Studies on floral biology, variability, divergence and adaptability of vanilla

Thesis submitted in part fulfillment of requirements for the Degree of Doctor of Philosophy in Botany of the University of Calicut

By

R.UMA MAHESWARI

GENETICS AND PLANT BREEDING DIVISION
DEPARTMENT OF BOTANY
UNIVERSITY OF CALICUT
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UNIVERSITY OF CALICUT

GENETICS AND PLANT BREEDING DIVISION

DEPARTMENT OF BOTANY

Calicut University (P.O.), Kerala- 673 635, India

Phone: 04942401144*406 Fax: 04942400269 Email: drkvmohanan@rediffmail.com

Dr.K.V.Mohanan
Reader & Research Guide

CERTIFICATE

Certified that this thesis entitled "**Studies on floral biology, variability, divergence and adaptability of vanilla"** embodies the results of a piece of bona fide research work carried out as part fulfillment of requirements for the degree of Doctor of Philosophy in Botany of the University of Calicut by Ms. R. Uma Maheswari under my guidance and supervision and that no part of the thesis has been submitted for any other degree.

I further certify that such helps or sources of information availed of in this connection have been duly acknowledged.

Calicut University (Dr.K.V.MOHANAN)

14 July 2008

DECLARATION

I, R. Uma Maheswari, hereby declare that this thesis entitled "**Studies on floral biology, variability, divergence and adaptability of vanilla"** being submitted in partial fulfillment of the requirements for the Degree of Ph.D. in Botany of University of Calicut embodies the results of a bona fide work done by me under the guidance of Dr.K.V.Mohanan, Reader & Research Guide, Genetics and Plant Breeding Division, Department of Botany, University of Calicut and that no part of it has been submitted for any other degree.

Calicut University R. UMA MAHESWARI

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PREFACE

Natural vanillin is a unique flavouring agent used world wide in food, beverage and pharmaceutical industries. It is obtained from the cured beans of the cultivated species of the orchid genus *Vanilla*. Among the three species cultivated, *Vanilla planifolia*, known as Mexican vanilla is the most popular. The other two species are *Vanilla tahitensis* and *Vanilla pompona*, the former known as Tahitian vanilla and the latter as West Indian vanilla. *Vanilla planifolia* is the only species cultivated in India and a few vines introduced for the purpose served as the source material for the entire vanilla cultivation in the country and as a result the genetic base of the crop is very narrow in India. The clonal nature of propagation of the crop is another reason for its narrow genetic base. *Vanilla tahitensis* is available in the germplasm of some national research institutes of the country.

Very often the differentiation between *Vanilla planifolia* and *Vanilla tahitensis* in the vegetative phase is difficult. Investigations carried out on *Vanilla planifolia* with a crop improvement perspective are only very few and systematic efforts are necessary to analyze the extent of variability in the field and also to partition its environmental and genetic components. The present experiments have been designed and carried out to study the floral biology and interspecific variability of *Vanilla planifolia* and *Vanilla tahitensis*, *ex situ* and *in situ* variability of *Vanilla planifolia* and also the interrelationship and association of characters and genetic divergence in *Vanilla planifolia*. An effort has also been made to study the adaptability of *Vanilla planifolia* to the different vanilla growing areas of Kerala State of India, since it is a source of supplementary income to the poor and marginal farmers of the areas in spite of the unexpected and undesirable fluctuations in the price of the crop.

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Chapter I

INTRODUCTION

Vanilla flavour is highly preferred all over the world in food and beverage industry. Vanilla fragrance is highly valued in perfumery. Vanillin (4-hydroxy 3-methoxy benzaldehyde) is the major chemical responsible for vanilla flavour and fragrance. Natural vanillin is preferred over synthetic vanillin eventhough it is highly expensive. Compounds like vanillic acid, vanillyl alcohol, para hydroxy benzaldehyde, para hydroxy benzoic acid, etc. also contribute towards the uniqueness of natural vanilla flavour. Only a very small percentage of the vanillin consumed annually is obtained from natural sources (Ramawat and Merillon, 1999). The remainder is produced synthetically mostly from petrochemicals and rarely from lignin (Clark, 1990). However, flavouring agents and food additives from natural sources are nowadays becoming popular rapidly due to the increasing awareness on health hazards caused by synthetic food additives.

Natural vanillin is obtained from three cutivated species of the orchid genus *Vanilla* namely *Vanilla planifolia* Andrews, *Vanilla tahitensis* J.W.Moore and *Vanilla pompona* Schiede. Of these, *Vanilla planifolia* is the most preferred and widely cultivated (Purseglove *et al*., 1981).

The vanilla plant is essentially monopodial in habit and the growth of the plant is accomplished by elongation from the apical meristem and the growth is indeterminate. With its vining habit and production of one root from each node it can climb many feet to the top of support plants (Withner *et al*., 1974).

Vanilla planifolia is native to the humid tropical rain forests of South Eastern Mexico, Central America, the West Indies and the northern part of South America. *Vanilla tahitensis* is indigenous to Tahiti, the French Oceania group of islands in the Pacific Ocean and *Vanilla pompona* is indigenous to South Eastern Mexico, Central America, Trinidad and North and South America (Correll, 1944; Purseglove *et al*., 1981).

In India only *Vanilla planifolia* is cultivated commercially. *Vanilla tahitensis* and *Vanilla pompona* are available in the germplasm repositories of the national institutes carrying out spices research in the country (Ravindran, 1999; Kuruvilla *et al*., 2004; Bhat and Sudharshan, 2004).

Attempts to introduce vanilla to India dated back to 1835 (Correll, 1953). However commercial cultivation of vanilla in India started by the end of the 19th century (Anonymous, 1992). The genetic base of vanilla in India is very narrow since vanilla cultivation most probably started in the country using very few cuttings introduced for the purpose (Anonymous, 2000).

Natural vanillin is obtained from the processed beans obtained from the commercial crop. Vanilla vines usually come to flower 4-5 years after planting. Inflorescences are axillary and they usually produce 20-25 flowers (Kuruvilla *et al*., 1996). Artificial pollination is necessary for fruit setting in the cultivated field. The ovaries of pollinated flowers develop into fruits and the fruits mature by about nine months. At maturity the beans are harvested and processed to convert the precursors of vanillin present in them to vanillin (Purseglove *et al*., 1981).

Different methods of processing (curing) of vanilla beans are used in different vanilla growing areas. However, the bourbon method of curing is the most popular (Krishnakumar *et al*., 2003).

Being an introduced and clonally propagated crop the genetic base of vanilla in India is very narrow (Anonymous, 2000). Preliminary investigations on exploration, collection and conservation of *Vanilla planifolia* in India have indicated the need of systematic efforts to assess the variability of vanilla both at *in situ* and *ex situ* levels and also efforts to study agronomical characters of the crop based on a crop improvement perspective. The objectives of the present experiment have been formulated from the above situation that demand studies that lead to the improvement of the planting materials that are made available to the farmers and also studies on the crop for further investigations on its genetics and genetic improvement.

The major objectives envisaged presently include study of the floral biology of two cultivated species of vanilla; study of interspecific variability between them; study of field level variability of *Vanilla planifolia*, the species commercially cultivated in India; study of its genetic variability, interrelationship of characters, character association and genetic divergence and also a study on the adaptability of *Vanilla planifolia* to different geographical regions of Kerala State of India.

The dawn of the twenty first century witnessed rapid popularization of vanilla in South India, especially in the State of Kerala. Even though unpredictable fluctuations in the price of the crop have recently resulted in adding fuel to the anxiety in the minds of farmers, the role of the crop in providing supplementary income especially to the small

and marginal farmers cannot be neglected. Ensuring the availability of consistently good yielding planting material is of utmost importance and hence efforts have been made presently to screen the available germplasm so as to identify superior genotypes.

Chapter II

REVIEW OF LITERATURE

2.1. The genus *Vanilla*

The genus *Vanilla* Mill (Plum. Ex.L) belongs to the orchid family, Orchidaceae, which is the largest family of flowering plants, with about 700 genera and 20,000 species. Orchidaceae comprises of a very natural, distinctive and highly advanced group of monocotyledons. They are perennial herbs which are widely distributed throughout the world with the greatest number in the tropics. They exhibit a wide range of life forms and have terrestrial, climbing, epiphytic and saprophytic species (Purseglove *et al*., 1981).

The genus *Vanilla* Mill. was first described by Miller in 1754 taking the name from the Spanish *vainilla* (small pod) in reference to the long, slender, pod like fruit. The generic name is often ascribed to O. Swartz who described it in 1799 (Anonymous, 2006).

The members of the genus are stout, terrestrial, climbing, branched herbs; branches giving rise to adventitious roots; leafy or leafless. Leaves when present are coriaceous or fleshy. Racemes usually axillary, sub sessile or peduncled. Flowers are large, sepals and petals sub equal, spreading. Lip is adnate by a claw to the base of the column and embracing it in its concave limb, entire or 3-lobed. Column is elongate. Anthers are incumbent, cells separate and pollen granular. Capsules are long, fleshy and one celled (Fischer, 1928).

The genus *Vanilla*, which is primitive in several characters like nature of the pollen, structure of the seed coat etc., has terminal unlimited

growth (Abraham and Vatsala, 1981). Withner *et al*. (1974) pointed out that evolutionary development of the vining habit of *Vanilla* perhaps depended up on the greater efficiency for water conduction by vessel elements as compared to the basic tracheids. Recent studies have indicated that interspecific hybridization and polyploidization might have played an important role in the evolution of the genus. Mating system diversity exists in *Vanilla* and this genus could be a good model to study the role of fragrance in orchid evolution (Bory *et al*., 2008).

2.2. Species of *Vanilla*

2.2.1. Cultivated species of *Vanilla*

The genus *Vanilla* consists of about 110 species. Three species of *Vanilla* namely *Vanilla planifolia* Andrews, *Vanilla tahitensis* J.W.Moore and *Vanilla pompona* Schiede are commercially exploited and cultivated. Of these, *Vanilla planifolia* is the most preferred commercially and hence widely cultivated and the two other species are occasionally cultivated and yield an inferior product (Purseglove *et al*., 1981).

2.2.1.1. *Vanilla planifolia*

Vanilla planifolia Andrews is a herbaceous perennial vine, climbing up trees or other supports to a height of 10-15 m by means of adventitious roots. In cultivation it is trained to a height which will facilitate hand pollination and harvesting. Long, whitish, aerial adventitious roots of about 2 mm in diameter are produced singly opposite the leaves and adhere firmly to the support plant. The roots at the base ramify in the humus or mulch layer. The stem is long, cylindrical, succulent and branched. It is 1-2 cm in diameter and is dark green and photosynthetic with stomata. The internodes are 5-15 cm in length. Leaves are large, flat, fleshy, sub sessile, alternate, oblong- elliptic to lanceolate.

They are 8-25 cm long and 2-8 cm broad. The tip is acute to acuminate and the base somewhat rounded. Venation is parallel and the veins are distinct. The petiole is thick, short and canalized above (Purseglove *et al*., 1981).

Flowers are large, waxy, fragrant, pale greenish yellow, bisexual and zygomorphic. Perianth lobes are six in number (3+3) and they look alike. The lower petal of the inner whorl is short, broad and it is modified into a labellum. The lower part of the labellum envelops a central structure called the 'column' (gynostemium) (Purseglove *et al*., 1981). Gynostemium is formed by the union of stamen, style and stigma (Lawrence, 1951). A tuft of hairs is seen in the middle of the disc. The tip of the column bears a single stamen with two pollen masses (pollinia) covered by a cap or hood like structure called 'rostellum' which prevents natural pollination. The slender stalk like portion is the ovary, which is 4-7 cm in length and 3-5 mm in diameter. The fruit is a capsule, which is dehiscent in *Vanilla planifolia* and in trade it is known as a bean. The bean is pendulous, narrowly cylindrical, obscurely three angled, 10-25 cm long and 5-15 mm in diameter. Each bean when ripe contains thousands of minute globose seeds, which are liberated by longitudinal splitting of the capsule (Purseglove *et al*., 1981). The product from *Vanilla planifolia* is known as Mexican vanilla, Bourbon vanilla or Indonesian vanilla based on the method of processing (George, 1989).

2.2.1.2. *Vanilla tahitensis*

Vanilla tahitensis J.W. Moore, Tahitian vanilla, is indigenous to Tahiti (the French Oceania group of Islands in the Pacific Ocean) and is cultivated in Hawaii also. The species is less robust than *Vanilla planifolia* with more slender stem and narrow leaves, which are12-14 cm long and

2.5-3 cm wide with longer perianth segments and a lip that is shorter, with beans 12-14 cm long and about 9-10 mm in width, broad in the middle and tapering towards the end (Purseglove *et al*., 1981).

2.2.1.3. *Vanilla pompona*

Vanilla pompona Schiede, the West Indian vanilla occurs wild in South Eastern Mexico, Central America, Trinidad, North America and South America. It is cultivated to a small extent in Guadeloupe Martinique and Dominica. It resembles *Vanilla planifolia*, except for that the leaves are larger and about 15-28 cm long and 4-11.5 cm wide. The flowers are greenish yellow and larger as well as more fleshy and the lip has a tuft of imbricated scales instead of hairs in the centre of the disc. The beans are three angled and fleshier. They show little tendency to split at maturity (Purseglove *et al*., 1981).

2.2.2. Other species of *Vanilla*

Wikipedia has listed 112 species of *Vanilla* (Table 2.1.). Their geographical distribution has also been mentioned.

Table 2.1. List of species of the genus *Vanilla* (Anonymous, 2008a).

1	*Vanilla abundiflora* (Borneo)
2	*Vanilla acuminata* (Gabon)
3	*Vanilla acuta* (N. South America)
4	*Vanilla africana* (W. & WC. Trop. Africa)
5	*Vanilla albida* (Taiwan, Indo-China to Malesia)
6	*Vanilla andamanica* (Andaman Is.)
7	*Vanilla angustipetala* (Brazil - São Paulo)
8	*Vanilla annamica* (SC. China to Vietnam)
9	*Vanilla aphylla* (Assam to Java)
10	*Vanilla appendiculata* (Guyana)
11	*Vanilla bahiana* (Brazil - Bahia)
12	*Vanilla bakeri* (Cuba)
13	*Vanilla bampsiana* (C. Zaire)

14	*Vanilla barbellata* (S. Florida to Caribbean)
15	*Vanilla barrereana* (French Guiana)
16	*Vanilla bertoniensis* (Paraguay)
17	*Vanilla bicolor* (Caribbean to N. & W. South America)
18	*Vanilla borneensis* (Borneo)
19	*Vanilla bradei* (Brazil - São Paulo)
20	*Vanilla calopogon* (Philippines - Luzon)
21	*Vanilla calyculata* (Colombia)
22	*Vanilla carinata* (Brazil)
23	*Vanilla chalottii* (Gabon)
24	*Vanilla chamissonis* (French Guiana to NE. Argentina)
25	*Vanilla claviculata* (Caribbean)
26	*Vanilla columbiana* (Colombia)
27	*Vanilla correllii* (Bahamas)
28	*Vanilla coursii* (Madagascar)
29	*Vanilla cristagalli* (N. Brazil)
30	*Vanilla cristatocallosa* (Guyana to N. Brazil)
31	*Vanilla cucullata* (Cameroon)
32	*Vanilla decaryana* (SW. Madagascar)
33	*Vanilla denticulata* (Brazil)
34	*Vanilla diabolica* (Indonesia - Sulawesi)
35	*Vanilla dilloniana* (S. Florida to Caribbean)
36	*Vanilla dubia* (Brazil - Minas Gerais)
37	*Vanilla duckei* (Brazil)
38	*Vanilla dungsii* (Brazil)
39	*Vanilla edwallii* (Brazil to Argentina)
40	*Vanilla fimbriata* (Guyana)
41	*Vanilla francoisii* (NE. Madagascar)
42	*Vanilla gardneri* (Brazil)
43	*Vanilla giulianettii* (New Guinea)
44	*Vanilla grandiflora* (S. Trop. America)
45	*Vanilla grandifolia* (Príncipe to Zaire)
46	*Vanilla griffithii* (W. Malaysia)
47	*Vanilla hallei* (Gabon)
48	*Vanilla hamata* (Peru)
49	*Vanilla hartii* (C. America)
50	*Vanilla havilandii* (Borneo)
51	*Vanilla helleri* (C. America)
52	*Vanilla heterolopha* (Gabon)
53	*Vanilla hostmannii* (Suriname)
54	*Vanilla humblotii* (Comoros)
55	*Vanilla imperialis* (W. Trop. Africa to Tanzania and Angola)

56	*Vanilla inodora* (Mexico to C. America)
57	*Vanilla insignis* (Honduras)
58	*Vanilla kaniensis* (New Guinea)
59	*Vanilla kempteriana* (New Guinea)
60	*Vanilla kinabaluensis* (Pen. Malaysia to Borneo)
61	*Vanilla latisegmenta* (Guyana)
62	*Vanilla leprieurii* (French Guiana)
63	*Vanilla lindmaniana* (Brazil - Mato Grosso)
64	*Vanilla madagascariensis* (N. & NW. Madagascar)
65	*Vanilla marowynensis* (Suriname)
66	*Vanilla methonica* (Colombia)
67	*Vanilla mexicana* (S. Florida, Mexico to Trop. America)
68	*Vanilla moonii* (Sri Lanka)
69	*Vanilla nigerica* (S. Nigeria to Cameroon)
70	*Vanilla ochyrae* (Cameroon)
71	*Vanilla odorata* (S. Mexico to Trop. America)
72	*Vanilla organensis* (Brazil - Rio de Janeiro)
73	*Vanilla ovalis* (Philippines)
74	*Vanilla ovata* (French Guiana)
75	*Vanilla palembanica* (Sumatra)
76	*Vanilla palmarum* (Cuba, S. Trop. America)
77	*Vanilla parvifolia* (S. Brazil to Paraguay)
78	*Vanilla penicillata* (Venezuela)
79	*Vanilla perexilis* (Paraguay)
80	*Vanilla perrieri* (NW. Madagascar)
81	*Vanilla phaeantha* (S. Florida to Caribbean, C. America)
82	*Vanilla phalaenopsis* (Seychelles)
83	*Vanilla pierrei* (Indo-China)
84	*Vanilla pilifera* (Assam to Borneo)
85	*Vanilla planifolia* Jacks. ex Andrews 1808 (S. Florida to Caribbean, Mexico to Paraguay)
86	*Vanilla platyphylla* (Indonesia - Sulawesi)
87	*Vanilla poitaei* (Caribbean)
88	*Vanilla pompona* Schiede (Caribbean)
89	*Vanilla polylepis* (Kenya to S. Trop. Africa)
90	*Vanilla porteresiana* (French Guiana)
91	*Vanilla purusara* (Brazil)
92	*Vanilla ramosa* (Ghana to Tanzania)
93	*Vanilla ribeiroi* (Brazil - Mato Grosso)
94	*Vanilla rojasiana* (Paraguay to NE. Argentina)
95	*Vanilla roscheri* (Ethiopia to NE. KwaZulu-Natal)
96	*Vanilla ruiziana* (Peru)

97	*Vanilla schwackeana* (Brazil - Minas Gerais)
98	*Vanilla seranica* (Maluku - Seram)
99	*Vanilla seretii* (WC. Trop. Africa)
100	*Vanilla siamensis* (China - S. Yunnan to Thailand)
101	*Vanilla sprucei* (Colombia)
102	*Vanilla sumatrana* (Sumatra)
103	*Vanilla surinamensis* (Suriname)
104	*Vanilla tahitiensis* (Tahiti)
105	*Vanilla trigonocarpa* (Costa Rica to N. Brazil.)
106	*Vanilla uncinata* (N. Brazil)
107	*Vanilla utteridgei* (W. New Guinea)
108	*Vanilla vellozii* (Brazil)
109	*Vanilla walkeriae* (S. India, Sri Lanka)
110	*Vanilla wariensis* (New Guinea)
111	*Vanilla weberbaueriana* (Peru)
112	*Vanilla wightii* (SW. India)

2.2.3. Indian species

Efforts have been made by different workers to explore *Vanilla* species from wild habitats of India. Hooker (1894) and Fischer (1928) reported two species of vanilla growing in South India namely *Vanilla walkeriae* Wt. and *Vanilla wightiana* Lindl. According to Borthakur and Hajra (1976), four species namely *Vanilla walkeriae* Wt., *Vanilla wightii* Lindl., *Vanilla andamanica* Rolfe and *Vanilla pilifera* Holtt. are present in India in the wild. Karthikeyan *et al*. (1989), in the Flora of India reported five species of *Vanilla* from India – *Vanilla andamanica* Rolfe, *Vanilla parishii* Reichb.f., *Vanilla pilifera* Holtt., *Vanilla walkeriae* Wt. and *Vanilla wightiana* Lindl.

Sathish kumar and Manilal (1993) reported *Vanilla aphylla* Blume from India. They have further indicated that the report of *Vanilla parishii* Reichb.f from India was also a misidentification of *Vanilla aphylla* Blume. However, as per Sathish kumar and Manilal (2004) the above report of *Vanilla aphylla* Blume by them itself was a misidentification of *Vanilla*

wightiana Lindl. Rasingam *et al*. (2007) reported a new species of *Vanilla* from the Andaman Islands of India. As per the above references, six species of *Vanilla* have been reported from India in the wild (Table 2.2). Sathish kumar and Manilal (2004) have reported two species of *Vanilla* from Kerala namely *Vanilla walkeriae* Wt. and *Vanilla wightiana* Lindl.

Table 2.2. Species of *Vanilla* reported from India

Sl. No.	Name of the species	Description	Reported by
1	*Vanilla walkeriae* Wt.	Stem very thick; internodes are 3-4 inches long; abortive leaves lanceolate, acuminate, 0.5-1.5 inches long; bracts ovate, acute, 0.25-0.4 inches long; flowers 2 inches long; sepals and petals oblongate- lanceolate, subobtuse, petals slightly the wider, undulate, lip entire, ovate-oblong, subacute, undulate, disk with 2 median puberulous lines (Fischer, 1928).	Hooker, 1894; Fischer 1928; Borthakur and Hajra, 1976; Sathish kumar and Manilal, 2004.
2	*Vanilla wightii* Lindl.	Could not be traced.	Borthakur and Hajra, 1976.
3	*Vanilla andamanica* Rolfe.	Scandent herbs with petiolate leaves. Leaves oblong lanceolate, acuminate, subcoriaceous, 14-19 cm long, 2.5-4 cm broad; petioles 1-1.5 cm long; racemes axillary, 2-3 cm long, multiflorous, bracts ovate-oblongate, obtuse, 4-5 mm long; pedicels 2 cm long. Flowers 5 cm long; sepals oblong-lanceolate, subobtuse, 5 cm long; petals oblanceolate- elliptoid, obtuse, 5 cm long. Labellum 5 cm long; column 3.5 cm long (Rolfe, 1918).	Borthakur and Hajra, 1976; Karthikeyan *et al*., 1989.
4	*Vanilla*	Terrestrial root- climbing leafy	Borthakur

	pilifera Holtt.	herb attaining a considerable height depending upon the supporting trees. Stem branched, terete, channeled, fleshy, green, 0.4-0.5 cm thick; internodes 5-14 cm long; leaves alternate, coriaceous, subsessile, elliptic, acuminate, dark green, many nerved, 6-14 cm x 0.8-2.5 cm; inflorescence an axillary raceme, 5-20 flowered, 3-5 cm long; bracts green, persistent, ovate, obtuse, clawed, 3-7 mm x 2-5 mm, pedicellate ovary, white, 2-3 mm thick, 5-7 cm long; flowers fragrant, green with pinkish white lip, sepals and petals subequal, 2.5-3.0 cm from dorsal sepal to lip; sepals pale green, subequal, lanceolate; apex obtuse; margin slightly reflexed inward, coriaceous; dorsal sepal 2.9-3 cm x 0.9-1.1 cm, 12-15 veined, laterals 2.9- 3.0 cm x 0.8 – 1.3 cm, 10-12 veined; lip white with pale pink veins on the upper surface, 2.5-3.0 cm x 2.7-3.2 cm, 3 lobed; side lobes overlapping and enfolding the end of the column; mid lobe with thin undulate edges and the mid part bearing erect hairs; mid- line of the lip raised above, grooved below, bearing opposite the anther a tuft of fine hairs directed towards the base of the lip; column white, jointed ¾ of its length with the claws of the lip, 0.9- 2.1 cm long; operculum pale yellow, 3 mm x 3 mm. (Borthakur and Hajra, 1976).	and Hajra, 1976; Karthikeyan *et al*., 1989.
5	*Vanilla wightiana*	Stem thick; internode 2-4 inches long; leafless; bracts broadly	Hooker 1894;

	Lindl.	ovate, subacute, about 0.2 inches long; flowers 1 inch or less long; sepals and petals oblong-lanceolate, subacute, lip 3- lobed, side lobes broad, rounded, midlobe rotund- ovate, subacute, disk with a median hirsute line and two densely retrorsely barbate crests (Fischer, 1928).	Fischer 1928; Karthikeyan *et al*., 1989; Sathish kumar and Manilal, 2004.
6	*Vanilla sanjappae* R.P.Pandey, J.J.Wood & K.K.Srivast.	Climber. Stems fleshy, terete, internodes 8 cm long, rooting at every node, glabrous green. Leaves 12-26 cm x 4-7.5 cm, blade oblong- elliptic to obovate, rounde at the base, apex obtusely acuminate, glabrous, greenish brown; petiole 0.5-1 cm long, channeled above. Inflorescences axillary, racemose, many-flowered, flowers opening successively from the base upwards; rachis upto 12 cm long, fractiflex, dark green; floral bracts 5 mm x 3 mm, broadly ovate to triangular, acute. Flower buds 2 cm x 0.8 cm, obovoid, dark green above, yellow below. Mature flowers articulate at the base, opening widely at first, later half closed, sepals and petals yellow flushed green, lip yellow with 5 pinkish lines on the inner surface of the side lobes. Pedicel with ovary up to 4.5 cm long, with numerous glandular thickenings, greenish yellow. Sepals 4 cm x 1.2 cm, oblanceolate, concave, acute, 12-nerved. Petals 4 cm x 1.6 cm, spathulate, gradually narrowed toward the base, concave, sub acute, 12 nerved. Lip forming a small 1.8 cm long narrow tube,	Rasingam *et al*., 2007.

		blade 3.7 cm long, 3 cm wide across the side lobes, distally 3-lobed; disc with a long- hairy median brush; side lobes broadly rounded, margin uneven, fused with the column for about three quarters of its length, glabrous, mid lobe oblong- triangular, acute, margin undulate, covered with dense long hairs upto 5 mm long arranged in a median band and continuing to the median brush, without a glabrous area in between. Column 2.5 cm long, slender, with two thickened and sparsely hairy apical lines; anther cap cucullate, base retuse. Young fruit capsules upto 11.2 cm x 0.5 cm, linear, fleshy (Rasingam *et al*., 2007.)	

2.3. Origin and history

Member of the genus *Vanilla* are found both in old and new worlds (Abraham and Vatsala, 1981). *Vanilla planifolia* Andrews [syn. *V. fragrans* (Salisb.) Ames] is native to the humid tropical rain forests of South Eastern Mexico, Central America, the West Indies and northern part of South America (Madhusoodanan *et al*., 2003). *Vanilla pompona* Schiede occurs wild in South Eastern Mexico, Central America, Trinidad and North and South America. *Vanilla tahitensis* J. W. Moore is indigenous to the French Oceania group of islands known as Tahitian islands (Purseglove *et al*., 1981).

It is not known with certainty how the vanilla bean was discovered as a flavouring agent or how the techniques for processing vanilla were developed. But several tribes living in the South Eastern Mexico might have discovered the use of vanilla at least 1000 years ago. Its use by the

Aztec tribe of Mexico was recorded by the Spanish conquistadors (Purseglove *et al*., 1981).

Bernel Diaz, a Spanish officer under Hernando Cortes, the Spanish conqueror of Mexico was perhaps the first white man to take note of this spice when he observed Montezuma, the intrepid Aztec emperor, drinking "chocolatl", a beverage prepared from pulverized seeds of the cacao tree, flavoured with ground vanilla beans which the Aztecs call "tlilxochitl", derived from "tlili", meaning "black" and "xochitl" interpreted here as meaning "pod". Vanilla beans were considered to be among the rarer tributes paid to the Aztec emperor by his subject tribes. Legend has it that Cortes in 1520 was given chocolate flavoured with vanilla by Montezuma, served in golden goblets. Hugh Morgan, apothecary to Queen Elizabeth I of England, suggested that vanilla could be used alone as flavouring. The Franciscon monk Bernardino de Sahagun, a Spanish missionary to Mexico is the first to document vanilla and its use for the preparation of cocoa beverage in his scripts in 1560. The first observation of botanical interest however was made by Carolus Clusius in 1605 who gave the name *Lobus oblongus aromaticus*. Since then several botanists attempted to describe the plant, give a botanical nomenclature and to record the methods of cultivation and curing the produce (Correll, 1953).

According to Correll (1953), attempts to introduce vanilla cultivation to India dated back to 1835; however, the effort was not successful. This crop was successfully introduced to India by the end of the 19th century (Anonymous, 1982). Documentary evidences show that the first vanilla farm of India was established at Kallar-Burliar Fruit Research Station, The Nilgiris which was established around 1945.

Vanilla cultivation was started in Kerala State of India by 1960 in and around Ambalavayal of Wayanad District (Anonymous, 1992).

2.4. Taxonomy

The detailed description of plants including orchids given by the German fathers of Botany like Otto Brunfels (1489-1534) and Leonhart Fuchs (1501-1566) provided materials for the later Botanists to work upon. The Swedish Botanist Linne (1707-1778) while classifying the plant kingdom included orchids under his group XX- Gynandria and Diandria. He described 8 genera and 69 species. Swartz (1766-1818) who later came to be honoured as the first orchidologist described 25 orchid genera under two divisions: i) orchids with one anther, ii) orchids with two anthers, thus giving shape to the great divisions of orchids recognized today as Monandrae and Diandrae. The Scottish Botanist Brown (1773-1858) clarified the comparative morphology of the orchid flower and established the principles of orchid systematics upon which the later orchidologists built the superstructure of their systems. The works of Blume (1825) on the flora of Java, Bateman (1837-1843) on the orchids of Mexico and Guatemala, Ruiz and Pavon (1797) on the flora of Peru and Chile and Thouars (1822) on the orchids of Africa were among the first of such works. The work of Lindley (1830-1840), 'Genera and Species of Orchidaceous Plants' embodies the results of years of study of orchids world over. The system of classification of Orchidaceae published in his work '*Folia Orchidacea*' (1852-1859) provides the first comprehensive classification of Orchidaceae (Abraham and Vatsala, 1981) (Table 2.3).

Table 2.3. The family Orchidaceae with details of Division Monandrae [as published in Folia Orchidacea by Lindley (1852-1859)].

Family Orchidaceae	
1. Division Monandrae	2. Division Diandrae

Tribe 1. Malaxideae: No caudicle or separable stigmatic gland.	Tribe 1. Cypripedieae: Ovary one or three-celled
Tribe 2. Epidendreae: A distinct caudicle but no separable stigmatic gland.	Tribe 2. Apostasieae: Ovary three-celled.
Tribe 3. Vandeae: A distinct caudicle attached to a deciduous stigmatic gland.	
Tribe 4. Ophrydeae: Anther terminal, erect or incumbent, adnate to the top of the column.	
Tribe 5. Arethuseae: Anther terminal, operculate.	
Tribe 6. Neottieae: Anther dorsal.	

For more than 30 years till his death in 1865 Lindley was the undisputed authority of orchids. Lindley (1830-1840) described 299 genera of orchids. Reichenbach (1824-1889) amassed a wealth of material and information on orchids and published it in the form of synopsis in *Annales Botanices Systematicae,* during the years 1861 to 1866 (Abraham and Vatsala, 1981).

Bentham and Hooker (1884) in *'Genera Plantarum'* recognized 5 tribes in the family Orchidaceae in the place of the 8 in Lindley's classification merging Malaxidae with Epidendreae, Arethuseae with Neottieae and Apostasieae with Cypripedieae (Table 2.4). They included 334 genera of orchids in their system.

Table 2.4. Classification of the family Orchidaceae as per Bentham and Hooker (1884)

Family Orchidaceae

Tribe 1. Epidendreae	9 sub tribes	Anther one, deciduous. Pollinia waxy, 1-4 in number, held together at the base by a viscid appendage. Not attached by a caudicle to the rostellum.
Tribe 2. Vandeae	8 sub tribes	Pollinia waxy, 2 or 4 in super-imposed pairs, attached to a gland or part of the rostellum which is carried away when pollinia are removed.
Tribe 3. Neottieae	6 sub tribes	Stem not bulbous. Anther erect or posticuous and persistent or opercular. Pollen granular, powdery or in small masses.
Tribe 4. Ophrydeae	4 sub tribes	Anther erect. Pollinia granular, produced into caudicles attached to a gland or to the rostellum. Terrestrial.
Tribe 5. Cypripedieae	3 genera	

Pfitzer (1887) treated the orders in the reverse order and gave as much importance to the vegetative characters as to the floral characters. He described 410 genera of orchids (Table 2.5).

Table 2.5. Classification of the family Orchidaceae as per Pfitzer (1887)

<table>
<tr><td colspan="4">Family Orchidaceae</td></tr>
<tr><td>1. Diandrae: Two fertile anthers</td><td colspan="3">2. Monandrae: One fertile anther</td></tr>
<tr><td rowspan="2">Cypripediloideae</td><td rowspan="2">Basitonae – Anther persistent. Pollinia developing caudicles towards the base of the anther.</td><td colspan="2">Acrotonae– Anther deciduous. Pollinia without appendage or developing it towards the top of the anther.</td></tr>
<tr><td>Acranthae– Inflorescence terminal.</td><td>Pleuranthae– Inflorescence lateral.</td></tr>
</table>

Schletcher (1926a) classified Orchidaceae into 2 sub families Diandrae and Monandrae. The sub family Diandrae included one tribe Cypripediloideae. The sub family Monandrae had two divisions Basitonae and Acrotonae (Table 2.6).

Table 2.6. Classification of the family Orchidaceae as per Schletcher (1926a)

<table>
<tr><td colspan="3">Family Orchidaceae</td></tr>
<tr><td>Sub family: Diandrae</td><td colspan="2">Sub family: Monandrae</td></tr>
<tr><td rowspan="3">Tribe 1. Cypripediloideae.</td><td>Division 1. Basitonae- Caudicle and viscid disc arising from the base of the anther</td><td>Division 2. Acrotonae- Caudicles and viscid disc aising from the top of the anther</td></tr>
<tr><td rowspan="2">Tribe 1. Ophrydoideae</td><td>Tribe 1. Polychondreae</td></tr>
<tr><td>Tribe 2. Kerosphaereae</td></tr>
</table>

Dressler and Dodson (1960) classified Orchidaceae in to three sub families based on the number of fertile anthers and sub divided each sub family into different number of tribes (Table 2.7).

Table 2.7. Classification of the family Orchidaceae as per Dressler and Dodson (1960)

Family Orchidaceae	
Sub family Cypripedioideae – Fertile anthers two or three. Filaments more or less united to the style but arising below the level of stigma. Stigma and terminal portion of the style free. No rosellum.	Sub family Orchidioideae – Fertile anther one. Filaments united with the style to form a distinct column, united for the full length of the style or the anther and stigma connected by a terminal rostellum.
Tribe 1. Apostasieae – Perianth regular. Lip never deeply saccate.	Tribe 1. Neottieae – Anther more or less erect, often dorsal. Stems

Fertile anthers two or three, elongate. Style slender.	without corms or other thickenings.
Tribe 2. Cypripedieae – Perianth irregular. Lip deeply saccate. Fertile anthers two. Subglobose. A conspicuous flattened median staminode. Style thick.	Tribe 2. Orchideae – Anther erect or reclinate (rarely incumbent). Persistent. Usually broadly joined to the column. Pollinia in soft masses (sectile). Caudicles arising from the base of the pollinia.
	Tribe 3. Epidendreae – Anther terminal and operculate, (incumbent) or rarely erect. Usually more or less versatile. Stems often with corms and corm like thickenings.

Holttum (1964) classified Orchidaceae into two sub families, Pleonandrae and Monandrae based on the number of the anthers and further subdivided Monandrae into sub groups (Table 2.8).

Table 2.8. Classification of the family Orchidaceae as per Holttum (1964)

Family Orchidaceae		
Sub family Pleonandrae – Anther more than one.	Sub family Monandrae– Anther one.	
	Basitonae– Anther attached by its base.	Acrotonae– Anther attached by the top.

In spite of repeated efforts of several systematists, classification of orchids is still incompletely studied. However the classification suggested by Dressler and Dodson (1960) seems to be comparatively appropriate for practical purposes. According to their classification the genus *Vanilla* belongs to the sub tribe Vanillinae of Tribe Epidendreae belonging to the sub family Orchidoideae of the family Orchidaceae. Vanilloid orchids consist of about 15 genera and 175 species. A majority of the species belong to the genus *Vanilla* (Cameron *et al*., 1999).

Certain recent investigations by different workers have contributed significantly towards the knowledge on the taxonomy of vanilloid orchids. Dressler (1993) included the vanilloid genera of orchids in the tribe Vanillaceae and he subdivided this into 3 sub tribes Vanillinae, Galeolinae and Lecanorchidinae. Szlachetko (1995) elevated most of the Vanilloid orchids to a higher taxonomic rank as sub family Vanilloideae. Studies on molecular analysis of vanilloid orchids also agree with the recognition of them as a sub family (Cameron *et al*., 1999; Cameron and Chase, 2000).

Floral characters, especially those relating to anther configuration and structure of pollina, have been the primary basis for classification of orchids (Dodson, 1962). These floral features are hypothesized to be especially prone to selection pressure from pollinators and hence are likely to display high levels of convergence or parallelism (Dodson, 1962; Atwood, 1986). Vegetative anatomical characters have some phylogenetic value in Vanilleae, but they are not useful in resolving placement of the large and polymorphic genus *Vanilla* (Stern and Judd, 2000). Classification of orchids based on embryogeny was attempted by Schletcher (1926b). Very few systematic studies of Orchidaceae have employed molecular techniques and only one (Chase *et al*., 1994) has addressed higher order relationships of this large and floristically important family. A recent study attempted to clarify the phylogenetic position of *Vanilla* by sequencing the plastid genes psb B and psb C. Nucleotide variation within each of these genes is sufficient to resolve the major relationships among Vanilloideae, and the combined two-gene tree is fully resolved at the genus level and highly supported (Cameron and Molina, 2006).

2.5. Morphology

The stem growth of *Vanilla planifolia* is essentially monopodial and the growth of the plant is accomplished by the elongation of the apical meristem, rather than by growth from yearly shoots. The growth is indeterminate. With its vining habit and a root emerging from each node to attach the plant as it grows, vanilla can climb many feet on the support. Probably it is the only orchid that behaves like a vine; and though its seedling stages may pass on the ground, it eventually achieves epiphytic status as the bases of the stems die and terminal growth and axillary branching occur (Withner *et al*., 1974).

Species of *Vanilla* are represented in two growth forms: green vines with leaves and those without leaves or with reduced leaves. Some of the leafless species may produce nodal scales, which soon fall off and others produce more expanded leaves, which nevertheless are still cauducous. The vines produce two kinds of roots, short unbranched aerial ones (variously termed as clasping and anchoring) which clasp the supporting structure and are usually limited in extension growth; and long, branched, terrestrial or absorbing roots which penetrate the substratum and are presumably of unlimited extension growth. Both root forms originate at the nodes of the same plant, usually one root at each node (Stern and Judd, 1999).

The herbaceous perennial vines climb up trees or other supports to a height of 10-15 m by means of adventitious roots. In cultivation it is trained to a height which will facilitate hand pollination and harvesting. Long, whitish, aerial adventitious roots about 2 mm in diameter are produced singly opposite the leaves and they adhere firmly to the support

plant. The roots at the base ramify in the humus or mulch layer. The stem is long, cylindrical, succulent and branched (Purseglove *et al*., 1981).

Inflorescences are axillary, usually simple and rarely branched racemes. Flowers are large, waxy, bisexual and zygomorphic. The lower part of the labellum envelops a central structure called the ‘column’ (gynostemium). The tip of the column bears a single stamen with two pollen masses (pollinia) covered by a cap or hood like structure called ‘rostellum’. The stigma is physically prevented from coming in contact with the anther by this flap like structure, rostellum (Purseglove *et al*., 1981).

Lawrence (1951) describes that gynostemium in Orchidaceae is formed by the union of stamen, style and stigma. All the orchid flowers represent a reduction from ancestral type in the androecium (originally six stamens in two whorls). In monandrous orchids which are advanced, two of the outer stamens have been suppressed and the third is present (fused with style and stigma) as one functional anther situated terminally on the column, while all the three stamens of the inner whorl are suppressed completely. The stigma and stamen of monandrous orchids are of particular significance taxonomically and morphologically. In this group the stigma is situated usually in a depressed cavity (recognized by its glossy, viscid surface) immediately below the terminal anther. Two stigmatic lobes or surfaces are present and often confluent; the third is modified in to a non functional lobe called rostellum (Lawrence, 1951). The rostellum separates the stamen from the functional part of the stigma (Dahlgren *et al*., 1985).

The ovary is slender and stalk like (Kuruvilla *et al*., 1996). A cross section of the ovary of an opened flower shows three carpels, three pairs of fibrovascular bundles and three pairs of placentae in *Vanilla planifolia*. The placentae extend throughout the length of the ovarian cavity (Madhusoodanan *et al*., 2003).

The fruit is a capsule which is dehiscent in *Vanilla planifolia* and in trade it is known as a bean (Purseglove *et al*., 1981). The bean is pendulous, narrowly cylindrical, obscurely three angled, 10-25 cm long and 5-15 mm in diameter in *Vanilla planifolia*. Each bean when ripe contains thousands of minute globose seeds (0.3 mm in diameter), which are liberated by longitudinal splitting of the capsule. In commercial production, the beans are harvested before they are fully ripe, *i.e.*, when the distal end of the beans turn to slight yellow in colour (Madhusoodanan *et al*., 2003).

2.6. Anatomy

Anatomical characters can be used to distinguish orchid species in the vegetative phase (Metcalfe, 1961). Although the genus *Vanilla* is easy to recognize, identification at the species level is difficult for a number of reasons including the rarity of finding plants in flower, the difficulty of drying specimens and the length of time required by plants in cultivation before they flower (Christenson, 1995).

The anatomy of vanilla stem is unique and deserves special mention because of the xylem. The xylem elements of vanilla are larger in diameter than is usual for orchids. The conducting cells are vessel elements, with gently sloping end walls and scalariform perforation plates. The walls of the elements show scalariform pitting; smaller protoxylar

elements have spiral thickenings. This might indicate a more advanced evolutionary state for the stems of vanilla, since it is generally accepted that vessel elements develop as a specialization of the primitive tracheids (Withner *et al*., 1974).

Stern and Judd (1999) studied the comparative vegetative anatomy and systematics of the species of *Vanilla* and found that vegetative anatomical characters have some phylogenetic value in them.

An anatomical study on the stems of *Vanilla planifolia* and *Vanilla siamensis* was made by Zhao and Wei (1999) and the results showed that the arrangement and structure of stem tissues of these two species were different. In the cross section of the stem of *Vanilla planifolia*, there was annular sclerenchyma composed of some particular cells which were small and with specialized thick walls between the cortex and vascular tissue region. But in the stem of *Vanilla siamensis*, this annular sclerenchyma was absent.

Vegetative anatomy of the leafless orchid *Vanilla wightiana* has been studied by Raju (1996) and he observed that anatomical characters of vegetative parts of this rare orchid can be utilized to distinguish the plant in vegetative phase from other leafless Indian species. While comparing the vegetative anatomy of *Vanilla pilifera* with that of *Vanilla wightiana*, there are found some significant anatomical variations between the two species (Baruah, 1998).

The leaves of *Vanilla planifolia* were found to be hypostomatous with crystals in all epidermal cells, except subsidiary and guard cells. The

stomatal apparatuses were largely tetracytic with some anomocytic (Nayar *et al*., 1976).

On reviewing the anatomy of orchids Withner *et al*. (1974) divided the leaves of orchids in to two major types (1) ribbed or plicate; and (2) leathery, soft or hard, often fleshy. Dressler and Dodson (1960) pointed out that the plicate type is ordinarily convolute and the leathery type conduplicate in development. Withner *et al*. (1974) emphasize that the conduplicate leaf probably evolved independently in several evolutionary lines and is strongly associated with the epiphytic habit. *Vanilla* seems to be intermediate, showing convolute formation during development and finally fleshy leaves with conduplicate appearance when mature.

French and Fischer (1977) studied the distribution of meristematic activity and cell length in the growing internodes of monocotyledonous *Vanilla* and observed that the loss of meristematic activity proceeded from the base to the top of the internode.

In the root cortex of *Vanilla planifolia*, there is direct continuity between raphide cell protoplasts. This is due to disintrigation of transverse walls of contiguous raphide bearing cells (Mollenhauer and Larson, 1966). The raphide bearing cells may loose their end walls forming unbranched tubes that weave among the cortical parenchyma in roots. There were cortical lysigenous lacunae of varying sizes opposite to phloem and endodermal cell wall thickenings were the heaviest in aerial roots (Alconero, 1968). For roots of *Vanilla planifolia* the transverse cell walls between crystal idioblasts were thin and attenuated progressively during idioblast maturation so that cortical syncytia developed via schizo-lysigenous breakdown of the cell walls (Kausch and Horner, 1983).

Engard (1944) studied the nature of velamen and exodermis in orchid roots. The anatomy of different species of *Vanilla* have been comparatively analysed by other workers also. The number of layers of velamen cells varies from species to species (Raju, 1996; Baruah, 1998).

Some epiphytes particularly the liane forming members of Vanilleae usually show a pattern of infection originating from the substrate and in turn leading to variations in root structure such as localized hypertrophy and hyperplasia of tissues. Enlargement of infected cells, hypertrophy of host cell nuclei and effects such as localized proliferation of epidermal hairs are probably quite common (Hadley and Williamson, 1971). Many fungi which are mycorrhizal with orchids have the potential for relationships with other plants. *Rhizoctonia solani*, for example is a well known pathogen of many hosts as well as an orchid endosymbiont and may be both on the same host as was reported by Alconero (1969) in green house experiments with *Vanilla planifolia* and *Vanilla phaentha*. Alconero (1969) while working with *Rhizoctonia solani* and the vanilla orchid found that separation into a fungal host cell layer and a digestion layer was not evident. Hyphal digestion occurred in cells scattered throughout the root, but it most frequently occurred in the peripheral cells. Once the fungal hyphae pierce through the epidermal cells of an orchid root, there is further penetration through passage cells of the exodermis. The passage cells in vanilla roots are found to be thin walled and more active metabolically than the other cells of the exodermis (Alconero, 1969).

Darwin (1884) while studying the paths of vascular bundles in flowers representing various orchid tribes, concluded that the labellum was a compound structure made of the median petal and two lateral

stamens of the outer whorl. In vanilla, the three vascular bundles each form two ovarian traces one to the lateral petal and lateral sepal and the other to the lateral petal and dorsal sepal respectively. The bundle that supplies the lateral petal and dorsal sepal branches off before diverging and provides the bundle for the bract (Withner *et al*., 1974). In examining the vasculature of the orchid flower Withner *et al*. (1974) considered two points: first, the origin of the six vascular bundles (called traces) which run along the ovary and second, the pattern of splitting of these traces to form the traces to the various parts of the flower. In vanilla, the traces of sepals and petals lie close together.

Odoux *et al*. (2003) examined the morphology, anatomy and histology of mature green vanilla beans by light and transmission electron microscopy and observed that vanilla beans have a triangular cross section with a central cavity containing seeds. Each angle is lined with tubular cells or papillae, while the cavity sides consist of placental laminae. The epicarp and endocarp are formed by one or two layers of very small cells, while the mesocarp contains large highly vacuolarised cells, the cytoplasm being restricted to a thin layer along the cell walls.

The epidermis of the syncarpous fruit of *Vanilla planifolia* contains isodiametric ground epidermal cells, which lack prominent chloroplasts. Each epidermal cell contains a rhomboidal crystal of calcium oxalate and is bounded by thickened, pitted cell walls. Stomata are widely spaced. In some varieties dozens of extrafloral nectaries occur on the fruit. In other varieties extrafloral nectaries are entirely absent. The fruit wall contains a ring of about 15 unbranched vascular bundles, each containing a strand of xylem and phloem with a sclerotic bundle sheath. Xylem consists of annular to helical and reticulate elements. The tissue outside

the ring of vascular bundles is composed of thin walled parenchyma cells several times longer than wide. Each ground parenchyma cell in the outer fruit wall contains chloroplasts and occasional rhomboidal calcium oxalate crystals. Raphide “vessels” are abundant in the outer fruit wall and they release mucilage containing raphides when the fruit is cut, which is highly irritating if it contacts skin. Compared with the outer fruit wall the wall tissue inside the ring of vascular bundles contains larger cells, with somewhat less abundant and smaller chloroplasts and so is much less green in freshly cut beans (Havkin–Frenkel *et al*., 2004).

2.7. Embryology

Structure of anther in orchids is very much the same as in other angiosperms. The sporogenous tissue is enclosed by the anther wall which is multilayered consisting of an epidermis, endothecium, two middle layers and a tapetum (Abraham and Vatsala, 1981). In the monandrous orchid vanilla, single pollen grains are formed (Davis, 1966). In vanilla individual tetrad cells round off investing themselves with a thick wall and behaving as the pollen of other angiosperms (Abraham and Vatsala, 1981). In vanilla, pollinia with simple pollen grains are united by viscous material and not by elastic threads (Veyret, 1974).

Embryogenesis is relatively a short stage, in comparison to other concurrent phenomena, in the formation of the fruit. It starts towards the middle of the period, occuring between pollination and the dehiscence of the ovary and lasts for about two weeks on the average. When the pod is ready to dehise, the internal integument of the ovule as well as the deepest layers of the external integument are generally found to be degenerated. The cuticle of the epidermis of the inner integument of the ovule persists

and this seems to have the effect of impeding the hydration of seeds and thus hindering their germination *in vitro* (Veyret, 1969).

The embryo in the absence of the mycorrhizal fungus *Rhizoctonia* may be in limited contact with the seed coat or may be more or less isolated in the centre according to the importance of the development of the external integument in the course of the embryogenesis. The cells of the coat are dead, empty, transparent and very thick in Vanilleae (Veyret, 1974). The orchid embryo has developed in a sac without the benefit of an endosperm and this has generally been interpreted as the cause of the rudimentary state of the embryo. Endosperm is not ordinarily formed in orchids, either due to a lack of fusion of the second sperm nucleus with the endosperm nuclei, or from an immediate degeneracy of the nucleus of the endosperm. Among a few species, however, the segmentation of the nucleus of the endosperm takes place, but it never leads to the production of a normal endosperm (Veyret, 1974).

Endosperm formation when it occurs is nuclear in vanilla (Davis, 1966). Swamy (1947) reported nuclear endosperm with twelve nuclei in *Vanilla planifolia*. Ten free nuclei have been reported in *Vanilla planifolia* by Davis (1966).

True polyembryony is frequent in Orchidaceae and has been reviewed by Wirth and Withner (1959). Swamy (1947) reported twin embryos in *Vanilla planifolia*. According to Philip and Nainar (1988a) orchid embryo at the shedding stage has been described as a mass of 10-100 similar undifferentiated cells, with organogenesis being initiated only after the seeds have been shed and brought under favourable conditions of germination. The specific chain of events of the embryo leading to

seedling formation has no parallel amongst other reported studies in angiosperms.

2.8. Cytology

Tanaka and Kamemoto (1974) provided a comprehensive tabulation of chromosome numbers of orchids including vanilla. The majority of these counts were derived by various workers from meristematic cells in root tips. In a few instances counts were made from shoot apex, young petal, embryo or protocorm. Somatic chromosome numbers and basic numbers show wide variation in the Orchidaceae. Studies have shown that the basic chromosome number of the genus *Vanilla* is 16 (x=16) and *Vanilla planifolia* is a diploid with 2n=32 (Hoffmann, 1929; Heusser, 1938; Eftimiu-Heim, 1950; and Martin, 1963). The somatic chromosome number of the other two cultivated species, viz., *Vanilla tahitensis* and *Vanilla pompona* is also 2n=32 (Eftimiu-Heim, 1950). However aneuploids having chromosome numbers 28 to 31 have been reported (Hurel Py, 1938). Nair and Ravindran (1994) reported that somatic chromosome number of *Vanilla planifolia* ranged from 2n= 20-32. Studies by different workers have revealed the occurrence of aneuploid, diploid and polyploid species in *Vanilla*. (Table 2.9).

Table 2.9. Chromosome numbers of different species of *Vanilla*

Species	2n	Reference
Vanilla aromatica	32	Eftimiu-Heim (1950)
Vanilla barbellata	32	Martin (1963)
Vanilla dilloniana	32	Martin (1963)
Vanilla hartii	32	Eftimiu-Heim (1950)
Vanilla imperialis	32	Eftimiu-Heim (1950)
Vanilla moonii	32	Eftimiu-Heim (1950)
Vanilla papeno	32	Martin (1963)
Vanilla phaentha	32	Eftimiu- Heim (1950)

Vanilla planifolia	20-32	Nair and Ravindran (1994)
	28-32	Hurel Py (1938)
	32	Hoffmann (1929); Martin (1963)
	30-32	Chardard (1963)
Vanilla pompona	32	Eftimiu-Heim (1950); Martin (1963)
Vanilla thaitii	32	Eftimiu-Heim (1950)
Vanilla roscheri	36	Krupko *et al*. (1954)
Vanilla haapape	64	Tonnier (1951)
Vanilla wightiana	64	Vatsala (1964)

In orchids, basic chromosome number is relatively high. It has been suggested, therefore, that orchids are ancient polyploids, but during the course of evolution there has been loss or gain of individual chromosomes coupled with structured alteration of sets, which has led to their now being effectively diploid (Jones, 1974).

Ravindran (1979) reported abnormalities in pollen grain mitosis combined with high pollen sterility. He also reported chromosome associations during pollen mitosis. The above observation indicates the possible occurrence of cytotypes in the seedling progenies of vanilla.

2.9. Physiology

2.9.1. Pattern of flowering

First flowering in vanilla depends upon the length of vine cutting used for planting. Vines of *Vanilla planifolia* having a length of one metre while planting flowered in the third year. The inflorescences emerge as light green protuberances from the leaf axils. Each inflorescence carries 20-25 large flowers. To form a developed flower it takes 50-60 days from bud initiation (Kuruvilla *et al*., 1996).

Flowering is annual and it extends for a period of 2 to 3 months. In the high range region of Kerala, it is from March to May (Kuruvilla *et*

al., 1996). According to Sasikumar *et al*. (1992) flowering season in vanilla is from December to March. Bhat and Sudharshan (2000a) reported blooming of vanilla in Karnataka from February to the end of April. Inflorescence initials were observed in January-February and the number of days taken from inflorescence initials to first bloom varied from 35 to 61 days. According to Madhusoodanan and Radhakrishnan (2001) flowering occurs in *Vanilla planifolia* from December to March depending upon altitude of the cultivated area and it takes about 45 days from inflorescence initiation to flowering.

Generally one and occasionally two flowers open simultaneously. Instances were noticed in a few cases where there were five day intervals between one flower to the next in the same inflorescence. Synchronized flowering is lacking and blooming period in an inflorescence continues for 14 to 30 days. In a season, seven to fifteen inflorescences are produced on a vine (Kuruvilla *et al*., 1996). Bhat and Sudharshan (2000a) observed that two flowers are produced in an inflorescence per day, occasionally. Blooming interval in different inflorescences varied from one to five days.

Bhat and Sudharshan (2002) also reported that blooming could take place every day, on alternate days, once in three days or at an interval of more than three days in different inflorescences. However in about 50% of the inflorescences blooming was observed every day.

Shadakshari *et al*. (2003a) observed that time taken from floral initiation to first flower varied from 30 to 45 days in different vines based on the health of the vine and number of inflorescences it produced. In a single inflorescence, the periodicity of flower opening is not regular. It

varies from a day to three; rarely two flowers open a day. Total blooming period varies depending on number of flowers produced.

2.9.2. Physiology of flowering

The factors that influence time and quantity of flower initiation in vanilla have not been studied fully (Childers *et al*., 1959). Factors such as drought, temperature, training of vines and pruning of shoot tips are found to influence flowering. In a study in Seychelles a dry period was found to be highly necessary for the formation of blossoms. The effect of this dry spell is explained as an increase in the ratio of C:N, which favours flowering (Lionnet, 1958). It has also been noticed that a good/abundant flowering season is alternated by a non/less flowering season. Of late, renewed interest is being shown by many researchers to investigate various factors responsible for optimal flowering and fruiting in vanilla, considering the high global demand for vanilla beans (Anonymous, 2000).

It is usually stated that as long as the vine can climb upwards, it will not flower. But Irvine and Delfel (1961) have reported flowering of vanilla under such conditions from Puerto Rico. The vines of *Vanilla planifolia* will climb to heights of 60 ft or more, if provided with a sufficiently tall support. The vine must therefore be trained to a height convenient for pollination and harvesting in the commercial production of vanilla. The prevention of the natural tendency to climb is thought to enhance the production of flowers (Irvine and Delfel, 1961). Childers *et al*. (1959) suggested that “bending of vines appears to be an important factor in causing it to flower and fruit beyond the bend, which may be due to an accumulation of carbohydrates and possibly other flow forming materials in this region of the vine.

Childers *et al*. (1959) stated that growers in Puerto Rico follow the practice of removing 10-15 cm of the growing shoot tip each year some time before flowering. The removal of this piece apparently results in the accumulation of carbohydrates and other substances thus encouraging production of inflorescence in the axils of the leaves on the hanging branches.

If vegetative growth of vanilla vine is arrested by pruning, it turns to reproductive phase (Potty and Krishnakumar, 2003). The method described by Lionnet (1958) which is followed in Seychelles is different from the practice of other areas and it involves preparing special bearing branches through pruning. Shoots when 1-1.2 m long are bent down round a branch of a support, slightly twisted in the process with tip pruned at about 45 cm from the soil. Any shoots appearing on the bearing branches themselves are cut off when 7.5-10 cm long but the shoots appearing on the rest of the plant before the bends are allowed to grow. They will constitute the bearing branches of the following year. As a result there is a decreased sap flow towards the bearing branches, which favours flower formation. After the harvesting of beans, the old branches are cut off. Meanwhile the following year’s bearing branches have already been prepared.

Fouch and Coumans (1995) reported that decapitated pending vines are the most responsive parts of vanilla plants. Inflorescence localization along training vines showed a decreasing gradient from the decapitated end to the base in sun locality and was irregular and showed opposite gradient in shade locality. Soluble and ionic peroxidase activities, determined in the leaves and in the internodes, showed a marked peak during the end of June and a gradient along training vines which was

inverse to the gradient of inflorescence localization and opposite between sun and shade conditions, supporting the utility of peroxidases as spatial and temporal biochemical markers of flowering processes.

Climate plays a dominant role in floral initiation. Under typical hill zone climatic condition with about 2000 mm rainfall distributed over six months, maximum temperature about 32^0C during summer, RH of >90% during rainy and winter mornings in most of the days, flowering starts by February/March, if proceeded with tip nipping by November followed by two months dry period. But in relatively drier climate with around 1000-1200 mm of rainfall distributed over 4 months, maximum temperature of 35^0C during summer with varying RH levels, it is being noticed from October itself (Shadakshari *et al*., 2003a).

Regulating the shade at the time of flowering by allowing more light to reach the plant by pruning the canopy was found to have a positive influence on flowering (Puthur and Krishnakumar, 2006). It was suggested by Childers *et al*. (1959) that heavy shade should be avoided because stem will become thin, leaves small and flowering and fruiting generally reduced.

Alconero *et al*. (1973) reported that heavy shade inhibited flower induction of vanilla in Uganda and suggested that pruning of branches of support trees at 1.8 m would be necessary for full scale bean production.

Although these techniques have been practiced over years, it is still unclear what physiological and biochemical changes take place concomitant with these manipulations of plant phenology. Puthur and Krishnakumar (2006) made an effort to analyze the role of nutrients (total

NPK) and metabolites (total soluble sugars and free amino acids) in flowering of vanilla and also studied the influence of inflorescence acting as strong sink on leaf photosynthesis. Along with flowering, an increase in total NPK and metabolites was noticed in different plant parts such as inflorescence peduncle, nodes bearing inflorescence and nodes bearing shoot, as compared to nodes devoid of inflorescence or shoot. Phosphorous and Potassium were found to be required in higher percentage as compared to N and influence of sugars was more prominent than free amino acids for flowering in *Vanilla planifolia.* The role of inflorescence as a strong sink for sugar is also made clear from the high oxygen evolution rate of the leaves bearing the inflorescence in their axis.

Nutrient and shade management for vanilla plantations should be programmed in such a way as to ensure proper nutrition and photosynthesis for resulting in optimal flowering of the crop (Puthur and Krishnakumar, 2006).

An experiment was conducted in China, to study the endohormone changes of *Vanilla fragrans* (*Vanilla planifolia*) cultivated under different conditions. In flower bud differentiation, there was a decline of endohormone level in the vine and an increase of endohormone level in the flower bud. Most of the flower buds are formed in the cernous vine. The decrease in growth regulators was greater in cernous vine than in antrose vine during flower bud differentiation (November-December) but the decrease in buds was greater in antrose vine. This change in the cernous vine might prompt flower bud differentiation. Apical dominance of *Vanilla fragrans* was excellent during growing. ZR, GA and IAA contents in lateral buds increased after the apex was removed. This might explain the induction of flowering in the antrose vine from November to December (Tian *et al*., 2004).

2.9.3. Photosynthesis

Total leaf area is closely associated with total photosynthesis and dry matter accumulation in vanilla (Krishnakumar *et al*., 1997). According to Purohit and Ranjan (2002) vanilla is intolerant to high light levels. Even though photosynthesis was effective in vanilla plants growing at sunlight of 300-800 $\mu Em^{-2}s^{-1}$, only plants receiving 600-800 $\mu Em^{-2}s^{-1}$ sunlight were able to effectively partition the accumulated carbon into fruiting structures. Therefore, light condition of 600-800 $\mu Em^{-2}s^{-1}$ is favourable for productivity while that of 300-600 $\mu Em^{-2}s^{-1}$ is favourable for vegetative growth. Sunlight above 800 $\mu Em^{-2}s^{-1}$ affected productivity negatively. It was observed that proline as well as carotenoids accumulated in vanilla plants with increasing light intensities. However, the protective mechanisms against the photodestructive high light intensity were not sufficient to protect vanilla plants from photoinhibitory damages. This was clearly manifested by the high levels of lipid peroxidation as judged by the malondialdehyde (MDA) levels, low chlorophyll content, low oxygen evolution rate and low productivity in plants exposed to sunlight above 800 $\mu Em^{-2}s^{-1}$. These results confirm that shade plants like vanilla do not have a well developed mechanism to counteract the after effects of photoinhibition. This study indicates that light has a profound influence on growth and productivity in *Vanilla planifolia* (Puthur, 2005).

Photosynthetic characteristics and antioxidant mechanisms in *Vanilla planifolia* exposed to varying intensities of solar radiation have been studied by Puthur and Rajan in 2006. The results showed that vanilla plants were acclimatized to varying intensities of solar radiations ranging from 2% to 100%. Solar radiations up to 64% did not significantly affect the photosynthetic pigment levels and oxygen evolution capacity of

vanilla plants. Sunlight above 64% brought about marked decrease in the levels of photosynthetic pigments as well as oxygen evolution capacity. Chlorophyll a degradation is at a higher level when compared to chlorophyll b in the case of plants exposed to higher light intensities. Degeneration of chlorophyll is higher when compared to carotenoid pigments, with increasing solar radiation. Acclimatization to very low solar radiation resulted in the temporary inactivation of the reaction centres as evidenced by the delayed oxygen evolution. The level of free radicals estimated from the extent of lipid peroxidation was high even at 64% solar radiation and above. The recovery of vanilla plants from the high light stress affected state was faster in the case of those acclimatized to 64% radiation as compared to higher light intensities. This result points out to the fact that a very effective antioxidant system may be functioning in vanilla plants acclimatized to 64% radiation. An enhancement of proline levels along with an increase in light intensities denotes its probable role as an antioxidant. Although superoxide dis mutase (SOD) and guaicol peroxidase (GPX) were showing an enhanced activity in vanilla plants at light intensities above 64%, the other enzymes involved in free radical scavenging [ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR) and glutathione reductase (GR)], showed a decreasing activity in these light conditions. These four enzymes involved in the ascorbate glutathione cycle (AGC) may be having a low profile due to the high energy dependence of these enzymes. Eventhough some antioxidative mechanisms may be functional, the failure of the enzymes involved in the AGC pathway may be the prime reason for the non performance of vanilla plants acclimatized to higher levels of solar radiation.

2.10. Floral biology

2.10.1. Structure of the flower

Kuruvilla *et al*. (1996), Bhat and Sudharshan (2002) and Shadakshari *et al*. (2003a) carried out studies on the floral biology of *Vanilla planifolia*. Phenology of flowering was studied by Bhat and Sudharshan (2002) and their study revealed that *Vanilla planifolia* vines come to flowering 3-4 years after planting. Flowering generally takes place on the branches which are one and a half years old. Inflorescence initials are generally activated in the leaf axils during December-January as light green protuberances. Bhat and Sudharshan (2002) observed that these buds attained a length of 3.56 cm in the first fortnight. After 30 days, the length was 4.59 cm. At this stage, the flower buds commence to emerge out from the base of the inflorescence. Inflorescence is of racemose type with a peduncle of 2.5 cm. About 45 days after formation, the flowers are fully formed and blooming commenced. The mean length of inflorescence at this stage is about 6.49 cm. Linear growth of inflorescence is observed upto 60 days, by which time it attains a mean length of 7.34 cm.

Vanilla planifolia flowers are large, bisexual, zygomorphic with inferior ovary of about 5 cm length and 1.4 cm girth. The three sepals and two of the petals are fleshy, linear, oblong, 5.2-5.8 cm in length and 1-1.4 cm wide. The third lower petal is short, broad, trumphet shaped and modified in to labellum. The labellum encloses the “column” (gynostemium) at the centre which is a little shorter than the perianth (4.6 cm) and also has a tuft of scales. The column has at its apex two pollen sacs (pollinia, 1.5 mm in length and 1.9 mm in breadth) covered by a hood like structure as their cap. The stigma is physically prevented from coming in contact with the anther by a flap like structure (4.1 mm in length and 3.0 mm in breadth) known as rostellum (Bhat and Sudharshan, 2002).

2.10.2. Anthesis

Nair and Mathew (1970) observed that flower opening in *Vanilla planifolia* was between 10.30 pm and 1.00 am and it was completed by 6.00 am. Shadakshari *et al*. (1996) observed that flowers opened around 1.15 am and the process was completed by 6.00 am. However, the calyx was completely spread by 9.00 am only. Closing of flowers began around 1.00 pm and was completed around 3.00 pm. Kuruvilla *et al*. (1996) reported that majority of the flowers opened at about 4 am. Flowers bloom from the base of the inflorescence upward and the opened flowers last for a day (Bhat and Sudharshan, 2002).

A study by Bhat and Sudharshan (2003) revealed that anthesis in *Vanilla planifolia* is a prolonged process of 11 hours, initiated at 7 pm and progressively ending at 6 am with complete opening of the flower. Anthesis commences as a longitudinal slit between two sepals on the dorsal side of the flower bud at 7 pm, 11 hours before opening. It slowly extends upwards and by 10 pm (8 hours before opening) the mouth of the labellum is visible at the tip of the perianth that further widens by 11 pm. The perianth segments begin to separate and the flowers start opening at 12 pm midnight. All the segments of the perianth separate by 4 am and the flower opens completely by maximum spreading of sepals at 1 pm and it extends up to complete closing at 4 pm.

2.10.3. Anther dehiscence

Observations on anther dehiscence showed that pollinia get released by longitudinal splitting of anther lobes. A longitudinal slit appears on the anther lobes 36 hours before opening of the flower (6 pm). The slit was more prominent and wider 24 h before opening.

Perpendicular to this slit, anther cleavage was observed at 4 pm (14 h before opening of the flower) and this results in the release of the masses of pollen grains (pollinia) at 8 pm (10 h before opening of the flower). The process of anther dehiscence commences 36 h prior to anthesis (Bhat and Sudharshan, 2003).

2.10.4. Palynology and pollen biology

All the normal mature (fertile) pollen grains are similar in morphological features. They are round in shape and possess two distinct coats. The size of the mature pollen grains varies from 60 µm to 90 µm and pollen fertility ranges from 72% to 87% (Kuruvilla *et al*., 1996).

Bhat and Sudharshan (2000a) found that the average weight of pollinia was about 2.25 mg (with a range of 2 to 2.5 mg) with about 2,32,916 to 3, 90, 410 pollen grains counted using haemocytometer (Bhat and Sudharshan, 2000b). Pollen grains are spherical in shape and possess two distinct coats. Size of mature pollen grains varies from 18.8 to 23.2 µm (Bhat and Sudharshan, 2000a).

Bhat and Sudharshan (2003) studied pollen viability by conducting pollination at different time intervals and they found that the flower buds pollinated with their own pollen 24 hours before complete flower opening failed to set fruits. Fruit set was observed from 23 hours before complete flower opening onwards. This indicates that pollen viability starts 23 hours before complete flower opening. Freshly opened flowers pollinated with pollen grains from older flowers set fruits with upto 34 hour old pollen, indicating the viability of pollen upto 34 hours after flower opening. Thus in *Vanilla planifolia* the total pollen viability period has been found to be 57 hours (23 h before flower opening and 34 h after flower opening).

Shadakshari *et al*. (2003a) and Shadakshari *et al*. (2003b) reported a total pollen viability period of 39 h 30 min (23 h before complete flower opening and 16 h 30 min after complete flower opening) in *Vanilla planifolia* under hill zone conditions.

2.10.5. Pollen germination

Kuruvilla *et al*. (1996) ascertained pollen fertility by germinating *Vanilla planifolia* pollen grains *in vitro* in a semi solid medium containing sucrose, boron and salts and found that pollen fertility ranged from 72% to 87%.

Bhat and Sudharshan (2000a) reported 60.3% pollen germination in a medium containing 8% sucrose and 10 ppm boric acid at ambient temperature.

In vitro pollen germination studies conducted by Muhammed Nissar *et al*. (2006) revealed that optimum pollen germination and tube growth of *Vanilla planifolia* pollen grains was in a medium supplemented with 5% sucrose, 150 ppm boron and at a temperature of 25^{O}C.

2.10.6. Pollination

The vanilla flower is so constructed that self pollination of the individual flower is impossible, unless hand pollinated, due to the separation of the stamen from the stigma by the rostellum (Purseglove *et al*., 1981).

Although vanilla plants grow well in the old world tropics, fruits are not produced because of the absence of natural pollinators. Professor

Charles Morren, a Belgian Botanist discovered the artificial means of hand pollination for the production of capsules in 1836 (Purseglove *et al*., 1981). In 1841 Edmond Albius, a former slave in the French Island of Reunion perfected a quick method of pollinating with the pointed tip of a small bamboo stick (Correll, 1953). The technique is still used and as a result commercial production of vanilla is possible in the eastern hemisphere away from the centre of origin (Purseglove *et al*., 1981).

In its native country of Mexico the flowers of vanilla are naturally fertilized by small bees of the genus *Melipona* and also by humming birds. But although there are plenty of bees in other parts of the world where vanilla is cultivated, for some reason they do not visit the flowers, or if they do so, fail to fertilize them, and there seems to be scarcely a case recorded of natural fertilization of the plant under cultivation. It is therefore necessary to fertilise the flowers of the plant by hand in order to produce fruits (Ridley, 1983).

Thus if the breeding barrier that segregates the population and so maintains the species is removed by hand pollination, it is not surprising that the seed is formed. The relevance of this to speciation and environmental adaptability under natural circumstances hinges on whether or not the barrier of pollinator specificity can be bridged in nature (Sanford, 1974).

Hand pollination is done with a splinter of bamboo or other material about the size of a tooth pick. The flower is held in one hand and the labellum is pushed down with the thumb releasing the column. The stamen cap is removed by the stick which is held in the other hand, which exposes the pollinia. Then the flap like rostellum is pushed up under the

stamens with the thumb and finger, the pollinia with the pollen mass are brought into contact with the stigma (Purseglove *et al.*, 1981).

In many countries vanilla flowers are pollinated manually (Torregrossa, 1989). Fouch and Coumans (1992) have summarized four techniques of pollination (Table 2.10).

Table 2.10. Methods of manual pollination in vanilla

1. Original or Albius method	Pull down the labellum with a needle to the base of the flower to free the gynostemium (column), rostellum and pollinia. Introduce the needle underneath the rostellum by pushing it up to the base of the anther. This will uncover the stigma. Hold the anther between the thumb and the index finger, and press gently onto the stigma in such a way that the pollinia stick to the stigma.
2. Tearing technique	The Tearing technique is a slight modification of the Albius method. It differs in that the labellum is torn away and collected by workers so that the number of pollinated flowers can be recorded. Pollination is facilitated through exposure of the sexual parts. The Tearing technique is utilized in the Malagasy Republic to eavaluate production and count the number of flowers pollinated. The labellum is held between the thumb and index finger and torn away to expose the sexual parts and pollinate the flower.
3. Boring technique	When done properly, the Boring technique is the least traumatic to the flower because the labellum, which remains intact, protects the pollinia and stigma completely from direct sun or rainfall after pollination. However, it is difficult to recognize pollinated flowers and for that reason this technique is rarely used. Introduce the needle through the side of the labellum. Position the tip of the needle exactly underneath the rostellum. Through the hole in the labellum, displace the rostellum to clear the stigma. Presss together the labellum and anther to be sure there is contact between the pollinia and stigma.

4. Tomorrow pollination technique	The latest and probably most original method is the Tomorrow pollination technique. It differs from the others by the fact that it is applied to buds, not flowers, and can be performed in the afternoon. The Tomorrow pollination technique is more sophisticated, requires greater skill for routine and efficient use and is more time consuming. It is beneficial when blooming is insufficient for day pollination. Combining open flower and tomorrow pollination, a worker can pollinate three times the number of flowers in one day. This requires one day of work every three days. More flowers can be processed per day by combining the two techniques: direct hand pollination in the morning and tomorrow pollination in the afternoon. Hold the base of a bud which is ready to flower within two days between the thumb, fore finger and middle finger rotating to the lower side. This is the side that will become the upper one following resurpination. Introduce a needle between the two sepals and separate them; tear open the labellum. Push the extremity of the bud to open it and expose the gynostemium (column), pollinia and rostellum. Excise the rostellum and remove with the needle tip. It is important to make sure that at this point the flower is not pollinated. Natural pollination will take place during the following day(s) after anthesis, when the pollinia will come into contact with the stigma after a backward folding of the anther.

According to Shadakshari *et al*. (1994) three methods are currently being used in hand pollination of vanilla: i) using a small pointed stick of tooth pick size, sharpened at both ends to transfer pollen from pollen sac to stigma, ii) using a thin flat stick to press the rostellum upward and pressing the anther cap with thumb to dislodge the pollen mass on the sticky stigma, iii) using cotton buds to transfer pollen from anther cap to stigma.

A new method of hand pollination has been perfected in *Vanilla planifolia* by Shadakshari *et al*. (1994). A fine tipped forceps of about 10

cm length is used in this method. The vanilla flower is held in the left hand and the rostellum pushed upwards beneath the anther cap by the right wing of the forceps held in the right hand. Later by using both wings of the forceps, the anther cap is pressed down to drop the pollen mass on the stigma. The rostellum is then freed to occupy its original position. This further holds the pollen mass firmly on the stigma.

According to Purseglove *et al*. (1981) flowers open from the base of the raceme upwards and usually one or more rarely two or three flowers open in an inflorescence one day. The flowers open early in the morning. They are receptive for eight hours and wither the following day. Sasikumar *et al*. (1992) reported that the ideal time for pollination is 6 am to 1 pm. Artificial pollination was found easy before 1 pm and thereafter the flowers loose their turgidity which makes hand pollination cumbersome (Kuruvilla *et al*., 1996). Hand pollination after closure of flowers by opening the closed flowers showed that flowers pollinated after 6.00 pm dropped indicating that pollination was effective from 1.15 am. to 6.00 pm only (Shadakshari *et al*., 1996). Fruit setting percentage was the maximum when pollinated at 8 am and showed a decreasing trend as pollination got delayed (Bhat and Sudharshan, 2000a).

A direct positive correlation between the amount of pollen transferred to the stigma and fruit growth was reported by Bhat and Sudharshan (2000b). Their study revealed that fruit growth was the maximum when 100 percent of the pollen mass was transferred to stigma at the time of pollination. The fruit length and girth got reduced considerably when less than 50% pollen mass was transferred. From this study, it is observed that the pollinators need to be trained to carry out pollination effectively by transferring complete pollinia to the stigmatic

surface to get maximum growth. The failure to transfer complete pollen mass to stigmatic surface during hand pollination could be one of the main reason for the differences observed in size of beans between vanilla gardens and to some extent between the different vines within the same garden (Bhat and Sudharshan, 2000b).

From a study on pollen viability and stigma receptivity Bhat and Sudharshan (2003) concluded that pollination could be done successfully in the flower bud even from 23 hours before opening. Since buds can be pollinated, the bud pollination technique can be adopted successfully in future pollination programmes in addition to already existing open flower pollination technique.

It seems that pollination has a stimulating effect in ovary prior to fertilization. Once fertilization takes place, the ovary elongates rapidly. In the failure of pollination, the flower falls within a day or two (Madhusoodanan *et al*., 2003).

According to Ridley (1983) better results may be obtained when pollen of another flower is used for fertilization (cross pollination). It is the natural method of fertilization, but it takes a little longer time and there is certainly the risk of dropping the pollen and or being accidentally brushed off during the transfer. But it would probably produce larger and finer fruits. After successful fertilization, the flower quickly commences to wither and soon the sepals and petals fall off but the column remains attached to the top of the ovary which is below the petal and so remains till the fruit is nearly ripe. If the operation has failed and the flower is not fertilized the column falls with petals. If a flower is fertilized after 3 pm (after starting to wither), the column falls off earlier and the fruit will be

shorter. In heavy rainfall, pollen masses become soft and though fruits may develop, they are not so large or fine and the column soon falls off. This may be due to spoilage of pollen grains as a result of withering of flowers or by rain resulting in the fertilization of very little amount of ovules.

A study conducted by Muhammed Nissar *et al*. (2006) indicates that pollination can be done with much success between 6 am and 12 noon. Their study revealed that cross pollination showed more fruit setting (94%) than self pollination (85%) and hence suitable techniques have to be evolved to carry out cross pollination for commercial production of beans.

2.10.7. Stigma receptivity and fruit set

Kuruvilla *et al*. (1996) reported that the stigma is highly receptive during morning hours. The flowers pollinated till noon had higher percentage of fruit set than those pollinated during afternoon. Their studies revealed that artificial pollination in vanilla till 2 pm resulted in better bean formation (>60%). George (1981) and Sasikumar *et al*. (1992) reported the ideal time for pollination as 6 pm to 1 pm. Shadakshari *et al*. (1996) observed that the stigma was receptive from 1.15 am to 6.00 pm and they suggested that hand pollination from 6.00 am to 11.00 am was ideal for quick and effective pollination. They could observe effective fertilization and fruit set in all the flowers when hand pollination was resorted to immediately after opening of flower around 3 pm at an interval of 30 minutes.

According to Bhat and Sudharshan (2000a), fruit setting percentage was maximum at 8 am (100%) and 66.7% at 6 am. After 8 am fruit setting percentage decreased gradually with 25% of fruit set at 6 pm.

Stigma receptivity in *Vanilla planifolia* has been reported to last for 24 hours (Torregrossa, 1989). However, Shadakshari *et al*. (2003 a & b) reported that stigma receptivity period was between 41 hours before complete flower opening and 17 hours after complete flower opening under hill zone conditions.

Observations on stigma receptivity by Bhat and Sudharshan (2003) showed that pollen from fresh opened flower was able to fertilize the buds, 40 hours before opening. Pollen of the same flower was able to set fruits upto 16 hours after opening of flowers. This indicates that stigmatic receptivity commenced 40 hours before anthesis and it lasted for 16 hours after anthesis. Thus, in *Vanilla planifolia* the total period of stigmatic receptivity is 56 hours. Natural fruit set has been reported in *Vanilla wightiana* Lindl., by Rao *et al*. (2000).

2.11. Fruits and seeds

The fruit of vanilla is a dehiscent capsule commercially known as bean. The bean is pendulous, narrowly cylindrical, obscurely three angled, 10-25 cm long and 5 to 15 mm in diameter. Pollination has a stimulating effect upon the ovary prior to fertilization. Once fertilization has taken place, the ovary elongates rapidly for 45 days until the full length and girth of the bean is attained (Madhusoodanan *et al*., 2003).

Bhat and Sudharshan (2000a) reported that at the time of pollination, the mean length and girth of the ovary was 5.08 cm and 1.29

cm, respectively. After pollination, pods had fast growth for 45 days and thereafter it declined. No increase in the length and girth of beans was observed beyond 75 days. Fruits were ready for harvest, 268 days after pollination. On an average each fresh bean weighed 15.2 g. Bhat and Sudharshan (2002) and Muhammed Nissar *et al*. (2006) also reported the growth period of vanilla beans as 75 days. Maturity is indicated by the yellowing of the beans starting from the distal end (Madhusoodanan *et al*., 2003). The harvesting period varies according to altitude and latitude and differs from one country to the other (Theodose, 1973).

Parthenocarpic development of pod can be induced in vanilla with growth regulating chemicals though they are poor both in quantitative and qualitative characters (Gregory *et al*., 1953).

Mature beans contain thousands of minute globose black seeds about 0.3 mm in diameter which are liberated by the longitudinal splitting of the capsule. As in other orchids, the seeds will not germinate under normal conditions. But now methods have been perfected for *in vitro* seed germination (Madhusoodanan *et al*., 2003).

Mature seeds of tropical *Vanilla* species and hybrids survive at least three years on synthetic media, germination occurring only when appropriate high temperature and low light levels are present (Knudson, 1950; Lugo, 1955). Knudson (1950), Lugo (1955) and Withner (1955) have reported that the sclerotic nature of seeds is not a strong barrier to germination.

Knudson (1950) was the first to produce hybrid seedlings of vanilla through seed germination. He reported that exposure of seeds to

green house conditions before incubation resulted in a higher percentage of germination. Nitrogen concentration of the culture medium was found to be the critical factor in the germination of vanilla seeds (Lugo, 1955).

Orchid seeds are characterized by their minute size and lack of any storage tissues, which is required during germination of seeds and development of seedlings. In nature, association with specific fungal partner is a pre requisite for orchid seed germination (Arditti, 1967). It has been suggested that vanilla seeds should be inoculated with *Rhizoctonia repens* to ensure the survival of seedlings. With modern techniques this would appear to be unnecessary (Purseglove *et al*., 1981).

Ridley (1983) has quoted a method for seed germination used by Dupont in 1902 at Government Botanic Station of Seychelles with alcohol treatment as highly successful. Fully ripened pods were allowed to blacken and seeds when removed were soaked in alcohol for 24 hours and shortly afterwards were thoroughly washed and planted. The seedlings are reported to have grown normally.

Seeds could successfully be germinated *in vitro* from the fourth month of fruit setting onwards. However, seeds harvested in the 6^{th} month and cultured *in vitro* gave the maximum (87%) germination according to Minoo (2002).

2.12. Cultivation of vanilla

Vanilla needs a warm and wet tropical climate. It grows well on a variety of soils, ranging from sandy loam to laterites. A moderate slope and good drainage aids its easy establishment. It requires partial shade. Being a climber it requires support to a height of about 150 cm. The

growth of standards is to be adjusted and shade regulated. Cuttings of 1 metre length with several nodes are used for planting. They are planted in rows at 2.7 m x 1.8 m distance. About 1500 vines are planted in a hectare. As the vines reach a height of 1-1.5 m they should be twisted or coiled on lateral support of branches. Horizontal coiling of vines gives profuse flowering. Vanilla vines begin yielding from the third year. The yield goes on increasing for a decade, and then declines (Madhusoodanan and Radhakrishnan, 2004).

Once established, the plantation has to be given constant attention. It should be frequently visited to train the vines to grow at a convenient level, prune the growing vines and tree supports, watch for diseased plants and to keep a watch on the surface of the ground, especially over the roots of the plants. Cultivation of the ground is not feasible since the roots grow at or near the surface and any disturbance of the soil would damage them (Potty and Krishnakumar, 2003).

In extremely dry years, irrigation should be provided at least once in four to five days (Potty and Krishnakumar, 2003). Mulching should be done two to three times a year. Coffee husks, banana leaves, sugarcane bagasse and any plant material make good mulch (Ranadive, 2003). Mulching with mango leaves (25 kg per vine per year) and watering once in four days was found to increase vanilla yield (Muraleedharan, 1975). Being a climber, vanilla requires a support or standard for climbing. Low branching trees with rough bark and small leaves are preferred as support trees. If the support tree selected is a leguminous one it can add nitrogen to the soil. A few of the common support trees suitable are *Glyricidia*, *Plumeria*, *Casuarina*, mulberry and *Erythrina* ((Madhusoodanan and

Radhakrishnan, 2001), *Garcinia maculata* (Elizabeth, 2002), *Coffea liberica* and *Artocarpus heterophyllus* (Muthuramalingam *et al*., 2004).

Support trees are planted at least six months prior to planting of vanilla for successful establishment (Madhusoodanan and Radhakrishnan, 2001). Judiciuos lopping of branches of the living supports is very important to give shade to vanilla plants (Potty and Krishnakumar, 2003). It was suggested by Childers *et al*. (1959) that the existing shades and trees should be pruned to admit as uniformly as possible, 30-50 percent sun light. It may be desirable to adjust the amount of light falling on the vanilla plants according to the season allowing more light during the flower bud formation and blossoming period.

Vanilla can also be grown on stone pillars inter connected with wires covered with PVC pipes or drip irrigation pipes. If it is grown in the open area on pillars, it is necessary to supply some form of partial shade by using shade nets (75% to 50% shade depending upon the stage of growth of vanilla vines) or by growing shade forming plants like banana which also gives side income (Hegde and Yadu kumar, 2002).

Vanilla can be cultivated as an intercrop in coconut, arecanut and similar other farms under filtered sunlight (Madhusoodanan and Radhakrishnan, 2001; Elizabeth, 2002). Vines are planted at the base of arecanut or coconut plants, allowed them to grow up to 1.5 m height on the stems of those plants and later, providing 1 m long sticks or clamps parallel to the ground tied at 1.5 m height on the stems of the palms and training on to them making vines hang down, which is congenial for flowering. Between the palms also, vanilla vines can be planted at 2 m to 3 m distance. In coconut and arecanut plantations, 50% light penetrates

through canopies and hence here vanilla can come up well under 50% shade (Hegde and Yadu kumar, 2002).

Studies conducted at Chikmagalore in Karnataka to investigate the economic feasibility of vanilla cultivation in coconut gardens revealed the viability of mixed cropping of vanilla in coconut gardens in plains and low rainfall areas under irrigation (Korikanthimath *et al*., 1999). In areas with optimum physiographical conditions such as high elevation (450-725 m above MSL), high humidity and uniform rainfall, vanilla could be a successful intercrop in coconut gardens (Subbiah *et al*., 2002). Nybe *et al*. (2004) reviewed the possibility of vanilla as one of the intercropping components under high density multispecies cropping system (HDMCS) in coconut plantations. Sefanaia *et al*. (1982) suggested vanilla as an intercrop in the coconut gardens of Fiji. Muthuramalingam *et al*. (2004) suggested vanilla as a golden crop in coffee plantations of Karnataka. Vanilla adoption in an already existing coffee farming system in Indonesia, illustrates how agroforestry can make tropical agriculture more sustainable by enabling its rapid adaptation to ecological and market changes through diversification and replanting (Ruf *et al*., 2004).

The way in which the vine is trailed has been observed to have an effect on flowering (Childers *et al*., 1959). Khan (1963) recommended to trail the vines erect to a height of 4 feet and then trailing them horizontally. He also reported a definite tendency in the vines to maintain an empty vegetative growth if they were not subjected to the coiling/ hanging treatment which was essential to promote flowering and fruiting. A field experiment was conducted at the Central Horticultural Research Station, Ambalavayal, Kerala for 8 years starting from 1960-61, to find out a suitable method of trailing vanilla vines. It was observed that trailing

the vines on dead wood posts to a vertical height of 6 feet and then trailing them horizontally on wooden trellis was better than trailing the vines on *Plumeria* standards to a height of 4 feet and then trailing or looping horizontally on wooden trellis. However this method required frequent replacing of posts and control against white ants in addition to the difficulty in pollinating the flowers from ground (Muraleedharan *et al*., 1974).

From the time of initial planting, the vines attached to the support are to be allowed to grow upward. However if they are permitted to grow up on the support tree itself, they will rarely blossom so long as they are growing upward. Moreover hand pollination will also be very difficult to be carried out. Therefore, the vines are allowed to grow up to a height of 1.2 to 1.5 metres and allowed to hang down on the branches. Such vines should be brought back to the ground, and a portion of it is placed under the mulch. Later it is coiled again on the same support tree. Thus the vine should be looped up and down adjusting the height in such a way that it is not more than the shoulder height of workers. This makes operations such as pollination, harvesting and pruning easy and will make the crop more productive. Vanilla vines can be trained from one support tree to the other within the same row by planting additional supports in between or on horizontal bars connecting two main support trees (Madhusoodanan and Radhakrishnan, 2001).

In coconut and arecanut plantations, farmers initially allow the vines to grow on the stem of these plants and later after reaching sufficient height before reaching the crown of the plant, vines are removed slowly by separating aerial roots touching the stem and coiling the vines without damaging. Such coiled vines are brought down without cutting and at 1.5

m height of the coconut or arecanut, vines are coiled on all the four sides of the clamps with hooks fixed (Hegde and Yadu kumar, 2002).

The main source of nutrients to vanilla is decomposed mulch (Muthuramalingam *et al*., 2004). Easily decomposable organic matter is applied around the plant base three to four times in a year. Spraying one percent 17:17:17 NPK complex on the foliage and stem has been found to be beneficial to enhance the growth of vines and for flower production (Madhusoodanan and Radhakrishnan, 2001; Elizabeth, 2002). Application of inorganic fertilizers at the rate of 40-60 g N, 20-30 g P_2O_5 and 60-100 g K_2O per vine per year in 2-3 split doses for efficient uptake is advisable according to Muthuramalingam *et al*. (2004) and Elizabeth (2002). Vanilla loves a lot of organic matter and plant basins should be mulched 3-4 times a year and timely irrigation should be provided. Organic manures such as vermicompost, oil cakes, poultry manure and wood ash are also applied (Elizabeth, 2002).

In a green house experiment, *Vanilla planifolia* cuttings were planted in poly bags of soil (1kg) to which 0.2 g urea, 0.05 g triple super phosphate and 0.08 g KCl per plant were applied alone or in combination at 0, 1, 2 or 3 weeks after planting. Application of N alone or of NPK gave the largest plants in terms of both weight and height (Asnawi, 1988). Rosman and Tasma (1988) conducted an experiment on the effect of farmyard manure rate on the growth of vanilla cuttings. Vanilla cuttings (2 nodes) with or without leaves were planted in soil+FYM mixtures of ratios 1:0, 1:1, 2:1, 3:1 or 4:1. Growth of cuttings in terms of the number of leaves produced and bud length at 3 or 4 months after planting was best in 1:1 soil+FYM mixtures and from cuttings with leaves retained.

In a trial carried out at the Regional Research Station, Indian Cardamom Research Institute, Sakleshpur, Karnataka, India in 1994, nine media were evaluated for rooting vanilla cuttings (20 cm in length with 3 nodes). Number of sprouts, sprout length, number of leaves per vine, leaf area per vine, number of roots and root length were assessed over 8 months. The best results were obtained with vermicompost or decomposed coir pith (Siddagangaiah *et al*., 1996).

A study was conducted in 1993-2000 in Kerala, India, to evaluate the performance of vanilla under different nutritional management practices: T1: control; T2: farmyard manure at 5 kg per plant; T3: NPK at 40:20:60 g per plant; T4: N:P:K at 60:30:80 g per plant; T5: urea 2% + superphosphate (SSP) 1% + muriate of potash (MOP) 3%; T6: urea 3% + SSP 2% + MOP 4%; T7: T3 (at 50% rate) + T5 (at 50% rate); and T8: T4 (at 50% rate) + T6 (at 50% rate), T5 resulted in the highest mean number of beans per plot followed by T7. T5 also produced the highest mean weight of beans in 1998 and 2000. In 1999, mean weight was the highest under the T7 treatment. The performance of plants in T6 was poor compared to the other fertilizer treatments with lower rates. T5 and T7 plants showed better growth in terms of vine length, number of nodes and yield than the other treatments (Krishnakumar and Potty, 2003).

Nutritional studies carried out at the Indian Cardamom Research Institute has indicated that vanilla yield can be enhanced by soil application of 20:10:30 g NPK per vine per year and foliar application of urea, single super phosphate and muriate of potash at the rate of 1.0%, 0.5% and 1.5% respectively during January, May and September (Potty and Krishnakumar, 2003).

An experiment on integrated nutrient management in vanilla was carried out at Horticultural Research Station, Thadiyankudisai situated in the Western Ghat region of India during 2001-2002. Experimental results revealed that the application of 25 g each of VAM, *Azospirillum* and *Phospobacteria* along with 100 g of NPK per vine per year recorded the maximum bean yield of 934 g per vine and maximum quantity of first grade vanilla beans.

2.13. Diseases and pests

As the extent of cultivation increased, the crop became susceptible to a number of fungal and viral pathogens which seriously affected the production of the crop (Suseela Bhai *et al*., 2006). Being succulent in nature, it is easily vulnerable to many of the plant pathogenic fungi, some of which causing destructive diseases such as bean rot due to *Phytophthora meadii* and *Sclerotium rolsfii* (Joseph Thomas and Suseela Bhai, 1999; Suseela Bhai and Joseph Thomas, 2000), root rot due to *Fusarium oxysporum* and *Phytophthora meadii* and stem rot due to *Fusarium* sp. (Joseph Thomas *et al*., 2003). Recently a new disease characterised by yellowing and bean dropping was reported from most of the vanilla plantations in lower elevations of Kerala and Karnataka States and *Colletotrichum vanillae* was involved in causing this (Suseela Bhai *et al*., 2006).

Viruses are known to cause highly significant yield reduction in many vanilla growing countries of the world (Pearson *et al*., 1991). Five different viruses belonging to three genera have been reported on vanilla from different parts of the world namely, Cucumber Mosaic Virus (CMV) (Wisler *et al*., 1987; Farryrol *et al*., 2001; Madhubala *et al*., 2005), Cymbidium Mosaic Virus (CyMV) (Wisler *et al*., 1987; Benzet *et al*.,

2000), Odontoglossum Ring Spot Virus (ORSV) (Pearson *et al*., 1991; Wisler *et al*., 1987; Pearson *et al*., 1993), Vanilla Mosaic Virus (VMV) (Wisler *et al*., 1987; Zettler and Wisler, 1990) and Vanilla Necrosis Virus (VNV) (Pearson and Pone, 1988; Wang *et al*., 1993). Besides, two uncharacterised viruses belonging to Poty Virus and Rhabdovirus genera have been reported (Pearson *et al*., 1991). Three poty Virus species have been reported in vanilla in the Pacific area: (i) Vanilla Mosaic Virus, a tentative species closely related to Dasheen Mosaic Virus (DsMV) that only infects *Vanilla* spp. and which is referred as DsMV- Van (Wisler *et al*., 1987); (ii) Watermelon Mosaic Virus (WMV), initially described on vanilla as Vanilla Necrosis Virus (Pearson *et al*., 1990; Wang *et al*., 1993); and Bean Common Mosaic Virus (BCMV) (Grisoni *et al*., 2004). Pathogenic Poty viruses that did not react with anti Van MV or anti WMV antibodies were also reported in vanilla in Tonga (Liefting *et al*., 1992). A mosaic inducing Poty Virus has been reported from India (Joseph Thomas *et al*., 2002). The first report on the occurrence of mosaic disease of vanilla from India was made by Suseela Bhai *et al*. (2003). Using the technique of direct sequencing of a short RT-PCR amplicon, Grisoni *et al*. (2006) identified four additional Poty Virus species that may infect vanilla: Bean Yellow Mosaic Virus, Cow Pea Aphid Borne Mosaic Virus, Ornithogalum Mosaic Virus and Wisteria Vein Mosaic Virus.

Many species of insect and non insect pests are reported on vanilla in India and in other countries (Purseglove *et al*., 1981; Anonymous, 2000; Prakash and Sudharshan, 2002 and Varadarasan *et al*., 2002). Survey conducted by Indian Cardamom Research Institute in vanilla plantations of South India indicated incidences of a few pests infesting vine, shoot tip, flower buds and roots (Varadarasan *et al*., 2003). Among the insect pests that damage vanilla, a hemipteran bug, a lepidopteran caterpillar and a

coleopteran weevil cause condiderable damage. The vanilla bug, *Halyomorpha picus* (Prakash and Sudharshan, 2002), the emrald bug, *Nezara viridula* (Varadarasan *et al*., 2002), *Triosa litseae* (Ridley, 1983), the aphid, *Cerataphis lataniae*, scale insects (unidentified) (Varadarasan *et al*., 2002), *Memmia vicina* (Varadarasan *et al*., 2003), *Riptortus pedestris* (Varadarasan *et al*., 2002) and *Cicada* (Duffels, 1988) are the hemipteran pests.

Six species of coleopteran insect pests have been recorded in vanilla. Among them, a weevil (vanilla vine weevil, *Sipalus* sp.) causes serious damage. *Perrissoderes oblongus* and *Perrissoderes ruficollis* are the other weevils (Varadarasan *et al*., 2002; Varadarasan *et al*., 2003).

Longicorn beetles, *Hoplia retusa* and *Enaria malanichtera* (Varadarasan *et al*., 2003), *Saula ferruginea* (Rai and Nayar, 1976), white grub and black weevil (Varadarasan *et al*., 2003) are the other Coleopteran pests found in vanilla.

Lepidopteran pests such as the caterpillars *Plusia aurifera*, *Conchylis vanillana* and *Simplica inarcualis* infest vanilla and feed on the young vegetative shoots (Varadarasan *et al*., 2003).

Non insect pests like snails, slugs and avian pests cause considerable damage to vanilla (Varadarasan *et al*., 2003). The mite, *Tyraphagus* sp. is found to infest beans in storage in India (Sasikumar *et al*., 1992). Varadarasan *et al*. (2005) reported that the causal factor for scab formation on vanilla beans was the mechanical damage during early stage of development of the bean and not thrips or mites. *Helopeltis theivora* has also been reported from vanilla (Prakash *et al*., 2006).

2.14. Curing

Fully matured beans (when yellow tinge appears at the distal end) are harvested for curing. Curing involves killing the beans within 5 days of harvesting to initiate enzymatic action for the development of vanillin. After harvesting, the beans are dipped in hot water (63^0C) for 3-5 minutes and then water is drained out. The beans are then raked in a woolen blanket to ensure heat retention and kept for the rest of the day in sweating boxes lined with blankets. The beans start acquiring chocolate brown colour by the following day. Raising of temperature (known as sweating) enhances enzymatic action in beans. They are then spread out in sun on dark coloured cotton for 3-4 hours and then rolled up to retain the heat. This is repeated for 6-8 days during which the beans lose some weight. Later, the beans are slow dried by spreading out in trays under shade in an airy location for over a month. By this time the beans turn dark brown in colour. Thereafter beans are bundled and stored in air tight containers for conditioning. Vanillin crystals often appear on the surface of the beans. Top grade beans should have 18-24 cm length, 25-30% moisture and above 2.4% vanillin and should be free from any sort of scars (Madhusoodanan and Radhakrishnan, 2004).

Sreedhar *et al*. (2007a) reported that specific pre treatments were found to reduce the curing period of vanilla beans. Based on the differences in the process of curing and the nature and source of the beans, different curing methods have been suggested. But all of them involve the four steps namely killing, sweating, slow drying and conditioning (Theodose, 1973). The method of curing discussed above is known as Bourbon method and the product obtained is called Bourbon vanilla. Other types of vanilla available in the market are Mexican vanilla produced by Mexican method of curing, Guianan vanilla produced by Guiana method

of curing and Peruvian vanilla produced by Peruvian method of curing (Purseglove, 1981).

A laboratory model curing has been described by Dignum *et al*. (2002) in which the cured vanilla beans are analyzed for enzyme activity and aroma. Studies on the botany of vanilla beans revealed that flavour precursors are found in the bean interior, where they are secreted onto the placental region around the seeds, whereas hydrolytic or other degradative enzymes which catalyze the release of the flavour precursors to flavour compounds are localized mostly in the outer fruit wall region. This suggests that the objective of killing, the first curing stage carried out by hot water scalding, freezing or by other methods, is to disorganize the bean tissue, such that contact is created between substrates and their respective enzymes. Sweating, a subsequent step in curing, entailing high temperatures and high humidity, provides conditions for enzyme catalyzed production of flavour compounds and also for non enzymatic reactions. The objective of the final curing steps, including drying and conditioning is to dry the cured beans so as to preserve the formed flavour compounds (Havkin- Frenkel *et al*., 2004).

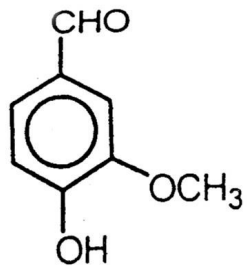
2.15. Phytochemistry

Vanillin, which contributes to about 2% of dry matter, is organoleptically the characteristic aroma component of the cured vanilla pod (Priefert *et al*., 2001). Chemically it is 4-hydroxy-3-methoxy benzaldehyde ($C_8H_8O_3$) (Fig. 2.1.) (Ramawat and Merillon, 1999). Isolated vanillin appears as white needle like crystalline powder with an intensely sweet and very tenacious creamy vanilla like odor. It is used in a broad range of flavours for foods, confectionary and beverages, as a fragrance ingredient in perfumes and cosmetics and for pharmaceuticals

(Priefert *et al*., 2001). Vanillin content of the cured product highly depends on the source of the crop, the species and the curing process. Moisture content of the cured product also has got a correlation with vanillin content (Vadiraj *et al*., 2002). Ranadive (1992) analysed vanilla beans from seven different vanilla growing regions of the world phytochemically, using extracts obtained from cured and uncured beans treated with β-glucosidase, and observed that the compounds like vanillin, p-hydroxybenzoic acid, p- hydroxybenzaldehyde and vanillic acid are present in green beans as glycosides and are released upon curing. Time of harvesting has also got influence on vanillin content (Larcher, 1989).

Leong *et al*. (1989) conducted a preliminary study for the synthesis, identification and determination of glucosides present in green vanilla beans and they could find out the presence of glucovanillin and other glucosides in it. The glucosides of vanillin, vanillic acid, p-hydroxybenzaldehyde and p-hydroxy benzoic acid were then synthesized and their structures confirmed by mass spectrometry and 13C–NMR spectroscopy. A reversed phase (C-18) HPLC method, using a photodiode array detector, enabled separation and characterization of the authentic substances and this method was applied to the determination of these glycosides in green pod extracts of various origins.

Fig. 2.1. Chemical structure of vanillin



Vanillin

Taylor (1993) perfected an improved technique for the determination of vanillin and related phenolic components in *Vanilla planifolia* by High Performance Liquid Chromatography. The compounds were separated on a silica column from ethanolic extracts of vanilla pods, bonded with penta fluorophenyl, using a mobile phase gradient of methanol-citric acid with diode-array detection at 275 nm. Vanillin, vanillic acid, p-hydroxy benzoic acid and p-hydroxy benzaldehyde were identified by the spectral data and retention times given by reference compounds. This worker has opined that this HPLC technique allows a more accurate mean of determining the vanillin content of vanilla than the ISO spectrophotometric assay.

Scharrer and Mosandl (2001) analysed the major components vanillin, vanillic acid, 4-hydroxy benzaldehyde and 4-hydroxy benzoic acid in 15 vanilla (*Vanilla planifolia*) pod samples of different origin and years of harvest. The pods were extracted with ethanol/water and with diethyl ether, respectively. Using ethyl vanillin and veratraldehyde as internal standards new HPLC and GC methods were developed and utilized to establish authenticity profiles. Extracting efficiency for vanillin was lower when diethyl ether was used.

Kaunzinger *et al.* (1997) discussed the genuineness of vanilla with regard to integral authenticity evaluation of vanillin and some characteristic minor compounds such as 4-hydroxy benzyl alcohol, vanillic acid, 4-hydroxy benzaldehyde, anisic alcohol, anisic acid and 4-hydroxy benzoic acid based on isotopic data as well as GC quantification of compounds (capillary GC on-line coupled with isotope ratio mass spectrometry). Authenticity profiles for *Vanilla planifolia* and *Vanilla tahitensis* were presented. Such integral authenticity profiles are new and have got promising perspectives in the authenticity evaluation of genuine vanilla.

John and Jamin (2004) reported that representative and validative samples taken from a 500 acre vanilla (*Vanilla planifolia* Andrews) plantation in India have shown significant deviations in aromatic profile, especially the relative amounts of vanillin (high) and p-hydroxybenzaldehyde (low) and the deuterium isotopic (SNIF-NMR) values when it was subjected to chemical investigation. However the carbon isotopic values (carbon 13 profiles) were generally in accordance with the previous findings on vanilla from other geographical regions.

When compared with the results obtained from beans of *Vanilla planifolia*, the Tahitian (*Vanilla tahitensis*) beans contained relatively low amounts of vanillin and vanillic acid, relatively high amounts of p-hydroxy benzoic acid and considerable amounts of anisic acid and anisyl alcohol (Ehlers *et al*., 1994). Ehlers and Pfister (1997) opined that, the typical heliotropin-like odour associated with *Vanilla pompona* was due to piperonal (heliotropin). The pods contained p-hydroxy benzoic acid, vanillic acid, p-hydroxy benzaldehyde, p-anisyl alcohol, p-anisic acid and p-anisaldehyde in addition to vanillin. Their chemical composition

resembled Tahiti vanilla (*Vanilla tahitensis*) more than Bourbon vanilla (*Vanilla planifolia*).

Analysis of the neutral lipids from the two vanilla species, *Vanilla planifolia* and *Vanilla tahitensis*, resulted in the identification of a new product family in this genus: long chain gamma pyrone compounds with an aliphatic chain containing a cis double bond at the n-9 position. These compounds represent 7-8% of the neutral lipids in mature beans (Ramaroson *et al*., 1999). Fifteen esters including two new natural compounds were separated and identified as volatile compounds from ground vanilla beans by Werkhoff and Guntert (1997).

Odoux *et al*. (2003) reported that the radial distribution of glucovanillin and β-glucosidase activity measured on p-nitrophenyl, β-glucopyranoside and glucovanillin are superimposable and show how β-glucosidase activity increases from the epicarp towards the placental zone, whereas glucovanillin is exclusively located in the placentae and papillae.

Ruiz- Teran *et al.* (2001) extracted glucovanillin from green pods and simultaneously transformed it in to vanillin by a combination of enzyme activities involving cell wall degradation and glucovanillin hydrolysis.

Efforts have been made for the biotechnological production of vanilla flavour metabolites by plant tissue/cell culture, microbial biotransformation and molecular approaches (Rao and Ravishankar, 2000). Alternative biotechnology based approaches for the production of vanillin are based on bioconversion of lignin, phenolic stilbenes, iso eugenol, eugenol, ferulic acid or aromatic amino acids and *de novo*

biosynthesis applying fungi, bacteria, plant cells or genetically engineered microorganisms (Priefert *et al*., 2001).

2.16. Crop improvement

Intensive programmes of conservation, selection and breeding are required to overcome the narrow genetic base of vanilla (Minoo *et al*., 2006b). Exploration of genetic variability and its exploitation for desirable traits such as yield and tolerance to biotic and abiotic stresses are targeted primarily in the varietal improvement programmes (Kuruvilla *et al*., 2004). The efforts carried out so far towards the collection and conservation of germplasm, study of genetic variability and efforts for genetic upgradation are reviewed below.

2.16.1. Germplasm

Although vanilla is cultivated throughout the tropics, its natural populations in Southern Mexico, the most critical sources of novel genetic diversity, are on the verge of disappearing due to deforestation and over collection (Lubinsky, 2003). The primary gene pool of *Vanilla planifolia* is heavily threatened in its centre of origin by habitat fragmentation and destruction (Grisoni *et al*., 2007). Since the narrow primary gene pool is evidently threatened, the secondary gene pools which are also equally threatened become important as sources of desirable traits (Rao *et al*., 2000; Minoo, 2002). In addition, *ex situ* conserved material is often endangered by viruses such as Cymbidium Mosaic Virus that easily spread by contact in plant collections.

Since the introduction of *Vanilla planifolia* to India, the crop has undergone selection processes for adaptation to various ecological niches and this would have perhaps resulted in the evolution of many

morphotypes/ecotypes. In early 1990s, a survey was undertaken by Indian Cardamom Research Institute (ICRI), Spices Board, India, to various habitats to collect germplasm accessions of vanilla. A total of 21 accessions of *Vanilla planifolia* were collected and deposited in the clonal repository of the institute and characterized based on morphological and phytochemical characters. A few allied species like *Vanilla tahitensis*, *Vanilla andamanica*, *Vanilla wightiana* and *Vanilla walkeriae* were also collected (Kuruvilla *et al*., 2004). Bhat and Sudharshan (2004) have reported that ICRI regional station at Sakleshpur maintains 56 accessions of *Vanilla planifolia*. Ravindran (1999) has reported that Indian Institute of Spices Research, Calicut, India maintains a germplasm collection of 45 accessions of vanilla. Recent advances in conservation have paved the way to safeguard plant biodiversity with a biotechnological approach, which can be regarded as complementary to the traditional clonal farms and seed banks. Effective procedures for micropropagation and *in vitro* conservation by slow growth in selected species of *Vanilla* have been developed by Minoo *et al*. (2006b). Synthetic seed technology and technology to maintain shoot cultures for more than one year without sub culture on slow growth medium were also standardized by them.

A germplasm collection of *Vanilla* species and varieties has been set up by CIRAD (Centre de Cooperation Internationale en Recherche Agronomique pour de Developpement) in Reunion Island since 2003. This collection gathers 250 accessions tentatively classified into 22 species. Most of the material belongs to the species *Vanilla planifolia* and originates from the south western Indian Ocean area. The tropical and contrasted climatic conditions of Reunion Island enable blooming and fruiting of most accessions, thus facilitating taxonomic identification and agronomic evaluation (Grisoni *et al*., 2007).

2.16.2. Variability

Continous clonal propagation has resulted in the existence of very little variability for crop improvement (Minoo *et al*., 2006b). Rao *et al*. (1993a) attempted to assess the genetic variability through poly acrylamide gel electrophoretic (PAGE) studies in six indigenous collections of *Vanilla planifolia* Andrews of ICRI. The study was undertaken with tissues from leaves and aerial roots. Qualitative and quantitative variabilities were encountered for esterase isozymes whereas no variability was observed for peroxidases and amylases.

Lubinsky (2005) has reported the results of a recent RAPD survey of cultivated *Vanilla planifolia* in Reunion which shows that the entire stock of cultivated *Vanilla planifolia* in the country consists of a single clone.

Schluter *et al*. (2007) investigated the RAPD genetic diversity and geographical structure within *Vanilla planifolia*. Multivariate analysis revealed three separate geographical groups.

Lubinsky and Kim (2007) reports that AFLP analysis of wild and cultivated accessions revealed low levels of diversity in cultivars. This research has identified unique genotypes of *Vanilla* in natural populations in southern Mexico, the most critical sources of novel genetic diversity.

Besse *et al*. (2004) conducted genetic diversity studies in cultivated vanilla from Reunion islands using RAPD markers. *Vanilla planifolia*, *Vanilla tahitensis* and *Vanilla pompona* could be neatly separated. Low levels of genetic diversity were detected in *Vanilla planifolia* in the

cultivation areas. Species specific RAPD markers were selected and used successfully to analyse putative *Vanilla planifolia* x *Vanilla tahitensis* hybrid specimens. It was also suggested that *Vanilla tahitensis* is probably not a species of direct hybrid origin (*Vanilla planifolia* x *Vanilla pompona*) but rather a species related to *Vanilla planifolia.* But Lubinsky and Kim (2007) have stated that on the basis of morphological characteristics, *Vanilla tahitensis* can be hypothesized to be a hybrid of either a recent provenance between *Vanilla planifolia* and *Vanilla pompona* or an F1 hybrid between *Vanilla planifolia* and *Vanilla odorata* Presl.

Duval *et al.* (2006) studied the interspecific and intraspecific diversity in vanilla using RAPD markers. Data analysis displayed a well structured diversity following the division between *Vanilla planifoila* and *Vanilla tahitensis*, the Mexican and oceanic species. A species endemic to Brazil, *Vanilla bahiana*, appeared the closest species to cultivated vanillas. There was no support to the hypothesis of a hybrid interspecific origin for *Vanilla tahitensis*, which appeared close to *Vanilla planifolia*. Among the three cultivated species, *Vanilla pompona* displayed the highest intra specific variability, accordingly to its wider distribution. Diversity levels were low within the two main cultivated species *Vanilla planifolia* and *Vanilla tahitensis*. The genetic diversity is probably due to the accumulation of punctual mutations arising from a few original introductions.

Minoo *et al*. (2006a) have reported that RAPD and AFLP profiles coupled with morphological characters can be utilized to assess the variability and hybrid nature of genotypes and of successful interspecific

hybridization and production of hybrids between *Vanilla planifolia* and *Vanilla aphylla*.

Minoo *et al*. (2008a) used RAPD polymorphism to estimate the level of genetic diversity and interrelationships among different collections of *Vanilla planifolia* Andrews and a few related species, including both leafy and leafless types such as *Vanilla tahitensis* J.W.Moore., *Vanilla andamanica* Rolfe, *Vanilla pilifera* Holtt. and *Vanilla aphylla* Blume. Studies revealed that there is very limited variation within collections of *Vanilla planifolia*, indicative of its narrow genetic base and *Vanilla tahitensis* was found to be the nearest to *Vanilla planifolia* among the species studied. The species studied are diverse and have a similarity ranging from 1.2% to 57.3%. *Vanilla andamanica* was the most divergent and there was also reasonable variability within its collections, indicating the possibility of natural seed set. Specific groupings were revealed by cluster analysis whereby the leafless forms and *Vanilla andamanica* formed a separate cluster.

Certain efforts have been carried out to induce genetic variability in vanilla. Induction of somaclonal variation combined with gamma irradiation has been used for the purpose by Sukmadjaja *et al*. (1996). Fifteen clones from *in vitro* culture induced by gamma irradiation were analysed for three isozyme patterns to identify the genetic variability of vanilla clones by the workers. Results of the analysis revealed that irradiation with gamma rays increased genetic variability as indicated by the esterase and peroxidase banding patterns of the clones.

An attempt to induce variability in *Vanilla planifolia* through *in vitro* techniques was made by Lovis *et al*. (2007) and great interclonal

variability was observed in the *in vitro* derived plantlets. The intraclonal variability was very low in tissue culture derived regenerants. Isozyme and RAPD assays confirmed genotypic variation.

Cameron (2006) used DNA bar coding techniques as a method for vanilla species identification. Several gene fragments and intergenic spacers were sequenced for the purpose in the case of more than 50 accessions. Each vanilla species has a unique DNA sequence and these DNA bar codes allow easy identification of the plants from just a small fragment of leaf, stem or fruit tissue. DNA test can provide the correct name of a plant with minimal effort. Furthermore, the ability to identify processed vanilla beans as being derived from plants of *Vanilla tahitensis*, *Vanilla planifolia*, *Vanilla pompona* or other wild *Vanilla* species could be an important application of this technique in the flavour and fragrance industry.

2.16.3. Selection

Evaluation of germplasm for the selection of appropriate genotypes for different crop improvement programmes has been carried out in vanilla only on a preliminary scale. Kuruvilla *et al*. (2004) conducted an evaluation of the germplasm of *Vanilla planifolia* available at ICRI resulting in the identification of four genotypes, MCV-1, MCV-2, MCV-3 and MCV-4 with consistently high yield.

2.16.4. Hybridization

Hybridization was initiated in *Vanilla* in recent years with an objective of transferring alien gene(s) responsible for natural bean formation and abiotic stress (Kuruvilla *et al*., 2004). Purseglove *et al*. (1981) reported that intra and inter specific hybrids are easy to make in *Vanilla*. *Vanilla planifolia* has been crossed with other species including

Vanilla phaentha, which is resistant to *Fusarium* root rot. Electrophoresis with seven polymorphic enzymes supported the finding of Nielsen and Siegismund (1999) about the chance of natural hybridization in localities where *Vanilla claviculata* and *Vanilla barbellata* co exist. Natural hybridization between *Vanilla claviculata* (W.Wright) Sw. and *Vanilla barbellata* Rchb.f. has been reported by Nielsen (2000).

Nybe (2001) reported more than 50% pod set in interspecific hybridization between *Vanilla planifolia* and *Vanilla valsalensis*. Seeds from these crosses were germinated *in vitro* and sub cultured for better proliferation.

Rao *et al*. (2002) reported successful crosses between *Vanilla planifolia* and *Vanilla wightiana* with an aim of transferring the characters like natural fruit set.

Minoo *et al*. (2006a) have made an attempt to increase the spectrum of variation in *Vanilla planifolia* by interspecific hybridization with *Vanilla aphylla*. Interspecific hybrids were successfully produced and morphological characters and molecular profiles revealed the true hybridity of the progenies.

Successful crosses were made between *Vanilla planifolia* and *Vanilla aphylla* by Muhammed Nissar *et al*. (2006). Hybridization between *Vanilla planifolia* (as female) and *Vanilla aphylla* (as male) resulted in 77.7% fruit set. The reciprocal cross (*Vanilla aphylla* as female and *Vanilla planifolia* as male) gave 88.9% fruit set. Beans resulting from both these crosses showed fruit growth similar to the selfed fruits. But the number of developed seeds was very less in the fruits produced by

interspecific hybridization than in the selfed fruits. Majority of the ovules were found aborted. *Vanilla aphylla* was found to possess long life of flowers (four days) compared to that of *Vanilla planifolia* in which the flowers withered in a day. *Vanilla aphylla* offers better chances to undertake interspecific hybridization due to its synchronized flowering with the cultivated species.

2.16.5. Mutation breeding

Mutation breeding is a viable method that can be adopted for inducing variability in vanilla (Kuruvilla *et al*., 2004). Sheji Mary *et al*. (2000) observed that vanilla seeds irradiated with gamma rays directly failed to germinate. Seeds collected from irradiated pods germinated based on the doze applied. Survival of seeds and seedling cultures after treatment was low. A mutant characterized by stout stem and dark green pointed leaf lamina was obtained by gamma ray treatment. The growth of the mutant was comparable to normal TC seedlings. EMS treated seeds showed delayed germination. Lovis *et al*. (2007) made an attempt to induce genetic variability in vanilla by *in vitro* induction of mutations. Great interclonal variability was observed in the plantlets derived. Intraclonal variability was very low in tissue culture derived plantlets. Isozyme and RAPD assay confirmed genotypic variation.

2.16.6. Biotechnological approaches

2.16.6.1. Micropropagation

Vanilla is commercially propagated by stem cuttings and the method is reportedly uneconomical as it involves sacrifice of the whole plant due to its monopodial growth. Tissue culture technique has emerged more or less as an alternative method for large scale production of planting materials within a relatively short span of time. As the growers are looking

for alternate sources of planting materials, micro propagated plantlets serve the purpose and are popularly used now (Geetha and Shetty, 2000).

Protocols on micro propagation of vanilla have been reported from early 1980s onwards. Nodal segments (Cervera and Madrigal, 1981; Kononowicz and Janick, 1984; Davidonis and Knorr, 1991; Rao *et al.*, 1993b; Ganesh *et al*., 1996) and aerial root tips (Philip and Nainar, 1986; Philip and Nainar, 1988c) were used as explants wherein shoot proliferation was achieved by axillary bud growth or protocorm formation.

Geetha and Shetty (2000) observed that axillary bud proliferation was initiated only when a longitudinal bisection injuring the shoot tip was made. This might be due to the strong apical dominance exerted by the shoot tip meristem with the consequent inhibition of axillary buds. Chithra *et al*. (2007) established a protocol for large scale propagation of vanilla through *in vitro* culture. Multiplication was at the rate of 40 shoots from a single axillary bud explant over a period of 140 days. Giridhar (2007) has opined that selection of plant material and explant preparation are crucial for vanilla *in vitro* culture and shoot tips and 4-5 month old nodal explants of 2-3 cm size with dormant axillary buds excised from three year old field grown vines are the best. MS medium with modified concentration of macro and micro nutrients and vitamins supplemented with optimal concentration of growth regulators was found to be the best for maximum response.

A study undertaken to determine the efficiency of *in vitro* axillary bud cultures of *Vanilla planifolia* proved that the growth of newly formed shoots was more vigorous on MS medium with NAA and Kinetin as growth hormones (Rao *et al*., 1993b). High rate of multiplication of

Vanilla planifolia clonal propagules was obtained from axillary bud explants using semi solid medium (MS supplemented with BA 2 mg/l and NAA 1 mg/l) successively (George and Ravishankar, 1997).

Attempts have been made to propagate vanilla from parts of the plant other than shoot apex (Philip and Nainar, 1986 & 1988c; Philip and Jose, 1989). Philip and Jose (1989) discussed the role of 1AA in the conversion of root meristems to shoot meristem. Scanning the root tip extracts for 1AA using UV, TLC, GLC and GC-MS showed higher levels of auxin in old aerial roots and also in young cultured root tips in which the root meristem had got transformed to shoots. Micropropagation through culture of callus masses was developed by Gu *et al*. (1987) and Davidonis and Knorr (1991).

Degeneration of the cap cells of vanilla roots in culture stimulated growth and division of cells of the quiescent center and the aerial root tips of the plantlets produced directly from the meristematic zone without a callus interphase, minimising the possibility of induced epigenetic changes in the resultant plants. Futher more, the root tips could perhaps be an ideal candidate for cryopreservation because of a small unit size, high regenerative potentiality, the constituent cells being vacuolated, meristematic, genetically uniform and without intercellular spaces (Philip and Jose, 1989).

Ganesh *et al*. (1996) investigated the effects of culture media and hormones on shoot proliferation *in vitro* in *Vanilla planifolia.* Good shoot proliferation was achieved only in the presence of BAP. An improved method of micropropagation of vanilla plants (*Vanilla planifolia*) was described by Giridhar *et al*. (2001). The effect of silver nitrate (an

inhibitor of ethylene activity) has been reported to be responsible for inducing positive response not only on shoot initiation and growth but also on increased root length of *Vanilla planifolia* when used in micro molar concentration.

Agrawal *et al*. (1992) devised a method of clonal propagation for *Vanilla walkeriae* which is restricted to the tropical forests of Tamilnadu and Kerala. A commercially viable protocol for mass propagation of *Vanilla tahitensis* was standarised by Mary *et al*. in 2000. A multiplication ratio of 1:47 was observed over a culture period of 60-70 days on benzyl amino purine (1 mgl^{-1}) and α- naphthalene acetic acid (0.1 $mg\ l^{-1}$).

Vanilla planifolia can be propagated using phenyl acetic acid (PAA) as a primary factor to induce multiple shoots from axillary buds. Shoots can be induced and then elongated in media containing MS salts, vitamins, 6-benzyladenine (BA) and PAA. Elongation of shoots and formation of new shoots from nodes was achieved on medium containing BA+PAA. The shoots could be rooted on a medium supplemented with IBA and 70-80% survied after hardening and transfer to the field (Giridhar *et al*., 2003).

For the first time, the influence of Zeatin, TDZ and coconut milk on shoot multiplication was studied by Giridhar and Ravishankar (2004). This protocol was effective in producing micro propagated vanilla plants characterized by successful hardening and field transfer. A high frequency simple and rapid regeneration protocol was developed from shoot tip and nodal explants of *Vanilla planifolia* on MS medium supplemented with 6-benzyl amino purine and coconut water (Kalimuthu *et al*., 2006).

2.16.6.2. Protoplast fusion

Isolation and fusion of protoplasts was attempted by Minoo *et al*. (2008b) in order to produce hybrids with desirable traits in *Vanilla* species. Protoplasts were successfully isolated from *Vanilla planifolia* and *Vanilla andamanica* and PEG mediated protoplast fusion was attempted. The fusion product could easily be identified since the protoplasts of the two species were different in size and arrangement of chlorophyll. This technique could be used for gene transfer for helpful traits.

2.16.6.3. Study of somaclonal variations

Occurrence of genetic variants during micropropagation is occasionally encountered when the cultures are maintained *in vitro* for a long period. Therefore, the micropropagated multiple shoots of *Vanilla planifolia* Andrews developed from axillary bud explants established 10 years ago were used to determine somaclonal variation using RAPD and ISSR by Sreedhar *et al*. (2007b). No difference was observed in the case of any of the samples for a particular primer, indicating the absence of variation among micropropagated plants.

2.16.6.4. On farm evaluation of TC plants

Performance of vanilla plants raised from tissue culture plantlets along with vegetative cuttings were assessed on a large scale in planters' field by Madhusoodanan *et al*. (2007). Obervations on growth and yield attributes such as vine length, number of leaves per vine, internodal length, number of beans per vine, bean size, yield, etc. revealed that performance of tissue culture plants was on par with that of conventional plants raised from vegetative cuttings of comparable length. It was also

observed that when good management practices were adopted, TC plants performed better at the full bearing stage of the plant.

2.16.6.5. *In vitro* seed germination

Vanilla produces numerous minute seeds that do not germinate under natural conditions. TC technique could be used to successfully germinate the seeds. Protocols for seed and embryo culture of vanilla have been standardized by different workers (Knudson, 1950; Withner, 1955; Hegarty, 1955; Philip and Nainar, 1988b; Minoo *et al*., 1997). Vanilla is a cross pollinated crop and is known to have many meiotic and post meiotic chromosomal abnormalities (Ravindran, 1979). As a result it is possible to get various cytotypes in the seed progenies. Culturing of seeds can thus give many genetically variant types. Minoo *et al.* (1997) reported morphological and biochemical variations in *in vitro* germinated seedlings. *In vitro* culture could be used for germination of seeds and selection of useful genotypes from segregating progenics and rapid multiplication for getting disease free planting material.

2.16.6.6. Synthetic seeds

Production of synthetic seeds (synseeds) by *in vitro* encapsulation of somatic embryos/ shoot tips /axillary buds has now emerged as an ideal system for conservation and exchange. Sajina *et al*. (1997) has reported the standardization of synseed production in many plant species including vanilla.

The above review of literature gives a bird's eye view of the earlier works on vanilla with special reference to floral biology and crop improvement. The review brings out the limited and fragmentary nature of the genetic resources of vanilla in India and it emphasizes the need for the assessment of the available variability so that the available resources

can be used for future breeding programmes in a better way. Under the above circumstances, the present experiments have been designed so that the floral biology of *Vanilla planifolia* and *Vanilla tahitensis* is analyzed, a comparative analysis of the variability between the two species is made and *in situ* and *ex situ* variability, correlation and interrelationship of characters and genetic divergence in *Vanilla planifolia* are assessed. Adaptability of *Vanilla planifolia* to different geographical regions of Kerala State of India has also been studied.

Chapter III
MATERIALS AND METHODS

Vanillin, one of the most popular flavouring agent used world wide in food, beverage, pharmaceutical and cosmetic industries is the commercial product obtained from three species of the orchid genus *Vanilla*. The most widely grown commercial species is *Vanilla planifolia* Andrews and two other cultivated species are *Vanilla pompona* Schiede and *Vanilla tahitensis* J.W. Moore. *Vanilla planifolia* is indigenous to the humid tropical rain forests of south eastern Mexico, Central America, the West Indies and the northern part of South America. *Vanilla tahitensis* is cultivated in the Tahiti islands of Polynesia and *Vanilla pompona* in the south pacific islands (Madhusoodanan *et al*., 2003). *Vanilla planifolia* is the species cultivated in India also. *Vanilla tahitensis* and *Vanilla pompona* are available in some germplasm repositories of the country but are not usually cultivated by Indian farmers.

The present experiments have been designed to study the floral biology of *Vanilla planifolia* Andrews and *Vanilla tahitensis* J.W. Moore, to study the interspecific variability between *Vanilla planifolia* and *Vanilla tahitensis* based on morphological and anatomical characters and vanillin content, to study the *in situ* intraspecific variability of *Vanilla planifolia* available in the vanilla growing areas of Kerala State of India, to study the genetic variability, character association and genetic divergence of *Vanilla planifolia* based on accessions collected from the major vanilla growing regions of South India and also to study the adaptability of *Vanilla planifolia* to different geographical regions of Kerala State of India.

3.1. The experimental field

For field experiments, observations were made from vanilla farms of Idukki, Calicut (Thamarassery area) and Wayanad districts of Kerala (Fig. 3.1) in the year of 2003. Experiments on floral biology of *Vanilla planifolia* and *Vanilla tahitensis*, inter specific variability between *Vanilla planifolia* and *Vanilla tahitensis* and genetic variability, correlation of characters, character association and genetic divergence of *Vanilla planifolia* were conducted in the Genetics and Plant Breeding Division of the Department of Botany of University of Calicut (Malappuram District), Kerala, India. Experiments on floral biology and interspecific variability were started in the month of September 2002 and experiments on genetic variability, correlation of characters, character association and genetic divergence in the month of September 2003. Anatomical study of the materials was carried out at Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, Kerala. For the analysis of vanillin content, solvent extraction of the volatile components of vanilla beans was carried out at Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, Kerala and GC-MS analysis was carried out in the Textiles Committee Laboratory (Ministry of Textiles, Government of India), Kannur, Kerala. Analysis of the soil samples collected from farmers' fields was done at Regional Coffee Research Station, Chundale, Wayanad, Kerala.

The geographical location and weather data of the experimental locations are presented in Table 3.1.

Fig. 3.1.

Table 3.1. Geographical location and weather data of the experimental locations (Anonymous, 2008b)

Parameter	Idukki District	Calicut District	Wayanad District	Malappuram District
Geographical location	9º15'-10º21' N 76º37'-77º25' E	11º08'-11º50' N 75º30'-76º08' E	11º27'-15º58' N 70º27'-75º47' E	10º-12º N 75º-77º E
Average annual rainfall	297 cm	343 cm	341 cm	283 cm

Distribution of rainfall (mm)												
	J	F	M	A	M	J	J	A	S	O	N	D
Idukki	20.3	25.0	46.3	129.4	226.9	565.9	684.8	459.4	273.5	302.3	171.9	63.8
Calicut	9.2	7.2	18.2	88.4	279.6	903.3	940.9	494.4	260.4	249.3	146.2	33.5
Wayanad	7.4	9.1	21.5	96.3	186.3	694.1	1163.6	639.6	258.7	206.6	101.4	26.7
Malappuram	5.4	6.7	20.8	90.9	220.2	645.8	786.8	395.1	213.3	274.5	145.4	28.2

3.2. The experimental materials

3.2.1. *Vanilla planifolia*

Vanilla planifolia Andrews is a herbaceous perennial vine, climbing up trees or other supports to a height of 10-15 m by means of adventitious roots. In cultivation it is trained to a height which will facilitate hand pollination and harvesting. Long, whitish, aerial adventitious roots about 2 mm in diameter are produced singly opposite the leaves and they help the plant to adhere firmly to the support. The roots at the base ramify in the humus or mulch layer. The stem is long, cylindrical, succulent and branched. It is 1-2 cm in diameter and is dark green and photosynthetic with stomata. The internodes are 5-15 cm in length. Leaves are large, flat, fleshy, sub sessile, alternate, oblong- elliptic to lanceolate, 8-25 cm long and 2-8 cm broad. The tip is acute to acuminate and the base somewhat rounded. Venation is parallel and the veins are distinct. The petiole is thick, short and canalized above (Purseglove *et al*., 1981).

Flowers are large, waxy, fragrant, pale greenish yellow, bisexual and zygomorphic. Perianth lobes are six in number (3+3) and they look alike. The lower petal of the inner whorl is short, broad and it is modified into a labellum. The lower part of the labellum envelops a central structure called the 'column' (gynostemium) (Purseglove *et al*., 1981). Gynostemium is formed by the union of stamen, style and stigma (Lawrence, 1951). A tuft of hairs is seen in the middle of the disc. The tip of the column bears a single stamen with two pollen masses (pollinia) covered by a cap or hood like structure called 'rostellum' which prevents natural pollination. The slender stalk like portion is the ovary, which is 4-7 cm in length and 3-5 mm in diameter. The fruit is a capsule, which is dehiscent in *Vanilla planifolia* and in trade it is known as a bean. The bean is pendulous, narrowly cylindrical, obscurely three angled, 10-25 cm long and 5-15 mm in diameter. Each bean when ripe contains thousands of

minute globose seeds, which are liberated by longitudinal splitting of the capsule (Purseglove *et al*., 1981).

3.2.2. *Vanilla tahitensis*

Vanilla tahitensis J.W. Moore, the Tahitian vanilla, is indigenous to Tahiti and cultivated in Hawaii also. The species is less robust than *Vanilla planifolia* with more slender stem and narrow leaves, which are 12 to 14 cm long and 2.5 to 3 cm wide with longer perianth segments and a lip that is shorter, with beans reddish brown and 12 to 14 cm long and about 9 to 10 mm in width, broad in the middle and tapering towards the end (Purseglove *et al*., 1981).

3.3. Experimental methods

3.3.1. Study of floral biology of *Vanilla planifolia* and *Vanilla tahitensis*

Study of the floral biology of a crop is an essentially important step towards further studies on the crop in terms of variability and improvement. Floral biology of *Vanilla planifolia* and *Vanilla tahitensis* were comparatively studied presently using the plants grown in the net house of the Genetics and Plant Breeding Division of the Department of Botany of University of Calicut, Kerala, India for the purpose. The planting material for both the species were made available by Indian Cardamom Research Institute (ICRI), Myladumpara, Idukki, Kerala. Nine plants each were grown in coloured polythene bags (nursery bags) of 38 cm x 32 cm size filled with garden soil, sand and enriched compost in 4:1:1 ratio and maintained as per the recommendations of Spices Board, India (Anonymous, 2002). 33 cm long vine cuttings were used for planting. The plants were trained on *Glyricidia* stumps grown adjacent to the pots. The experiment was started in September 2002. The plants were

allowed to grow on the support up to a height of 150 m and then the vines were trained in such a way that they grow downwards and reach the soil level. When the plants reached the soil level, they were made to grow upwards on the support. This type of training and hanging the vines from the branches of the support by way of bending the vines when they reach a particular level reportedly cause a stimulus required for flower induction (Childers, 1959). Also, the tips of the hanging vines were pruned by removing 1-2 nodes from their tip so as to induce flowering as suggested by Lionnet (1958). This operation was carried out by the end of the third year and fourth year of growth after the north east monsoon in the beginning of the month of December.

The plants were observed for flowering in the third and fourth years of growth and data wererecorded on 24 inflorescence/flower characters as detailed in Table 3.2.

Table 3.2. Characters observed for the study of floral biology of *Vanilla planifolia and Vanilla tahitensis*

Sl. No.	Characters
1	Percentage of plants that flowered at the end of three years of growth
2	Percentage of plants that flowered at the end of four years of growth
3	Period of inflorescence initiation
4	Period of flower opening
5	Mean duration from inflorescence initiation to first flower opening
6	Mean duration from the opening of the first flower to the opening of the last flower in an inflorescence
7	Number of inflorescences per metre
8	Number of flowers per inflorescence
9	Inflorescence length (cm)
10	Inflorescence rachis diameter (cm)
11	Flower length (cm)
12	Bract length (cm)

13	Bract breadth (cm)
14	Sepal length (cm)
15	Sepal breadth (cm)
16	Petal length (cm)
17	Petal breadth (cm)
18	Labellum length (cm)
19	Labellum breadth (cm)
20	Gynostemium length (cm)
21	Ovary length (cm)
22	Ovary diameter (cm)
23	Rostellum length (cm)
24	Rostellum breadth (cm)

3.3.2. Study of interspecific variability between *Vanilla planifolia* and *Vanilla tahitensis*

A comparative analysis of *Vanilla planifolia* and *Vanilla tahitensis*, two cultivated species of *Vanilla*, was made presently based on vegetative and floral morphology, anatomy and vanillin content. Plants grown in the experimental net house of the Genetics and Plant Breeding Division of University of Calicut were used for the study. The planting material for both the species was made available by Indian Cardamom Research Institute, Myladumpara, Idukki, Kerala. The experiment was designed with the objective of comparing and differentiating the two species at morphological, anatomical and phytochemical levels. The plants were grown in polythene bags of 38 cm x 32 cm size filled with garden soil, sand and enriched compost in 4:1:1 ratio and maintained as per the recommendations of Spices Board, India (Anonymous, 2002). Vanilla vine cuttings of 33 cm length were used for planting. The plants were trained on *Glyricidia* stumps grown adjacent to the pots. The experiment was started in September 2002. The plants were allowed to grow on the support up to a height of 150 m and then the vines were trained in such a way that they grow downwards and reach the soil level. When the plants reached the soil level, they were made to grow upwards on the support.

This type of training and hanging the vines from the branches of the support by way of bending the vines when they reach a particular level reportedly cause a stimulus required for flower induction (Childers, 1959). Also, the tips of the hanging vines were pruned by removing 1-2 nodes from their tip so as to induce flowering as suggested by Lionnet (1958). This operation was carried out by the end of the third year and fourth year of growth after the north east monsoon in the beginning of the month of December. Observations were made starting from September 2006 when the plants attained a maturity of four years.

3.3.2.1. Vegetative and floral morphology

Vanilla planifolia and *Vanilla tahitensis* were compared presently in terms of the variations in vegetative and floral morphology (Table 3.3). Three replications arranged in randomized block were used for the study. Analysis of variance was carried out to test the significance of variability between the two species in the case of the different characters.

Table 3.3. Morphological characters of *Vanilla planifolia* and *Vanilla tahitensis* studied for the analysis of interspecific variability

Sl. No.	Characters
1	Vine length (cm)
2	Vine girth (cm)
3	Number of nodes per metre
4	Leaf length (cm)
5	Leaf breadth (cm)
6	Leaf thickness (mm)
7	Leaf area (cm^2) (Leaf area was calculated using the following formula: Leaf area = Leaf length x Leaf breadth x Conversion factor. Conversion factor was calculated by graphical method, replicating the observation 10 times in the

	case of both the species and calculating the mean)
8	Internodal length (cm)
9	Length of velamen roots (cm)
10	Thickness of velamen roots (cm)
11	Number of inflorescences per metre
12	Number of flowers per inflorescence
13	Inflorescence length (cm)
14	Flower length (cm)
15	Inflorescence rachis diameter (cm)
16	Bract length (cm)
17	Bract breadth (cm)
18	Sepal length (cm)
19	Sepal breadth (cm)
20	Petal length (cm)
21	Petal breadth (cm)
22	Labellum length (cm)
23	Labellum breadth (cm)
24	Gynostemium length (cm)
25	Ovary length (cm)
26	Ovary diameter (cm)
27	Rostellum length (cm)
28	Rostellum breadth (cm)
29	Length of three month old beans

3.3.2.2. Anatomy

Fresh materials of the species were collected from the net house of the Genetics and Plant Breeding Division of the Department of Botany of University of Calicut, and transections of leaf, stem and velamen root were made with the help of a sharp razor blade by free hand section. Sections and peelings of the stem, leaf and velamen root were double stained and anatomical observations were made and photo micrographs prepared using Canon digital camera fitted to Zeiss Axioftar+ microscope. The anatomical observations were made using the facilities available in the Centre for Medicinal Plants Research, Kottakkal, Kerala.

3.3.2.3. Phytochemistry

Phytochemical analysis of the dried beans of *Vanilla planifolia* and *Vanilla tahitensis* was carried out using GC-MS to locate and quantify vanillin, the major flavour producing volatile compound present in them.

3.3.2.3.1. Preparation of extract

About 4 gm of coarsely powdered air dried material was accurately weighed and placed in a glass stoppered conical flask. Macerated with 100 ml of petroleum ether (60-80ºC) for 6 hours, shaking frequently and then allowed to stand for 18 hours. Filtered rapidly taking care not to loose any solvent. Dried beans of 9 month age were used for the purpose.

3.3.2.3.2. GC-MS analysis

GC-MS analysis was carried out with Hewlett Packard 6890 series. The carrier gas was Nitrogen and the column used was HP-5 (column length 30 m, diameter 320 µm and film thickness 0.25 µm). The sample was injected in a splitless mode at an initial temperature of 220ºC. The maximum temperature was 325ºC. The mass spectra were recorded at 70 eV in EI mode. The scan range was 40-400 amu. Mass spectra correlations were done using NIST library.

3.3.3. Study of field level variability of *Vanilla planifolia*

Assessment of the existing variability of a crop under field conditions is the most important initial step in any effort to study the genetic variability and related parameters under experimental conditions especially when the genetic base of the crop is critically narrow. The present experiment was carried out to assess the variability of *Vanilla planifolia* at field level in the important vanilla growing regions of Kerala State of India. Data were collected for the purpose from fifty vanilla farms distributed in Idukki, Calicut and Wayanad districts of Kerala (Fig. 3.1)

with a minimum area of 0.40 hectare (Table 3.4). Nine plants of 5-6 years of age were selected from each field by simple random sampling and observations on vine girth, leaf length, leaf breadth, leaf thickness, leaf area, number of nodes per metre, internodal length, number of inflorescences per plant and yield per plant (beans) were recorded in the year 2003. Yield per plant was calculated on the assumption that each inflorescence produced ten beans and eighty beans weighed one kilogram (Krishnakumar *et al*., 2003). Mean yield per hectare was calculated considering the number of plants that can be planted per hectare as 2000 (Potty and Krishnakumar, 2003). Mean, standard deviation and coefficient of variation were calculated to analyse the extent of variability among the plants and analysis of variance carried out to test the significance of variation.

Table 3.4. Vanilla farms included in the study of field level variability of *Vanilla planifolia*

Sl. No.	Particulars
VF 1	Sunny Mathew, Karinattu, Pulloorampara, Kodenchery, Calicut, Kerala.
VF 2	Ambat Williams, Ambat Padi, Kodenchery, Calicut, Kerala.
VF 3	A.J.Joseph, Alackal, Meenmutty, Kodenchery, Calicut, Kerala.
VF 4	Mathew.T.A., Kanniyil, Meenmutty, Kodenchery, Calicut, Kerala.
VF 5	K.M.Mathew, Kuttippoovathingal, Chembukadavu, Kodenchery, Calicut, Kerala.
VF 6	K.M.Mathew, Kaniamkunnel, Nellipoyil, Kodenchery, Calicut, Kerala.
VF 7	Chackochan Ezhanikkatt, Ambat Padi, Kodenchery, Calicut, Kerala.
VF 8	M.P.Chacko, Muttathu, Santhinagar, Kodenchery, Calicut, Kerala.
VF 9	John Perumbananickal, Chembukadavu, Kodenchery, Calicut, Kerala.
VF 10	Sebastian Kunnelveedu, Chembukadavu, Kodenchery,

	Calicut, Kerala.
VF 11	Augustian Kunnelveedu, Chembukadavu, Kodenchery, Calicut, Kerala.
VF 12	M.C.Johny, Mannoor, Meenmutty, Kodenchery, Calicut, Kerala.
VF 13	I.C.Jose, Iyrattil, Meenmutty, Kodenchery, Calicut, Kerala.
VF 14	Antony Neeruvelil, Kodenchery, Calicut, Kerala.
VF 15	N.M.Varghese, Neeruvelil, Kodenchery, Calicut, Kerala.
VF 16	Biju Chanthamkulam, Mannuvayal, Kodenchery, Calicut, Kerala.
VF 17	P.V.John, Pekuzhiyil, Mannuvayal, Kodenchery, Calicut, Kerala.
VF 18	Mathew Karinatttu, Pulloorampara, Kodenchery, Calicut, Kerala.
VF 19	I.C.Alexander, Iyrattil, Pulloorampara, Kodenchery, Calicut, Kerala.
VF 20	Sony Sebastian, Mathalikunnel, Thiruvampady, Calicut, Kerala.
VF 21	Joseph Tharappel, Thiruvampady, Calicut, Kerala.
VF 22	Joseph T.M., Mannoor, Thiruvampady, Calicut, Kerala.
VF 23	Tomy Francis, Kallekulangara, Thiruvampady, Calicut, Kerala.
VF 24	M.J.Chacko, Madickakel, Ambalavanna, Thiruvampady, Calicut, Kerala.
VF 25	Manoj Sebastian, Vazheparambil, Muthappanpuzha, Calicut, Kerala.
VF 26	Rajmohan Kalladayil, Mylampadi, Wayanad, Kerala.
VF 27	Baburaj Kalladayil, Mylampadi, Wayanad, Kerala.
VF 28	K.G.Damodaran, Kunnumpurathu, Mylampadi, Wayanad, Kerala.
VF 29	Vaidhyanathan, Sreyas, Mylampadi, Wayanad, Kerala.
VF 30	Thiruppathy Gowder, Kottakkunnu, Meenangadi, Wayanad, Kerala.
VF 31	Jagadeesh, Sreevardha Estate, Karani, Wayanad, Kerala.

VF 32	Thomas Joseph, Nellikunnel, Nadavayal, Wayanad, Kerala.
VF 33	Dr.Cyriac, Nellikunnel, Nadavayal, Wayanad, Kerala.
VF 34	Baby Ickara, Nadavayal, Wayanad, Kerala.
VF 35	Charlie Ickara, Nadavayal, Wayanad, Kerala.
VF 36	John Potass, Chattupara, Adimali, Idukki, Kerala.
VF 37	K.V.Abraham, Kunnarath, Adimali, Idukki, Kerala.
VF 38	Mathew Zacharia, Thekkumkattil, Adimali, Idukki, Kerala.
VF 39	Xavier Kannatt, Adimali, Idukki, Kerala.
VF 40	Zacharia Abraham, Kailasanadu Estate, Kailasanadu, Idukki, Kerala.
VF 41	Jacob Daniel, Combayar, Idukki, Kerala.
VF 42	Anandan Pillai, Combayar, Kallar, Idukki, Kerala.
VF 43	A.J. Lal, Sanniasiyoda, Idukki, Kerala.
VF 44	Tomy Kottarathil, Balagram, Idukki, Kerala.
VF 45	T.C.Zachariah, Thombil, Chembalam, Idukki, Kerala.
VF 46	Abraham, Nedumparai Estate, Kailasanadu, Idukki, Kerala.
VF 47	P.J.Joseph, Palathinal (H), Purappuzha, Thodupuzha, Idukki, Kerala.
VF 48	P.C.Chacko, Ottallur, Thodupuzha, Idukki, Kerala.
VF 49	George Thomas, Kochukudi, Thodupuzha, Idukki, Kerala.
VF 50	A.M.Satheesh Kumar, Arakkal (H), West Kodikulam, Thodupuzha, Idukki, Kerala.

3.3.4. Genetic variability of *Vanilla planifolia*

Vanilla planifolia, the species of *Vanilla* that is commercially cultivated in India has got a very narrow genetic base. However, no scientific assessment of it has been carried out on a considerable scale, eventhough some preliminary investigations have been made in the direction. Hence, the present experiment was designed to assess the genetic variability of the crop based on 20 accessions collected from the major vanilla growing areas of Kerala State and adjacent states of India (Table 3.5). The plants were grown in polythene bags of 38 cm x 32 cm size filled with garden soil, sand and enriched compost in 4:1:1 ratio and maintained as per the recommendations of Spices Board, India (Anonymous, 2002). Vanilla vine cuttings of 33 cm length were used for planting. *Gliricidia* stumps planted adjacent to the pots were used as standard. The experiment was laid out in randomized block design, with three replications per accession. The experiment was started in September 2003. Observations were made starting from September 2007 when the plants attained a maturity of four years. Thirteen morphological characters (Table 3.6) were observed and analysed for significance of variation between accessions using ANOVA. Phenotypic and genotypic variances, phenotypic and genotypic coefficients of variation, heritability (%) and genetic advance (%) were calculated to assess the extent of variability between the accessions. However, only 11 accessions could be analysed for floral/yield characters since the other accessions did not flower by the fourth year (Table 3.7).

Table 3.5. Accessions used for the study of genetic variability of growth characters in *Vanilla planifolia*

Accession number	Source
CUV 1	Ambalavayal, Wayanad, Kerala
CUV 2	Kalpetta, Wayanad, Kerala
CUV 3	Kallar- Purliar, Tamil Nadu
CUV 4	Kalpetta, Wayanad, Kerala

CUV 5	Kallar, Tamil Nadu
CUV 6	Kuzhithodu, Idukki, Kerala
CUV 7	Thirthahalli, Karnataka
CUV 8	Sringeri, Karnataka
CUV 9	Sringeri, Karnataka
CUV 10	Puthoor, Karnataka
CUV 11	Kodenchery, Calicut, Kerala
CUV 12	Combayar, Idukki, Kerala
CUV 13	Combayar, Idukki, Kerala
CUV 14	Combayar, Idukki, Kerala
CUV 15	Combayar, Idukki, Kerala
CUV 16	Kallar, Idukki, Kerala
CUV 17	Sanniasiyoda, Idukki, Kerala
CUV 18	Mundiyeruma, Idukki, Kerala
CUV 19	Kailasanadu, Idukki, Kerala
CUV 20	Udumpanchola, Idukki, Kerala

Table 3.6. Characters of *Vanilla planifolia* accessions studied for variability analysis

Sl. No.	Characters
1	Vine length (cm)
2	Vine girth (cm)
3	Number of nodes per metre
4	Leaf length (cm)
5	Leaf breadth (cm)
6	Leaf thickness (mm)
7	Leaf area (cm^2)
8	Internodal length (cm)
9	Length of velamen roots (cm)
10	Thickness of velamen of roots (cm)
11	Number of inflorescences per plant
12	Number of flowers per inflorescence
13	Yield per plant (kg)

Table 3.7. Accessions used for the study of genetic variability of yield characters in *Vanilla planifolia*

Accession number	Source
CUV 5	Kallar, Tamil Nadu
CUV 6	Kuzhithodu, Kerala
CUV 9	Sringeri, Karnataka
CUV 10	Puthoor, Karnataka
CUV 12	Combayar, Kerala
CUV 14	Combayar, Kerala
CUV 15	Combayar, Kerala
CUV 17	Sanniasiyoda, Kerala
CUV 18	Mundiyeruma, Kerala
CUV 19	Kailasanadu, Kerala
CUV 20	Udumpanchola, Kerala

3.3.4.1. Analysis of variance

Analysis of variance (ANOVA) was carried out to test the significance of variations between the accessions in terms of the different characters studied. Significance of F value was tested with reference to

standard F table (Fischer and Yates, 1963). CD was calculated using the formula

$$CD = t_{0.05} \times \sqrt{\frac{2VE}{r}}$$

Where $t_{0.05}$ is $t_{0.05}$ for error degree of freedom, VE is the error mean square and r is the number of replications.

3.3.4.2. Genotypic and phenotypic variances and coefficients of variation

Phenotypic and genotypic variances give estimates of total and heritable components of variation of quantitative characters. Phenotypic and genotypic variances of the different characters studied were estimated as per Singh and Choudhary (1985).

$$\text{Genotypic variance } (\sigma^2 g) = \frac{\text{MSS for treatment-MSS for error}}{\text{Number of replications}}$$

Phenotypic variance $(\sigma^2 p) = \sigma^2 g + \sigma^2 e$ where $\sigma^2 e$ is error variance.

Coefficients of variation provide information on the extent of variability of characters as percentages of the corresponding mean values of the characters. Phenotypic and genotypic coefficients of variation of the characters studied were estimated following Burton and Devane (1953).

$$\text{Phenotypic coefficient of variation (PCV)} = \frac{\sigma p \times 100}{\overline{X}}$$

where σp = the phenotypic standard deviation and $\overline{X}$ = grand mean of the character.

$$\text{Genotypic coefficient of variation (GCV)} = \frac{\sigma g \times 100}{\overline{X}}$$

where σg = the genotypic standard deviation.

3.3.4.3. Heritability

Heritability (broad sense) is the fraction of the total variance that is heritable and it depends on the number of alleles involved in the control of the character and also on the extent of influence of environment. It is estimated as the percentage of genotypic variance over phenotypic variance (Jain, 1982).

$$\text{Heritability (broad sense) } (H^2) = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

3.3.4.4. Genetic advance

Genetic advance under selection is the extent of improvement possible in the case of the character by selection. Genetic advance was calculated using the following formula:

$$GA = \frac{KH^2}{\overline{X}} \sigma p \quad \text{(Singh and Choudhary, 1985)}$$

where H^2 = heritability (broad sense); σp = phenotypic standard deviation; K= selection differential which is 2.06 at 5% intensity of selection (Allard, 1960).

3.3.4.5. Study of genetic control of characters

The 13 morphometric plant characters of *Vanilla planifolia* studied presently have been subjected to analysis of their genetic control based on the nature of their frequency distribution. Data on 60 plants selected at random from the study population were grouped together for frequency distribution analysis.

3.3.5. Study of correlation of characters

Quantitative characters of crop plants show different levels of interrelationship due to the common sharing of alleles between them. Correlation of the quantitative characters of *Vanilla planifolia* studied for genetic variability presently have been analyzed for correlation of characters as suggested by Rangaswamy (1995). Data collected from the 11 accessions that flowered in the 4th year have been used for correlation analysis based on 13 characters (Tables 3.6 and 3.7).

3.3.6. Study of character association

Quantitative characters of organisms show association between them. Study of association of characters is being carried out to find out related characters and the findings are used for data reduction so as to find out the lead characters useful in selection and other plant breeding programmes. Factor analysis by means of principal component analysis has been done for the purpose presently using the statistical software STATISTICA, based on 8 growth characters and 3 yield characters (Table 3.8) recorded from the 11 accessions of *Vanilla planifolia* that flowered in the 4th year.

Table 3.8. Characters of *Vanilla planifolia* accessions studied for character association and genetic divergence analysis

Sl. No.	Characters
1	Vine length (cm)
2	Vine girth (cm)
3	Number of nodes per metre
4	Leaf thickness (mm)
5	Leaf area (cm^2)
6	Internodal length (cm)
7	Length of velamen roots (cm)
8	Thickness of velamen of roots (cm)
9	Number of inflorescence per plant
10	Number of flowers per inflorescence
11	Yield per plant (kg)

3.3.7. Study of genetic divergence

Different genotypes of plant species can be grouped into different clusters based on genetic divergence studies. The 11 *Vanilla planifolia* accessions that flowered in the 4th year were subjected to cluster analysis based on 11 phenotypic characters (Table 3.8) using the software STATISTICA following UPGMA procedure (unweighted pair group mathematical average procedure) (Sneath and Sokal, 1973).

3.3.8. Study of comparative performance of the *Vanilla planifolia* accessions

The overall performance of 11 accessions of *Vanilla planifolia* (Table 3.7) has been comparatively analysed presently based on performance indices calculated for each agronomic character and the cumulative performance index derived from them in the case of each accession as suggested by Amaravenmathy and Srinivasan (2003) using the following formula:

$$\text{Performance index} = \frac{\text{Accession mean of the character}}{\text{Grand mean of the character}}$$

However, in the case of internodal length, the formula

$$\text{Performance index} = \frac{\text{Grand mean}}{\text{Accession mean}}$$

has been used since low internodal length is the desirable form of the character.

3.3.9. Study of adaptability of *Vanilla planifolia* to different geographical regions of Kerala

Adaptability of *Vanilla planifolia* to three geographical regions of Kerala was analysed in the present study based on data collected from 10 vanilla farms each observed from three vanilla growing districts of Kerala (Table 3.9). Data were collected on 12 characters (Table 3.10) and comparatively analysed in relation to the geographical regions. Yield per plant has been calculated as suggested by Krishnakumar *et al*. (2003) and yield per hectare as suggested by Potty and Krishnakumar (2003) as described elsewhere.

Table 3.9. Details of the vanilla farms studied for adaptability

1. Thamarassery (Calicut)	
VAP 1	Ambat Williams, Ambat Padi, Kodenchery, Calicut, Kerala.
VAP 2	A.J.Joseph, Alackal, Meenmutty, Kodenchery, Calicut, Kerala.
VAP 3	K.M.Mathew, Kaniamkunnel, Nellipoyil, Kodenchery, Calicut, Kerala.
VAP 4	Augustian Kunnelveedu, Chembukadavu, Kodenchery, Calicut, Kerala.
VAP 5	N.M.Varghese, Neeruvelil, Kodenchery, Calicut, Kerala.
VAP 6	Biju Chanthamkulam, Mannuvayal, Kodenchery, Calicut, Kerala.
VAP 7	I.C.Alexander, Iyrattil, Pulloorampara, Kodenchery, Calicut, Kerala.
VAP 8	Tomy Francis, Kallekulangara, Thiruvampady, Calicut,

	Kerala.
VAP 9	M.J.Chacko, Madickakel, Ambalavanna, Thiruvampady, Calicut, Kerala.
VAP 10	Manoj Sebastian, Vazheparambil, Muthappanpuzha, Calicut, Kerala.
2. Idukki	
VAP 11	John Potass, Chattupara, Adimali, Idukki, Kerala.
VAP 12	K.V.Abraham, Kunnarath, Adimali, Idukki, Kerala.
VAP 13	Mathew Zacharia, Thekkumkattil, Adimali, Idukki, Kerala.
VAP 14	Zacharia Abraham, Kailasanadu Estate, Kailasanadu, Idukki, Kerala.
VAP 15	Jacob Daniel, Combayar, Idukki, Kerala.
VAP 16	A.J. Lal, Sanniasiyoda, Idukki, Kerala.
VAP 17	Abraham, Nedumparai Estate, Kailasanadu, Idukki, Kerala.
VAP 18	P.J.Joseph, Purappuzha, Thodupuzha, Idukki, Kerala.
VAP 19	George Thomas, Kochukudi, Thodupuzha, Idukki, Kerala.
VAP 20	A.M.Satheesh Kumar, West Kodikulam, Thodupuzha, Idukki, Kerala.
3. Wayanad	
VAP 21	Rajmohan Kalladayil, Mylampadi, Wayanad, Kerala.
VAP 22	Baburaj Kalladayil, Mylampadi, Wayanad, Kerala.
VAP 23	K.G.Damodaran, Kunnumpurathu, Mylampadi, Wayanad, Kerala.
VAP 24	Vaidhyanathan, Sreyas, Mylampadi, Wayanad, Kerala.
VAP 25	Thiruppathy Gowder, Kottakkunnu, Meenagadi, Wayanad, Kerala.
VAP 26	Jagadeesh, Sreevardha Estate, Karani, Wayanad, Kerala.
VAP 27	Thomas Joseph, Nellikunnel, Nadavayal, Wayanad, Kerala.
VAP 28	Dr.Cyriac, Nellikunnel, Nadavayal, Wayanad, Kerala.

VAP 29	Baby Ickara, Nadavayal, Wayanad, Kerala.
VAP 30	Charlie Ickara, Nadavayal, Wayanad, Kerala.

Table 3.10. Characters of *Vanilla planifolia* studied for adaptability

Sl. No.	Character
1	Vine girth (cm)
2	Number of leaves per metre
3	Leaf length (cm)
4	Leaf breadth (cm)
5	Leaf thickness (cm)
6	Leaf area $(cm)^2$
7	Number of nodes per metre
8	Internodal length (cm)
9	Number of inflorescences per plant
10	Yield per plant (number of beans)
11	Yield per plant (kg)
12	Yield per hectare (kg)

Chapter IV

RESULTS AND DISCUSSION

Commercial vanillin is obtained from three species of the orchid genus *Vanilla* namely *Vanilla planifolia* Andrews, *Vanilla tahitensis* J.W.Moore and *Vanilla pompona* Schiede. Of these, *Vanilla planifolia* is the most widely cultivated and used and it is usually called Mexican vanilla. The other two species are cultivated and used in certain regions of the world only and hence they are known as Tahitian vanilla and West Indian vanilla respectively.

Vanilla planifolia is the cultivated species in India. *Vanilla tahitensis* is available in the germplasm of the national institutes carrying out researches on spices and also rarely as a mixture in *Vanilla planifolia* plantations. The genetic base of vanilla in India is very narrow since vanilla cultivation most probably started in the country using very few cuttings introduced to the country (Anonymous, 2000). The possibility of origin of new variation is also limited since the crop is clonally propagated. However, some extent of variability at field level has been reported in recent years (Ravindran *et al.*, 1996, Kuruvilla *et al*., 2003 and Umamaheswari and Mohanan, 2004). The study of existing variability, isolation of superior lines and studies based on genetic control of characters, character association, genetic divergence of genotypes and adaptability of the crop to different geographical regions are important since the crop can provide significant additional income to farmers coming especially under small scale and marginal categories.

With the above objective some experiments have been carried out presently to study the floral biology of *Vanilla planifolia* and *Vanilla*

tahitensis, to study the interspecific variability between *Vanilla planifolia* and *Vanilla tahitensis* based on vegetative and floral morphology, anatomy and phytochemistry, to study the intraspecific variability of *Vanilla planifolia* both *in situ* and *ex situ* and to study the genetic control of growth and yield characters and the correlation of characters, character association and genetic divergence in *Vanilla planifolia*. An attempt has been made to compare the accessions of *Vanilla planifolia* based on their overall performance and also to study the adaptability of *Vanilla planifolia* to three major vanilla growing regions of Kerala State of India. The observations are presented and discussed below under appropriate heads.

4.1. Study of floral biology of *Vanilla planifolia* and *Vanilla tahitensis*

Study of floral biology of a crop is a significant step towards any study on variability and improvement. Floral biology of *Vanilla planifolia* and *Vanilla tahitensis* has been studied presently based on the data generated from an experiment carried out for the purpose as described elsewhere.

Twenty four characters were observed for the purpose and the data are presented in Table 4.1. Only 20% of both *Vanilla planifolia* and *Vanilla tahitensis* flowered by the end of the 3rd year of growth. However, 67% of *Vanilla planifolia* plants and 56% of *Vanilla tahitensis* flowered in the fourth year of growth. Inflorescence initiation occurred in the months of February-March in *Vanilla planifolia* and in the months of January-February in *Vanilla tahitensis*. Flowers opened from March to May in *Vanilla planifolia* and from January to March in *Vanilla tahitensis*. The average duration taken from inflorescence initiation to opening of the first flower was 45 days in *Vanilla planifolia* and 40 days in *Vanilla tahitensis*.

The average duration taken from the opening of the first flower to the opening of the last flower was 30 days *Vanilla planifolia* and 28 days on the average in *Vanilla tahitensis*.

Table 4.1. Observations on the characters of *Vanilla planifolia* and *Vanilla tahitensis* plants studied for floral biology

Sl. No.	Character	*Vanilla planifolia*	*Vanilla tahitensis*
1	Percentage of plants that flowered at the end of three years of growth	20%	20%
2	Percentage of plants that flowered at the end of four years of growth	67%	56%
3	Period of inflorescence initiation	February-March	January-February
4	Period of flower opening	March-May	February- March
5	Mean duration from inflorescence initiation to first flower opening	45 days	40 days
6	Mean duration from the opening of first flower to the opening of last flower in the inflorescence	30 days	28 days
7	Number of inflorescences per metre	7-9	6-8
8	Number of flowers per inflorescence	10- 16	6-9
9	Inflorescence length (cm)	3.3-7.3	6.5-13
10	Inflorescence rachis diameter (cm)	0.5-0.7	0.5-0.7
11	Flower length (cm)	9.5-10.5	8-10
12	Bract length (cm)	1.4-1.7	0.7-1.3
13	Bract breadth (cm)	0.6-0.7	0.5-0.7
14	Sepal length (cm)	5.7-5.9	4.6-5.9

15	Sepal breadth (cm)	1.1-1.3	0.9-1.2
16	Petal length (cm)	5.6-5.8	4.5-5.7
17	Petal breadth (cm)	1	1-1.2
18	Labellum length (cm)	5-5.5	4.4-4.9
19	Labellum breadth (cm)	2.5-2.9	1.6-2.3
20	Gynostemium length (cm)	4	3.3-3.5
21	Ovary length (cm)	4.3-4.7	2.5-4.5
22	Ovary diameter (cm)	0.4-0.5	0.3-0.4
23	Rostellum length (cm)	0.3	0.3
24	Rostellum breadth (cm)	0.3	0.3

Photographs of *Vanilla planifolia* and *Vanilla tahitensis* in blossom are given in Figs. 4.1. and 4.2. The flowers and floral parts are presented in Figs. 4.3 and 4.4.

In *Vanilla planifolia*, number of inflorescences ranged from 7 to 9, number of flowers per inflorescence from 10 to 16, inflorescence length from 3.3 cm to 7.3 cm, inflorescence rachis diameter from 0.5 cm to 0.7 cm, flower length from 9.5 to 10.5 cm, bract length from 1.4 cm to 1.7 cm, bract breadth from 0.6 to 0.7 cm, sepal length from 5.7 cm to 5.9 cm, sepal breadth from 1.1 to 1.3 cm and petal length from 5.6 to 5.8 cm. Petal breadth was 1 cm, labellum length 5 to 5.5 cm, labellum breadth 2.5 to 2.9 cm, gynostemium length 4 cm, ovary length 4.3 to 4.7 cm, ovary diameter 0.4 to 0.5 cm, rostellum length 0.3 cm and rostellum breadth 0.3 cm.

Figs. 4.1. and 4.2.

Fig. 4.3.

Fig. 4.4.

In *Vanilla tahitensis*, number of inflorescences per metre ranged from 6 to 8, number of flowers per inflorescence from 6 to 9, inflorescence length from 6.5 to 13 cm, inflorescence rachis diameter from 0.5 to 0.7 cm, flower length from 8 to 10 cm, bract length from 0.7 to 1.3 cm, bract breadth from 0.5 to 0.7 cm, sepal length from 4.6 to 5.9 cm, sepal breadth from 0.9 to 1.2 cm, petal length from 4.5 to 5.7 cm, petal breadth from1 to 1.2 cm, labellum length from 4.4 to 4.9 cm, labellum breadth from 1.6 to 2.3 cm, gynostemium length from 3.3 to 3.5 cm, ovary length from 2.5 to 4.5 cm, ovary diameter from 0.3 to 0.4 cm, rostellum length 0.3 cm and rostellum breadth 0.3 cm.

Floral biology of *Vanilla planifolia* has been studied by earlier workers like Kuruvilla *et al*., 1996; Bhat and Sudharshan, 2002 and Shadakshari *et al*., 2003a. The findings of the present experiment agree with those of these workers. However, some variations in flowering period have been reported based on climatic and geographical variations. In the case of *Vanilla tahitensis* no similar works could be traced.

4.2. Study of interspecific variability between *Vanilla planifolia* and *Vanilla tahitensis*

Vanilla planifolia is the commercially cultivated species of vanilla in India. However, *Vanilla tahitensis* is being cultivated in some vanilla growing countries, especially in the pacific islands. It fetches a comparatively lower price in the market (Correll, 1944 and Madhusoodanan *et al*., 2003). In India, it occasionally gets mixed up with planting materials of *Vanilla planifolia* (Radhakrishnan *et al*., 2005). Radhakrishnan *et al*. (2005) have developed a method to differentiate the two species based on leaf length, leaf breadth and internodal length.

Umamaheswari *et al*. (2003) has analyzed the variation between the two species based on morphometric characters of juvenile plants. The present attempt is to explore the variability between the two species based on vegetative and floral morphology, anatomy and vanillin content so that better tools are developed to differentiate them.

4.2.1. Vegetative and floral morphology

Observations on the variability in vegetative and floral morphology in the case of *Vanilla planifolia* and *Vanilla tahitensis* have been presented in Table 4.2. *Vanilla planifolia* and *Vanilla tahitensis* plants, their vines, inflorescences and 3 month old fruits have been presented in Figs. 4.5, 4.6, 4.7, 4.8, 4.9, 4.10, 4.11 and 4.12. Comparative analysis of twenty nine morphometric characters of *Vanilla planifolia* and *Vanilla tahitensis* has shown that the two species differ significantly in the case of many characters whereas no such difference has been noticed in the case of other characters (Table 4.2). Data were collected by the end of the fourth year of growth of the plants.

Table 4.2. Comparison of morphological characters of *Vanilla planifolia* and *Vanilla tahitensis*

Character	Statistic	*Vanilla planifolia*	*Vanilla tahitensis*	CD @5%	CD @1%
1. Vine length (cm)	Mean	1999.4	1225	763.22	NS
	Range	1208-2955	855-1534		
	Standard deviation	475.49	26.06		
	Coefficient of variation	23.78	2.13		
2. Vine girth (cm)	Mean	0.87	0.6	0.26	NS
	Range	0.6-1.1	0.5-0.6		

	Standard deviation	0.15	0.06		
	Coefficient of variation	17.63	10.19		
3. Number of nodes per metre	Mean	10	10.9	NS	NS
	Range	8-13	10-12		
	Standard deviation	1.48	0.60		
	Coefficient of variation	14.80	5.51		
4. Leaf length (cm)	Mean	18.8	16.6	NS	NS
	Range	17.1-20.3	14-18.9		
	Standard deviation	0.93	1.42		
	Coefficient of variation	4.93	0.85		
5. Leaf breadth (cm)	Mean	4.8	2.9	0.56	0.93
	Range	4.2-5.5	2.3-3.2		
	Standard deviation	0.91	0.2		
	Coefficient of variation	19.15	6.90		
6. Leaf thickness (mm)	Mean	1.6	1.46	NS	NS
	Range	1.23-1.78	1.3-1.77		
	Standard deviation	0.17	0.08		
	Coefficient of variation	10.90	5.59		
7. Leaf area $(cm)^2$	Mean	63.3	35.27	0.86	14.69
	Range	56.93-71.45	30.55-41.19		
	Standard deviation	7.95	5.42		
	Coefficient	12.57	15.36		

	of variation				
8. Internodal length (cm)	Mean	11	11.3	NS	NS
	Range	7-14	8.5-12		
	Standard deviation	2.44	2.11		
	Coefficient of variation	22.13	18.75		
9. Length of velamen roots (cm)	Mean	66.3	48.5	NS	NS
	Range	17-161	22.7-91.7		
	Standard deviation	29.81	22.32		
	Coefficient of variation	44.98	46.00		
10. Thickness of velamen roots (cm)	Mean	0.3	0.2	NS	NS
	Range	0.2-0.4	0.1-0.2		
	Standard deviation	0.06	0.06		
	Coefficient of variation	21.65	34.64		
11. Number of inflorescences per metre	Mean	8.1	6.7	0.97	NS
	Range	7-9	6-8		
	Standard deviation	0.17	0.58		
	Coefficient of variation	2.14	8.7		
12. Number of flowers per inflorescence	Mean	13	7.1	1.98	3.28
	Range	10- 16	6-9		
	Standard deviation	0.89	0.85		
	Coefficient of variation	6.84	12.03		
13. Inflorescence length	Mean	5.1	10.3	0.77	1.28
	Range	3.3-7.3	6.5-13		

(cm)					
	Standard deviation	0.32	0.36		
	Coefficient of variation	2.03	3.50		
14. Inflorescence rachis diameter (cm)	Mean	0.6	0.6	NS	NS
	Range	0.5-0.7	0.5-0.7		
	Standard deviation	0.08	0.08		
	Coefficient of variation	13.33	13.33		
15. Flower length (cm)	Mean	9.9	8.8	0.76	NS
	Range	9.5-10.5	8-10		
	Standard deviation	0.25	0.40		
	Coefficient of variation	2.54	4.59		
16. Bract length (cm)	Mean	1.5	1.0	0.09	0.15
	Range	1.4-1.7	0.7-1.3		
	Standard deviation	0.06	0.24		
	Coefficient of variation	3.85	24		
17. Bract breadth (cm)	Mean	0.7	0.6	NS	NS
	Range	0.6-0.7	0.5-0.7		
	Standard deviation	0.06	0.06		
	Coefficient of variation	8.57	9.6		
18. Sepal length (cm)	Mean	4.1	5.4	NS	NS
	Range	5.7-5.9	4.6-5.9		
	Standard deviation	2.80	0.38		
	Coefficient of variation	68.3	7.01		

19. Sepal breadth (cm)	Mean	1.2	1.1	NS	NS
	Range	1.1-1.3	0.9-1.2		
	Standard deviation	0.1	0.06		
	Coefficient of variation	8.33	5.25		
20. Petal length (cm)	Mean	4.1	5.3	NS	NS
	Range	5.6-5.8	4.5-5.7		
	Standard deviation	2.92	0.31		
	Coefficient of variation	71.12	5.76		
21. Petal breadth (cm)	Mean	1	1.1	NS	NS
	Range	1-1	1-1.2		
	Standard deviation	0	0.14		
	Coefficient of variation	0	12.73		
22. Labellum length (cm)	Mean	5.4	4.6	0.31	0.51
	Range	5-5.5	4.4-4.9		
	Standard deviation	0.15	0.12		
	Coefficient of variation	2.83	2.51		
23. Labellum breadth (cm)	Mean	2.7	1.9	0.26	0.43
	Range	2.5-2.9	1.6-2.3		
	Standard deviation	0.12	0.12		
	Coefficient of variation	4.28	6.07		
24. Gynostemium length (cm)	Mean	4	3.4	0.09	0.15
	Range	4-4	3.3-3.5		
	Standard deviation	0	0.06		
	Coefficient	0	1.70		

	of variation				
25. Ovary length (cm)	Mean	4.4	3.2	0.65	1.07
	Range	4.3-4.7	2.5-4.5		
	Standard deviation	0.06	0.40		
	Coefficient of variation	1.31	12.51		
26. Ovary diameter (cm)	Mean	0.4	0.35	0.09	NS
	Range	0.4-0.5	0.3-0.4		
	Standard deviation	0.06	0.07		
	Coefficient of variation	14.4	20		
27. Rostellum length (cm)	Mean	0.3	0.3	NS	NS
	Range	0.3-0.3	0.3-0.3		
	Standard deviation	0	0		
	Coefficient of variation	0	0		
28. Rostellum breadth (cm)	Mean	0.3	0.3	NS	NS
	Range	0.3-0.3	0.3-0.3		
	Standard deviation	0	0		
	Coefficient of variation	0	0		
29. Length of 3 month old beans (cm)	Mean	14.7	10.3	0.93	2.18
	Range	14.0-15.3	9.6-11.1		
	Standard deviation	0.06	0.41		
	Coefficient of variation	0.41	7.28		

Figs. 4.5. and 4.6

Figs. 4.7. and 4.8

Figs.4.9 and 4.10

Figs. 4.11 and 4.12.

Among the growth characters, vine length and vine girth of plants of four years of maturity, leaf breadth and leaf area showed statistically significant differences. Vine growth reached a length of 1208 cm to 2955 cm in the case of *Vanilla planifolia* and 855 cm to 1534 cm in *Vanilla tahitensis* within a growth period of four years under uniform conditions. Mean vine length produced within four years by *Vanilla planifolia* starting from 33 cm long planting material was about 20 metres and it was 12.25 metres in *Vanilla tahitensis*. Vines of *Vanilla planifolia* showed significantly higher girth when compared to *Vanilla tahitensis*. Mean vine girth was 0.87 cm in *Vanilla planifolia* and 0.60 cm in *Vanilla tahitensis*. Mean leaf area was 63.30 cm^2 in *Vanilla planifolia* and 35.27 cm^2 in *Vanilla tahitensis*, showing that the leaves of *Vanilla planifolia* were significantly larger (Table 4.2). Leaf length and leaf breadth have been suggested as distinctive features that could help in differentiating the two species by Radhakrishnan *et al*. (2005). Leaf area and vine girth have been reported as characters significantly different between the two species in the case of juvenile plants by Umamaheswari *et al*. (2003).

Study of variability of the floral characters of *Vanilla planifolia* and *Vanilla tahitensis* has been carried out presently based on 18 characters (Table 4.2). Number of inflorescences per metre, number of flowers per inflorescence, inflorescence length, flower length, bract length, labellum length, labellum breadth, gynostemium length, ovary length and ovary diameter showed significant differences between the species. No significant difference was observed in the case of inflorescence rachis diameter, bract breadth, sepal length, sepal breadth, petal length, petal breadth, rostellum length and rostellum breadth between the two species. Number of inflorescences per metre, number of flowers

per inflorescence, flower length, bract length, gynostemium length, ovary length and ovary diameter were significantly higher in *Vanilla planifolia* whereas inflorescence length was significantly higher in *Vanilla tahitensis*. Sepal length and petal length were higher in *Vanilla tahitensis* even though the difference was not statistically significant. The fruits of *Vanilla tahitensis* were shorter and stouter when compared to the fruits of *Vanilla planifolia* as evidenced by the observation on 3 month old fruits (Table 4.2. and Fig. 4.5).

Efforts have been made by earlier workers to study the floral biology of *Vanilla planifolia* to some extent (Kuruvilla *et al*., 1996, Bhat and Sudharshan, 2000a, Bhat and Sudharshan, 2002, Shadakshari *et al*., 2003a and Muhammed Nissar *et al.,* 2006). However no such efforts have been made to study the floral biology of *Vanilla tahitensis*. The present study has revealed that significant variability occurred in the case of many characters between the two species and they could be differentiated based on number of inflorescences per metre, number flowers per inflorescence, inflorescence length, flower length, bract length, bract breadth, labellum length, labellum breadth, gynostemium length, ovary length and ovary diameter. Thicker fruits have been reported in *Vanilla tahitensis* compared to *Vanilla planifolia* by Lubinsky and Kim (2006). Nybe *et al*. (2007) has reported that the perianth segments are longer in *Vanilla tahitensis*.

4.2.2. Anatomy

Stem, leaf and velamen root anatomy of *Vanilla planifolia* and *Vanilla tahitensis* have been comparatively analyzed presently so as to differentiate the two species based on anatomical differences (Table 4.3 and Figs 4.13, 4.14, 4.15, 4.16, 4.17 & 4.18).

Table 4.3. Comparative anatomy of *Vanilla planifolia* and *Vanilla tahitensis*

Tissue	*Vanilla planifolia*	*Vanilla tahitensis*
1. Stem anatomy		
Epidermal cells	Epidermal cells are tangentially elongated and all cells contain prismatic crystals. Mean cell size is 22.24 µm	Epidermal cells are small and rounded. Prismatic crystals are present in them. Mean cell size is 15.8 µm
Granular contents	Some cells near endodermis contain granular content	Granular content is usually absent
Vascular bundles	4 rings; Mean number of bundles is 25	4 rings; 40 in number
Bundle cap	3-6 layered bundle cap is present. Made of well developed sclerenchyma	2-3 layered; Made of feebly thickened cells
2. Leaf anatomy		
Main vascular bundle	Well developed when compared to *Vanilla tahitensis*	Not so much developed
Bundle cap	Complete	Not complete
3. Velamen root anatomy		
Velamen tissue	Outer layer shows distorted cells with wavy radial points	Inner tangential walls of the outer velamen layer highly thickened
Cortex	14-18 layered	10-14 layered
Air cavities	Inner cortical layer consists of 10-12 schizogenous air cavities	Absent
Stele	Metaxylem is very large compared to *Vanilla tahitensis*	Medium sized
Pith	Comparatively smaller than *Vanilla tahitensis*	Very large

Fig. 4.13

Fig. 4.14

Fig. 4.15

Fig. 4.16

Fig. 4.17

Fig. 4.18

The epidermal cells of the stem of *Vanilla tahitensis* are smaller when compared to that of *Vanilla planifolia*. Mean cell size has been found to be 22.24 μm in *Vanilla planifolia* and 15.8 μm in *Vanilla tahitensis*. The collenchymatous hypodermis was single layered in both the cases. Cortex was parenchymatous in both with lesser number of cells in *Vanilla tahitensis*. Some of the cells near the endodermis contain granular content in *Vanilla planifolia* and it was absent in *Vanilla tahitensis*. Needle like crystals of Calcium oxalate were present in the cortex of both. Endodermis was single layered in both the cases. Vascular bundles were present in four rings in both the species, but the number of bundles was lesser in *Vanilla planifolia*. Bundle number was around 25 in *Vanilla planifolia* and around 40 in *Vanilla tahitensis*. Bundle cap was complete and sclerenchymatous with 3-6 layers of cells in *Vanilla planifolia* whereas it was 2-3 layered and made of feebly thickened cells in the case of *Vanilla tahitensis*.

Leaf is isobilateral with no palisade layer in both the cases. Epidermis is single layered in both with stomata only on the lower epidermis. Hypodermis is single layered and collenchymatous in both the cases and present throughout the lamina. The main vascular bundle is comparatively well developed in *Vanilla planifolia* when compared to *Vanilla tahitensis*. Bundle sheath is complete in *Vanilla planifolia* and incomplete in *Vanilla tahitensis*. Needle like crystals of Calcium oxalate are seen in some of the mesophyll cells in both the cases.

Velamen tissue is two layered in both the species. The outer layer of the velamen tissue shows distorted cells with wavy radial points in the case of *Vanilla planifolia*. Inner tangential walls of the outer velamen

layer are highly thickened in both the cases. Cortex is 14-18 layered in *Vanilla planifolia* and 10-14 layered in *Vanilla tahitensis*. Inner cortical layer of *Vanilla planifolia* contains 10-12 schizogenous air cavities and those are absent in *Vanilla tahitensis*. Root endodermis is single layered in both the cases. Twelve groups of exarch xylem and phloem bundles are arranged radially in the stele of *Vanilla planifolia* and 13 groups in *Vanilla tahitensis*. Pith is large in *Vanilla tahitensis* and comparatively small in *Vanilla planifolia*.

Anatomical characters can be used to distinguish species in their vegetative phase (Metcalfe, 1961). Recent reports on vegetative anatomical studies on certain endemic species of *Vanilla* have confirmed that anatomical features are significant in distinguishing the species and this information can be used to screen different parents and hybrids (Raju, 1996; Baruah, 1998; Zhao and Wei, 1999). The study of Zhao and Wei (1999) showed that the arrangement and structure of the stem tissues of *Vanilla planifolia* and *Vanilla siamensis* could be used to differentiate between them. Stern and Judd (1999) found that vegetative anatomy had certain phylogenetic values. The hypostomatous nature of the leaves of *Vanilla planifolia* has been reported by Nayar *et al*. (1976). Khasim and Mohana Rao (1984; 1986) who studied the nature of velamen tissue of some epiphytic orchid roots have opined that the number of velamen layers, wall thickening and lignification of velamen and endodermal cells are correlated with habitat tolerance in orchids. Screening of vanilla genotypes based on the nature and thickening of velamen tissue may provide potential for selection of drought tolerant lines.

4.2.3. Phytochemistry

Phytochemical analysis of dried beans of *Vanilla planifolia* and *Vanilla tahitensis* was carried out presently to compare the vanillin content in them using GC-MS analysis as described elsewhere. The study showed high difference in the case of vanillin content between the species (Table 4.4 and Figs. 4.19 and 4.20).

Table 4.4. Vanillin content in the dried beans of *Vanilla planifolia* and *Vanilla tahitensis*

Species	Retention time of vanillin	Percentage
1. *Vanilla planifolia*	20.610	50.89
2. *Vanilla tahitensis*	20.530	2.08

Vanillin content was found to be 50.89% of the total volatile compounds in the case of *Vanilla planifolia* and only 2.08% in the case of *Vanilla tahitensis*. Ehlers *et al*. (1994) has reported the presence of lesser amount of vanillin in *Vanilla tahitensis* when compared with *Vanilla planifolia*.

4.3. Study of field level variability of *Vanilla planifolia*

The genetic base of *Vanilla planifolia* is very narrow in India since it is an introduced crop. However it has developed certain levels of variability in spite of its vegetative mode of propagation. Assessment of the existing level of variability in the field is the first step towards any crop improvement programme. The results of an experiment carried out to assess the variability of the crop at field level in three important vanilla growing regions of Kerala State of India are presented and discussed below. Fifty vanilla plantations of 5-6 years of age with a minimum area of 0.40 ha were selected for the study (Table 3.4). Nine plants were

selected at random from each field and analyzed for major growth and yield characters.

Fig. 4.19 V.Planifolia chr.

Fig. 4.20 V. tahitensis chr.

Field level variability of *Vanilla planifolia* was assessed presently based on seven growth characters and four yield characters (Tables 4.5 & 4.6) utilizing the data collected from 50 vanilla farms of Kerala (Table 3.4). All the growth characters studied presently showed statistically significant variation between the fields selected. Among the growth characters the highest coefficient of variation was shown by leaf area (11.84%) and the lowest by leaf breadth (7.11%). Among the yield characters studied, yield per plant (number of beans) showed a CV of 62.45%, number of inflorescences per plant showed a CV of 61.95%, yield per plant (kg) showed a CV of 61.26% and extrapolated yield per hectare showed a CV of 61.10%. The variation was statistically significant in all the cases. This shows that characters like number of inflorescences per plant, yield per plant and yield per hectare show very high variation between the different vanilla fields studied, even though the vegetative characters showed comparatively lesser variability.

Table 4.5. Field level variability of growth characters in *Vanilla planifolia*

Plots	Vine girth (cm)	Leaf length (cm)	Leaf breadth (cm)	Leaf thick ness (cm)	Leaf area $(cm)^2$	Number of nodes per metre	Inter nodal length (cm)
VF1	1.1 ±0.15	20.1 ±2.54	5.4 ±0.64	1.81 ±0.10	76.89 ±19.07	8.8 ±0.76	11.4 ±0.98
VF2	1.2 ±0.12	22 ±0.84	6 ±0.36	1.71 ±0.09	93.13 ±8.76	10.2 ±0.76	9.9 ±0.70
VF3	1.2 ±0.1	23.6 ±0.84	6.6 ±0.21	1.97 ±0.21	110.48 ±7.29	8.5 ±0.5	11.8 ±0.7
VF4	1.2 ±0.06	21.1 ±1.74	5.9 ±0.2	1.85 ±0.05	87.38 ±5.34	9 ±0.87	10.3 ±1.15
VF5	1.3 ±0.06	21.9 ±1.29	6.3 ±0.23	2.13 ±0.65	95.41 ±7.53	9.8 ±0.76	10.3 ±0.76

VF6	1.1 ±0.06	23.2 ±1.72	6.1 ±0.1	1.88 ±0.09	93.36 ±4.08	8.7 ±0.29	11.6 ±0.29
VF7	1.3 ±0.06	20 ±1.15	5.6 ±0.67	1.99 ±0.10	80.34 ±15.59	9.3 ±0.76	10.8 ±0.92
VF8	1.2 ±0.06	21.3 ±1.66	5.6 ±0.21	1.72 ±0.23	82.34 ±7.90	9.5 ±0.87	10.6 ±1.04
VF9	0.9 ±0.06	19.2 ±1.35	4.6 ±0.72	1.59 ±0.19	67.31 ±8.00	10.5 ±0	9.6 ±0.08
VF10	1.1 ±0.10	21.1 ±1.72	6 ±0.31	1.75 ±0.18	90.78 ±9.80	11.2 ±0.76	9.01 ±0.64
VF11	1.1 ±0.06	22.7 ±0.55	6.1 ±0.15	1.78 ±0.11	97.07 ±4.43	9.8 ±0.29	10.2 ±0.32
VF12	1± 0.06	21± 1.36	5.8 ±0.26	1.72 ±0.17	85.72 ±8.82	10.8 ±0.29	9.4 ±0.29
VF13	1.1 ±0.12	21.1 ±2.00	6 ±0.38	1.89 ±0.32	88.89 ±13.20	9.5 ±0.5	10.6 ±0.51
VF14	1.1 ±0.06	22.6 ±0.94	6 ±0.35	1.77 ±0.11	91.49 ±4.98	9.8 ±1.04	10.3 ±1.01
VF15	1.1 ±0.03	20.7 ±0.61	5.8 ±0.28	1.64 ±0.20	84.67 ±6.22	9.7 ±0.76	10.4 ±0.79
VF16	1.1 ±0.03	19.9 ±0.52	5.6 ±0.08	1.79 ±0.08	78.96 ±3.59	10.5 ±0.87	9.6 ±0.75
VF17	1.1 ±0.05	20.6 ±0.59	6.1 ±0.78	1.84 ±0.15	88.65 ±13.99	9.5 ±0.87	10.6 ±0.94
VF18	1.3 ±0.1	17.5 ±3.09	5.4 ±0.46	1.70 ±0.15	72.5 ±5.74	9.7 ±0.29	10.4 ±0.26
VF19	1.2 ±0.08	20.7 ±1.08	5.9 ±0.48	1.89 ±0.13	84.77 ±4.61	9.2 ±0.76	10.9 ±0.91
VF20	1.2 ±0.06	21.6 ±0.55	5.9 ±0.23	2.01 ±0.15	91.02 ±5.69	8.8 ±0.29	11.3 ±0.40
VF21	1.2 ±0.18	22.6 ±0.66	6.3 ±0.05	2.37 ±0.46	103.33 ±0.67	9.2 ±0.29	11.01 ±0.40
VF22	1.2 ±0.13	20.5 ±1.57	5.8 ±0.25	2.25 ±0.48	83.9 ±3.91	9.2 ±0.58	11 ±0.72
VF23	1.2 ±0.05	20.5 ±0.43	5.8 ±0.15	1.9 ±0.05	84.90 ±2.92	10 ±0.5	10.2 ±0.54
VF24	1.2 ±0.10	20.3 ±1.45	5.6 ±0.43	1.84 ±0.11	80.91 ±11.81	9.8 ±0.29	10.2 ±0.32
VF25	1.1 ±0.13	19.3 ±0.49	5.7 ±0.43	2.27 ±0.45	77.95 ±7.04	8.8 ±0.58	11.4 ±0.72

VF26	1.2 ±0.2	21.3 ±0.67	6.3 ±0.61	1.94 ±0.10	94.58 ±11.38	9.3 ±1.26	10.9 ±1.65
VF27	1.2 ±0.06	20.8 ±1.56	6.3 ±0.59	1.97 ±0.17	90.5 ±14.16	9.3 ±0.58	10.7 ±0.64
VF28	1.2 ±0.08	19.5 ±0.86	6.2 ±0.63	1.87 ±0	85.22 ±6.35	9.2 ±0.76	11 ±0.91
VF29	1.2 ±0.05	19.4 ±0.79	5.7 ±0.1	1.96 ±0.29	78.11 ±4.30	8.5 ±0.5	11.8 ±0.7
VF30	1.1 ±0.03	21.0 ±0.68	5.8 ±0.75	1.93 ±0.15	86.63 ±12.67	9.8 ±0.58	10.2 ±0.58
VF31	1.1 ±0.06	22.4 ±2.28	6.8 ±0.26	1.77 ±0.28	103.07 ±6.13	10.2 ±0.76	9.9 ±0.76
VF32	1.2 ±0.06	20.7 ±1.69	6 ±0.3	1.96 ±0.10	85.95 ±2.76	9.7 ±1.15	10.5 ±1.22
VF33	1.3 ±0.06	21 ±0.61	6.9 ±0.61	2.12 ±0.03	101.29 ±8.05	9.3 ±0.29	10.9 ±0.36
VF34	1.2 ±0.06	20 ±1.11	5.8 ±0.45	1.71 ±0.10	81.38 ±6.94	10 ±0	10.03 ±0.06
VF35	1.1 ±0	19 ±1.25	5.6 ±0.36	1.75 ±0.02	75.13 ±9.84	10.2 ±0.76	9.9 ±0.77
VF36	1.1 ±0.06	20.9 ±1.91	6 ±0.57	1.75 ±0.06	83.75 ±15.88	10.2 ±0.29	9.9 ±0.32
VF37	1.0 ±0.1	19.5 ±1.65	5.4 ±0.32	1.73 ±0.08	73.58 ±9.16	9.3 ±0.58	10.7 ±0.64
VF38	1.1 ±0.06	19.5 ±1.78	5.9 ±0.57	1.68 ±0.37	81.8 ±14.85	9.5 ±0.87	10.6 ±1.04
VF39	1.2 ±0.06	17.7 ±6.16	6.5 ±0.71	1.86 ±0.12	97.16 ±13.37	9.7 ±0.29	10.4 ±0.35
VF40	1.3 ±0.1	18.2 ±0.2	6 ±0.12	1.96 ±0.05	76.93 ±1.18	9 ±1	11.2 ±1.25
VF41	1 ±0.06	15.4 ±3.54	5.5 ±0.64	1.71 ±0.05	73.18 ±17.64	12.3 ±3.62	9.7 ±0.25
VF42	1 ±0.06	18.8 ±1.04	5.2 ±0.40	1.59 ±0.13	69.44 ±9.63	10.8 ±0.29	9.5 ±0.25
VF43	1.1 ±0.06	19.8 ±1.61	5.9 ±0.23	1.75 ±0.1	69.14 ±17.95	10.5 ±1.80	9.8 ±1.53
VF44	1 ±0.15	21.2 ±0.87	5.9 ±0.26	1.70 ±0.05	87.71 ±7.05	10.3 ±1.15	10.1 ±1.10
VF45	1.1 ±0	21.5 ±0.55	6.3 ±0.32	1.89 ±0.08	95.02 ±2.07	9.8 ±0.29	10.2 ±0.76

VF46	1 ±0.1	18 ±2.87	5.2 ±0.25	1.62 ±0.26	66.41 ±10.59	9.8 ±0.58	10.3 ±0.7
VF47	1.2 ±0.12	20.8 ±1.93	5.8 ±0.55	1.7 ±0.24	84.22 ±16.49	11.5 ±0.5	8.7 ±0.45
VF48	1.3 ±0.15	23.3 ±2.86	6.6 ±0.65	1.58 ±0.64	107.05 ±21.12	9.7 ±1.04	10.4 ±1.21
VF49	1.2 ±0.06	21.1 ±0.96	6.1 ±0.40	1.73 ±0.05	91.2 ±9.20	11.2 ±0.29	7.7 ±2.57
VF50	1.2 ±0.1	22.7 ±2.52	6.1 ±0.17	1.76 ±0.07	96.39 ±14.94	10.5 ±0	9.5 ±0.03
Mean	1.15	20.57	5.91	1.84	86.14	9.79	10.35
Range	0.92-1.3	15.4-23.6	4.62-6.9	1.58-2.37	66.41-110.48	8.5-12.3	7.72-11.8
SD	0.09	1.60	0.42	0.17	10.20	0.78	0.77
CV	7.83	7.78	7.11	9.24	11.84	7.97	7.44
CD@ 1%	1.85	3.79	0.93	0.47	22.13	1.85	1.82

Table 4.6. Field level variability of yield characters in *Vanilla planifolia*

Plots	Number of inflorescence per plant	Yield per plant (number of beans)	Yield per plant (kg)	Yield per hectare (kg)
VF1	8.3±3.55	83.3±35.47	1.04±0.45	2086.7±892.7
VF2	8.7±4.31	86.7±43.11	1.08±0.54	2166.7±1075.9
VF3	18.5±1.5	18.5±15	2.31±0.19	4626.7±192.2
VF4	6.7±0.58	78.3±28.43	0.98±0.36	1966.7±712.8
VF5	11.3±7.59	113.3±75.88	1.42±0.95	2833.3±1902.0
VF6	5±3.12	50±31.22	0.62±0.39	1246.7±779.8
VF7	8.5±4.77	85±47.70	1.06±0.60	2126.7±1195
VF8	2.8±1.89	28.3±18.93	0.37±0.24	713.3±477.2
VF9	5.3±1.04	53.3±10.41	0.67±0.13	1333.3±257.9
VF10	2.3±0.58	23.3±5.77	0.29±0.08	586.7±150.1
VF11	5.6±1.26	56.7±12.58	0.71±0.16	1420±321.9
VF12	1.3±0.58	13.3±5.77	0.17±0.07	340±138.6

VF13	6.7±2.89	66.7±28.87	0.84±0.36	1673.3±715.9
VF14	7.8±4.75	78.3±47.52	0.98±0.59	1960±1180.5
VF15	2.3±1.26	23.3±12.58	0.29±0.16	586.7±311.3
VF16	4.5±0	45±0	0.56±0	1120±0
VF17	4.7±0.76	46.7±7.64	0.58±0.10	1166.7±194.3
VF18	11.8±8.22	118.3±82.21	1.48±1.03	2960±2060
VF19	8.3±1.53	83.3±15.28	1.04±0.19	2086.7±377.5
VF20	6.3±3.51	63.3±35.12	0.79±0.44	1586.7±873.2
VF21	9.5±4.44	95±44.44	1.19±0.56	2380±1119.5
VF22	10.8±4.62	153.3±108.67	1.92±1.36	3840±2719.3
VF23	5.5±0.5	55±5	0.69±0.06	1380±120
VF24	2.2±0.29	21.7±2.89	0.27±0.03	540±69.3
VF25	30±13.43	300±134.26	3.75±1.68	7500±3360.7
VF26	11.2±2.93	128.3±33.29	1.60±0.42	3206.7±840.1
VF27	12.8±4.80	128.3±14.43	1.60±0.60	3206.7±3801.1
VF28	8.7±3.40	86.7±34.03	1.08±0.42	2166.7±847.2
VF29	5.3±0.29	71.7±33.29	0.9±0.42	1800±833.5
VF30	8.7±3.69	86.7±36.86	1.08±0.46	2166.7±926.1
VF31	6.8±1.04	68.3±10.41	0.85±0.13	1706.7±261.0
VF32	18.5±4.58	185±45.83	2.31±0.58	4626.7±1150.0
VF33	13.8±4.04	138.3±40.41	1.73±0.50	3460±1009.6
VF34	14.8±3.69	148.3±36.86	1.86±0.46	3713.3±926.1
VF35	9.5±1.32	95±13.23	1.19±0.17	2380±336.5
VF36	2.8±0.76	28.3±7.64	0.36±0.10	713.3±194.3
VF37	2±0.87	20±8.66	0.25±0.10	500±207.8
VF38	7.8±1.53	90.3±23.35	1.13±0.29	2260±589.2
VF39	3.3±1.61	33.3±16.07	0.42±0.20	833.3±397.2
VF40	14.2±0.58	141.7±5.77	1.77±0.07	3540±138.6
VF41	10.6±3.82	106.7±38.19	1.33±0.48	2666.7±957.9
VF42	5.8±3.33	58.3±33.29	0.73±0.41	1466.7±829.5
VF43	14.2±3.06	141.7±30.55	1.77±0.38	3546.7±763.8
VF44	11±3.5	110±35	1.38±0.45	2760±892.9
VF45	2.5±0.5	25±5	0.31±0.07	626.7±130.1
VF46	5.2±1.53	51.715.28±	0.65±0.19	1293.3±377.5
VF47	13.8±4.54	138.3±45.37	1.73±0.57	3460±1141.8
VF48	10±5.29	100±52.92	1.25±0.66	2500±1322.9

VF49	16±3.12	160±31.22	2±0.39	4000±783.1
VF50	7.2±2.47	71.7±24.66	0.90±0.31	1793.3±614.9
Mean	8.62	85.07	1.11	2212.41
Range	1.3-30	13.3-300	0.17-3.75	340-7500
SD	5.34	53.13	0.68	1351.77
CV	61.95	62.45	61.26	61.10
CD@1%	7.99	86.55	1.08	2167.43

Only very few works have been carried out in India to assess the variability of *Vanilla planifolia* at field level. Parthiban *et al*. (2003) has evaluated eleven accessions of vanilla collected from different parts of Western Ghats and reported some degree of variability among them even though most of the variations were not statistically significant.

However, efforts have been made to assess the variability of *Vanilla planifolia* in their natural wild and agricultural habitats in other parts of the world. The workers have opined that the level of variability is critically low especially in the case of agricultural habitats. A report from Reunion shows that the entire stock of cultivated *Vanilla planifolia* of the country consisted of a single clone as revealed by RAPD analysis (Lubinsky, 2005). Lubinsky and Kim (2007) have also reported low variability of *Vanilla planifolia* in agricultural habitats.

Under the above circumstances, the observation of statistically significant variability between the cultivated populations of *Vanilla planifolia* in India is highly significant. Further screening of the variability at genetic level and its conservation and improvement are very important for the safety of the genetic base of the crop in the country. The significant level of variability observed presently may be the result of both genetic and environmental factors. The unique environmental situations in the vanilla farms might have resulted in the induction of variations thus

leading to the origin of variability of characters at quantitative and qualitative levels. Further investigations and partitioning of the variability in to genetic and environmental categories is needed to find out the extent of genetic variability of the populations of *Vanilla planifolia* in the country.

4.4. Study of genetic variability of *Vanilla planifolia*

4.4.1. Phenotypic and genotypic variability

The above observation has revealed the existence of statistically significant variability among the vanilla plants cultivated in the farmers' field. However, the cause of this variability may be both genetic as well as environmental. Hence an effort has been made presently to collect vanilla accessions from farmers' fields distributed in Idukki, Kozhikode and Wayanad districts of Kerala and also from some fields in Tamil Nadu and Karnataka (Tables 3.5 and 3.7). 33 cm long cuttings were planted as described elsewhere in the month of September, 2003 for genetic variability studies. Observations made in this connection are presented and discussed below under appropriate heads.

Among the ten growth characters studied in the case of four year old plants, seven showed statistically significant variability between the accessions (Tables 4.7 & 4.8). Leaf length, leaf area and length of velamen root did not show statistically significant variation whereas, vine length, vine girth, number of nodes per metre, leaf breadth, leaf thickness, internodal length and thickness of velamen root showed statistically significant variation. This observation indicates the existence of variability at genetic level in the case of vine length, vine girth, number of nodes per metre, leaf breadth, leaf thickness, internodal length and thickness of velamen root. Differential vine length attained by different accessions during a particular growth period is to be studied and exploited

scientifically so that fast growing accessions are identified and used for further improvement protocols especially since longer vines means higher yield in vanilla. The velamen roots of vanilla are the sole agencies to absorb moisture and mineral nutrients, since there is usually no root system other than velamen roots in vanilla under cultivation. The velamen roots of lower nodes reach the soil and ramify in the humus and absorb water and nutrition. The hanging velamen roots directly absorb moisture from atmosphere and that is why aerial irrigation to increase the humidity around the plant both in the soil and above is important (Purseglove *et al*., 1981). Well developed velamen tissue is one of the important parameters that help epiphytic plants to survive under conditions of water stress (Khasim and Mohana Rao, 1986).

Table 4.7. Genetic variability of growth characters in *Vanillla planifolia*

Acce-ssions	Vine length (cm)	Vine girth (cm)	Number of nodes per metre	Leaf length (cm)	Leaf breadth (cm)
CUV1	1315±438.4	0.7±0.06	11±1	18.4±1.57	5±0.59
CUV2	1574±11	0.7±0	10±2.08	17.6±2.36	4.1±0.49
CUV3	1269±250	0.8±0.06	10±0	19±1.42	5±0.45
CUV4	1611±47.43	0.7±0.12	10±0.58	18.1±1.74	4.4±0.67
CUV5	2032±303.7	0.9±0.06	8.3±0.58	20±0.55	5±0.42
CUV6	1993±90.9	0.9±0.15	9±0	20±2.48	5±0.71
CUV7	2101±01.7	0.8±0.06	10±0	19±0.95	4.2±0.52
CUV8	1864±552.6	0.9±0.06	9±1.15	19.4±0.25	4.8±0.1
CUV9	1946±420.7	0.8±0.1	10±0.58	18.5±1.54	4.4±0.45
CUV10	2114±1251.1	0.8±0.06	9±1.15	19.1±0.70	4.7±0.23
CUV11	1137±15.63	0.9±0.15	9.3±0.58	18.5±0.67	5.1±0.1
CUV12	1527±9.28	1±0.12	13±2.08	18±1.46	4.8±0.15
CUV13	1106±107.5	1±0.06	12±1	18.4±0.38	5.7±0.1
CUV14	1405±216.9	1±0.15	10.3±0.58	18±1.81	5.2±0.3
CUV15	1917±414	1.1±0.06	13±0	19±0.38	5.2±0.3
CUV16	803±359	0.9±0.15	12±1.73	17.3±3.10	5±0.31
CUV17	1046±244.3	0.9±0.15	11±0.58	18±2.87	5.1±0.74
CUV18	1441±87.20	1.1±0.1	11.3±1.15	18.6±3.08	5.2±0.93
CUV19	1385±429.9	1±0.1	11.3±1.53	19±1.66	5.2±0.06

CUV20	1597±554.9	0.9±0.06	12±1	17.4±1.92	4.6±0.15
Mean	1559.15	0.89	10.58	18.57	4.89
Range	803-22114	0.7-1.1	8.3-13	17.3-20	4.1-5.7
SD	385.24	0.12	1.36	0.76	0.39
CV	24.71	13.48	12.85	4.09	7.98
CD@ 5%	691.38	0.01	1.75	NS	0.75
CD@ 1%	919.53	0.23	2.33	NS	NS

Table 4.8. Genetic variability of growth characters in *Vanillla planifolia*- contd.

Accessions	Leaf thick ness (mm)	Leaf area $(cm)^2$	Internodal length (cm)	Length of velamen roots (cm)	Thick ness of velamen roots (cm)
CUV1	1.4±0.18	62.33±12.13	11.8±0.29	64.9±34.4	0.3±0.06
CUV2	1.6±0.16	52.00±13.44	11.8±0.58	30.4±17.6	0.2±0
CUV3	1.4±0.21	60.82±9.95	11.3±2.08	37.8±9.3	0.2±0.06
CUV4	1.4±0.30	47.19±18.90	12.3±1.25	33.2±10.4	0.2±0.06
CUV5	1.5±0.09	65.09±7.07	12.5±0.87	75±11.1	0.2±0.06
CUV6	1.6±0.09	67.20±17.99	12±1.5	60±39.1	0.3±0
CUV7	1.5±0.17	56.47±9.53	13.2±1.04	57.6±8.2	0.3±0.06
CUV8	1.7±0.10	65.86±2.01	12±2.5	44.3±9.2	0.3±0.06
CUV9	1.6±0.05	58.11±9.44	9.8±1.26	23.7±4.9	0.2±0.06
CUV10	1.6±0.04	63.11±5.25	12.2±0.29	33.9±15.2	0.2±0.06
CUV11	1.7±0.07	66.52±3.53	10±1	57.7±17.6	0.3±0
CUV12	1.5±0.31	59.87±3.85	8.3±1.26	88.6±62.7	0.3±0.06
CUV13	1.8±0.10	74.32±2.12	9.3±1.53	36.2±9.2	0.3±0.06
CUV14	1.7±0.06	64.69±9.62	8.7±0.76	64.4±35.9	0.3±0
CUV15	1.7±0.08	68.12±2.97	8.3±1.53	72.7±23.7	0.4±0
CUV16	1.6±0.13	61.91±14.63	9.3±2.25	49.5±13.4	0.3±0.06
CUV17	1.4±0.05	64.17±19.87	11.2±0.29	72.7±23.6	0.3±0.06
CUV18	1.7±0.28	69.19±24.21	9±2.18	56.2±8.2	0.3±0.06
CUV19	1.7±0.10	70.08±6.25	9.2±0.76	65.4±27.9	0.3±0
CUV20	1.6±0.06	57.01±7.58	6.7±1.26	44.2±6.1	0.3±0
Mean	1.59	62.70	10.45	53.42	0.28

Range	1.4-1.8	41.45-74.32	6.7-13.2	23.7-88.6	0.2-0.4
SD	0.12	6.40	1.80	17.56	0.06
CV	7.55	10.21	17.22	32.87	21.43
CD@5%	0.25	NS	2.26	NS	0.08
CD@1%	NS	NS	3.00	NS	0.10

The three yield characters studied presently, number of inflorescences per plant, number of flowers per inflorescence and yield per plant showed statistically significant variation among the eleven accessions studied for yield characters (Table 4.9). All the yield characters showed comparatively high coefficients of variation ranging from 35.89 % in the case of number of inflorescences per plant and 35.48% in the case of yield per plant to 23.53% in the case of number of flowers per inflorescence.

Table 4.9. Genetic variability of yield characters in *Vanilla planifolia*

Accessions	Number of inflorescences per plant	Number of flowers per inflorescence	Yield per plant (kg)
CUV5	2±0	9±1	0.25±0
CUV6	3±0	14±1	0.38±
CUV9	3±1	11.7±5.51	0.38±0.13
CUV10	2±1	17.7±1.15	0.25±0.13
CUV12	3±0	15±1	0.38±0
CUV14	2.3±1.15	8.7±3.79	0.30±0.46
CUV15	4.3±1.53	13±1.73	0.54±0.19
CUV17	3±1	11.7±3.51	0.38±0.13
CUV18	1.7±0.58	13±4.36	0.21±0.07
CUV19	1±0	16±2	0.13±0
CUV20	2±0	9±1	0.25±0
Mean	2.48	12.62	0.31
Range	1-4.3	8.7-17	0.13-0.54
SD	0.89	2.97	0.11
CV	35.89	23.53	35.48
CD@5%	1.30	4.63	0.16
CD@1%	0.73	6.15	0.22

In the case of all the characters that showed statistically significant variability, phenotypic coefficients of variation was higher than genotypic coefficients of variation, thus indicating the additive nature and polygenic control of the characters (Tables 4.10 & 4.11). Among the growth characters the highest PCV and GCV were shown by vine length, the lowest PCV by leaf breadth and lowest GCV by leaf thickness. Polygenic characters show different levels of variability. Among the three yield characters number of inflorescences per plant and yield per plant showed almost the same PCV and GCV. PCV in the case of number of inflorescences per plant was 44.76% and in the case of yield per plant it was 44.65%. GCV in the case of number of inflorescences per plant was 31.13% and that in the case of yield per plant was 31.15%. Number of flowers per inflorescence showed a lower PCV and GCV. In all the cases PCV was higher than GCV (Table 4.11).

Table 4.10. Phenotypic variance, genotypic variance, phenotypic coefficient of variation, genotypic coefficient of variation, heritability (broad sense) and genetic advance in the case of the growth characters of *Vanilla planifolia*

Characters	Pheno-typic variance	Geno-typic variance	PCV (%)	GCV (%)	Herita-bility (%)	Genetic advance (%)
1. Vine length (cm)	267847.63	88595.63	33.19	19.09	33.08	22.62
2. Vine girth (cm)	0.02	0.01	16.67	11.88	50.79	17.44
3. Number of nodes per metre	2.71	1.56	15.57	11.82	57.61	18.48
4. Leaf length (cm)	Variation not significant statistically					
5. Leaf breadth (cm)	0.29	0.08	11.00	5.78	27.54	6.24

6. Leaf thickness (cm)	0.03	0.01	11.32	5.68	25.19	5.87
7. Leaf area (cm^2)	Variation not significant statistically					
8. Inter nodal length (cm)	4.49	2.58	20.27	15.36	57.40	23.97
9. Length of velamen roots (cm)	Variation not significant statistically					
10. Thick ness of velamen roots (cm)	0.004	0.002	21.76	14.05	41.65	18.67

Table 4.11. Phenotypic variance, genotypic variance, phenotypic coefficient of variation, genotypic coefficient of variation, heritability (broad sense) and genetic advance in the case of the yield characters of *Vanilla planifolia*

Characters	Phenotypic variance	Genotypic variance	PCV (%)	GCV (%)	Heritability (%)	Genetic advance (%)
1. Number of inflorescences per plant	1.23	0.60	44.76	31.13	48.36	44.59
2. Number of flowers per inflorescence	14.19	6.16	29.85	19.67	43.42	26.70
3. Yield per plant (kg)	0.02	0.01	44.65	31.15	48.67	44.76

No efforts to study the genetic variability of vanilla using biometric methods of analysis of morphometric characters could be traced. However studies to detect variability of vanilla using chemical and molecular markers have been carried out by earlier workers. Rao *et al*. (1993a) tried to detect genetic variability in *Vanilla planifolia* through PAGE studies. They analyzed isozymes namely, amylase, esterase and

peroxidase using Poly Acrylamide Gel Electrophoresis in six indigenous collections of *Vanilla planifolia*. Qualitative and quantitative variabilities were encountered for esterase isozymes, whereas no variability was observed for peroxidases and amylases. Genetic diversity in the case of cultivated and wild collections of vanilla has been assessed by Lubinsky (2005) using AFLP analysis. Duval *et al*. (2006) used AFLP markers to study interspecific diversity between *Vanilla planifolia* and *Vanilla tahitensis*. Schluter *et al*. (2007) studied genetic diversity of *Vanilla planifolia* using RAPD analysis. They could separate *Vanilla planifolia* into three geographical groups in Central America. Minoo *et al*. (2008a) studied the RAPD polymorphism of *Vanilla planifolia* accessions based on materials collected from India. They found that variation was very narrow in the species. Efforts to assess the genetic variability of crop species using biometric analysis of morphometric characters have been made by different workers in crops like soybean (Nirmala Kumari and Balasubramanian, 1993), turmeric (Narayanpur and Hanamashetti, 2003), coriander (Tripathi *et al*., 2000), betel vine (Reddy, 1994), mango ginger (Jayasree *et al*., 2006) and cardamom (Radhakrishnan *et al*., 2006a). In all the cases PCV was found to be higher than GCV.

4.4.2. Heritability (broad sense)

Heritability (broad sense) is the ability of a character to get inherited to the progeny. Oligogenic characters show high heritability whereas the heritability of polygenic characters is controlled by the number of polygenes involved and the influence of environment on their expression. Among the seven growth characters that showed statistically significant variability, number of nodes per metre and internodal length showed the highest heritability followed by vine girth (Table 4.10). Number of nodes per metre showed a heritability (broad sense) of 57.61%,

internodal length showed a heritability (broad sense) of 57.40%, vine girth showed a heritability (broad sense) of 50.79%, thickness of velamen roots showed a heritability (broad sense) of 41.65%, vine length showed a heritability (broad sense) of 33.08%, leaf breadth showed a heritability (broad sense) of 27.54% and leaf thickness showed a heritability (broad sense) of 25.19%.

The yield characters studied showed comparatively high heritability ranging from 48.67% to 48.36% (Table 4.11). Yield per plant showed heritability (broad sense) of 48.67%, number of inflorescences per plant showed heritability (broad sense) of 48.36% and number of flowers per inflorescence showed heritability (broad sense) of 43.42%. High heritability of characters indicates low influence of environment on these characters. Vine girth, number of nodes per metre, internodal length, thickness of velamen roots, number of inflorescences per plant and yield per plant of *Vanilla planifolia* showed high heritability in the present experiment. It shows that these characters are influenced comparatively to a lesser extent by environment. Studies on heritability in the case of other crops by earlier workers also indicate similar results (Radhakrishnan *et al*., 2006a; Tripathi *et al*., 2000; Narayanpur and Hanamashetti, 2003).

4.4.3. Genetic advance

Percentage of genetic advance is the measure of the quantum of improvement that is possible under selection (Allard, 1960). The growth and yield characters of *Vanilla planifolia* studied presently have been subjected to the study of genetic advance so as to find out the quantum of improvement that is possible in the case of the growth and yield characters

of *Vanilla planifolia*. Among the growth characters, internodal length (23.97%) and vine length (22.62%) showed the highest genetic advance followed by thickness of velamen roots (18.67%), number of nodes per metre (18.48%) and vine girth (17.44%) (Table 4.10). Among the yield characters yield per plant and number of inflorescences per plant showed genetic advance of 44.76% and 44.59% respectively and number of flowers per inflorescence showed a genetic advance of 26.70% (Table 4.11). The genetic advance possible in the case of yield characters is high when compared to that of growth characters. Studies on genetic advance in the case of *Vanilla planifolia* are scanty. However, such studies carried out in other crops like cardamom (Radhakrishnan *et al*., 2006a) and mango ginger (Jayasree *et al*., 2006) have revealed the possibility of differential levels of genetic advance under selection in the case polygenic agronomic characters of crop species.

4.4.4. Genetic control of characters

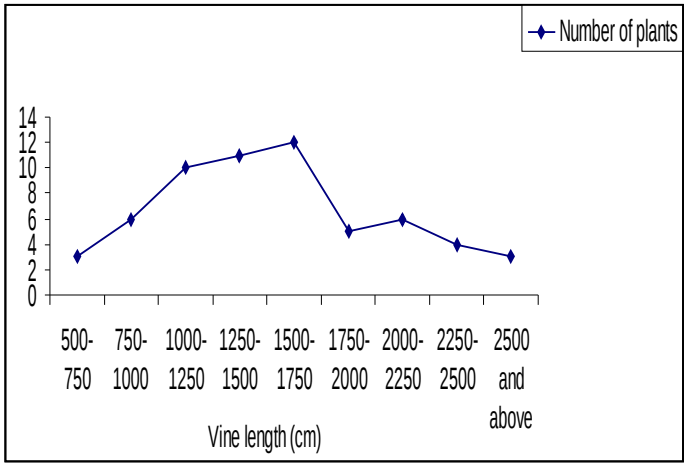
A preliminary study of the genetic control of ten growth characters and three yield characters in the case of *Vanilla planifolia* has been attempted presently based on frequency distribution analysis (Table 4.12). Sixty plants of four years of age were observed and analyzed for the purpose. Vine length showed normal frequency distribution with higher frequency of plants around the mean but the frequency of plants with higher vine length was comparatively low. Vine girth varied from 0.7 cm to 1.2 cm. However, majority of the plants were distributed in the central frequency class. Number of nodes per metre showed normal frequency distribution with higher number of plants towards the left half of the distribution. Leaf length varied from 14 cm to 24 cm and majority of the plants belonged to the central frequency class. In the case of leaf breadth also, majority of the plants belonged to the central frequency class with

low frequency of plants towards higher leaf breadth. Leaf thickness also showed continuous distribution with majority of plants belonging to the central frequency class. Leaf area ranged from 30 cm^2 to 105 cm^2 with majority of plants belonging to the central frequency class, thus showing continuous distribution of the character. However, number of plants with leaf area above the upper limit of the median class was very few. Internodal length of the vines varied from 5-15 cm but the distribution was skewed towards the right with higher frequency of plants with higher internodal length. Distribution of length of velamen roots in the study population showed continuous distribution but it was skewed towards the left showing higher frequency of plants with smaller velamen roots. Length of velamen roots varied from 10 cm to more than 130 cm. Majority of the plants studied showed velamen root thickness distributed around the central frequency class of the distribution. However, number of plants with higher thickness of velamen roots was scanty in number. Number of inflorescences per plant varied from 1-7 in the case of the four year old plants studied presently. Most of the plants were distributed towards the left side of the distribution indicating that the frequency of plants with higher number of inflorescences was only few in number. On the contrary number of flowers per inflorescence in majority of the plants belonged to the central frequency class or the post central classes of the distribution thus showing that plants with higher number of inflorescences was higher in the study population. In the case of yield per plant majority of plants were distributed in the pre central classes of the distribution showing that plants with comparatively higher yield were only few in number in the distribution.

Table 4.12. Frequency distribution of growth characters in *Vanilla planifolia*

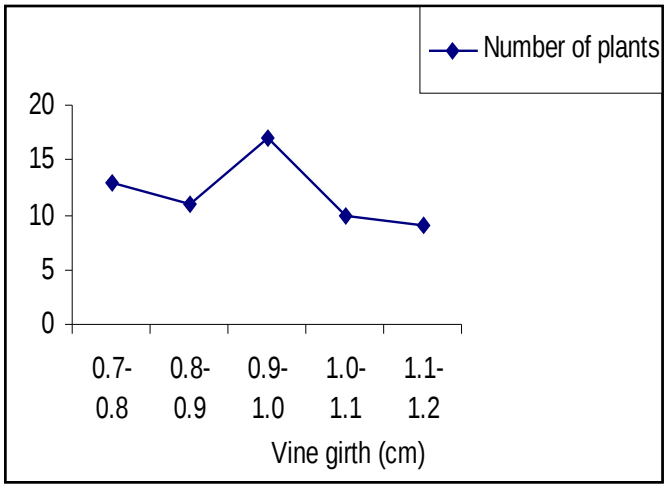
1. Vine length

Vine length (cm)	Number of plants
500-750	3
750-1000	6
1000-1250	10
1250-1500	11
1500-1750	12
1750-2000	5
2000-2250	6
2250-2500	4
2500 and above	3
Total	60



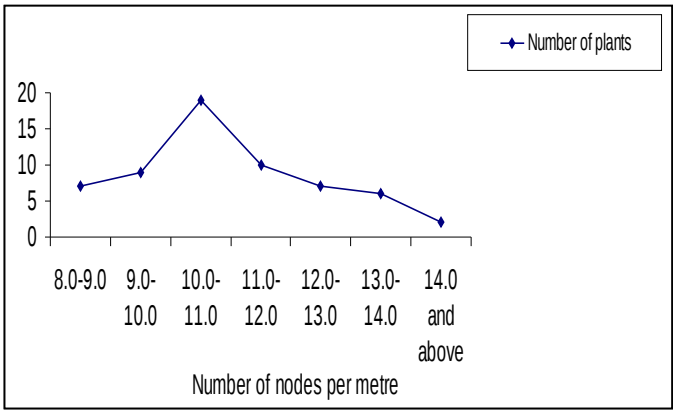
2. Vine girth

Vine girth (cm)	Number of plants
0.7-0.8	13
0.8-0.9	11
0.9-1.0	17
1.0-1.1	10
1.1-1.2	9
Total	60



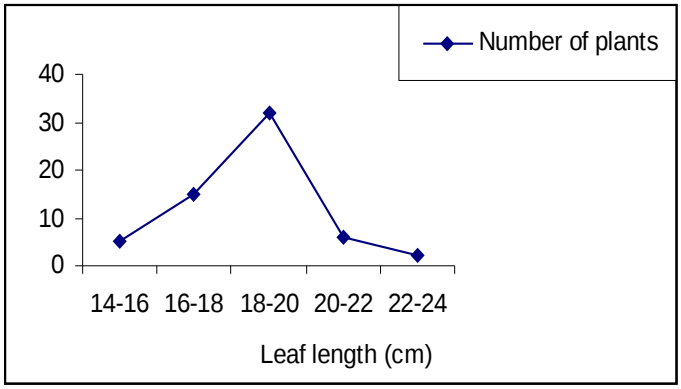
3. Number of nodes per metre

Number of nodes per metre	Number of plants
8-9	7
9-10	9
10-11	19
11-12	10
12-13	7
13-14	6
14 and above	2
Total	60



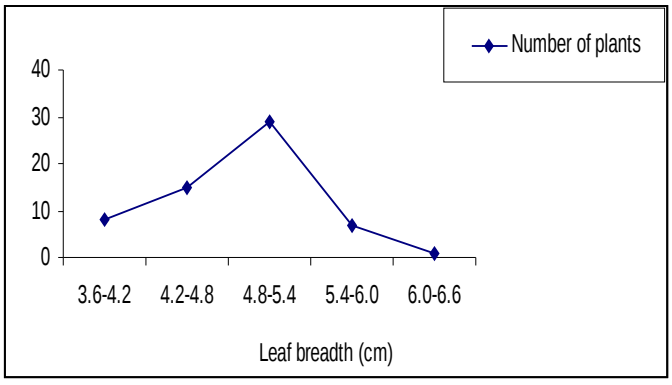
4. Leaf length

Leaf length (cm)	Number of plants
14-16	5
16-18	15
18-20	32
20-22	6
22-24	2
Total	60



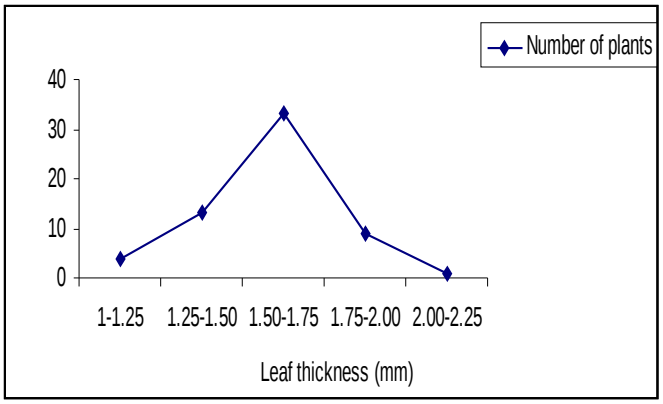
5. Leaf breadth

Leaf breadth (cm)	Number of plants
3.6-4.2	8
4.2-4.8	15
4.8-5.4	29
5.4-6.0	7
6.0-6.6	1
Total	60



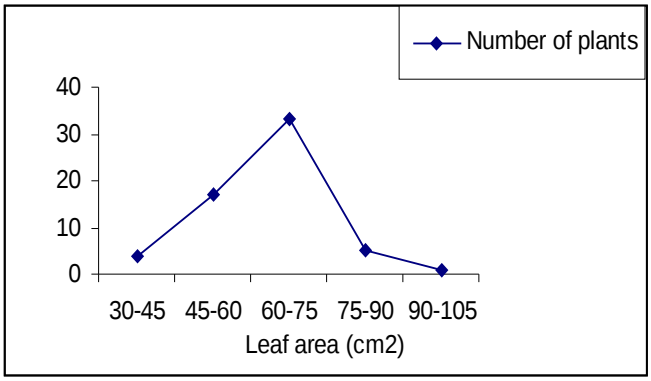
6. Leaf thickness

Leaf thickness (mm)	Number of plants
1-1.25	4
1.25-1.50	13
1.50-1.75	33
1.75-2.00	9
2.00-2.25	1
Total	60



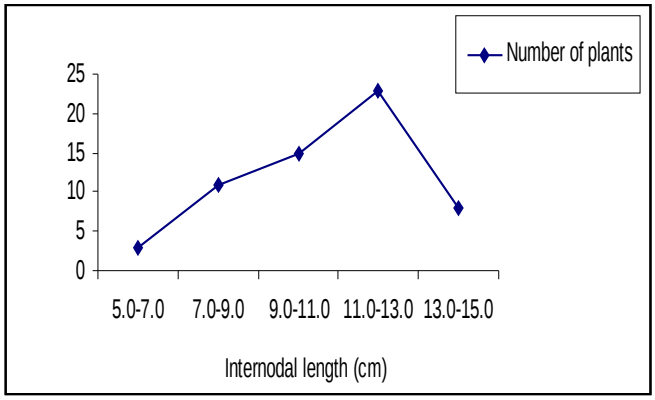
7. Leaf area

Leaf area $(cm)^2$	Number of plants
30-45	4
45-60	17
60-75	33
75-90	5
90-105	1
Total	60



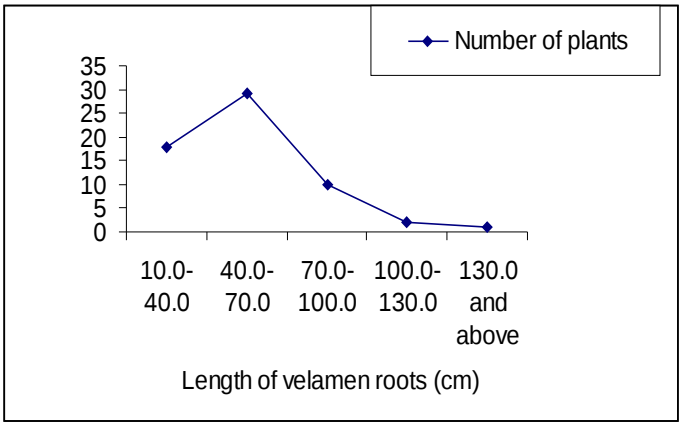
8. Internodal length

Internodal length (cm)	Number of plants
5-7	3
7-9	11
9-11	15
11-13	23
13-15	8
Total	60



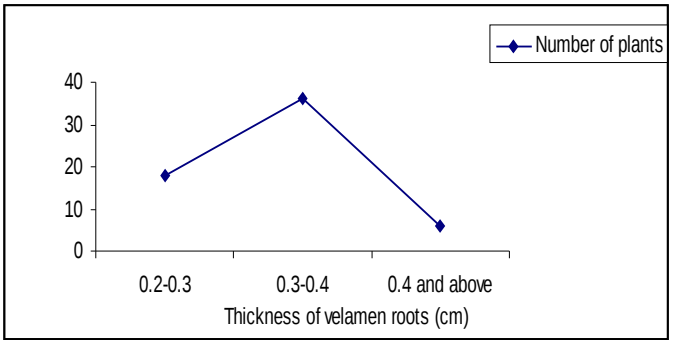
9. Length of velamen roots

Length of velamen roots (cm)	No. of plants
10-40	18
40-70	29
70-100	10
100-130	2
130 and above	1
Total	60



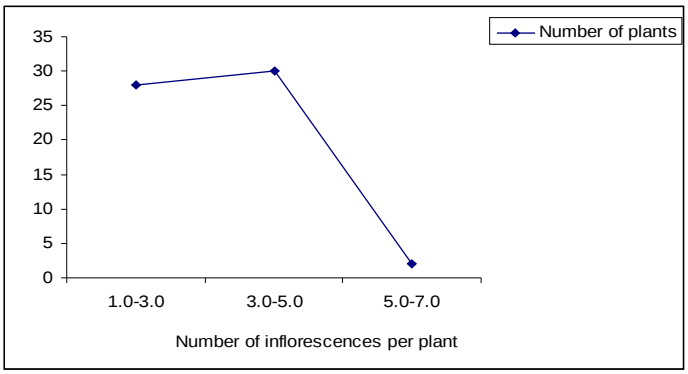
10. Thickness of velamen roots

Thickness of velamen roots (cm)	No. of plants
0.2-0.3	18
0.3-0.4	36
0.4 and above	6
Total	60



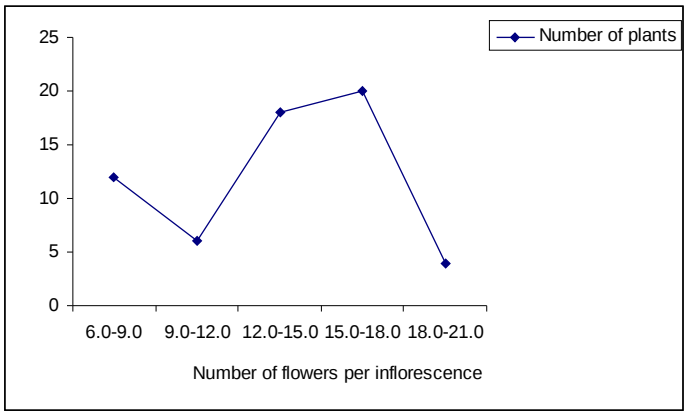
11. Number of inflorescences per plant

Number of inflorescences per plant	Number of plants
1-3	28
3-5	30
5-7	2
Total	60



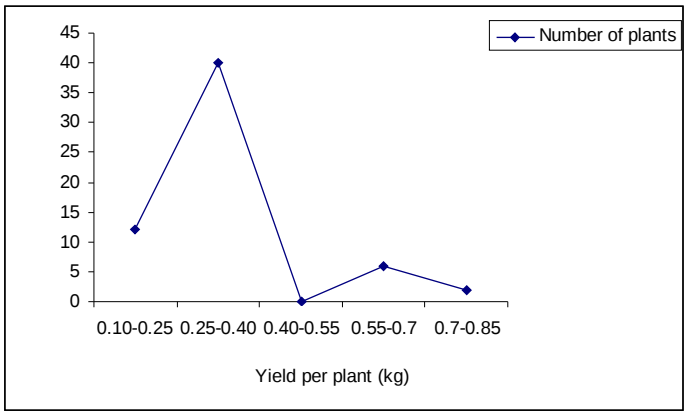
12. Number of flowers per inflorescence

Number of flowers per inflorescence	Number of plants
6-9	12
9-12	6
12-15	18
15-18	20
18-21	4
Total	60



13. Yield per plant

Yield per plant (kg)	Number of plants
0.10-0.25	12
0.25-0.40	40
0.40-0.55	0
0.55-0.7	6
0.7-0.85	2
Total	60



The above study shows that in the case of vine length, vine girth, number of nodes per metre, leaf area, internodal length, length of velamen roots, thickness of velamen roots, number of inflorescences per plant and yield per plant, higher number of plants are distributed towards the undesirable side of the distribution. Moreover the frequency of plants with desirable traits is lesser in all the cases. This shows that selection programmes for the identification of plants with desirable traits and

developing new planting materials from them should be carried out immediately so that better performing vanilla genotypes are being cultivated by farmers in future.

Umamaheswari and Mohanan (2004) have reported the polygenic nature of agronomic traits of *Vanilla planifolia* while studying the field level variability of the crop in Kerala. Some studies have been carried out in other crops like plantation crops (Dharmaraj and Sreenivasan, 1992; Nikhila, 2007; Raghu *et al*., 2003), cereals (Paramasivan and Sreerangasamy, 1988) and medicinal plants (Chandramohanan and Mohanan, 2005) in this direction.

4.5. Study of correlation of characters

Most of the agronomic characters of crop plants are polygenic in nature. These characters show different levels of interrelationship between them. This relationship is mainly due to the sharing of genes between the characters. Correlation analysis is an efficient tool to identify the relationship between characters. Correlation between thirteen agronomic characters of *Vanilla planifolia* has been studied presently so as to bring out this interrelationship. Data collected from eleven accessions of *Vanilla planifolia* grown for the purpose have been used for this study (Table 3.7).

Correlation analysis revealed that vine girth and number of nodes per metre showed significant positive correlation with maximum number of characters followed by leaf length, leaf breadth and thickness of velamen roots (Tables 4.13 and 4.14). Vine girth showed significant positive correlation with number of nodes per metre, leaf breadth, leaf area, length of velamen roots and thickness of velamen roots. Number of nodes per metre showed significant positive correlation with vine girth,

leaf length, internodal length, length of velamen roots and thickness of velamen roots. Leaf length showed significant positive correlation with vine length, number of nodes per metre, leaf area and internodal length. Leaf breadth showed significant positive correlation with vine girth, leaf area, length of velamen roots and thickness of velamen roots. Thickness of velamen roots showed significant positive correlation with vine girth, number of nodes per metre, leaf breadth and internodal length. Leaf area showed significant positive correlation with vine girth, leaf length and leaf breadth. Internodal length showed significant positive correlation with number of nodes per metre, leaf length and thickness of velamen roots. Length of velamen roots showed significant positive correlation with vine girth, number of nodes per metre and leaf breadth. Vine length showed significant positive correlation with leaf length. Number of inflorescences per plant and yield per plant showed significant positive correlation with each other. Leaf thickness and number of flowers per inflorescence did not show significant positive correlation with any of the characters studied. Characters with maximum interrelationship with other characters show high level of gene sharing and they can be used as lead characters in selection and other crop improvement programmes. Correlation between biometrical characters in *Vanilla planifolia* has been studied by Sankaran *et al.* (1994). Vine length, number of leaves, number of nodes, internodal length, leaf area and vine girth showed significant positive correlation with each other. A regression model for yield prediction in *Vanilla planifolia* has been proposed by Priya *et al*. (2002). They found that yield was positively correlated with yielding area of vine, vine girth, bean length and number of beans per vine.

Table 4.13. Correlation of characters in *Vanilla planifolia*- correlation coefficients

racters	Vine	Vine girth	Number of	Leaf	Leaf	Leaf thick	Leaf area	Inter	Length of	Thick	Number of	Numbe

	length		nodes per metre	length	breadth	ness		nodal length	velamen roots	ness of velamen roots	inflorescences per plant	flowers inflores
length	1											
ine irth	-0.38982	1										
nber of es per etre	-0.47693	0.636367 **	1									
length	0.641839 **	-0.09912	-0.62643 *	1								
eaf eadth	-0.42177	0.747649 **	0.140698	0.233149	1							
eaf kness	0.144156	0.482753	0.163631	0.07208	0.28512	1						
f area	-0.11156	0.588993 *	-0.07314	0.555374 *	0.87485 **	0.426843	1					
rnodal ngth	0.395877	-0.52014	-0.8344 **	0.735958 **	0.031006	-0.42065	0.253194	1				
gth of amen oots	-0.38919	0.60456 *	0.365618 *	0.066735	0.651441 **	-0.26235	0.37591	-0.06127	1			
hick ss of amen oots	-0.39875	0.786 **	0.750487 **	-0.24268	0.585649 *	0.331662	0.387841	-0.57391 *	0.51478	1		
nber of escenc er plant	0.214636	0.096678	0.30763	-0.00565	-0.06045	-0.19107	-0.1501	-0.05249	0.192342	0.421901	1	
nber of ers per escenc e	0.167572	-0.02569	0.07497	0.275704	-0.00589	0.111018	0.291127	0.233998	-0.06235	-0.00913	-0.07057	1
ld per lant	0.20131	0.096312	0.306087	-0.01354	-0.05347	-0.18734	-0.15012	-0.05599	0.19824	0.424983	0.999366 **	-0.076

*: significant at 5% level; **: significant at 1% level

Table 4.14. Correlation of characters in *Vanilla planifolia*- characters and cases of significant positive correlation with other characters

Characters	Characters to which positively	Number

	correlated	of characters
1. Vine length	Leaf length	1
2. Vine girth	Number of nodes per metre, leaf breadth, leaf area, length of velamen roots, thickness of velamen roots	5
3. Number of nodes per metre	Vine girth, leaf length, internodal length, length of velamen roots, thickness of velamen roots	5
4. Leaf length	Vine length, number of nodes per metre, leaf area, internodal length	4
5. Leaf breadth	Vine girth, leaf area, length of velamen roots, thickness of velamen roots	4
6. Leaf thickness		Nil
7. Leaf area	Vine girth, leaf length, leaf breadth	3
8. Internodal length	Number of nodes per metre, leaf length, thickness of velamen roots	3
9. Length of velamen roots	Vine girth, number of nodes per metre, leaf breadth	3
10. Thickness of velamen roots	Vine girth, number of nodes per metre, leaf breadth, internodal length	4
11. Number of inflorescences per plant	Yield per plant	1
12.Number of flowers per inflorescence		Nil
13. Yield per plant	Number of inflorescence per plant	1

4.6. Study of character association

Polygenic characters that are quantitative in inheritance usually show different levels of association between them. This is mainly due to

the influence of same sets of alleles on different characters. Grouping of characters based on this relationship is an effective tool to group the variables and to identify lead variables so that further breeding programmes could be focused on those characters, thus reducing the bulk of variables being studied.

Character association in *Vanilla planifolia* has been studied presently based on factor analysis using eleven variables (Tables 4.15, 4.16 and 4.17). The analysis has been carried out as suggested by Sneath and Sokal (1973) using the statistical software STATISTICA. The eleven characters studied could be grouped into three factors. The first factor group consisted of the characters internodal length and vine length. The second factor group consisted of three characters namely, number of inflorescences per plant, yield per plant and number of nodes per metre. Six characters namely, leaf area, number of flowers per inflorescence, length of velamen roots, vine girth, thickness of velamen roots and leaf thickness were grouped under the third factor. Internodal length showed the maximum factor loading in the first group, number of inflorescences per plant in the second group and leaf area in the third group. These characters are the lead characters in each group and it shows that internodal length, number of inflorescences per plant and leaf area can be considered the most important characters in *Vanilla planifolia* while practicing selection and other crop improvement programmes. Vine length is also an important character in *Vanilla planifolia* since it also shows comparatively high factor loading. The percentage of variance contributed by the characters coming under the first factor is 34.68, that contributed by the characters coming under the second factor is 20.55 and that contributed by the characters coming under the third factor is 15.21. These

three factors cumulatively contribute 70.45 % of the total variance in the case of the characters studied presently in the vanilla population.

Table 4.15. Factor analysis in the case of *Vanilla planifolia*- factor loadings

Character	Factor1	Factor 2	Factor 3
Vine length	**.494857**	.276944	.386037
Vine girth	-.868670	-.302535	**.187451**
Number of nodes per metre	-.846768	**.106079**	-.299318
Leaf thickness	-.312193	-.517858	**.099677**
Leaf area	-.311232	-.529812	**.725625**
Internodal length	**.684611**	.067987	.618451
Length of velamen roots	-.581599	.042266	**.289229**
Thickness of velamen roots	-.922776	.051473	**.135590**
Number of inflorescences per plant	-.346053	**.862237**	.265687
Number of flowers per inflorescemce	.058413	-.199173	**.489805**
Yield per plant	-.349886	**.860402**	.261314

Table 4.16. Factor analysis in the case of *Vanilla planifolia*- eigen values, percentages of total variance, cumulative eigen values and cumulative percentages of variance

Factors	Eigenvalue	% of total variance	Cumulative eigenvalue	Cumulative % of variance
1	3.814866	34.68060	3.814866	34.68060
2	2.260827	20.55297	6.075694	55.23358
3	1.673524	15.21386	7.749218	70.44743

Table 4.17. Factor analysis in the case of *Vanilla planifolia*- characters associated as per factor analysis

Factors	Characters
1	**Internodal length, Vine length**
2	**Number of inflorescences per plant**, yield per plant, number of nodes per metre

3	**Leaf area,** number of flowers per inflorescence, length of velamen roots, vine girth, thickness of velamen roots, leaf thickness

Factor analysis has been used as an efficient tool to study character association and also to group variables so as to effect data reduction by identifying the lead variables of each group. This method has been used for data reduction, grouping of variables and also to find out the lead variables in different crops like rubber (Abraham *et al*., 2002), cardamom (Radhakrishnan *et al*., 2004), tea (Ramasubramanian, 2005), rice (Mini, 2006), chillies (Hrideek *et al*., 2006), coconut (Abdulkader *et al*., 2007) and coffee (Nikhila, 2007).

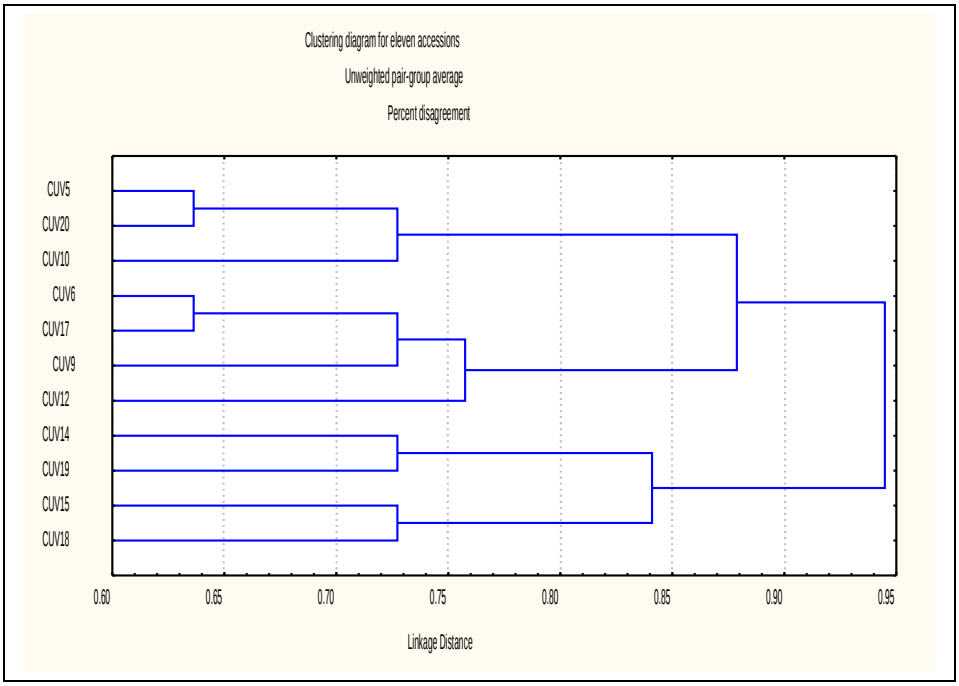
4.7. Study of genetic divergence

Different genotypes of a plant species will show differential interrelationships between them based on their genetic set up and habitats. Evolution is highly habitat specific and hence there is ample scope for the origin of genetic differences between genotypes that are spatially and reproductively separated, but scope for genetic recombinations that lead to mixing up of genes does not exist in vegetatively reproduced plant populations. However there is scope for the origin of variations due to different reasons and the development of lines with variant characters. When pooled up, this may result in the accumulation of comparatively high levels of variability even in vegetatively propagated species. Study of genetic divergence provides an effective tool to classify genotypes based on such similarities and variations. The results of cluster analysis attempted presently in eleven accessions of *Vanilla planifolia* based on eleven phenotypic characters are presented in Fig 4.21. The eleven genotypes studied presently could be grouped into two clusters at a linkage distance of 0.945, the first cluster consisting of seven genotypes

namely CUV 5, CUV 20, CUV 10, CUV 6, CUV 17, CUV 9 and CUV 12 and the second cluster consisting of four genotypes namely CUV 14, CUV 19, CUV 15 and CUV 18. At a linkage distance of 0.88 the first cluster could be again divided into two, the first group consisting of three genotypes and the second group consisting of four genotypes. At a linkage distance of 0.84 the second cluster could be divided into two groups, each group consisting of two genotypes. The present study showed that CUV 5 and CUV 20 and CUV 6 and CUV 17 are the closest genotypes (Fig 4.19). CUV 5 and CUV 20 and CUV 6 and CUV 17 come from different geographical regions. Hence it shows that geographical separation is not a major reason for proximity or distance between genotypes. Similarly the two clusters obtained at the linkage distance 0.945 consisted of genotypes collected from nearby areas. For example, CUV 12 collected from Combayar, Kerala and CUV 14 collected from Combayar, Kerala showed high linkage distance between them.

The above study shows that origin of variability in *Vanilla planifolia* occurs confined to each and every population separately and there are no geographical factors involved in the process. Moreover, since the populations are vegetatively propagated there is no mixing up of the gene pools of nearby populations. This shows that search for *in situ* variability of *Vanilla planifolia* should be carried out taking each niche as a unit. The first step towards the exploration and exploitation of variability in vanilla is the establishment of a germplasm with accessions from each and every agriculture unit and subsequent screening of them under identical conditions.

Fig. 4.21. Clustering diagram of the 11 accessions of *Vanilla planifolia* studied for genetic divergence



Studies on genetic divergence have been used as an effective tool to analyze the genetic distance between different genotypes of crop plants by earlier workers (Mathew *et al*., 2004; Nair and Thomas, 2005; Lenka *et al*., 1999; Radhakrishnan *et al*., 2006b; Sodani *et al*., 1990; Misra *et al*., 1990; Pandey and Dobhal, 1993).

Efforts to study *in situ* variability of vanilla, to establish *ex situ* collections of germplasm and also to study their genetic variability and divergence are only few in India. Twenty one accessions of *Vanilla planifolia* have been collected and conserved by the Central Research Station of Indian Cardamom Research Institute (ICRI) at Myladumpara in Idukki District of Kerala (Kuruvilla *et al*., 2004). The ICRI Regional Research Station at Sakleshpur, Karnataka has a collection of fifty six accessions of *Vanilla planifolia* (Bhat and Sudharshan, 2004) and Indian Institute of Spices Research (IISR), Calicut, Kerala has got a collection of

forty five accessions (Ravindran, 1999). Twenty accessions of *Vanilla planifolia* from Kerala, Karnataka and Tamil Nadu states have been collected and conserved in Calicut University for the present experiment. *In vitro* conservation of different species of *Vanilla* has been attempted by IISR, Calicut, Kerala recently (Minoo *et al*., 2006b). Serious efforts to collect and conserve genetic resources of vanilla have been made in other parts of the world. In 1991, Pearson *et al*. developed the FAO/IBPGR technical guidelines for the collection and movement of vanilla germplasm. In 1999, Arenas pointed out that the genetic resources of *Vanilla planifolia* are threatened by deforestation, over collection and bad management. Grisoni *et al*. (2007) collected 250 accessions of vanilla from south western ocean area and *ex situ* collection of the same has been maintained at CIRAD in Reunion. Bory *et al*. (2008) attempted to study the genetic diversity and reproductive biology of *Vanilla planifolia* and they have raised certain questions regarding the origin of phenotypic diversity in a vegetatively propagated crop like vanilla. Their studies have demonstrated the urgent need for preservation of genetic resources of *Vanilla planifolia*.

4.8. Performance analysis of *Vanilla planifolia* accessions

Study of overall performance of eleven accessions (Table 3.7) of *Vanilla planifolia* has been attempted presently based on performance index and cumulative performance index calculated as described elsewhere. The study showed that the accession CUV 15 ranked first among the eleven accessions based on the cumulative performance index calculated based on thirteen characters including ten growth characters and three yield characters. CUV 15 was followed by CUV 12 and CUV 6 in their relative performance (Tables 4.18; 4.19 and Figs. 4.22; 4.23; 4.24). CUV 15 was collected from farmer's field at Combayar, Idukki, Kerala,

CUV12 also from Combayar, Idukki, Kerala and CUV 6 from Kuzhithodu, Kerala. These accessions can be further analyzed for consistency in yield in the coming years, subjected to further protocols and developed as new varieties since no commercial variety has been released so far in vanilla.

4.9. Study of adaptability of *Vanilla planifolia* to different geographical regions of Kerala

Vanilla planifolia is a comparatively new crop introduced to India and in Kerala most probably its cultivation started around 1945 (Anonymous, 1992). Presently vanilla is cultivated in several districts of Kerala. However, Idukki and Wayanad districts and the hilly tracts of Kozhikode district are very important geographical regions of the state where vanilla plantations were established decades ago. Vanilla was a major source of income to the farmers, especially to the small and marginal holders (Hegde and Yadu kumar, 2002; Muthuramalingam *et al*., 2004). In spite of unexpected fluctuations in the price of the crop, farmers look forward to better days with good and remunerative price for the crop.

Table 4.18. Performance analysis of the accessions of vanilla studied- character means and grand means

Character	Accession means											Grand mean
	CU V5	**CU V6**	CU V9	CU V10	**CU V12**	CU V14	**CU V15**	CU V17	CU V18	CU V19	CU V20	
1.Vine length (cm)	2032	**1993**	1946	2114	**1527**	1405	**1917**	1046	1441	1385	1597	1673
2. Vine girth (cm)	0.9	**0.9**	0.8	0.8	**1.0**	1.0	**1.1**	0.9	1.1	1.0	0.9	0.95
3. Number of	8.3	**9.0**	10.0	9.0	**13.0**	10.3	**13.3**	11.0	11.3	11.3	12.0	10.77

nodes per metre												
4. Leaf length (cm)	20.0	**20.0**	18.5	19.1	**18.0**	18.0	**19.0**	18.0	18.6	19.0	17.4	18.69
5. Leaf breadth (cm)	5.0	**5.0**	4.4	4.7	**4.8**	5.2	**5.2**	5.1	5.2	5.2	4.6	4.95
6. Leaf thickness (mm)	1.5	**1.6**	1.6	1.6	**1.5**	1.7	**1.7**	1.4	1.7	1.7	1.6	1.6
7. Leaf area $(cm)^2$	65.09	**67.20**	58.11	63.11	**59.87**	64.69	**68.12**	64.17	69.19	70.08	57.01	64.24
8. Internodal length (cm)	12.5	**12.0**	9.8	12.2	**8.3**	8.7	**8.3**	11.2	9.0	9.2	6.7	9.81
9. Length of velamen roots (cm)	75.0	**60.0**	23.7	33.9	**88.6**	64.4	**72.7**	72.7	56.2	65.4	44.2	59.71
10. Thickness of velamen roots (cm)	0.2	**0.3**	0.2	0.2	**0.3**	0.3	**0.4**	0.3	0.3	0.3	0.3	0.28
11. Number of inflorescences per plant	2.0	**3.0**	3.0	2.0	**3.0**	2.3	**4.3**	3.0	1.7	1.0	2.0	2.48
12. Number of flowers per inflorescence	9.0	**14.0**	11.7	17.7	**15.0**	8.7	**13.0**	11.7	13.0	16.0	9.0	12.62
13. Yield per plant (kg)	0.25	**0.38**	0.38	0.25	**0.38**	0.30	**0.54**	0.38	0.21	0.13	0.25	0.31

Table 4.19. Performance analysis of the accessions of vanilla studied- performance indices

Characters	Performance indices of accessions										
	CUV 5	CUV 6	CUV 9	CUV 10	CUV 12	CUV 14	CUV 15	CUV 17	CUV 18	CUV 19	CUV20
1.Vine length (cm)	1.21	1.19	1.16	1.26	0.91	0.84	1.15	0.63	0.86	0.83	0.95
2. Vine girth (cm)	0.95	0.95	0.84	0.84	1.05	1.05	1.1.6	0.95	1.16	1.05	0.95
3. Number of nodes per	0.77	0.84	0.93	0.84	1.21	0.96	1.23	1.02	1.04	1.04	1.11

metre											
4. Leaf length (cm)	1.07	1.07	0.99	1.02	0.96	0.96	1.02	0.96	1.00	1.02	0.93
5. Leaf breadth (cm)	1.01	1.01	0.89	0.95	0.97	1.05	1.05	1.03	1.05	1.05	0.93
6. Leaf thickness (mm)	0.94	1.00	1.00	1.00	0.94	1.06	1.06	0.88	1.06	1.06	1.00
7. Leaf area $(cm)^2$	1.01	1.05	0.90	0.98	0.93	1.01	1.56	1.00	1.08	1.09	0.89
8. Internodal length (cm)	0.78	0.82	1.00	0.84	1.18	1.13	1.18	0.88	1.09	0.11	1.46
9. Length of velamen roots (cm)	1.26	1.00	0.40	0.57	1.48	1.08	1.22	1.22	0.94	1.10	0.74
10. Thickness of velamen roots (cm)	0.71	1.07	0.71	0.71	1.07	1.07	1.43	1.07	1.07	1.07	1.07
11. Number of inflorescences per plant	0.81	1.21	1.21	0.81	1.21	0.93	1.73	1.21	0.69	0.40	0.81
12. Number of flowers per inflorescence	0.71	1.11	0.93	1.40	1.19	0.69	1.03	0.93	1.03	1.27	0.71
13. Yield per plant (kg)	0.81	1.23	1.23	0.81	1.23	0.97	1.74	1.23	0.68	0.42	0.81
Total Performance index	12.04	13.55	12.19	12.03	14.33	12.8	15.4	13.01	12.75	11.51	12.36
Rank of performance	IX	III	VIII	X	II	V	I	IV	VI	XI	VII

Fig. 4.22.

Fig.4.23.

Fig. 4.24.

An attempt has been made presently to study the adaptability of *Vanilla planifolia* to three geographical regions of Kerala where vanilla is cultivated on a considerable scale. Ten vanilla farms each from Thamarassery area of Kozhikode district, Idukki district and Wayanad district of Kerala were studied presently to investigate into the adaptability of *Vanilla planifolia* to these areas.

4.9.1. Soil parameters of the regions studied

The soil parameters, pH, nitrogen content (in terms of percentage of organic carbon), available phosphorous content (kg/ha) and available potassium content (kg/ha) were studied as a pre requisite for the study. pH of the soil of the vanilla farms is acidic in all the cases in Thamarassery and Idukki whereas in Wayanad it is acidic or neutral. In the vanilla farms of Thamarassery, the pH varies from 4.0 to 6.0 and in Wayanad it varies from 4.5 to 6.5 (Tables 4.20 and 4.21). In Thamarassery and Wayanad all the farms studied showed medium availability of nitrogen whereas in Idukki 80% of the farms show medium availability and 20% of the farms show low availability of nitrogen. Availability of phosphorous was high in 10% of the farms in Thamarassery and Idukki, medium in 30% of the farms in Thamarassery and 20% of the farms in Idukki and low in 60% of the farms in Thamarassery and 70% of the farms in Idukki. All the farms in Wayanad studied presently showed low availability of phosphorous. 20% of the farms in Thamarassery and 40% of the farms in Idukki showed high availability of potassium. 10% of the farms in Thamarassery and 40% farms in Idukki showed medium availability of potassium. 70% of the farms in Thamarassery and 20% of the farms in Idukki showed low availability of potassium. However in Wayanad 80% of the farms showed high availability of potassium and 20% of the farms showed medium availability of potassium.

Table 4.20. Soil parameters of the three vanilla growing regions studied

Plot No.	pH	N (percentage of organic Carbon)	P (kg/ hectare)	K (kg/ hectare)
1. Thamarassery (Kozhikode)				
VAP1	4.4 (A)	0.73 (M)	7 (L)	95 (L)
VAP2	4.4 (A)	1.29 (M)	12 (M)	57 (L)
VAP3	4.5 (A)	0.94 (M)	10(M)	62 (L)
VAP4	4.6 (A)	0.67 (M)	2 (L)	286 (H)
VAP5	5.1 (A)	1.30 (M)	2 (L)	162 (M)
VAP6	5.3 (A)	1.31 (M)	22 (M)	107 (L)
VAP7	5.2 (A)	0.97 (M)	27 (H)	95 (L)
VAP8	4.7 (A)	1.71 (M)	2 (L)	57 (L)
VAP9	5.4 (A)	1.13 (M)	2 (L)	57 (L)
VAP10	4.9 (A)	1.11 (M)	5 (L)	530 (H)
2. Idukki				
VAP11	5.4 (A)	0.84 (M)	5 (L)	144 (M)
VAP12	4.4 (A)	1.22 (M)	7 (L)	431 (H)
VAP33	4.8 (A)	0.71 (M)	2 (L)	261 (M)
VAP14	5.4 (A)	0.43 (L)	7 (L)	996 (H)
VAP15	5.7 (A)	0.58 (M)	10 (M)	249 (M)
VAP16	5.8 (A)	0.58 (M)	15 (M)	157 (M)
VAP17	5.7 (A)	0.04 (L)	47 (H)	299 (H)
VAP18	4.6 (A)	1.85 (M)	2 (L)	510 (H)
VAP19	4.6 (A)	0.97 (M)	7 (L)	87 (L)
VAP20	4.5 (A)	1.08 (M)	2 (L)	100 (L)
3. Wayanad				
VAP21	5.5 (A)	0.44 (M)	5 (L)	237 (M)
VAP22	5.5 (A)	0.61 (M)	7 (L)	996 (H)
VAP23	6.2 (N)	1.78 (M)	7 (L)	921 (H)
VAP24	6.3 (N)	0.78 (M)	5 (L)	996 (H)
VAP25	4.9 (A)	0.73 (M)	5 (L)	261 (M)
VAP26	4.9 (A)	1.05 (M)	2 (L)	610 (H)
VAP27	5.9 (A)	0.95 (M)	5 (L)	369 (H)
VAP28	5.8 (A)	0.57 (M)	7 (L)	431 (H)
VAP29	5.7 (A)	0.87 (M)	7 (L)	448 (H)
VAP30	5.4 (A)	0.59 (M)	5 (L)	311 (H)

Table 4.21. Variation in soil parameters in the vanilla plots of different geographical regions studied

Geogra-phical region	pH		N		P		K	
1.Thamarassery (Calicut)	4.0-4.5	20%	Low	0%	Low	60%	Low	70%
	4.5-5.0	40%	Medium	100%	Medium	30%	Medium	10%
	5.0-5.5	40%	High	0%	High	10%	High	20%
2. Idukki	4.0-4.5	10%	Low	20%	Low	70%	Low	20%
	4.5-5.0	40%	Medium	80%	Medium	20%	Medium	40%
	5.0-5.5	20%	High	0%	High	10%	High	40%
	5.5-6.0	30%						
3. Wayanad	4.5-5.0	20%	Low	0%	Low	100%	Low	0%
	5.0-5.5	10%	Medium	100%	Medium	0%	Medium	20%
	5.5-6.0	50	High	0%	High	0%	High	80%
	6.0-6.5	20%						

4.9.2. Performance of *Vanilla planifolia* in the three geographical regions

Out of the eleven growth and yield characters studied based on the region of cultivation, eight showed no statistically significant differences and three showed statistically significant differences at 5% level. The variation in vine girth, leaf breadth, leaf area, internodal length, number of inflorescences per plant and yield were not statistically significant. This shows that *Vanilla planifolia* performs almost equally in these three areas of cultivation (Table 4.22). Yield per plant showed a variation from 0.27 kg to 3.75 kg at Thamarassery, 0.25 kg to 2 kg at Idukki and 0.85 kg to 2.31 kg at Wayanad. This shows that yield per plant is the highest at Thamarassery when compared to the other areas. However, the variability

in yield was also the highest in Thamarassery as revealed by the highest coefficient of variation (96.46%). Highest yield per hectare (extrapolated) was also observed at Thamarassery. Yield per hectare reached up to 7500 kg in this region. The above observation shows that vanilla plants perform better in the Thamarassery area when compared to other areas of cultivation studied presently. However, since this difference is not statistically significant, the study shows that vanilla cultivation can be successfully practiced in all the three regions studied presently.

Table 4.22. Adaptability of *Vanilla planifolia* to different vanilla growing regions of Kerala State

Character	Statistic	Thamarassery (Kozhikode)	Idukki	Wayanad	CD 5%	CD 1%
1.Vine girth (cm)	Mean	1.15	1.12	1.17	NS	NS
	Range	1.08-1.23	1.0-1.3	1.08-1.33		
	SD	0.05	0.10	0.07		
	CV (%)	4.35	8.93	5.98		
2.Number of nodes per metre	Mean	9.52	10.38	9.55	0.23	NS
	Range	8.50-10.50	9.0-12.33	8.5-10.17		
	SD	0.68	1.05	0.52		
	CV (%)	7.14	10.12	5.45		
3.Leaf length (cm)	Mean	21.29	19.50	20.49	0.45	NS
	Range	19.32-23.57	15.43-22.67	19.03-22.37		
	SD	1.48	1.97	1.03		
	CV (%)	6.95	10.10	5.03		
4.Leaf breadth (cm)	Mean	5.92	5.79	6.13	NS	NS
	Range	5.58-6.63	5.23-6.13	5.60-6.87		
	SD	0.31	0.32	0.44		
	CV (%)	5.25	5.53	7.18		
5.Leaf thickness (cm)	Mean	1.87	1.74	1.90	0.04	NS
	Range	1.64-2.27	1.62-1.96	1.71-2.12		
	SD	0.17	0.09	0.12		

	CV (%)	9.09	5.17	6.32		
6.Leaf area (cm^2)	Mean	88.62	79.66	88.19	NS	NS
	Range	77.95-110.50	66.41-96.39	75.13-103.07		
	SD	10.02	9.59	9.27		
	CV (%)	11.31	12.04	10.51		
7.Internodal length (cm)	Mean	10.63	9.81	10.59	NS	NS
	Range	9.57-11.80	7.66-11.20	9.90-11.80		
	SD	0.77	1.03	0.60		
	CV (%)	7.24	10.50	5.67		
8.Number of inflorescence-nces per plant	Mean	9.07	9.38	11.02	NS	NS
	Range	2.17-30.00	2.00-16.00	5.33-18.50		
	SD	8.71	5.09	4.01		
	CV (%)	96.03	54.26	36.39		
9.Yield per plant (number of beans)	Mean	90.67	95.03	113.67	NS	NS
	Range	21.67-300.00	20.00-160.00	68.33-185.00		
	SD	87.08	50.67	37.94		
	CV (%)	96.04	53.32	33.38		
10. Yield per plant (kg)	Mean	1.13	1.19	1.42	NS	NS
	Range	0.27-3.75	0.25-2.00	0.85-2.31		
	SD	1.09	0.63	0.47		
	CV (%)	96.46	52.94	33.10		
11.Yield per hectare (kg)	Mean	2013.34	2377.33	2843.34	NS	NS
	Range	540.00-7500.00	500.00-4000.00	1706.67-4626.67		
	SD	2013.30	1266.28	948.24		
	CV (%)	99.99	53.26	33.35		

Chapter V

SUMMARY AND CONCLUSION

Vanillin (4-hydroxy 3-methoxy benzaldehyde) is the major chemical responsible for vanilla flavour and fragrance. Natural vanillin is obtained from the processed beans of the three cultivated species of the orchid genus *Vanilla* namely *Vanilla planifolia* Andrews, *Vanilla tahitensis* J.W.Moore and *Vanilla pompona* Schiede. Of these, *Vanilla planifolia* is the most preferred and widely cultivated.

Vanilla planifolia is native to the humid tropical rain forests of South Eastern Mexico, Central America, the West Indies and the northern part of South America. *Vanilla tahitensis* is indigenous to Tahiti, the French Oceania group of islands in the pacific ocean and *Vanilla pompona* is indigenous to South Eastern Mexico, Central America, Trinidad and North and South America. In India only *Vanilla planifolia* is cultivated commercially. *Vanilla tahitensis* is available in the germplasm repositories of the national institutes carrying out spices research in the country.

Attempts to introduce vanilla to India dated back to 1835. However commercial cultivation of vanilla in India started by the end of the 19th century. The genetic base of vanilla in India is very narrow since vanilla cultivation most probably started in the country using very few cuttings introduced for the purpose and it is clonally propagated.

Preliminary efforts on exploration, collection and conservation of *Vanilla planifolia* in India have indicated the need of systematic efforts to assess the variability of vanilla both at *in situ* and *ex situ* levels and also to

study agronomical characters of the crop based on a crop improvement perspective. The objectives of the present experiment have been formulated from the above situation that demand studies that lead to the improvement of the planting materials that are made available to the farmers and also studies on the crop for further investigations on its genetics and genetic improvement.

The major objectives envisaged presently include study of the floral biology of two cultivated species of vanilla; study of interspecific variability between them; study of field level variability of *Vanilla planifolia*, the species commercially cultivated in India; study of its genetic variability, interrelationship of characters, character association and genetic divergence and also a study on the adaptability of *Vanilla planifolia* to different geographical regions of Kerala State of India.

For field experiments, observations were made from vanilla farms of Idukki, Calicut (Thamarassery area) and Wayanad districts of Kerala in the year of 2003. Experiments on floral biology of *Vanilla planifolia* and *Vanilla tahitensis* and inter specific variability between *Vanilla planifolia* and *Vanilla tahitensis* were carried out from 2002 to 2008 and experiments on genetic variability, correlation of characters, character association and genetic divergence of *Vanilla planifolia* from 2003 to 2008 in the Genetics and Plant Breeding Division of the Department of Botany of University of Calicut, Kerala.

Twenty four characters were observed for the study of floral biology of *Vanilla planifolia* and *Vanilla tahitensis*. Only 20% of both the species flowered by the end of the 3rd year of growth in the case of the present experiments in which 33 cm vine cuttings were used as the

planting material. However, 67% of *Vanilla planifolia* plants and 56% of *Vanilla tahitensis* flowered in the fourth year of growth. Inflorescence initiation occurred in the months of February-March in *Vanilla planifolia* and in the months of January-February in *Vanilla tahitensis*. Flowers opened from March to May in *Vanilla planifolia* and from January to March in *Vanilla tahitensis*. The average duration taken from inflorescence initiation to opening of the first flower was 45 days in *Vanilla planifolia* and 40 days in *Vanilla tahitensis*. The average duration taken from the opening of the first flower to the opening of the last flower was 30 days in *Vanilla planifolia* and 28 days in *Vanilla tahitensis*.

Study of floral biology showed that in *Vanilla planifolia*, number of inflorescences ranged from 7 to 9, number of flowers per inflorescence from 10 to 16, inflorescence length from 3.3 cm to 7.3 cm, inflorescence rachis diameter from 0.5 cm to 0.7 cm, flower length from 9.5 to 10.5 cm, bract length from 1.4 cm to 1.7 cm, bract breadth from 0.6 to 0.7 cm, sepal length from 5.7 cm to 5.9 cm, sepal breadth from 1.1 to 1.3 cm and petal length from 5.6 to 5.8 cm. Petal breadth was 1 cm, labellum length 5 to 5.5 cm, labellum breadth 2.5 to 2.9 cm, gynostemium length 4 cm, ovary length 4.3 to 4.7 cm, ovary diameter 0.4 to 0.5 cm, rostellum length 0.3 cm and rostellum breadth 0.3 cm. In *Vanilla tahitensis*, number of inflorescences per metre ranged from 6 to 8, number of flowers per inflorescence from 6 to 9, inflorescence length from 6.5 to 13 cm, inflorescence rachis diameter from 0.5 to 0.7 cm, flower length from 8 to 10 cm, bract length from 0.7 to 1.3 cm, bract breadth from 0.5 to 0.7 cm, sepal length from 4.6 to 5.9 cm, sepal breadth from 0.9 to 1.2 cm, petal length from 4.5 to 5.7 cm, petal breadth from 1 to 1.2 cm, labellum length from 4.4 to 4.9 cm, labellum breadth from 1.6 to 2.3 cm, gynostemium

length from 3.3 to 3.5 cm, ovary length from 2.5 to 4.5 cm, ovary diameter from 0.3 to 0.4 cm, rostellum length 0.3 cm and rostellum breadth 0.3 cm.

Study of interspecific variability between *Vanilla planifolia* and *Vanilla tahitensis* showed that among the growth characters, vine length, vine girth, leaf breadth and leaf area showed statistically significant difference between the species. Vine growth reached a length of 1208 cm to 2955 cm in *Vanilla planifolia* and 855 cm to 1534 cm in *Vanilla tahitensis* within a growth period of four years under uniform conditions. Mean vine length produced within four years by *Vanilla planifolia* starting from 33 cm long planting material was about 20 metres and it was 12.25 metres in *Vanilla tahitensis*. Vines of *Vanilla palnifolia* showed significantly higher girth when compared to *Vanilla tahitensis*. Mean leaf area was 63.30 cm^2 in *Vanilla planifolia* and 35.27 cm^2 in *Vanilla tahitensis*, showing that the leaves of *Vanilla planifolia* were significantly larger. Number of inflorescences per metre, number of flowers per inflorescence, flower length, bract length, gynostemium length, ovary length and ovary diameter were significantly higher in *Vanilla planifolia* whereas inflorescence length was significantly higher in *Vanilla tahitensis*. Sepal length and petal length were higher in *Vanilla tahitensis* even though the difference was not statistically significant. The fruits of *Vanilla tahitensis* were shorter and stouter when compared to the fruits of *Vanilla planifolia* as evidenced by the observation on 3 month old fruits.

The present study has revealed that significant variability occurred in the case of many morphological characters between the two species and they could be differentiated based on number of inflorescences per metre, number flowers per inflorescence, inflorescence length, flower length,

bract length, bract breadth, labellum length, labellum breadth, gynostemium length, ovary length and ovary diameter.

Anatomical comparison showed that in the stem the epidermal cells of *Vanilla tahitensis* were smaller when compared to that of *Vanilla planifolia*. The collenchymatous hypodermis was single layered in both the cases. Cortex was parenchymatous in both with lesser number of cells in *Vanilla tahitensis*. Some of the cells near the endodermis contain granular content in *Vanilla planifolia* and it was absent in *Vanilla tahitensis*. Needle like crystals of Calcium oxalate were present in the cortex of both. Endodermis was single layered in both the cases. Vascular bundles were present in four rings in both the species, but the number of bundles was lesser in *Vanilla planifolia*. Bundle number was around 25 in *Vanilla planifolia* and around 40 in *Vanilla tahitensis*. Bundle cap was complete and sclerenchymatous with 3-6 layers of cells in *Vanilla planifolia* whereas it was 2-3 layered and made of feebly thickened cells in the case of *Vanilla tahitensis*.

Leaf is isobilateral with no palisade layer in both the cases. Epidermis is single layered in both with stomata only on the lower epidermis. Hypodermis is single layered and collenchymatous in both the cases and present throughout the lamina. The main vascular bundle is comparatively well developed in *Vanilla planifolia* when compared to *Vanilla tahitensis*. Bundle sheath is complete in *Vanilla planifolia* and incomplete in *Vanilla tahitensis*. Needle like crystals of Calcium oxalate are seen in some of the mesophyll cells in both the cases.

Velamen tissue is two layered in both the species. The outer layer of the velamen tissue shows distorted cells with wavy radial points in the

case of *Vanilla planifolia*. Inner tangential walls of the outer velamen layer are highly thickened in both the cases. Cortex is 14-18 layered in *Vanilla planifolia* and 10-14 layered in *Vanilla tahitensis*. Inner cortical layer of *Vanilla planifolia* contains 10-12 schizogenous air cavities and those are absent in *Vanilla tahitensis*. Root endodermis is single layered in both the cases. Twelve groups of exarch xylem and phloem bundles are arranged radially in the stele of *Vanilla planifolia* and 13 groups in *Vanilla tahitensis*. Pith is large in *Vanilla tahitensis* and comparatively small in *Vanilla planifolia*. Screening of vanilla genotypes based on the nature and thickening of velamen tissue may provide potential for selection of drought tolerant lines.

Study of vanillin content in the beans of *Vanilla planifolia* and *Vanilla tahitensis* showed that vanillin content was very high in *Vanilla planifolia*.

Field level variability of *Vanilla planifolia* was assessed presently based on seven growth characters and four yield characters. All the growth characters studied presently showed statistically significant variation between the fields selected. Among the growth characters the highest coefficient of variation was shown by leaf area and the lowest by leaf breadth. Among the yield characters, number of inflorescences per plant, yield per plant and yield per hectare showed significantly high variation between the different vanilla fields studied.

Further screening of the variability at genetic level and its conservation and improvement are very important for the safety of the genetic base of the crop in the country. The significant level of variability observed above may be the result of both genetic and environmental

factors. The unique environmental situations in the vanilla farms might have resulted in the induction of variations thus leading to the origin of variability of characters at quantitative and qualitative levels.

An experiment was conducted to assess the genetic variability of *Vanilla planifolia* using 20 accessions collected from farmer's field. Among the ten growth characters studied in the case of four year old plants, seven showed statistically significant variability between the accessions. The observation indicated the existence of variability at genetic level in the case of vine length, vine girth, number of nodes per metre, leaf breadth, leaf thickness, internodal length and thickness of velamen root. Differential vine length attained by different accessions during a particular growth period is to be studied and exploited scientifically so that fast growing accessions are identified and used for further improvement protocols especially since longer vines means higher yield in vanilla. The velamen roots of vanilla are the sole agencies to absorb moisture and mineral nutrients, since there is usually no root system other than velamen roots in vanilla under cultivation. The velamen roots of lower nodes reach the soil and ramify in the humus and absorb water and nutrition. The hanging velamen roots directly absorb moisture from atmosphere also. Number of inflorescences per plant, number of flowers per inflorescence and yield per plant showed statistically significant variation among the accessions studied for yield characters. All the yield characters showed comparatively high coefficients of variation. In the case of all the characters that showed statistically significant variability, phenotypic coefficients of variation was higher than genotypic coefficients of variation, thus indicating the additive nature and polygenic control of the characters. Among the growth characters the highest PCV and GCV were shown by vine length, the lowest PCV by leaf breadth and lowest GCV by leaf thickness. Among the three yield characters number

of inflorescences per plant and yield per plant showed almost the same PCV and GCV.

Heritability (broad sense) is the ability of a character to get inherited to the progeny. Oligogenic characters show high heritability whereas the heritability of polygenic characters is controlled by the number of polygenes involved and the influence of environment on their expression. Among the seven growth characters that showed statistically significant variability, number of nodes per metre and internodal length showed the highest heritability followed by vine girth. The yield characters studied showed comparatively high heritability ranging from 48.67% to 48.36%. High heritability of characters indicates low influence of environment on these characters.

Percentage of genetic advance is the measure of the quantum of improvement that is possible under selection. Among the growth characters of *Vanilla planifolia* studied presently, internodal length and vine length showed the highest genetic advance followed by thickness of velamen roots, number of nodes per metre and vine girth. Among the yield characters yield per plant and number of inflorescences per plant showed comparatively high genetic advance. The genetic advance possible in the case of yield characters is high when compared to that of growth characters.

A preliminary study of the genetic control of ten growth characters and three yield characters in the case of *Vanilla planifolia* has been attempted presently based on frequency distribution analysis. Vine length showed normal frequency distribution with higher frequency of plants around the mean but the frequency of plants with higher vine length was

comparatively low. Vine girth varied from 0.7 cm to 1.2 cm. Number of nodes per metre showed normal frequency distribution with higher number of plants towards the left half of the distribution. Leaf length varied from 14 cm to 24 cm and majority of the plants belonged to the central frequency class. In the case of leaf breadth also, majority of the plants belonged to the central frequency class with low frequency of plants towards higher leaf breadth. Leaf thickness also showed continuous distribution with majority of plants belonging to the central frequency class. Leaf area ranged from 30 cm^2 to 105 cm^2 with majority of plants belonging to the central frequency class. However, number of plants with leaf area above the upper limit of the median class was very few. Internodal length of the vines varied from 5-15 cm but the distribution was skewed towards the right with higher frequency of plants with higher internodal length. Length of velamen roots showed continuous distribution but it was skewed towards the left showing higher frequency of plants with smaller velamen roots. Majority of the plants studied showed velamen root thickness distributed around the central frequency class of the distribution. However, number of plants with higher thickness of velamen roots was scanty in number. Number of inflorescences per plant varied from 1-7 in the case of the four year old plants studied presently. Most of the plants were distributed towards the left side of the distribution indicating that the frequency of plants with higher number of inflorescences was only little in number. On the contrary number of flowers per inflorescence in majority of the plants belonged to the central frequency class or the post central classes of the distribution thus showing that plants with higher number of inflorescences was higher in the study population. In the case of yield per plant majority of plants were distributed in the pre central classes of the distribution showing that plants

with comparatively higher yield were only few in number in the distribution.

The above study shows that in the case of vine length, vine girth, number of nodes per metre, leaf area, internodal length, length of velamen roots, thickness of velamen roots, number of inflorescences per plant and yield per plant, higher number of plants are distributed towards the undesirable side of the distribution. Moreover the frequency of plants with desirable traits is lesser in all the cases. This shows that selection programmes for the identification of plants with desirable traits and developing new planting materials from them should be carried out immediately.

Correlation analysis revealed that vine girth and number of nodes per metre showed significant positive correlation with maximum number of characters followed by leaf length, leaf breadth and thickness of velamen roots. Characters with maximum interrelationship with other characters show high level of gene sharing and they can be used as lead characters in selection and other crop improvement programmes.

Character association in *Vanilla planifolia* has been studied presently based on factor analysis using eleven variables. The characters studied could be grouped into three factors. The first factor group consisted of the characters internodal length and vine length. The second factor group consisted of three characters namely, number of inflorescences per plant, yield per plant and number of nodes per metre. Six characters namely, leaf area, number of flowers per inflorescence, length of velamen roots, vine girth, thickness of velamen roots and leaf thickness were grouped under the third factor. Internodal length showed

the maximum factor loading in the first group, number of inflorescences per plant in the second group and leaf area in the third group. These characters are the lead characters in each group and it shows that internodal length, number of inflorescences per plant and leaf area can be considered the most important characters in *Vanilla planifolia* while practicing selection and other crop improvement programmes.

Different genotypes of a plant species will show differential interrelationships between them based on their genetic set up and habitats. Evolution is highly habitat specific and hence there is ample scope for the origin of genetic differences between genotypes that are spatially and reproductively separated, but scope for genetic recombinations that lead to mixing up of genes does not exist in vegetatively reproduced plant populations. However there is scope for the origin of variations due to different reasons and the development of lines with variant characters. When pooled up, this may result in the accumulation of comparatively high levels of variability even in vegetatively propagated species. Study of genetic divergence provides an effective tool to classify genotypes based on such similarities and variations. By cluster analysis, the eleven genotypes studied presently could be grouped into two clusters at a linkage distance of 0.945, the first cluster consisting of seven genotypes and the second cluster consisting of four genotypes. At a linkage distance of 0.88 the first cluster could be again divided into two, the first group consisting of three genotypes and the second group consisting of four genotypes. At a linkage distance of 0.84 the second cluster could be divided into two groups, each group consisting of two genotypes. The present study showed that geographical separation is not a major reason for proximity or distance between genotypes.

Study of overall performance of eleven accessions of *Vanilla planifolia* has been attempted presently and three superior accessions could be identified based on the study. These accessions can be further analyzed for consistency of yield in the coming years, subjected to further protocols and developed as new varieties since no commercial variety has been released so far in vanilla.

An attempt has been made presently to study the adaptability of *Vanilla planifolia* to three geographical regions of Kerala where vanilla is cultivated on a considerable scale. Out of the eleven growth and yield characters studied based on the region of cultivation, eight showed no statistically significant differences and only three showed statistically significant differences. The variation in important characters like leaf area, internodal length, number of inflorescences per plant and yield were not statistically significant. This shows that *Vanilla planifolia* performs almost equally in these three areas of cultivation. However, the variability in yield was the highest in Thamarassery area.

The present study has helped to analyze the floral biology of *Vanilla planifolia* and *Vanilla tahitensis*, to study the interspecific variability between the two species, to study the field level variability of *Vanilla planifolia*, and to study its genetic variability, correlation of characters, character association, genetic divergence and adaptability. Three superior accessions of *Vanilla planifolia* have been identified and the same can be used for further breeding programmes. It is hoped that the findings may become useful to the farmers of the country and the plant breeders in their future endeavours to ensure better returns from this crop.

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