesia* and *C. xanthorrhiza*, which were collected and conserved in the previous months, were planted at the Experimental farm and used in the study. The experiment was laid out in Completely Randomized Design (CRD). All the observations were recorded from three random plants per plot.

A total of twenty-four morphological characters including eight qualitative and sixteen quantitative characters were used to study the variation present in the four *Curcuma* species. Aerial morphological characterization was carried out on fully grown plants (180-200 days after planting) and rhizome characters were recorded after the harvest. Morphological data were analysed using one-way ANOVA using SPSS and XLSTAT software. Associations among accessions and species were investigated by the Principal Component Analysis (PCA) and cluster analysis. The genetic parameters were calculated by using standard formula.

Four *Curcuma* species exhibited significant variation for morphological characters. While quantitative morphological characters showed both inter and intraspecific variation, the qualitative characters did not exhibit intraspecific variations except for pseudostem colour variation; Acc.751 and Acc.1001 of *C. caesia* showed intraspecific variation for pseudostem colour. These two accessions showed a purple tinge on their pesudostem, whereas rest of the accessions of *C. caesia* were devoid of purple tinge.

A strikingly high intraspecific variation observed in quantitative morphological characters was governed by both genetic and non-genetic factors. For the aerial morphological characters, major source of variation was from within the species source. For half of the

rhizome characters, within species source contributed maximum variability while for other half, the major source of variation resulted from variability among the species.
Quantitative morphological characters were subjected to Principal Component Analysis and cluster analysis. The loading plot and dendrogram revealed a close association between *C. amada* and *C. aromatica*. *C. amada* and *C. aromatica* were almost similar for visual aerial morphological characters.

The cluster analysis and Principal Component Analysis revealed prominent intraspecific variation in morphological characters in almost all the species. In each individual species, some accessions showed a strikingly high variation. Acc. 6390 (Vijayawada, Andhra Pradesh) of *C. amada*, Acc. 1124 (Ernakulam, Kerala) and Acc. 711(Thrissur, Kerala) of *C. aromatica,* Acc.1001(Lohit, Arunachal Pradesh) and Acc. 1154 (Thrissur, Kerala) of *C. caesia* and Acc.1168 (Malappuram, Kerala) and Acc.760 (Kalpetta, Kerala) of *C. xanthorrhiza* maintained a unique status from its counterparts.

By and large none of the accessions from the four *Curcuma* species showed any location specificity in the dendrogram except a few accessions. Accessions like Acc.752 & Acc.753 (Pundibari, West Bengal) of *C. amada*; Acc.1518 & Acc.1520 (Anachal & Thekkady of Kerala) of *C. aromatica*; Acc. 1108 & Acc. 1122 (Parambikulam, Kerala) and Acc.1164 and Acc.1167 (Nilambur, Malappuram, Kerala) of *C. xanthorrhiza* showed a closer association with each other for morphological characters based on their place of collection.
The estimate of genetic parameters among the accessions of the four species revealed that the Phenotypic Coefficient of Variation (PCV) was more or less same or a little bit higher than Genotypic Coefficient of Variation (GCV) for morphological characters. The characters like plant height, number of tillers per clump, number of leaves per tillers, rhizome weight, number and weight of mother, primary, secondary rhizomes etc. which exhibit high heritability, as well as genetic advance, indicating the scope of selection in improving these traits.

6.2. Biochemical Characterization of four *Curcuma* species

Eight biochemical characters were considered to study the inter and intraspecific variation present among the four *Curcuma* species. Biochemical characterization was carried out using standard protocols and analysed using one-way ANOVA and data profiles were subjected to Principal Component Analysis (PCA) and cluster analysis. Analysis of variance revealed significant variation among the four species for quality traits studied except for total

protein content. The study showed significant intraspecific variation for almost all quality traits except for one or two traits in each species. The observed variation was believed to be attributed to both genetic and non-genetic factors.

Accessions of *C. aromatica* recorded highest amount of oleoresin (10.68%) and essential oil (5.01%) followed by *C. xanthorrhiza* (9.29%, 3.16%, respectively) and lowest percentage of oleoresin was present in *C. caesia* (6.92%) and essential oil in *C. amada* (2.37%). Presence of high amount of essential oil and oleoresin in *C. aromatica* may be the reason behind their high camphoraceous aroma of the rhizomes and thus grabbed an important place in the aromatherapy and perfumery. Percentage of curcumin, total phenol and total curcuminoids was found maximum in the *C. xanthorrhiza*. Low percentage of curcumin, phenol and total carbohydrates were found in *C. caesia*, *C. amada* and *C. aromatica* respectively. Starch and total carbohydrate content was found maximum in the accessions of *C. amada*.

Significant inter and intraspecific variation was observed among the four *Curcuma* species for essential oil content and its constituents. GC-MS analysis of essential oil identified a total of 30 compounds in four *Curcuma* species and some of the volatile oil constituents were unique to certain *Curcuma* species studied. Beta-ocimene was specific to *C. amada*, highest amount of curdione was present in the species *C. aromatica*, 2-nonanol, β-caryophyllene, epicurzerenone, isocurcumenol were found in *C. caesia*, xanthorrhizol was recorded in *C. xanthorrhiza*.

HPLC analysis was done to separate out and measure total curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) present in the accessions of four *Curcuma* species. Different types and composition of mobile phase were tested and desired resolution of curcuminoids peaks was obtained by using Acetonitrile and 0.1% orthophosphoric acid (60:40 and 1ml minute^{1}). Although we could distinguish three different compounds of total curcuminoids successfully, amount of total curcuminoids present in *C. amada*, *C. aromatica* and *C. caesia* was very low and *C. xanthorrhiza* was the only species with appreciable amount of total curcuminoids showing considerable variation.

Starch granules extracted from four *Curcuma* species were subjected to quality analysis and size and shape of the starch granules were assessed with the help of Scanning Electron Microscopy. Although the four *Curcuma* species did not differ significantly in terms of

starch yield and moisture content, they did show qualitative differences for solubility, swelling power, water-holding capacity, and ash content. *C. amada* topped in terms of both solubility and swelling power. The starch granules of the four *Curcuma* species varied greatly in shape and size. *C. aromatica* granules were the largest, showed surface ornamentation, and were different from the granules of the rest of the species in having concentric rings.

The inter and intraspecific variation for quality traits were further confirmed in multivariate analysis. The result of the Principal Component Analysis proved the existence of similarity between the accessions of *C. amada* and *C. caesia* as loading plot showed an admixture of accessions from both the species whereas accessions of *C. xanthorrhiza* and *C. aromatica* grouped separately and showed clear differentiation from the rest of the species. Acc. 521 and Acc. 752 of *C. amada*; Acc. 1025, Acc. 1520 and Acc. 711 of *C. aromatica*; Acc. 292 and Acc. 751 of *C. caesia* and Acc. 1167 and Acc. 1168 of *C. xanthorrhiza* were found distinct from their counterparts. Both Principal Component Analysis and Cluster analysis could not draw a location specific grouping of the accessions.

The estimate of genetic parameters showed a narrow difference between genotypic and phenotypic variation for most of the traits. The total carbohydrate and total starch content had very little genotypic and phenotypic coefficient variations. Traits such as total phenol content, essential oil content and total curcumin content recorded high phenotypic and genotypic coefficient of variation (PCV and GCV) across all the four species. High heritability coupled with genetic advance was observed for traits like total starch, total carbohydrates, essential oil, protein, curcumin and oleoresin among the four *Curcuma* species.

6.3. Phenological variation in two species of *Curcuma* at three growth stages

In order to assess the effect of maturity on growth, yield and quality traits, a study was conducted in two economically important *Curcuma* species *viz*., *C. amada* and *C. aromatica,* at three different stages of growth *i.e*. 90,140 and 180 days after planting. The study revealed significant variations for the parameters such as plant height, yield plant^{-1}, dry recovery and for the biochemical characters such as starch, curcumin, crude fiber and oil content. However, protein and tiller number did not show any significant variation over three growth stages and remained almost same. The two species differed significantly for plant height, dry recovery, yield, oil, curcumin and starch between the species as well as among the

growth stages. Species x growth stage interaction was also significant in these cases. Tiller number and protein content did not vary with the growth stages in both the species. Considerable accumulation of photosynthates even after 140DAP in both the species was evidenced by the increase in fresh yield and dry recovery. The study implied the need for standardizing the growth stage for sampling of the species to assess the intra and interspecific variation.

6.4. Molecular characterization of four *Curcuma* species

High quality of genomic DNA was isolated from fresh leaves of four *Curcuma* species and their accessions using modified CTAB protocol. The yield of the DNA ranged from 108µg g^{-1}(Acc.265 of *C. amada*) to 210 µg g^{-1} (Acc.1168 of *C. xanthorrhiza*).

Two dominant molecular markers (RAPD and ISSR) and one codominant molecular marker (SSR) were used for the molecular characterization of four *Curcuma* species. Simple Matching similarity coefficient was used for constructing dendrogram for individual species whereas Simple Matching, Jaccard's and Sorensen-Dice were used to construct dendrogram of four species combined. Nei's genetic distance based dendrogram was also used to further confirm the data.

Twenty-six RAPD Primers yielded a total of 994 bands, out of which 648 were polymorphic. The percentage of polymorphic bands ranged from 37% (OPA 19) to 86% (OPA5) with an average percentage of polymorphism of 65%. Nei's genetic variance showed 75.07% of variation in *C. caesia* followed by *C. aromatica* (67.37%) and least in *C. xanthorrhiza* (54.65%). Nei's genetic distance and Simple matching similarity based dendrogram showed closer association of *C. amada* and *C. caesia* whereas Jaccard's and Sorensen-Dice showed similarity between *C. aromatica* and *C. xanthorrhiza*. Prominent intraspecific variation was observed in RAPD analysis as accessions from different species formed two or more than two groups in each dendrogram. Acc.6390 of *C. amada*, Acc.1124 of *C. aromatica*, Acc.1135 of *C. caesia* and Acc.1168, Acc.1108 and Acc.465 of *C. xanthorrhiza* showed a distinct status from their counterparts as they formed lone group in the dendrogram.

Twenty-one ISSR primers resulted in the amplification of 784 bands of which 440 were polymorphic bands. The percentage of polymorphism ranged from 26% (ISSR 12) to 72% (ISSR 8) with an average value of 55%. Nei's genetic variance showed 64.54% of variation in *C. amada*, followed by *C. caesia* (60.46%) and least in *C. aromatica* (44.50%).

Dendrogram based on Nei's genetic distance and cluster analysis showed closer association of *C. amada* and *C. xanthorrhiza*. Dendrogram generated using ISSR profiles formed three to four groups in cluster analysis showing intraspecific variation. Among the individual species, Acc.6390 of *C. amada*, Acc. 751 and Acc.1171 of *C. caesia*, Acc.465 and Acc.1108 of *C. xanthorrhiza* maintained a unique status in the dendrogram. Acc.1518 and Acc.1520 of *C. aromatica* which was collected from Idukki district formed a separate group in the dendrogram. Acc.1124, which showed distinct status in RAPD analysis showed a closer association with Acc.1132 in ISSR analysis. Acc. 1135 and Acc. 1154 which were collected from Thrissur (Kerala) showed maximum similarity. Eight accessions of *C. xanthorrhiza* were clustered in to three groups. First and third group were formed by one accession each; Acc. 465 and Acc. 1108, respectively. Acc.1164 and Acc.1167 collected from Nilambur (Kerala) were closely related to each other. Nei's genetic distance and UPGMA based dendrogram showed closer association of *C. amada* and *C. xanthorrhiza.*

Twenty-three SSR markers resulted in the amplification of 484 bands of which 362 were polymorphic bands. The bands amplified were in the range of 90 bp to 300 bp. The percentage of polymorphic bands ranged from 48% (CuMiSat 16) to 100% (CuMiSat 17) with an average percentage of polymorphism of 75%. Estimates of Nei's genetic distance based genetic variance showed 82.39% of polymorphism in *C. amada* followed by *C. aromatica* (78.98%) and least in *C. xanthorrhiza* (62.91%).

In the dendrogram generated using SSR marker, *C. amada* showed close affinity with *C. caesia*. Among the individual species, ten accessions of *C. amada* were clustered in to four groups. The first and fourth groups were formed by single accessions – Acc. 265 and Acc. 6390, respectively. Maximum similarity was between Acc.752 and Acc.848. Seven accessions of *C. aromatica* were clustered in to four groups. First, third and fourth groups were formed by single accessions; Acc. 711, Acc.1520 and Acc.1124 respectively. Seven accessions of *C. caesia* were clustered in to four groups. The second and fourth groups were formed by single accessions each, Acc. 1006 and Acc.1171 respectively. Maximum similarity was found between Acc.1135 and Acc.1154, which were collected from Thrissur (Kerala). Eight accessions of *C. xanthorrhiza* were separated in to four groups. Acc. 465, Acc.1168 and Acc. 1108 showed distinct status in the dendrogram.

Combined molecular markers (RAPD+ISSR+SSR) generated a total of 2262 bands, out of which 1450 bands were polymorphic in nature with an average polymorphic percentage of

65%. Dendrogram constructed using simple matching similarity matrix and Nei's genetic distance showed a closer relationship of *C. amada* and *C. caesia* whereas in the dendrogram constructed using Jaccard's and Sorensen-Dice similarity, *C. aromatica* and *C. xanthorrhiza* showed a closer relationship. Combined molecular marker also revealed distinct status of Acc. 6390 of *C. amada*, Acc.711 and Acc.1124 of *C. aromatica*, Acc.1171 and Acc. 751 of *C. caesia* and Acc.1168, Acc.1108 and Acc.465 of *C. xanthorrhiza* in the dendrogram. Acc.6390, Acc.1124, Acc.711 and Acc.1168 also showed distinct profile for morphological characters as well. Although Acc.1001 and Acc.1154 of *C. caesia* exhibited distinct morphological profile from their counterparts, they failed to exhibit the distinction in their molecular profile. Acc.751 of *C. caesia*, which showed distinct status in molecular markers analysis also exhibited unique purple pseudostem colour. The divergent accessions such as Acc.6390 of *C. amada*, Acc.1124 and Acc.711 of *C. aromatica*, Acc.751 *C. caesia* and Acc.1168 of *C. xanthorrhiza* which showed distinct status in morphological, biochemical and molecular parameters, can be considered as a possible subspecies.

6.5. Population Diversity studies in four *Curcuma* species

A population diversity study was initiated with an aim to assess the level of genetic diversity and relationship among different population of four *Curcuma* species acquired from various parts of India. In order to achieve a comprehensive analysis of genetic diversity RAPD, ISSR and SSR profiles were pooled together to generate a cumulative dendrogram based on Nei's genetic distance. The dendrogram based on Nei's genetic distance separated four populations of *C. amada* in to two clusters. Population from south and western part of India were closely related and formed the first cluster. Population from Northeast and east formed the second cluster. Nei's genetic diversity indices showed a higher polymorphism in population 2 which was from Northeast part of India. However, AMOVA couldn't generate a significant variation among and within the population, the reason may be the low sample size in each population.

In *C. aromatica*, the dendrogram showed closer association of population 1 collected from Thrissur region and population 2 collected from Pathanamthitta, Ernakulam and Tirunelveli. The lower genetic diversity was observed in the population consisting closely located accessions from Idukki. The intrapopulation (85%) variation was found to be higher than interpopulation (15%) variation. The observed variation might be attributed to seed setting nature of *C. aromatica* besides other genetic and non-genetic factors.

Among the three populations of *C. caesia*, higher genetic diversity was detected in population 3 collected from Meghalaya and West Bengal regions, followed by population 2 which consists of accessions from the Southern region. The lowest genetic variation was seen in Population 1, which were collected from Arunachal Pradesh. The low genetic differentiation observed in *C. caesia* population may be related to closely placed collection sites as majority of the accessions were obtained from North eastern part of India which enabled exchange or transfer of same plant material of the same state/population. Although Nei's genetic diversity revealed higher intra-population variation than inter-population variation, AMOVA studies couldn't support this significant variation.

Among the three population of *C. xanthorrhiza*, population 2 collected from Palakkad and Kollam exhibited higher genetic diversity than rest of the population. The dendrogram showed very close association between population 2 (Palakkad and Kollam) and population 3 collected from Wayanad and Malappuram region owing to their low geographical distance. Population 1 (Assam) was distantly related to population 2 and population 3. Nei's genetic diversity indices detected higher genetic differentiation among the populations. AMOVA analysis didn't show any significant variation among and within the population.

In nutshell, following conclusions could be drawn from the present study: -

1. Despite being vegetatively propagated, four *Curcuma* species showed wide range of variation not only at the interspecific level but also at the intraspecific level for morphological, biochemical and molecular parameters. Two accessions of *C. caesia*, Acc.751 and Acc.1001 showed purple tinge on their pesudostem. Among these two accessions, Acc.751 exhibited distinct status in molecular analysis as well.
2. Among the four species, *C. amada* showed high genetic diversity than rest of the species which may be attributed to its long term cultivation and distribution to wide range of geographical conditions which enabled them to adapt to the growing environment by genetically modifying themselves. *C. amada* and *C. aromatica* showed closer association in morphological characters whereas for biochemical and molecular parameters, *C. amada* showed close affinity with *C. caesia*.
3. Some of the accessions in each *Curcuma* species found more diverse than their counterparts. The divergent accessions such as Acc.6390 of *C. amada*, Acc.1124 and Acc.711 of *C. aromatica*, Acc.751 and Acc.1171 of *C. caesia* and Acc.1168, Acc.1108 and Acc.465 of *C. xanthorrhiza* should be further confirmed at the

genomic sequence level and for any variation at the chromosomal level to get a clear picture of their status.

4. The four *Curcuma* species appear to be promising unconventional alternative starch source for commercial purpose not only because they offer many desirable physiochemical properties but also because they are easy to cultivate, widely adapted and resilient to adverse climatic conditions. *C. amada* with favourable starch properties like high solubility and swelling power, can find a place in food industries. Whereas starch from *C. caesia*, given its low solubility and small granule size, will be useful in metabolic products and textile industries.
5. The four *Curcuma* species can be utilized for bioprospecting economically / pharmacologically important phytoconstituents like essential oil, oleoresin, phenol, starch etc.
6. Phenological studies revealed possible accumulation of photosynthates even after 140 days in *C. amada* and *C. aromatica*. The information can be useful in devising / rescheduling the fertilizer requirement for these *Curcuma* species.
7. The lack of sufficient sample size in each population acted as a limiting factor for the proper evaluation of intra and inter-population variation in all the four *Curcuma* species. May be in future, a study involving more sample size in each population may shed some light towards the population diversity among these species.
8. High heritability coupled with genetic advance was observed for morphological characters like plant height, number of tillers per clump, number of leaves per tillers, rhizome weight, number and weight of mother, primary, secondary rhizomes and quality traits like total starch, total carbohydrates, essential oil, protein, curcumin and oleoresin in four *Curcuma* species. Effective selection can be made for these characters.
9. The study indicated the feasibility of using molecular markers along with morphological and biochemical parameters for assessing the genetic diversity. Among the three marker system tested, SSR marker proved to be more advantageous in predicting the intraspecific variation.
10. The information on genetic variability generated in present study can be utilized to select superior genotypes from the germplasm collections of these underutilized economically important *Curcuma* species.

Chapter 7

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ANNEXURE

Annexure 1. Reagent preparation for manual DNA isolation protocol

Sl. No	Reagents	Preparation protocol
1	1M Tris (pH 8)	Weighed and dissolved 121.1 g of Tris base (Sigma, USA) to 800ml of double distilled water and adjusted the pH to 8, using conc. HCl. The total volume was made up to 1000ml after adjusting the pH. The solution was sterilised by autoclaving.
2	0.5 M EDTA (pH 8)	186.1 g of Ethylene diamine tetra acetate. $2H_2O$ (Sigma, USA) was weighed and dissolved into 800ml of distilled water. A magnetic stirrer can be used intermediately for dissolving the salt. NaOH pellets were used to adjust the pH to 8.0, and the final volume was made up to 1000ml with double distilled water. The solution was sterilised by autoclaving.
3	5M NaCl	292.2g of NaCl (Sigma, USA) was weighed and dissolved into 800ml of double distilled water. The final volume was adjusted to 1000ml after the salt completely dissolved. The solution was sterilised by autoclaving.
4	Extraction buffer/ CTAB buffer	100mM Tris HCl :100ml 20mm EDTA: 40ml 1.5M NaCl: 300ml 3% CTAB: 30g
5	Chloroform: isoamyl alcohol (24:1)	96ml of chloroform (Merck, India) was mixed with 4ml of isoamyl alcohol (Merck, India). The solution was stored in reagent bottles at 4℃.
6	Phenol: Chloroform: isoamyl alcohol (25:24:1).	Mixed 25ml of Tris saturated phenol (pH>6.8) (Merck, India) with 25ml of Chloroform: isoamyl alcohol (24:1). The solution was stored in brown coloured reagent bottles at 4℃.
7	1% PVP (Poly Vinyl Pyrrolidone)	1g of PVP was dissolved in 100ml of autoclaved double distilled water
8	70% ethanol (Sigma, USA)	70ml of 100% absolute ethanol was mixed with 30ml of autoclaved double distilled water.
9	β- mercaptoethanol (Sigma, USA)	0.3% β- mercaptoethanol was freshly added to the extraction buffer
10	RNase A (Genei, Bangalore, India)	Ready to use RNase A (10mg/ml) were used.

Annexure 2. Reagents used for electrophoresis

Sl. No	Reagents	Preparation protocol
1	10X TBE buffer (Tris borate buffer)	Dissolved 108g of Tris base (Sigma, USA), 55g of boric acid (Himedia, India) in 800ml of distilled water. 40ml of 0.5 M EDTA (pH-8) (Sigma, USA) was added to it. The final volume was made up to 1000ml. A working stock of 1X TBE buffer (1000ml) was made by mixing 100ml of 10X TBE with 900ml of water.
2	0.8% agarose	0.8g of agarose (Merck, India) was dissolved in 100ml of TBE buffer
3	6X gel loading buffer	Dissolved 25g of Bromophenol blue (Himedia, India) and 25 mg Xylene Cynol FF(Himedia, India) in 74ml of water and mixed with 26ml of 87% Glycerol (Himedia, India) using a magnetic stirrer. The solution was stored at 4°C.
4	Ethidium bromide	1g of ethidium bromide (Himedia, India) was added to 100ml of distilled water and stirred using a magnetic stirrer. The solution was stored in dark reagent bottle at room temperature.
5	PAGE gel loading buffer	98 % formamide 10 mM EDTA (pH 8.0) 0.05 % xylene cyanol 0.05% bromophenol blue
6	Urea-Acrylamide mix – 500 ml	40 % acrylamide solution 22.5 ml 10 X TBE 6ml Urea 25.2. g All the above components were mixed, dissolved and made up to 500 ml using distilled water. The solution was filter sterilized and stored at 4 ^{0}C in amber coloured bottle.
7	10 % APS	To 1 g of ammonium per sulphate, 1 ml of distilled water was added, mixed and stored at 4 ^{0}C.

Annexure 3. Reagents used for silver staining

Sl. No	Reagents	Preparation protocol
1	Fixing solution (18-25 ^{o}C) Glacial acetic acid (CH_3COOH)	Glacial acetic acid (CH_3COOH) (Himedia, India) was dissolved to 7.5% (v/v) with deionized water.
2	Formaldehyde solution (18-25 ^{o}C)	25 ml of formaldehyde (Sigma, USA) was dissolved in 75 ml deionized water
3	Impregnating solution (18-25 ^{o}C) Silver nitrate ($AgNO_3$)	0.1 g silver nitrate ($AgNO_3$) (Sigma, USA) dissolved in deionized water
4	Developer solution (8 ^{o}C) Sodium carbonate (Na_2CO_3)	3 g of Sodium carbonate (Na_2CO_3) dissolved in 100 ml deionized water.
5	Developer stop solution (4 ^{o}C) Glacial acetic acid (CH_3COOH)	Glacial acetic acid was diluted to 7.5% with deionized water.