Functional imprints of high intensity light and UV-B radiation in selected rice (*Oryza sativa* L.) varieties

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DOCTOR OF PHILOSOPHY IN BOTANY

By

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CERTIFICATE

This is to certify that the thesis entitled **"Functional imprints of high intensity light and UV-B radiation in selected rice (*Oryza sativa* L.) varieties"** submitted by **Faseela, P.,** in partial fulfilment of the requirements for the degree of **Doctor of Philosophy** in **Botany** of the **University of Calicut**, is a *bona fide* record of the research work undertaken by her in this department under my supervision and guidance and no part thereof has been submitted for the award of any other degree.

Calicut University Campus **Dr. Jos T. Puthur**

DECLARATION

I, **Faseela, P.,** do hereby declare that the Ph. D thesis entitled **"Functional imprints of high intensity light and UV-B radiation in selected rice (*Oryza sativa* L.) varieties"** is a research work accomplished by me under the supervision of **Dr. Jos T. Puthur, Reader, Plant Physiology and Biochemistry Division, Department of Botany, University of Calicut,** in partial fulfilment of the requirements for the award of **Doctor of Philosophy** in **Botany, University of Calicut.** I also declare that this has not been submitted by me for the award of any other degree or diploma, and it represents original work done by me.

Calicut University Campus **Faseela, P.**

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Faseela, P.

Dedication

To my mother,
for raising me to believe that anything was possible

to my better half Ippu and my little princess Ithoos,
for making everything possible

ABBREVIATIONS

^{1}Chl	-	singlet chlorophyll
$^1O_2$	-	singlet oxygen
^{3}Chl	-	triplet chlorophyll
A	-	antheraxanthin
ABS/CSo	-	absorption flux per cross section
ABS/RC	-	absorption flux per reaction center
APX	-	ascorbate peroxidase
AsA	-	ascorbate
BSA	-	bovine serum albumin
CAT	-	catalase
CFCs	-	chlorofluorocarbons
DCMU	-	3-(3,4-dichlorophenyl)-1,1-dimethyl urea
DCPIP	-	2,6-dicholrophenolindophenol
DHAR	-	dehydroascorbate reductase
DIo/CSo	-	dissipated energy flux per cross section
DIo/RC	-	dissipated energy flux per reaction center
DTNB	-	5 , 5-dithio-bis(2-nitrobenzoic acid)
DW	-	dry weight
E	-	transpiration rate
EDTA	-	ethylenediaminetetraacetic acid
ETo/CSo	-	electron transport flux per cross section
ETo/RC	-	electron transport flux per reaction center
FAO	-	Food and Agriculture Organization
Fm	-	maximum Chl *a* fluorescence
Fm´	-	maximal fluorescence level in the light-adapted state
Fo	-	initial Chl *a* fluorescence
Fo´	-	minimal fluorescence level in the light adapted state
FT-IR	-	fourier transform infrared spectrometry
Fv	-	variable Chl *a* fluorescence

Fv/Fm	-	maximum quantum yield of PSII
Fv/Fo	-	the ratio of photochemical to non photochemical quantum efficiencies
FW	-	fresh weight
GPOX	-	guaiacol peroxidase
GR	-	glutathione reductase
g_s	-	stomatal conductance
GSH	-	glutathione
H_2O_2	-	hydrogen peroxide
HL	-	high intensity light
HPLC	-	high performance liquid chromatography
HSPs	-	heat-shock proteins
L	-	lutein
LHC	-	light harvesting complex
Lx	-	lutein epoxide
MDA	-	malondialdehyde
MDHA	-	monodehydroascorbate
MDHAR	-	monodehydroascorbate reductase
MV	-	methyl viologen
NaN_3	-	sodium azide
NBT	-	nitroblue tetrazolium
NPQ	-	non-photochemical quenching
$O_2^{\bullet -}$	-	superoxide
OEC	-	oxygen evolving complex
$OH^{\bullet}$	-	hydroxyl radical
PAL	-	phenylalanine ammonia lyase
PAR	-	photosynthetically active radiation
pBQ	-	parabenzoquinone
Φ_{Eo}	-	electron transport quantum yield
$PI_{(abs)}$	-	performance index on absorption basis
p_n	-	net photosynthetic rate

PPFD	-	photosynthetic photon flux density
PSI	-	photosystem I
PSII	-	photosystem II
Ψ_o	-	the yield of electron transport per trapped exciton
RC/CSo	-	reaction center per cross section
ROS	-	reactive oxygen species
SEM	-	scanning electron microscopy
$SFI_{(abs)}$	-	PSII structure-function-index
SOD	-	superoxide dismutase
TAL	-	tyrosine ammonia lyase
TBA	-	thiobarbituric acid
TCA	-	trichloroacetic acid
TEM	-	transmission electron microscope
Tf(max)	-	the time when maximum fluorescence value is reached
TRo/CSo	-	trapping flux per cross section
TRo/RC	-	trapping flux per reaction center
UV	-	ultraviolet
V	-	violaxanthin
VDE	-	violaxanthin de-epoxidase
Vj	-	relative variable fluorescence at J step
Z	-	zeaxanthin

CONTENTS

INTRODUCTION

Nowadays, global food security is being haunted by the rapid increase in population and severe climate changes which have the potential to threaten global food security, according to the latest edition of FAO's flagship annual report, 'The State of Food and Agriculture' (FAO, 2016). Changes in climate condition is mainly attributed to the tremendous increase in greenhouse gases, including carbon dioxide (CO_2), methane, fluorinated gases and nitrous oxide, which causes changes in rain fall, temperature and negative effects on water and land resources (Kreft et al., 2017). Even though climate change is a global phenomenon, its impacts are more affected in the developing countries as reported by Stockholm International Peace Research Institute (Bremberg, 2018). Because most of the developing countries have agriculture-based economies, their agricultural zone is highly affected by climate change. Moreover, the variability in climate change becomes a major challenge to agricultural production, as it affects approximately 2.5 billion people who are partially or completely dependent on agriculture (Ali et al., 2017).

Climate change affects various crops and areas differently, but it is generally expected that agricultural productivity will decline. As climate change will negatively affect the crop productivity, it causes food security problems worldwide (Tripathi et al., 2016). World population is growing day by day and by 2050 it is expected to reach 9.1 billion, but agricultural production is not rising at a parallel pace. To feed the world population, productivity must be increased by 70% for an additional 2.3 billion people by 2050 (Rosenzweig et al., 2014). As far as India is considered, the country is highly sensitive to changes in climate because of high physical exposure to climate related parameters and also the India's economy and population depends on climate sensitive sectors like agriculture, forests and fisheries.

Low productivity is the main problem of Indian agriculture. Moreover, to meet India's growing food demand, there is an acute need for increasing productivity in all sectors of agriculture. Agricultural policy should focus on improving crop productivity and developing safety nets to cope with the risks of climate change to feed the overall population (Chakrabarty, 2016).

Agriculture production is dwindled mainly due to various biotic and abiotic stresses resulting in 30-60% yield losses globally each year. The major abiotic stresses like temperature (heat, cold chilling/frost), water (drought, flooding), chemicals (heavy metals/pesticides, gaseous toxins), radiation (UV, ionizing radiation), mineral deficiency/excess, light (high/low), mechanical (wind, soil movement, submergence) are responsible for over 50% reduction in agricultural production (Pereira, 2016). In the world only 9% of the total land area is useful for crop production, while 91% is under stress. The area under stress is likely to increase further due to land degradation, climate change and urbanization. It is anticipated that if present scenario persists, 50% of current cultivated land will be lost by 2050 (Gomiero, 2016). Added pressure to the above is that global population is likely to reach 7 billion by 2025 and 10 billion by 2050, whereas the area of cultivable land is diminishing and the necessity for enhancing the crop production is increasing (Wani and Sah, 2014). Therefore, the whole world is on the lookout for developing various technologies to enhance crop production and also to counter various abiotic stresses, so that achievements out of these attempts will help to cope up with the food demand of the increasing population (Gimenez et al., 2018).

Sunlight fuels plant growth and development through a process called photosynthesis, which converts light energy into chemical energy in plants. In natural conditions, plants have to respond to diurnal change in the light

environment due to their sessile character (Darko et al., 2014). Sunlight is an electromagnetic spectrum with an array of visible and non-visible energy with different wavelengths and it consists of packages of light (photons). Visible light to human's eye is only a small portion of the electromagnetic spectrum that includes radiation wavelengths ranging from gamma rays to TV and radio. Photons travel directly through space as long as nothing obstructs them, but different particles such as aerosols, clouds, gases, dust and other particles present in the atmosphere affect the quantity and quality of incoming radiation. Photons are absorbed, scattered and reflected by the ozone layer in the stratosphere and the particles present in the troposphere. As a result, the portion of the total solar radiation reaching Earth's surface is different (Munawwar, 2006).

Mainly sunlight is composed of three most important parts; ultraviolet radiation (UV), visible light and infrared radiation. Among them, photosynthetically relevant solar energy that reaches the surface of Earth is divided into two main spectrums; photosynthetically active radiation (PAR) (400 to 700 nm) and UV (100 to 400 nm). Because of the short wavelength, UV radiation has high energy and frequency. In biological research, UV band has been divided as UV-A (320 to 400 nm), UV-B (280 to 320 nm) and UV-C (100 to 280 nm). Among these UV-A and PAR radiation are affected by light scattering. In contrast, UV-B region is selectively absorbed by the ozone layer present in the stratosphere (Tarasick et al., 2003). UV-C, the most biologically damaging wavelength, is not a significant factor for biological processes under natural conditions since it is completely absorbed by atmosphere (Bais et al., 2018).

The main role of sunlight in plant's life is to provide the energy for photosynthesis and for the regulation of plant growth and development at different stages, now termed as photomorphogenesis (Folta and Childers,

2008). The majority of developmental processes throughout the entire life cycle of plants are influenced by light: seed germination, vegetative growth, sensing neighboring plants, circadian rhythm, de-etiolation, shade avoidance, stomatal development, phototropism and induction of flowering (Franklin et al., 2005). Plants are able to detect the quality, quantity and direction of light and respond to it as an external signal, even though both intensity and duration of light have an additive relationship in plants. Light intensity is the total amount of light received each day, each hour or each minute and the duration is the period of time that light strikes the plant's leaves each day, generally measured in hours and termed as day length (Franklin and Quail, 2010).

Light stress in plants is defined as the exposure to insufficient/excess levels of light that negatively affect plant growth and development. Exposure to insufficient light limits photosynthetic activity, whereas exposure to excess light energy can damage the photosynthetic apparatus in plant system. The type of stress response in plant system induced by light is depend on the factors like fluence rate, intensity, exposure time, age of the plants and whether plants have been acclimated by prior exposure to light (Gururani et al., 2015). High intensity light (HL) or excessive light can reduce photosynthetic activity and efficiency known as photoinhibition (Adir et al., 2003; Fiorucci and Fankhauser, 2017). In HL situations, plants absorb more light than can be used for photosynthesis, resulting in over-excitation of the photochemical reaction centers in the chloroplast, which can lead to serious damage to the photosynthetic apparatus and ultimately causes plant growth reduction (Derks et al., 2015). This excess energy has the potential to be transferred to oxygen, leading to formation of reactive oxygen species (ROS), which can cause damage to cells and inhibit growth. Since photosynthetic CO_2 assimilation is a major sink for absorbed light energy, the difference in

photosynthetic capacity depends on light intensity and plant type (Kami et al., 2010).

The stratospheric ozone layer is a high altitude expanse of oxygen molecules that protects life on Earth from damaging solar UV radiation. During the last few decades, stratospheric ozone has been catalytically broken down due to high concentration of greenhouse gases and halogenated species, resulting in significantly decreased ozone levels (Shanklin, 2010). Depletion of the stratospheric ozone layer has occurred mainly by emissions of chlorofluorocarbons (CFCs), methyl bromide, nitrogen oxides, sulphur oxides and some other substances released by human activities (Portmann et al., 2012). The depletion of the ozone layer has been on the recovery for the past decade or so. But industrial emissions of chemicals commonly used in solvents, paint removers, and the production of pharmaceuticals have doubled in the past few years, which could slow the healing of the ozone layer (Hossaini et al., 2017).

Bais et al. (2015) assessed the factors that determine the intensity of UV radiation reaching at Earth's surface and found that stratospheric ozone, which absorbs UV radiation, is of considerable importance, but other constituents of the atmosphere, as well as certain consequences of climate change, can also be major influences. In addition to ozone effects, the UV changes in the last two decades have been influenced by changes in aerosols, clouds, surface reflectivity and solar activity. The main reason for positive trends of UV radiation observed after the mid-1990s over northern mid-latitudes are decreases in clouds and aerosols. Since the beginning of the 1980s, an ozone hole has developed over Antarctica resulting in a decrease in ozone level up to 70% during the southern spring season. Ozone depletion is more severe in Antarctica than at the North Pole because high wind speeds cause a fast rotating vortex of cold air, resulting to low temperatures (Harris et al., 2008). Over the Arctic, the

northern hemisphere's irregular landmasses and mountains normally prevent the build-up of strong circumpolar winds and so the effect is far less pronounced. Ozone depletion over the southern hemisphere means that people living there are more exposed to cancer-causing UV radiation (Cerrone et al., 2017).

Continuous observations since the 1980's have shown that ozone content of stratospheric zone have decreased by 3 to 6%, resulting in a 6 to 14% increase of UV-B radiation at Earth's surface. The stratospheric ozone depletion in southern hemisphere is dangerous, where an annual reduction in ozone density occurs in each spring, than high latitudes of the northern hemisphere where it is less pronounced (Rowland, 2006). Each CFCs molecule may cause the destruction of many molecules of ozone as the half life of CFCs ranges from 50 to 150 years. Therefore it is expected that decreased ozone levels will only recover to pre-1970 levels after several decades as the occurrence of CFCs will remain in the upper atmosphere for a long time. The generally expected forecast is that the stratospheric ozone layer will recover by 2050, even though the interactive effects of global climate change will remain (McKenzie et al., 2011). International agreements on protecting the ozone layer - particularly the Montreal Protocol - have stopped the increase of ozone depleting substances mainly CFCs, and a drastic fall has been observed since the mid-1990s. However, the progression of the ozone layer is affected by the interplay between atmospheric chemistry and factors like wind and temperature. Unfortunately, the ozone hole formed over Antarctica grew to about 8.9 million square miles in 2016 before starting to recover, according to scientists from NASA and the National Oceanic and Atmospheric Administration (NOAA) who are monitoring this annual phenomenon**.**

Scenarios based on chemistry-climate models shows that in the middle of 21st century, UV-B radiation at ground level is enhanced and resulted in alteration of the normal spectral UV composition reaching the surface of Earth, which is predicted to continue in the future (Taalas et al., 2000; McKenzie et al., 2007). It has been found that thinning of the stratosphere ozone layer has caused significant increase in the UV-B radiation and change in the spectral UV composition that reaches Earth's surface. Since 1980s and 1990s, increases in UV-B due to decreasing ozone were observed, where ozone depletion was more pronounced, particularly at high latitudes (>60^{o}) (Caldwell et al., 1998). Though, only a small part of the total solar spectrum (0.5%), it has the potential to cause photobiological damage in biological system in the case of not only major increases, but also minor increases in its content, due to its high energy. It is mandatory to understand the mechanisms of biological processes such as negative effects, repair or protection caused by UV-B, in order to understand the eco-physiological role of UV-B radiation. This is of special importance in plants which have sessile and photosynthetic characters. Plants monitor changes in their environment and are able to memorize and respond to these changes (Hollósy, 2002).

The UV-B range of solar spectrum is absorbed by many components of the cell which leads to harmful consequences. UV-B radiation is cytotoxic, at the cellular level it initially causes an increase in ROS levels, which subsequently oxidizes proteins, lipids, photosynthetic pigments and other biomolecules, and thus damages the structural integrity and functionality of enzymes and membranes in the cell (Schuch et al., 2017). UV-B induced pyrimidine dimer formation in DNA strands becomes mutagenic and genotoxic by blocking the action of DNA polymerase. As a result, the exposure of plants to UV-B can lead to cell damage and often cell death also may occurs (Robson et al., 2015; Köhler et al., 2017).

HL and UV-B irradiation is expected to be adverse on plant growth and development even at relatively small doses and it can induce several plant photomorphogenic responses. HL and UV-B induces various morphological, physiological and biochemical stress responses in plants, which are species specific and different, even for closely related cultivars (Cramer et al., 2011). The morphological effects of HL and UV-B in plants includes many changes, such as the reduction of biomass, plant height, root growth and leaf area, curling of cotyledons and leaves, increased auxiliary branching, chlorosis and necrotic spots, shortened internodes, altered flowering, *etc.* Decreased plant height was mainly due to shorter internodes rather than reduction in number of nodes (Kakani et al., 2003a; Fedina et al., 2010).

The physiological effects of HL and UV-B include reduction in photosynthetic and respiratory efficiency, destruction of chlorophyll and carotenoids pigments and the effects on stomatal activity. Reduction in the photosynthetic activity due to HL exposure was found in several plant species which ultimately reduced the crop yield (Zlatev et al., 2012; Tian et al., 2017). UV-B inhibits photosynthesis at several levels, including photosystem I (PSI) and photosystem II (PSII) photochemistry, maximum net photosynthetic rate, the activity of carbon fixing enzymes and electron transport (Rousseaux et al., 2004; Sztatelman et al., 2015). Many components have been summarized as primary targets of HL and UV-B action on photosynthesis, including the reaction centre itself, oxygen evolving Mn cluster, quinone acceptors and other components on the donor and acceptor sides of PSII (Kataria et al., 2014).

The biochemical effects of HL and UV-B include anthocyanin and flavonoids accumulation and they function as UV absorbing compounds by providing a shield to protect plants from harmful radiation and excess visible light (Guo et al., 2008; Zoratti et al., 2014). In order to reduce the impact of

ROS generated by HL and UV-B exposure, plant produces non enzymatic antioxidants such as glutathione, ascorbic acid, α-tocopherol and evokes antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR) and guaiacol peroxidase (GPOX). Moreover, leaf surfaces of higher plants develop an epicuticular wax coating which provides the first line of outermost defence against external influences such as air pollutants, UV and HL irradiation, restriction of the water vaporization and attack by pathogens together with some other leaf surface structures such as glandular hairs or trichomes (Yeats and Rose, 2013; Biswas et al., 2017).

Cereals are the most important source of calories to humans. According to Khush (2001), rice, wheat and maize are globally offer 23%, 17% and 10% calories respectively. Rice is a well known cost-effective cereal; also staple food included in the diet of humans which is feeding more than 2.7 billion people worldwide. More than 28% of the world's population live in Asia-Pacific region, where, the current 524 million tonnes of rice produced annually will have to be increased to 700 million tonnes by the year 2025, which is an immense task (Papademetriou, 2000). It is a chief and most vital source of food for more than half of the population and more than 90% of the world's rice is grown and consumed in Asia, where 60% of Earth's people live. It is expected that we will have to produce 25% more rice by the year 2030 (Khush, 2012).

As the 21st century unfolds, global rice production has showed signs that it may no longer be stable in the future. Globally, the traditional agricultural practices are not enough to produce rice for the needs of an ever-increasing population. Thus, the global population continues to increase, although with lower growth rate, while the resources for rice production are diminishing (Papademetriou, 2000). There are many challenges to reduce rice food shortage and poverty within the rice-based systems. Climatic change casts a huge shadow in the horizon of agricultural productivity. A variety of

factors including shrinkage of arable land, declining yields and labour, scarcity of irrigation water, effects of economic growth, pressure on land use, climate change and the related abiotic stress situation, environmental pollution *etc.* make threats to future rice production (Korres et al., 2017).

Based on the facts stated above, the focus of the present study is on thirteen rice varieties (Aathira, Aiswarya, Annapoorna, Harsha, Jyothi, Kanchana, Karuna, Mangalamahsuri, Mattatriveni, Neeraja, Swarnaprabha, Swetha and Varsha) which are the commonly cultivated high yielding rice varieties in Kerala with an emphasis on the effects of HL and UV-B radiation on the physiological, biochemical processes and the mechanisms of its tolerance which can aid in distinguishing the tolerant and sensitive rice varieties towards these two environmental stresses. Therefore, the present study was designed for fulfilling the following objectives.

1) Screening of thirteen commonly cultivated high yielding *Oryza sativa* varieties grown in nutrient solution for analyzing the effect of HL and UV-B irradiation.

2) Evaluation of the effect of HL and UV-B irradiation on the photosynthesis of selected *O. sativa* varieties.

3) Revealing the physiological and biochemical adaptations in the seedlings of selected *O. sativa* varieties towards HL and UV-B irradiation.

4) Identify the HL/UV-B stress tolerant and sensitive *O. sativa* varieties based on their HL/UV-B stress tolerance potential.

REVIEW OF LITERATURE

Food security, food production and climate change are interlinked factors hence the changes in one would have negative impacts on the other two factors. Climate change is severely affecting the food security at the local, regional and global level thus it can disrupt the food availability and also affect food quality (Islam and Wong, 2017). Nowadays, the effects of climate change on agriculture and food availability are likely to be more severe throughout the world. Moreover, other factors such as increasing population growth also magnify the effects of climate change on food security. Climate change and its drivers already affect the food production and food security through increased temperatures, changed precipitation patterns, CO_2 and ozone. According to Chen et al. (2017), these effects are expected to continue for the foreseeable future worldwide and this will cause deleterious changes to agricultural production with regional consequences for global food security. Recently, UN FAO reported that 795 million people suffered from food insecurity in 2016, on a global basis (FAO, 2017).

The impacts of climate change on agriculture, nutrition and health will be particularly felt by the most vulnerable segments of the world population and who appear to contribute the less to the root causes of climate change. Likewise, the nutritious quality of crops and their safety will also be affected by climate change (Noiret, 2016; Springmann et al., 2016). Khan and Hasan (2017) studied the effects of climate change in developing countries such as India on a number of economic, environmental and social issues, comprising agricultural yield and food security. It was observed that two billion people are deficient in one or more micronutrients, 160 million children under the age of 5 years are too short for their age, 50 million children under the age of five years are dangerously thin for their height, and 790 million people have

insufficient daily dietary energy intake. Thus climate change is a threat multiplier, particularly when it relates with food security and nutrition and it aggravates the risks of hunger and undernutrition through extreme weather events as well as long term and gradual climate risks (Chen et al., 2017).

2.1 Climate change and agriculture

Climate change and agriculture are interrelated processes and their relationship is of particular importance as the imbalance between population and food production increases worldwide. Climate change will negatively affect the various aspects of agriculture and influence various factors such as weeds, soils, insects and disease with regard to agriculture crops. The most important climatic variables are solar radiation, temperature, salinity, water availability and atmospheric CO_2 level (Holzkämper, 2017; Iizumi et al., 2017). As a result of global climate change, weather patterns are also changing much faster than anticipated. Weather condition will affect water distribution, daily temperatures, wind direction and patterns of salinity. The global environmental changes have become a serious threat for crop yield and quality, directly or indirectly. The stressful factors that affect agriculture production are drought, flooding, excess/low solar radiation, non optimal pH, high salinity, low/ high temperatures, etc. (Pereira, 2016; Verma and Deepti, 2016; Pandey et al., 2017a).

It is estimated by 2050 that the world population will reach about nine billion, therefore there is a huge demand to increase crop production to feed an additional two billion people during the next 40 years (Abewoy, 2018). According to a report by United Nations Department of Economic and Social Affairs (UN DESA, 2015), the world population of 7.3 billion is projected to reach 8.5 billion by 2030, 9.7 billion in 2050 and 11.2 billion in 2100. Therefore, to feed the increasing population, agricultural food production must be increased to the extent of 70 percent by 2050 (FAO, 2009). Given

that increase of land suitable for agriculture cannot be expected in the future, producing more of the desired products per unit area of land will be an important task. Hence, it is very urgent to develop crops that can yield under extreme climatic and soil growing conditions for improving the qualities and quantities of the crop against multiple environmental stresses (Wang et al., 2016a; Yohannes, 2016).

2.2 Abiotic stress and agriculture

Abiotic stresses are the major ones which limit crop productivity, reduce average yields of major crop plants worldwide. They are the primary cause of crop loss worldwide, reducing average yields by more than 50% for most major crop plants (Bray et al., 2000). Plant crops reduce growth and development during exposure to various environmental stress conditions, which ultimately affect the crop yield negatively. Crop plants come across various environmental stresses (both abiotic and biotic stresses) and they are usually exposed to abiotic stresses at any stage of their growth and development. As a consequence of abiotic stress, the crop sector suffers the most within agriculture and the highest damage and losses to crops were 42% followed by those to livestock (36%) (FAO, 2015).

Due to the sessile nature of plants, they must endure adverse environmental conditions and consequently evolve a variety of responses towards the various adverse environmental factors. The plant species, which are able to withstand such environmental stresses, have more importance today (Verma and Deepti, 2016). Abiotic stresses, such as solar radiation, high temperature, drought, chilling, frost, salinity, toxic chemicals and nutrient deficiencies adversely affect plant growth and productivity and trigger a series of morphological, biochemical and molecular changes in plants. In addition to this aspect, more than one abiotic stress can occur simultaneously on the crop production such as high temperature and high

photon irradiance often accompanied by low water supply, which can in turn be aggravated by heavy metal toxicities that constrain mainly root growth. Moreover, a single abiotic stress can decrease a plant's ability to resist a second stress (Tester and Bacic, 2005).

India is the fourth largest country in the world and accounts 2.4% of the total world's land area and 17.5% of the world's population. India's food production has remained relatively constant, but the population has increased dramatically in the last 20 years. The reason for this condition is environmental disasters, such as UV radiation, drought, heat stress, salinity, etc. and these stresses limit crop production; the situation has aggravated due to the drastic and rapid changes in climate globally (Halford et al., 2015).

The development of crop varieties which can tolerate environmental stresses without affecting their productivity would be of major advantage to agriculture in areas where abiotic stresses are a chronic problem. However, no transgenic crop variety with tolerance to abiotic stress has so far been reported to be released for cultivation, even though some transgenic varieties exhibiting tolerance to various abiotic stresses are under field testing (Verma and Deepti, 2016). The ability of crops to tolerate abiotic stresses such as an excessive or inadequate light or supply of water, high winds, extreme temperatures, frost, salt, and other osmotic stresses is a key aspect of yield production and its improvement has long been a target for plant breeders since these factors potentially affects crop yields, farmer earnings, reliability of the food supply, quality and food safety (Bray et al., 2000; Curtis and Halford, 2014). Moreover, the issue now has more significance than ever because of the anticipated effects of climate change, an increase in the frequency and severity of extreme weather events and ultimately a rise in temperature of up to 5°C by the end of this century, which is predicted by the International Panel for Climate Change (IPCC) (Stocker et al., 2013).

2.3 Sunlight as energy source for plants

Solar radiation is radiant (electromagnetic) energy from the sun and it provides light and heat for Earth and energy for photosynthesis. The part of the electromagnetic spectrum that reaches Earth from the sun is between 100 nm and 1 mm. The three important bands or ranges in the solar radiation are UV, visible (PAR) and infrared spectrum and each of these bands has a different impact on the environment globally. UV contains wavelengths between 100-400 nm, visible light contains wavelengths from 400-700 nm and infrared falls within the range of 700 nm to over 1 mm. Among them infrared radiation makes up 49.4%, visible light provides 42.3% and UV radiation makes up just over 8% of the total solar radiation (Moan, 2001). Moreover, the amount and intensity of solar radiation that reaches Earth surface depends on various factors and mainly include elevation above sea level, altitude, the angle of the sun (due to latitude, season and time of day) and scattering elements such as cloud coverage and aerosols. Visible light is the only spectrum of light to be considered photosynthetically active for plants (Chen and Blankenship, 2011).

Photosynthesis is a photobiochemical process of successive redox reactions that occur when the light-harvesting complexes (LHCs) absorb photonic energy and transfer it to photosystem reaction centers via excitons and produce ATP and NADPH (Baker, 2008; Solymosi and Keresztes, 2012). Exposure of photosynthetic organisms to some environmental factors such as drought, HL, UV, heat, heavy metal toxicity, results in over-reduction of the electron transport chain, which in turn leads to inhibition of photosynthetic process by the reduction of PSII activity and this phenomenon is referred to as photoinhibition. Since light energy is the fundamental force for photosynthesis, photoinhibition is unavoidable in photosynthetic

organisms (Murata et al., 2007; Nishiyama and Murata, 2014; Gururani et al., 2015).

2.4 High intensity light as the major stress factor for plants

Plants require light for photosynthetic process, although absorption of excess light can lead to photooxidative damage to the photosynthetic apparatus and decrease the photosynthetic efficiency. Thus absorbed solar energy may become excessive for plants when it exceeds the capacity to utilize the same for photosynthesis. When light energy exceeds cell's needs it results in the production of ROS molecules which damage cell components including photosynthetic apparatus causing light induced photoinhibition (Nishiyama et al., 2006; Gururani et al., 2015). Over reduction of the electron transport chain results in the generation of large amounts of ROS and that are responsible for damage to the photosynthetic apparatus. The most common reactive byproducts of photosynthesis are ROS which include radical molecules such as superoxide ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), singlet oxygen ($^1O_2$) and non-radicals hydrogen peroxide (H_2O_2). Moreover, they are produced in plant cells by the over-reduction of the photosynthetic electron transport chain and severely damage the cellular homeostasis (Karuppanapandian et al., 2011; Das and Roychoudhury, 2014).

Under HL irradiation in plants, limitation in energy transfer occurs, when the excess energy absorbed by chlorophyll in the PSII antennae complex is not fully utilized in the PSII reaction center by charge separation (Vialet-Chabrand et al., 2017). Under these conditions, singlet chlorophyll (^{1}Chl) might be converted to triplet chlorophyll (^{3}Chl) which is highly deleterious and transfers energy to O_2 forming $^1O_2$. However, to prevent generation of triplet chlorophyll in the photosynthetic apparatus, quenching of ^{1}Chl to heat is achieved directly by xanthophylls or indirectly by the

rearrangement of LHCII protein by PsbS (Hakala et al., 2006; Ruban et al., 2012).

Photoinhibition directly affect the performance of the photosynthetic apparatus and respiration in plants (Aniszewski et al., 2001; Ashraf and Harris, 2013; Faseela and Puthur, 2018). Hence, the plants have to adapt with high light conditions by several metabolic and morphological changes and such changes in morphological and physiological level may be crucial for survival in heterogeneous and variable light conditions (Pospíšil, 2016). The adaptation at physiological and biochemical level helps the plants to overcome the adverse effects induced by the HL exposure and maintains the normal metabolic activities of the plants even under stressed conditions (Vaz and Sharma, 2011; Szymańska et al., 2017).

2.5 UV-B stress in plants

Global changes in the chemical composition of the atmosphere with a substantial reduction of the protective O_3 layer have led to a notable increase in the solar UV radiation reaching Earth's surface. Photosynthetic organisms are inevitably exposed to UV-B radiation along with sunlight, which is required for normal growth (Bornman and Teramura, 1993). UV-B is an important component of the sunlight acting as an ecophysiological factor with the potential to alter growth and development in plants. All living organisms of the biosphere are exposed to UV-B at intensities that vary with the solar angle and the thickness of the stratospheric ozone layer. Although UV-B is only a minor component of sunlight, which accounts for <0.5% of total light energy, its potential for causing biological damage is exceptionally high due to its high energy and even small increases could lead to a substantial impact on the biosphere (Pfeifer et al., 2006; Fountoulakis et al., 2014).

Earlier studies have clearly demonstrated the extent of damage caused by UV-B radiation and it was shown to significantly affect on the morphological, physiological, biochemical and molecular characters of many crop plants (Agrawal and Rathore, 2007; Mishra et al., 2008; Kataria et al., 2014). At the cellular level, production of a variety of ROS was demonstrated in response to high UV-B doses, which directly oxidize many biomolecules such as phospholipids in the plasma membranes, proteins, and nucleic acids, leading to severe damage to plant cells and compromising the functionality and integrity of enzymes and cell membranes (Hideg et al., 2013; Köhler et al., 2017). ROS generated upon UV-B irradiation seems to be involved in plant adaptation to UV radiation by functioning as UVR8 photoreceptor mediated signaling molecules, for the effective regulation of cellular redox-homeostasis in plants (Dat et al., 2000; Das and Roychoudhury, 2014). Moreover, computer modeling proved that UV-B radiation is capable of increasing the possibility of cellular damage in plant cells by modifying the ROS profile through partial photo-conversion of H_2O_2 to OH radicals (Czégény et al., 2014).

The responses of UV-B radiation on plant cells depends upon various experimental growth conditions, plant development stages, the ratio of PAR to UV-B radiation, UV-B dosage, as well as on the interaction with other environmental stress factors such as temperature, drought, nutrient availability and salinity (Hunt and McNeil, 1998; Fedina et al., 2003), UV-B radiation was reported to provoke clear impacts at various levels, including visual symptoms (e.g., tissue chlorosis and necrosis), chloroplast ultrastructure and leaf anatomy (e.g., changes in thickness of epidermal and palisade mesophyll layers), photosynthetic pigments, lipid peroxidation, stomatal functioning, photosynthesis, transpiration and respiration (Kakani et al., 2003a; He et al., 2006; Jansen et al., 2010). In addition, the impact of high doses of UV-B in

Arabidopsis leaves depending on their light status after UV treatment (Sztatelman et al., 2015).

2.6 Adaptations of plants to HL and UV-B irradiation

2.6.1 Morphological adaptations

Photosynthetic organisms need sunlight and are inevitably exposed to high visible light and UV-B radiation, which has the potential to alter plant growth and photosynthesis (Müller-Xing et al., 2014). Light acclimatization strategies in plants include mechanisms for effective light harvesting at low light conditions (Chen et al., 2016) and protection against HL and UV-B stress (Lichtenthaler and Burkart, 1999). The morphological changes or adaptations facilitate the plants to overcome the adverse effects induced by HL and UV-B exposure and thus maintains the normal metabolic activities of the plant even under severe HL and UV-B irradiation. Plants express plasticity for a number of morphological traits towards changing light conditions, such as changes in leaf area and shoot length. To optimize light absorption, plants may alter the structure and orientation of leaves, especially in shade plants. To cope with HL and UV-B stress, the adaptation on the level of whole organism involves adjustment in the leaf orientation and this adaptation helps plants to manage with excess irradiation particularly during the midday (Björkman and Powles, 1984; Ruban, 2009).

Plants growing in HL and UV-B stress conditions tend to have a conservative leaf morphological pattern to avoid the production of structures too expensive to be sustained (Zhang et al., 2012). The leaves of many plants re-orientate their laminae photonastically in response to non-directional light signals, and/or phototropically in response to directional light signals, by flexing of pulvini bases. Physiological and structural specializations in the pulvinus enable for differential and repeatedly reversible axial volume

changes (expansion/contraction) in opposite sectors of pulvinus motor tissue (Koller, 1990).

The various leaf traits such as size, dimension, margin and venation characteristics can provide a link between various environmental factors and leaf functions in plants. Light condition plays crucial role in the morphology of leaves and the plasticity in the leaf morphology of plants under various light conditions and it helps the plants to cope up with the change in light conditions (Schneider et al., 2006; Xu et al., 2009). One of the main morphological features which changes in response to long term HL and UV-B exposure is the specific leaf area (ratio between leaf area and leaf dry mass) which reflects the leaf thickness and relative proportion of various type of tissue in plant leaves. It has been reported earlier that HL and UV-B irradiance generally leads to smaller leaves with increased thickness (Teramura and Sullivan, 1991: Dale and Causton, 1992; Valladares et al., 2000). Stem elongation was more sensitive to UV-B irradiation than leaf expansion and UV-B induced reduction in plant height was mainly due to shorter internodes rather than fewer nodes (Reddy et al., 2003). Moreover, high UV-B irradiation results in formation of glazing, chlorosis and necrotic spots or patches in leaves (Kakani et al., 2003a; Hectors et al., 2007). The increased total leaf thickness in sun leaves compared to shade leaves is mainly due to the greater palisade parenchyma, spongy parenchyma, and epidermal tissue thickness, suggesting that leaf internal structure may play an important role in light capture (Gratani, 2014).

2.6.2 Physiological adaptations

2.6.2.1 Photosynthesis

HL and UV-B can induce severe damage to the photosynthetic apparatus or cause photoinhibition by generation of harmful ROS in plant

leaves (Ort, 2001). HL induced production of ROS is further increased under the influence of other environmental stress conditions, due to the excess absorption of light energy relative to the photosynthetic activity. Excited chlorophyll molecules in the triplet state results in the production of $^1O_2$ in photosystems and this is the major ROS produced in photosynthetic apparatus under HL and UV-B conditions (Krieger-Liszkay, 2005; Lu et al., 2017).

The cell organelle most affected under unfavorable light conditions is chloroplasts. As one of the most light-sensitive component in plants, the photosynthetic pigments in leaves affect the capacity of light absorption during HL and UV-B radiation and associated with this a series of physiological processes occurs during photosynthesis (Lu et al., 2003; Zhang et al., 2016a). Light stress is frequent under tropical conditions, and variations in chlorophyll as well as carotenoid concentrations are indicators of plant responsiveness to light intensity (Li et al., 2014). Chlorophyll tends to be photooxidized at HL and UV-B irradiance and carotenoids can prevent the chlorophyll photooxidation in plants. Thus the ratio between chlorophyll and carotenoids in plant leaves may be used as a potential indicator of photooxidative damages caused by high light and UV-B exposure (Salama et al., 2011).

Specific negative effects of strong visible and UV-B light stress on photosynthetic pigments, photosynthetic electron transport, gas exchange, Chl *a* fluorescence, chloroplast ultrastructure, physiological and photochemical processes related to electron transport, have been established (Lichtenthaler and Burkart, 1999; Kalaji et al., 2012). The exposure to high solar radiation has a significant negative impact on the photosynthetic light reactions in the thylakoid membrane system of chloroplasts. However, any other abiotic or biotic stress that depresses photosynthesis will further reduce the

photosynthetic process and is regarded as the underlying cause of irreversible photoinhibition (Kirchhoff, 2014). In addition, energy distribution through photosystems and thylakoid membrane integrity are negatively affected by HL and UV-B irradiation in plants (Essemine et al., 2012; Kataria et al., 2014). Under photoinhibitory conditions induced by excess light, the rate of photosynthetic linear electron flow is inhibited by the regeneration rates of both $NADP^+$ and ADP. As a result of this limitation, the photosynthetic electron transport system becomes filled up with excess electrons and results in overexcitation (Krumova et al., 2014; Walawwe, 2014).

When PSII is exposed to excess light, triplet chlorophyll is formed in the acceptor side and $O_2^{\bullet-}$ is formed in the donor side of PSII (Yadav and Pospíšil, 2012). HL and UV-B induced damage to the PSII reaction center occurs primarily in the water-oxidizing manganese (Mn) cluster, the quinine electron acceptors, tyrosine electron donors and the reaction centers of the D1 and D2 protein which results in the inactivation of the electron transport chain (Ihle, 1997; Szilárd et al., 2007; Vass, 2012). Moreover, PSII is more vulnerable to most abiotic stresses such as HL, high-temperature and UV-B as compared to PSI (Scheller and Haldrup, 2005). However, Zhang et al. (2016a) reported that UV-B could indirectly affect PSI under chilling-light conditions (200 $\mu molm^{-2}s^{-1}$ and 6°C).

Moreover, electron paramagnetic resonance spin-trapping spectroscopy revealed that both $O_2^{\bullet-}$ and $HO^{\bullet}$ are involved in the HL induced photoinhibition. Recently, tandem mass spectroscopy analysis indicates that oxidative modifications in D1 and D2 residues are associated with formation of $OH^{\bullet}$ at both the Mn_4O_5Ca cluster and the nonheme iron and $O_2^{\bullet-}$ is formed by the reduction of O_2 at either $Pheo_{D1}$ or Q_A (Kale et al., 2017). Similarly, Sicora et al. (2006) reported that the primary UV-B damage occurs at the

catalytic Mn cluster of water oxidation, which is most likely sensitized by the UV absorption of Mn(III) and Mn(IV) ions ligated by organic residues. Moreover, the presence of visible light enhances the photodamage to PSII induced by UV-B irradiation, but has no synergistic interaction with UV radiation.

It is widely accepted that inactivation and protein damage due to HL exposure in plants can be caused by two distinct mechanisms i.e., acceptor and donor side mechanism (Aro et al., 1993; Vass, 2012). The excess light energy absorbed by photosynthetic pigments and the photodamage to PSII initially happens at the site of PSII reaction centre. During the donor-side photoinhibition, PSII becomes damaged because electron donation from the Mn_4Ca cluster of oxygen evolving complex (OEC) is disrupted and is unable to keep up with the rate of withdrawal of electrons by $P680^+$ (Aro et al., 1993; Murata et al., 2007). This results in the accumulation of long-lived, oxidizing radicals at the PSII donor side leading to rapid inactivation of electron transport and thus causing protein damage. Donor side photoinhibition has been directly observed in isolated thylakoid membrane systems in which the OEC of PSII is artificially inhibited or destroyed in an active PSII unit (Chen et al., 1992; Henmi et al., 2004).

According to acceptor side photoinhibition of PSII, strong light causes double reduction of the primary electron acceptor Q_A which leads to dissociation of Q_A electron acceptor from PSII. In such PSII centers, the electron transfer from Q_A^- to Q_B is limited due to reduced status of plastoquinone pool (Vass et al., 1992; Adir et al., 2003). Moreover, in vitro spectral and kinetic study of photobleaching of chlorophyll and amino acids under photoinhibitory conditions in pea seedlings revealed that antenna chlorophyll, rather than chlorophyll of the reaction center, is the sensitizer for both acceptor and donor side photoinhibition of PSII (Strizh and Neverov,

2007). Hakala et al. (2006) hypothesized that light absorption by the Mn(III) and/or Mn(IV) ions of the OEC leads to release of an Mn ion from the OEC, which inhibits electron donation to the oxidized primary donor of PSII under strong light induced photoinhibition.

Kale et al. (2017) reported that proteins and lipids might be oxidized by ROS formed in PSII under HL exposure and PSII proteins were oxidatively modified in the following order D1 > D2 > Cyt b559 > CP43 > CP47 > Mn_4O_5Ca cluster. The D1 reaction-centre protein of PSII is a target of HL and UV-B induced damage to the PSII structure and turnover of the D1 protein is enhanced by increasing the exposure period (Rintamäki et al., 1995). Recent report by Michoux et al. (2016) found that D1 protein is the main target site for photoinhibition among more than 25 subunits of PSII. Initially, PSII protein oxidation occurs which subsequently leads to cleavage and aggregation of the D1 subunit, although to a lesser extent, in the D2 protein, which degrades in a similar manner to the D1 protein. Likewise, Frankel et al. (2012) identified a number of natively oxidized residues in the vicinity of the Mn_4O_5Ca cluster on the D1, D2, and CP43 subunits of the PSII electron donor side.

2.6.2.2 Chl *a* fluorescence

The light energy absorbed by Chl molecules in plants can be utilized in three ways: energizing photosynthesis, dissipation as heat or remission as fluorescence. Chl *a* fluorescence measurements have been used to study the function and performance of photosynthetic machinery of various plants and to identify the stress factor that mostly affects or/and limits plant growth (Kalaji et al., 2012, 2017). In order to evaluate the physiological status of a certain plant and to determine the photosynthetic damage as affected by various environmental stresses, Chl *a* fluorescence assay is a rapid and non invasive technique (Lu et al., 2002; Faseela and Puthur, 2017).

When the photosynthetic samples, which have been kept in darkness, are exposed to light, a fluorescence induction is observed, known as the Kautsky effect, after Hans Kautsky, the discoverer of the phenomenon and it is based on the theory of energy flow in thylakoid membranes (Oukarroum et al., 2004). The Chl induction curve is characterized by three apparent kinetic steps denoted as OJ, JI, and IP i.e., the Chl fluorescence intensity rises from a minimum level (O level), in less than 1 s to a maximum level (P level) via two intermediate steps labeled J and I and these phases were correlated with peak accumulation of different reduced species on the acceptor side of PSII and photosynthetic efficiency of the leaf sample (Maxwell and Johnson, 2000; Stirbet and Govindjee, 2011).

HL and UV-B exposure reflects to a substantial increase in the dissipation of excitation energy, probably mediated by the zeaxanthin and Chl fluorescence emission (Olsson et al., 2000; Ripoll et al., 2016). In order to evaluate the effect of light intensity on cold tolerance in soybean cultivars, Jenabiyan et al. (2015), utilized the feasibility of using Chl *a* florescence technique and showed that cultivar 032 was more tolerant than cultivar BP towards light stress (2000 or 8000 lux). Similarly, HL and UV-B stress causes a significant alteration in minimum fluorescence (Fo), maximum fluorescence (F_m), variable fluorescence (F_v), Fv/Fm and photochemical efficiency of PSII (Φ_{PSII}) in various plant species (Hazrati et al., 2016; Reyes et al., 2018). Moreover, slow and fast Chl *a* fluorescence induction analyses revealed the major functional differences between barley leaves grown under sun and shade light regimes *i.e.*, the lower connectivity among PSII, decreased number of electron carriers, and limitations in electron transport between PSI and PSII in the shade leaves contributes towards functional differences between the sun and the shade leaves (Zivcak et al., 2014).

Exposure to full natural sunlight caused an inactivation of the primary photochemistry associated with PSII in *Nerium oleander* when subjected to water stress and it was presumed that HL induced photoinhibition is might be due to the lower energy transfer efficiency from reaction centers to PSII (Björkman and Powles, 1984). UV-B also has a negative effect on maximum quantum yield of PSII and the expression of genes associated with photosynthesis in *Arabidopsis* leaves (Sztatelman et al., 2015). According to van Rensen et al. (2007) UV-B induced damage occurs first on the acceptor side of PSII and later on the donor side, revealed by Chl *a* fluorescence analysis. The decrease in electron transport activity can also be correlated with an increase in the peroxidation of thylakoid lipids upon HL and UV-B exposure in plants. Thus, it seems likely that lipids are required for photosynthetic electron transport activity and under HL and UV-B induced photoinhibition, a loss in activity is registered as the thylakoid lipids are degraded (Zhu et al., 2008). Similarly, Mishra and Singhal (1992) determined that the decrease in photosynthetic electron transport activity in isolated chloroplasts of wheat leaves exposure to HL stress was accompanied by an increase in the oxidative degradation of polyunsaturated fatty acyl residues of the thylakoid lipids.

2.6.2.3 Gas exchange parameters

Gas exchange measurements are a non-destructive, non-invasive technique to monitor accurate photosynthetic carbon assimilation rate in plants. Measurements are rapid and can be used to examine the altered photosynthetic rates under different environmental stress conditions (Souza et al., 2004; Massacci et al., 2008; Wang et al., 2016b). The main gas exchange parameters such as the photosynthetic rate (P_n), transpiration rate (E) and the stomatal behavior, generally fluctuates at varying degrees when plants are

subjected to HL and UV-B irradiation and these variations are species specific (Sanusi et al., 2011; Lu et al., 2017).

Studies on the physiological attributes of *Platycerium bifurcatum* and *Olea europaea* by Sanusi et al. (2011) and Koubouris et al. (2015) revealed that reduction in the P_n upon HL and UV-B exposure, respectively, might point towards lower value of stomatal conductance (g_s). As previously reported by Larcher (2003), HL induced stomatal closure generally reduced the diffusion of ambient CO_2 into mesophyll, thus resulting in the reduction of photosynthetic rate. Both stomatal (closure of stomata) and non-stomatal (including damage to photosynthetic apparatus) limitations may be involved in reduction of photosynthetic rate in plants (Jiang et al., 2006; Zervoudakis et al., 2012). Moreover, an exposure to light regime from high to low light levels caused a decrease of CO_2 assimilation rate as influenced by gas exchange parameters in *Phaseolus vulgaris* leaves and this reduction was correlated with the decline in activity of ribulose-1,5-bisphosphate (RuBP) carboxylase and reduction in the RuBP-regeneration capacity (von Caemmerer and Farquhar, 1984).

Stomatal regulation of photosynthesis and its role in controlling the decline of net CO_2 uptake leading to decreases in leaf internal CO_2 concentrations during various abiotic stress has been well documented (Souza et al., 2004; Saravanan et al., 2008). The reduction in CO_2 assimilation rate imposed by stomatal closure may result in the decline of photochemical efficiency of PSII and electron transport rate, leading to an overexcitation and subsequent photoinhibitory damage of PSII reaction centers (Long et al., 1994). Under natural conditions, where HL exposure is often associated with other abiotic factors such as high temperature or UV, the protective mechanisms may be insufficient to dissipate the excess of excitation energy and a chronic photoinhibition, characterized by a slow-reversible reduction in

the photosynthetic efficiency in the photosynthetic apparatus and successive programmed cell death may result in plants. Consequently, damages to the photosynthetic machinery may eventually occur, imposing an additional non-stomatal limitation to the process, which may add to the HL and UV-B induced metabolic alterations (Osmond, 1994; Zlatev et al., 2012).

UV-B treatment impaired the net photosynthetic rate, stomatal conductance, transpiration rate, the light-saturated assimilation rate, assimilation capacity, light compensation point and uptake rate of CO_2 for photosynthesis (Yao et al., 2008). The time taken for stomatal closure is rapid even at low UV-B levels due to a loss of turgor pressure with ion leakage from the guard cells. Wright and Murphy (1982) have shown that UV-B radiation can induce stomatal closure directly by inhibiting K^+ accumulation, and Nogués et al. (1999) demonstrated that UV-B indirectly affect the stomatal conductance by reducing pore size through UV-B-induced changes in the elasticity of the cell walls or the cytoskeleton of guard cells.

2.6.2.4 Choloroplst ultrastructure

Chloroplasts are serving as structural and functional skeleton for photosynthetic performance in plants. Decreases in photosynthetic rate in response to different environmental factors are mainly corresponding with ultrastructural alterations in the chloroplast. HL and UV-B exposure in plants induces architectural alterations of the chloroplast ultrastructure in terms of shape and thylakoid folding (Duan et al., 2014). Buchner et al. (2007) experimentally proved that chloroplasts of mesophyll cells in nine different alpine plant species after exposure to HL stress have the ability to form marked, stroma-filled protrusions that do not contain thylakoids. Moreover, the chloroplasts of guard cells in UV-B irradiated potato leaves showed a deviation from the control cells and resulted in the reduced size and smaller starch granules (Santos et al., 2004).

HL chloroplasts composed of fewer thylakoids, grana stacks and a higher proportion of exposed photosynthetic membranes, Chl α/β -carotene-proteins CPI and CPIa as compared to low light chloroplasts (Lichtenthaler et al., 1983). Recently, Schumann et al. (2017) compared the changes in ultrastructure of chloroplasts when acclimated to fluctuating light conditions and found that plants exposed to HL exhibited more unstacking of grana after 30 min of illumination, whereas tight packing of grana stacks was observed in low light and natural light conditions. HL exposure results in two main ultrastructural changes that work synergistically to improve the accessibility between damaged PSII in grana and its repair machinery in stroma lamellae: lateral shrinkage of grana diameter and increased protein mobility in grana thylakoids (Herbstová et al., 2012).

Chloroplast membrane alterations due to HL and UV-B induced photoinhibition may affect the chloroplast/thylakoid related photochemical processes, such as light-harvesting, electron transport and ion movement (Caasi-Lit et al., 1997; Gururani et al., 2015). Likewise, the chloroplast swelling and starch depletion upon exposure to strong light can be interconnected and it support the line of evidence that starch degradation products could be osmotically active agents involved in the higher swelling of chloroplasts and it provide higher stress tolerance also. *Anoectochilus roxburghii* leaves from high irradiance treatments was possessed with grana containing less thylakoids than those of leaves from 30, 20 and 5% shade treatments, and, as a consequence, had lower photosynthetic rates. Also found that leaves grown in 30% irradiance environments exhibited better-developed chloroplasts, grana, and stroma lamellae (Shao et al., 2014). Similarly, UV-B radiation resulted in the dilation of thylakoid membranes, progressive disruption of the thylakoid structure, disintegration of the double membrane envelope surrounding the chloroplast and the accumulation of large starch grains in pea leaves. The higher concentration of starch in supplementary UV-

B treated leaves is due to its immobilization, rather than to any increase in starch synthesis.

2.7 HL and UV-B tolerance mechanisms

2.7.1 Oxidative stress and antioxidation mechanisms

Oxidative stress is induced by a wide range of environmental factors including HL and UV conditions that stimulate photoinhibition of photosynthesis and the negative effects of environmental stresses may be partially due to the generation of ROS and/or inhibition of the processes that scavenges them. ROS are toxic molecules found in various subcellular compartments due to oxidative stress in plants and they rapidly inactivate enzymes, pigments, proteins, lipids and nucleic acids and damage vital cellular organelles in plants (Karuppanapandian et al., 2011; Yadav and Sharma, 2016). They not only serve as agents of damages in plants, but also act as signals in signal transduction pathways and also as a secondary messenger in various developmental processes in plants. Moreover, ROS trigger the lipid peroxidation chain reactions to produce peroxyl radical in plant cells. Peroxyl radical causes a chain reaction and transforms polyunsaturated fatty acids into lipid hydroperoxides and easily decompose to secondary products, such as aldehydes and malondialdehydes (MDA). Thus ROS results in the formation of a complex form of lipid degradation products such as MDA (Foyer and Shigeoka, 2011; Birben et al., 2012). MDA content is an important parameter for measuring the peroxidation of membrane lipids induced by oxidative stress since it disturbs the integrity of cell membranes and leads to rearrangement of membrane structure (Tewari et al., 2002; You and Chan, 2015). Moreover, Swarna et al. (2012) revealed that UV-B induced reduction in the integrity of the thylakoid membranes is closely related to the extent of lipid peroxidation in maize leaves.

Distelbarth et al. (2013) investigated the HL tolerance in four natural accessions of *Arabidopsis* (C24, Nd, Rsch, Te) and found that Te appeared most sensitive to oxidative stress, having higher MDA levels under high light. Moreover, Fu et al. (2012) suggested the range of 400 to 600 $\mu molm^{-2}s^{-1}$ is a recommendable light intensity for lettuce production by analyzing oxidative stress and induced lipid peroxidation after HL treatment of lettuce plants with different intensities of light (100, 200, 400, 600 and 800 $\mu molm^{-2}s^{-1}$). Similarly, UV-B radiation significantly increased the levels of H_2O_2 and MDA in leaves of rice (Du et al., 2011), wheat (Feng et al., 2007), *Prunella vulgaris* (Zhang et al., 2017). Recently, the interaction effects between nitrogen and UV-B radiation (8 Wm^{-2} for 7 h/d) were studied in sunflower by Cechin et al. (2018) and found that the gas exchange parameters after UV-B radiation were highly dependent of nitrogen supply, whereas, the rate of lipid peroxidation upon UV-B exposure was independent of nitrogen level.

Plants however, are able to cope with these adverse environmental effects by inducing antioxidant machinery in the cells and its organelles like chloroplast, mitochondria and peroxisomes. The equilibrium between the production and elimination of ROS is maintained by various enzymatic and nonenzymatic antioxidants. They have an innate ability to biosynthesize a wide range of antioxidants and compatible solutes capable of attenuating ROS induced oxidative damage. Thus, ROS production and antioxidant system are interwoven with various abiotic stress conditions and the consequences of ROS formation and detoxification by ROS depend on the intensity or severity of stress and on the physicochemical conditions in the cell. Foyer and Shigeoka (2011) reviewed the persistence of ROS in stressful conditions which limit the capacity of photosynthesis in situations where energy producing and energy utilizing processes are not well balanced and explained the methods for enhancing the activities of antioxidant enzymes and/or the

accumulation of low molecular weight antioxidants by genetic manipulation to provide tolerance to a variety of stresses through more efficient removal of ROS.

2.7.1.1 Enzymatic free radical scavenging mechanisms

Plants respond to oxidative damage induced by HL and UV-B through activating their enzymatic antioxidant metabolism and it is an important protective mechanism to minimize the ROS levels upon high sunlight irradiation. In stressed condition of photo irradiation, the free radical species may be increased, which will enhance the activities of the antioxidant enzymes (Wingsle et al., 1999). The activation of CAT, GPOX, SOD and APX, which scavenge ROS and offer photoprotection to nucleic acids, lipids and proteins, were mainly found to be induced in plant species owing to overexposure of visible and UV radiation (Jain et al., 2004; Zu et al., 2010; Foyer and Shigeoka, 2011).

It is generally considered that high doses of visible or UV light induce oxidative stress and antioxidation mechanism in plant cells (Watanabe et al., 2006). Moreover, Younis et al. (2010) reported that exposure of dark or ambient visible light grown broad bean seedlings to low and high visible light intensities, UV-A, or UV-C either alone or in combination, enhanced the activity of antoxidant enzymes such as SOD, CAT and GPOX. Under normal conditions of photo irradiation, level of ROS remains optimum because of the ROS scavenging activity of these various antioxidative enzymes (Mittler, 2002; Gill and Tuteja, 2010).

SOD, a metalloenzyme, is considered as an important antioxidant enzyme that plays a key role in the defense of plants for protecting molecules against oxidation damage induced by high irradiation of visible and UV-B range (Zhang et al., 2005; Distelbarth et al., 2013). The SOD scavenge $O_2^{\bullet-}$

by catalyzing its dismutation in two different ways, one being its reduction to less harmful H_2O_2 and another by its oxidization to O_2. Several classes of peroxidases and catalase scavenge the H_2O_2 produced by converting it into H_2O and O_2 (Anjum et al., 2016). These key enzymes work together with the aid of other antioxidant enzymes in the regulation of ROS levels in plants through their role in the water-water cycle and water-ascorbate-glutathione cycle under oxidative stress (Mittler, 2002). Many instances of SOD's action in controlling the oxidative damage induced by HL and UV-B have been reported revealing its central protective role in the $O_2^{\bullet-}$ and H_2O_2 scavenging process (Alscher et al., 2002; Rai et al., 2011; Vuleta et al., 2016; Köhler et al., 2017). For instance, adaptation to HL stress was manifested by increases in the levels of SOD in the drought tolerant varieties as compared to drought sensitive varieties of *Sorghum bicolor* (Jagtap and Bhargava, 1995).

Peroxidase activity levels also have been found as one of the defensive mechanisms providing protection against excessive light absorption and UV-B radiation in plants. A significant positive correlation of APX activity in a number of plant species with enhanced photooxidative stress induced by HL and UV-B exposure was reported earlier. According to the report by Sofo et al. (2015), ascorbate-dependent peroxidase, APX has a higher affinity for H_2O_2 than other peroxidases and CAT removes H_2O_2 generated in the peroxisomal respiratory pathway without the need of any reducing power. APX reduces H_2O_2 to H_2O after utilizing ascorbate in the thylakoid lumen as an alternative electron donor to PSII (Dat et al., 2000; Anjum et al., 2016). Interestingly, in addition to induction of peroxidase activity in *Arabidopsis* following UV exposure, it also increased NADPH-oxidase activity, leading to peroxide production (Rao et al., 1996).

Recently, the photooxidative damages induced by HL and UV-B were found to get alleviated by highly significant increase of APX activity in rice

cultivar Kanchana (Faseela and Puthur, 2018). For instance, Vuleta et al. (2016) tested the adaptiveness of high light induced variation in isoform patterns and activities of important antioxidant enzymes SOD, APX and CAT in the leaves of *Iris pumila* and found that APX as the primary H_2O_2 scavenger. In addition, treatment of *Prunella vulgaris* with UV-B radiation significantly increased POD and SOD activity which was positively correlated with accumulation of secondary metabolites (Zhang et al., 2017). However, the activity of the antioxidant enzymes CAT, GPOX and glutathione dehydrogenase were increased at 15 and 30 $kJm^{-2}d^{-1}$ of UV-B radiation, while APX and GR activities were not altered in sunflower cotyledons (Costa et al., 2002).

2.7.1.2 Non enzymatic scavenging mechanisms

The non enzymatic antioxidants protect the cell from damage by directly detoxifying the ROS by donating electron or hydrogen or they can reduce substrates for antioxidant enzymes and they include ascorbate, tocopherol, carotenoids, glutathione and phenolic compounds such as flavonoids and polyphenols (Ahmad et al., 2009; Gill and Tuteja, 2010). Ascorbate or vitamin C, the most abundant and extensively studied antioxidant in plants (Arrigoni and de Tullio, 2000), is considered as a most powerful ROS scavenger in plants because of its ability to donate electrons in a number of enzymatic and non-enzymatic reactions. It protects PSII against donor side photoinhibition by acting as an alternative electron donor of PSII in leaves with inactive oxygen evolving complex (Tóth et al., 2011). Also it acts as a cofactor of violaxanthin de-epoxidase (VDE) and thus can dissipate the excess excitation energy from chloroplasts under photoinhibition induced by HL and UV-B radiation. Gao and Zhang (2008) experimentally revealed that the ascorbate-deficient *Arabidopsis* mutant *vtc1* is more sensitive to supplementary UV-B treatment than wild type plants.

Glutathione, a low molecular weight thiol (γ-glutamyl-cysteinyl-glycine), functions as a reductant in scavenging of ROS under excess irradiation. Glutathione along with ascorbate plays a central role in antioxidation under HL exposure in *Arabidopsis* (Heyneke et al., 2013) and in pea upon UV radiation (Saleh, 2007). Normally, chloroplast membranes of higher plants are well protected against photooxidative damage since they contain α-tocopherol and α-tocotrienols (Fryer, 1992; Blokhina et al., 2003). There is also evidence that α-tocopherol quinone existing in chloroplast membranes under photoinhibition shows antioxidant properties similar to those of α-tocopherol (Kruk et al*.,* 1997). In alpine plants of high altitude, as day temperature decreases and light intensity increases, it was found that ascorbate, α-tocopherol and glutathione content increased, thus playing pivotal role by these antioxidants in stress tolerance (Wildi and Lütz, 1996).

Other than the above compounds, carotenoids or lipophilic antioxidants have a primary role in photoprotection by quenching ROS before oxidative damage can occur, or by activating NPQ of excess light energy. Carotenoids have a structural role also in the PSI assembly and the stabilization of light harvesting complex proteins and thylakoid membrane (Lu and Li, 2008). Many studies have described the physiological role of carotenoids in plants under various stresses by quenching triplet state chlorophyll molecules and scavenging $^1O_2$ formed within the photosynthetic apparatus (Ramel et al., 2012; Kang et al., 2017). The carotenoids exert their protective function as antioxidants to inactivate HL and UV-B induced radicals in the photosynthetic membrane (White and Jahnke, 2002; Zhang et al., 2005; McElroy and Kopsell, 2009).

Plant phenolic compounds have the ability to reduce ROS and also function as metal chelators and they act synergistically with other physiological antioxidants such as ascorbate or tocopherol to amplify their

biological effects. The antioxidant capacity of phenolics in plants under stress conditions will increase with the number of free hydroxyls and conjugation of side chains to the aromatic rings (Bergmann et al., 1994). Flavonoids are UV screening compounds and effectively scavenge $^1O_2$ after exposure to excess light and UV radiation (Agati et al., 2012). According to Dai and Mumper (2010), phenolics and flavonoids have great antioxidant property and are more effective than Vitamin C, E and carotenoids. Thus maintenance of antioxidant components (including enzymatic and non-enzymatic mechanisms) and oxidative damage (including lipid peroxidation) reflect a steady state metabolic process in plants which could potentially be exploited as a sensitive and reliable early-warning biomarker for HL and UV-B induced photoinhibition (Jansen et al., 1998; Blokhina et al., 2003).

2.7.1.3 Compatible solutes

Accumulation of compatible solutes in response to oxidative stress is known to correlate with increased tolerance in plants. This compatible solutes or osmolytes includes amino acids (e.g. proline), sugar alcohols (e.g. pinitol), sucrose, quartemary ammonium compounds (e.g. glycine betaine, alanine betaine), polyols, trehalose and polyamines (Rhodes and Hanson, 1993). These are low molecular weight, highly soluble compounds and are nontoxic even at high cellular concentrations and maintain the cellular homeostasis in plants (Ashraf and Foolad, 2007). Certain compatible solutes such as sorbitol, mannitol and proline that accumulate in plant cells mainly in response to environmental stress and protect cellular components from injury, mitigate oxidative damage caused by free radicals and maintain the enzyme activities under stressed conditions.

Proline is a major compatible solute in plants which is also found to have significant beneficial functions in response to photoinhibition induced by environmentally relevant doses of UV-B and PAR. In addition to

functioning as a compatible osmolyte, it plays a significant role in scavenging harmful ROS molecules, stabilizing subcellular structures and maintaining intracellular redox homeostasis in subcellular structures. It has been reported that accumulation of proline in wheat and have been related to the protection from UV-B radiation (Yang et al., 2000). However, UV-B induced increases in proline levels was not linked to UV-B tolerance or plant productivity in populations of white clover (Hofmann et al., 2003). Whereas, higher tolerance to drought and HL stress for *Arabidopsis* plants grown under UV-B radiation was attributed to increased proline content (Poulson et al., 2006). Proline can stimulate the primary photochemical activity by reducing the lipid peroxidation during exposure to strong light (Alia et al., 1997).

Soluble sugars accumulated in plants during different abiotic stress conditions related to oxidative stress and stress induced ROS accumulation (Couée et al., 2006). Sucrose and hexoses both play active role in stress related metabolism of plants by the up-regulation of growth-related genes and down-regulation of stress-related genes (Rosa et al., 2009). Under UV-B irradiation significant increase occurred in the total soluble sugar content in maize (Erram et al., 2017) and soybean (Gao and Yang, 2016) cultivars. The efficient role of sugars as true ROS scavengers during abiotic stress has been proved and the synergistic interaction of sugars and phenolic compounds functions as an integrated redox system, providing the stress tolerance in plants (Keunen et al., 2013). Moreover, acclimation to HL exposure in *Arabidopsis* was obtained by the high accumulation of sugars through the expression of a glucose 6-phosphate/phosphate translocator, GPT2 protein (Dyson et al., 2015).

2.7.2 Epidermal responses

2.7.2.1 Epidermal reflectance: cuticular wax as a photoprotective layer

Cuticle constitutes the first line of defense and which interact with solar radiation and plays a key role in maintaining the plant's integrity within an inherently hostile environment. It is formed by a mixture of compounds with different physico-chemical properties such as waxes, cutin and/or cutan, polysaccharides, phenolics and some mineral elements (Fagerström et al., 2013; Fernández et al., 2016). The chemical composition, architecture and thickness of cuticular wax in plants is depending on species, organ, developmental stages, plant physiological status, or environmental conditions during growth (Oliveira and Salatino, 2000; Kitagami et al., 2013; Guzmán-Delgado et al., 2016). Epidermal reflectivity is strongly influenced by the surface topography of leaves which is primarily determined by leaf hairs and the cuticular wax layer, particularly at visible and UV wavelengths. The importance of cuticular wax in stress tolerance has been shown in various studies by removal of intracuticular and epicuticular waxes from waxy leaved species after washing of the foliar surface with low polarity solvents (Shepherd and Wynne Griffiths, 2006).

The cuticle contains a biopolymer called as cutin and two types of waxes: the intracuticular waxes which embeds in the cutin matrix and the epicuticular waxes which is deposited on the surface of cuticle. The crystalline microstructure of cuticular wax have the potential to induce and modulate the properties of light reflectance in leaves under high irradiation and regulate the temperature and also limit the transpiration in leaves by regulating the water vaporization (Guhling et al., 2005; Biswas et al., 2017). Kakani et al. (2003b) reported that photoprotective capacity of the cuticle is related to its ability to moderate the wavelengths of light that penetrate into

the tissues and the wax content on the leaf adaxial surface is known to increase when cotton plants were exposed to 8 and 16 $kJm^{-2}d^{-1}$ of UV-B.

From a chemical viewpoint, the cuticular waxes are generally composed of complex chemical mixtures and form a tubular or planar morphology (Koch et al., 2006). The cuticular waxes in plants are composed of long chain aliphatic groups, such as hydrocarbons (mainly *n*-alkanes), esters of fatty acids and fatty alcohols, aldehydes, primary and secondary alcohols and free fatty acids. Different cyclic classes, such as triterpenoids, flavonoids, and tocopherols, can also be found in cuticular wax of some plant species (Jetter et al., 2006; Yeats and Rose, 2013; Schuster, 2016). Within the long chain aliphatic groups, primary alcohols mainly accumulate in the intracuticular wax layer, while free fatty acids and *n*-alkanes accumulate in the epicuticular layer (Buschhaus and Jetter, 2011).

Cuticular wax generally gets accumulated in leaf surface under drought stress, such as alfalfa (Jefferson et al., 1989), banana (Surendar et al., 2013), sesame (Kim et el., 2007) and wheat (Adamski et al., 2013) indicating that it is related to drought tolerance. Guo et al. (2016) studied the cuticular wax accumulation in glaucous (with wax) and non-glaucous (without wax) wheat cultivars in response to drought stress and revealed that cuticular wax can maintain a relatively high water potential in leaves, a relatively high photosynthetic rate, PSII activity and grain yield in glaucous lines than non-glaucous cultivars under drought stress conditions and they suggested that leaf cuticular wax content can be an effective selection criterion in the development of drought-tolerant wheat cultivars. Moreover, Zhou et al. (2013) genomically proved that *OsGL1-6* is involved in the decarbonylation pathways in wax biosynthesis and directly influence the drought tolerance in rice.

Even though many studies were reported of UV-B effects on wax profile, only a few studies have been reported with regard to HL effects on epicuticular waxes of crop plants. According to Caldwell et al. (1983), wax deposition might provide a protective mechanism as the epicuticular wax reflects about 10 to 30% of the incident UV-B radiation in eucalyptus. The structure and composition of surface waxes were examined in leaves of barley, bean and cucumber seedlings and found that the main wax components were primary alcohols in barley, primary alcohols and monoesters in bean, and alkanes in cucumber leaves. Irradiation with UV-B stress caused an increase of total wax by about 25% on leaf area basis in barley, bean and cucumber seedlings. Likewise, aldehydes detected as a minor constituent of cucumber and barley wax, increased two fold (Steinmüller and Tevini, 1985).

Zu et al. (2011) studied the physiological and biochemical responses of Korean pines under 1.4, 2.81, and 4.22 $kJm^{-2}d^{-1}$ of UV-B and recorded that the most prominent substance class of Korean pine waxes were alkanes (50%) and the percentage of alkanes and phenols decreased with the enhanced UV-B radiation. However, the percentage of long-chain esters in Korean pines was increased with the increase in UV-B radiation and maximum increase was noticed after 4.22 $kJm^{-2}d^{-1}$ of UV-B radiation. Long et al. (2003) tested the sun-screening ability of maize epicuticular waxes using the *glossy1* mutant, which is specifically defective in juvenile wax production and recorded a significant difference in UV induced leaf rolling and DNA damage levels under enhanced UV levels of glossy1 mutant as compared to the wild type. In contrast, Semerdjieva et al. (2003) suggested that there was no significant difference in leaf cuticle structure in four variants of dwarf shrubs upon irradiation with UV-B.

2.7.2.2 Epidermal absorption by screening or absorbing compounds

Changes in the optical properties of outer epidermal layers are considered to play an important role in plants, and much attention has been paid to the ability of the epidermis to act as a wavelength-specific filter of high photon irradiation through the accumulation of phenyl propanoid type compounds such as flavonoids, flavones and anthocyanins. In higher plants, these compounds are concentrated mainly in the superficial structures, such as cuticle or epidermis and/or distributed within various cells and tissues (Jansen et al., 1998; Frohnmeyer and Staiger, 2003). Epidermal absorbance of UV-B by epidermal pigments appears to be a major UV-B screening mechanism, functioning to absorb UV-B radiation, while minimizing the absorption of photosynthetically active wavelengths. Therefore, the tolerance and avoidance of high irradiation in plant leaves is manifested with increasing photoprotective screen or UV screening compounds, once formed could be maintained with minimal expenditure of energy providing a long term protection against photodamage (Reuber et al., 1996; Bilger et al., 2007; Agati et al., 2012).

The monohydroxy B-ring-substituted flavonoids may effectively attenuate UV irradiance as well as attack of pathogens. However, the accumulation of dihydroxy over monohydroxy flavonoids in response to HL is likely to reduce photo-oxidative damage in plants. The accumulation of mesophyll flavonoids and the ratio of orthodihydroxy to monohydroxy B-ring-substituted flavonoids reduced the extent of oxidative damage in barley (Schmitz-Hoerner and Weissenböck, 2003). Even though, the best candidates for UV-B related oxidative damage attenuators are hydroxycinnamic acid derivatives (e.g. ferulic and caffeic acid), the flavonoid content increased as compared to hydroxycinnamic acids upon exposure to UV-B or strong sunlight (Burchard et al., 2000; Agati et al., 2002). In addition to the ability to

absorb UV wavelengths, flavonoids function in metal chelating and ROS scavenging activities in plants under exposure to HL. The developmental responses in plants under HL exposure is generally under the control of auxins and flavonoids have been reported to be the most effective regulators of auxin transport *in vivo* (Taylor and Grotewold, 2005). Thus, flavonoids regulate the development of plants growing under different intensities of sunlight irradiance by controlling the development of the whole plant and individual shoot organs through modulating the auxin movement (Peer and Murphy, 2007).

In addition, exposure to HL and UV-B radiation may increase the concentration of UV-B absorbing compounds in the epidermis, rendering some plants less susceptible to photosynthetic damage due to HL and UV-B irradiation. Levels of flavonoids vary considerably between plant species or varieties, with developmental stage and with differing environmental conditions such as visible radiation levels, water, temperature and nutrient supply (Murali and Teramura, 1985). Visible light, UV-A and UV-B radiation influences the accumulation as well as qualitative and quantitative nature of UV screening phenyl propanoid compounds. Carotenoids, betalains and anthocyanins also play an important role in protection of plants against HL and UV-B induced photodamage (Booji-James et al., 2000; Solovchenko and Schmitz-Eiberger, 2003).

The accumulation of phenolic compounds including flavonoids that selectively absorb UV radiation, which probably aid in the long-term adaptation of regular and prolonged exposure to elevated levels of solar radiation, including its UV-B component. Plants tolerant to UV-B and HL irradiation alleviate the damaging effects of photo-irradiation using absorbing pigments that reduce the penetration of UV-B into mesophyll tissue in leaves and fruits (Cen and Bornman, 1990; Krauss et al*.,* 1997; Solovchenko and

Merzlyak, 2008). Physiological parameters such as UV-B absorbing compounds and chlorophylls have been found to be useful indicators of UV-B sensitivity and tolerance in plants (Greenberg et al., 1997). In contrast, Smith et al. (2000) found that the contents of chlorophyll and UV absorbing compounds did not correlate with resistance at the time of UV screening in varieties of vegetable crops. Moreover, the molecular mechanisms of protection against photoirradiation and the potential contribution of UV screening compounds to tolerate UV radiation in plants is well explained by mutant studies. Li et al. (1993) studied the protection strategy by flavonoids and other phenolic compounds such as monocyclic sinapic acid esters against UV-B irradiation using *tt5* and *tt6* mutants of *Arabidopsis*, which have reduced levels of UV absorptive leaf flavonoids and the monocyclic sinapic acid ester phenolic compounds, are highly sensitive to the damaging effects of UV-B radiation.

Phenylalanine ammonia lyase (PAL) is a regulatory enzyme of the secondary metabolite synthesis that catalyzes the formation of trans-cinnamate from L-phenylalanine by non-oxidative deamination occurring in most plants (Gitz et al., 1998). Thus, PAL activity is important in plants as it catalyses the first committed step in the biosynthesis of defense related phenylpropanoids. Therefore the PAL in the plants can play an extremely important role to run the resistance mechanisms against the toxic effects of stress conditions (Dixon and Paiva, 1995; Khan et al., 2003; Edreva et al., 2008). The products of PAL and TAL (tyrosine ammonia lyase) such as lignin, flavonoids, pigments and phytoalexins plays an important role in imparting tolerance against a wide range of environmental stresses (Morrison and Buxton, 1993). It was found that anthocyanins, flavonoids content, PAL activity and TAL activity of *Vigna mungo* (Shaukat et al., 2013) *and Triticum aestivum* (Balouchi et al., 2009) was enhanced under UV-B irradiation. Moreover, the expression profiling of PAL and chalcone synthase genes, *pal*

and *chs* respectively, in *Brassica juncea* indicated an upregulation under UV-B treatment compared to that of control, suggesting that these enzymes ensured an efficient induction of flavonoid biosynthetic pathway (Pandey et al., 2012).

2.7.3 Non-photochemical quenching (NPQ)

One of the most important protection mechanisms is the dissipation of harmful excessive excitation energy as thermal energy by quenching the excitation of chlorophyll harmlessly within the light-harvesting complexes of the photosystems and this process is known as non-photochemical quenching (NPQ). The importance of NPQ for the protection of the photosynthetic apparatus is supported by its ubiquity in the plant system and it requires a structural change of the photosynthetic antenna complexes that are normally optimized with regard to efficient light-harvesting for photosynthesis (Müller et al., 2001; Goss and Lepetit, 2015). Enhanced dissipation of excess excitation energy as thermal energy in the antenna systems of the photosystems is accompanied by a strong quenching of the Chl *a* fluorescence and has thus been termed non-photochemical quenching of Chl *a* fluorescence, NPQ (Niyogi and Truong, 2013).

In the process of NPQ, the excessive excitation energy which cannot be utilized for photosynthesis is dissipated as heat. NPQ is provoked rapidly on a time-scale of seconds to a few minutes and is thus perfectly suited to cope with sudden variations in the light intensity. Based on the kinetics of Chl fluorescence relaxation in darkness following a period of illumination, at least three different components of NPQ have been distinguished i.e., the sum of different mechanisms which contribute to the overall heat dissipation (Azzabi et al., 2012; Allorent et al., 2016). The first and main component of NPQ is the energy dependent component or the high-energy-state quenching qE, which is triggered by the proton gradient across the thylakoid membrane and

relaxes within seconds. The second component, fluorescence quenching caused by state transitions qT, forms and relaxes on a timescale of 10 min., is predominantly observed in low light and is related to the balancing of excitation energy between PSII and PSI. The last component is the most slowly forming and relaxing NPQ component, photoinhibitory quenching qI, which was originally ascribed to the photoinhibitory damage of PSII reaction centers, which can persist for several hours in the dark following illumination (Alonso et al., 2017).

For the energy dependent component qE, the formation of a pH gradient across the thylakoid membrane is the primary driving step. Specialized members of the light harvesting complex (LHC) protein family, such as PSII subunit S (PsbS) in higher plants or members of the LHC Stress-Related (LHCSR) family in mosses and algae, are the central proteins to qE (Jahns and Holzwarth, 2012; Rochaix, 2014). The acidification of the thylakoid lumen leads to the activation of the PsbS protein and further the activation of violaxanthin de-epoxidase (VDE), which transforms violaxanthin through de-epoxidation to antheraxanthin and finally to zeaxanthin (Goss and Lepetit, 2015; Quaas et al., 2015; Zhao et al., 2017). It was well known that zeaxanthin acts as an allosteric modulator in the thylakoid membrane, enhancing LHCII proton binding affinity. Thus the transformation of these xanthophylls bounded to the LHC proteins, induces conformational changes in these proteins and activates qE. PsbS proteins maintain the mobility of PSII membrane complexes leading to qE generation and relaxation (Crouchman et al., 2006; Schaller et al., 2012). However, Betterle et al. (2009) concluded that irrespective of the presence of the PsbS protein or zeaxanthin, the formation of NPQ requires a structural reorganization of the thylakoid membrane.

Recently, Barczak-Brzyżek et al. (2017) experimentally demonstrated that excess energy is dissipated as heat in *Arabidopsis* mutants through the mechanism known as NPQ of Chl fluorescence. Goss and Lepetit (2015) reviewed about NPQ in higher plants and different algal groups with a special focus on the molecular mechanisms that lead to the structural rearrangements of the antenna complexes and enhanced heat dissipation in higher plants and algae. The relative contribution of the different components to heat dissipation depends on growth conditions and age of the plants (Müller et al., 2001).

2.7.4 Interconversions of xanthophyll pigments

Xanthophylls are accessory light harvesting pigments in the photosynthetic apparatus of green plants and they have very short excited state lifetimes, approximately 500 times shorter than Chl, thus it become effective light harvesters (Dall'Osto et al., 2006; Ruban et al., 2012). One of the most ubiquitous mechanisms involves modulation in the efficiency of the conversion of solar energy into chemical energy is xanthophyll cycle - a process of enzymatic reactions of epoxidation and de-epoxidation of xanthophylls (Niyogi et al., 1997; Latowski et al., 2011; Jahns and Holzwarth, 2012). Xanthophyll cycle is one of the most efficient mechanisms protecting plants and other photosynthesizing organisms under overexcitation conditions resulted by various environmental factors (Kromdijk et al., 2016; Junker et al., 2017).

Much of the research work was performed by scheming HL irradiance, drought, UV, nutrient availability of greenhouse or growth chamber environments to examine the role of the xanthophyll cycle in the photoprotection of plants under stressful conditions (Lokstein et al., 2002; Szôllôsi et al., 2008). The deactivation of $^1O_2$ formed in the PSII reaction center under photoinhibition is carried out by β-carotene while xanthophylls

are involved in the NPQ of excitation energy in light harvesting antenna proteins under conditions of excessive illumination (Jahns and Holzwarth, 2012). In vascular plants two xanthophyll cycles have been discovered, the violaxanthin cycle (V cycle) or xanthophyll cycle and lutein epoxide cycle (Lx cycle) which seems to be specific to certain species, to convert excess excitation energy into harmless thermal energy (Leonelli et al., 2017; Leuenberger et al., 2017).

2.7.4.1 Violaxanthin cycle (V cycle)

It is well documented that plants under increasing HL and UV-B stress employ a greater level of xanthophyll cycle interconversion, whereby conversion of particular group of carotenoids, violaxanthin, antheraxanthin and zeaxanthin occurs by enzyme catalyzed de-epoxidations. Under moderate stress conditions, violaxanthin function as the most abundant antenna pigment by transferring energy to Chl *a*. With increasing stress dosage or intensity, violaxanthin is biochemically converted to pigment zeaxanthin *via* the intermediate antheraxanthin by the activity of VDE and excess excitation energy can be harmlessly dissipated as heat through the formation of zeaxanthin or antheraxanthin in the antenna pigment complexes of PSII (Chen and Gallie, 2012). In darkness, this reaction is reversible by the action of the enzyme zeaxanthin epoxidase (ZE). VDE requires reduced ascorbate as co-substrate to reduce the epoxy group, which is then eliminated as H_2O (Grouneva et al., 2006; Jahns and Holzwarth, 2012). As a consequence, quenching of excess energy helps to keep PSII reaction centers open and maintain the electron transport and protects the reaction center from photoinhibition (Goss and Jakob, 2010).

In response to excess light, the de-epoxidation of xanthophyll cycle pigments increased and was found to be associated with high levels of NPQ (Ware et al., 2015; Magney et al., 2017). Joubert et al. (2016) studied the role

of specific xanthophylls which responded to UV-B acclimation in field grown grapevine berries and identified that zeaxanthin as being the most responsive to UV-B during the early stages of development. Likewise, Reyes et al. (2018) evaluated the capacity of *Chenopodium quinoa* to resist UV-B irradiation by daily exposure of 1.69 Wm^{-2} UV-B for 30 or 60 min. It was found that 30 min UV-B exposure did not cause severe alterations in the de-epoxidation of xanthophyll cycle pigments. On the other hand, 60 min UV-B increased the de-epoxidation of xanthophyll cycle pigments and showed a gradual increase in the content of antheraxanthin and zeaxanthin during the increase in the dosage of UV-B radiation.

2.7.4.2 Lutein epoxide cycle (Lx cycle)

Lx cycle typically functions in parallel with the V cycle involves a light-driven de-epoxidation of lutein-5,6-epoxide (Lx) to lutein (L) catalyzed by VDE and the accumulation of L is thought to be beneficial to plants when encountered with photoinhibition because it has been shown to increase NPQ in various species subjected to stress (García-Plazaola et al., 2003; Dall'Osto et al., 2006). Lutein is the most abundant xanthophyll in higher plants and is bound to L1 and L2 binding sites of LHC. The Lx cycle is found in species that accumulate lutein epoxide (Lx) in shade conditions and convert it to lutein (L) when exposed to high light by VDE. The main functions of L include light harvesting, structural stabilization of antenna proteins and quenching of ^{1}Chl and ^{3}Chl states (Esteban et al., 2009; Jahns and Holzwarth, 2012). The Lx cycle was identified in large numbers of woody plant species and it has also been involved in the thermal dissipation of energy in some plant species (García-Plazaola et al., 2007; Mastubara et al., 2007).

However, the contribution of the Lx cycle to photoprotection has been difficult to dissect because the V cycle often functions in parallel and responds more rapidly than Lx cycle. In fact, V cycle and Lx cycle differ in

the epoxidation kinetics in the dark, the rate of dark epoxidation is lower (or zero) in the Lx cycle (García-Plazaola et al., 2003, 2004). Notably, both *in vitro* and *in vivo* studies have recently shown that accumulation of Lx can increase the light harvesting efficiency in the antennae of PSII and play a photoprotective role in leaves growing in a more irradiated environment (Esteban et al., 2008; Avenson et al., 2009; Abney et al., 2013). Recently Leonelli et al. (2017) engineered the Lx cycle into *Arabidopsis thaliana* and created this engineered lines as a model system to study the role of NPQ as thermal dissipation in response to light stress.

MATERIALS AND METHODS

3.1 Plant material

Rice is a cereal grain belonging to the family Poaceae. Seeds of 13 high yielding common rice varieties (Table 1) were collected from Regional Agricultural Research Station (RARS) of Kerala Agricultural University, Pattambi, Kerala, India. Healthy and uniform sized seeds of rice were selected and pre-washed for 1 min with 0.25% Triton X-100 to remove the dirt. The seeds were surface sterilized with 0.1% $HgCl_2$ solution for 5 min and further seeds were washed thoroughly with distilled water. The seedlings were raised in plastic bottles (27×11 cm) containing absorbent cotton soaked with modified half strength Hoagland medium (Epstein, 1972). Seedling growth was carried out in a plant growth chamber (INLABCO) set at 14/10 h light-dark cycles at 300 $\mu molm^{-2}s^{-1}$, 24±2^{o}C temperature and 55±5% relative humidity.

Table 1: *O. sativa* varieties used in this study.

Sl. No.	Variety	Developed in year	Parental lines/pedigree
1	Ptb 35 - Annapoorna	1966	Ptb 10 x Taichung 1
2	Ptb 39 - Jyothi	1974	Ptb 10 x IR 8
3	Ptb 43 - Swarnaprabha	1985	Bhavani x Triveni
4	Ptb 45 - Mattatriveni	1990	Reselection from Triveni
5	Ptb 47 - Neeraja	1990	IR 20 x IR 5
6	Ptb 50 - Kanchana	1993	IR 36 x Pavizham
7	Ptb 51 - Aathira	1993	BR 51-46-1 x Cul 23332-2
8	Ptb 52 - Aiswarya	1993	Jyothi x BR 51
9	Ptb 53 - Mangalamahsuri	1998	Reselection from Mashuri
10	Ptb 54 - Karuna	1998	CO.25 x H4
11	Ptb 55 - Harsha	2001	Ptb 10 x Ptb 28
12	Ptb 56 - Varsha	2002	M210 x Ptb 28
13	Ptb 57 – Swetha	2002	IR 50 x C14-8

3.2 Composition and preparation of Hoagland nutrient solution

Hoagland nutrient solution was prepared according to Epstein (1972) with some modifications and used for growing rice seedlings (Table 2). A stock solution for each nutrient was prepared separately and appropriate volume of each was mixed together to make up the final volume and concentration of the nutrient solution. pH of the nutrient solution was adjusted to 6.8 using 0.1 N HCl or NaOH.

Table 2: Composition of modified Hoagland nutrient solution employed in the present study.

Compound		Molecular weight	Concentration of stock solution	Concentration of stock solution	Volume of stock solution/L of Hoagland solution
		$gmol^{-1}$	mM	gL^{-1}	ml
Macronutrients	KNO_3	101.10	1,000	101.10	6.0
	$Ca(NO_3)_2.4H_2O$	236.16	1,000	236.16	4.0
	$NH_4H_2PO_4$	115.08	1,000	115.08	2.0
	$MgSO_4.7H_2O$	246.48	1,000	246.48	1.0
Micronutrients	KCl	74.55	25	1.864	2.0
	H_3BO_3	61.83	12.5	0.773	
	$MnSO_4.H_2O$	169.01	1	0.169	
	$ZnSO_4.7H_2O$	287.54	1	0.288	
	$CuSO_4.5H_2O$	249.68	0.25	0.062	
	H_2MoO_4	161.97	0.25	0.040	
	NaFeEDTA	558.50	53.7	30.0	0.3

3.3 Chemicals

Chemicals of analytical reagent (AR) or guaranteed grade (GR) from Merck, Himedia, Qualigens, BDH, Spectrochem and SRL companies were used. Chemicals like gluteraldehyde, potassium bromide (FT-IR grade), 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU), riboflavin, methyl viologen

(MV), sodium azide (NaN_3), bovine serum albumin (BSA), lutein, zeaxanthin and β-carotene from Sigma-Aldrich Co., USA were used.

3.4 Determination of pre-growth period for imparting HL stress in rice seedlings

For the standardization of growth period in rice seedlings for imparting HL treatment, they were analyzed for better performance in terms of total chlorophyll content and Fv/Fm ratio. Thirteen varieties of rice seedlings were germinated in half strength Hoagland medium and they were analyzed for best performance after 8, 9, 10, 11 and 12 d of germination.

3.5 Experimental design and stress treatments

Two types of stress treatments in thirteen rice varieties were performed in this study: HL and UV-B treatments.

3.5.1 High intensity light treatment

Thirteen varieties of rice seeds were germinated and grown in plastic bottles containing absorbent cotton soaked with half strength Hoagland solution. After 10 d of growth, rice seedlings were exposed to HL (2000 $\mu molm^{-2}s^{-1}$), provided by 1000 W PAR64 metal halide lamps (Philips, Netherlands). A trough of transparent glass (20 cm depth) with circulating water was placed under the lamp to protect the seedlings from the heat generated by the lamp. Air was circulated around the seedlings and thus the temperature was maintained at 24 ± 2ºC (Page no. 54). Various physiological and biochemical parameters were analyzed after rice seedlings were exposed to HL stress at different time intervals (0, 2, 4, 6 and 8 h). The detailed scheme of work is illustrated in Chart 1.

3.5.2 UV-B treatment

For imparting UV-B stress, rice seedlings were exposed to UV-B radiation (280-320 nm) for one week after 4 d of germination. The seedlings

were irradiated with UV-B in the presence of continuous white fluorescent illumination of 300 μmol$m^{-2}s^{-1}$ held in mobile adjustable frames over the plants in a UV chamber (ROTEK). The UV-B tubes were covered with 0.13 mm thick cellulose diacetate filters to avoid transmission of wave length below 280 nm. The rice seedlings were exposed to UV-B stress (1.93±0.29 Wm^{-2}) for 1, 2, 3 and 4 h. The intensity of 1 h UV-B irradiation was equivalent to UV-B dose of 7 $kJm^{-2}d^{-1}$, 2 h treatment was equivalent to a dose of 14 $kJm^{-2}d^{-1}$, 3 h UV-B irradiation was equivalent to 21 $kJm^{-2}d^{-1}$ and 4 h treatment was equivalent to 28 $kJm^{-2}d^{-1}$ dose of irradiation. Various morphological, physiological, biochemical and anatomical parameters were analyzed in rice seedlings after irradiating with various level of UV-B on 10 d of germination. The detailed scheme of work is illustrated in Chart 2.

A **B**

Experimental set up for HL (A) and UV-B (B) treatment of rice seedlings

3.5.3 PAR (photosynthetically active radiation) and UV-B measurement

Photosynthetically active radiation in terms of light intensity at the surface of the leaves at one hour interval was measured by a solar radiation

monitor (EMCON, India). The intensity of PAR is referred as PPFD (Photosynthetic Photon Flux Density) which is measured in $\mu molm^{-2}s^{-1}$. The UV-B radiation just above the plant inside the UV chamber at 2 h interval was measured using spectroradiometer (Solar Light Co., PMA 2200, USA) and expressed as Wm^{-2}.

3.6 Growth parameters

The shoot length of rice seedlings was measured using a graduated scale and was expressed in centimeters. The fresh weight of rice seedlings were recorded by weighing them immediately after harvesting using an electronic weighing balance after blotting and wrapping of seedlings separately in pre-weighed labeled aluminium foils. For dry weight measurements, the weighed seedlings were kept in a hot air oven at 100°C for one h and further kept in oven set at 60°C. On the next day, the samples were allowed to cool in a desiccator with vaccum and then weighed. Drying and weighing were repeated at regular intervals (24 h) until the values of dry weight became constant. The dry weight percentage was calculated by using the following formula:

$$Dry\ weight\ percentage = \frac{Dry\ weight}{Fresh\ weight} \times 100$$

3.7 Physiological parameters

3.7.1 Photosynthetic pigment analysis

The chlorophyll and carotenoid pigments in the leaves of rice seedlings were estimated following the method of Arnon (1949) by using 80% acetone as the extracting medium. Fresh leaves of rice seedlings were washed out thoroughly with distilled water and blotted dry with filter paper. To estimate pigment contents, two hundred milligram of fresh leaf sample was weighed, homogenized and extracted in 80% acetone (v/v). Then the homogenate was

centrifuged at 5000 rpm for 10 min at 4°C and the supernatant was collected. The residue was re-extracted with the same extracting medium and centrifuged at 5000 rpm for 10 min. This process was repeated until the pellet became colourless. The final volume of the acetone extract was noted and the optical density was read at 663, 646, 750 and 470 nm against the solvent blank (80% acetone) using a UV-VIS spectrophotometer (Systronics 2201). The concentration of the total chlorophyll (Chl a+b) and carotenoids contents were expressed in mg chlorophyll/carotenoids g^{-1} dry weight of leaf sample.

$$Total\ chlorophyll\ (a+b) = \frac{20.12\ (A_{646} - A_{750})\ +\ 8.02\ (A_{663} - A_{750})}{Fresh\ weight\ of\ the\ sample} \times Volume$$

$$Carotenoid\ = \frac{1000\ (A_{470}) + 3.27\ (Chl\ a - Chl\ b)}{Fresh\ weight\ of\ the\ sample \times 229} \times Volume$$

Where,

$$Chlorophyll\ a\ \mu g/g\ = \frac{12.69\ (A_{663} - A_{750}) - 2.69\ (A_{646} - A_{750})}{Fresh\ weight\ of\ the\ sample} \times Volume$$

$$Chlorophyll\ b\ \mu g/g\ = \frac{22.9\ (A_{646} - A_{750}) - 4.68\ (A_{663} - A_{750})}{Fresh\ weight\ of\ the\ sample} \times Volume$$

3.7.2 Estimation of photosynthetic electron transport activities

The photosynthetic electron transport activities were analyzed polarographically using Oxygraph Plus oxygen electrode system (DW1/AD, Hansatech, Norfolk, UK) which consists of a highly sensitive S1 Clark Type polarographic oxygen electrode disc mounted within a DW1/AD electrode chamber and connected to the Oxygraph Plus electrode control unit (OXYG1, Hansatech). The DW1/AD electrode chamber provides a highly versatile solution to measurements of dissolved oxygen in liquid phase samples with clear cast acrylic construction providing excellent sample visibility and

uniform illumination. Precise temperature control of the sample and electrode disc can be achieved by connecting the water jacket of the DW1/AD to a thermoregulated circulating water bath. Thylakoids from rice leaves after HL and UV-B irradiation were isolated at 4^{o}C and photosystem I (PSI) (O_2 uptake) and photosystem II (PSII) (O_2 evolution) activities were measured as described by Puthur (2000). The light dependent O_2 uptake/evolution was measured by irradiating the thylakoid suspension with white light (1800 $\mu molm^{-2}s^{-1}$) continuously, provided by a 100W halogen lamp (LS2, Hansatech). The activities of PSI and PSII was expressed in terms of µmol of O_2 consumed (PSI)/evolved (PSII) $min^{-1}mg^{-1}$ chlorophyll.

3.7.2.1 Preparation of thylakoid membranes

Thylakoids membranes were isolated from rice leaves according to standard method (Puthur, 2000). The fresh leaves were cut into pieces and one hundred milligram of fresh leaf tissue was gently homogenized with a chilled mortar and pestle in an ice-cold isolation buffer containing 400 mM sucrose, 20 mM tricine (pH 7.8) and 10 mM NaCl. The homogenate was filtered through 6 layers of Mira cloth to remove large debris and the filtrate was centrifuged at 5000 rpm for 6 min at 4^{o}C. The supernatant was discarded and the thylakoid pellets were suspended in 500 µl suspension buffer (pH 7.5) containing 10 mM NaCl, 20 mM HEPES [N-(2-Hydroxyethyl) piperazine-N-(2-Ethanesulphonic acid)], 100 mM sucrose and 2 mM $MgCl_2$ and it was transferred to a clean chilled tube and stored on ice for hours with minimal loss of activity.

3.7.2.2 Estimation of the total chlorophyll concentration in the thylakoid suspension

To compare results obtained using different chloroplast preparations, the total chlorophyll content of the thylakoid samples was estimated

according to the method of Arnon (1949). 20 µl of the thylakoid suspension was added to the test tube containing 3 ml of 80% acetone. The tube was covered with parafilm and the contents of the tubes were mixed thoroughly using a vortex mixer to dissolve the chlorophyll and the homogenate was centrifuged at 5000 rpm for 5 min to pellet any particulate material and the supernatant was collected. The absorbance of the supernatant was measured at 645, 663 and 750 nm against the solvent blank (80% acetone). The total chlorophyll concentration was calculated from the following equation:

$$20.12\ (A_{646} - A_{750}) + 8.02\ (A_{663} - A_{750}) \times \text{dilution factor}$$

3.7.2.3. Assay of photosystem I and II activities

PSI and PSII activities were analyzed using Oxygraph Plus oxygen electrode system (DW1/AD, Hansatech, Norflok, UK) according to the protocol of Puthur (2000). PSI activity was measured in terms of oxygen consumption after PSII activity was blocked initially by adding DCMU to the medium. The reaction mixture consisted of reaction buffer, reduced 2,6-dichlorophenolindophenol (DCPIP) (0.1 mM), ascorbate (600 µM), MV (500 µM), NaN_3 (1 mM) and DCMU (5 µM). Thylakoid suspension equivalent to 20 µg chlorophyll was added and the volume was made up to 1 ml with reaction buffer. Electron transport to PSI was maintained by artificial electron donors, ascorbate and DCPIP in the medium. Ascorbate acted as reductant by donating electrons to DCPIP and further the electrons supplied by reduced DCPIP to plastocyanin were transferred to PSI. Electrons from PSI are bypassed to an artificial electron acceptor, MV in the reaction mixture instead of being accepted by FeS centre. Finally MV reacts with oxygen molecules in the medium and produce H_2O_2. The dissociation of H_2O_2 into oxygen and H_2O by the action of catalase in the plant tissue is arrested by NaN_3 added in the reaction mixture. Thus the oxygen consumption by activity of PSI alone is measured by oxygen electrode system.

PSII activity was measured in terms of oxygen evolution by using para-benzo quinone (pBQ) as an artificial electron acceptor and it will scavenge the electrons from plastoquinone. The transfer of electron from plastoquinone to cytochrome is terminated and so the activity of PSII alone can be measured. Splitting of water for transferring of electrons to PSII result in evolution of oxygen molecules in the medium and it was measured by Oxygraph Plus system. The reaction mixture (1 ml) in DW1/AD electrode chamber consisted of the reaction buffer, pBQ (500 μM) and isolated thylakoid suspension equivalent to 20 μg chlorophyll.

3.7.3 Assay of mitochondrial activity

3.7.3.1 Isolation of mitochondria

Mitochondrial isolation from the seedlings was carried out according to the method of Kollöffel (1967). The plant materials were gently homogenized with a chilled mortar and pestle at 4^{o}C in ice cold 0.05 M phosphate buffer (isolation buffer, pH 7.2) containing 0.4 M sucrose and 5 mM ethylenediaminetetraacetic acid (EDTA). The homogenate was filtered through four layers of Mira cloth and the filtrate was centrifuged at 5000 rpm for 10 min. The supernatant was again centrifuged at 20,000 rpm for 15 min after removing the pellets. The resultant pellet, containing mitochondria was re-suspended in known volume of suspension buffer (0.05 M phosphate buffer with 0.2 M sucrose, pH 7.6). The protein content in the mitochondrial preparations was determined by the method of Bradford (1976).

3.7.3.2. Estimation of mitochondrial electron transport activity

Oxygen consumption by mitochondria was measured at 25^{o}C using a Oxygraph Plus oxygen electrode system (DW1/AD, Hansatech, Norflok, UK) as per the protocol of Schmitt and Dizengremel (1989). Reaction medium contained 935 μl of assaying buffer (0.3 M sucrose, 10 mM potassium

phosphate, 10 mM Tris, 5 mM $MgCl_2$ and 10 mM KCl, pH 7.2), 40 µl mitochondrial preparations (equivalent to 0.3 mg protein) and 25 µl of 100 mM NADH. The substrate was added very last, so that oxygen uptake rate measurements were started immediately. The oxidation rate of NADH was calculated in terms of µmol O_2 consumed $min^{-1}mg^{-1}$ protein.

3.7.4 Chlorophyll *a* fluorescence parameters

Chl *a* fluorescence parameteres were analyzed by using Plant Efficiency Analyzer (Handy PEA; Hansatech Ltd., King's Lynn, Norflok, UK), which is a portable fluorometer having high resolutions (Strasser et al., 2004). All measurements were performed on the upper surfaces of the first formed leaves after dark adapted for a period of 20 min using the leaf exclusion clips and then they were illuminated with continuous red light of high intensity (3000 $\mu molm^{-2}s^{-1}$). All measurements were recorded up to 1s with a data acquisition rate of 10 µs for the first 2 ms and at 1 ms thereafter.

The various fluorescence parameters, maximal fluorescence (Fm), area above the fluorescence curve, the activity of the water-splitting complex on the donor side of the PSII (Fv/Fo), PSII structure-function-index [$SFI_{(abs)}$], relative variable fluorescence at J step (Vj), performance index [$PI_{(abs)}$], the electron transport quantum yield (Φ_{Eo}), the time when maximum fluorescence value is reached [Tf(max)] and the yield of electron transport per trapped exciton (Ψ_o) were measured. The phenomenological energy fluxes were figured as energy pipeline leaf model [RC/CSo (concentration of the active reaction centres per cross section), ABS/CSo (the number of photons absorbed per cross section), TR/CSo (the maximal trapping rate of an exciton measured per cross section), ET/CSo (the electron transport flux per cross section) and DI/CSo (the dissipation rate per cross section)]. PSII energy fluxes per reaction center (RC) [flux of absorption per reaction center

(ABS/RC), trapping flux per reaction center (TR_o/RC), electron transport flux per reaction center (ET_o/RC) and dissipated energy flux per reaction center (DI_o/RC)] was figured as specific membrane model. Data were analyzed; radar plot, energy pipeline and specific membrane models were deduced using Biolyzer HP3 software (Chl fluorescence analyzing program by Bioenergetics Laboratory, University of Geneva, Switzerland).

For calculating NPQ, Chl *a* fluorescence was measured at room temperature with a Modulated Chl fluorometer (OPTI-SCIENCES, OS1p), according to van Kooten and Snel (1990). After determining Fm, the leaf was continuously illuminated with a white actinic light of 1000 $\mu molm^{-2}s^{-1}$. Then the steady-state value of fluorescence (Fs) was recorded after 5 min and a second saturating pulse at 8000 $\mu molm^{-2}s^{-1}$ was imposed to determine maximal fluorescence level in the light-adapted state (Fm'). Thereafter the minimal fluorescence level in the light adapted state (Fo´) was determined by illuminating the leaf with a 3 s pulse of far-red light and NPQ was calculated from the following equation:

$$NPQ = (Fm/Fm') - 1$$

3.7.5 Leaf gas exchange parameters

Leaf gas exchange parameters were analyzed by using a LI-6400 portable photosynthesis system (Infra-red gas analyzer, LI-COR, Lincoln, Nebraska, USA). Leaf surfaces were cleaned and dried using tissue paper before being enclosed in the leaf chamber for gas exchange measurements. All measurements were record on fully expanded first formed rice leaves and reading was taken between 9.00 to 10.00 am at growth temperature and ambient CO_2 conditions. The internal light source in LI-6400 was set at an intensity of 1500 $\mu molm^{-2}s^{-1}$ to ensure a constant and uniform light across all measurements. The various leaf gas exchange parameters, net photosynthetic

rate, P_n ($\mu molm^{-2}s^{-1}$), stomatal conductance, g_s ($\mu molm^{-2}s^{-1}$) and transpiration rate, E ($mmolm^{-2}s^{-1}$) was measured in rice leaves after exposure with HL and UV-B irradiation. The measurements were done in the absence of HL and UV-B to avoid instantaneous effects of HL and UV-B radiation. P_n, g_s and E were calculated using the equations derived by von Caemmerer and Farquhar (1981).

3.8 Biochemical parameters

3.8.1 Metabolites

3.8.1.1 Rate of lipid peroxidation

The malondialdehyde content (MDA) estimation was done according to the method of Heath and Packer (1968).

Extraction: Two hundred milligrams of plant tissue was weighed in triplicate and homogenized in 5 ml of 5% trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 12,000 rpm for 15 min. The supernatant was collected and used for the estimation of MDA.

Estimation: The MDA content estimation was done according to Heath and Packer (1968). Two millilitre of the supernatant was mixed with an equal aliquot of 0.5% of thiobarbituric acid (TBA) in 20% TCA and the solution was heated at 95^oC for 24 min, cooled and then centrifuged at 3000 rpm for 2 min. The absorbance of the supernatant was measured at 532 and 600 nm against reagent blank using UV-VIS spectrophotometer (Systronics 2201). The absorbance value at 532 nm was corrected for non-specific turbidity by subtracting absorbance value at 600 nm. Then the MDA content was calculated using its extinction coefficient of 155 $mM^{-1}cm^{-1}$.

3.8.1.2 Proline

Proline content in the experimental and control of rice seedlings was estimated according to the method of Bates et al. (1973).

Extraction: Two hundred milligrams of experimental and control plant tissues were weighed and homogenized in 10 ml of 3% (w/v) aqueous sulfosalicylic acid using a clean glass mortar and pestle. The homogenate was transferred to centrifuge tubes and centrifuged for 10 min at 10,000 rpm and the supernatant was collected and estimation of proline was done using acid ninhydrin.

Estimation: Two ml of supernatant was taken in test tubes in triplicate and equal volume of glacial acetic acid and 2.5% acid ninhydrin (1.25 g of ninhydrin dissolved in a mixture of 30 ml of glacial acetic acid and 20 ml of 6 M ortho phosphoric acid) were added to it. The tubes were then heated in a boiling water bath for 1 h and then the reaction was terminated by placing the tubes in ice bath. Four ml of toluene was added to the reaction mixture and stirred well using a vortex mixer. The chromophore-toluene layer was separated carefully and the optical density of the separated solution was measured at a wavelength of 520 nm using spectrophotometer (Genesis 20). L-proline was used as the standard.

3.8.1.3 Total soluble sugars

The total soluble sugar was estimated using the method proposed by Dubois et al. (1956).

Extraction: Two hundred milligrams of plant tissue was homogenized in 80% ethyl alcohol using a clean glass mortar and pestle. The homogenate was transferred to centrifuge tubes and then centrifuged at 10000 rpm for 10 minutes at 4^{o} C, the supernatant was collected and re-extract the pellet using

80% alcohol. The supernatant was collected and estimation of total soluble sugars was done.

Estimation: From the supernatant, a known volume of aliquot was taken in the test tube and made upto 1 ml with distilled water. To this, 0.1 ml of 5% (v/v) phenol was added and mixed well. Add 5 ml of concentrated sulphuric acid was added to the tube quickly from a burette. After cooling, the optical density of the resultant solution was measured at 490 nm using a spectrophotometer. D-glucose was used as the standard.

3.8.1.4 Total protein

Total protein content of the plant material was estimated using Folin-Ciocalteau reagent according the method of Lowry et al., (1951).

Extraction*:* Two hundred mg of leaf tissue was homogenized in 5 ml of phosphate buffer using pre-chilled glass mortar and pestle. A known volume of the homogenate was pipetted in to a centrifuge tube and equal volume of 10% TCA was added. This mixture was kept in a refrigerator (4^oC) for 1 h for flocculation. The protein precipitate was collected by centrifugation at 5000 rpm for 10 min at 4^oC. The supernatant was decanted off. The residue was washed twice with cold 2% TCA followed by washing with 30% perchloric acid to remove starch. Diethyl ether was used to extract lipids and 80% acetone to remove the pigments.

Estimation*:* The pellet obtained after centrifugation was dried and later digested in 5 ml 0.1 N sodium hydroxide by heating in a water bath for 10 min. After cooling the suspension was cleared by centrifugation (5000 rpm for 10 min at 4ºC) and the supernatant was collected. Known volume of aliquot was pipetted and made up to 1 ml with distilled water. To the aliquots, 5 ml of alkaline copper reagent was added and shaken well. After 10 min, 0.5 ml of 1 N Folin-Ciocalteu's phenol reagent was added and

shaken well immediately. The tubes were kept for 30 min for colour development. The optical density of the solution was read at 700 nm using a UV-VIS spectrophotometer (Systronics 2201). BSA fraction V powder was used as standard. Total protein was expressed as mg of protein g^{-1} dry weight of plant tissue.

3.8.1.5 Total free amino acids

Total free amino acids were determined by following the method of Moore and Stein (1948).

Extraction*:* Five hundred milligrams of fresh samples were homogenized in a clean mortar and pestle with 80% (v/v) ethanol. The extract was centrifuged at 10,000 rpm for 15 min at 4^{o}C and the supernatant was made up to 10 ml with 80% ethanol.

Estimation: One ml of the resultant supernatant was mixed with 1 ml of ninhydrin reagent in a test tube. Tubes were kept in boiling water bath for 20 min and further 5 ml of diluent (equal volume of water and n-propanol) was added to it. This mixture was incubated at room temperature for 15 min and absorbance was read at 570 nm using a UV-VIS Spectrophotometer (Systronics 2201) against a reagent blank and the results were expressed as mgg^{-1} sample. Standard curve was plotted by using leucine in 0.1 M citrate buffer at pH 5.

***Preparation of reagent*:** Reagent solution was prepared by dissolving 20 g of ninhydrin and 3 g of hydrindantin in 750 ml of methyl cellosolve. The solution was stirred carefully to avoid air bubbles into the solution. 250 ml of sodium acetate buffer (pH 5.5) was added to this solution and the resulting reddish reagent solution was immediately transferred to a 1 L dark glass bottle. The reagent was used freshly without storage.

3.8.2 UV-B absorbing compounds

Estimation of UV-B absorbing or screening compounds, anthocyanin and flavonoid content, in the leaf material of rice seedlings were done spectrophotometrically.

Anthocyanin content was determined according to the method of Mancinelli et al. (1975) with some modifications. Fresh rice leaf samples (0.2 g) were homogenized and extracted in 5 ml of acidified methanol (1: 99, HCl: methanol, v/v) using a mortar and pestle. The extract was kept at 4°C for 24 h and the content was made up to 10 ml. The amount of anthocyanin was estimated from the absorbance at 530 nm using UV-VIS spectrophotometer (Systronics 2201). Anthocyanin content was expressed as $\mu molg^{-1}$ DW and the concentration of anthocyanin was calculated using its extinction coefficient of 33 $mM^{-1}cm^{-1}$.

Flavonoids were extracted and measured according to the method of Mirecki and Teramura (1984). Two hundred mg of fresh leaf samples were homogenized in a clean mortar and pestle with 5 ml of solvent containing acidified methanol:HCl:H_2O (79:1:20) and UV-B absorbing compounds were extracted after keeping the homogenate for 24 h at room temperature. The flavonoid content was determined from the absorbance of the supernatant at 315 nm using UV-VIS spectrophotometer (Systronics 2201). Flavonoid content was expressed as $\mu molg^{-1}$ DW and the concentration of flavonoids was calculated using its extinction coefficient of 33 $mM^{-1}cm^{-1}$.

3.8.3 Phenylalanine ammonia lyase (PAL, EC 4.3.1.24)

PAL activity in the fresh plant samples was determined in accordance to the methodology of Zucker (1965).

Extraction: For PAL enzyme analysis, 0.2 g of fresh leaves were homogenised in 3 ml borate buffer (pH 8.8) containing 23 µl of mercaptoethanol. Extraction was performed at 2°C. The homogenate was centrifuged at 8,500 rpm for 20 min at 4°C in refrigerated centrifuge (Thermo scientific X1R). The supernatant was transferred to a clean test tube and stored in an ice bath and used for enzyme assay.

Enzyme Assay: The PAL assay system contained 1 ml of the supernatant, 1 ml of buffer, 1 ml of 0.05 M L-phenylalanine as substrate and it was incubated at 37°C for 1 h. The reaction was stopped with 30% trichloroacetic acid and absorbance of the formed trans-cinnamic acid was measured at 290 nm wavelength. The spectrophotometric determination of PAL activity in rice leaves is based on changes of optical density at 290 nm compared to the resulting mixture with stopped enzyme reaction at the beginning by heating, which was taken as blank. Standard curve was prepared using trans-cinnamic acid and PAL activity was expressed as µmoles of trans-cinnamic acid formed min^{-1} mg^{-1} of protein. Protein content in the PAL enzyme extract was determined by the method of Bradford (1976).

3.8.4 ROS scavenging mechanism

3.8.4.1 Non-enzymatic antioxidants

3.8.4.1.1 Ascorbate (AsA) content

For the estimation of AsA content, the method of Chen and Wang (2002) was adopted.

Extraction*:* Two hundred mg of plant tissue was weighed using an electronic balance and homogenized with 5 ml 5% (w/v) TCA. The homogenate was transferred to centrifuge tube and centrifuged at 12,000 rpm for 15 min at 4^oC. The supernatant was collected and used for the estimation of AsA content.

***Estimation**:* An aliquot of 0.1 ml of the supernatant was mixed well with 0.3 ml of 200 mM NaH_2PO_4. To this mixture, 0.5 ml of 10% (v/v) TCA, 0.4 ml of 42% (v/v) H_3PO_4, 0.4 ml of 4% (w/v) bipyridyl (dissolved in 70% alcohol) and 0.2 ml of 3% $FeCl_3$ (w/v) was added. The mixture was incubated at 42^oC for 15 min. The absorbance was measured immediately after incubation at 524 nm and AsA content was calculated from a standard curve prepared using different concentrations of AsA.

3.8.4.1.2 Glutathione (GSH) content

The GSH content estimation was done as per the protocol of Chen and Wang (2002).

***Extraction**:* Two hundred mg of plant tissue was weighed using an electronic balance and homogenized in 5 ml of 5% TCA (w/v). The homogenate was filtered through a filter paper and centrifuged at 12,000 rpm for 15 min at 4^oC. The supernatant was collected and used for the estimation of reduced glutathione content.

***Estimation**:* To an aliquot of 0.5 ml of the supernatant, 2.6 ml of 150 mM NaH_2PO_4 buffer (pH 6.8) and 0.18 ml of 3 mM 5,5-dithio-bis(2nitrobenzoic acid) (DTNB) were added (DTNB was dissolved in 100 mM phosphate buffer, pH 6.8) and kept for 5 min. Then the absorbance was read at 412 nm and GSH content was calculated from a standard curve using varying concentrations of reduced glutathione.

3.8.4.1.3 Total phenolics

Total phenolic was estimated using Folin-Denis reagent according to the method of Folin and Denis (1915).

***Extraction**:* One hundred mg of fresh tissue was weighed using an electronic balance and homogenized in 80% ethanol (v/v) in a clean mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 20 min and the

supernatant was collected. The residue was reextracted with 80% ethanol. The homogenate was again centrifuged and supernatant was pooled. Pooled supernatant was then dried in an oven and the residue was dissolved in 5 ml of distilled water.

Estimation: Aliquots of 50 µl in triplicate were pipetted out and made up to 2 ml with distilled water. Equal volume of Folin-Denis reagent was added to it. The contents were thoroughly mixed and after 3 min, 2 ml of 1N sodium carbonate was added. This mixture was kept for 1 h after thorough mixing for colour development. The optical density of the resultant solution was measured at 700 nm and total phenolic content in the plant tissue was calculated using tannic acid as standard.

3.8.4.2 Enzymatic antioxidant system assay

3.8.4.2.1 Superoxide dismutase (SOD, E C 1.15.1.1)

Estimation of SOD activity in the rice seedlings was done as per the modified protocol of Giannopolitis and Ries (1977).

Extraction*:* Five hundred mg of leaf tissue was weighed and homogenized gently in 50 mM phosphate buffer of pH 7.8 with pre-chilled mortar and pestle. The homogenate was centrifuged at 16,000 rpm for 15 min in refrigerated centrifuge (REMI C-24 BL) at 4ºC. The supernatant was used for enzyme assay.

Enzyme Assay*:* SOD activity was carried out by monitoring the ability of SOD to inhibit the photochemical reduction of nitro blue tetrazolium chloride (NBT). The reaction mixture consisted of 0.1 ml of 1.5 M sodium carbonate, 0.3 ml of 0.13 M methionine, 0.3 ml of 10 µM EDTA, 0.3 ml of 13 µM riboflavin and 0.3 ml of 0.63 mM NBT and 0.1 ml ezyme extract. The reaction mixture was made up to 3.0 ml using phosphate buffer (50 mM, pH 7.8). Different assay systems were set, *viz.* dark-control, light-control and test samples. Test tubes containing

only assay mixture without enzyme extract were illuminated under fluorescent lamp for 30 min (light-controls). Test samples (tubes containing assay mixtures with enzyme extract) were also illuminated and other set (tubes containing assay mixtures with enzyme extract) was kept in dark (dark-control). The formazan accumulation in different tubes was quantified using UV-VIS spectrophotometer (Systronics 2201) by recording the absorbance of the developed blue colour at 560 nm against the blank (reaction mixture without NBT). Results were expressed as units SOD $mg^{-1}protein^{-1}$. One unit of SOD was defined as the enzyme activity that inhibited the photo reduction of NBT to blue formazan by 50%.

3.8.4.2.2 Guaiacol peroxidase (GPOX, EC 1.11.1.7)

GPOX activity in the fresh samples was measured by adopting the method of Gaspar et al. (1975).

Extraction*:* Five hundred mg of fresh plant tissue was weighed and gently homogenized in 50 mM Tris-HCl buffer (pH 7.5) using a chilled mortar and pestle. The extract was filtered through two layered muslin cloth. The filtrate was transferred to centrifuge tube and centrifuged at 15,000 rpm for 15 min at 4°C in refrigerated centrifuge (Thermo scientific X1R). The supernatant was transferred to a test tube and stored in an ice bath and used for enzyme assay.

Enzyme Assay*:* GPOX activity was measured following the H_2O_2 dependent oxidation of guaiacol (extinction coefficient 26.6 $mM^{-1}cm^{-1}$) at 420 nm. Three ml assay mixture consisted of 2.86 ml 100 mM phosphate buffer (pH 7.8), 30 µL 1% guaiacol and 100 µl enzyme extract. The blank was prepared by adding 50 mM Tris-HCl (pH 7.5) to the reaction mixture instead of enzyme extract. All the components were mixed thoroughly and 12 µL of H_2O_2 was added to initiate the enzyme activity. Immediately after the addition of H_2O_2, the increase in absorbance due to oxidation of guaiacol was measured at 420

nm using a UV-VIS spectrophotometer (Systronics 2201) for 3 min at intervals of 30 sec. One unit of GPOX activity was defined as the amount of enzyme that caused the formation of 1 μM of tetraguaiacol per min.

3.8.4.2.3 Ascorbate peroxidase (APX, EC 1.11.1.11)

APX activity in the fresh samples was assayed by following the method of Nakano and Asada (1981).

Extraction*:* Leaf extraction for enzyme assay was prepared as per the protocol of Zhang and Kirkham (1996). Five hundred mg of fresh plant tissue was ground with a pre-chilled mortar and pestle in 10 ml of extraction medium. The extraction buffer consisted of 50 mM sodium phosphate buffer (pH 7.0), containing 0.33 M sorbitol, 1 mM $MgCl_2$, 2 mM EDTA, 10 mM NaCl, 0.5 mM KH_2PO_4 and 1 mM ascorbate. The homogenate was filtered through two layers of cheese cloth and centrifuged at 4^oC for 4 min at 2000 rpm. The pellet was discarded and the supernatant was centrifuged again at 5000 rpm for 15 min at 4^oC and the supernatant was saved. It was again centrifuged at 15000 rpm for 15 min at 4^oC and the resulting supernatant was used as the cytosolic fraction for APX assay.

Enzyme Assay*:* Cytosolic APX activity was assayed by monitoring the decrease in absorbance at 290 nm due to AsA oxidation. The 3 ml assay system consisted of 0.5 mM AsA, 0.1 mM EDTA in 50 mM sodium phosphate buffer (pH 7.0). Twenty μL of cytosolic enzyme extract was added to the buffer and the enzyme reaction was initiated by adding 10 μL of 100 mM H_2O_2 to reach a concentration of 0.1 mM H_2O_2 in the final reaction mixture. H_2O_2 dependent oxidation of AsA (ε=2.8 $mM^{-1}cm^{-1}$) was followed by monitoring the decrease in absorbance at 290 nm. One unit was defined as the amount of enzyme that oxidized one μmol of AsA min^{-1} at room temperature under the above conditions.

3.9 Foliar micromorphology and stomatal characteristics

The foliar micromorphology of rice seedlings was examined and the stomatal characteristics such as stomatal length and width of stomata on both abaxial and adaxial epidermis of the leaf blade were studied using a scanning electron microscopy **(**SEM). Modified method of Li et al. (2014) was primarily used for this microscopic technique. Samples of the middle region of young rice leaves treated with HL or UV-B were collected for SEM analyses. Leaf segments of control and treated plants were fixed in 2.5% gluteraldehyde, prepared in 0.1 M phosphate buffer (pH 7.4) for 12 h. Fixed specimens were washed twice with 0.1 M phosphate buffer and dehydrated by passing through an ascending acetone series. Ten minutes incubation time was provided in each acetone series. Dehydrated leaf samples were mounted on to grooves cut on aluminium stubs using double side adhesive conducting carbon tapes to expose the sections. Then the specimens were sputter coated with gold palladium and further photomicrographs were taken using the photographic attachment of the SEM (Make: JEOL Model JSM-6390LV, magnification: 5x to 300,000 x, probe current: 1 pA to 1 mA).

3.10 Ultrastructure of chloroplasts

The ultrastructure of the chloroplasts was observed as described by Wang et al. (2014) using transmission electron microscope (TEM). Samples of the middle region of young rice leaves treated with HL or UV-B radiation were collected for TEM analyses. Fresh leaf segments were cut into pieces of approximately 1-2 mm^2 and fixed in 2.5% gluteraldehyde, prepared in 0.1 M phosphate buffer (pH 7.4) for 12 h (primary fixation). Leaf samples were then washed in phosphate buffer (pH7.4) for two times and post-fixed in 1% osmium tetroxide prepared in 0.1 M phosphate buffer (pH 7.4) for 12 h at the room temperature (secondary fixation). The fixed leaf samples were washed twice with 0.1 M phosphate buffer and dehydrated in an ascending series of

acetone (50%, 60%, 70%, 80%, 90% and 100%; 10 min each). After dehydration, leaf samples were embedded in epoxy resin (Epon 812) and allowed to harden by heat treatments (50ºC for overnight, then at 60 ºC for two days), ultra-thin sections were cut using ultra microtome (Leica, UC6), double stained with uranium acetate and lead citrate in series, and examined under a Jeol/JEM 2100 TEM (Make: Jeol Ltd., USA, magnification 2000x-1500000x, voltage 200 kV).

3.11 Functional group analysis of cuticular wax deposition

Fourier transform infrared spectrometry (FT-IR) analysis was used to analyze the important functional group in the chloroform extract of cuticular wax deposition with the help of IR spectrometer.

Epicuticular wax extraction and wax content determination: For extraction of cuticular wax from the leaf blades of rice seedlings, the method of Walton (1990) was adopted with minor modifications. Fresh leaf samples were washed and 0.5 g of leaf tissue was weighed and cut into pieces. The leaves were immersed two times repeatedly for 30 s each in a test tube with 25 ml chloroform for extraction at room temperature. During each extraction, the solvent was agitated for 30 s by pumping with a Pasteur pipette. Wax extracts from leaves were pooled and were left to dry overnight under hood. After evaporation of the chloroform, the test tubes were weighed using precision balance (Sartorius, Germany). The wax content was calculated by subtracting the initial weight of the test tube from its final weight and expressed as mgg^{-1} dry weight of rice leaves. All extraction experiments were carried out as entirely independent triplicates, results are given as mean values.

Infra-red analysis of the epicuticular wax deposition: The waxy deposition, accurately weighed and it was mixed with potassium bromide powder to make a pellet at high pressure. The disk was placed in instrument beam to

measure the solid state spectrum in a FT-IR (JASCO-4100). Infra-red analysis of the wax extracts were recorded in the 400 to 4000 cm^{-1} range with 2 cm^{-1} resolution.

3.12 Analysis of xanthophyll cycle pigments

High performance liquid chromatography (HPLC) analysis of xanthophylls cycle pigments was done by the method of Gopalakrishnan and Annamalainathan (2016).

***Extraction*:** Fresh leaf samples were weighed (1 g) and processed immediately with liquid nitrogen. Samples were homogenized in 6 ml of 100% ice cold acetone containing 0.1% butylated hydroxytoluene (w/v) by using a pre-cold mortar and pestle. The homogenate was centrifuged for 25 min at 4^{o}C for 8000 rpm and the supernatant was collected. The pellets were re-extracted with 6 ml of acetone and the supernatant was pooled. The extracts were analyzed on the same day to reduce loss of pigments during analysis. All analytical procedures were carried out under dim light.

***Analysis*:** Identification and separation of pigments were done on a reverse phase column, Waters Spherisorb ODS-5 µm column (250x4.6 mm) in HPLC. Samples were injected with a Rheodyne 7010 injector, with a 20 µl loop at 30^{o}C for 60 min. The solvent system consisted of acetonitrile:methanol:water (solvent A, 84:14:2) and methanol:ethyl acetate (solvent B, 68:32). The mobile phases were pumped with a Waters 600 high pressure pump at a flow rate of 0.5 ml per min. The column was equilibrated with mobile phase prior to injecting each sample and the analysis done in gradient mode. The gradient used was 0-20.5 min in 100% solvent A, 20.5-25.5 min decreasing to 0% solvent A, 25.5-40 min 100% solvent B, 40.0-40.1 min increasing to 100% solvent A and finally from 40.1-60 min 100% solvent A. Peaks were detected at 450 nm with waters 996 photodiode array detector

and integrated using Empower software and peaks were identified using standard methods. Analysis was done in triplicates.

Standards of xanthophylls (lutein, zeaxanthin and β-carotene) from M/s. Sigma Aldrich were used for the study. Pigments were identified by comparing their absorption spectra and retention time with standards. Standard curves for quantification of pigments were made by plotting concentration against absorbance responses. Lutein, zeaxanthin and β-carotene were quantified according to their respective standards. The response factor of lutein standard was used for quantifying the pigments viz. neoxanthin, violaxanthin and antheraxanthin. The concentration of pigment was determined using linear regression and expressed as $\mu g g^{-1}$ fresh weight. Level of de-epoxidation and epoxidation state of xanthophyll cycle pigments was calculated as (Z + A)/(V + A + Z) and (V+A)/(V+A+Z), respectively.

Where, Z- zeaxanthin

A- antheraxanthin

V- violaxanthin

3.13 Statistical analysis

Statistical analyses were carried out according to Tukey's studentized range (HSD) test at 5% probability level. One-way ANOVA were applied using the SPSS software (Version 16.0, SPSS Inc., Chicago, USA) to analyze the consequences of HL and UV-B irradiation in rice varieties. The data is an average of recordings from three independent experiments each with three replicates (*i.e.* n=9). The data represent mean±standard error (SE).

RESULTS

4.I High intensity light stress

4.1.1 Determination of optimal growth period for imparting HL stress in rice seedlings

Determination of the optimal growth period for imparting HL stress in rice seedlings was analyzed by estimating shoot length, total chlorophyll content and Chl *a* fluorescence parameter Fv/Fm (maximum quantum yield of PSII) after 8, 9, 10, 11 and 12 d of germination. The shoot length in all rice varieties was increased from 8 d onwards until 12 d of germination. However, the rate of increase in shoot length was maximum on 10 d of germination in all rice varieties. The maximum growth in terms of total chlorophyll content was recorded on 10 d of germination in Aathira, Aiswarya, Annapoorna, Harsha, Jyothi, Karuna, Neeraja, Swarnaprabha, Swetha and Varsha. However, the maximum chlorophyll accumulation was observed in Mattatriveni, Mangalamahsuri and Kanchana on 9, 11 and 12 d of germination respectively. Similarly, the best growth period in terms of Fv/Fm was recorded on 10 d of germination in Aathira, Aiswarya, Annapoorna, Harsha, Neeraja, Swarnaprabha, Swetha and Varsha. However, the maximum Fv/Fm ratio was recorded on 11 d of germination in Jyothi and Karuna seedlings (Table 3).

4.1.2 Primary screening for identifying the sensitive and tolerant rice varieties towards HL exposure

4.1.2.1 Photosynthetic pigment composition

A significant increase in total chlorophyll content recorded in Aathira, Jyothi, Annapoorna, Aiswarya, Mattatriveni and Harsha when treated with different periods of HL exposure as compared to the control plants. Among

them, Aathira, Jyothi and Annapoorna showed maximum enhancement in total chlorophyll content upon 6 h of HL exposure (32-53%), and no further increase was observed thereafter at 8 h. In contrast, on imparting HL, the total chlorophyll content in Swetha, Karuna, Mangalamahsuri, Varsha, Swarnaprabha, Kanchana and Neeraja showed a decreasing pattern upon increasing the exposure period and the decrease was maximum at 8 h as compared to the control seedlings. Among them, Swarnaprabha, Kanchana and Neeraja recorded maximum reduction in total chlorophyll content (>40%) than other varieties (<30%) (Table 4).

Significant increase in the total carotenoid content was recorded in all thirteen rice varieties studied except in Kanchana where the carotenoid content was significantly decreased upon 6 (20%) and 8 h (42%) of HL exposure even though it was found to be enhanced after two initial treatment (2 and 4 h) of HL exposure as compared to the control plants. The carotenoid content was much higher in Aathira, Jyothi, Annapoorna, Aiswarya and Mattatriveni and the maximum accumulation was recorded after 8 h of HL exposure in Aathira (162%), Jyothi (167%), Annapoorna (195%) and Aiswarya (75%), whereas in Mattatriveni, the maximum carotenoid accumulation was recorded upon 6 h (71%) of HL exposure and beyond which the trend remains stable as compared to the control plants. Moreover, the rice varieties Harsha, Swetha, Karuna, Mangalamahsuri and Varsha exhibited an intermediate type with regard to carotenoid accumulation and the rate of enhancement was in the range of 30-50% with respect to the control plants. However, the increase in carotenoid content was not prominent in Swarnaprabha and Neeraja and the percentage of increase was much lower (<20%) as compared to other varieties and the rate of reduction was more upon 6 h of HL exposure (Table 4).

4.1.2.2 Fv/Fo

It was observed that the efficiency of the water-splitting complex on the donor side of PSII (Fv/Fo) was lowered with increase in period of exposure to HL in all thirteen rice varieties. By 8 h of exposure to HL stress, all rice varieties showed 32-60% reduction in Fv/Fo as compared to control plants. However, only a negligible decrease in Fv/Fo was recorded in the seedlings of Aathira, Jyothi, Annapoorna, Aiswarya and Mattatriveni (<15%) upon 2 h of HL exposure as compared to leaves of control plants. On 2 h of HL exposure, about 20-25% reduction in Fv/Fo was registered in rice varieties Harsha, Swetha, Karuna, Mangalamahsuri and Varsha as compared to control seedlings. On the other hand, after 2 h of HL exposure, the reduction in Fv/Fo was found to be more significant in Swarnaprabha, Kanchana and Neeraja (30, 37 and 49%, respectively) than other varieties (Table 5).

4.1.2.3 Malondialdehyde content

Significant increase in MDA content due to HL exposure of different time intervals was recorded in all thirteen rice varieties studied. Even though MDA content was increasing upon increasing the treatment period in Aathira, Jyothi, Annapoorna, Aiswarya and Mattatriveni and reached maximum after 8 h of HL exposure (<22%), the percentage of increase was lower than that recorded in other rice varieties. Significant increase in MDA content was noticed in the seedlings of Harsha, Swetha, Karuna and Varsha from 2 h onwards and the enhancement was more upon 8 h of HL treatments i.e., 87-97% increase of MDA content in Harsha, Swetha, Karuna and Varsha treated with 8 h of HL stress as compared to the control plants. The increase in MDA content of Mangalamahsuri was highest upon 6 h of HL exposure (48%) and thereafter the increase recorded was not significant. A sudden increase in MDA content was recorded in Swarnaprabha, Kanchana and Neeraja from 2 h

itself (50-73%) and reached maximum at 6 h of HL exposure in Kanchana, Swarnaprabha (120 and 150% respectively) and upon 8 h of HL exposure in Neeraja (202%) over the control plants (Table 5).

4.1.3 Secondary screening for identifying the sensitive and tolerant rice varieties towards HL exposure

4.1.3.1 Primary metabolites

4.1.3.1.1 Total protein

Treatment with HL stress for different time intervals (0-8 h) induced significant increase of total protein content and the increase was coinciding with increase in HL exposure period in all rice varieties. However compared to other varieties, HL exposure induced accumulation of higher protein content in Aathira and Jyothi and in these varieties accumulation of protein was recorded from the initial periods of HL stress application (2 h) and maximum protein content was recorded upon 8 h of HL exposure (145 and 85% in Aathira and Jyothi, respectively) over the untreated plants. Similarly HL treatment enhanced protein content in Annapoorna, Aiswarya and Mattatriveni as compared to the control plants; the maximum protein content was registered upon 8 h in Mattatriveni (10%) and upon 6 h of HL exposure in Annapoorna and Aiswarya (25-30%) as compared to the control seedlings. However in the case of Swarnaprabha, Kanchana and Neeraja, a decrease in the total protein content was recorded with increase in HL treatment period and maximum reduction was noticed at 8 h of HL exposure (13, 30 and 40% of reduction in Neeraja, Swarnaprabha and Kanchana respectively) as compared to the control seedlings (Fig. 1).

4.1.3.1.2 Total free amino acids

In general, total free amino acid content of all rice varieties was showing enhancement with the HL treatment period as compared to the control plants and maximum amino acid content was recorded in Aathira seedlings. A sharp increase in the accumulation of amino acid was recorded in HL treated Aathira from 2 h itself, i.e., about 80% increase over the control plants and maximum accumulation was recorded at 8 h of HL exposure (231%). In the seedlings of Jyothi, amino acid accumulation was found to be maximum at 6 h of HL exposure (72%) and thereafter no significant increase was observed. Amino acid content in Annapoorna, Aiswarya and Mattatriveni was showing an increasing pattern with the treatment period and the increase was maximum at 8 h of HL exposure, i.e., 85, 57 and 54% in Annapoorna, Aiswarya and Mattatriveni respectively over the control plants. However, the rate of increase in amino acid content was lower in Neeraja, Swarnaprabha and Kanchana with respect to the control plants. In Swarnaprabha and Kanchana, the maximum accumulation of total amino acids was recorded upon 8 h of HL exposure (20-22%) and in the case of Neeraja it was observed at 6 h (8%), and beyond which no further increase was recorded over the control plants (Fig. 2).

4.1.3.1.3 Total soluble sugar

Significant increase in the accumulation of soluble sugar content was recorded in all eight rice varieties subjected to different time intervals of HL exposure and the trend was more or less similar in all varieties. However, the sugar content was much higher in Aathira and Jyothi than other varieties as compared to control plants. The accumulation started from 2 h onwards up to 8 h of HL exposure, i.e., about 210 and 235% of increase in the sugar content was recorded upon 8 h in Aathira and Jyothi respectively over their control seedlings. In the plants treated with varying HL treatment periods, total sugar

content in Annapoorna, Aiswarya and Mattatriveni increased gradually from 2 h onwards upto 8 h as compared to untreated plants and the increase at 8 h was in the range of 130-166%. Compared to other varieties, Kanchana, Neeraja and Swarnaprabha showed accumulation of maximum sugar content at 6 h of HL exposure and the increase was 95, 110 and 150% respectively over their control plants and which further reduced to 63, 12 and 127% respectively upon 8 h of HL treatment over their control plants (Fig. 3).

4.1.3.1.4 Proline

Generally, the proline content of seedlings treated with HL stress of various time intervals did not vary much in most rice varieties. Significant increase in the accumulation of proline content was recorded in Aathira and Jyothi subjected to different periods of HL exposure, 96 and 40% of increase was recorded upon 8 h of HL exposure in the seedlings of Aathira and Jyothi respectively over their control plants. Whereas, the increase of proline content was lower in other six varieties as compared to the untreated plants. Moreover, a negligible increase in the accumulation of proline content was recorded in the seedlings of Annapoorna, Mattatriveni and Neeraja subjected to 4-8 h of HL treatment (4-12%) over the control plants. In contrast, other three rice varieties (Aiswarya, Swarnaprabha and Kanchana) exhibited a negligible decrease after HL treatment as compared to the control plants (Fig. 4).

4.1.3.2 Non enzymatic antioxidants

4.1.3.2.1 Ascorbate

HL stress of various exposing periods (0-8 h) imparted to eight rice varieties resulted in the increased accumulation of ascorbate when compared to the control plants. Treatment with HL resulted a significant increase in the ascorbate content even in the initial time period (2 h) of treatment in all rice

varieties studied except in Kanchana where it was slightly decreased (15%) as compared to control seedlings. A steep increase in the accumulation of ascorbate content was recorded in Jyothi and Aathira from 2 h (142 and 175%) onwards and maximum accumulation was recorded upon 8 h of HL treatment, i.e., 447 and 795% increase over their control seedlings. Likewise, ascorbate content increased progressively in Mattatriveni, Annapoorna and Aiswarya from 2 h onwards and maximum accumulation of ascorbate content was noticed after 6 h of HL exposure, i.e., 122, 160 and 202%, which further reduced to 90, 97 and 167% respectively upon 8 h of HL treatment over their control plants. Even though 90 and 185% increase in the accumulation of ascorbate content was recorded in Neeraja and Swarnaprbha subjected to 4 h of HL treatment over their untreated plants, no further increase was observed thereafter upto 8 h. However in the case of Kanchana, initial period of HL exposure (2 h) resulted in decreased ascorbate content and further there was gradual increase in ascorbate content. Maximum accumulation of ascorbate content in Kanchana was recorded after 6 h of HL exposure (30%), and beyond which no further increase was registered with respect to the control seedlings (Fig. 5).

4.1.3.2.2 Glutathione

Significant increase in the accumulation of glutathione content was recorded in all rice varieties subjected to HL stress of various treatment periods. A steep increase in the glutathione content was recorded in both Aathira and Jyothi right from 2 h of imparting HL stress and reached maximum upon 8 h of HL exposure (310 and 203% of increase in Aathira and Jyothi, respectively) over their control plants. Likewise, significant increase in glutathione content was recorded from 4 h onwards in Annapoorna, Aiswarya and Mattatriveni and maximum accumulation was observed upon 8 h of HL exposure in Aiswarya and Mattatriveni (57-60%) with respect to their

untreated plants. Unlike Aiswarya and Mattatriveni, maximum accumulation of glutathione was recorded upon 6 h of HL exposure in Annapoorna (55%) and beyond which no further increase was observed. Although, an increase in glutathione content was recorded in Swarnaprabha, Kanchana and Neeraja seedlings, but the rate of increase was not much significant like Aathira and Jyothi. The maximum rate of enhancement in glutathione content was recorded in Swarnaprabha and Kanchana at 6 h (31% for both varieties) and further no significant increase was noticed for both varieties. But in the case of Neeraja, maximum accumulation was noticed upon 4 h of HL exposure (12%) and beyond which no further increase was observed. Moreover, upon 8 h of treatment, significant reduction of glutathione content in Neeraja was observed in the seedlings over the control plants (Fig. 6).

4.1.3.2.3 Phenolics

Eight rice varieties subjected to different time intervals of HL stress exposure induced an increased accumulation of phenolic content over their control plants. Total phenolic content increased progressively with increasing period of HL stress and maximum accumulation was recorded in Aathira (171%), Jyothi (103%) and Mattatriveni (87%) seedlings after 8 h of HL exposure with respect to their control plants. Even though a 49% increase in the accumulation of phenolic content was recorded in the seedlings of Annapoorna subjected to 4 h of HL treatment over the control plants, no further significant increase was observed thereafter upto 8 h. In the seedlings of Aiswarya, maximum accumulation was noticed after 8 h (47%) of HL treatment with respect to the control plants. Similarly, in Swarnaprabha and Neeraja more or less similar percentage of increase (20-30%) was noticed after 6 h of HL exposure, and beyond which no further increase was noticed. In contrast, HL exposure resulted in a significant reduction of the phenolic content in Kanchana seedlings and the maximum reduction of 14% was

recorded upon severe stress of HL exposure (8 h) with respect to the control plants (Fig. 7).

4.1.4 Tertiary screening for identifying the most tolerant and sensitive rice varieties towards HL exposure

4.1.4.1 PSI activity

PSI activity after various time periods of HL treatment exhibited a gradual reduction in the leaves of Jyothi, Swarnaprabha, Kanchana and Neeraja. However, a gradual increase in PSI activity was recorded in the leaves of Aathira exposed to various time periods of HL and the maximum activity was recorded on 6 and 8 h of HL treatment, i.e., 29% higher than control leaves. The maximum reduction of PSI activity (77%) was recorded in seedlings of Neeraja exposed to 8 h of HL as compared to the control plants. Likewise, in HL exposed seedlings of Jyothi and Swarnaprabha, the PSI activity tend to be decreased and maximum reduction was recorded after 8 h of HL exposure (27 and 50%, respectively) with respect to their untreated leaves. Similarly, significant decrease in PSI activity was recorded from 2 h onwards in Kanchana and maximum rate of reduction was observed after 6 h of HL exposure (44%) and beyond which no further decrease was recorded with respect to the control plants (Fig. 8).

4.1.4.2 PSII activity

Significant increase in PSII activity of Aathira and Jyothi was recorded upon 2 h of HL exposure (48 and 18%, respectively) over the control plants. Further, a gradual decrease in the PSII activity was observed in HL exposed seedlings of these two rice varieties and exhibited the maximum reduction upon 6-8 h (16 and 44%, respectively) of HL stress. PSII activity of Swarnaprabha, Kanchana and Neeraja was decreasing upon increasing the HL

exposing period and maximum reduction (63-73%) was recorded after 8 h of HL treatment (Fig. 9).

4.1.4.3 Mitochondrial activity

Activity of mitochondria in Aathira was showing an increasing trend upon 2 and 4 h of HL treatment and maximum activity was recorded upon 2 h (50%) and further it was decreased upon 6 and 8 h of HL exposure as compared to the control seedlings. Compared to other varieties, the rate of mitochondrial activity was found much lower in Swarnaprabha and Kanchana from the initial period (2 h) onwards and the trend remained same throughout the entire treatment period (2-8 h). The percentage of decrease in the mitochondrial activity was more at 8 h, i.e., about 87 and 71% decrease in Swarnaprabha and Kanchana respectively over the control plants. However, in the case of Jyothi and Neeraja, the mitochondrial activity exhibited a decreasing trend from the initial period (2 h) onwards and maximum reduction was recorded upon 6 h (43 and 60%, respectively) of treatment (Fig. 10).

4.1.4.4 Enzymatic antioxidants

4.1.4.4.1 Superoxide dismutase (SOD, EC 1.15.1.1)

A steep increase in the activity of SOD was recorded in Aathira, Jyothi, Kanchana and Neeraja throughout the HL treatment period (0-8 h). However, the maximum rate of enhancement in SOD activity was observed in Aathira upon 8 h and Jyothi upon 6 h (445-460%) of HL treatment over the control plants. Likewise, significant increase in SOD activity was recorded in Kanchana and Neeraja from 2 h onwards and the increase was maximum (150 and 192%, respectively) upon 8 h of HL treatment. Even though a 40% increase in SOD activity upon 2 h of HL exposure was recorded in Swarnaprabha seedlings over the control plants, a steep decrease was

observed from 4 h onwards and maximum rate of reduction (30%) was noticed at 8 h of HL exposure as compared to the control plants (Fig. 11).

4.1.4.4.2 Guaiacol peroxidase (GPOX, EC 1.11.1.7)

In general GPOX activity of rice varieties treated with HL stress for 0-8 h showed an enhanced activity profile over their control plants. Among all the varieties studied, Jyothi exhibited maximum GPOX activity (830%) and Swarnaprabha showed less GPOX activity (78%) after 8 h of exposure as compared to their control plants. Unlike other varieties, in Jyothi treated with HL stress, a sudden increase in GPOX activity was recorded in 6 h (542%) from 4 h (55%) of HL exposure. Likewise in the seedlings of Aathira, Kanchana and Neeraja maximum GPOX activity was recorded on 6 h of treatment i.e., an increase of 363, 230 and 86% in Aathira, Kanchana and Neeraja respectively over the control plants (Fig. 12).

4.1.4.4.3 Ascorbate peroxidase (APX, EC 1.11.1.11)

On imparting HL stress, the activity of APX was also significantly increased in all five rice varieties like other antioxidant enzymes studied. The activity of APX was significantly enhanced in Aathira and Jyothi upon increasing the treatment period and maximum activity (740 and 615% respectively) was noticed at 8 h of HL exposure. Similarly, HL treatment enhanced APX activity in Kanchana and Neeraja from 2 h onwards and maximum activity was recorded in Neeraja (414%) and Kanchana (330%) after 8 h of treatment over the control plants. The least APX activity was recorded in Swarnaprabha as compared to other varieties and maximum activity observed after 6 h of HL treatment (88%) (Fig. 13).

4.1.5 Final analysis of the most tolerant and sensitive rice varieties towards HL exposure

4.1.5.1 Chl *a* fluorescence parameters

Various Chl *a* fluorescence parameters were analyzed in the leaves of Aathira and Swarnaprabha to study the effect of different time intervals (0-8 h) of HL treatment on the PSII photochemistry. Pronounced changes were observed in the Chl *a* fluorescence parameters of both rice varieties as compared to the control seedlings. Exposure to different periods of HL stress reduced the plant vitality as assessed by performance index calculated on absorption basis [$PI_{(abs)}$] in both rice varieties. However, the rate of decrease of $PI_{(abs)}$ was faster and higher in Swarnaprabha leaves at 2 h of HL exposure (97%) and there was no further decrease with increase in HL exposure period as compared to the control leaves. Whereas, the rate of decrease in $PI_{(abs)}$ of Aathira was only 27% upon 6 h of HL exposure as compared to the control leaves, beyond which the decrease was not significant. In the case of maximum fluorescence (Fm), Aathira and Swarnaprabha seedlings treated with 8 h of HL stress showed a reduction of 10 and 55% respectively over the control plants. $SFI_{(abs)}$, 'structure-function-index' or vitality index refers to structural and functional PSII events leading to electron transport within photosynthesis. An increase in the duration of HL exposure causes a significant decrease in the $SFI_{(abs)}$ in both varieties studied and the maximum percentage of reduction was recorded on 6 h of HL treatment and further it decreased significantly i.e., about 67 and 90% in Aathira and Swarnaprabha respectively over the control plants (Fig. 14).

Treatment with HL resulted in significant increase in the relative variable fluorescence at J step (Vj) even in the initial time period (2 h) of treatment in both Aathira and Swarnaprabha as compared to control seedlings. With severity of HL (8 h) exposure, the rate of increase in Vj reached up to 53

and 93% in Aathira and Swarnaprabha leaves respectively. Area above the fluorescence curve was found to be lowering upon increasing HL exposing period and the decrease was found to be more significant in Swarnaprabha as compared to Aathira seedlings. The maximum rate of reduction was noticed in Swarnaprabha at 2 h (52%), whereas in Aathira after 4 h (45%) of HL exposure and thereafter no further decrease was noticed (Fig. 14).

Likewise, the electron transport quantum yield (Φ_{Eo}) and the yield of electron transport per trapped exciton (Ψ_o) were significantly decreased and it was predominant in Swarnaprabha as compared to Aathira leaves. In HL exposed seedlings of Swarnaprabha, the maximum reduction in Φ_{Eo} was recorded after 8 h (89%) and the decrease in Ψ_o was maximum after 6 h of HL exposure (61%) with respect to their untreated leaves. Similarly, maximum rate of reduction in Φ_{Eo} and Ψ_o of Aathira was observed after 6 h of HL exposure (64% and 34%, respectively) and beyond which no further decrease was recorded with respect to the control plants. The parameter Tf(max) which is used to indicate the time when maximum fluorescence value (Fm) is reached and it was decreased in both varieties over the control leaves. However, a minor increase (8%) in Tf(max) was observed at 8 h of HL exposure in Aathira as compared to the control plants (Fig. 14).

The energy pipeline leaf model of the photosynthetic apparatus was used to visualize the phenomenological energy fluxes per cross section of PSII upon HL exposure of varying periods in Aathira and Swarnaprabha leaves (Fig. 9). A maximum decrease in electron transport per cross section (ETo/CSo) was recorded after exposure with 8 h of HL exposure and this decrease was to the extent of 15 and 85% in Aathira and Swarnaprabha respectively with respect to their control leaves. However, the energy absorbed per excited cross section (ABS/CSo) and the ratio of total dissipation per cross section (DIo/CSo) was increased with increasing HL

exposure period (0-8 h) in both rice varieties. In Aathira, maximum rate of enhancement in ABS/CSo and DIo/CSo was recorded at 6 h of HL exposure (15 and 60%, respectively) over their untreated plants. Likewise, the rate of increase in the parameters ABS/CSo and DIo/CSo was maximum at 8 h of HL exposure in Swarnaprabha (30 and 120% respectively) seedlings. However, there was no significant change in trapping flux (TRo/CSo) during HL treatment in both rice varieties. Moreover, the density of the active reaction centers (RC/CSo) were highly decreased in Swarnaprabha after 6 h (50%) of HL exposure than that recorded in Aathira (2%) as compared to their control leaves (Fig. 15).

An alteration of PSII energy fluxes per reaction center (RC) of PSII in response to HL treatment in rice varieties was visualized by specific membrane models of photosynthetic apparatus. It was found that the flux of absorption (ABS/RC) was increased in Aathira and maximum enhancement was noticed at 8 h (10%), but it was decreased in Swarnaprabha and maximum reduction was recorded at 6 h of HL exposure (43%) and beyond which no significant decrease was observed with respect to the control plants. However, the trapping per reaction center (RC) of PSII, symbolized as TR_o/RC, was lowered in Swarnaprabha with increase in high light exposing period and it did not change significantly in Aathira leaves. Likewise, the electron transport flux (ET_o/RC) decreased over the control plants in both varieties and maximum reduction was recorded at 8 h (25 and 90% in Aathira and Swarnaprabha, respectively) of HL exposure. Moreover, dissipated energy (DI_o/RC) increased over the control plants in both rice varieties and it was increased to the extent of 32 and 192% in Aathira and Swarnaprabha leaves respectively with 8 h of exposure to HL as compared to the control plants (Fig. 16).

Exposure to different time intervals of HL stress increased the NPQ in both rice varieties. However, the rate of increase in NPQ was faster and higher in Aathira leaves from 2 h onwards and maximum rate of enhancement was observed after 6 h of HL exposure (105%) and beyond which no further increase was recorded with respect to the control plants. Whereas, the maximum rate of increase in NPQ of Swarnaprabha was recorded upon 8 h of HL exposure (72%) as compared to their control leaves (Fig. 17A).

4.1.5.2 Leaf gas exchange parameters

4.1.5.2.1 Net photosynthetic rate (P_n)

Treatment with HL stress for different time intervals (0-8 h) significantly decreased the P_n in both rice varieties over the control plants. However, the rate of reduction of P_n was more prominent in Swarnaprabha than Aathira seedlings upon treatment with HL stress as compared to the control leaves. In Aathira, the rate of reduction in P_n was maximum upon 8 h (29%), whereas in Swarnaprabha, the maximum reduction was recorded upon 6 h (59%) of HL exposure as compared to the control plants (Fig. 17B).

4.1.5.2.2 Stomatal conductance (g_s)

Significant decrease in g_s was recorded in both Aathira and Swarnaprabha leaves when they were subjected to various time periods of HL treatment as compared to the control leaves. A gradual decrease was observed in g_s from 2 h onwards and maximum decrease in g_s was observed after 6 h of HL exposure in both rice varieties (30 and 63% in Aathira and Swarnaprabha, respectively) and beyond which no further decrease was recorded with respect to the control plants (Fig. 17C).

4.1.5.2.3 Transpiration rate (E)

In HL exposed seedlings of Aathira and Swarnaprabha, the parameter E tend to be decreased upon increasing the HL exposing period with respect to the control plants. In these two varieties, upon 2 h of HL treatment period, E was found to decrease (5 and 13% in Aathira and Swarnaprabha, respectively) and maximum reduction was recorded after 8 h of HL stress (48 and 81% in in Aathira and Swarnaprabha, respectively) with respect to the control leaves (Fig. 17D).

4.1.5.3 Anthocyanin content

Significant increase in the anthocyanin content was recorded in Aathira and Swarnaprabha, on being subjected to different time intervals of HL treatment from 2 h onwards. In Aathira seedlings subjected to HL stress, the anthocyanin content tends to increase upto 6 h (165%) and beyond which no further increase was recorded with respect to the untreated plants. In the case of Swarnaprabha seedlings, the increase in anthocyanin content was found to be highest upon 4 h (40%) and thereafter the increase was not significant. However, at 8 h of HL treatment, the anthocyanin content was decreased significantly (27%) in Swarnaprabha leaves as compared to the control leaves (Fig. 18A).

4.1.5.4 Flavonoid content

HL exposure induced significant increase of total flavonoid content in both Aathira and Swarnaprabha seedlings. However compared to Swarnaprabha, Aathira showed higher accumulation of flavonoid content upon treatment with HL stress as compared to the control leaves. In Aathira, a sharp increase in the accumulation of flavonoids was recorded from the 2 h itself, i.e., 55% increase and maximum accumulation was recorded upon 8 h (210%) of HL treatment over the control plants. Even though a negligible

decrease in flavonoid content was recorded at 2 h of HL treatment in the leaves of Swarnaprabha, the accumulation was found to be maximum upon 8 h (50%) of HL exposure as compared to the control plants (Fig. 18B).

4.1.5.5 PAL activity

The activity of PAL enhanced in the seedlings of Aathira and Swarnaprabha when exposed to HL treatment for a time period of 8 h and the increase was significant in both varieties from the initial period of stress (2 h). In the case of Aathira subjected to HL treatment, about 90% increase in the PAL activity was recorded upon 8 h of HL exposure over that of the control plants. But in Swarnaprabha only 33% increases was recorded over the control leaves upon 6 h of HL treatment and no further increase was recorded beyond 6 h (Fig. 18C).

4.1.5.6 Foliar micromorphology and stomatal characteristics

In general, the SEM images of epidermal cells of both abaxial and adaxial surface of Aathira and Swarnaprabha leaves showed that the stomata consisted of homogeneous guard cells. Stomatal pores were quite evident in the control leaves of both varieties in both surfaces, while the stomata of the rice leaves of both varieties subjected to 8 h of HL stress showed several abnormalities, sometimes without ostiole, or very small in size. However, the stomata on the leaves of Swarnaprabha treated with HL stress were more closed in number and damaged than those of Aathira leaves. Moreover, SEM images of epidermal cells of the leaf surfaces showed some deposition of cuticular waxes and it was more pronounced in the adaxial surfaces of Aathira leaves as compared to Swarnaprabha leaves (Fig. 19-22).

Table 6: Stomatal length and width (µm) on the adaxial and abaxial epidermis of Aathira and Swarnaprabha leaves after exposure to 8 h of HL treatment (2000 µmolm^{-2}s^{-1}). Values given are mean of 3 independent experiments, each with a minimum of 3 replicates (*i.e.* n=9)±S.E.

Rice variety	HL stress (h)	Adaxial epidermis		Abaxial epidermis	
		Stomatal length (µm)	Stomatal width (µm)	Stomatal length (µm)	Stomatal width (µm)
Aathira	0	28.91±1.11	7.29±0.25	26.89±1.15	11.21±0.52
	8	24.44±1.21	6.1±0.13	25.99±0.96	10.21±0.57
Swarnaprabha	0	23.48±1.12	6.49±0.17	24.58±0.78	8.91±0.34
	8	18.49±0.56	4.74±0.12	20.3±0.89	7.2±0.31

The stomatal length and width of the adaxial and abaxial epidermis of both rice varieties were obviously lowered after exposure to 8 h of HL stress. However, the rate of reduction in stomatal length and width was higher in Swarnaprabha as compared to Aathira leaves. HL exposure lowered the stomatal length of adaxial and abaxial epidermis in Aathira (15 and 3%, respectively) and Swarnaprabha seedlings (21 and 17%, respectively). Likewise, the rate of reduction in stomatal width of both adaxial and abaxial epidermis of Aathira was lower (16 and 9%, respectively) than Swarnaprabha leaves (26 and 19%, respectively) (Table 6).

4.1.5.7 Chloroplast ultrastructure

TEM images of ultrathin sections from untreated leaves of Aathira and Swarnaprabha varieties revealed that the chloroplasts exhibited normal ultrastructure; most were of lens-like ellipsoid in shape, with a typical close arrangement of granal and stromal thylakoids (Fig. 23A & C). In contrast, chloroplasts from the 8 h of HL treated leaves showed remarkable differences, with the chloroplast appearing to be swollen and exhibiting a round shape. The alteration in shape of chloroplasts to form round shape was observed very

well in Swarnaprabha leaves (Fig. 23D); however the change in shape of chloroplast in Aathira leaves was not severe like Swarnaprabha leaves (Fig. 23B). In addition to this, the chloroplast membranes and thylakoids were damaged with a loss of grana stacking after 8 h of HL stress in rice seedlings. It was observed that the internal lamellar system including lamellae of stromal thylakoids and stacked granal thylakoids were damaged in Aathira and Swarnaprabha leaves after exposure to 8 h of HL stress (Fig. 24).

4.1.5.8 Cuticular wax content and functional group anlysis of the epicuticular wax deposition

The treatment with long term HL exposure (8 h) resulted in an enhanced accumulation of epicuticular wax deposition in both Aathira and Swarnaprabha leaves as compared to their control leaves. The average wax content was 1.17 and 0.66 mgg^{-1} FW in the control seedlings of Aathira and Swarnaprabha, respectively. Under HL exposure of 8 h treatment, the rate of increase in the wax content of Aathira (1.66 mgg^{-1} FW) and Swarnaprabha (0.92 mgg^{-1} FW) leaves were more or less similar (39-42%) as compared to their control plants (Table 7).

Table 7: Epicuticular wax content (mgg^{-1} FW) extracted from Aathira and Swarnaprabha leaves after exposure to 8 h of HL (2000 $\mu molm^{-2}s^{-1}$) treatment. Values given are mean of 3 independent experiments, each with a minimum of 3 replicates (*i.e.* n=9)±S.E.

HL stress (h)	Aathira	Swarnaprabha
0	1.17±0.18	0.66±0.01
8	1.66±0.19	0.92±0.11

In rice seedlings, the major component of the leaf surface epicuticular waxes of both rice varieties are primary alcohols as calculated from the IR spectra, i.e., the absorbance of the main peak at 3600-3000 cm^{-1} corresponds

to hydroxyl group. FT-IR spectra of epicuticular wax in untreated Aathira leaves showed six important peaks at 3455, 2963, 2850, 1631, 1223 and 776 cm^{-1} which corresponds to hydroxyl group, symmetrical and asymmetrical stretching of methyl groups, C–H alkanes, C=C alkenes, amide C–N stretching and C–H alkenes, respectively. On imparting 8 h of HL treatment in Aathira, a strong peak appeared at 2924 cm^{-1}, corresponding to symmetrical and asymmetrical stretching of methylene groups, as compared to control leaves. Likewise, the epicuticular wax of untreated Swarnaprabha showed five peaks at 3455, 2963, 2358, 1220 and 772 cm^{-1} which corresponds to hydroxyl group, symmetrical and asymmetrical stretching of methyl groups, C–H stretching, amide C–N stretching and C–H alkenes, respectively. However, on imparting 8 h of HL treatment in Swarnaprabha resulted in disappearance of peak at 2358 which corresponds to C–H stretching, as compared to the control plants (Fig. 25).

4.1.5.9 Interconversions of xanthophyll pigments

The xanthophyll pigment profile analyzed by HPLC showed significant difference between Aathira and Swarnaprabha leaves after exposure to 8 h of HL irradiation. Fig. 26 represents HPLC elution profile of the photosynthetic xanthophyll pigments extracted from rice seedlings. Apart from the major fractions of the xanthophylls pigments, including lutein, some other carotenoids - α and β carotenes could also be detected. Neoxanthin (N) was eluted first followed by violaxanthin (V), antheraxanthin (A), lutein (L), zeaxanthin (Z), Chl *b*, Chl *a*, α and β carotenes. It was found that the content of pigments N, A and Z registered a steady increase after exposure to 8 h of HL stress in both rice varieties. In contrast, the content of V was found to decrease after HL treatment in both rice varieties as compared to the control leaves. It was showed that the Z content was not traceable in control seedlings of Aathira and Swarnaprabha leaves, while it was increased significantly after

exposure to 8 h of HL treatment and reached to an extent of 7.7 μgg^{-1} FW in both Aathira and Swarnaprabha leaves. Likewise, the content of A was also enhanced to an extent of 2.3 μgg^{-1} FW in Aathira leaves after 8 h of HL exposure, but A content was not detected in untreated leaves of this variety. In Swarnaprabha leaves, A was found to increase from 1.3 μgg^{-1} FW (control) to 2.4 μgg^{-1} FW after 8 h of HL treatment (Fig. 27).

A prominent reduction of lutein content in Aathira and Swarnaprabha was recorded after 8 h of HL (55 and 60%) with respect to their untreated leaves. The content of N was enhanced in Aathira seedlings (35%) after exposure to 8 h of HL treatment, but not in Swarnaprabha as compared to the respective control seedlings. Likewise, a significant variation in both α and β carotene content was registered after HL stress in both rice varieties. A significant increase in the accumulation of β-carotene content was registered in the leaves of Aathira (170%) and the increase in Swarnaprabha leaves was negligible (12%) upon 8 h of HL treatment over the control plants. Moreover, α-carotene was also enhanced in Aathira leaves, whereas in Swarnaprabha it was not increased after treatment with HL exposure (Fig. 28A).

The de-epoxidation state of xanthophyll cycle pigments (DEPS=Z+A/V+A+Z) generally exhibited higher values in HL treated Aathira and Swarnaprabha seedlings (37 and 33%) as compared with controls. In contrast, a significant decrease was found in the epoxidation state of xanthophyll cycle pigments (EPS=V+A/V+A+Z) after exposure to HL irradiation in both rice seedlings and the rate of decrease was more or less similar in both varieties (23-28%). Parallel to this, the proportion of the xanthophyll cycle pool (V+A+Z) to total Chl and carotenoids became more significant in rice seedlings upon HL exposure. The ratio of xanthophyll cycle pool to total Chl (*a*+*b*) was highly increased in Aathira (983%) and to a lesser extent in Swarnaprabha leaves (54%) after exposure to 8 h of HL stress over

the control leaves. However, the ratio of xanthophyll cycle pool to total carotenoids was decreased in both Aathira and Swarnaprabha leaves after 8 h of HL exposure with respect to the control seedlings (Fig. 28B).

4.2. UV-B stress

4.2.1 Primary screening for identifying the sensitive and tolerant rice varieties towards UV-B irradiation

4.2.1.1 Morphological parameters

Various doses of UV-B treatments resulted in a decrease of shoot length in all rice varieties studied, but the lower dose of UV-B (7 $kJm^{-2}d^{-1}$) exhibited a negligible increase in shoot length of Aathira, Mangalamahsuri, Kanchana, Jyothi and Annapoorna (<5%) as compared to the control plants. In the seedlings of Aathira, Mangalamahsuri, Kanchana, Jyothi and Annapoorna, maximum reduction of shoot length was noticed at high dose of UV-B exposure (28 $kJm^{-2}d^{-1}$). However, Mattatriveni, Karuna, Harsha and Varsha exhibited a reduction in shoot length which was in the range of 15-25% with respect to the untreated control plants. Moreover, Neeraja, Swetha, Aiswarya and Swarnaprabha showed maximum reduction in the shoot length of seedlings when subjected to various doses of UV-B radiation (>40% with respect to the control plants) with maximum decrease observed at 21 $kJm^{-2}d^{-1}$, with no further decrease observed thereafter at 28 $kJm^{-2}d^{-1}$ (Table 8, Fig. 29A-M).

The leaves of both Aiswarya and Swarnaprabha seedlings showed necrotic spots of yellow to brown spots and stripes on the leaf lamina after exposure to 21 and 28 $kJm^{-2}d^{-1}$ of UV-B radiation. In contrast, no visible UV-B damage symptoms were observed in other varieties of rice seedlings (Fig. 23N-Q).

4.2.1.2 Fresh weight

After imparting the various doses of UV-B, the fresh weight of seedlings in all thirteen rice varieties declined and the least reduction was found after 21-28 $kJm^{-2}d^{-1}$ of UV-B exposure in Aathira, Mangalamahsuri, Jyothi, Kanchana and Annapoorna (11-19%) with respect to the control plants. In the case of UV-B (28 $kJm^{-2}d^{-1}$) treated seedlings of Mattatriveni, Karuna, Harsha and Varsha, a reduction of 38-55% was recorded with respect to the control plants. Moreover, among thirteen rice varieties, the decrease in percentage of fresh weight was highest in Neeraja, Swetha, Aiswarya and Swarnaprabha (60-75%) and showed maximum reduction at 21 $kJm^{-2}d^{-1}$ with respect to the control plants (Table 8).

4.2.1.3 Photosynthetic pigment composition

Total chlorophyll content recorded significant increase on imparting low dose (7 $kJm^{-2}d^{-1}$) of UV-B in Kanchana, Jyothi and Annapoorna followed by a gradual decrease over the control plants upto 28 $kJm^{-2}d^{-1}$. During the higher dose of UV-B irradiation (28 $kJm^{-2}d^{-1}$) these varieties exhibited 4-20% reduction in total chlorophyll content. Unlike these three varieties, the other ten rice varieties showed a decreasing pattern from the initial dose onwards upto 28 $kJm^{-2}d^{-1}$ of UV-B treatment. Out of these ten varieties, in Aathira and Mangalamahsuri, the percentage of reduction in total chlorophyll content was much lower (<25%) as compared to control leaves and the rate of reduction was higher at 21-28 $kJm^{-2}d^{-1}$ of UV-B. Significant decline of chlorophyll content was also registered in UV-B treated leaves of Mattatriveni, Karuna, Harsha and Varsha and it was 39, 32, 60 and 62% respectively upon exposure to 28 $kJm^{-2}d^{-1}$ of UV-B as compared to control plants. The rate of reduction in chlorophyll content was much more in Neeraja, Swetha, Aiswarya and Swarnaprabha (76-90%) after exposure to 21-28 $kJm^{-2}d^{-1}$ of UV-B and the

decline was initiated from 7 $kJm^{-2}d^{-1}$ onwards with respect to the control plants (Table 9).

Unlike total chlorophyll content, the carotenoid content was showing a different pattern of accumulation under various doses of UV-B treatments and the carotenoid accumulation was initiated in plants exposed to UV-B dosage of 7 $kJm^{-2}d^{-1}$ onwards. In most of the rice varieties treated with UV-B, carotenoid content was showing an increasing pattern upon increasing the treatment dosage when compared to the control plants. A sharp increase in the accumulation of carotenoids was recorded in seedlings of UV-B irradiated Aathira, Annapoorna, Mangalamahsuri, Kanchana and Jyothi from 7 $kJm^{-2}d^{-1}$ itself and maximum accumulation was noticed at 21-28 $kJm^{-2}d^{-1}$ of UV-B irradiation, i.e., 355, 371, 508, 168 and 196% increase respectively over the control plants. Similarly the carotenoid accumulation was also noticed in Mattatriveni, Karuna, Harsha and Varsha and it was about 25-50% after exposure to UV-B dosage of 21-28 $kJm^{-2}d^{-1}$ over the untreated plants. However, in rice seedlings of Swetha, Aiswarya, Neeraja and Swarnaprabha, the carotenoid accumulation was significantly very less (<10%) and it was noticed upon 14-21 $kJm^{-2}d^{-1}$ of UV-B treatment and beyond which it decreased significantly (Table 9).

4.2.1.4 Malondialdehyde content

Under various doses of UV-B exposure, the significant increase in the rate of lipid peroxidation as assessed by MDA content was recorded in all thirteen rice varieties studied. The increase of MDA content was lower in Aathira, Mangalamahsuri, Kanchana, Jyothi and Annapoorna (<25%) as compared to the control plants and the maximum increase was recorded after 21-28 $kJm^{-2}d^{-1}$ of UV-B application. Moreover, MDA content was increased in seedlings of Aathira and Mangalamahsuri when irradiated with lower dose of UV-B (7 $kJm^{-2}d^{-1}$) to the extent of 25% as compared to control plants.

Likewise in Mattatriveni, Karuna, Harsha and Varsha, the MDA content was also showing an increasing pattern with increase in UV-B dose and the increase was found maximum at 21-28 $kJm^{-2}d^{-1}$ of UV-B treatment (80-98%) over the control seedlings. Significant increase in MDA content was recorded in rice seedlings of Neeraja, Swetha, Aiswarya and Swarnaprabha in the low dose (7 $kJm^{-2}d^{-1}$) of UV-B treatments and maximum MDA content was recorded after 21 $kJm^{-2}d^{-1}$ of treatment, an increase of 199, 164, 450 and 229%, respectively over the control plants and beyond which the trend remains stable (Table 9).

4.2.2 Secondary screening for identifying the sensitive and tolerant rice varieties towards UV-B irradiation

4.2.2.1 Primary metabolites

4.2.2.1.1 Total protein

Significant increase in the total protein content was recorded in all nine rice varieties studied on being subjected to various doses of UV-B. In Aathira, Mangalamahsuri and Kanchana subjected to various doses of UV-B, a sharp increase in the total protein content was recorded from 7 $kJm^{-2}d^{-1}$ (108, 80 and 26%, respectively) onwards and the trend was constant upto 28 $kJm^{-2}d^{-1}$ (172, 132 and 128%) as compared to the control plants. A lower level of increase in total protein content (30-72%) was recorded in Annapoorna, Neeraja, Swetha and Jyothi seedlings after 21-28 $kJm^{-2}d^{-1}$ over the control plants. A still lower level of total protein content of Aiswarya and Swarnaprabha seedlings was found at UV-B dosage of 21 $kJm^{-2}d^{-1}$ (17%) and thereafter the increase recorded was very negligible (Fig. 30).

4.2.2.1.2 Total free amino acids

Treatment with various doses of UV-B (0-28 $kJm^{-2}d^{-1}$) induced significant increase of total free amino acids in all rice varieties studied and the percentage of increase was different in all rice varieties. Among all rice varieties studied, maximum amino acid content was recorded in Aathira, Mangalamahsuri and Kanchana after 28 $kJm^{-2}d^{-1}$ of UV-B irradiation over the control plants. A considerable increase in the accumulation of total free amino acids was recorded in plants subjected to 7 $kJm^{-2}d^{-1}$ of UV-B (45, 10 and 22% in Aathira, Mangalamahsuri and Kanchana, respectively) and maximum accumulation of total free amino acids was recorded after 28 $kJm^{-2}d^{-1}$ of UV-B treatment, i.e., 196, 186 and 140% in Aathira, Mangalamahsuri and Kanchana respectively over the control plants. Likewise in Jyothi, Annapoorna, Neeraja and Swetha, the total free amino acid content increased gradually from 7 $kJm^{-2}d^{-1}$ of UV-B stress (13-20%) and the increase was found maximum at 28 $kJm^{-2}d^{-1}$ of treatment (86-93%) over the control seedlings. Even though 32 and 19% increase in the accumulation of total free amino acid content was recorded in the seedlings of Aiswarya and Swarnaprabha subjected to 7 $kJm^{-2}d^{-1}$ of UV-B treatment over the control plants, no further increase was observed thereafter upto 28 $kJm^{-2}d^{-1}$ of UV-B treatment (Fig. 31).

4.2.2.1.3 Soluble sugar

Significant increase in the soluble sugar content was noticed upon various dose of UV-B irradiation in all rice varieties studied and the percentage of increase was more prominent in Aathira, Mangalamahsuri and Kanchana (230, 253 and 288%, respectively) after 21-28 $kJm^{-2}d^{-1}$ of UV-B irradiation as compared to other varieties. The varieties Neeraja, Swetha and Jyothi exhibited an increase of soluble sugar content upto 180, 175 and 97% respectively as compared to the control plants at 14-28 $kJm^{-2}d^{-1}$ of UV-B

exposure and it was in an intermediate range as compared to the varieties described above. However, soluble sugar content recorded in Annapoorna showed a significant decrease in the two initial doses (7 and 14 $kJm^{-2}d^{-1}$) of UV-B followed by a gradual increase over the control plants upto 28 $kJm^{-2}d^{-1}$ (144%). However, soluble sugar content of Aiswarya and Swarnaprabha did not vary much during the entire treatment period. On imparting lower dose of UV-B (7 $kJm^{-2}d^{-1}$), significant decrease was recorded in the soluble sugar content followed by a gradual increase over the control plants upto 21 $kJm^{-2}d^{-1}$ (43 and 37%, respectively). During the higher dose (28 $kJm^{-2}d^{-1}$) of UV-B exposure, there was no further increase of soluble sugar content in these two varieties (Fig. 32).

4.2.2.1.4 Proline

In general proline content of all nine rice varieties was enhanced significantly with various doses of UV-B and the increase was most prominent in Aathira, Mangalamahsuri and Kanchana as compared to other rice varieties. However compared to Mangalamahsuri and Kanchana, Aathira seedlings showed higher accumulation of proline content after exposure to 28 $kJm^{-2}d^{-1}$ of UV-B treatment (212%) over the control plants. In Mangalamahsuri and Kanchana also, the maximum proline accumulation was recorded after higher dose of UV-B treatment (28 $kJm^{-2}d^{-1}$) and it was 105 and 116%, respectively. Moreover, an increase in proline content was recorded in Annapoorna, Neeraja, Swetha and Jyothi subjected to UV-B treatment and reached maximum at a dose of 21-28 $kJm^{-2}d^{-1}$, i.e., 32-78% increase over the control plants. Similarly in rice varieties Aiswarya and Swarnaprabha, proline accumulation was less as compared to other varieties and maximum accumulation occurred on exposure to 21 $kJm^{-2}d^{-1}$ of UV-B treatment in Aiswarya (19%) and Swarnaprabha (15%) and further no significant increase was observed in both varieties (Fig. 33).

4.2.2.2 Non enzymatic antioxidants

4.2.2.2.1 Ascorbate

Various doses of UV-B treatment imparted to nine rice varieties resulted in the increased accumulation of ascorbate when compared to control plants. Significant increase in ascorbate content was recorded in Aathira, Mangalamahsuri and Kanchana even after the initial dose of UV-B treatments (7 $kJm^{-2}d^{-1}$) and maximum accumulation was recorded upon 21-28 $kJm^{-2}d^{-1}$ of treatment (an increase of 11, 19 and 14 folds, respectively) over the control plants. Exposure to various dose of UV-B resulted in an enhancement in ascorbate content in all other rice varieties also but it was only about 3-5 fold increase over their control plants. In Jyothi and Aiswarya seedlings, ascorbate content was showing an increasing pattern upon increasing the UV-B dosage and reached maximum at 28 $kJm^{-2}d^{-1}$ of UV-B, but in the case of Annapoorna and Swarnaprabha seedlings, the maximum ascorbate content was recorded on exposure to 21 $kJm^{-2}d^{-1}$ of UV-B treatment and thereafter decreased. Moreover, the maximum ascorbate content was recorded upon 14 $kJm^{-2}d^{-1}$ of UV-B treatment in Neeraja and Swetha seedlings (Fig. 34).

4.2.2.2.2 Glutathione

In general, glutathione content in rice seedlings increased gradually with the increase of UV-B treatment dose and the accumulation was higher in Aathira, Mangalamahsuri and Kanchana as compared to other six rice varieties. A significant increase in glutathione content was recorded even at 7 $kJm^{-2}d^{-1}$ of UV-B treatment in the seedlings of Aathira, Mangalamahsuri and Kanchana (2, 4 and 3 fold increase respectively) and the increase of this antioxidant was maximum upon exposure to 28 $kJm^{-2}d^{-1}$ of UV-B (9, 8 and 6 fold increase respectively). It was found that in Jyothi, Annapoorna, Neeraja, Swetha and Aiswarya seedlings, significant increase in the glutathione content (2 fold) was recorded even in the lower dose of UV-B treatment over the control seedlings. The rate of increase in glutathione content of Jyothi and

Swetha was maximum upon 28 $kJm^{-2}d^{-1}$ of UV-B treatment. However, maximum accumulation of glutathione content in Annapoorna and Neeraja was recorded after 21 $kJm^{-2}d^{-1}$ of UV-B treatment as compared to control plants. Compared with other rice varieties, glutathione accumulation in Aiswarya and Swarnaprabha was much lower and the rate of accumulation was maximum at 21 $kJm^{-2}d^{-1}$ of UV-B treatment (1 fold) (Fig. 35).

4.2.2.2.3 Phenolics

Significant differences were observed in total phenolic content of all rice varieties upon exposure to various dose of UV-B irradiation. However, phenolic content was found to be greatly enhanced in Aathira, Mangalamahsuri and Kanchana when subjected to UV-B treatments and the trend remained same throughout various treatments (0-28 $kJm^{-2}d^{-1}$). Phenolic content exhibited significant increase over the control plants in both Aathira and Mangalamahsuri from 7 $kJm^{-2}d^{-1}$ of UV-B treatment (36 and 28% respectively) and maximum increase was registered after 28 $kJm^{-2}d^{-1}$ of UV-B exposure, i.e., about 203 and 245% respectively over the untreated control plants. In rice seedlings of Kanchana, the total phenolic content was highly increased even after lower dose of UV-B (7 $kJm^{-2}d^{-1}$) itself (147%) and it was found to be highest at 14 $kJm^{-2}d^{-1}$ (237%) and thereafter no further increase was recorded as compared to control plants. In Jyothi, Annapoorna and Swetha, a gradual increase in phenolics content was observed and the maximum was registered upon 21-28 $kJm^{-2}d^{-1}$ of UV-B exposure i.e., about 72, 121 and 138% increase over the control plants. However unlike the above varieties, the phenolic accumulation recorded in Swarnaprbha, Aiswarya and Neeraja was less and reached maximum at 21-28 $kJm^{-2}d^{-1}$ of UV-B exposure, i.e., only 7-40% with respect to the control seedlings (Fig. 36).

4.2.3 Tertiary screening for identifying the most tolerant and sensitive rice varieties towards UV-B irradiation

4.2.3.1 PSI activity

Various doses of UV-B imparted to five rice varieties resulted in varied responses with respect to PSI activity. Treatment with UV-B resulted in significant enhancement of PSI activity of Aathira and the increase was in correlation with increament of UV-B dosage. In the case of Mangalamahsuri, it was noticed that PSI activity was found to increase after imparting two initial doses of UV-B (7 and 14 $kJm^{-2}d^{-1}$) and maximum activity was at 14 $kJm^{-2}d^{-1}$ of UV-B treatment (46%) and thereafter no further increase was recorded as compared to control plants. In contrast, a prominent enhancement in PSI activity of Kanchana leaves treated with UV-B was recorded from 7 $kJm^{-2}d^{-1}$ (64%) onwards upto 28 $kJm^{-2}d^{-1}$ of UV-B (125%) over their untreated seedlings. Unlike these three varieties, a rapid reduction of PSI activity was recorded in the other two rice varieties (Aiswarya and Swarnaprabha). In Aiswarya, maximum reduction was recorded on exposure to 21 $kJm^{-2}d^{-1}$ (35%) of UV-B, and beyond which no further decrease was observed. PSI activity in the leaves of Swarnaprabha was exhibiting a gradual decrease with increase in UV-B dosage (7-28 $kJm^{-2}d^{-1}$), and maximum reduction was recorded (75%) after exposure to 28 $kJm^{-2}d^{-1}$ (Fig. 37).

4.2.3.2 PSII activity

PSII activity was showing a decreasing pattern upon increasing the UV-B dosage in all rice varieties (Aathira, Mangalamahsuri, Kanchana, Aiswarya and Swarnaprabha). PSII activity of Aathira and Kanchana was decreasing upon increasing the irradiation dosage and reduction to the extent of 18-25% was recorded after 28 $kJm^{-2}d^{-1}$ of UV-B treatment. However, PSII activity of Mangalamahsuri exhibited 15% reduction even at initial dose (7

$kJm^{-2}d^{-1}$) of UV-B and thereafter no further decrease was recorded with respect to control seedlings. However, significant reduction in PSII activity of Aiswarya and Swarnaprabha was recorded from the initial dose of UV-B (7 $kJm^{-2}d^{-1}$) treatment and it continued upto 28 $kJm^{-2}d^{-1}$ of UV-B irradiation (71% for both varieties) over their control plants (Fig. 38).

4.2.3.4 Mitochondrial activity

The mitochondrial activity after imparting various doses of UV-B treatment exhibited a varied profile in all rice varieties. A gradual increase in the mitochondrial activity was recorded in UV-B exposed seedlings of Aathira over the control plants during the entire range of UV-B treatment (0-28 $kJm^{-2}d^{-1}$) and a 4 fold increase was recorded after 28 $kJm^{-2}d^{-1}$ of UV-B treatment. Likewise, in UV-B treated seedlings of Kanchana, the mitochondrial activity increased significantly at 14 $kJm^{-2}d^{-1}$ of UV-B treatment (5 fold); however, during the higher dose of treatments (21 and 28 $kJm^{-2}d^{-1}$), the increase in mitochondrial activity recorded was lower than that of 14 $kJm^{-2}d^{-1}$ of UV-B exposure. Initially there was very less enhancement in mitochondrial activity of Mangalamahsuri seedlings upto 14 $kJm^{-2}d^{-1}$ of UV-B stress (1 fold) and beyond which the activity was decreased gradually. In contrast to the above varieties, Aiswarya and Swarnaprabha seedlings exhibited a decreasing trend of mitochondrial activity right at low dosage (7 $kJm^{-2}d^{-1}$) and least activity was registered upon 21 $kJm^{-2}d^{-1}$ of UV-B treatment, i.e., 0.4 fold lower than control plants in both rice varieties (Fig. 39).

4.2.3.5 Enzymatic antioxidants

4.2.3.5.1 Superoxide dismutase (SOD, EC 1.15.1.1)

The activity of SOD was found to increase upon treatment with various doses of UV-B in all five rice varieties studied except in Swarnaprabha where

high doses (21 and 28 $kJm^{-2}d^{-1}$) of UV-B irradiation resulted in a decreased activity of SOD as compared to the control plants. Among all five rice varieties, Aathira and Mangalamahsuri showed maximum SOD activity under UV-B stress. In these two varieties, the SOD activity was gradually enhanced with increase in UV-B dosage and reached maximum upon exposure to 28 $kJm^{-2}d^{-1}$ of UV-B irradiation (190 and 277%, respectively) over their control seedlings. Likewise treatment with UV-B dosage in Kanchana seedlings registered a maximum activity of SOD at 14 $kJm^{-2}d^{-1}$ of UV-B (177%) and thereafter the activity was gradually decreased. While in Aiswarya and Swarnaprabha, the increase of SOD activity was least (10 and 36%) and the maximum enhancement was recorded after 28 and 14 $kJm^{-2}d^{-1}$ of UV-B exposure respectively as compared to their untreated plants (Fig. 40).

4.2.3.5.2 Guaiacol peroxidase (GPOX, EC 1.11.1.7)

On imparting UV-B irradiation, the activity of GPOX was significantly increased in all five rice varieties. Among all rice varieties, higher GPOX activity was recorded in Aathira and the rate of increase was upto 250% upon exposure to 7 $kJm^{-2}d^{-1}$ of UV-B and it was gradually increased and the highest activity was recorded after exposure to 28 $kJm^{-2}d^{-1}$ of UV-B (857%) over that of control plants. Similarly, UV-B treatment enhanced GPOX activity in Mangalamahsuri and Kanchana from 7 $kJm^{-2}d^{-1}$ onwards and maximum activity was recorded after exposure to 28 $kJm^{-2}d^{-1}$ of UV-B over the control plants. Even though a 140% increase in the GPOX activity was recorded in the seedlings of Aiswarya subjected to 21 $kJm^{-2}d^{-1}$ of UV-B irradiation over the control plants, no further increase was observed upon exposure to 28 $kJm^{-2}d^{-1}$ of UV-B. The increase in GPOX activity was least in Swarnaprabha as compared to other varieties i.e., 82% increase after exposure to 14 $kJm^{-2}d^{-1}$ of UV-B and no further increase was recorded beyond 14 $kJm^{-2}d^{-1}$ as compared to control plants (Fig. 41).

4.2.3.5.3 Ascorbate peroxidase (APX, EC 1.11.1.11)

In general, APX activity enhanced in all five rice varieties when exposed to various doses of UV-B and the increase was significant from the lower dosage of UV-B (7 $kJm^{-2}d^{-1}$). In the case of Aathira, a steep increase in the APX activity was recorded from 7 $kJm^{-2}d^{-1}$ (11 fold) onwards and maximum activity was recorded on exposure to 28 $kJm^{-2}d^{-1}$ of UV-B, i.e., 22 fold increase over the control plants. Similarly, in UV-B treated plants of Mangalamahsuri, Kanchana, Aiswarya and Swarnaprabha maximum activity of APX was recorded upon 21 $kJm^{-2}d^{-1}$ of UV-B irradiation, i.e., 11, 8, 2 and 3 fold increases in Mangalamahsuri, Kanchana, Aiswarya and Swarnaprabha respectively over the control plants and further no significant increase was observed in these varieties of rice seedlings with increase in UV-B irradiation (Fig. 42).

4.2.4 Final analysis of the most tolerant and sensitive rice varieties towards UV-B irradiation

4.2.4.1 Chl *a* fluorescence transients

Chl *a* fluorescence parameters were analyzed by using Plant Efficiency Analyzer in dark adapted Aathira and Swarnaprabha leaves after exposure to various level of UV-B (0-28 $kJm^{-2}d^{-1}$) to study the effect of UV-B induced photoinhibition on the photochemistry. Different dose of UV-B reduced the parameter $PI_{(abs)}$ in both rice varieties and the decrease was dose dependent in both varieties. The rate of decrease of $PI_{(abs)}$ was maximum at 28 $kJm^{-2}d^{-1}$ in both varieties (40 and 98% in Aathira and Swarnaprabha, respectively) with respect to their control leaves. UV-B treatment decreased the rate of Fm in both varieties and the maximum rate of reduction was recorded upon imparting 28 $kJm^{-2}d^{-1}$ of UV-B irradiation. The overall PSII activity (Fv/Fo) tends to decrease in both rice varieties (Aathira and

Swarnaprabha) as compared to the control and the decrease was more prominent in Swarnaprabha than Aathira treated with 28 $kJm^{-2}d^{-1}$ treated plants. $SFI_{(abs)}$ decreased in rice leaves upon treating with various dose of UV-B as compared to the control plants. However the rate of decrease was maximum upon 28 $kJm^{-2}d^{-1}$ of UV-B in Aathira (32%) and in the case of Swarnaprabha, maximum reduction was recorded after imparting 21 $kJm^{-2}d^{-1}$ of UV-B irradiation (92%), and thereafter the decrease recorded was not significant (Fig. 43).

The relative variable fluorescence at J (Vj) step increased in both rice varieties upon increase in the dosage of UV-B when compared to the control plants. However, the rate of increase was more in Swarnaprabha as compared to Aathira seedlings. Aathira and Swarnaprabha seedlings treated with 21 $kJm^{-2}d^{-1}$ of UV-B stress were showing an enhancement of 48 and 87% respectively over the control plants. On the other hand, area above the fluorescence curve between Fo and Fm reduced significantly over the control plants in both rice cultivars when subjected to various level of UV-B. The decrease in area above the fluorescence curve of Aathira and Swarnaprabha seedlings was found to be highest upon exposure to 21 $kJm^{-2}d^{-1}$ UV-B (10 and 38%, respectively) with respect to their control leaves (Fig. 43).

An increase in the dose of UV-B exposure causes a decrease in the parameter Φ_{Eo} in both varieties studied and the maximum percentage of reduction was recorded on 21 $kJm^{-2}d^{-1}$ of UV-B treatment in Aathira (63%) and after 28 $kJm^{-2}d^{-1}$ UV-B treatment in Swarnaprabha (90%) leaves and further it decreased significantly over the control plants. Likewise, treatment with UV-B resulted in significant decrease in the Ψ_o even in the initial UV-B dose (7 $kJm^{-2}d^{-1}$) in both Aathira and Swarnaprabha as compared to control seedlings. With severity of UV-B exposure (28 $kJm^{-2}d^{-1}$), the rate of increase in Ψ_o reached up to 35 and 85% in Aathira and Swarnaprabha leaves

respectively. However, Tf(max) was found to be lowering upon increase in the dose of UV-B and the increase was found to be more significant in Swarnaprabha as compared to Aathira seedlings. The maximum rate of reduction was noticed in Swarnaprabha at 21 $kJm^{-2}d^{-1}$ (72%) and further it was decreased significantly. But in the case of Aathira, the reduction was noticed upon 28 $kJm^{-2}d^{-1}$ of UV-B exposure (18%) with respect to their control seedlings (Fig. 43).

An alteration of PSII energy fluxes per cross section in response to various dose of UV-B photoinhibition in Aathira and Swarnaprabha leaves was visualized by phenomenological leaf models of photosynthetic apparatus. The electron transport flux (ETo/CSo) decreased with increase in UV-B dose in Swarnaprabha and maximum reduction was at 21 $kJm^{-2}d^{-1}$ of UV-B treatment (90%). However the rate of decrease in Aathira was more or less similar (25%) in all doses of UV-B. About 22 and 36% decrease was recorded in the energy absorbed per excited cross section (ABS/CSo) in Aathira and Swarnaprabha treated with 21 $kJm^{-2}d^{-1}$ of UV-B respectively as compared to the control plants. Similarly, the ratio of total dissipation to the cross section (DIo/CSo) was increased with increase in UV-B dose in both rice varieties and the maximum rate of enhancement in Aathira and Swarnaprabha was recorded at 21 $kJm^{-2}d^{-1}$ of UV-B (23 and 145%, respectively) over their untreated plants. UV-B treatment did not cause a major change in the trapping flux (TRo/CSo) of the rice leaves of both varieties as compared to the respective control seedlings. Similarly, the density of active reaction centres as represented by RC/CSo decreased corresponding to the increase in UV-B dose and it was more severely decreased in the Swarnaprabha (78%), whereas in the case of Aathira, the reduction recorded was only 4% (Fig. 44).

Specific membrane models of PSII energy fluxes per reaction center (RC) in response to UV-B irradiation was recorded in both rice varieties.

About 5 and 53% decrease was recorded in the flux of absorption (ABS/RC) in Aathira and Swarnaprabha after 28 $kJm^{-2}d^{-1}$ of UV-B with respect to their control leaves. However, the trapping flux per reaction center (TR_o/RC) was lowered in both Aathira and Swarnaprabha and maximum reduction was registered at 21 $kJm^{-2}d^{-1}$ of UV-B treatment (12 and 52% in Aathira and Swarnaprabha respectively). Similarly, the electron transport flux (ET_o/RC) decreased in both varieties as compared to the control plants and maximum reduction was recorded in Swarnaprabha (81%) than Aathira (34%) after 21 $kJm^{-2}d^{-1}$ of UV-B treatment. Moreover, dissipated energy (DI_o/RC) increased significantly in both rice varieties upon treating the rice seedlings with various doses of UV-B and the maximum increase in Aathira was recorded at 14 $kJm^{-2}d^{-1}$ (40%), whereas in the case of Swarnaprabha the maximum increase was recorded at 28 $kJm^{-2}d^{-1}$ (215%) of UV-B treatment over the control plants (Fig. 45).

NPQ exhibited significant increase over the control plants in both Aathira and Swarnaprabha from 7 $kJm^{-2}d^{-1}$ of UV-B treatment (35 and 9% respectively). In rice seedlings of Aathira, NPQ was found to be highest at 21 $kJm^{-2}d^{-1}$ (225%) and further no significant increase was observed after 28 $kJm^{-2}d^{-1}$ of UV-B irradiation. However, the NPQ recorded in Swarnaprabha was less and reached maximum at 28 $kJm^{-2}d^{-1}$ of UV-B exposure, i.e., only 60% increase over the control seedlings (Fig. 46A).

4.2.4.2 Leaf gas exchange parameters

4.2.4.2.1 Net photosynthetic rate, (P_n)

Various doses of UV-B imparted to Aathira and Swarnaprabha resulted in decrease of P_n with respect to their control leaves. Significant reduction in P_n of Swarnaprabha was recorded on imparting low dose of UV-B and it continued upto 28 $kJm^{-2}d^{-1}$ of UV-B irradiation (46%) as compared to the

control plants. However, the rate of reduction in P_n of Aathira seedlings was less and the maximum decrease was recorded after exposure to 21 $kJm^{-2}d^{-1}$ of UV-B (10%) as compared to control plants (Fig. 46B).

4.2.4.2.2 Stomatal conductance (g_s)

The parameter g_s showed a decreasing pattern upon increasing the UV-B dosage in Aathira and Swarnaprabha varieties and reached more or less similar percentage of reduction after exposure to 28 $kJm^{-2}d^{-1}$ of UV-B (50-54%) as compared to control plants. In the case of Aathira, there was no significant change in g_s after two initial doses of UV-B treatments (7 and 14 $kJm^{-2}d^{-1}$) over their control plants. In contrast, a prominent reduction in g_s of Swarnaprabha was recorded from 7 $kJm^{-2}d^{-1}$ (38%) onwards upto 28 $kJm^{-2}d^{-1}$ of UV-B irradiation with respect to their untreated leaves (Fig. 46C).

4.2.4.2.3 Transpiration rate (E)

A gradual decrease in the parameter E was recorded in UV-B exposed seedlings of Aathira and Swarnaprabha over their control plants. It was decreased progressively in UV-B treated leaves of Aathira from 7 $kJm^{-2}d^{-1}$ onwards and maximum reduction was recorded after exposure to 28 $kJm^{-2}d^{-1}$ of UV-B exposure (47%). Even though 84% reduction in E was recorded in Swarnaprabha subjected to 21 $kJm^{-2}d^{-1}$ of UV-B over their control leaves, no further decrease was observed thereafter upon 28 $kJm^{-2}d^{-1}$ of UV-B treatment (Fig. 46D).

4.2.4.3 Anthocyanin

In general, anthocyanin content in leaf tissue of Aathira was showing significant enhancement with the UV-B dose, but such an enhancement was not recorded in the leaves of Swarnaprabha. In the leaves of Aathira treated with UV-B, anthocyanin accumulation was found much higher when

compared to the control plants and significant enhancement in accumulation was recorded from 7 $kJm^{-2}d^{-1}$ onwards. About 122% increase in the accumulation of anthocyanin content was registered in the leaves of Aathira variety upon 28 $kJm^{-2}d^{-1}$ of UV-B treatment when compared to the control plants. In Swarnaprabha subjected to UV-B treatment, initially there was a significant reduction of total anthocyanin accumulation upto 14 $kJm^{-2}d^{-1}$ UV-B irradiation and beyond which the rate of accumulation increased gradually and maximum accumulation was registered after 28 $kJm^{-2}d^{-1}$ (23%) of UV-B stress as compared to the control plants (Fig. 47A).

4.2.4.4 Flavonoids

Rice varieties treated with various doses of UV-B induced an accumulation of flavanoids both in Aathira and Swarnaprabha leaves over their control plants. Flavanoid content increased progressively in UV-B treated leaves of Aathira from 7 $kJm^{-2}d^{-1}$ onwards (124%) and maximum accumulation was recorded after exposure to 28 $kJm^{-2}d^{-1}$ (263%). Even though a 45% increase in the accumulation of flavonoid content was recorded in Swarnaprabha subjected to 14 $kJm^{-2}d^{-1}$ of UV-B over their control leaves, no further increase was observed thereafter upto 28 $kJm^{-2}d^{-1}$ (Fig. 47B).

4.2.4.5 PAL activity

UV-B treatment imparted to Aathira and Swarnaprabha varieties resulted in the increased activity of PAL when compared to the control seedlings. Treatment with various doses of UV-B resulted in significant increase of the PAL activity in Aathira even in the low dose of UV-B treatment (7 $kJm^{-2}d^{-1}$). Maximum activity of PAL in Aathira subjected to UV-B treatment was observed on exposure to 21 $kJm^{-2}d^{-1}$ (140%) over their control plants and further it decreased. However, the rate of PAL activity was lower in Swarnaprabha subjected to UV-B treatment when compared to

Aathira seedlings, i.e., only 44% increase at 14 $kJm^{-2}d^{-1}$ of UV-B stress. Further, a gradual decreasing trend of PAL activity in Swarnaprabha was recorded at 28 $kJm^{-2}d^{-1}$ of UV-B treatment, i.e., 17% lower than control seedlings (Fig. 47C).

4.2.4.6 Foliar micromorphology and stomatal characteristics

The SEM image of epidermal cells of Aathira and Swarnaprabha leaves subjected to 28 $kJm^{-2}d^{-1}$ UV-B treatment showed that the stomata were closed in both abaxial and adaxial surfaces. However, the stomata were opened and functional in the untreated plants of both varieties. The epidermal cells of the Swarnaprabha leaves subjected to 28 $kJm^{-2}d^{-1}$ of UV-B treatment showed several damaged and closed stomata than Aathira leaves. The SEM images of abaxial and adaxial leaf surfaces showed some deposition of cuticular waxes and it was more pronounced in the adaxial surfaces as compared to the abaxial surfaces of leaves in both rice varieties. The wax deposition on the adaxial surface of Aathira leaves was higher than Swarnaprabha after 28 $kJm^{-2}d^{-1}$ of UV-B treatment. In the adaxial surface of Swarnaprabha leaves, the stomata were more closed and damaged than those of abaxial surface (Fig. 48-51).

The stoma parameters such as stomatal length and width of both adaxial and abaxial surfaces of both Aathira and Swarnaparbha leaves were significantly decreased after 28 $kJm^{-2}d^{-1}$ of UV-B treatment, except the stomatal width on adaxial epidermis of Aathira leaves. The decrease in stomatal length in the adaxial and abaxial epidermis of Swarnaprabha upon 28 $kJm^{-2}d^{-1}$ of UV-B irradiation was higher as compared to Aathira rice variety. Moreover, the rate of decrease in stomatal width of abaxial epidermis was lower in Aathira (13%) than recorded in Swarnaprabha leaves (43%) after higher dose of UV-B treatment (28 $kJm^{-2}d^{-1}$). A slight increase in the stomatal width on adaxial epidermis of Aathira leaves (7%) was recorded after UV-B

irradiation, while that of Swarnaparbha was greatly decreased (35%) as compared to untreated leaves (Table 10).

Table 10: Stomatal length and width (µm) on the adaxial and abaxial epidermis of Aathira and Swarnaprabha leaves after exposure to 28 kJm^{-2}d^{-1} of UV-B irradiation. Values given are mean of 3 independent experiments, each with a minimum of 3 replicates (*i.e.* n=9)±S.E.

Rice variety	UV-B stress (kJm^{-2}d^{-1})	Adaxial epidermis		Abaxial epidermis	
		Stomatal length (µm)	Stomatal width (µm)	Stomatal length (µm)	Stomatal width (µm)
Aathira	0	28.91±1.11	7.29±0.25	26.89±1.15	11.21±0.52
	28	27.13±0.95	7.85±0.28	26.44±0.65	9.67±0.38
Swarnaprabha	0	23.48±1.12	6.49±0.17	24.58±0.78	8.91±0.34
	28	20.91±0.82	4.21±0.11	22.52±0.46	5.01±0.07

4.2.4.7 Chloroplast ultrastructure

The images of chloroplasts from the leaves of Aathira and Swarnaprabha seedlings using TEM analysis revealed that UV-B stress induced alterations in the structure of chloroplasts and thylakoids as compared to the control leaves. The shape of chloroplasts changed from elliptical to nearly round and this change in shape was highly prominent in Swarnaprabha than Aathira leaves (Fig. 52). Chloroplast membranes were notably disorganized in the rice leaves upon UV-B irradiation and the chloroplasts showed disarranged thylakoids and these changes were prominent in Swarnaprabha than Aathira leaves. Furthermore, the lamellae of stromal thylakoids and stacked granal thylakoids were damaged in both rice leaves after exposure to 28 kJm^{-2}d^{-1} of UV-B radiation (Fig. 53).

4.2.4.8 Cuticular wax content and functional group analysis of the epicuticular wax deposition

The accumulation of epicuticular wax content in Aathira and Swarnaprabha leaf surface was increased upon 28 $kJm^{-2}d^{-1}$ of UV-B treatment over their control plants. The wax content in control seedlings of Aathira and Swarnaprabha was 1.17 and 0.66 mgg^{-1} FW of leaf samples. Wax content increased significantly in the leaf surface of Aathira (2.61 mgg^{-1} FW) as compared to Swarnaprabha (1.21 mgg^{-1} FW) after exposure to 28 $kJm^{-2}d^{-1}$ of UV-B treatment. Moreover, in Aathira, there were fissures in the wax layer on the adaxial surface of leaf lamina after exposure to 28 $kJm^{-2}d^{-1}$ of UV-B irradiation (Table 11).

Table 11: Epicuticular wax content (mgg^{-1} FW) extracted from Aathira and Swarnaprabha leaves after exposure to 28 $kJm^{-2}d^{-1}$ of UV-B treatment. Values given are mean of 3 independent experiments, each with a minimum of 3 replicates (*i.e.* n=9)±S.E.

UV-B stress ($kJm^{-2}d^{-1}$)	Aathira	Swarnaprabha
0	1.17±0.08	0.66±0.01
28	2.61±0.09	1.21±0.06

FT-IR spectra of epicuticular wax in untreated Aathira leaves showed six important peaks and it was detailed in 4.1.5.8. However, the peak at 2963 cm^{-1} corresponding to symmetrical and asymmetrical stretching of methyl groups was negligible and a new peak appeared at 1472 cm^{-1} corresponding to methylene C–H bending vibration after exposure to 28 $kJm^{-2}d^{-1}$ of UV-B treatment in Aathira seedlings. Similarly, the epicuticular wax of untreated Swarnaprabha leaves showed five peaks as detailed in 4.1.5.8. Upon imparting 28 $kJm^{-2}d^{-1}$ of UV-B treatment in Swarnaprabha leaves, peak at 2358 cm^{-1} which corresponds to C–H stretching was highly reduced but

resulted a new peak appeared at 1740 cm^{-1}, which corresponds to carbonyl ester vibrations associated with methyl esterified lipids (Fig. 54).

4.2.4.9 Interconversions of xanthophyll pigments

The HPLC elution components of the photosynthetic xanthophyll pigments extracted from rice seedlings recorded significant difference in Aathira and Swarnaprabha leaves after exposure to 28 $kJm^{-2}d^{-1}$ of UV-B irradiation. The major photosynthetic pigments eluted from rice leaves were violaxanthin (V), antheraxanthin (A), luetin (L), zeaxanthin (Z), lutein (L), neoxanthin (N), α and β carotenes (Fig. 55). The content of pigments N, A and Z were increased, whereas the content of V was found to decrease after 28 $kJm^{-2}d^{-1}$ of UV-B treatment in both rice varieties as compared to the control leaves. It was showed that the Z content was not traceable in control seedlings of Aathira and Swarnaprabha leaves, while it was increased significantly to an extent of 6.1 and 7 $\mu g g^{-1}$ FW in Aathira and Swarnaprabha, respectively, after 28 $kJm^{-2}d^{-1}$ of UV-B treatment. Likewise, the content of A was also enhanced to an extent of 2.2 $\mu g g^{-1}$ FW in Aathira leaves after 28 $kJm^{-2}d^{-1}$ of UV-B treatment, from a negligible level in untreated leaves. Similarly, A was found to increase from 1.3 $\mu g g^{-1}$ FW (control) to 1.8 $\mu g g^{-1}$ FW after 28 $kJm^{-2}d^{-1}$ of UV-B treatment in Swarnaprabha leaves (Fig. 56).

The lutein content was reduced upon 28 $kJm^{-2}d^{-1}$ of UV-B irradiation in Aathira and Swarnaprabha leaves (28 and 39%) with respect to their untreated leaves. In contrast, the content of neoxanthin was enhanced in Aathira and Swarnaprabha seedlings after exposure to UV-B irradiation (98 and 19%, respectively) as compared to the control seedlings. Likewise, a significant increase in both α and β carotene content in Aathira and Swarnaprabha leaves was registered after UV-B treatment over their control seedlings (Fig. 57A). In addition to this, the de-epoxidation state of xanthophyll cycle pigments (DEPS=Z+A/V+A+Z) enhanced in UV-B treated

Aathira (33%) and Swarnaprabha (31%) when compared with respective control seedlings. However, the epoxidation state of xanthophyll cycle pigments (EPS=V+A/V+A+Z) was found to decrease after exposure to 28 $kJm^{-2}d^{-1}$ of UV-B irradiation in both rice seedlings (24-26%). Likewise, the ratio of xanthophyll cycle pool (V+A+Z) to total Chl was enhanced in Aathira after exposure UV-B stress (213%), while it was decreased in Swarnaprabha leaves (23%) as compared with the control seedlings. Moreover, the ratio of xanthophyll cycle pool to total carotenoids was slightly increased in Aathira leaves, while it was decreased in Swarnaprabha seedlings after exposure to 28 $kJm^{-2}d^{-1}$ of UV-B irradiation (Fig. 57B).

DISCUSSION

5.1 High intensity light stress

Excess light from sun leads to increased production of ROS, which may cause photooxidative damage to plant system. Most of the plant species have the ability to develop morphological, anatomical, physiological and biochemical variations in response to different light intensities (Peri et al., 2007; Langkamp et al., 2015). Rice is usually grown in a high intensity lighting condition (300-2000 $\mu molm^{-2}s^{-1}$) and summer sunlight during mid noon is around 1800-2000 $\mu molm^{-2}s^{-1}$ in natural condition. Therefore 2000 $\mu molm^{-2}s^{-1}$ was imparted to rice seedlings to generate HL stress. In the present study, the rice seedlings were exposed to 0-8 h of HL (2000 $\mu molm^{-2}s^{-1}$) because above this period of exposure to high light intensity seems to be highly deleterious.

The determination of optimal growth period for imparting HL stress in rice varieties was assessed based on shoot length, chlorophyll content and the efficiency of the water-splitting complex on the donor side of PSII (Fv/Fo). Even though, the shoot length of rice seedlings was found to further increase upto 11 and 12 d of germination, the optimal growth for imparting HL stress was found to be on 10 d of germination. Moreover, in most of the rice varieties, leaf chlorosis was observed on 11 d, which got intensified on further exposure to HL stress. There are many instances of HL stress studies being carried out on 1-3 week old seedlings of different plant species as seedlings of this age could give responses which are very similar to that of mature plants. Yang et al. (2007) treated two week old rice seedlings with 6, 12 and 24 h of HL stress. Likewise, Janda et al. (2015) studied the effects of HL stress on one week old *Arabidopsis* seedlings and 18-21 d old *Nicotiana benthamiana*.

In the case of *Salvia officinalis,* plants were exposed to HL (1400 $\mu molm^{-2}s^{-1}$) after 15 d of germination (Zervoudakis et al., 2012).

5.1.1 Primary screening for identifying the tolerant and sensitive rice varieties towards HL exposure

The 10 d old seedlings of 13 rice varieties (Aathira, Aiswarya, Annapoorna, Harsha, Jyothi, Kanchana, Karuna, Mangalamahsuri, Mattatriveni, Neeraja, Swarnaprabha, Swetha and Varsha) raised under half strength Hoagland nutrient medium were subjected to HL stress (2000 $\mu molm^{-2}s^{-1}$) for different time intervals (0-8 h). Various physiological and biochemical parameters were analyzed in detail in the control and HL treated plants, which can aid in distinguishing the HL tolerant and sensitive rice varieties. The primary screening was done on the basis of their performance after exposure to HL for different time intervals (0-8 h) by analyzing photosynthetic pigments, Fv/Fo and the rate of lipid peroxidation.

5.1.1.1 Photosynthesis

Chlorophyll reduction in HL treated seedlings of Swetha, Karuna, Mangalamahsuri, Varsha, Swarnaprabha, Kanchana and Neeraja was due to the interference of HL irradiation on photosynthetic pigments either through inhibition of chlorophyll biosynthetic pathways, due to deleterious effects on the enzymes involved in the chlorophyll biosynthetic pathways and/or faster degradation of pigment molecules. Chlorophyll content was declined with increasing light intensity in *Phaseolus vulgaris* and severe symptoms of chlorosis and necrosis were observed at HL stress of 600 $\mu molm^{-2}s^{-1}$ (Marschner and Cakmak, 1989). Among all the varieties of *O. sativa* subjected to HL treatment of different time intervals, maximum decrease of chlorophyll pigments was observed in the seedlings of Swarnaprabha, Kanchana and Neeraja. The decrease in total chlorophyll content was

maximum at 8 h of HL exposure in these three varieties. Degradation of LHCII under HL exposure should potentially release chlorophyll molecules and thus cause decrease of total chlorophyll content in leaves (Tzvetkova-Chevolleau et al., 2007). However, the total chlorophyll content was enhanced in Aathira, Jyothi, Annapoorna, Aiswarya, Mattatriveni and Harsha when treated with different periods of HL exposure and this could be due to the activation of chlorophyll biosynthesis under high illumination. Thus, the rate of chlorophyll synthesis overtakes the degradation rate upon HL exposure, therefore, a higher chlorophyll concentration is observed in these rice varieties as observed in *Hymenaea stilbocarpa* seedlings when treated with HL stress (Malavasi and Malavasi, 2001).

The increased carotenoid content triggered by HL irradiation in all thirteen rice varieties studied except in Kanchana after 8 h of HL stress, protects the thylakoid membrane from ROS mediated photooxidative destruction by quenching the singlet oxygen and peroxy radicals. Aathira, Jyothi, Annapoorna, Aiswarya and Mattatriveni seedlings accumulated more carotenoids after HL stress. Highly enhanced chlorophyll and carotenoid content in these five varieties would ensure better photosynthesis and further growth of the rice seedlings. The total carotenoids in plants include xanthophyll pigments and carotenes and the accumulation of these upon HL stress in rice seedlings will be discussed later in 5.1.4.4. The acclimation of photosynthesis to high irradiance in two rice varieties, IR72 and NPT, revealed the shift in carotenoid composition in favor of the operation of xanthophyll cycle, which is responsible for high-energy state quenching of Chl fluorescence, which is indicative of photoprotection (Murchie et al., 2002). In contrast, the carotenoid content was decreased in Kanchana after long term HL exposure (8 h) and such changes were generally observed in sensitive plants. Kusumi et al. (2000) experimentally proved that a mutant

line of rice with less content of chlorophyll and carotenoids displayed high susceptibility towards HL stress.

The Chl *a* fluorescence parameter Fv/Fo, denoting the efficiency of the water-splitting complex on the donor side of PSII, measured in leaves of rice seedlings subjected to HL treatment showed significant variation between varieties and treatments. However, the rate of reduction in Fv/Fo after long term of HL exposure (8 h) was not varying between different rice varieties. Indeed, the difference in Fv/Fo level among thirteen rice varieties was predominant after short term HL treatment (2 h). The reduction in Fv/Fo of rice seedlings indicated that HL exposure resulted in a decrease in efficiency of the water-splitting complex on the donor side of PSII and thus led to reduction of photosynthetic efficiency. The reduction in Fv/Fo in HL treated plants of rice seedlings could also be due to the result of electron transport impairment of photosynthesis (Pereira et al., 2000).

The varietal differences in reduction of Fv/Fo, the efficiency of the water-splitting complex on the donor side of PSII, is a means to assess the tolerance potential of different rice varieties towards different time intervals of HL treatment (Kalaji et al., 2012; Gautham et al., 2014). Swarnaprabha, Kanchana and Neeraja varieties recorded the highest photoinhibition on the donor side of PSII. However, the decrease in Fv/Fo was less in the seedlings of Aathira, Jyothi, Annapoorna, Aiswarya and Mattatriveni, attributing to high level of photoprotection mechanisms getting operated in them under HL irradiation. Kalaji et al. (2012) revealed that HL stress caused a reduction of Fv/Fo in two barley cultivars and found that Arabi Aswad was more tolerant than Arabi Abiad cultivar in terms of various Chl *a* fluorescence parameters, including Fv/Fo.

5.1.1.2 Lipid peroxidation

The increased level of ROS in plants due to HL exposure damages the biomolecules such as lipids and results in MDA formation as the breakdown product of polyunsaturated fatty acids of membranes. This is widely accepted as an important criterion for making the assessment of the severity of the stress induced oxidative damages (Abedini et al., 2017). Even though, significant increase in MDA content due to HL exposure of various levels was recorded in all thirteen rice varieties studied, the rate of lipid peroxidation was higher in Swarnaprabha, Kanchana and Neeraja seedlings and it was primarily ascribed to the oxidative damage induced by excessive ROS production and membrane damage.

HL exposure induced the production of $^1O_2$ and endogenous cationic radicals at the reaction center which results in the lipid peroxidation and further damages the PSII subunits, mainly D1 protein (Chan et al., 2013). The lower rate of MDA content in Aathira, Jyothi, Annapoorna, Aiswarya and Mattatriveni seedlings upon different time intervals of HL exposure denotes the powerful activation of antioxidant system so as to mitigate the lipid peroxidation (role of antioxidants will be discussed later in 5.1.2.2 and 5.1.3.3) or a state wherein the situation leading to lipid peroxidation was minimal. Rastogi et al. (2014) revealed that HL induced production of antioxidants can act as efficient $^1O_2$ scavengers and protect effectively lipids against photooxidative damage in *Arabidopsis* plants.

In general, after primary screening of thirteen rice varieties based on photosynthetic pigments content, the efficiency of the water-splitting complex on the donor side of PSII and the rate of lipid peroxidation after 0-8 h of HL exposure, screened them into sensitive and tolerant types. It was clear that Aathira, Jyothi, Annapoorna, Aiswarya and Mattatriveni were probable tolerant types; Swarnaprabha, Kanchana and Neeraja were the probable

sensitive ones. The remaining five rice varieties, Harsha, Swetha, Karuna, Mangalamahsuri and Varsha were showing intermediate level of photoinhibitory damages and tolerance potential. Therefore, for further studies only the tolerant and sensitive varieties were considered to have a clear understanding on the difference of tolerance mechanisms functional in them.

5.1.2 Secondary screening for identifying the tolerant and sensitive rice varieties towards HL exposure

The selected five tolerant (Aathira, Jyothi, Annapoorna, Aiswarya and Mattatriveni) and three sensitive (Swarnaprabha, Kanchana and Neeraja) rice varieties from primary screening was further screened for the selection of the most HL tolerant and sensitive ones among them by analyzing primary metabolites and non enzymatic antioxidants after exposure to HL stress (2000 $\mu molm^{-2}s^{-1}$) of various exposure periods (0-8 h).

5.1.2.1 Primary metabolites

The high protein content was recorded in Aathira and Jyothi varieties after long term HL exposure (8 h), which would certainly aid these varieties in encountering HL stress. The rice varieties Annapoorna, Aiswarya and Mattatriveni responded to HL exposure of 0-8 h by increasing their total protein content, but the increase was very less as compared to Aathira and Jyothi seedlings. Induction of protein synthesis is an important and immediate effect of HL stress in plants (Roach and Krieger-Liszkay, 2012). A general response of plants to various stresses is the increased synthesis of various HSPs or molecular chaperons (Lee and Vierling, 2000; Sun et al., 2001). Induction of HSPs in response to oxidative stress is thought to play a crucial role in protecting plants against stress by preventing the misfolding and degradation of proteins under stress conditions (Sun et al., 2002). The

expression of chloroplast chaperones was highly expressed in HL tolerant *Sorghum* variety than susceptible variety and it was responsible for the stability of photosynthetic components in tolerant variety (Jagtap et al., 1998).

Even though, a slight increase in protein content was noticed at 2 h of HL exposure in Swarnaprabha, Kanchana and Neeraja seedlings, higher time intervals of HL exposure reduced the total protein content and maximum reduction was noticed at 8 h of HL exposure in these rice varieties. This could be attributed to enhanced protein degradation or inactivation than the synthesis rate that occurs in plants under conditions that induce oxidative stress. The reductive effect of HL exposure on protein content is related to amino acid destruction and enzymes inactivation induced by HL induced oxidative stress. A number of reports have shown that cells exhibit increased rates of proteolysis following exposure to oxidative stress (Salama et al., 2011; Zhang et al., 2013).

As in the case of proteins, increased accumulation of soluble sugar content was recorded in Aathira and Jyothi seedlings as compared to Annapoorna, Aiswarya and Mattatriveni, Swarnaprabha, Kanchana and Neeraja after exposure to HL stress, which could aid in maintaining the cell structure and membrane integrity under stressed conditions. Several studies suggested that abiotic stresses enhanced the total sugar content in plant cells (Djilianov et al., 2011; Bano et al., 2017). Moreover, it maintains the protein structure and normalizes intracellular macromolecules in membrane structures from the damage caused by stress (Couée et al., 2006). A steep increase in the accumulation of soluble sugar content was recorded in Aathira and Jyothi from 2 h onwards and maximum accumulation was recorded upon 8 h of HL treatment. Moreover, the role of soluble sugar content on alleviating HL stress is probably through its role in protection of photosynthetic components from HL induced damages and stabilizing the

chloroplast structure. The enhanced PSI activity and reduced decrease of PSII activity in Aathira leaves is discussed in 5.1.3.1.

The amino acid content increased significantly in all rice varieties studied upon HL exposure and it is considered as precursors and constituents of proteins, which play an important role in plant stress metabolism. The maximum amino acid content was recorded in Aathira seedlings after 8 h of HL exposure. Likewise, HL stress enhanced the total amino acid content in poplar plants and the major amino acids enhanced under HL stress were glutamate, aspartate, glutamine, asparagine, serine and glycine (Noctor et al., 1997). According to Chen et al. (2018), differences in light intensity produce relatively large changes in amino acid levels. In wheat seedlings, response to simultaneous HL and salinity stress involves reconfiguration of amino acid metabolism and resulted in high accumulation of γ-amino butyric acid, amides and minor amino acids (Woodrow et al., 2017). However, the rate of increase in amino acid content of Jyothi, Annapoorna, Aiswarya and Mattatriveni was not significantly higher as in the case of Aathira seedlings. The total amino acid content in Neeraja, Swarnaprabha and Kanchana seedlings upon exposure to HL stress was very less as compared to control plants.

Plants synthesize a variety of osmolytes such as proline, sucrose and polyols which are low molecular weight, highly soluble compounds which function as compatible solutes in plant cells and are nontoxic even when accumulated at high cellular concentrations (Hare et al., 1998; Singh et al., 2017). Upon irradiating rice seedlings with HL stress, significant increase in the accumulation of proline was recorded in Aathira and Jyothi, and maximum increase was recorded upon 8 h of HL exposure. However, a negligible increase in the accumulation of proline content was recorded in the seedlings of Annapoorna, Mattatriveni and Neeraja seedlings. The

overproduction of proline upon 0-8 h of HL exposure in Aathira and Jyothi seedlings is certainly an adaptive response of seedlings to HL stress. Proline accumulation is one of the important non enzymatic defence systems which are known to occur under HL induced oxidative stress (Hayashi et al., 2000; Puthur and Rajan, 2006).

In contrast to the above, a negligible decrease in proline content of Aiswarya, Swarnaprabha and Kanchana was recorded after HL treatment and the reduction in proline content could have been due to greater proline utilization than production caused by HL exposure. Surprisingly, Bandurska et al. (2012) also noticed such an effect of UV-B treatment on proline accumulation in barley leaves and it might be due to the high rate of lipid peroxidation and membrane damages which negatively influences the proline biosynthesis in plants. Likewise, the findings on proline content in three rice varieties were consistent with the results reported by Ayala-Astorga and Alcaraz-Meléndez (2010) in two *Paulownia* species subjected to salt stress. Experiments with two different species of *Paulownia* revealed that proline content in *Paulownia imperialis* (tolerant species) was increased while that of *Paulownia fortunei* (sensitive species) was highly decreased under salt stress.

5.1.2.2 Non enzymatic antioxidants

Antioxidants like ascorbate, glutathione and phenolics work hand in hand with enzymatic antioxidants to scavenge toxic ROS produced under unfavorable environmental conditions and prevent the oxidation of vital components in the plant cells (Kurutas, 2015). A steep increase in the accumulation of ascorbate in Aathira and Jyothi varieties substantiates the significant increase in APX activity recorded in these rice varieties subjected to different time intervals of HL exposure. The ascorbate detoxifies tocopheroxyl radicals produced as a result of $^1O_2$ induced lipid peroxidation in PSII (Havaux, 2003). In addition to this, ascorbate content in Annapoorna,

Aiswarya and Mattatriveni was also increased with increase in duration of HL exposure and this would help these varieties to minimize ROS production. However, the increase in ascorbate content in Kanchana, Swarnaprabha and Neeraja seedlings upon HL exposure was very less as compared to other rice varieties. Thylakoid bound ascorbate is involved in the xanthophyll cycle as a cofactor for violaxanthin de-epoxidase, which is involved in the NPQ of excess excited energy in PSII (Niyogi, 1999; Müller-Moulé et al., 2003). The increase of xanthophyll and NPQ upon HL exposure is being discussed in 5.1.4.4. Indeed, ascorbate also supports a sustained electron transport activity in stressed leaves by acting as an alternative electron donor to PSII and thus can alleviate the donor side induced photoinhibition of PSII (Tóth et al., 2011). Moreover, Page et al. (2012) compared the responses of six *Arabidopsis* ecotypes and showed an accumulation of ascorbate upon HL treatment.

Another powerful antioxidant other than ascorbate is glutathione - a non protein sulphur containing tripeptide which is termed as 'ubiquitous' or 'universal redox buffer', plays an important role in scavenging of ROS molecules (Foyer and Noctor, 2005). A steep increase in the glutathione content was recorded in both Aathira and Jyothi right from 2 h of imparting HL stress and reached maximum upon 8 h of HL exposure. As in the case of ascorbate, this antioxidant too was over produced in the same two rice varieties. The sensitive rice varieties Swarnaprabha, Kanchana and Neeraja on HL exposure of 0-8 h showed lower accumulation of glutathione in the seedlings. Heyneke et al. (2013) found that plants accumulated ascorbate and glutathione in *Arabidopsis* after exposure to different light intensities (300, 700 and 1500 $\mu molm^{-2}s^{-1}$) for a short term exposure of 4 h and a long term exposure of 14 d. The rate of reduction in glutathione content of Neeraja variety upon exposure to long term HL stress (8 h) might be due to the

deterioration effects of strong light upon progressive regeneration of the glutathione pool in rice seedlings.

In addition to ascorbate and glutathione, phenolic compounds are abundantly present in plant cells, which have the antioxidant potential thus reduce ROS accumulation and also function as metal chelators (Ksouri et al., 2008). These are the most widely distributed secondary metabolites in plants which possess one or more aromatic rings with one or more hydroxyl groups and the antioxidant capacity of phenolics will increase with the number of free hydroxyls and conjugation of side chains to the aromatic rings (Bergmann et al., 1994). The maximum accumulation of phenolic content was recorded in Aathira, Jyothi and Mattatriveni seedlings on exposure to 8 h of HL, while that of Swarnaprabha and Neeraja was much lower than former three rice varieties. In an earlier study, the antioxidant activity of two varieties of ginger plants was found to be linearly proportional with total phenolic content upon exposure to HL stress (310, 460, 630 and 790 $\mu molm^{-2}s^{-1}$) (Ghasemzadeh et al., 2010). Recently Pérez-López et al. (2018) also reported the differential accumulation of phenolics and the antioxidant capacity of the same in response to HL exposure in two lettuce cultivars.

Thus, after secondary screening of rice varieties (Aathira, Jyothi, Annapoona, Aiswarya, Mattatriveni, Swarnaprabha, Kanchana and Neeraja) based on accumulation of primary metabolites and non enzymatic antioxidants after 0-8 h of HL exposure, differentiated them into sensitive and tolerant types. It was recorded that Aathira and Jyothi were most probable tolerant; Swarnaprabha, Kanchana and Neeraja were the most probable sensitive varieties towards HL exposure. The remaining three varieties (Annapoona, Aiswarya and Mattatriveni) were showing intermediate tolerance characters and therefore they were not considered for the next level

screening for the selection of most tolerant and sensitive ones towards HL treatments.

5.1.3 Tertiary screening for identifying the most tolerant and sensitive rice varieties towards HL exposure

The selected two highly tolerant (Aathira and Jyothi) and three sensitive (Swarnaprabha, Kanchana and Neeraja) rice varieties after two successive screening were further analyzed for the determination of the most tolerant and sensitive varieties on the basis of their performance after exposure to HL treatments (0-8 h) by analyzing photosystems activity, mitochondrial activity and various enzymatic antioxidants.

5.1.3.1 PSI and PSII activity

In agreement with earlier reports (Kono et al., 2014; Yamori et al., 2016), it was found that PSI activity was suppressed in Jyothi, Swarnaprabha, Kanchana and Neeraja seedlings upon treating with HL exposure. However, short term HL treatment (2 h) influences Jyothi seedlings by increasing the PSI activity. The reduction in PSI activity in rice leaves can be attributed to the disruptive action of HL exposure on the structure of thylakoid membrane protein complexes resulting in the inhibition of PSI. Normally under HL exposure, PSI is very rarely damaged, but when occurring, the damage is practically irreversible. HL treatment of 2000 $\mu molm^{-2}s^{-1}$ in *Psychotria rubra* resulted in photoinhibition to PSI (Huang et al., 2016). In Aathira, there was significant enhancement in PSI activity after 6-8 h of HL treatment. It was already reported that the PSI activity enhances under stress condition to cope up with abiotic stress and thus meet the additional demand of ATP for countering stress (Sudhir et al., 2005). Moreover, the cyclic electron flow around PSI can generate a large trans-thylakoid proton gradient that can

induce the xanthophyll cycle primarily operating for heat dissipation under HL exposure (Huang et al., 2016).

The higher rate of reduction in the PSII activity recorded for Swarnaprabha, Kanchana and Neeraja as compared to Aathira and Jyothi after exposure to HL treatment is due to the direct negative effect of HL stress on the oxygen evolving complex or due to the degradation of thylakoid membrane proteins. Mainly, HL induced photoinhibition occurs at the manganese cluster of the OEC on the donor side of PSII (Ohnishi et al., 2005; Murata et al., 2007). Moreover, HL stressed conditions causes inactivation of active reaction centers in the rice leaves, which directly reduces the photosynthetic activity of the rice seedlings, as also evidenced from the Chl *a* fluorescence data which will be discussed in 5.1.4.1. Indeed, the rate of reduction in PSII activity was very less in Aathira leaves upon HL exposure as compared to other rice varieties. In addition to this, the increase in PSII activity of Aathira and Jyothi after exposure to short term HL stress (2 h) might be due to the possible involvement of alternative electron transport pathways in inducing greater resistance to PSII damage as reported earlier in *Arabidopsis* leaves after HL stress (Ivanov et al., 2012).

5.1.3.2 Mitochondrial activity

In Jyothi, Swarnaprabha, Kanchana and Neeraja rice varieties, significant decrease in the mitochondrial activity was recorded after exposure to HL stress (0-8 h) and it may due to the impacts on mitochondrial membrane protein complexes or various mitochondrial enzymes in rice seedlings. However, the rate of reduction in Aathira variety was very less during 6-8 h of HL exposure. Stress conditions in plants promote directly or indirectly the mitochondrial inner membrane permeabilization and results in reduction of mitochondrial activity (Li and Xing, 2011). In accordance with Grigorova et al. (2012), mitochondria were affected in a variety-specific

manner under HL exposure and this organelle of the stress tolerant varieties were better preserved than those in the sensitive varieties. The high reduction of mitochondrial activity in Swarnaprabha seedlings was recorded after 8 h of HL treatment. In contrast, mitochondrial activity was found to be enhanced under mild stress of HL exposure (2-4 h) in Aathira seedlings, even though further increase in HL exposure leads to the reduction of respiratory O_2 evolution. This increase in respiration as referred in the case of mild stress of HL irradiation would also probably be interrelated to the increased mitochondrial electron flow through alternative electron pathway, a general response in plants towards various stressful conditions (Atkin and Macherel, 2009).

5.1.3.3 Enzymatic antioxidants

The enzymatic antioxidant molecules are discussed as one of the defensive mechanisms in plant cells against ROS and free radicals and providing protection against excessive light (Sharma et al., 2012). Metalloenzyme SOD is the most effective intracellular antioxidant enzyme and provides the first line of defense against the toxic effects of elevated levels of ROS in plant cells. The SOD scavenge $O_2^{\bullet-}$ in two different ways, one being its reduction to H_2O_2 and another by its oxidization to O_2. Further, the activity of GPOX, APX and CAT were activated, as the rate of O_2 and H_2O_2 radicals increases in cells by the activation of SOD (Gill and Tuteja, 2010).

In the present study, 0-8 h of HL treatment caused an enhancement in the activity of SOD in all the five rice varieties studied, Aathira, Jyothi, Kanchana, Neeraja and Swarnaprabha seedlings. However, the maximum rate of enhancement in SOD activity was observed in Aathira upon 8 h and in Jyothi upon 6 h of HL treatment over the control plants. Vuleta et al. (2016) reported that SOD activity was significantly greater in *Iris pumila* growing in

the HL exposed habitat, than that in the shaded one and it might indicate that the SODs have a prominent role in maintaining intracellular ROS and redox balance in plants exposed to HL stress. In addition to this, the rate of enhancement in SOD activity in Kanchana and Neeraja varieties upon exposure to HL stress was less as compared to other varieties. In the case of Swarnaprabha, even though a slight increase in SOD activity was noticed after short term exposure of HL stress (2 h), it was highly decreased after 4-8 h of HL exposure. This reduction in the enzyme activity of sensitive rice varieties may be due to the fact that the SOD is either degraded or the synthesis process of the same is inhibited in response to HL stress.

GPOX activity of rice varieties treated with HL stress for 0-8 h showed an enhanced activity profile and Athira as well as Jyothi varieties exhibited maximum GPOX activity, while Swarnaprabha showed less GPOX activity. Moreover, the seedlings of Kanchana and Neeraja recorded maximum GPOX activity after 6 h of HL exposure. This is to counter the increase of H_2O_2 (arising out of the action of SOD) generated in rice seedlings as a result of HL treatment. It was reported that GPOX activity was enhanced along with other antioxidant enzymes upon exposure to HL stress due to increase in H_2O_2 accumulation (Morita et al., 1999). Like SOD and GPOX, the ascorbate-glutathione cycle also plays a key role in scavenging ROS and protecting plants from oxidative stress and it is catalyzed by the enzymes APX, MDHAR, DHAR, and GR. APX reduces H_2O_2 to H_2O upon various environmental stresses after utilizing ascorbate as specific electron donor (Caverzan et al., 2016; Pandey et al., 2017b). The activity of APX was significantly enhanced in all five rice varieties studied and maximum activity was recorded in Aathira and least APX activity was recorded in Swarnaprabha upon HL exposure as compared to other rice varieties. Vuleta et al. (2016) experimentally proved that APX as the primary H_2O_2 scavenger in *Iris pumila* leaves exposed to HL stress.

From the results obtained, it was clearly evident that HL treatments caused increase in activation of various free radical scavenging enzymes, SOD, GPOX and APX in rice seedlings and it was higher in Aathira and lower activities of these enzymes was recorded in Swarnaprabha seedlings after 0-8 h of HL exposure. Thus the tolerant rice varieties is equipped to counter the oxidative stress more rapidly and vigorously, by taking advantage of the enhanced activities of SOD, GPOX and APX. Thus, it was clear that Aathira was the most tolerant variety and Swarnaprabha was the most sensitive variety after tertiary screening of five rice varieties (Aathira, Jyothi, Swarnaprabha, Kanchana and Neeraja) towards HL exposure with respect to PSI, PSII, mitochondrial activity and various antioxidant enzyme activities.

5.1.4 Final analysis of the most tolerant and sensitive rice varieties towards HL exposure

Aathira (most tolerant) and Swarnaprabha (most sensitive) - the two varieties with totally contrasting features with respect to HL tolerance potential was finally characterized after exposure to HL stress (2000 $\mu molm^{-2}s^{-1}$) of various exposure periods (0-8 h) by analyzing various parameters such as Chl *a* fluorescence parameters, leaf gas exchange parameters, UV-B absorbing compounds and NPQ. For analyzing the impact of extreme HL stress (8 h) on both Aathira and Swarnaprabha seedlings, analysis of chloroplast ultrastructure, xanthophyll cycle pigments and leaf wax was carried out.

5.1.4.1 Photosynthesis

The effect of high photon flux densities causes the reduction of photosynthetic capacity in plants and it can be characterized by quantifying the changes in PSII characteristics *via* measurements of the Chl *a* fluorescence parameters (Strasser et al., 1999, 2000). Different periods of HL

exposure negatively influenced the Chl *a* fluorescence parameters in the leaves of Aathira and Swarnaprabha leaves and the reduction rate was dose dependent in both rice varieties. Under 0-8 h of HL treatment, the values of $PI_{(abs)}$, Fm, $SFI_{(abs)}$, Fv/Fo, area above the fluorescence curve, Φ_{Eo}, Ψ_o and Tf(max) were significantly decreased and it was predominant in Swarnaprabha as compared to Aathira leaves. The rate of decrease in Chl *a* fluorescence parameters was faster and higher in Swarnaprabha leaves at short term HL exposure (2 h) and there was no further decrease observed with increase in HL exposure period. In contrast, Vj was found to be increased even in the initial time period of HL treatment (2 h) in both Aathira and Swarnaprabha seedlings and therefore could conclude that short term HL exposure is adequate for studying the effect of photoinhibition in rice varieties and characterize them as tolerant and sensitive varieties.

The higher decrease of area above the fluorescence curve in Swarnaprabha and comparatively lower rate of decrease in Aathira seedlings suggests that HL stress highly inhibits the electron transfer rates at the donor side of PSII in Swarnaprabha leaves than Aathira and it might induce a structural damage of reaction center leading to decreased excitation energy transfer from the antenna to the reaction center which ultimately leads to drastic reduction of photochemistry in rice seedlings (Larsson et al., 1998; Strasser et al., 2000). Similar observations were also reported earlier in two barley cultivars treated with either HL or salt and based on the effect the cultivars were differentiated into tolerant and sensitive type (Kalaji et al., 2011, 2012). Under HL treatment, the electron transport quantum yield (Φ_{Eo}) and the yield of electron transport per trapped exciton (Ψ_o) were greatly decreased in sensitive rice variety Swarnaprabha than tolerant variety Aathira and it proved that HL exposure negatively influenced the electron transport at the acceptor side of PSII. Likewise, HL treatment decreased the rate of Fm in both Aathira and Swarnaprabha leaves and the maximum rate of reduction

was recorded upon imparting 8 h of HL stress and it indicates that the donor side of PSII, especially the oxygen evolving complex was damaged and it resulted in the reduction of electron transport (Phyo and Chung, 2017).

Plant vitality as assessed by $PI_{(abs)}$ decreased significantly in both varieties after 8 h of HL treatments and the extremely reduced $PI_{(abs)}$ recorded in Swarnaprabha over Aathira on exposure to HL indicates the greater loss of plant vitality in Swarnaprabha leaves under HL treatment. $PI_{(abs)}$ is the product of three independent characteristics: the concentration of reaction centers per Chl, a parameter related to primary photochemistry and a parameter related to electron transport (Strasser et al., 2004). Moreover, treatment with HL impaired the PSII structure and electron transport system of Swarnaprabha more severely than that of Aathira leaves as inferred from the parameter $SFI_{(abs)}$. Gautham et al. (2014) suggested that the use of Chl *a* fluorescence measurement as an effective tool to screen wheat genotypes for their stress tolerance and they found that Tf(max) which is used to indicate the time when maximum fluorescence value (Fm) is reached decreased significantly in low yielding wheat genotypes during heat stress conditions while in high yielding genotypes only a slight decrease or a negligible increase was observed. Likewise, the rate of light trapping and electron transport was highly decreased in Swarnaprabha seedlings upon HL exposure. Similarly, the photosynthetic efficiency of two barley cultivars, Arabi Aswad and Arabi Abiad, was negatively influenced after HL exposure as concluded by various Chl *a* fluorescence parameters and it was found that former was more tolerant while latter was more sensitive to HL stress (Kalaji et al., 2012). Further, they found that $PI_{(abs)}$ is a sensitive parameter to explore the effect of HL exposure on PSII activity after a single day of stress application.

An alteration of PSII energy fluxes per CSo in response to HL stress in Aathira and Swarnaprabha was visualized by phenomenological leaf models

of photosynthetic apparatus and it was found that Swarnaprabha had a lower level of calculated ETo/CSo and RC/CSo and higher values of DIo/CSo and ABS/CSo as compared to Aathira leaves after exposure to various extents of HL irradiation. There was no significant change in trapping flux (TRo/CSo) during HL treatment in both rice varieties. Similar findings were also noticed in *Alhagi sparsifolia* (Li et al., 2014) and in camellia leaves (Krüger et al., 2006) where ETo/CSo and RC/CSo was reduced while DIo/CSo and ABS/CSo was enhanced upon exposed to HL stress. High susceptibility of Swarnaprabha seedlings towards HL exposure negatively influenced the PSII energy fluxes per reaction center (RC) as revealed from specific membrane model of PSII. The expression ABS/RC can be taken as a calculated average amount of chlorophyll which channels excitation energy into reaction centers (Strasser et al., 2004). Therefore, the reduction in ABS/RC can be taken as the decrease in an average antenna size in rice plants after exposure to HL irradiation. TRo/RC represents the maximal rate by which an exciton is trapped by the RC resulting in the reduction of Q_A and decrease of this ratio in Swarnaprabha indicates that all the Q_A^- has been reduced but it is not able to oxidize back due to further hindrance in electron transport. Therefore, Q_A^- cannot transfer electrons efficiently to Q_B and as a result maximum energy is lost in dissipation without any effective utilization (Srivastava et al., 1998; Mathur et al., 2011, 2013).

Correspondingly, energy dissipation, as indicated by DIo/CSo and DIo/RC increased in HL treated rice varieties and dissipation occurs as fluorescence and energy transfer to other systems. As the inactive centers increased, the DIo/CSo and DIo/RC also increased because the inactive centers were unable to trap the photon so the untrapped photons increased which is in correlation with the results obtained in rice leaves of Aathira and Swarnaprabha (Jiang et al., 2008; Yan et al., 2013). The estimated high electron fluxes (ETo/CSo and ETo/RC) in Aathira seedlings reflects the

potential proportions of the flux of electrons which result in higher light reaction. Indeed, $PI_{(abs)}$, Φ_{Eo}, ETo/CSo, DIo/CSo, ETo/RC and DIo/RC were found to be more reliable for exploring the effect of changes in PSII activity and are important for assessing the HL tolerance potential in the seedlings of different rice varieties.

HL exposure also altered the fundamental processes of photosynthesis as evidenced by various gas exchange parameters. The reduction of net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration rate (E) was more prominent in Swarnaprabha than Aathira seedlings upon treatment with 0-8 h of HL irradiation. The diffusive (stomatal and mesophyll) limitations are most influential for HL induced alteration of gas exchange parameters in rice leaves. The reduction in photosynthetic efficiency induced by HL was first triggered by stomatal closure as evidenced by SEM images, resulting in limitation of ambient CO_2 diffusion to the mesophyll cells and thus reduction in photosynthetic efficiency (Franck and Vaast, 2009; Sanusi et al., 2011). According to Zhang et al. (2016b), HL irradiation induced deactivation of rubisco, which suppressed P_n and E in *Platanus orientalis*, *Melia azedarach* and *Solanum lycopersicum* seedlings. Thus the enhancement rate of inhibition in photosynthesis under HL exposure in Swarnaprabha, followed by Aathira could be due to reduced carbon assimilation and inhibition of PSII photochemistry (both donor and acceptor side of PSII) as evidenced by Fv/Fo and area over the fluorescence curve respectively.

In addition to this, most of the stomata were fully closed and damaged in both abaxial and adaxial surfaces of Aathira and Swarnaprabha leaves subjected to 8 h of HL treatment. However, the HL induced stomatal closure was more severe in Swarnaprabha than Aathira leaves, attributing high sensitivity of the former one towards HL exposure. However, the stomata

were fully opened and functional in the untreated plants of both varieties. Thus, the high reduction of stomatal conductance in Swarnaprabha and comparatively lower reduction in Aathira, results in reduction of gaseous exchange, ultimately resulting in decline of photosynthesis in Swarnaprabha leaves. It has been reported that H_2O_2 can act as a local or systemic signal for leaf stomata closure and finally leaf acclimation to HL irradiaton (Karpinska et al., 2000). Tani et al. (2001) concluded that the stomatal limitation to photosynthesis is responsible for the limited photosynthetic activity of *Pteridophyllum racemosum* to increased light stress conditions.

Stomatal parameters such as stomatal length and width on both adaxial and abaxial epidermis were greatly reduced in Swarnaprabha leaves upon exposure to 8 h of HL irradiation. In the case of Aathira leaves, the stomatal length and width was maintained as that of untreated leaves, suggesting that stomatal limitation to photosynthesis was nil in Aathira as compared to Swarnaprabha leaves. Previously, it was reported that stomatal length was decreased with increasing HL irradiance in *Eucalyptus globulus* leaves (James and Bell, 2000). Likewise, Xu-yang et al. (2017) reported that stomatal length, stomatal width and stomatal frequency were reduced under exposure to HL treatments (300 and 400 $\mu molm^{-2}s^{-1}$) in *Brassica* seedlings.

Maintaining structural integrity and organization of chloroplast in plants is necessary for the conversion of light energy and the photosynthetic machinery is mainly localized in thylakoid membranes of the chloroplasts. It was reported earlier that the abiotic stresses decreases the photosynthetic efficiency and electron transport activity that might be associated with the changes in the structure of photosynthetic apparatus (Mittal et al., 2012; Shu et al., 2013). On exposure to 8 h of HL irradiation, the chloroplast ultrastructure was altered slightly in highly tolerant variety, Aathira and to a greater extent in most sensitive variety, Swarnaprabha as evidenced by TEM

images of ultrathin sections of rice leaves. The ellipsoid chloroplasts in control leaves appeared as swollen and round in shape after HL treatment in rice seedlings of Swarnaprabha. However, the shape of chloroplasts in Aathira after HL exposure was almost maintained as in control leaves and this was justified with the high PSI and PSII activity recorded in Aathira variety.

The susceptibility of plant leaves to photoinhibition is linearly related to the degree of thylakoid membrane stacking. According to Anderson and Aro (1994), plants with greater membrane stacking are more susceptible to photoinhibition and have higher D1 protein degradation. Swarnaprabha was more sensitive to HL induced photoinhibition, which had the presence of thick and numerous stacks in control leaves than untreated leaves of Aathira. These findings were in accordance with Zhao et al. (2017), wherein they observed that the degree of thylakoid membrane stacking in chloroplasts were higher in susceptible rice variety Zhefu802 as compared to tolerant variety Chl-8. The thick and numerous thylakoid stackings get destacked on exposure to HL and it was at a higher rate in Swarnaprabha. The chloroplast ultrastructure of the leaves, including a regular thylakoid arrangement and dense stacking of grana, are destroyed during HL condition in watermelon seedlings (Duan et al., 2014). It was demonstrated that HL exposure (500 $\mu molm^{-2}s^{-1}$) in *Arabidopsis* leaves resulted in unstacking of the thylakoid membranes and the structural flexibility of the thylakoid membrane provides the basis for a high NPQ formation and high levels VAZ pool size of plants (Schumann et al., 2017).

5.1.4.2 UV-B absorbing compounds

One of the best documented protective responses to HL exposure is the increased accumulation of compounds such as flavonoids and anthocyanins in the vacuoles of epidermal cells which can be correlated with the protection of the leaf mesophyll tissue. Significant increase in anthocyanin content was

recorded in Aathira as compared to Swarnaprabha seedlings, on being subjected to different time intervals of HL treatment (0-8 h). Albert et al. (2009) found that HL induced anthocyanin accumulation in *Petunia* plants protects photosynthetic machinery from ROS mediated oxidative stress without reducing the maximal photosynthetic assimilation rate. The results indicated that Aathira seedlings could accumulate flavonoids than Swarnaprabha even at short term of HL exposure (2 h) and maximum accumulation was recorded after 8 h of HL stress and therefore could ensure protection even under long term HL stress. However, the flavonoid content accumulated in Swarnaprabha seedlings even after 8 h of HL exposure was very less, revealing its high susceptibility. Flavonoids have high antioxidant activity and can stabilize and protect the lipid phase of the thylakoid membrane by quenching the excited ^{3}Chl and $^{1}O_2$ (Agawal and Rathore, 2007).

In addition, it has been reported that the photoinduced accumulation of flavonoids occurs by an activity of several enzymes of phenylpropanoid biosynthetic pathway such as PAL and chalcone synthase of the flavonoid biosynthetic pathway (Kubasek et al., 1992; Dixon and paiva, 1995; Rigano et al., 2016). The activity of PAL enhanced in the seedlings of Aathira and Swarnaprabha and the increase was significant in both varieties from the initial period of HL exposure. But the rate of enhancement was more in Aathira than Swarnaprabha seedlings. Etiolated pea seedlings respond to HL illumination by changing the activity of phenylpropanoid and flavonoid synthesizing enzymes mainly by PAL activity (Hrazdina and Parsons, 1982). Su et al. (2016) demonstrated that remarkable tolerance to HL irradiation in radish sprouts was provided by the increased accumulation of anthocyanins and PAL activity.

5.1.4.3 Cuticular wax deposition

One of the most important functions of cuticular wax is to protect plants from excessive visible light irradiation. Wax content and composition in plants can vary significantly with species or genotypes, developmental stage and environmental growth conditions (Jenks and Ashworth, 1999). It was found that the treatment with 8 h of HL exposure resulted in accumulation of epicuticular wax deposition in both Aathira and Swarnaprabha leaves. According to Olascoaga et al. (2014), wax layer contribute to photoprotection in the needles of *Pinus sylvestris* from HL induced photodamage. SEM images of epidermal cells of the leaf surfaces showed some deposition of cuticular waxes and it was more pronounced in the adaxial surfaces as compared to the abaxial surfaces of leaves in both Aathira and Swarnaprabha varieties. Hess et al. (2002) reported that *Sophora microphylla* adapted to HL are tolerant to the effects of UV-B than plants growing in lower light intensities and this might be due to the enhanced accumulation of epicuticular wax deposition in the leaves of sunny plants than shady plants.

FT-IR spectra provided additional evidence corroborating the SEM data, about the accumulation and biochemistry of epicuticular wax in rice leaves after HL treatment and it revealed that the major components of the leaf surface epicuticular waxes are primary alcohols in both Aathira and Swarnaprabha rice seedlings. Hess et al. (2002) also found that the major component of the *Sophora microphylla* leaf surface cuticular waxes was primary alcohols as recorded by FT-IR analysis. A strong peak corresponding to symmetrical and asymmetrical stretching of methylene groups appeared on imparting 8 h of HL treatment in Aathira as compared to control leaves and it could be related to changes in the aliphatic lipid chain and finally it provide the additional protection towards HL exposure. Recently, Willick et al. (2018)

studied the composition and structure of cuticular wax by FTIR-ATR analysis in wheat leaves of drought tolerant and sensitive cultivars and found that the most significant changes between cultivars were related to changes in the aliphatic lipid chain (represented by the asymmetrical and symmetrical methylene vibrational peaks) and the ester region represented by the carbonyl ester peaks. In addition, the concentration of aliphatic lipid chains observed on the adaxial surface of leaves in drought resistant cultivar was greater than that of sensitive cultivar under control conditions. However, there was no variation in the functional groups of epicuticular wax in Swarnaprabha on imparting 8 h of HL treatment, though a peak corresponds to C–H stretching was highly reduced as compared to the control plants, which might not have any significance as far as HL tolerance is concerned.

5.1.4.4 Non-photochemical quenching and xanthophyll cycle

NPQ protect plants from photodamage induced by HL exposure (Zhao et al., 2017). Exposure to 0-8 h of HL illumination increased the NPQ in both Aathira and Swarnaprabha varieties. However, in contrast to Swarnaprabha, Aathira showed higher and faster NPQ under HL treatments, which suggested that the higher rate of NPQ in Aathira might be responsible for higher P_n and electron transport in HL treatments. Earlier reports have showed that NPQ was getting enhanced under HL exposure in various plant species (Zhou et al., 2007; Betterle et al., 2015; Poulson and Thai, 2015; Barczak-Brzyżek et al., 2017). NPQ have a prominent role in photoprotection of rice plants that are adapted to higher light intensities and it has a regulatory role in buffering the electron transport chain against rapid fluctuations in light (Murchie et al., 2015). The NPQ induction in Aathira is much higher than in Swarnaprabha and this would be in agreement with the higher levels of zeaxanthin and the higher de-epoxidation state of xanthophyll cycle pigments found in 8 h of HL irradiated Aathira leaves.

Xanthophyll cycle is one of the most efficient mechanisms protecting plants under over excitation conditions of HL stress and some components of this cycle act as quenchers of singlet chlorophyll, thus preventing the formation of ROS. The concomitant increase of some xanthophylls and carotenes with 8 h of HL exposure (2000 $\mu molm^{-2}s^{-1}$) in Aathira and Swarnaprabha rice leaves can be attributed to the involvement of these pigments in dissipation of HL induced excess energy. HL exposure in rice leaves led to the reduction of violaxanthin and concomitant accumulation of antheraxanthin and zeaxanthin in both Aathira and Swarnaprabha seedlings. However, conversion of violaxanthin to zeaxanthin was maximum in Aathira than Swarnaprabha leaves upon exposure to HL irradiation. The results were corroborated with the findings obtained by HPLC analysis in the leaves of *Secale cereale* leaves exposed to HL illumination (1200 $\mu molm^{-2}s^{-1}$) wherein it was found that zeaxanthin content was highly enhanced after HL treatment (Janik et al., 2008). Similar pattern of changes in xanthophyll pigments have been reported in *Hevea brasiliensis* after exposure to HL conditions (Gopalakrishnan and Annamalainathan, 2016).

Lutein is the most abundant xanthophyll pigment in higher plants and it has a primary role in quenching of triplet chlorophyll states (Estaban et al., 2008). HL irradiated rice seedlings showed the lowest epoxidation state of xanthophyll pigments and lutein content, which suggested that the absence of lutein is compensated by increased levels of other xanthophylls derived from β-carotene (Pogson et al., 1996). High NPQ formation in Aathira leaves upon exposure to HL stress than Swarnaprabha could be correlated with the high xanthophyll cycle pigments accumulated in Aathira since xanthophylls are involved in the NPQ of excitation energy in LHCII of the PSII. In addition to this, zeaxanthin presumably exert a further photoprotective effect as an antioxidant in the lipid phase of the thylakoid membrane and related to the

stabilization of thylakoid membrane lipids under extreme HL stress (Gruszecki and Strzałka, 2005).

The de-epoxidation state of xanthophyll cycle pigments was enhanced while the epoxidation state of xanthophyll cycle pigments was decreased upon exposure to 8 h of HL irradiation in rice leaves. Likewise, the proportion of the xanthophyll cycle pool to total Chl was tremendously increased in Aathira than Swarnaprabha upon HL exposure, revealing the superior nature of Aathira variety in terms of tolerance potential towards thermal dissipation of excess energy after HL induced photooxidative stress. Photoprotection of the photosynthetic machinery to HL conditions through xanthophyll pigments was observed in *Plantago media* plants (Golovko et al., 2011). Moreover, the extent of de-epoxidation of xanthophyll cycle pigments and the content of total xanthophyll cycle pigments expressed per chlorophyll was greatly enhanced in *Vinca minor* upon HL exposure (Verhoeven et al., 1999). Previously it was reported that oxidative stress in plants leads to an increase in β-carotene content since the deactivation of singlet oxygen is provided by β-carotene in plants (Ramel et al., 2012). Similarly, HL (8 h) exposure induced accumulation of α and β-carotene content was higher in the leaves of Aathira and comparatively lower in Swarnaprabha leaves upon 8 h of HL treatment.

5.2 UV-B stress

The dose of ambient UV-B radiation in sunlight varies with latitude and is relatively higher in tropical regions where rice is grown as the major food crop than in temperate regions. Previous studies from the last two decades suggest that nearly 50% of crop plants of India are affected by elevated levels of solar UV-B as India lies in a low ozone belt and receives more UV-B radiation than those of temperate regions with higher latitudes (Mitra, 1991; Madronich et al., 1995). Temporal observation of the

UV-B intensity in natural solar condition of Kerala recorded from time to time during 2014-2015 found that maximum UV-B recorded was on 12 noon of March (3.581Wm^{-2}) and the lowest value of UV-B ranged from 1.31 to 1.67 Wm^{-2} at 4 pm in all the months (Sahebrao, 2015). Reduction in rice yield during puncha season in Kerala with an increase in UV-B absorbing compounds in the leaves of rice plants gives an indication on the negative effect of UV-B radiation on rice yield (Nandini et al., 2014).

The oxidative stress effects induced by UV-B in plants varies between varieties to varieties and, in thirteen rice varieties the tolerance level of UV-B was found to be 28 $kJm^{-2}d^{-1}$ and above this high dosage, nearly immediate, visually observable stress symptoms on the metabolic processes was observed. Therefore in the present study, the various doses of UV-B exposure was fixed as 0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$ for one week. The dosage of UV-B applied in the present study coincides with some previous studies in rice seedlings. Dai et al. (2006) studied the effect of UV-B radiation on 10 d old rice seedlings of two varieties and oxidative stress effects were evaluated after irradiation with 6 and 13 $kJm^{-2}d^{-1}$ for 4 weeks. In this study, although dosage was less, the period of exposure was higher. Likewise, Du et al. (2011) fixed the UV-B doses of 14.4, 28.7 and 57.5 kJm^{-2}, for irradiating rice seedlings and they noticed the appearance of light brown patches on leaves after exposure to severe dose of UV-B.

5.2.1 Primary screening for identifying the tolerant and sensitive rice varieties towards UV-B irradiation

Various morphological, anatomical, physiological and biochemical effects of UV-B irradiation on thirteen prominent rice varieties (Aathira, Jyothi, Aiswarya, Annapoorna, Mattatriveni, Swetha, Karuna, Mangalamahsuri, Harsha, Varsha, Swarnaprabha, Neeraja and Kanchana) were studied, which helped to classify them into probable sensitive and

tolerant types. The primary screening was done by analyzing the growth parameters (shoot length and fresh weight), photosynthetic pigments and the rate of lipid peroxidation after exposure to various doses of UV-B irradiation (7, 14, 21, and 28 $kJm^{-2}d^{-1}$) for one week.

5.2.1.1 Growth parameters

First line of evidence for UV-B stress in plants was visualized in terms of morphological alterations including leaf chlorosis, necrosis, wilting, reduced shoot length etc. (Dai et al., 1995; Hopkins et al., 2002; Kakani et al., 2003a). Various doses of UV-B treatments resulted in a decrease of shoot length and fresh weight in all rice varieties studied and Neeraja, Swetha, Aiswarya and Swarnaprabha showed maximum reduction in the shoot length and fresh weight. However, the lower dose of UV-B (7 $kJm^{-2}d^{-1}$) exhibited a negligible increase in shoot length of Aathira, Mangalamahsuri, Kanchana, Jyothi and Annapoorna (<5%) as compared to the control plants, revealing the priming effect of low dose of UV-B in rice seedlings (Thomas and Puthur, 2017). Likewise, the least reduction in fresh weight was found in Aathira, Mangalamahsuri, Jyothi, Kanchana and Annapoorna after 0-28 $kJm^{-2}d^{-1}$ of UV-B exposure. The reduced growth rate measured in terms of shoot length and fresh weight in few varieties is the sum effect of the deleterious effects of UV-B irradiation. According to Cechin et al. (2007), the decrease in the biomass and further physiological disorders in crop plants upon UV-B irradiation is the result of photosynthesis inhibition and carbon accumulation. Zuk-Golaszewska et al. (2003) reported that the different doses of UV-B radiation (4, 8, 12 $kJm^{-2}d^{-1}$) applied to *Avena fatua* and *Setaria viridis* resulted in a decrease of plant height and fresh weight, as well as it caused the leaf curling in both of the species. It was showed that the leaves of both Aiswarya and Swarnaprabha seedlings showed necrotic spots of yellow to brown spots and stripes on the leaf lamina after exposure to 21 and 28 $kJm^{-2}d^{-1}$ of UV-B

radiation and it was coinciding with chlorophyll depletion, which could be due to several reasons such as excessive photodegradation and disintegration of chloroplasts (Henriques, 2008; Zhou et al., 2013).

5.2.1.2 Photosynthetic pigments

Among the rice varieties studied, Neeraja, Swetha, Aiswarya and Swarnaprabha showed higher reduction of chlorophyll content under various doses of UV-B irradiation; whereas in Aathira, Mangalamahsuri, Kanchana, Jyothi and Annapoorna the reduction of chlorophyll content was least after UV-B irradiation (0-28 $kJm^{-2}d^{-1}$). The remaining varieties, Mattatriveni, Karuna, Harsha and Varsha showed an intermediate level of reduction in photosynthetic pigments. The reduction in chlorophyll content upon abiotic stress has been discussed in 5.1.1.1. Sahebrao (2015) experimentally proved that UV-B radiation reduced the Chl *a*, *b* and total chlorophyll content in two rice varieties of Kerala, Jyothi and Uma at tillering and flowering stages. Li et al. (2011) studied the intraspecific variation in sensitivity towards UV-B stress in 10 wheat cultivars and found that the reduction of total chlorophyll content was higher in sensitive cultivars and that could be due to the changes in plant hormones. Surprisingly, the chlorophyll content of Kanchana, Jyothi and Annapoorna was enhanced on imparting low dose (7 $kJm^{-2}d^{-1}$) of UV-B irradiation and was obviously due to the increase in leaf density as previously reported in *Colophosphermum* tree ecotypes and in the shrubs *Leucadendron* and *Phylica* upon UV-B irradiation (Musil et al., 2002).

Among the rice varieties Aathira, Mangalamahsuri, Kanchana, Jyothi and Annapoorna recorded the highest accumulation of carotenoid content after UV-B treatment. The carotenoid accumulation in Mattatriveni, Karuna, Harsha and Varsha was not to the extent as in the case of Aathira, Mangalamahsuri, Kanchana, Jyothi and Annapoorna at various doses of UV-B radiation (0-28 $kJm^{-2}d^{-1}$). The photo-protective role of carotenoids in plants

is well established in 5.1.1.1. The carotenoids could enhance the total antioxidant capacity of tobacco leaves after UV-B stress (Shen et al., 2017). Likewise, increased carotenoid accumulation was earlier reported by Zhang et al. (2005) in two cultivars of *Poa pratensis* after UV-B irradiation and suggested that tolerant cultivar with high carotenoid accumulation might be an effective choice to breeders for cultivating in the environments with high UV-B radiation. However, the reduction of carotenoid content in Swetha, Aiswarya and Swarnaprabha upon high doses of UV-B treatment (21 and 28 $kJm^{-2}d^{-1}$) has obviously proved their sensitivity towards UV-B induced photooxidative damage as reported in *Plantago major* (Salama et al., 2011).

5.2.1.3 Lipid peroxidation

The rate of lipid peroxidation was increased in Neeraja, Swetha, Aiswarya and Swarnaprabha after UV-B stress and reached maximum at 21 $kJm^{-2}d^{-1}$ of UV-B irradiation. Oxidative stress induced MDA production in plants is the final product of lipid peroxidation which was discussed in 5.1.1.2. Lipid peroxidation was significantly higher when rice seedlings were subjected to higher doses of UV-B stress (21 and 28 $kJm^{-2}d^{-1}$) than that of lower doses of UV-B irradiation (7 and 14 $kJm^{-2}d^{-1}$). Among the rice varieties studied, Aathira, Mangalamahsuri, Kanchana, Jyothi and Annapoorna exihibited lower accumulation of MDA content as compared to Mattatriveni, Karuna, Harsha and Varsha seedlings upon various extents of UV-B irradiation. Moreover, the lower rate of lipid peroxidation in these rice varieties indicates its increased tolerance to oxidative damage achieved through increased activities of antioxidant function, which is the most common mechanism for detoxifying ROS during UV-B stress (Köhler et al., 2017; Shen et al., 2017). The results obtained in this study were agreeing with several other findings made by Tripathi et al. (2011) in *Linum usitatissimum*, Takshak and Agrawal (2014) in *Withania somnifera*, Hagh et al. (2012) in

sunflower cultivars and Ma et al. (2016) in poplar plants upon UV-B irradiation.

In general, after primary screening of thirteen rice varieties by analyzing growth parameters, photosynthetic pigments and the rate of lipid peroxidation under various doses of UV-B irradiation, they were classified into probable sensitive and tolerant types. It was found that Aathira, Mangalamahsuri, Kanchana, Jyothi and Annapoorna were probable tolerant types and Neeraja, Swetha, Aiswarya and Swarnaprabha were the probable sensitive ones. The remaining four varieties (Mattatriveni, Karuna, Harsha and Varsha) were showing the intermediate characters and they were eliminated after the primary screening for selection of highly tolerant and sensitive types.

5.2.2 Secondary screening for identifying the tolerant and sensitive rice varieties towards UV-B irradiation

The selected five tolerant (Aathira, Mangalamahsuri, Kanchana, Jyothi and Annapoorna) and four sensitive (Neeraja, Swetha, Aiswarya and Swarnaprabha) rice varieties from primary screening was further screened for selecting the highly tolerant and sensitive ones among them on the basis of their performance after exposure to various doses of UV-B irradiation (0, 7, 14, 21, and 28 $kJm^{-2}d^{-1}$) for one week by analyzing various primary metabolites (total protein, total free amino acids, soluble sugar and proline content) and non enzymatic antioxidants (ascorbate, glutathione and phenolics).

5.2.2.1 Primary metabolites

Significant increase in the total protein content was recorded in Aathira, Mangalamahsuri, Kanchana, Annapoorna, Neeraja, Swetha and Jyothi as compared to untreated plants. However, UV-B irradiation induced

accumulation of protein content was very less in Aiswarya and Swarnaprabha seedlings. These findings were consistent with the results of proteomic studies by Du et al. (2011), who proposed that UV-B radiation (14.4, 28.7 and 57.5 $kJm^{-2}d^{-1}$) resulted in induction of proteins involved in detoxification/ antioxidation and defense mechanism in rice seedlings. It was reported that UV radiation increased protein content in *Brassica napus* which was related to the synthesis of defense proteins such as HSPs (Nasibi and Kalantari, 2005) and the role of HSPs upon oxidative stress has been discussed in 5.1.2.1. However, lower dose of UV-B radiation (7 $kJm^{-2}d^{-1}$) reduced the total protein content slightly in Aiswarya seedlings as previously reported by Salama et al. (2011) in some desert plants. Proteolysis or inactivation of proteins and enzymes can be caused directly by UV-B induced photolysis of aromatic amino acids or disulfide groups in plants.

The accumulation of soluble sugar content was very high in Aathira, Mangalamahsuri and Kanchana seedlings on exposure to various doses of UV-B irradiation. However, the accumulation of soluble sugars in Aiswarya and Swarnaprabha after irradiation with UV-B treatment was very less. The other rice varieties exhibited an intermediate level of sugar accumulation upon UV-B irradiation. The vital role of soluble sugars in maintaining the properties of biological molecules under stressed conditions was discussed in 5.1.2.1. Moreover, sucrose might have a protective role against oxidative stress due to its capacity to act synergistically with phenolic compounds and function as an integrated redox system in plants and thus contributing to stress tolerance (Bolouri-Moghaddam et al., 2010). In contrast to these findings, UV-B radiation decreased the soluble sugar content in three soybean cultivars which indicated a greater movement of assimilates from the source to sink without utilizing the same for maintaining the cellular integrity (Gao and Yang, 2016).

In addition to this, the content of total amino acids after UV-B irradiation was enhanced in Annapoorna, Neeraja, Swetha and Jyothi seedlings and maximum accumulation was registered at highest dose of UV-B (28 $kJm^{-2}d^{-1}$) and at the same time amino acid content was not as higher like Aathira, Mangalamahsuri and Kanchana varieties upon UV-B treatment. However, the amino acid content in Aiswarya and Swarnaprabha was not much enhanced throughout the various doses of UV-B treatment. Amino acid metabolic pathways play an important role in stress related metabolism in plants. Likewise, stress-associated defensive metabolites such as flavonoids synthesized from amino acid metabolic pathways demonstrate the vital roles of amino acid metabolism in response to UV-B stress (Häusler et al., 2014). Moreover, amino acid accumulation was enhanced in response to different durations of UV-B irradiation (12, 24, 48 and 96 h) in a medicinal herb, *Glycyrrhiza uralensis* (Zhang et al., 2018). In *Arabidopsis*, UV-B treatment dramatically increased the glutamate, glutamine, histidine, lysine, asparagine, aspartate, and threonine and the accumulation of specific amino acids in oxidative stress tolerance is generally considered as a species specific trait (Kusano et al., 2011).

UV-B treatment of various doses differentially influenced the proline biosynthesis in various rice varieties. The increased proline content in the seedlings of Aathira, Mangalamahsuri and Kanchana after UV-B irradiation, which was distinctly higher than other varieties, indicates that the antioxidative activity of proline could be particularly involved in the protection of these rice varieties against injury caused by UV-B exposure. The proline has got a potential to detoxify ROS molecules and imparts stress tolerance in plants which was discussed in 5.1.2.1. Likewise, the proline accumulation in Annapoorna, Neeraja, Swetha and Jyothi seedlings was significantly higher than Aiswarya and Swarnaprabha seedlings. This augmentation of proline accumulation in Annapoorna, Neeraja, Swetha and

Jyothi plants results in a lesser degree of injury as compared to Aiswarya and Swarnaprabha by the multiple protective role of proline such as decreasing the rate of lipid peroxidation and stabilizing membranes and protein machinery. The higher contents of proline together with other antioxidants imparted greater tolerance to *Vigna* species on exposure to UV-B stress (Dwivedi et al., 2015). However, the proline content was not found to be enhanced under UV-B radiation in lettuce plants and it was probably due to the low dosage of UV-B radiation applied, which did not influence proline overproduction (Rajabbeigi et al., 2013).

5.2.2.2 Non enzymatic antioxidants

The increase in ascorbate content of Aathira, Mangalamahsuri and Kanchana was higher even after the initial dose (7 $kJm^{-2}d^{-1}$) of UV-B treatments and maximum accumulation was recorded upon 28 $kJm^{-2}d^{-1}$ of UV-B treatment. The free radicals generated as a result of UV-B induced oxidative stress would have effectively scavenged by ascorbate as indicated by the low levels of MDA in seedlings of Aathira, Mangalamahsuri and Kanchana varieties. Considering the multifold overproduction of this antioxidant, this molecule can be recommended as a biomarker for notifying the increased tolerance or susceptibility of rice varieties towards UV-B stress. The potential of ascorbate towards ROS scavenging in response to abiotic stress was discussed in 5.1.2.2. But in the case of rice varieties Annapoorna, Aiswarya and Swarnaprabha the ascorbate content recorded upon UV-B irradiation was very less than other rice varieties and that would enhance the sensitivity of these varieties towards UV-B. Gao and Zhang (2008) suggested that *Arabidopsis* plants with an impaired production of ascorbate are more sensitive to UV-B treatment.

Similar to ascorbate, the glutathione content was enhanced gradually with the increase of UV-B dose and the high rate of accumulation was

observed in Aathira, Mangalamahsuri and Kanchana upon 28 $kJm^{-2}d^{-1}$ of UV-B as compared to other rice varieties. Similar results were observed in previous studies by Wu et al. (2001) in rice, Gao (2007) in *Arabidopsis* and Zhang et al. (2017) in *P. vulgaris* upon UV-B irradiation. Even though, an increase in glutathione content was recorded in Annapoorna, Neeraja, Swetha and Jyothi seedlings after UV-B exposure, the rate of accumulation was not much higher as recorded in former ones. However, in Aiswarya and Swarnaprabha, the rate of accumulation was much lower upon UV-B irradiation. In the case of carotenoids and α-tocopherol, antioxidative effects in plants are restricted to the chloroplasts, whereas ascorbate and glutathione molecules can be found in different cell compartments and thus they can protect plants from oxidative damage in other cell compartments besides chloroplasts as well (Zechmann and Müller, 2010; Foyer and Noctor, 2011).

In the case of *O. sativa* varieties, Aathira, Mangalamahsuri and Kanchana were found to be superior to other varieties with respect to enhanced phenolic content upon UV-B irradiation. Likewise, the phenolic content was found to be greatly enhanced in Annapoorna, Neeraja, Swetha and Jyothi seedlings as compared to Swarnaprabha, Aiswarya and Neeraja after exposure to UV-B radiation right from 7 $kJm^{-2}d^{-1}$ and maximum accumulation was observed upon 28 $kJm^{-2}d^{-1}$ of UV-B irradiation. This is in agreement with the results obtained for *Vicia faba* seedlings under UV-B radiation, i.e., the enhancement of accumulation of phenolic compounds appeared to provide protection to photosynthetic components under UV-B irradiation (Younis et al., 2010). Previously, various authors reported that increased antioxidant capacity of plants under UV-B stress and it is correlated to total phenolic content (Zavala et al., 2001; Ravindran et al., 2008; Shaukat et al., 2013; Escobar-Bravo et al., 2017). Dwivedi et al. (2015) studied physiological and biochemical aspects of two *Vigna* species under UV-B radiation and concluded that the higher contents of total phenolics together

with other antioxidants recorded in *V. acontifolia* as compared to *V. mungo* provided greater tolerance to the former species.

After secondary screening of nine rice varieties based on their performance under various doses of UV-B irradiation, they were classified into most probable sensitive and tolerant types. It was observed that Aathira, Mangalamahsuri and Kanchana were highly tolerant and Aiswarya and Swarnaprabha were the highly sensitive varieties towards various doses of UV-B exposure. The remaining four varieties (Jyothi, Annapoorna, Neeraja and Swetha) were showing intermediate tolerance characters and they were eliminated after the secondary screening for the selection of most tolerant and sensitive types.

5.2.3 Tertiary screening for identifying the most tolerant and sensitive rice varieties towards UV-B irradiation

The selected three highly tolerant (Aathira, Mangalamahsuri and Kanchana) and two sensitive (Aiswarya and Swarnaprabha) rice varieties after two successive screening was further analyzed for the determination of most tolerant and sensitive varieties among them on the basis of their performance after exposure to various doses of UV-B irradiation (0-28 $kJm^{-2}d^{-1}$) by analyzing photosystems activity (PSI and PSII activity), mitochondrial activity and activity of various enzymatic antioxidants (SOD, GPOX and APX).

5.2.3.1 PSI and PSII activity

The decrease in PSI activity as evidenced by reduced O_2 consumption in the leaves of Aiswarya and Swarnaprabha from 7 $kJm^{-2}d^{-1}$ onwards upto 28 $kJm^{-2}d^{-1}$ of UV-B may be due to an enhanced charge recombination in the reaction center, which might represent an incipient inactivation of PSI as found in tropical trees (Krause et al., 2003). As far as the PSI activities of rice

varieties were concerned, UV-B radiation caused more decrease in PSI activity of Swarnaprabha leaves. In contrast, the increase in PSI activity of Aathira, Mangalamahsuri and Kanchana upon exposure to stressful UV-B conditions was found to be related to the increased demand for ATP necessary for repairing processes of damaged thylakoid membrane protein complexes (Fedina et al., 2009; León-Chan et al., 2017). Moreover, UV-B treated Aathira, Mangalamahsuri and Kanchana leaves dissipated the excess photon energy through NPQ by enhancing the ability of cyclic electron flow around PSI to induce acidification of the thylakoid lumen since induction of NPQ requires a pH gradient across the thylakoid membranes.

Similarly, UV-B irradiation resulted in highly reduced PSII activity in Aiswarya and Swarnaprabha leaves as compared to Aathira, Kanchana and Mangalamahsuri leaves. Olsson et al. (2000) experimentally proved that UV-B irradiation damaged the D1 protein of PSII and inhibits the *de novo* synthesis of thylakoid proteins during the UV-B radiation treatment. UV-B radiation has a more negative impact on PSII because the D1 and D2 proteins are very sensitive due to the chemical transformations taking place in amino acids of these proteins with regard to double bonds (Zlatev et al., 2012; Kale et al., 2017). In all five rice varieties, the high rate of reduction in PSII activity recorded upon severe dose of UV-B irradiation (28 $kJm^{-2}d^{-1}$) implies that UV-B irradiation negatively influences on the photosynthetic efficiency.

5.2.3.2 Mitochondrial activity

In Aathira and Kanchana varieties, treatment with various doses of UV-B exhibited an enhanced mitochondrial activity over the control seedlings. Likewise, the lower doses of UV-B (7 and 14 $kJm^{-2}d^{-1}$) in Mangalamahsuri seedlings slightly enhanced the mitochondrial activity, while high doses of UV-B exhibited a reduction in mitochondrial activity. Indeed, a reduced photophosphorylation in the chloroplasts of UV-B treated varieties

may enhance the demands on mitochondria-based energy supply (Baier and Dietz, 2005). Thus increased rate of respiration recorded in Aathira and Kanchana even when imparted with high dose of UV-B irradiation would be aiding the seedlings to tolerate the adverse condition induced by UV-B irradiation. In contrast to the above, treatment with various doses of UV-B highly reduced the mitochondrial activity in Swarnaprabha and Aiswarya seedlings. The reduction in mitochondrial activity might be due to the increased destruction of mitochondrial membranes and/or due to the destruction of a number of mitochondrial proteins by ROS molecules generated under oxidative stress in plants (Taylor et al., 2004).

5.2.3.3 Enzymatic antioxidants

ROS scavenging antioxidant enzymes, such as SOD, GPOX and APX, play a vital role in removing destructive oxygen species and was extensively discussed in 5.1.3.3. In rice varieties, various doses of UV-B stress triggered the enhancement in activities of GPOX and APX which detoxifies the harmful H_2O_2 produced by the SOD, the most effective intracellular metalloenzyme that plays a key role in protecting molecules in plants from oxidation damage (Ahmad et al., 2010; Dai et al., 2006). In the present study, Aathira and Mangalamahsuri showed higher SOD activity after 0-28 $kJm^{-2}d^{-1}$ of UV-B stress than other three rice varieties and maximum SOD activity in these two varieties was recorded upon severe dose of UV-B irradiation (28 $kJm^{-2}d^{-1}$). The higher doses of UV-B treatments (21 and 28 $kJm^{-2}d^{-1}$) lead to decrease in SOD activity in Swarnaprabha seedlings and this result in high accumulation of MDA content in this variety and finally it leads to its high susceptibility towards UV-B radiation. The reduced SOD activity under UV-B irradiation showed that ROS balance was affected to a certain extent as reported in poplar plants upon UV-B irradiation (Ma et al., 2016). Higher activity of SOD suggests that Aathira and Mangalamahsuri possessed more

efficient superoxide dismutation so that more effective scavenging of superoxide radicals into H_2O_2 takes place and the H_2O_2 produced by SOD was then rapidly removed by GPOX and APX in thse rice seedlings.

Likewise, GPOX activity in Aathira seedlings was tremendously increased than other rice varieties and maximum activity resulted after exposure to 28 $kJm^{-2}d^{-1}$ of UV-B irradiation. However, the increase in GPOX activity was least in Swarnaprabha as compared to other varieties. This underlines the potential of Aathira for ROS scavenging upon UV-B irradiation as compared to other rice varieties. Dai et al. (2006) investigated the impact of elevated UV-B radiation (6, 13 $kJm^{-2}d^{-1}$ for 14 and 28 d) on enzymatic antioxidants in the leaves of two UV-B susceptible rice cultivars (IR74 and Dular) and found that activities of antioxidant enzymes were enhanced by 13 $kJm^{-2}d^{-1}$ after 14 d of UV-B exposure. Likewise, Zhang et al. (2017) reported that SOD and GPOX activities increased in *Prunella vulgaris* subjected to UV-B radiation. A similar study reported markedly greater SOD, GPOX and APX activities after UV-B exposure in leaves of *Artemisia annua* (Rai et al., 2011).

Similar to SOD and GPOX activity, APX activity was also significantly enhanced in all rice varieties under 0-28 $kJm^{-2}d^{-1}$ of UV-B irradiation. Of different antioxidant enzymes studied, the APX with enhanced activity was found to be a reliable marker for assessing UV-B tolerance of these rice varieties since it has significantly enhanced in rice seedlings upon UV-B irradiation. APX activity in Aathira seedlings was significantly enhanced right from 7 $kJm^{-2}d^{-1}$ onwards and the maximum APX activity was noticed after 28 $kJm^{-2}d^{-1}$ of UV-B irradiation. However, the enhancement in APX activity of Mangalamahsuri and Kanchana was not higher as in the case of Aathira seedlings. The APX activity of Aiswarya and Swarnaprabha seedlings was very less than other rice varieties after UV-B irradiation,

pointing the higher susceptibility of these two varieties towards harmful UV-B radiation. It was reported that APX activity was enhanced in *Arabidopsis* after UV-B treatment (Rao et al., 1996). He et al. (2014) studied the differential responses of two rice cultivars (Baijiaolaojing and Yuelianggu) towards various doses of UV-B irradiation (2.5, 5 and 7 $kJm^{-2}d^{-1}$) and found that a highly significant correlation between leaf H_2O_2 and the activity of SOD, CAT and GPOX in these two cultivars.

From the results obtained, it was clearly evident that UV-B treatments caused activation of various free radical scavenging enzymes, such as SOD, GPOX and APX in rice seedlings and it was higher in Aathira and less in Swarnaprabha seedlings. Thus, it became more evident that Aathira was the most tolerant variety and Swarnaprabha was the most sensitive variety after tertiary screening of five rice varieties (Aathira, Mangalamahsuri, Kanchana, Aiswarya and Swarnaprabha) towards various doses of UV-B irradiation with respect to PSI, PSII, mitochondrial activity and various antioxidant enzyme activities.

5.2.4 Final analysis of the most tolerant and sensitive rice varieties towards UV-B irradiation

The selected most tolerant rice variety, Aathira and the most sensitive variety Swarnaprabha were finally characterized after exposure to various doses of UV-B (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$) and various parameters were analyzed (Chl *a* fluorescence parameters, leaf gas exchange parameters, UV-B absorbing compounds and NPQ). Further, it was found that UV-B treatment (28 $kJm^{-2}d^{-1}$) was a dosage, quite good enough to evaluate the negative impacts of a highest dosage on some morphological and ultralevel changes in these two rice varieties. Hence for the further evaluation of UV-B stress tolerance potential in Aathira and Swarnaprabha, analysis of

chloroplast ultrastructure, xanthophyll cycle pigments and leaf wax was carried out after imparting higher dose of UV-B (28 $kJm^{-2}d^{-1}$).

5.2.4.1 Photosynthesis

Analysis of various Chl *a* fluorescence parameters revealed that exposure to various doses of UV-B (0-28 $kJm^{-2}d^{-1}$) induced photoinhibition of the photochemistry of Aathira and Swarnaprabha leaves. Different doses of UV-B reduced $PI_{(abs)}$, area above the fluorescence curve, Fv/Fo, maximal fluorescence (Fm), $SFI_{(abs)}$, Φ_{Eo} and Ψ_o in both rice varieties and the decrease was dose dependent in both varieties. The rate of decrease in these Chl *a* fluorescence parameters was maximum at 28 $kJm^{-2}d^{-1}$ in both varieties. However, the rate of reduction was higher in Swarnaprabha than Aathira as compared to control leaves, attributing the tolerant potential of latter towards UV-B irradiation. The similar findings were reported by Lidon and Ramalho (2011) in rice leaves after exposure to UV-B irradiation of 20.8 $kJm^{-2}d^{-1}$ for one week. A decrease in fluorescence yield of rice leaves in response to oxidative stress can be attributed to an inhibition of electron flow at oxidizing site of PSII and it might be due to the inhibition of electron transport at donor side of the PSII which was already discussed in 5.1.4.1. However, Vj was found to increase upon UV-B irradiation and the rate of increase was more in Swarnaprabha as compared to Aathira seedlings upon increase in the dosage of UV-B. The increase in Vj is an indication that UV-B irradiation impaired the PSII electron transport at acceptor side of PSII in Swarnaprabha leaves more severely than that of Aathira variety as reported earlier by Kalaji et al. (2012) in barley culivars upon HL illumination. Ma et al. (2016) reported that the damage caused by UV-B irradiation was prominent at the donor side of PSII in two poplar species, *Populus alba* and *Populus russkii*. In addition to this, Šprtovä et al. (2000) found that UV-B irradiation had a negative impact

on the primary photosynthetic reactions as revealed by Chl *a* fluorescence measurements in *Picea abies*.

The flux of absorption (ABS/CSo and ABS/RC) and electron transport (ETo/CSo and ETo/RC) was decreased in both Aathira and Swarnaprabha with increasing doses of UV-B and the reduction was higher in Swarbnaprabha than Aathira leaves. Similar findings were reported recently by Kalaji et al. (2018) in barley cultivars exposed to various stress factors (salt, heavy metal, high temperature, low temperature, HL and low light). TR_o/RC was lowered in both Aathira and Swarnaprabha upon UV-B radiation and maximum reduction was registered at 21 $kJm^{-2}d^{-1}$ of UV-B treatment in Swarnaprabha as recorded in *Salix arctica* upon UV-B irradiation (Albert et al., 2005). UV-B irradiation showed an increase in fluorescence dissipation (DIo/CSo and DIo/RC) in Swarnaprabha than Aathira leaves and it reflects the loss of connectivity between PSII heterogeneous units in leaves (Force et al., 2003). Conclusively it was found that Fm, Ψ_o, $SFI_{(abs)}$, ETo/CSo, DIo/CSo and RC/CSo were highly altered under various doses of UV-B irradiation (0-28 $kJm^{-2}d^{-1}$) and these are important parameters for screening the UV-B tolerance potential in the seedlings of different rice varieties.

UV-B treatment caused the reduction of net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration rate (E) in leaves of rice varieties, Aathira and Swarnaprabha. Even though, the effect of UV-B radiation on g_s was more or less similar in both rice varieties, the rate of reduction in P_n and E was higher in Swarnaprabha after UV-B irradiation as compared to Aathira leaves. Recently, Reyes et al. (2018) observed that UV-B treatment (18.24 $kJm^{-2}d^{-1}$) negatively affected g_s, which led to a reduced assimilation rate and carboxylation capacity in *Quinoa* plants. Moreover, UV-B induced reduction of P_n can result from reduced Calvin cycle enzymes content and activity, decreased thylakoid integrity and altered

chloroplast structure (Ma et al., 2016) and similar observations were made in the case of rice seedlings upon HL exposure and the same was discussed in 5.1.4.1. Direct inhibition of UV-B on photosynthetic rates and transpiration through stomatal inhibition has been reported in various plants (Zhao et al., 2004; Cechin et al., 2008; Basahi et al., 2014), which occurs due to both abaxial and adaxial epidermal stomatal damage/closure which reduced the diffusion of ambient level of CO_2 into mesophyll as evidenced by SEM images in both Swarnaprabha and Aathira leaves and it was more severe in the former.

Plants regulate the stomatal movements to maintain CO_2 influx and water loss in leaves which critically affect photosynthetic efficiency and plant growth. The reduction in photosynthetic efficiency of Swarnaprabha than Aathira leaves under UV-B radiation was as a result of reduced stomatal conductance and/or stomatal closure in the former. Previous studies have revealed that UV-B induced stomatal closure in *Vicia faba* and *Arabidopsis* leaves (Jansen and van den Noort, 2000; He et al., 2013). The SEM analysis of rice leaves treated with UV-B radiation (30 d) revealed that the stomata and silica cells appeared with irregular shape and damages to the epicuticular wax was also observed (de Almeida et al., 2012). Moreover, H_2O_2 and nitric oxide regulated the UV-B induced stomatal closure by acting as signaling molecules through UVR8 mediated signaling pathway (He et al., 2005; Tossi et al., 2014).

Stomatal length and width in both adaxial and abaxial epidermis of Swarnaprabha was highly reduced than Aathira variety. Kakani et al. (2003b) reported that UV-B irradiation decreased stomatal length and width on both adaxial and abaxial leaf surfaces of *Gossypium*. In the case of Aathira also, a slight increase in the stomatal width on the adaxial epidermis was registered, suggesting that it has the potential for leaf cooling by permitting

evapotranspiration than sensitive variety Swarnaprabha. Likewise, UV-B induced oxidative stress leads to alteration in stomatal length and stomatal width in melon plants, even though stomatal frequency was unchanged by exposure to UV-B radiation (Sosa-Flores et al., 2014).

UV-B treatment (28 $kJm^{-2}d^{-1}$) in rice leaves resulted in damage to the structure of chloroplasts in Aathira and Swarnaprabha seedlings, as manifested by disruption of thylakoid membranes and disintegration of the double membrane of the chloroplast as reported previously in pea leaves after UV-B irradiation (He et al., 1994). The chloroplasts became more rounded after UV-B irradiation in both rice varieties, while the control leaves of both Aathira and Swarnaprabha varieties were more elliptical in shape. Yu et al. (2013) reported that UV-B irradiation resulted in a change of chloroplast shape from elliptical to more spherical and a gradual disturbance of thylakoid organization, including a distortion of granal arrangement, accompanied by an increase in the starch granules also observed in a high yielding rice variety (Liangyoupeijiu). UV-B treatment (14.4 and 22.5 $kJm^{-2}d^{-1}$) induced the alteration in the shape of chloroplast of *Betula* seedlings (Wulff et al., 1999).

5.2.4.2 UV-B absorbing compounds

The tremendous increase in anthocyanin and flavonoid content in leaves of Aathira seedlings after UV-B irradiation, indicates that anthocyanin and flavonoids are involved in the alleviation of ROS generation as a result of oxidative burst which was discussed in 5.1.4.2. The relatively higher anthocyanin and flavonoid content in Aathira seedlings as compared to Swarnaprabha seedlings subjected to various doses of UV-B irradiation ensures better protection which reflects in higher photosynthetic rate. Thus, anthocyanins and flavonoids can reduce the UV-B penetration and protect the photosynthetic apparatus, and the content of these protective compounds may vary in different varieties (Feng et al., 2007). The increased rate of UV-B

absorbing compounds in Aathira, followed by Swarnaprabha seedlings in response to UV-B irradiation corroborates with the findings of Mahdavian et al. (2008) in *Capsicum annuum*, Ravindran et al. (2010) in *Indigofera tinctoria* and Pandey et al. (2012) in *Brassica juncea*.

The different varieties of the plant species respond differentially to UV radiation leading to their tolerance potential towards various extents of irradiation. For example, in soybean, increasing UV_{A+B} radiation doses induced a significant increase in PAL activity, anthocyanins and flavonoid content in the three soybean cultivars and found that Giza-111 cultivar was most tolerant to UV_{A+B} radiation followed by Giza-22, while Giza-35 was the most sensitive cultivar to enhanced UV_{A+B} radiation (SalehDina et al., 2006). The PAL activity in Aathira seedlings enhanced upto 21 $kJm^{-2}d^{-1}$ of UV-B treatment. However, the rate of PAL activity was lower in Swarnaprabha subjected to UV-B treatment as compared to Aathira variety and maximum activity in the latter was registered after 14 $kJm^{-2}d^{-1}$ of UV-B irradiation. Shaukat et al. (2013) concluded that the accumulation of anthocyanins, flavones and increased PAL activity in UV-B treated *Vigna* provide a protective mechanism to UV-B radiations.

5.2.4.3 Cuticular wax deposition

Epicuticular wax on leaf surfaces provided the first line of defense by acting as a barrier from the adverse conditions of the environment which was already discussed in 5.1.4.3. The epicuticular wax content increased significantly in the leaf surface of both Aathira and Swarnaprabha varieties. However, Aathira leaves accumulated more epicuticular wax content on the leaf surfaces than Swarnaprabha leaves after exposure to 28 $kJm^{-2}d^{-1}$ of UV-B radiation and it suggested that Aathira variety was highly protected against UV-B irradiation. Steinmüller and Tevini (1985) reported that UV-B radiation enhanced the wax content in barley and bean leaves. Moreover, UV-B

irradiation enhanced the epicuticular wax content in tolerant genotype of tobacco while it was reduced in the more sensitive genotype (Barnes et al., 1996). Moreover, this data was additional evidence corroborating the SEM analysis wherein there were clear visual differences in thickness of epicuticular wax content on both abaxial and adaxial leaf surfaces and the thickness of the wax layer varied between rice varieties after exposure to 28 $kJm^{-2}d^{-1}$ of UV-B irradiation, which was higher in Aathira and lower in Swarnaprabha leaves. Previously, Kumari and Agrawal (2010) reported the presence of dense waxy covering on the adaxial surface of UV-B exposed leaves of Indian aromatic plant *Cymbopogon citrates* by SEM analysis after irradiation with UV-B stress (9.6 $kJm^{-2}d^{-1}$).

The major component of the leaf surface epicuticular waxes of both rice varieties, Aathira and Swarnaprabha are primary alcohols as calculated from the IR spectra and as reported by Hess et al. (2002), wherein they found that the major component of the *Sophora microphylla* waxes are primary alcohols. In addition to this, a peak correspond to methylene C–H bending vibration was recorded after exposure to 28 $kJm^{-2}d^{-1}$ of UV-B treatment in Aathira seedlings and it may be a necessary modification to provide enhanced protection in Aathira leaves upon UV-B irradiation. Likewise, a peak corresponds to C–H stretching was highly reduced and a new peak corresponds to carbonyl ester vibrations associated with methyl esterified lipids was appeared upon imparting 28 $kJm^{-2}d^{-1}$ of UV-B treatment in Swarnaprabha leaves. This was corroborated by recent research by Willick et al. (2018) on wax composition of wheat cultivars upon drought stress. They also found that drought stress caused a significant increase in the concentration of carbonyl esters due to the alterations in the methyl esterification of the subcuticular pectin on the leaf surface of the susceptible cultivar.

5.2.4.4 Non-photochemical quenching and xanthophyll cycle

Indeed, NPQ is a process in which absorbed light energy is dissipated as heat which was discussed in 5.1.4.4. Under various doses of UV-B irradiation (0-28 $kJm^{-2}d^{-1}$), Aathira had high NPQ induction than in Swarnaprabha leaves, which correlated with the greater tolerance potential of the former variety to harmful doses of UV-B irradiation. Previously, it was proved that NPQ was getting enhanced in plants under UV-B induced oxidative stress and it was higher in those which showed higher tolerance potential (Szôllôsi et al., 2008; Hassan et al., 2011; Moon et al., 2011). Moreover, the high NPQ in Aathira seedlings was again associated with a greater increase in antheraxanthin and zeaxanthin contents, indicating that a higher level of thermal dissipation involved in the xanthophyll cycle occurred in Aathira leaves when exposed to various doses of UV-B irradiation. The findings were consistent with the results of *Scutellaria baicalensis* under conditions of enhanced UV-B radiation (Quan et al., 2018).

The involvement of the xanthophyll cycle pigments for enhancing the photoprotective capacity in response to oxidative stress in rice seedlings has been discussed in 5.1.4.7. The increase in de-epoxidation state of the xanthophylls (as determined by the DEPS ratio) in Aathira and Swarnaprabha rice leaves upon irradiation with 28 $kJm^{-2}d^{-1}$ of UV-B stress is due to higher zeaxanthin content than violaxanthin in the xanthophyll pool, and is indicative of a photosynthetic system that is utilizing non-photochemical quenching for the dissipation of excess energy. Thus, the conversion of violaxanthin to zeaxanthin was maximum in Aathira leaves than Swarnaprabha after UV-B irradiation revealing the significant adaptation of Aathira leaves to high dose of UV-B. The higher de-epoxidation of xanthophyll pigments in *Quercus petraea* seedlings exposed to UV-B radiation suggested that de-epoxi compounds of V+A+Z cycle is involved in photo protective processes (Szôllôsi et al., 2008). The de-epoxidation state of the xanthophylls was linearly correlated with NPQ and it was also in respect to the rice variety.

These findings indicated that the de-epoxidation state of the xanthophyll cycle pigments are closely related to thermal energy dissipation from the antenna of PSII in Aathira to a greater extent and to a lesser extent in Swarnaprabha leaves. In addition to this, the content of lutein was decreased after UV-B irradiation in both rice varieties as reported in tomato and *Arabidopsis* leaves after UV-B treatment and it might be due to the more contribution of violaxanthin cycle in heat dissipation which often functions in parallel and responds more rapidly than lutein epoxide cycle (Moon et al., 2011).

In addition to this, the results point to a pronounced increase in xanthophyll cycle pool (V+A+Z) to total chlorophyll and total carotenoids in Aathira leaves with an induction of the de-epoxidation state, where these pigments are involved in thermal dissipation of excitation energy in plants after exposure to 28 $kJm^{-2}d^{-1}$ of UV-B irradiation. However, the xanthophyll cycle pool to total carotenoids reduced in Swarnaprabha leaves, correlating to the lower rate of NPQ formation and less photoprotection towards harmful UV-B irrdiation. Recently, Reyes et al. (2018) reported that the xanthophyll pigments might be relevant in the protection of chloroplasts from UV-B induced oxidative damages in *Chenopodium quinoa* seedlings. The UV-B induced accumulation of both α and β carotenes are higher in Aathira than Swarnaprabha seedlings revealing the importance of carotenes in photoprotection and permitting maximum energy consumption by photosynthesis. Kirchgebner et al. (2003) reported that high content of α and β carotenes in *Picea abies* protect the photosynthetic machinery from harmful excited state of Chl. Conclusively it was shown that the primary photoprotective mechanism in adaptation of rice seedlings to high dose of UV-B is the increase in the degree of the de-epoxidation state of the xanthophyll cycle pigments and finally it provides the harmless dissipation of excess excitation energy as heat in the photochemical system of rice leaves.

SUMMARY AND CONCLUSIONS

Studies on thirteen commonly cultivated high yielding rice varieties (Aathira, Aiswarya, Annapoorna, Harsha, Jyothi, Kanchana, Karuna, Mangalamahsuri, Mattatriveni, Neeraja, Swarnaprabha, Swetha and Varsha) was carried out for studying the functional imprints of HL (0-8 h) and UV-B irradiation (0-28 $kJm^{-2}d^{-1}$) on these rice varieties and thus to identify the HL and UV-B tolerant and sensitive varieties based on their HL and UV-B stress tolerance potential. The effects of various levels of HL and UV-B irradiation on the morphological, physiological, anatomical and biochemical processes were varied in different rice varieties. Some of these parameters were part of the mechanism of tolerance which can aid in distinguishing the tolerant and sensitive rice varieties towards HL and UV-B induced oxidative stress. Thirteen varieties of rice seeds were germinated and grown in half strength Hoagland solution. After 10 d of growth, rice seedlings were exposed to HL stress (2000 $\mu molm^{-2}s^{-1}$), at different time intervals (0, 2, 4, 6 and 8 h). For imparting UV-B treatment, rice seedlings were exposed to UV-B radiation after 4 d of germination so as to impart various doses (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$).

The major conclusions derived from the present study on functional imprints of HL and UV-B irradiation in thirteen rice varieties are summarized below:

- Two successive screenings revealed that Aathira and Jyothi were tolerant ones while Swarnaprabha, Kanchana and Neeraja were sensitive varieties towards various treatment periods of HL exposure. Likewise Aathira, Mangalamahsuri and Kanchana varieties have high tolerance potential and Aiswarya and Swarnaprabha were sensitive varieties towards various doses of UV-B irradiation.

- Based on various morphological, physiological, anatomical and biochemical parameters, Aathira was found to be most superior in terms of tolerance potential whereas Swarnaprabha was the most sensitive variety towards both HL and UV-B irradiation.

- Kanchana and Aiswarya varieties responded to HL and UV-B signals independently and tolerance mechanisms to individual stress factors were also differentially modulated in these two varieties. Kanchana was found to be tolerant to UV-B irradiation while susceptible to HL exposure. In contrast Aiswarya was tolerant to HL exposure and susceptible to UV-B irradiation.

- Exposure of rice seedlings to HL and UV-B radiation caused significant increases in the accumulation of various metabolites such as total protein, free amino acids and soluble sugar content, which confirms that the observed tolerance towards HL/UV-B stress is partially due to the increased production of above metabolites.

- High tolerance nature of Aathira seedlings towards HL and UV-B irradiation is also due to the enhanced activities of enzymatic (SOD, APX and GPOX) and enhanced accumulation of non enzymatic antioxidants (ascorbate, glutathione and total phenolics).

- Mainly ascorbate, GPOX and APX provided the protection against oxidation and related harm towards photosynthetic and mitochondrial machinery in rice seedlings under HL exposure. Whereas, total phenolics, ascorbate, glutathione, APX and GPOX provided the protection towards harmful effects of UV-B exposure in rice seedlings.

- The photoinhibition induced by HL and UV-B irradiation (as assessed by the PSI, PSII, Chl *a* fluorescence analysis, gas exchange parameters and chloroplast ultrastructure) in rice seedlings irradiated with HL and

UV-B is mainly due to the decrease in photosynthetic pigments content and the impaired gaseous exchange caused by the partially opened/closed stomata, and the damages on thylakoid membranes as revealed by TEM analysis.

- Chl *a* fluorescence analysis revealed that short term effect of HL (2 h) is more effective for studying the consequence of HL on photosynthetic efficiency and tolerance potential towards HL stress as compared to long term (8 h) in rice varieties.

- Performance index, Φ_{Eo}, ETo/CSo, DIo/CSo, ETo/RC and DIo/RC were found to be more reliable for exploring the effect of changes in photochemistry and important for assessing the HL tolerance potential in the seedlings of different rice varieties. Similarly, Ψ_o, Fm, $SFI_{(abs)}$, ETo/CSo, DIo/CSo and RC/CSo were highly altered under UV-B exposure and these parameters are important for screening the UV-B tolerance potential in the seedlings of different rice varieties.

- The decline in photosynthetic activities of rice seedlings exposed to HL and UV-B irradiation could also be contributed by a decrease in gaseous exchange as a result of reduction in stomatal conductance, which had occurred due to stomatal closure or damages to stomata as evidenced by SEM images.

- HL and UV-B irradiation in rice seedlings resulted in a change of chloroplast shape from elliptical to more spherical and a gradual disturbance of thylakoid organization, including distortion of granal and stromal arrangement as revealed by TEM images.

- The very low rate of disturbance in thylakoid organization of tolerant variety (Aathira) could be the part of structural flexibility of the

thylakoid membrane, which provides the basis for a high NPQ status and the high levels xanthophyll pigment pool size.

- UV-B absorbing compounds such as anthocyanins and flavonoids, PAL activity and cuticular wax deposition and its modification (revealed by FT-IR spectrometry) were highly significant in tolerant rice variety Aathira than susceptible variety Swarnaprabha after exposure to HL and UV-B irradiation.

- Thermal dissipation of excess absorbed energy known as NPQ is one of the most important of the rapidly activated regulatory mechanisms in rice seedlings involved in HL and UV-B conditions and it has a regulatory role in buffering the electron transport chain against oxidative stress induced by HL and UV-B irradiation. Aathira showed higher and faster NPQ than Swarnaprabha leaves under HL and UV-B treatments and it might be responsible for higher P_n and electron transport after exposure to HL and UV-B treatments.

- The accumulation and biochemistry of epicuticular wax in leaves protected the rice seedlings from excessive visible light and UV-B irradiation. FT-IR spectra revealed that Aathira leaves had increased symmetrical and asymmetrical methylene groups and provided an additional protection towards HL exposure. The methylene C–H bending vibration was recorded after exposure to 28 $kJm^{-2}d^{-1}$ of UV-B treatment in Aathira seedlings and this condition may provide an enhanced protection in Aathira leaves upon UV-B irradiation, while the sensitivity of Swarnaprabha was related to the increase in the concentration of carbonyl esters on its leaf surface under UV-B irradiation.

- The magnitude of variation in xanthophyll pigments (violaxanthin, zeaxanthin and antheraxanthin) and carotenes (α and β carotenes) after HL and UV-B irradiation in Aathira as compared to Swarnaprabha leaves is a clear indicator to analyze the excess energy dissipation as heat by xanthophyll cycle dependent mechanism in the former as compared to latter .

- The higher levels of zeaxanthin and the lower level of epoxidation state of xanthophyll pigments after HL and UV-B irradiation largely contributes towards thermal dissipation of excess light energy within the LHC of PSII and thus avoiding the imbalance between light absorption and the energy required for photosynthesis.

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Table 3: Shoot length, total chlorophyll content and Fv/Fm of thirteen *O. sativa* varieties after germination. Different alphabetical letters indicate statistically different means at p > 0.05.

Days	Aathira	Jyothi	Annapoorna	Aiswarya	Mattatriveni	Harsha	Swetha	Karuna	Mangalamahsuri	Varsha	Swarnaprabha	Kanchana	Neeraja
Shoot length plant^{-1} (cms)													
8	13.67±0.58^{a}	16.40±0.36^{a}	10.88±0.43^{a}	14.04±0.53^{a}	10.91±0.53^{a}	14.86±0.60^{a}	17.32±1.07^{a}	10.36±0.97^{a}	15.50±0.50^{a}	9.97±0.45^{a}	17.77±0.64^{a}	13.80±0.55^{a}	11.03±1.18^{a}
9	15.67±0.58^{b}	18.83±0.76^{b}	13.90±0.56^{b}	17.85±1.26^{b}	16.52±0.95^{b}	19.73±050^{b}	21.42±2.30^{b}	15.14±1.1^{b}	19.85±1.30^{ab}	13.02±0.63^{b}	21.71±1.22^{b}	17.36±0.77^{b}	16.17±1.56^{b}
10	20.53±0.81^{c}	25.55±0.70^{c}	17.28±0.28^{c}	23.10±0.75^{c}	24.23±0.20^{c}	24.03±0.45^{c}	25.87±0.81^{c}	21.97±0.58^{c}	22.10±0.30^{ab}	17.30±0.26^{c}	25.67±0.70^{c}	20.40±0.30^{c}	23.67±0.83^{c}
11	23.67±0.58^{d}	27.33±0.58^{d}	20.58±1.29^{d}	25.01±0.52^{d}	27.93±0.50^{d}	27.15±0.65^{d}	29.36±0.52^{d}	24.76±0.47^{d}	23.77±0.64^{ab}	19.06±0.67^{d}	27.74±0.60^{d}	23.04±0.53^{d}	25.13±1.50^{c}
12	25.50±0.50^{e}	28.58±0.37^{e}	21.27±0.87^{d}	26.85±1.03^{e}	28.93±0.50^{d}	27.92±0.52^{d}	29.65±0.15^{d}	26.95±0.42^{e}	27.12±0.50^{b}	20.98±0.77^{e}	29.10±0.58^{d}	24.73±0.59^{e}	28.33±0.76^{d}
Total chlorophyll content (mgg^{-1} DW)													
8	8.28±0.73^{c}	4.81±0.63^{b}	3.42±0.18^{c}	6.34±0.60^{c}	10.20±0.52^{a}	8.01±0.51^{c}	10.91±0.46^{b}	5.97±0.84^{b}	4.05±0.65^{c}	6.60±0.09^{a}	7.30±0.69^{d}	8.38±1.60^{b}	6.00±0.87^{b}
9	12.02±0.55^{b}	5.34±0.30^{ab}	3.90±0.56^{bc}	9.90±0.99^{b}	11.22±0.51^{a}	10.20±0.58^{ab}	12.03±0.92^{ab}	7.10±0.56^{a}	5.90±0.52^{b}	6.32±0.28^{a}	14.68±1.11^{c}	9.57±0.51^{b}	6.23±0.68^{b}
10	14.24±1.67^{a}	5.99±0.31^{b}	4.82±0.31^{a}	13.51±1.17^{a}	10.79±0.63a	10.69±0.72^{a}	12.75±1.07^{a}	7.61±0.18^{a}	7.24±1.04^{a}	6.75±0.60^{a}	19.80±0.56^{a}	12.77±0.37^{a}	9.23±0.89^{a}
11	11.36±1.01^{b}	5.05±0.56^{a}	4.61±0.60^{ab}	13.20±1.13^{a}	11.05±1.12^{a}	10.03±1.42^{ab}	11.52±0.91^{ab}	7.51±0.41^{a}	7.90±0.48^{a}	5.32±0.63^{b}	18.67±1.53^{ab}	13.41±0.93^{a}	8.63±0.71^{a}
12	10.78±0.55^{b}	4.59±0.05^{a}	4.58±0.18^{ab}	12.19±0.57^{a}	7.19±0.37^{b}	8.96±0.49^{bc}	11.98±0.57^{ab}	7.02±0.59^{a}	5.55±0.51^{b}	4.90±0.61^{b}	17.87±0.81^{b}	13.63±0.78^{a}	5.13±1.03^{b}
Fv/Fm (Relative Unit)													
8	0.77±0.02^{bc}	0.71±0.04^{c}	0.73±0.02^{b}	0.69±0.07^{b}	0.76±0.02^{b}	0.81±0.01^{a}	0.52±0.05^{b}	0.62±0.05^{b}	0.74±0.03^{b}	0.54±0.00^{c}	0.74±0.03^{b}	0.62±0.04^{c}	0.76±0.05^{a}
9	0.80±0.01^{a}	0.74±0.02^{bc}	0.79±0.01^{a}	0.69±0.07^{b}	0.81±0.01^{a}	0.80±0.01a	0.77±0.01^{a}	0.67±0.06^{b}	0.77±0.02^{ab}	0.80±0.01^{a}	0.79±0.01^{a}	0.73±0.05^{b}	0.81±0.01^{a}
10	0.81±0.01^{a}	0.81±0.01^{a}	0.79±0.00^{a}	0.80±0.01^{a}	0.82±0.01^{a}	0.81±0.00	0.78±0.01^{a}	0.80±0.02^{a}	0.82±0.01^{a}	0.82±0.01^{a}	0.81±0.01^{a}	0.82±0.01^{a}	0.82±0.00^{a}
11	0.79±0.01^{ab}	0.79±0.01^{a}	0.80±0.02^{a}	0.75±0.01^{ab}	0.79±0.01^{a}	0.80±0.01a	0.75±0.01^{a}	0.81±0.01^{a}	0.63±0.02^{c}	0.76±0.01^{b}	0.78±0.01^{a}	0.73±0.05^{b}	0.79±0.02a
12	0.74±0.02^{c}	0.78±0.01^{ab}	0.74±0.01^{b}	0.72±0.01^{ab}	0.67±0.02^{c}	0.76±0.02b	0.73±0.03a	0.75±0.01a	0.58±0.07^{c}	0.76±0.02^{b}	0.71±0.03^{b}	0.67±0.01^{bc}	0.67±0.07^{b}

Table 4: Total chlorophyll and carotenoid content of thirteen *O. sativa* varieties after exposure to different time intervals of HL treatments (0, 2, 4, 6 and 8 h). Different alphabetical letters indicate statistically different means at p > 0.05.

HL (h)	Aathira	Jyothi	Annapoorna	Aiswarya	Mattatriveni	Harsha	Swetha	Karuna	Mangalamahsuri	Varsha	Swarnaprabha	Kanchana	Neeraja
Total chlorophyll content (mgg^{-1} DW)													
0	14.24 ± 1.67^{c}	5.99 ± 0.31^{c}	4.82 ± 0.31^{a}	13.51 ± 1.17^{b}	10.79 ± 0.63^{b}	10.69 ± 0.72^{a}	12.75 ± 1.07^{a}	7.61 ± 0.18^{a}	7.24 ± 1.04^{ab}	6.75 ± 0.60^{a}	19.80 ± 0.56^{a}	12.77 ± 0.37^{a}	9.23 ± 0.89^{a}
2	14.79 ± 0.65^{c}	5.83 ± 0.10^{c}	5.06 ± 0.19^{a}	13.06 ± 0.53^{b}	10.33 ± 0.70^{b}	11.00 ± 0.58^{a}	11.70 ± 0.16^{ab}	7.58 ± 1.13^{a}	7.25 ± 0.61^{ab}	5.46 ± 0.29^{b}	19.72 ± 0.63^{a}	10.96 ± 0.51^{b}	6.76 ± 0.58^{b}
4	17.10 ± 0.41^{b}	6.70 ± 0.64^{bc}	5.01 ± 0.37^{a}	14.18 ± 0.63^{ab}	11.04 ± 0.79^{b}	10.23 ± 0.50^{a}	10.81 ± 0.55^{a}	7.81 ± 0.57^{a}	7.57 ± 0.30^{a}	5.62 ± 0.34^{b}	17.79 ± 1.63^{a}	10.23 ± 0.52^{b}	5.10 ± 0.60^{c}
6	21.77 ± 1.13^{a}	8.03 ± 0.69^{a}	6.36 ± 0.45^{a}	14.34 ± 0.08^{ab}	11.37 ± 0.95^{b}	11.01 ± 0.62^{a}	10.72 ± 0.53^{a}	7.20 ± 0.55^{ab}	6.30 ± 0.14^{bc}	5.05 ± 0.14^{bc}	12.85 ± 2.19^{b}	7.25 ± 0.61^{c}	4.77 ± 0.37^{c}
8	18.86 ± 0.79^{b}	7.16 ± 0.57^{ab}	5.69 ± 0.27^{b}	15.10 ± 0.58^{a}	13.14 ± 0.70^{a}	11.30 ± 0.23^{a}	10.07 ± 1.09^{a}	6.27 ± 0.23^{b}	6.07 ± 0.31^{c}	4.78 ± 0.20^{c}	11.74 ± 0.52^{b}	6.76 ± 0.57^{c}	3.04 ± 0.56^{d}
Total carotenoid content (mgg^{-1} DW)													
0	0.65 ± 0.04^{c}	0.47 ± 0.04^{b}	0.53 ± 0.02^{e}	2.31±0.28c	0.41 ± 0.08^{c}	4.44 ± 0.19^{c}	2.41 ± 0.10^{c}	2.37 ± 0.06^{d}	0.40 ± 0.05^{b}	0.82 ± 0.07^{b}	1.20 ± 0.15^{b}	0.77 ± 0.03^{a}	1.40 ± 0.07^{b}
2	0.69 ± 0.05^{c}	0.44 ± 0.01^{b}	0.69 ± 0.05^{d}	2.59 ± 0.17^{c}	0.48 ± 0.02^{bc}	4.45 ± 0.15^{c}	2.40 ± 0.17^{c}	2.61 ± 0.06^{c}	0.36 ± 0.07^{b}	0.91 ± 0.13^{b}	1.14 ± 0.05^{b}	0.83 ± 0.06^{a}	1.63 ± 0.13^{a}
4	1.07 ± 0.15^{b}	0.53 ± 0.12^{b}	0.91 ± 0.06^{c}	3.10 ± 0.11^{b}	0.54 ± 0.11^{b}	4.60 ± 0.10^{c}	2.50 ± 0.00^{c}	2.71 ± 0.09^{c}	0.58 ± 0.06^{a}	1.13 ± 0.01^{a}	1.23 ± 0.03^{b}	0.80 ± 0.07^{a}	1.39 ± 0.13^{b}
6	1.49 ± 0.25^{c}	1.03 ± 0.18^{a}	1.22 ± 0.10^{b}	3.81 ± 0.16^{a}	0.70 ± 0.04^{a}	5.48 ± 0.16^{b}	3.65 ± 0.09^{a}	2.98 ± 0.00^{b}	0.44 ± 0.10^{b}	1.21 ± 0.01^{a}	1.44 ± 0.01^{a}	0.61 ± 0.13^{b}	1.59 ± 0.05^{a}
8	1.70 ± 0.16^{c}	1.26 ± 0.24^{a}	1.55 ± 0.10^{a}	4.05 ± 0.14^{a}	0.55 ± 0.01^{b}	5.80 ± 0.08^{a}	3.21±0.02b	3.51 ± 0.05^{a}	0.59 ± 0.04^{a}	1.14 ± 0.01^{a}	1.44 ± 0.03^{a}	0.44 ± 0.10^{c}	1.58 ± 0.08^{a}

Table 5: Fv/Fo and malondialdehyde content of thirteen rice varieties after exposure to different time intervals of HL treatments (0, 2, 4, 6 and 8 h). Different alphabetical letters indicate statistically different means at p > 0.05.

HL (h)	Aathira	Jyothi	Annapoorna	Aiswarya	Mattatriveni	Harsha	Swetha	Karuna	Mangalamahsuri	Varsha	Swarnaprabha	Kanchana	Neeraja
Fv/Fo (Relative Unit)													
0	2.75±0.08^{a}	2.81±0.23^{a}	2.77±0.10^{a}	2.74±0.24^{a}	2.77±0.18^{a}	1.71±0.07^{a}	2.79±0.09^{a}	2.44±0.10^{a}	2.91±0.04^{a}	1.73±0.05^{a}	2.72±0.20^{a}	2.78±0.11^{a}	2.56±0.03^{a}
2	2.52±0.07^{a}	2.41±0.10^{b}	2.41±0.02^{b}	2.52±0.02^{a}	2.35±0.02^{ab}	1.19±0.03^{b}	2.21±0.15^{b}	1.88±0.12^{b}	2.34±0.14^{b}	1.31±0.09^{b}	1.91±0.51^{b}	1.74±0.09^{b}	1.31±0.09^{b}
4	1.54±0.11^{c}	1.86±0.03^{c}	1.75±0.03^{c}	1.87±0.06^{b}	1.94±0.06^{bc}	1.26±0.05^{b}	2.03±0.06^{b}	1.72±0.23^{b}	1.68±0.05^{c}	1.17±0.15^{b}	1.54±0.19^{bc}	1.89±0.11^{b}	1.35±0.19
6	1.77±0.13^{b}	1.95±0.05^{c}	1.69±0.08^{c}	1.56±0.08^{c}	1.88±0.08^{c}	1.21±0.18^{b}	1.52±0.16^{c}	1.72±0.06^{b}	1.62±0.07^{c}	1.13±0.12^{b}	1.37±0.02^{c}	1.26±0.20^{c}	1.30±0.18^{b}
8	1.97±0.20^{b}	1.55±0.02^{d}	1.72±0.10^{c}	1.55±0.06^{c}	1.41±0.06^{d}	1.16±0.07^{b}	1.44±0.09^{c}	1.31±0.06^{c}	1.58±0.08^{c}	0.97±0.01^{c}	1.49±0.10^{bc}	1.30±0.17^{c}	1.02±0.15^{c}
Malondialdehyde content (µmolg^{-1} DW)													
0	8.06±1.41^{a}	38.29±1.02^{c}	19.69±0.29^{c}	5.60±0.23^{c}	23.47±1.56^{c}	6.29±0.55^{c}	5.64±0.99^{c}	6.65±1.16^{c}	22.62±2.45^{b}	20.14±2.72^{d}	11.56±0.99^{d}	33.91±0.79^{d}	15.21±1.52d
2	7.36±0.05^{a}	39.38±1.04^{c}	19.77±0.72^{c}	5.58±0.15^{bc}	22.66±1.28^{c}	8.17±1.23^{b}	6.77±0.58^{c}	7.58±1.13^{c}	22.39±1.57^{b}	24.74±0.08^{c}	17.79±1.63^{c}	50.43±1.99^{c}	26.27±2.27^{c}
4	8.70±1.10^{a}	39.30±1.28^{c}	21.00±0.84^{bc}	5.81±0.57^{bc}	23.69±0.44^{c}	8.52±1.13^{b}	8.48±0.06^{b}	7.81±0.57^{c}	27.00±3.22^{b}	34.16±1.25^{b}	25.02±3.35^{b}	63.11±2.58^{b}	35.00±3.00^{b}
6	9.08±0.20^{a}	41.51±0.83^{b}	22.71±0.37^{ab}	6.21±0.34^{b}	26.70±0.69^{b}	11.34±1.00^{a}	10.72±0.53^{a}	11.37±0.95^{b}	33.45±3.63^{a}	35.73±1.61^{ab}	28.72±1.03^{a}	75.19±3.20^{a}	43.63±6.61^{a}
8	9.36±0.08^{a}	44.23±0.73^{a}	23.99±1.99^{a}	6.83±0.10^{a}	28.70±0.53^{a}	11.76±0.58^{a}	11.07±1.09^{a}	13.14±0.70^{a}	27.37±1.85^{b}	38.31±0.28^{a}	25.00±1.57^{b}	67.10±4.17^{b}	46.02±1.48^{a}

Table 8: Shoot length and fresh weight of thirteen *O. sativa* varieties after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$). Different alphabetical letters indicate statistically different means at $p > 0.05$.

UV-B ($kJm^{-2}d^{-1}$)	Aathira	Mangalamahsuri	Kanchana	Jyothi	Annapoorna	Mattatriveni	Karuna	Harsha	Varsha	Neeraja	Swetha	Aiswarya	Swarnaprabha
Shoot length plant^{-1} (cms)													
0	20.53±0.81^{ab}	22.10±0.30^{b}	20.40±0.30^{a}	19.20±0.70^{a}	17.28±0.28^{ab}	23.80±0.20^{a}	21.97±0.58^{a}	24.03±0.45^{a}	17.30±0.26^{a}	23.67±0.83^{a}	24.50±0.81^{a}	23.10±0.75^{a}	24.50±0.70^{a}
7	21.47±0.81^{a}	23.07±0.51^{a}	20.83±0.15^{a}	19.22±0.92^{a}	18.24±0.86^{a}	23.20±0.79^{ab}	21.00±1.13^{a}	22.87±0.12^{b}	16.23±0.40^{b}	21.77±0.32^{b}	22.40±0.29^{b}	19.87±0.75^{b}	20.60±0.58^{b}
14	20.60±0.46^{ab}	21.10±0.53^{c}	19.20±0.35^{b}	18.20±0.78^{b}	16.70±0.67^{bc}	22.84±0.80^{b}	18.66±0.60^{b}	23.13±0.61^{b}	15.03±0.55^{c}	19.77±0.25^{c}	17.80±0.72^{c}	17.17±0.21^{c}	19.20±0.58^{c}
21	19.93±0.32^{b}	20.20±0.72^{d}	19.00±0.20^{bc}	18.00±0.50^{b}	15.62±0.54^{d}	20.50±0.67^{b}	20.47±0.92^{a}	21.00±0.53^{c}	13.73±0.95^{d}	13.47±0.45^{d}	13.70±0.45^{d}	11.87±0.61^{e}	12.20±0.29^{e}
28	19.53±0.50^{b}	20.10±0.26^{d}	18.60±0.46^{c}	17.7±0.32^{b}	15.76±0.01^{cd}	20.13±0.23^{c}	17.67±0.70^{b}	20.57±0.38^{c}	12.87±0.50^{d}	14.80±0.70^{e}	16.80±0.70^{c}	13.47±0.50^{d}	13.90±0.38^{d}
Fresh weight plant^{-1} (g)													
0	0.121±0.01^{a}	0.153±0.00^{a}	0.122±0.01^{a}	0.159±0.01^{a}	0.096±0.01^{a}	0.122±0.01^{a}	0.141±0.01^{a}	0.177±0.01^{a}	0.141±0.010^{a}	0.088±0.01^{a}	0.164±0.00^{a}	0.125±0.01^{a}	0.148±0.00^{a}
7	0.114±0.01^{ab}	0.143±0.00^{b}	0.11±0.01^{ab}	0.15±0.00^{b}	0.094±0.00^{a}	0.114±0.00^{ab}	0.142±0.01^{a}	0.163±0.01^{b}	0.123±0.00^{b}	0.072±0.00^{b}	0.139±0.01^{b}	0.107±0.00^{b}	0.121±0.01^{a}
14	0.112±0.00^{ab}	0.142±0.00^{b}	0.116±0.01^{ab}	0.142±0.00^{c}	0.085±0.00^{b}	0.102±0.00^{bc}	0.121±0.01^{b}	0.133±0.00^{c}	0.106±0.00^{c}	0.045±0.00^{c}	0.077±0.00^{c}	0.085±0.01^{c}	0.066±0.05^{b}
21	0.108±0.01^{b}	0.135±0.01^{c}	0.102±0.01^{c}	0.136±0.00^{c}	0.079±0.00^{b}	0.088±0.01^{cd}	0.099±0.01^{c}	0.116±0.01^{d}	0.09±0.01^{d}	0.034±0.00^{d}	0.043±0.01^{e}	0.032±0.01^{e}	0.051±0.01^{b}
28	0.107±0.00^{b}	0.124±0.00^{d}	0.109±0.01^{bc}	0.129±0.00^{d}	0.083±0.00^{b}	0.075±0.01^{d}	0.064±0.01^{d}	0.097±0.00^{e}	0.083±0.00^{e}	0.033±0.00^{d}	0.054±0.00^{d}	0.047±0.01^{d}	0.066±0.01^{b}

Table 9: Total chlorophyll and carotenoid content in thirteen *O. sativa* varieties after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$). Different alphabetical letters indicate statistically different means at $p > 0.05$.

UV-B ($kJm^{-2}d^{-1}$)	Aathira	Mangalamahsuri	Kanchana	Jyothi	Annapoorna	Mattatriveni	Karuna	Harsha	Varsha	Neeraja	Swetha	Aiswarya	Swarnaprabha
Total chlorophyll content (mgg^{-1} DW)													
0	14.24±1.67^{a}	7.24±1.04^{a}	12.77±0.37^{b}	5.99±0.31^{ab}	4.82±0.31^{b}	10.79±0.63^{c}	7.61±0.18^{a}	10.69±0.72^{a}	6.75±0.60^{a}	9.23±0.89^{a}	12.75±1.07^{a}	13.51±1.17^{a}	19.80±0.56^{a}
7	12.85±2.19^{ab}	5.36±0.85^{ab}	14.09±1.15^{a}	6.30±0.15^{a}	5.85±0.27^{a}	9.71±0.30^{b}	6.30±0.14^{b}	10.69±1.50^{a}	4.66±0.29^{b}	7.35±0.63^{b}	7.60±0.86^{b}	7.50±0.77^{b}	15.29±1.18^{b}
14	11.74±0.52^{ab}	6.01±0.86^{ab}	12.60±0.69^{b}	5.41±0.46^{b}	4.81±0.18^{b}	8.91±0.55^{b}	5.35±0.08^{c}	8.05±0.34^{b}	2.78±0.23^{c}	5.46±0.29^{c}	5.43±0.91^{c}	4.75±0.20^{c}	7.77±0.58^{c}
21	10.69±1.76^{b}	6.02±0.29^{b}	11.22±0.54^{c}	5.41±0.48^{b}	4.63±0.22^{b}	7.25±0.61^{a}	4.95±0.53^{cd}	4.68±0.31^{c}	2.75±0.72^{c}	1.75±0.15^{c}	3.04±0.56^{d}	1.42±0.47^{d}	7.10±0.58^{c}
28	11.05±0.53^{b}	5.76±0.18^{b}	10.23±0.52^{c}	5.28±0.43^{b}	4.61±0.71^{b}	7.57±0.30^{a}	4.62±0.43^{d}	4.19±0.18^{c}	2.53±0.44^{c}	3.68±0.37^{d}	6.39±0.40^{bc}	1.48±0.32^{d}	4.04±0.70^{d}
Total carotenoid content (mgg^{-1} DW)													
0	0.65±0.04^{c}	0.40±0.05^{c}	0.77±0.03^{d}	0.47±0.04^{c}	0.53±0.02^{d}	0.41±0.08^{ab}	2.37±0.06^{c}	4.44±0.19^{c}	0.82±0.07^{b}	1.40±0.07^{c}	2.41±0.10^{a}	2.31±0.28^{ab}	1.20±0.15^{a}
7	0.95±0.05^{d}	0.52±0.06^{c}	1.20±0.14^{c}	0.48±0.07^{c}	0.54±0.01^{d}	0.40±0.11^{b}	2.64±0.13^{b}	4.42±0.13^{c}	1.18±0.08^{a}	1.43±0.04^{c}	2.55±0.19^{a}	2.54±0.00^{a}	1.26±0.05^{a}
14	1.37±0.25^{c}	1.13±0.07^{b}	1.12±0.09^{c}	0.87±0.12^{b}	0.85±0.06^{c}	0.52±0.04^{ab}	2.55±0.01^{b}	4.66±0.01^{bc}	1.22±0.12^{a}	1.51±0.03^{ab}	2.62±0.56^{a}	2.54±0.00^{a}	1.33±0.03^{a}
21	2.58±0.16^{b}	2.10±0.58^{a}	1.45±0.14^{b}	1.40±0.11^{a}	1.15±0.07^{b}	0.52±0.06^{ab}	2.94±0.04^{a}	4.96±0.07^{b}	1.10±0.11^{a}	1.55±0.04^{a}	1.55±0.10^{b}	2.24±0.10^{ab}	0.83±0.13^{b}
28	2.95±0.07^{a}	2.43±0.39^{a}	2.06±0.16^{a}	0.80±0.16^{b}	2.48±0.06^{a}	0.30±0.01^{a}	3.07±0.13^{a}	6.44±0.48^{a}	1.24±0.07^{a}	1.44±0.01^{bc}	1.70±0.16^{b}	1.99±0.25^{b}	0.41±0.12^{c}
Malondialdehyde content (µmolg^{-1} DW)													
0	8.06±1.41^{b}	22.62±2.45^{b}	33.91±0.79^{a}	38.29±1.02^{a}	19.69±0.29^{b}	23.47±1.56^{c}	6.65±1.16^{c}	6.29±0.55^{c}	20.14±2.72^{d}	15.21±1.52^{d}	5.64±0.99^{c}	5.60±0.23^{c}	11.56±0.99^{b}
7	6.10±0.57^{c}	16.92±2.24^{c}	36.85±1.44^{b}	43.10±1.39^{a}	19.77±0.20^{b}	32.03±1.46^{b}	6.77±0.58^{c}	6.57±0.15^{c}	28.72±1.60^{c}	19.47±2.45^{cd}	12.88±0.59^{b}	24.88±1.50^{b}	36.81±1.40^{a}
14	8.37±0.06^{ab}	25.92±4.14^{ab}	36.93±2.09^{b}	46.01±0.73^{a}	22.60±0.72^{ab}	35.00±1.15^{b}	8.48±0.06^{b}	6.48±0.17^{c}	33.66±2.68^{b}	23.40±2.29^{c}	13.06±0.73^{b}	27.48±1.18^{ab}	37.93±0.71^{a}
21	8.07±0.74^{b}	28.25±1.58^{a}	40.46±1.02^{a}	40.80±7.77^{a}	23.07±0.64^{ab}	45.66±2.31^{a}	12.06±1.16^{a}	11.70±0.64^{a}	36.64±0.77^{ab}	45.50±3.73^{a}	14.88±0.60^{a}	30.81±5.18^{a}	38.03±0.61^{a}
28	9.78±0.58^{a}	26.58±0.95^{ab}	39.61±074^{ab}	47.84±7.83^{a}	24.14±4.32^{a}	42.63±3.15^{a}	10.74±0.51^{a}	9.44±0.18^{b}	39.84±1.57^{a}	36.68±3.25^{b}	14.66±0.92^{a}	30.43±1.77^{a}	37.70±0.55^{a}

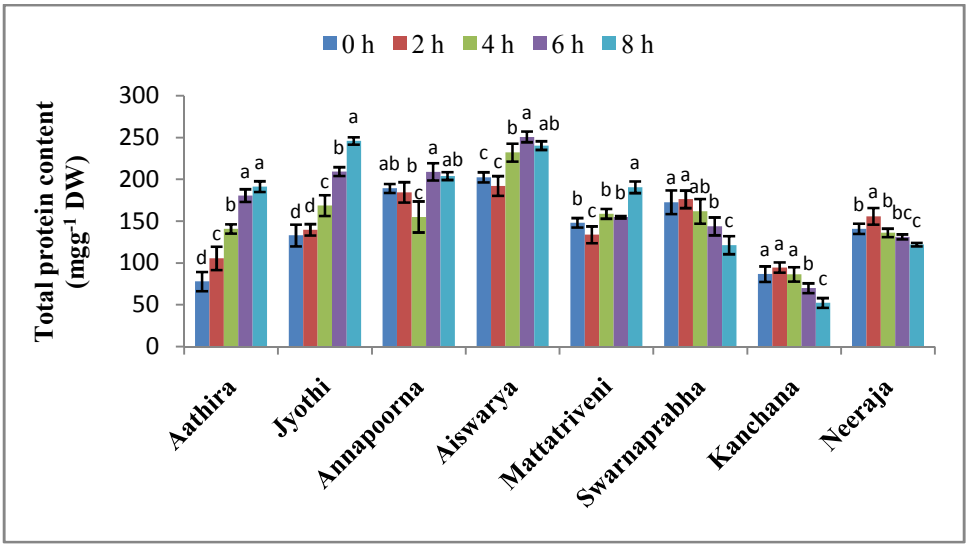

Figure 1: Total protein content in eight *O. sativa* varieties after exposure to HL stress (2000 µmolm^{-2}s^{-1}) for different time intervals (0, 2, 4, 6 and 8 h). Different alphabetical letters indicate statistically different means at p > 0.05.

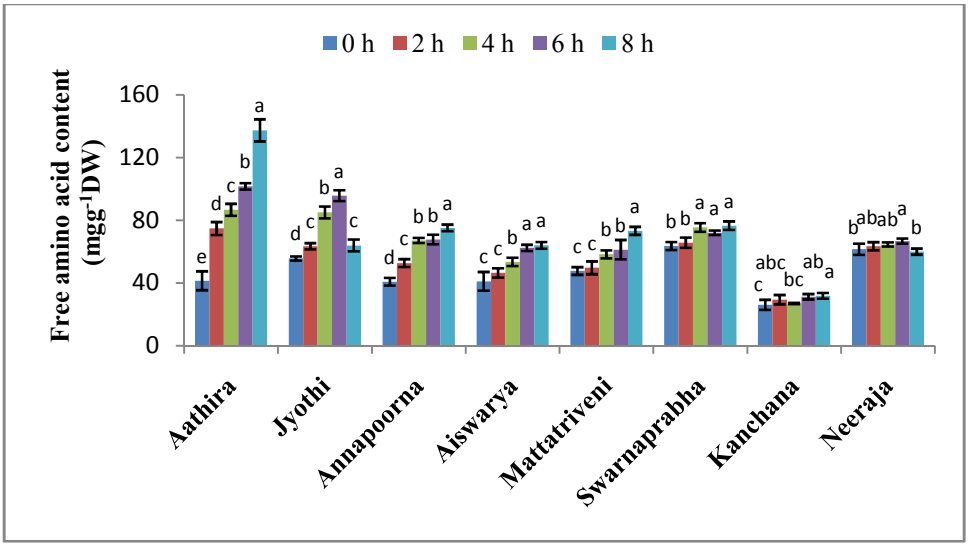

Figure 2: Free amino acid content in eight *O. sativa* varieties after exposure to HL stress (2000 µmolm^{-2}s^{-1}) for different time intervals (0, 2, 4, 6 and 8 h). Different alphabetical letters indicate statistically different means at p > 0.05.

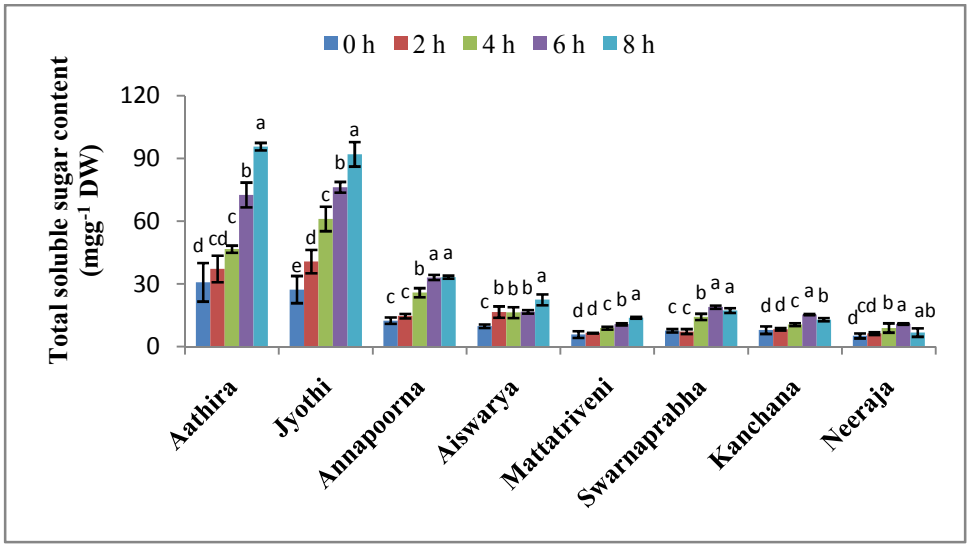

Figure 3: Total soluble sugar content in eight *O. sativa* varieties after exposure to HL stress (2000 µmolm^{-2}s^{-1}) for different time intervals (0, 2, 4, 6 and 8 h). Different alphabetical letters indicate statistically different means at p > 0.05.

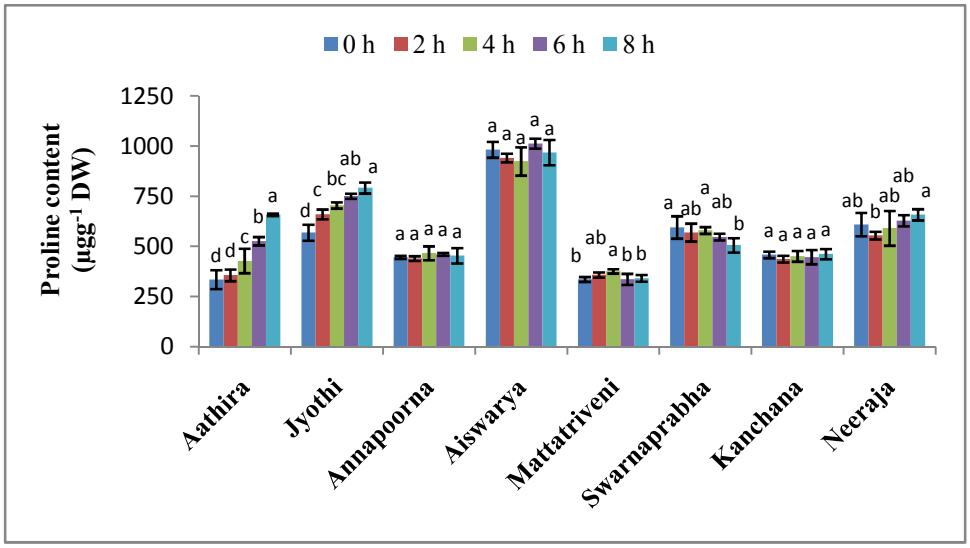

Figure 4: Proline content in eight *O. sativa* varieties after exposure to HL stress (2000 µmolm^{-2}s^{-1}) for different time intervals (0, 2, 4, 6 and 8 h). Different alphabetical letters indicate statistically different means at p > 0.05.

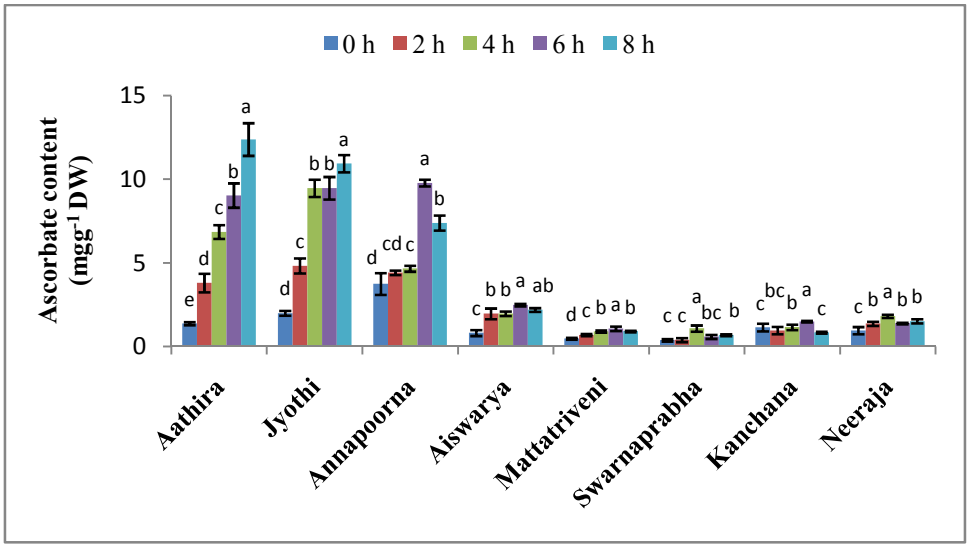

Figure 5: Ascorbate content in eight *O. sativa* varieties after exposure to HL stress (2000 µmolm^{-2}s^{-1}) for different time intervals (0, 2, 4, 6 and 8 h). Different alphabetical letters indicate statistically different means at p > 0.05.

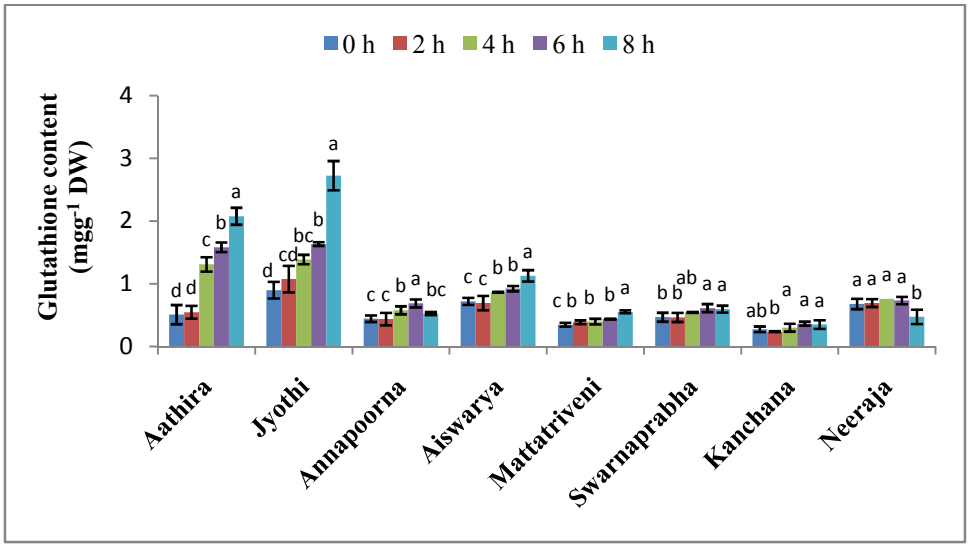

Figure 6: Glutathione content in eight *O. sativa* varieties after exposure to HL stress (2000 µmolm^{-2}s^{-1}) for different time intervals (0, 2, 4, 6 and 8 h). Different alphabetical letters indicate statistically different means at p > 0.05.

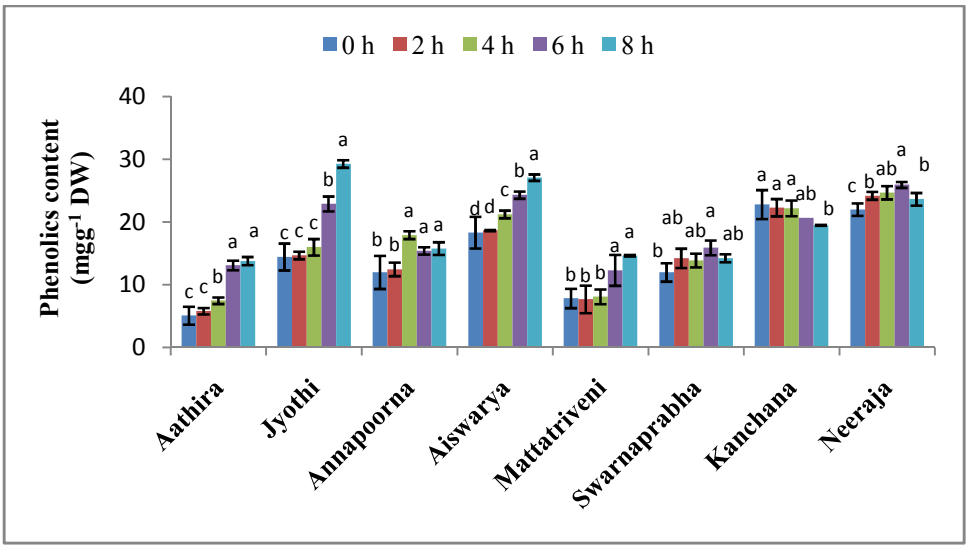

Figure 7: Phenolics content in eight *O. sativa* varieties after exposure to HL stress (2000 µmolm^{-2}s^{-1}) for different time intervals (0, 2, 4, 6 and 8 h). Different alphabetical letters indicate statistically different means at p > 0.05.

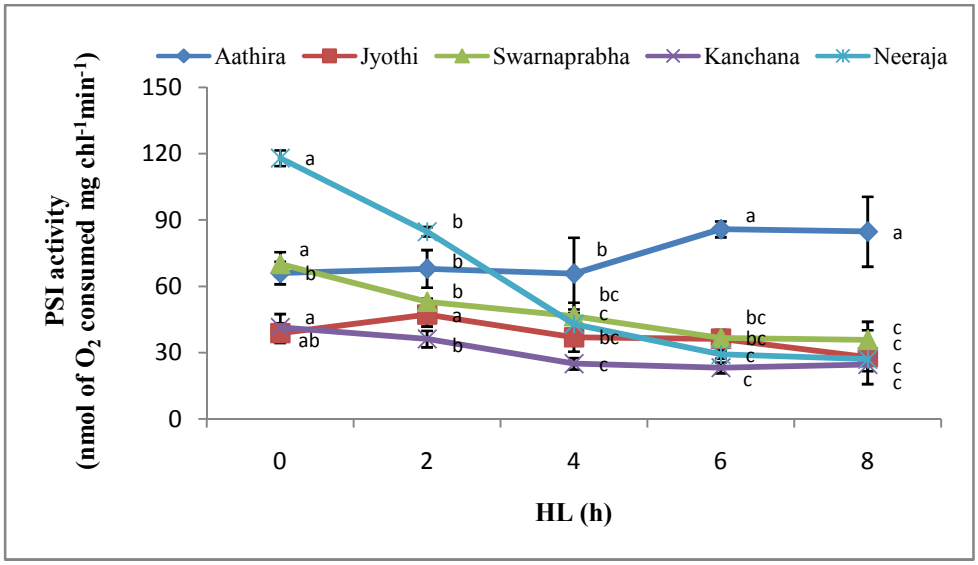

Figure 8: PSI activity in leaves of five *O. sativa* varieties after exposure to HL stress (2000 µmolm^{-2}s^{-1}) for different time intervals (0, 2, 4, 6 and 8 h). Different alphabetical letters indicate statistically different means at p > 0.05.

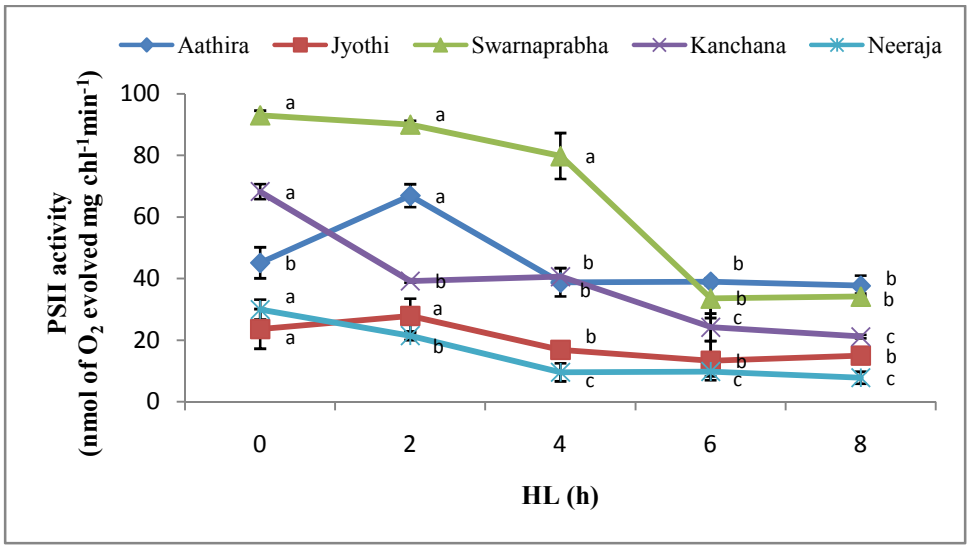

Figure 9: PSII activity in leaves of five *O. sativa* varieties after exposure to HL stress (2000 µmolm^{-2}s^{-1}) for different time intervals (0, 2, 4, 6 and 8 h). Different alphabetical letters indicate statistically different means at p > 0.05.

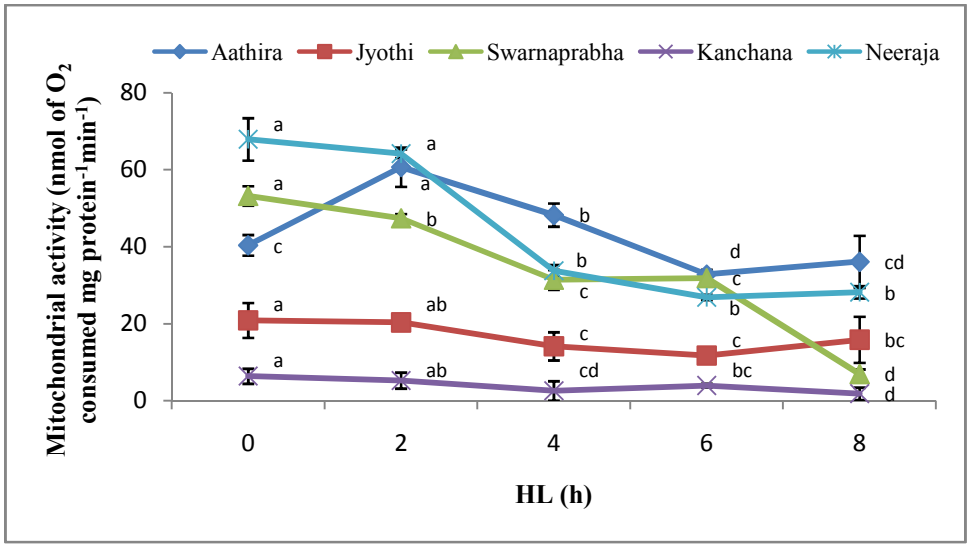

Figure 10: Mitochondrial activity in five *O. sativa* varieties after exposure to HL stress (2000 µmolm^{-2}s^{-1}) for different time intervals (0, 2, 4, 6 and 8 h). Different alphabetical letters indicate statistically different means at p > 0.05.

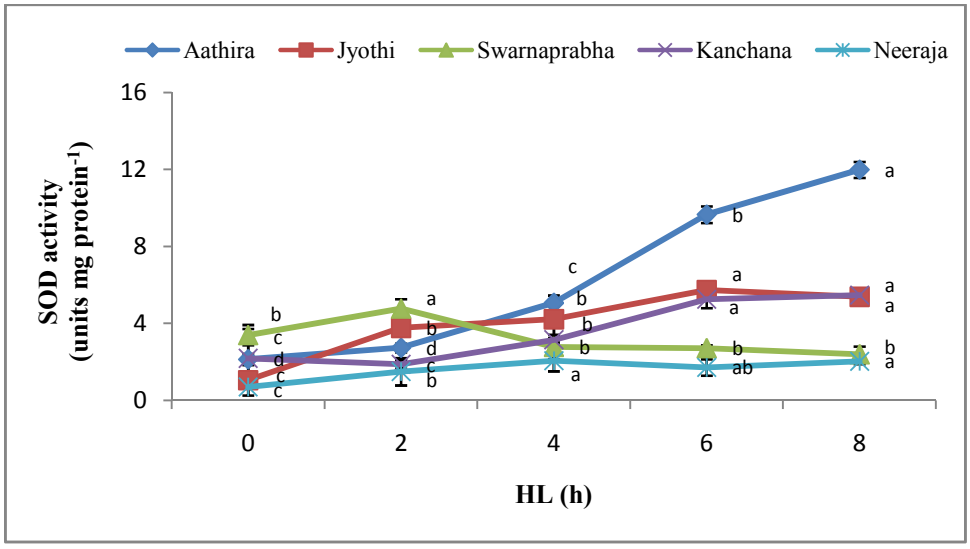

Figure 11: SOD activity in five *O. sativa* varieties after exposure to HL stress (2000 µmolm^{-2}s^{-1}) for different time intervals (0, 2, 4, 6 and 8 h). Different alphabetical letters indicate statistically different means at p > 0.05.

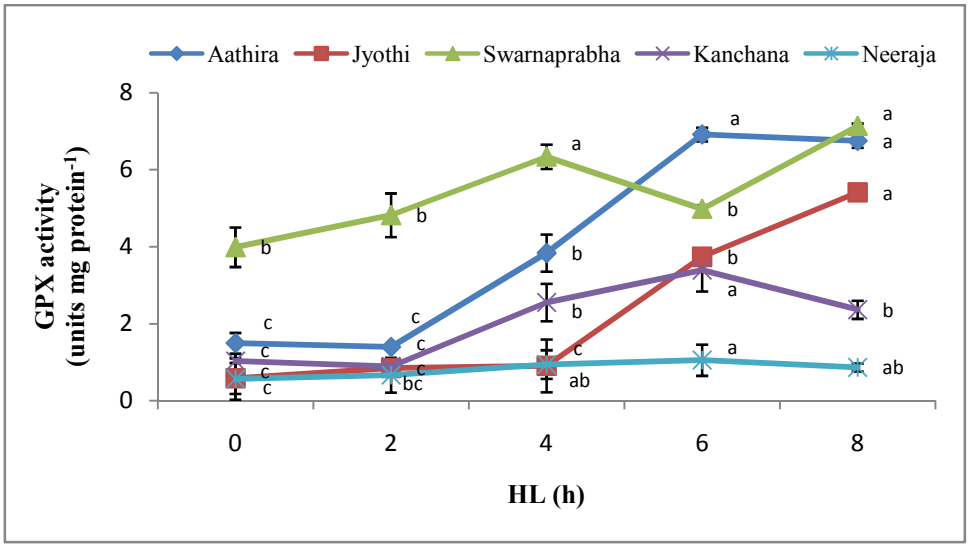

Figure 12: GPOX activity in five *O. sativa* varieties after exposure to HL stress (2000 µmolm^{-2}s^{-1}) for different time intervals (0, 2, 4, 6 and 8 h). Different alphabetical letters indicate statistically different means at p > 0.05.

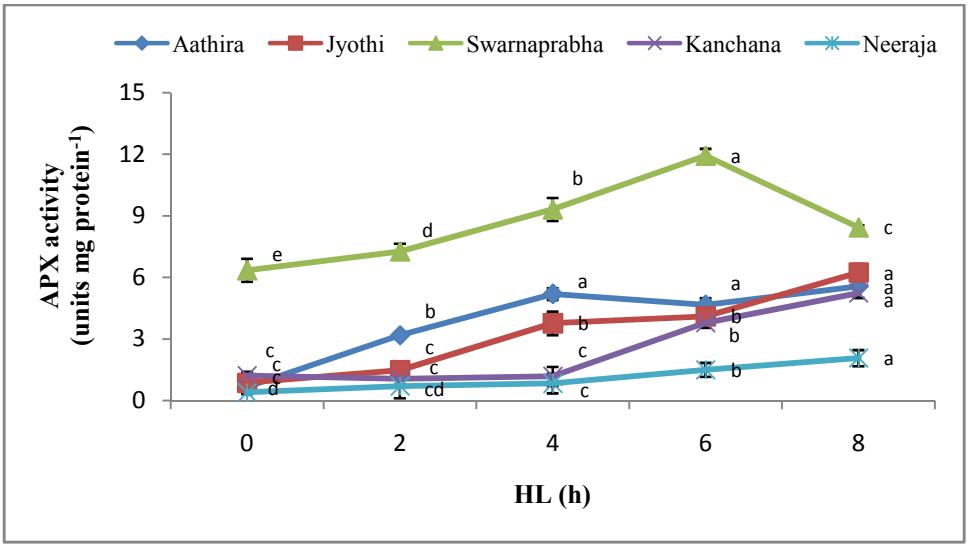

Figure 13: APX activity in five *O. sativa* varieties after exposure to HL stress (2000 µmolm^{-2}s^{-1}) for different time intervals (0, 2, 4, 6 and 8 h). Different alphabetical letters indicate statistically different means at p > 0.05.

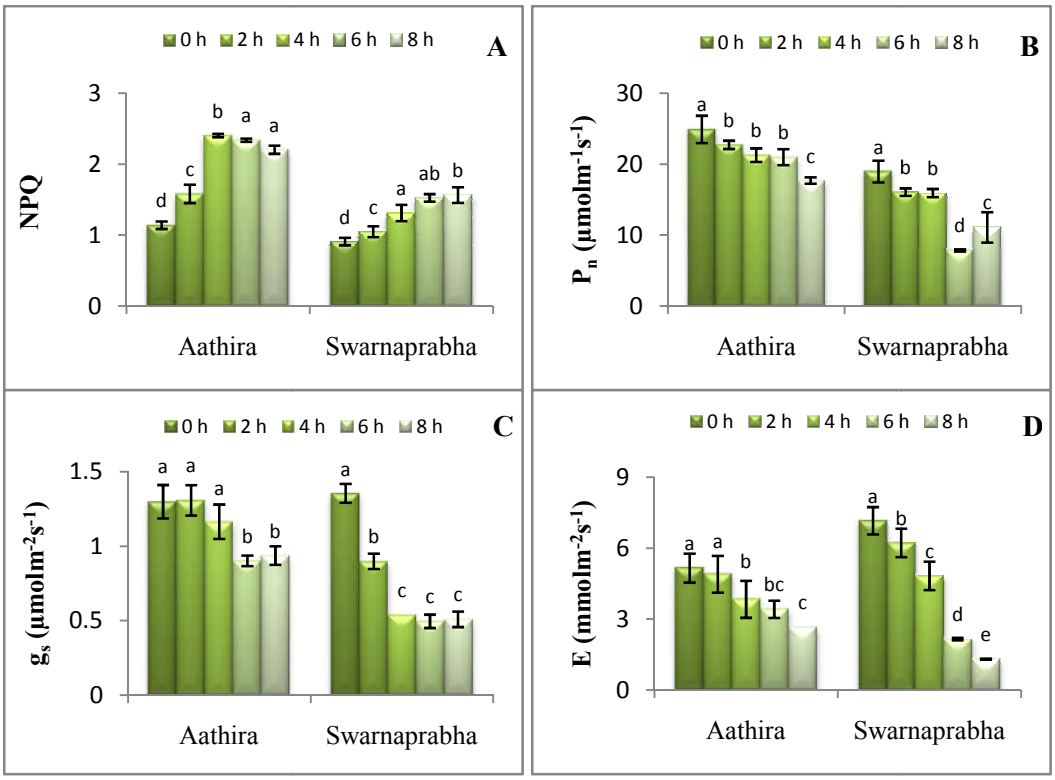

Figure 17: NPQ (A), P_n (B), g_s (C) and E (D) of *O. sativa* var. Aathira and *O. sativa* var. Swarnaprabha after exposure to HL stress (2000 µmolm^{-2}s^{-1}) for different time intervals (0, 2, 4, 6 and 8 h). Different alphabetical letters indicate statistically different means at $p > 0.05$.

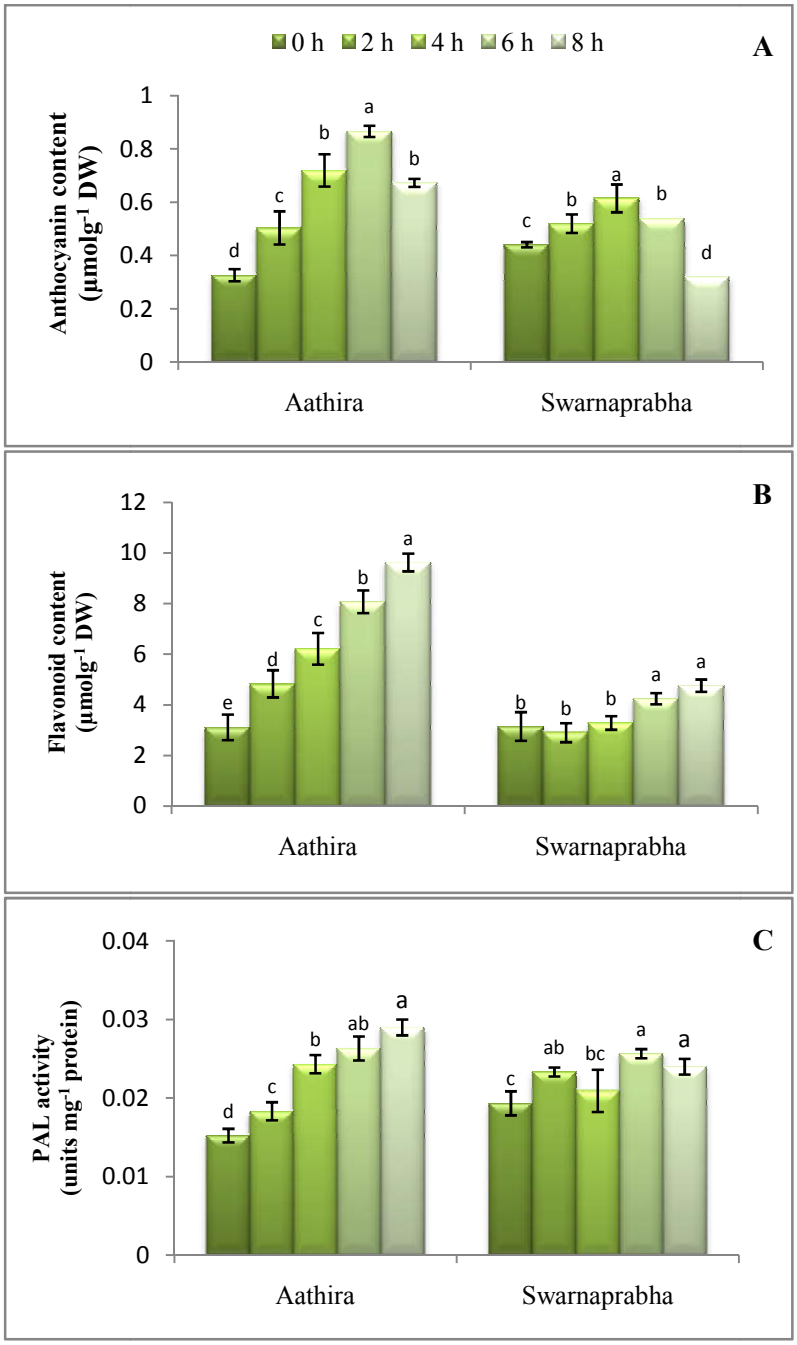

Figure 18: Anthocyanin (A), flavonoid content (B) and PAL activity (C) of *O. sativa* var. Aathira and *O. sativa* var. Swarnaprabha after exposure to HL stress (2000 µmolm^{-2}s^{-1}) for different time intervals (0, 2, 4, 6 and 8 h). Different alphabetical letters indicate statistically different means at p > 0.05.

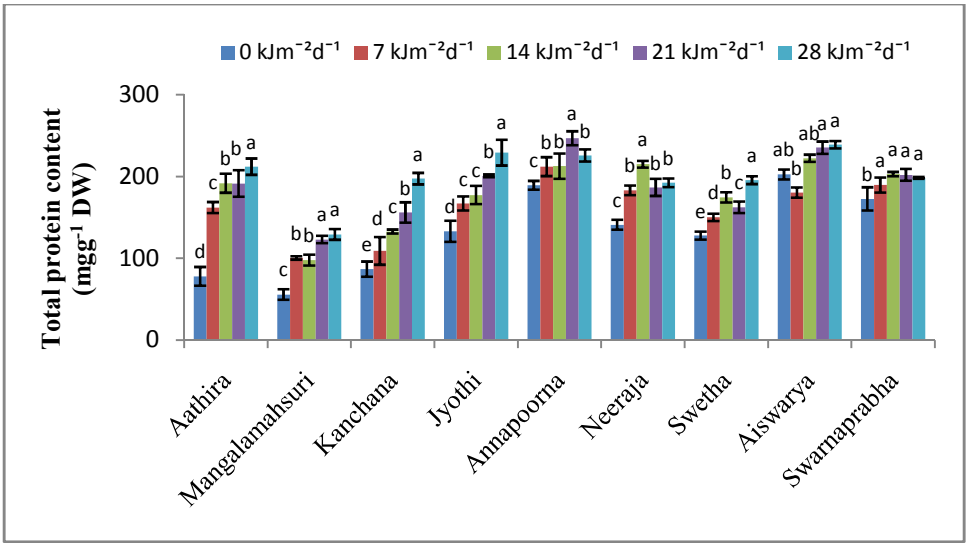

Figure 30: Total protein content in nine *O. sativa* varieties after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$). Different alphabetical letters indicate statistically different means at $p > 0.05$.

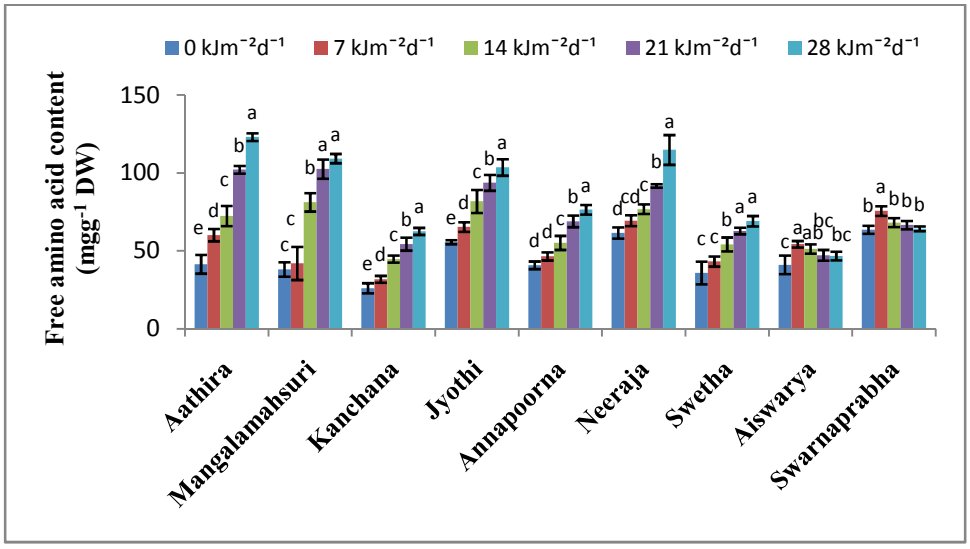

Figure 31: Free amino acid content in nine *O. sativa* varieties after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$). Different alphabetical letters indicate statistically different means at $p > 0.05$.

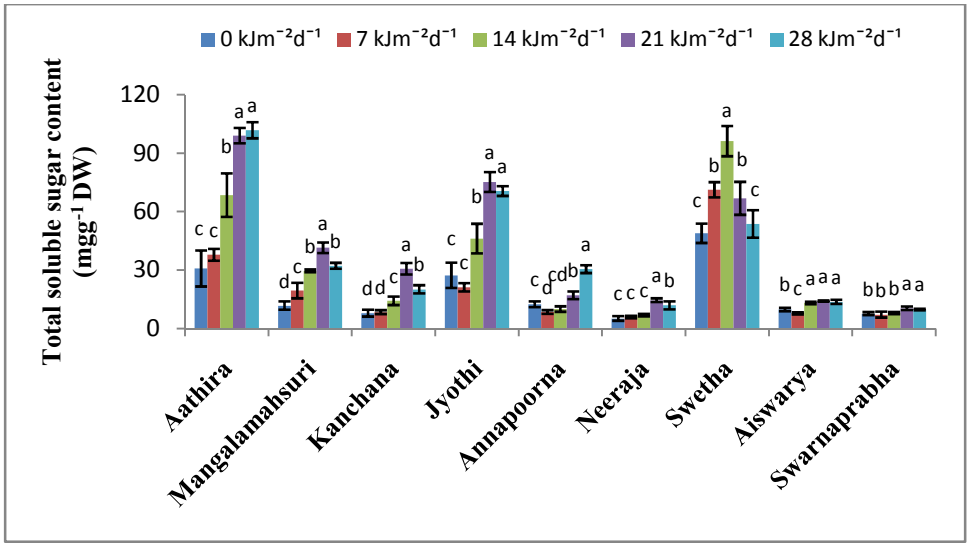

Figure 32: Total soluble sugar content in nine *O. sativa* varieties after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$). Different alphabetical letters indicate statistically different means at $p > 0.05$.

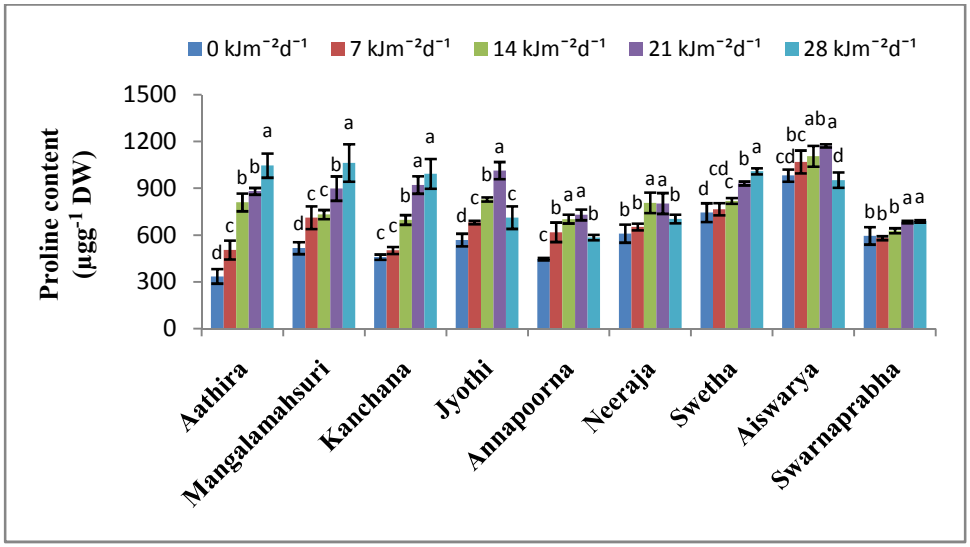

Figure 33: Proline content in nine *O. sativa* varieties after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$). Different alphabetical letters indicate statistically different means at $p > 0.05$.

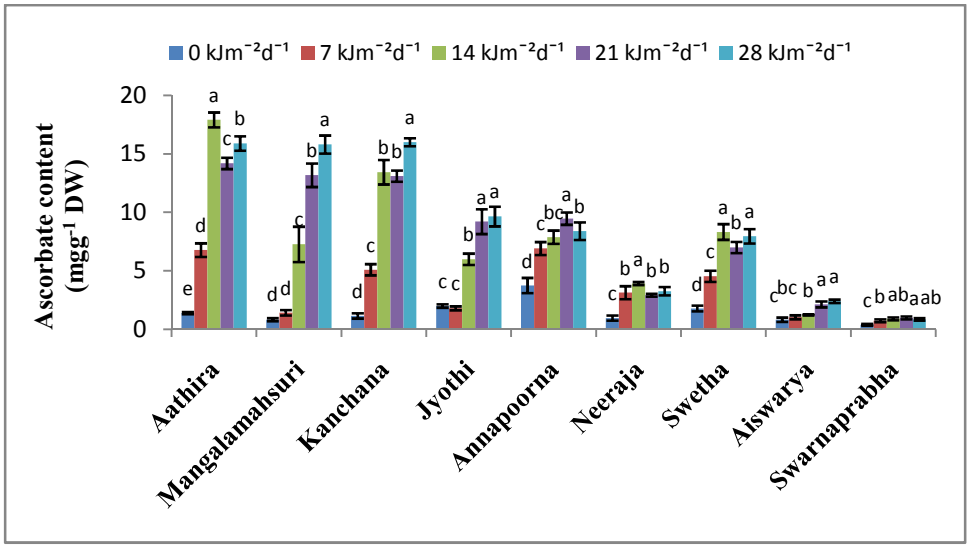

Figure 34: Ascorbate content in nine *O. sativa* varieties after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$). Different alphabetical letters indicate statistically different means at $p > 0.05$.

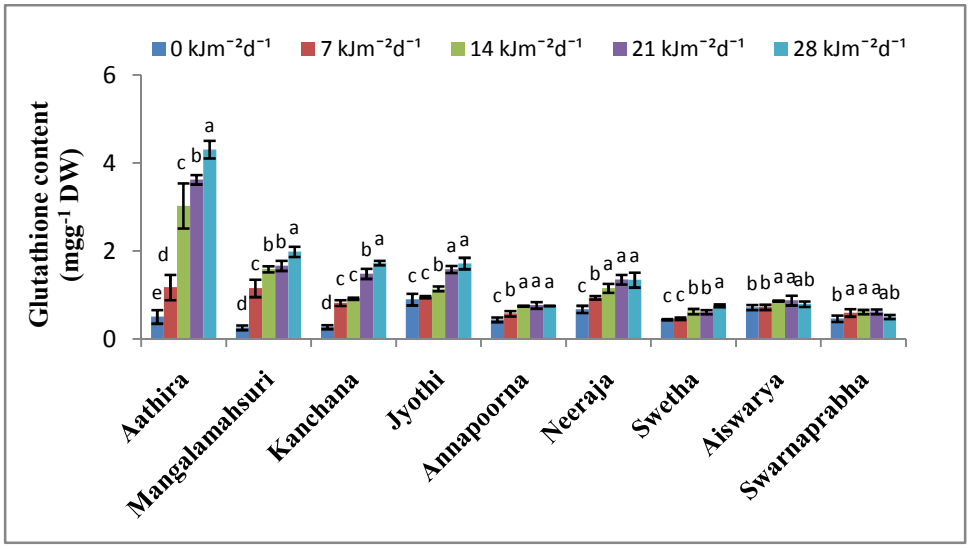

Figure 35: Glutathione content in nine *O. sativa* varieties after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$). Different alphabetical letters indicate statistically different means at $p > 0.05$.

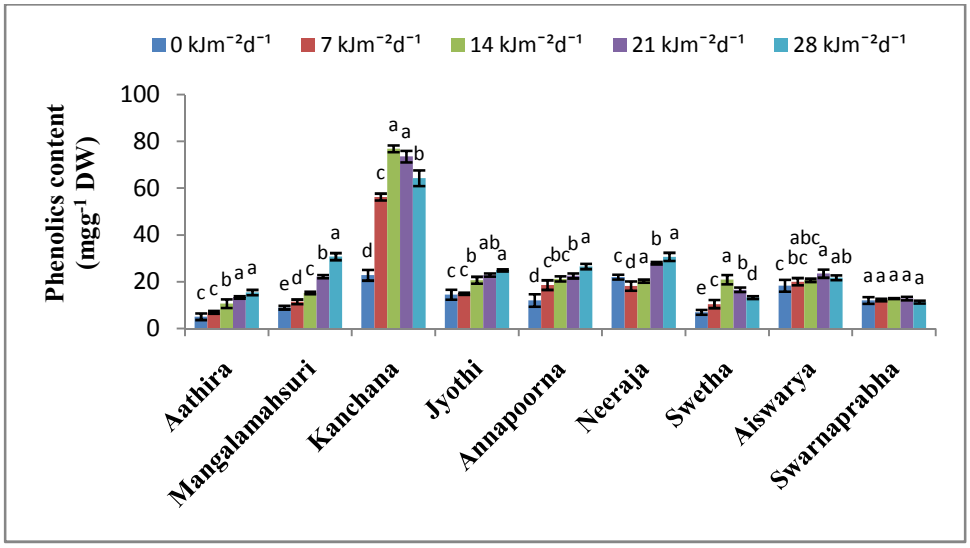

Figure 36: Phenolics content in nine *O. sativa* varieties after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$). Different alphabetical letters indicate statistically different means at $p > 0.05$.

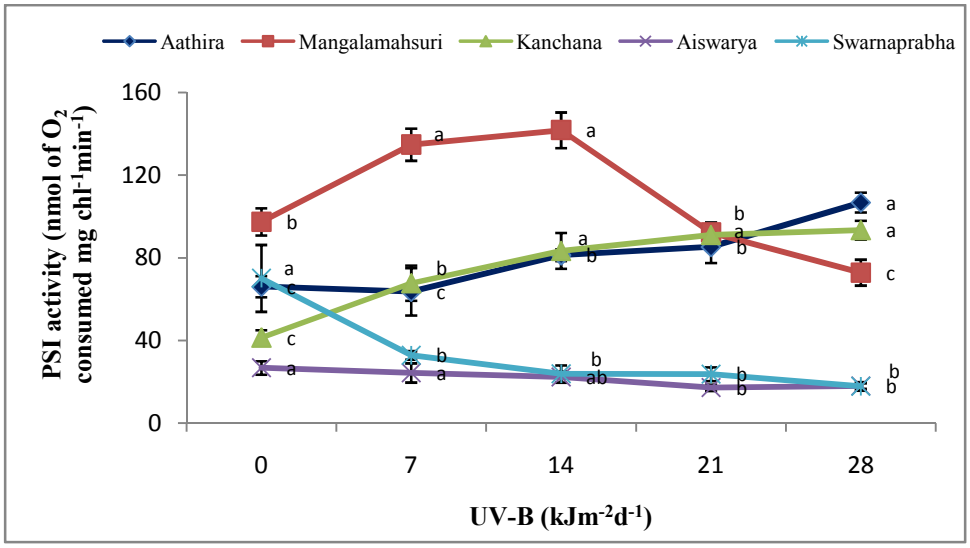

Figure 37: PSI activity in leaves of five *O. sativa* varieties after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$). Different alphabetical letters indicate statistically different means at $p > 0.05$.

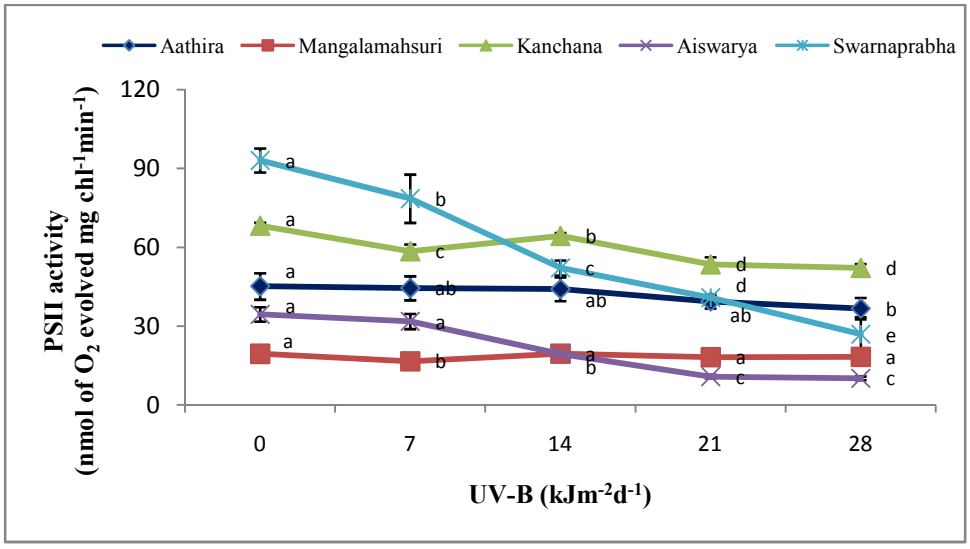

Figure 38: PSII activity in leaves of five *O. sativa* varieties after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$). Different alphabetical letters indicate statistically different means at $p > 0.05$.

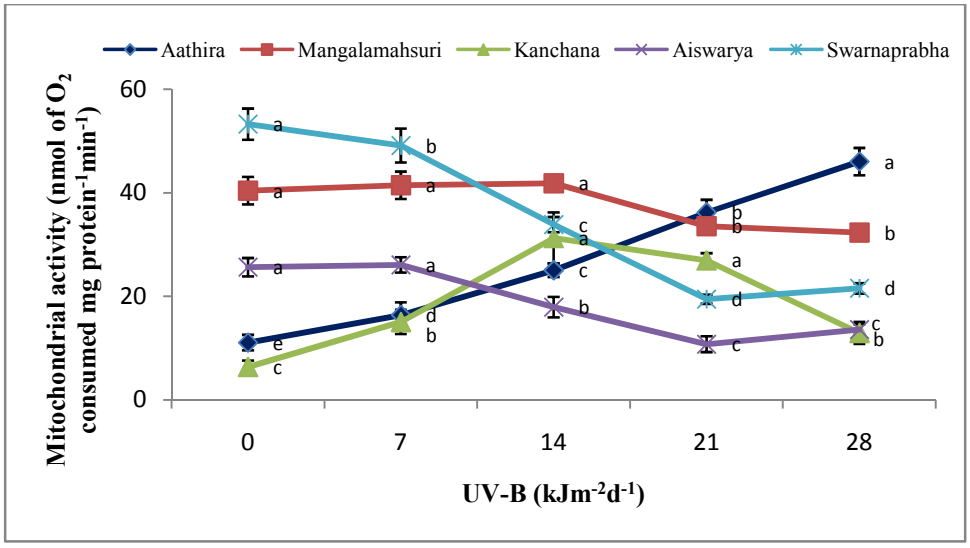

Figure 39: Mitochondrial activity in five *O. sativa* varieties after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$). Different alphabetical letters indicate statistically different means at $p > 0.05$.

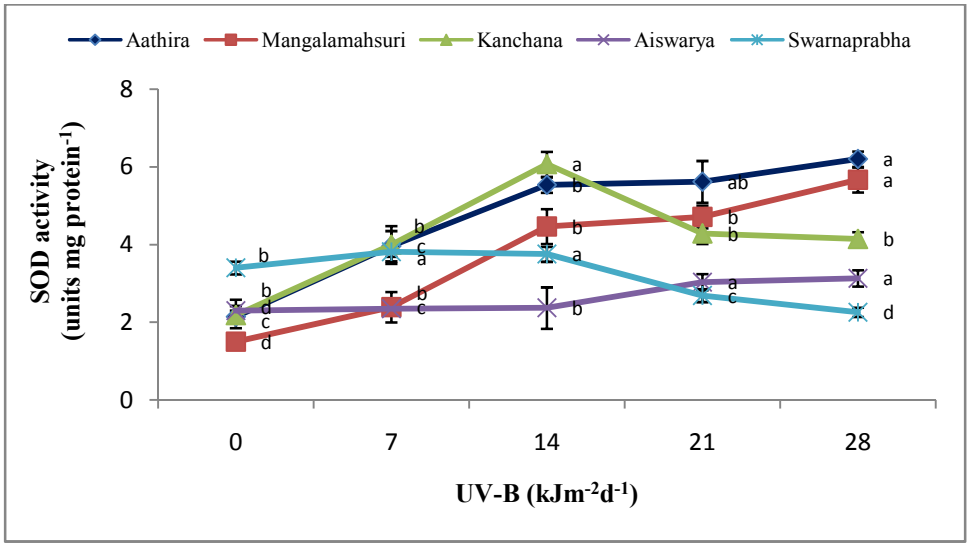

Figure 40: SOD activity in five *O. sativa* varieties after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$). Different alphabetical letters indicate statistically different means at $p > 0.05$.

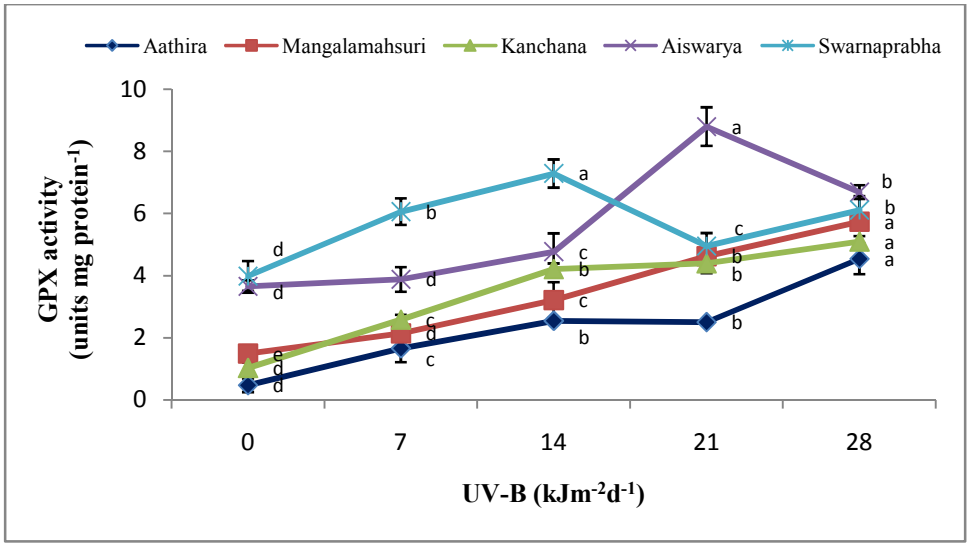

Figure 41: GPOX activity in five *O. sativa* varieties after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$). Different alphabetical letters indicate statistically different means at $p > 0.05$.

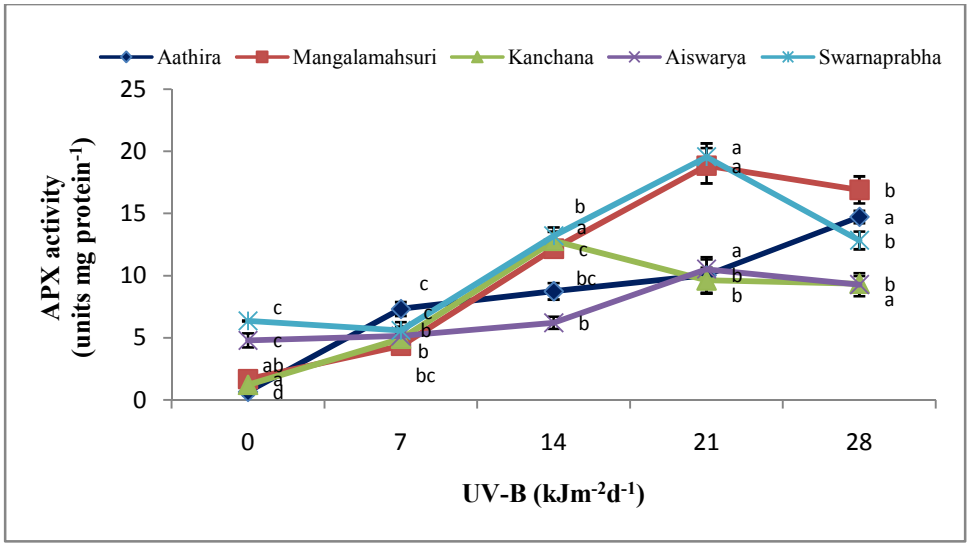

Figure 42: APX activity in five *O. sativa* varieties after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$). Different alphabetical letters indicate statistically different means at $p > 0.05$.

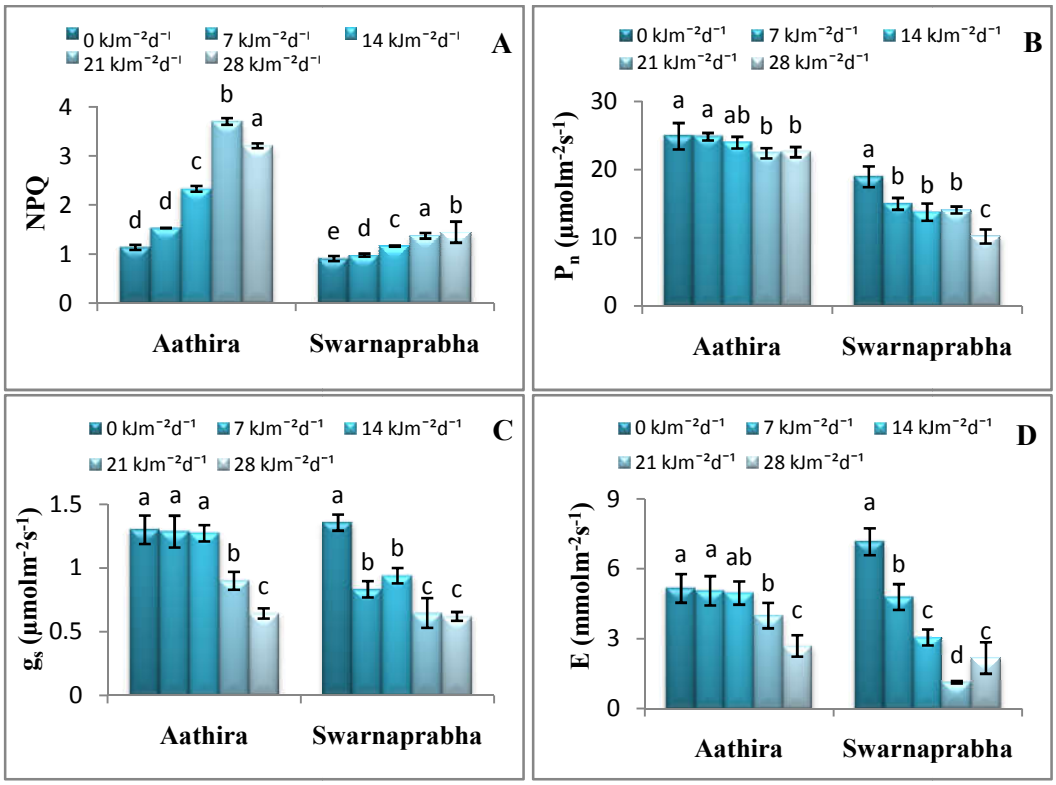

Figure 46: NPQ (A), P_n (b), g_s (C) and E (D) of *O. sativa* var. Aathira and *O. sativa* var. Swarnaprabha after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$). Different alphabetical letters indicate statistically different means at $p > 0.05$.

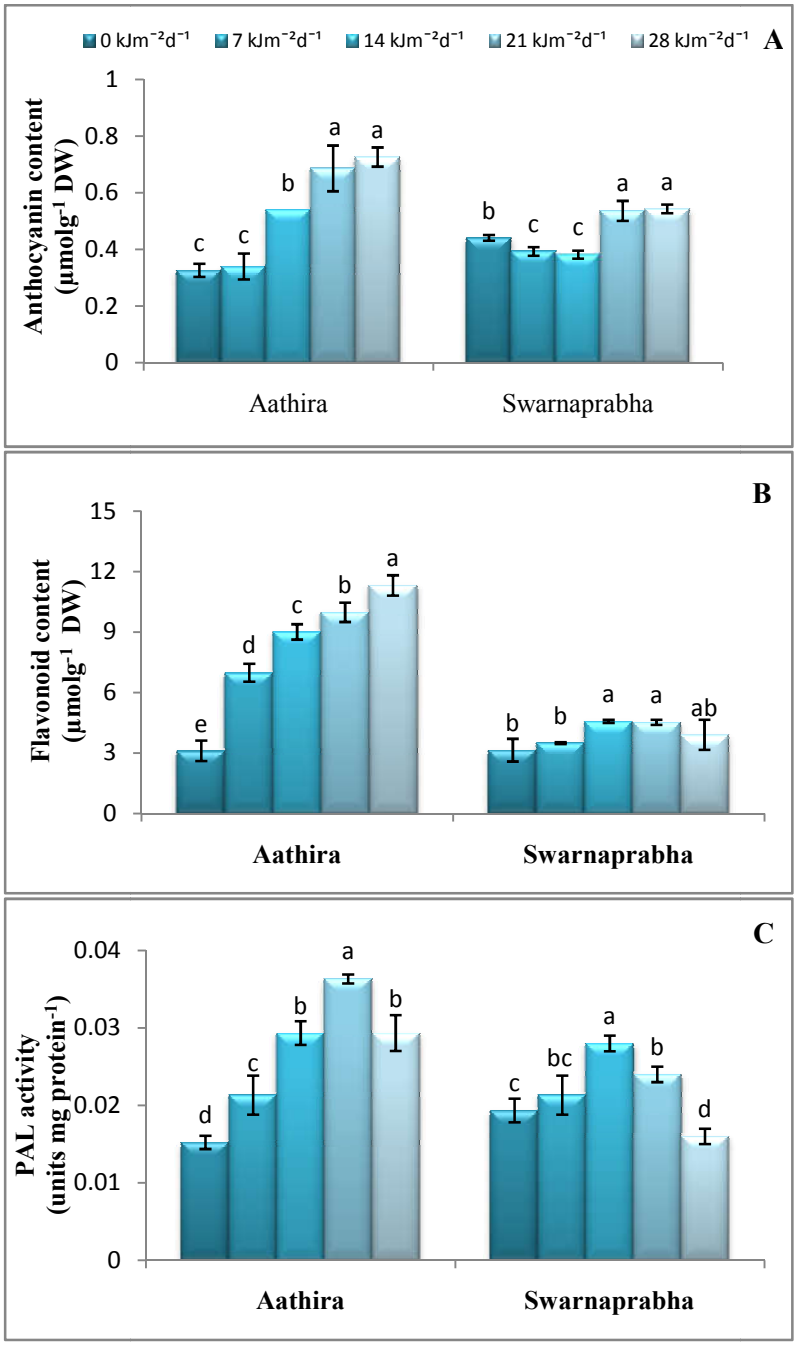

Figure 47: Anthocyanin (A), flavonoid content (B) and PAL activity (C) of *O. sativa* var. Aathira and *O. sativa* var. Swarnaprabha after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$). Different alphabetical letters indicate statistically different means at $p > 0.05$.

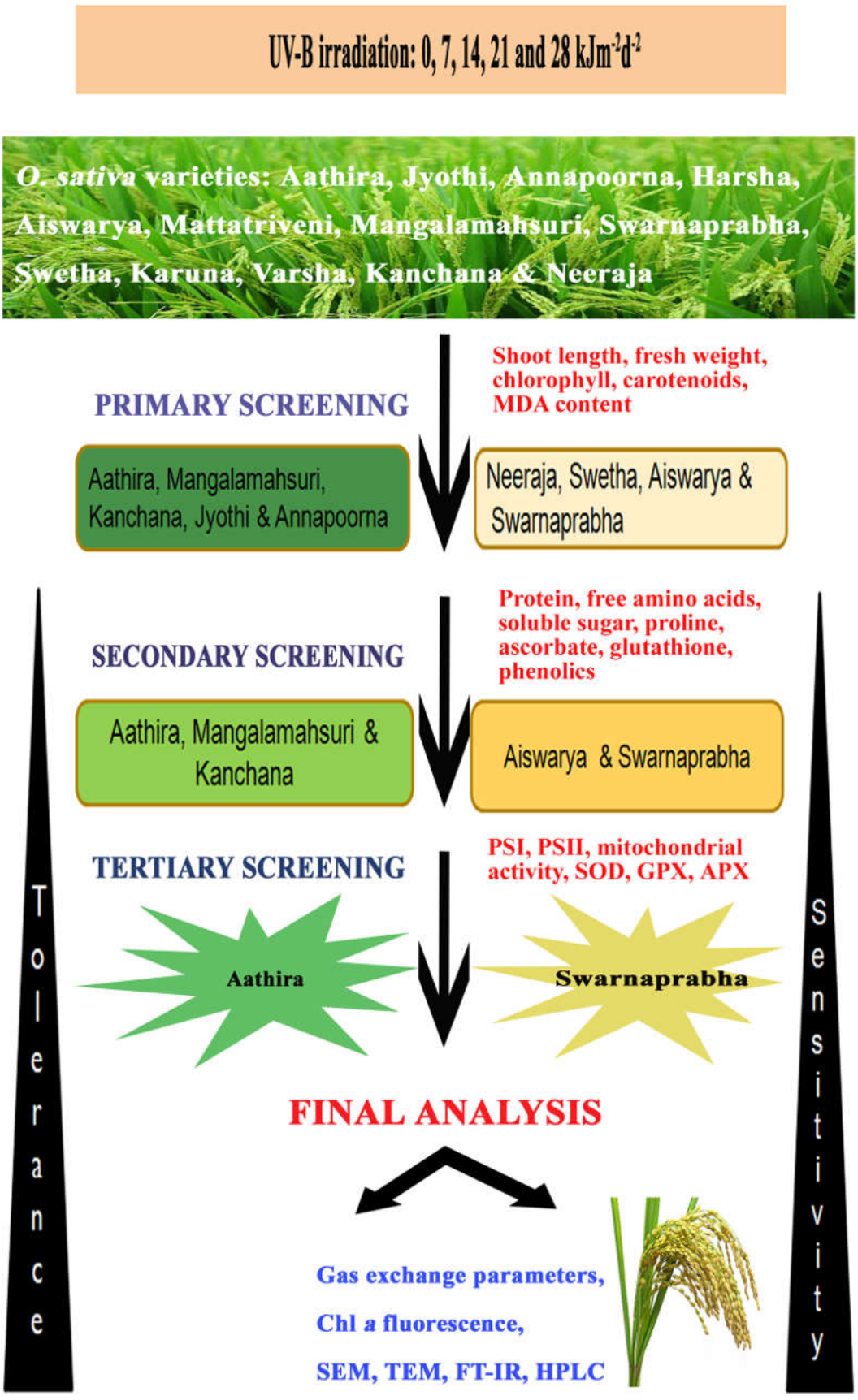

Chart 2: Experimental design of the work on imparting UV-B irradiation to thirteen varities of *O. sativa* and diffrent stages of screening with the parameters analysed at each stage.

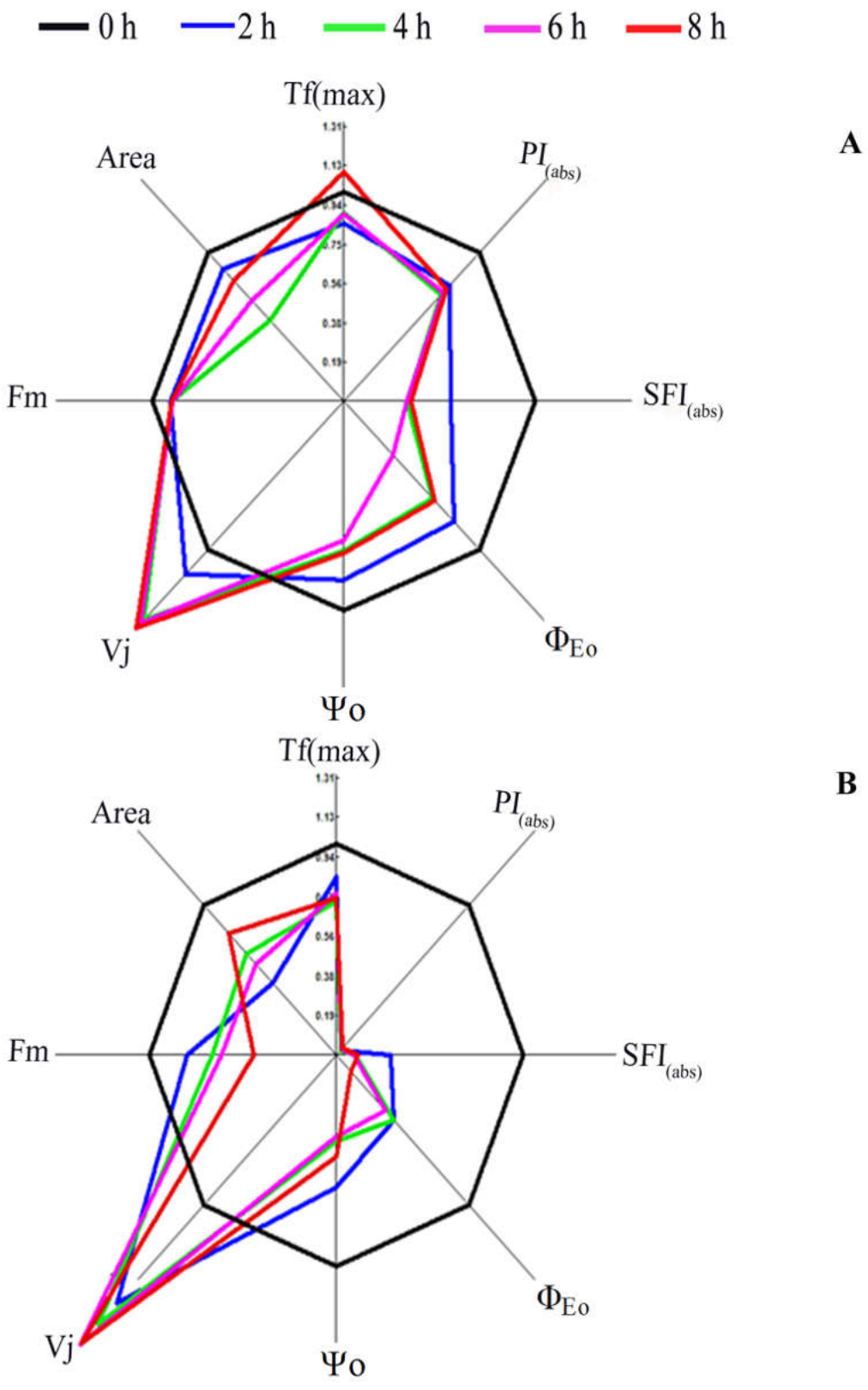

Figure 14: Radar plot of selected Chl *a* fluorescence parameters recorded in *O. sativa* var. Aathira (A) and *O. sativa* var. Swarnaprabha (B) leaves after exposure to HL stress (2000 µmolm^{-2}s^{-1}) for different time intervals (0, 2, 4, 6 and 8 h).

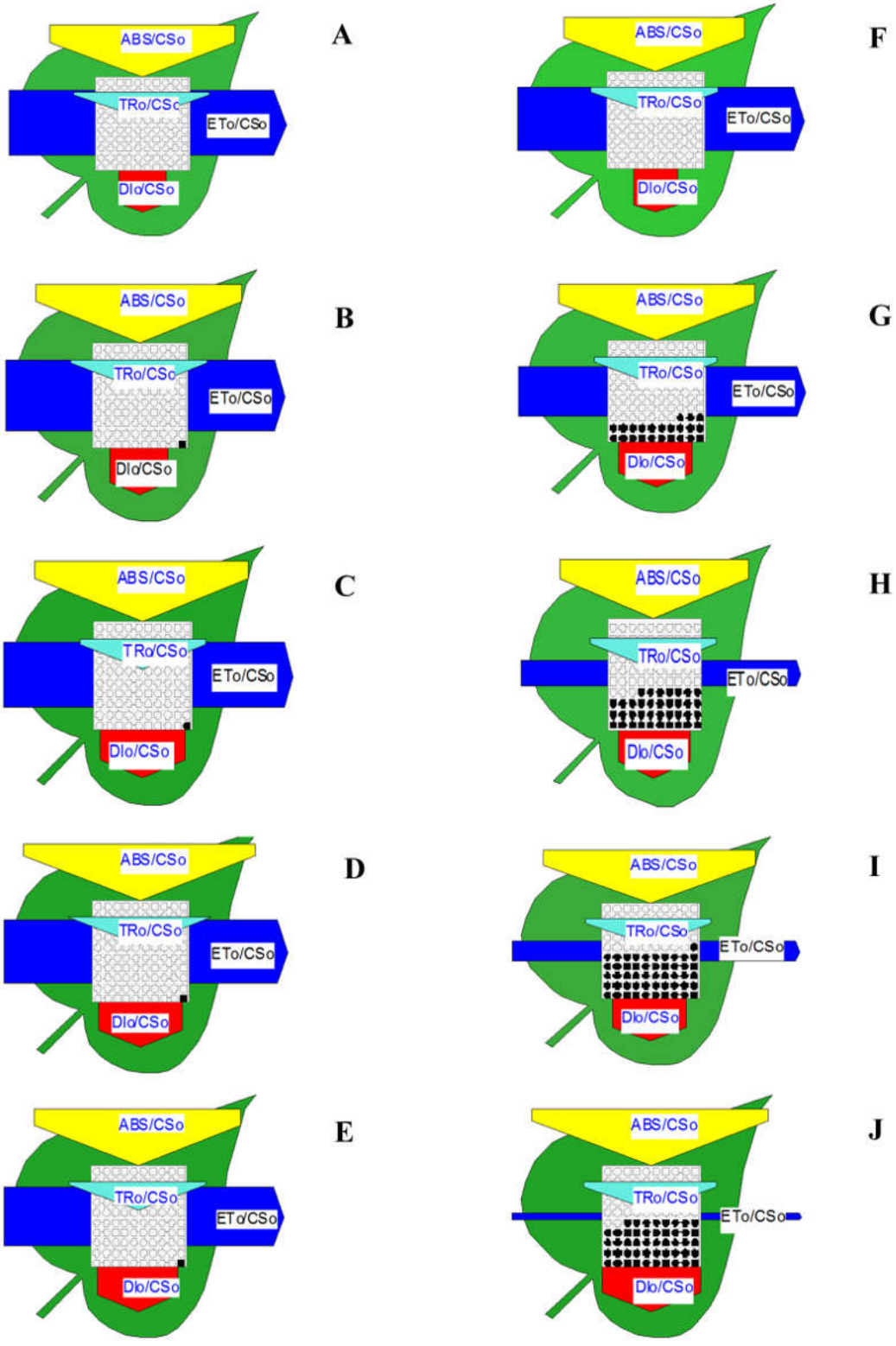

Figure 15: Energy pipeline leaf model of phenomenological energy fluxes per cross section (CSo) in *O. sativa* var. Aathira (A, B, C, D & E) and *O. sativa* var. Swarnaprabha leaves (F, G, H, I & J) after exposure to HL stress (2000 µmolm^{-2}s^{-1}) for different time intervals (0, 2, 4, 6 and 8 h). The value of each parameter can be seen in relative changes in width of each arrow.

A&F-0 h; B&G-2 h; C&H-4 h; D&I-6 h; E&J-8 h

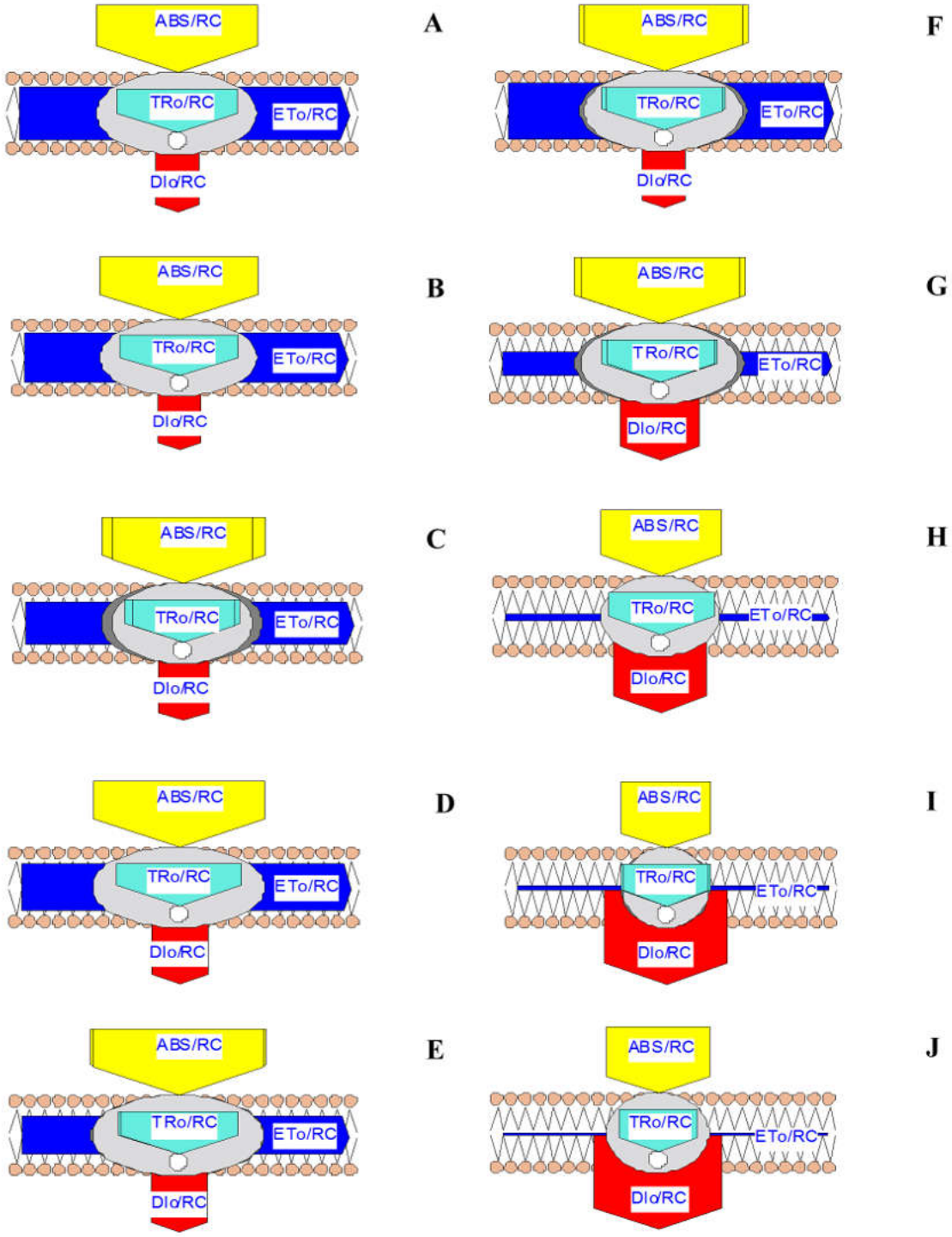

Figure 16: Specific membrane model of energy fluxes per reaction centre (RC) in leaves of O. *sativa* var. Aathira (A, B, C, D & E) and O. *sativa* var. Swarnaprabha (F, G, H, I & J) after exposure to HL stress (2000 µmolm^{-2}s^{-1}) for different time intervals (0, 2, 4, 6 and 8 h). The value of each parameter can be seen in relative changes in width of each arrow.

A&F-0 h; B&G-2 h; C&H-4 h; D&I-6 h; E&J-8 h

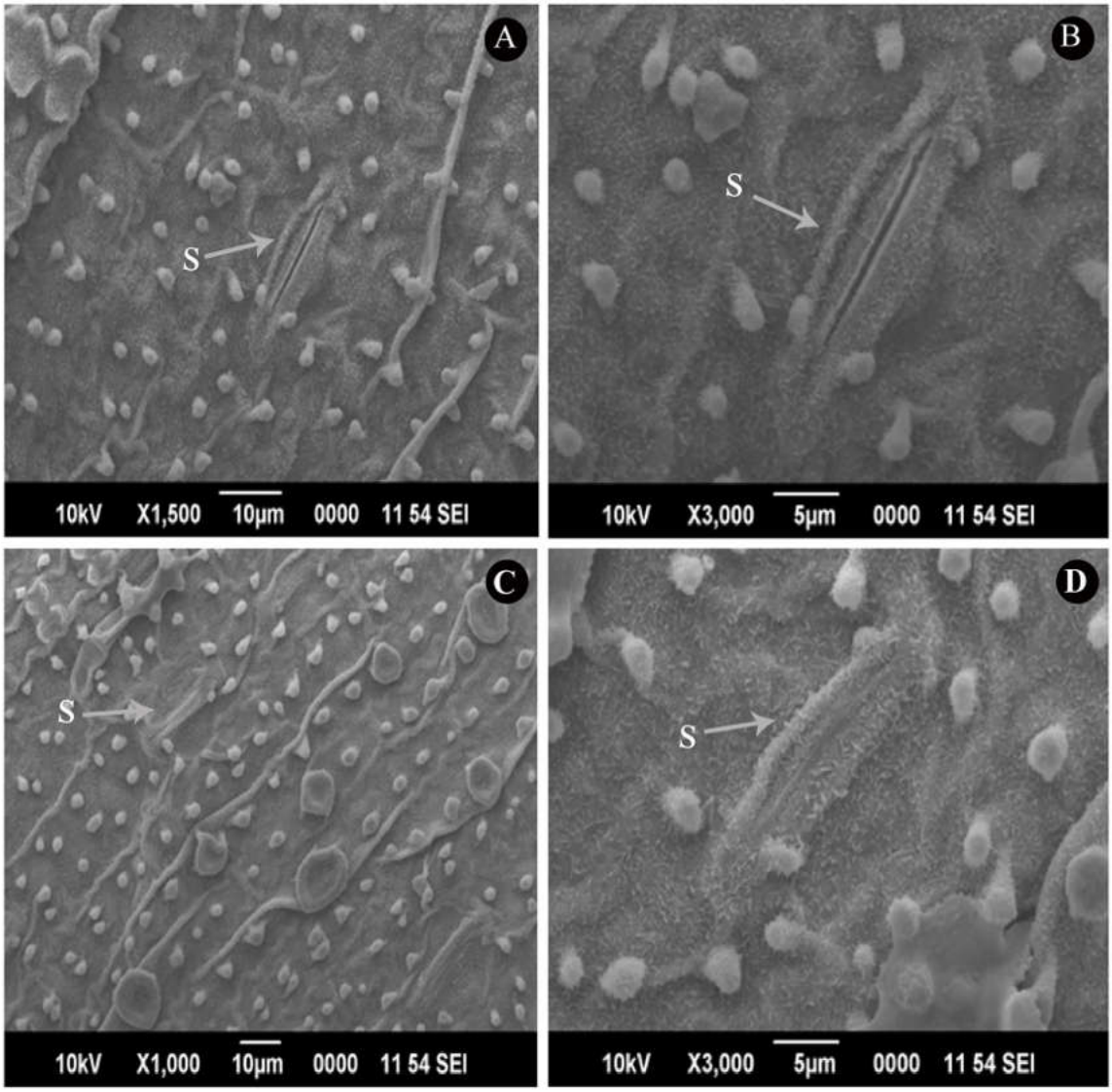

Figure 19: Scanning electron micrograph of the adaxial epidermis of *O. sativa* var. Aathira leaves after exposure to 0 (A&B) and 8 h (C&D) of HL stress (2000 µmolm^{-2}s^{-1}).

S-stomata

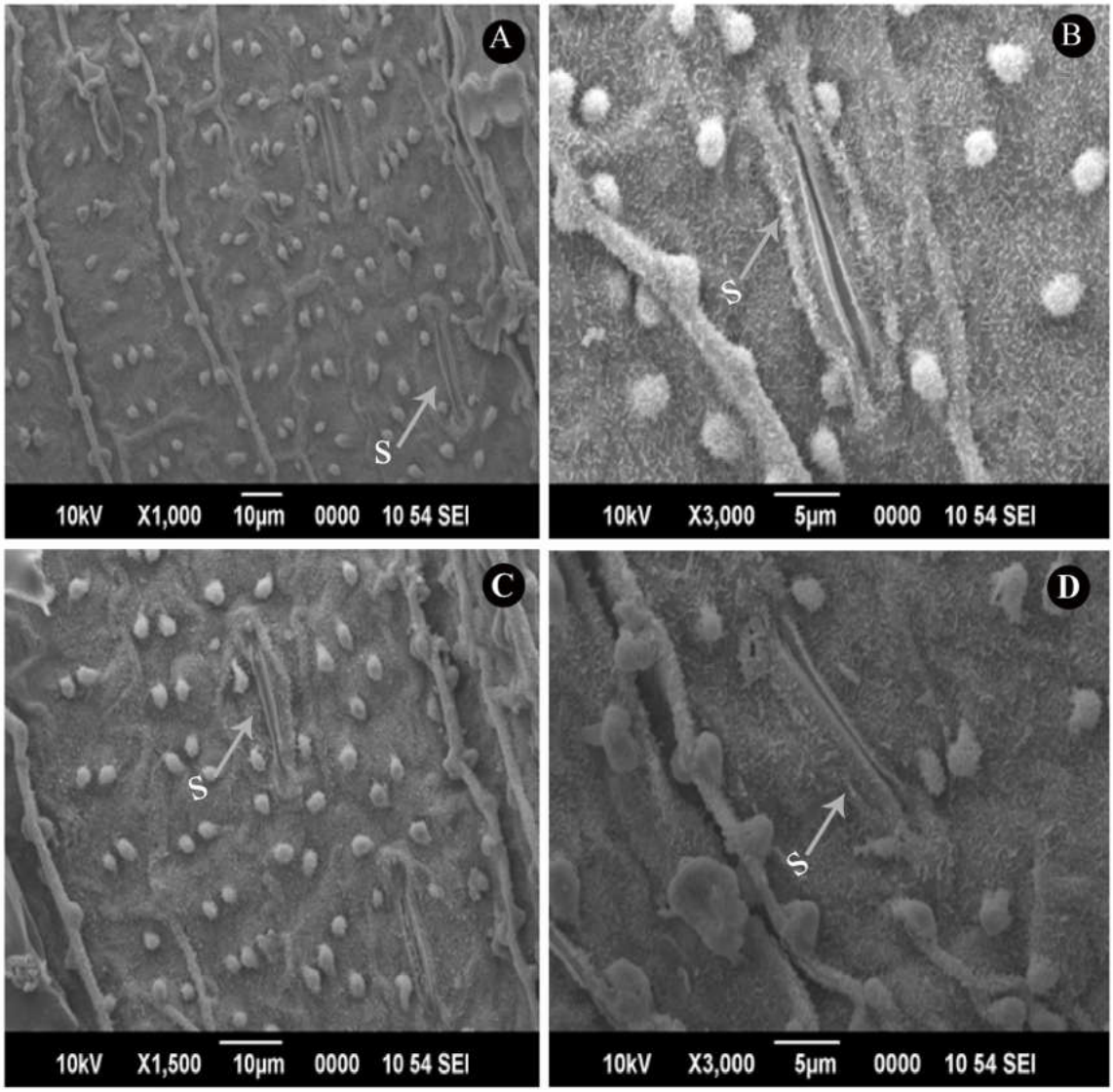

Figure 20: Scanning electron micrograph of the adaxial epidermis of *O. sativa* var. Swarnaprabha leaves after exposure to 0 (A&B) and 8 h (C&D) of HL stress (2000 µmolm^{-2}s^{-1}).

S-stomata

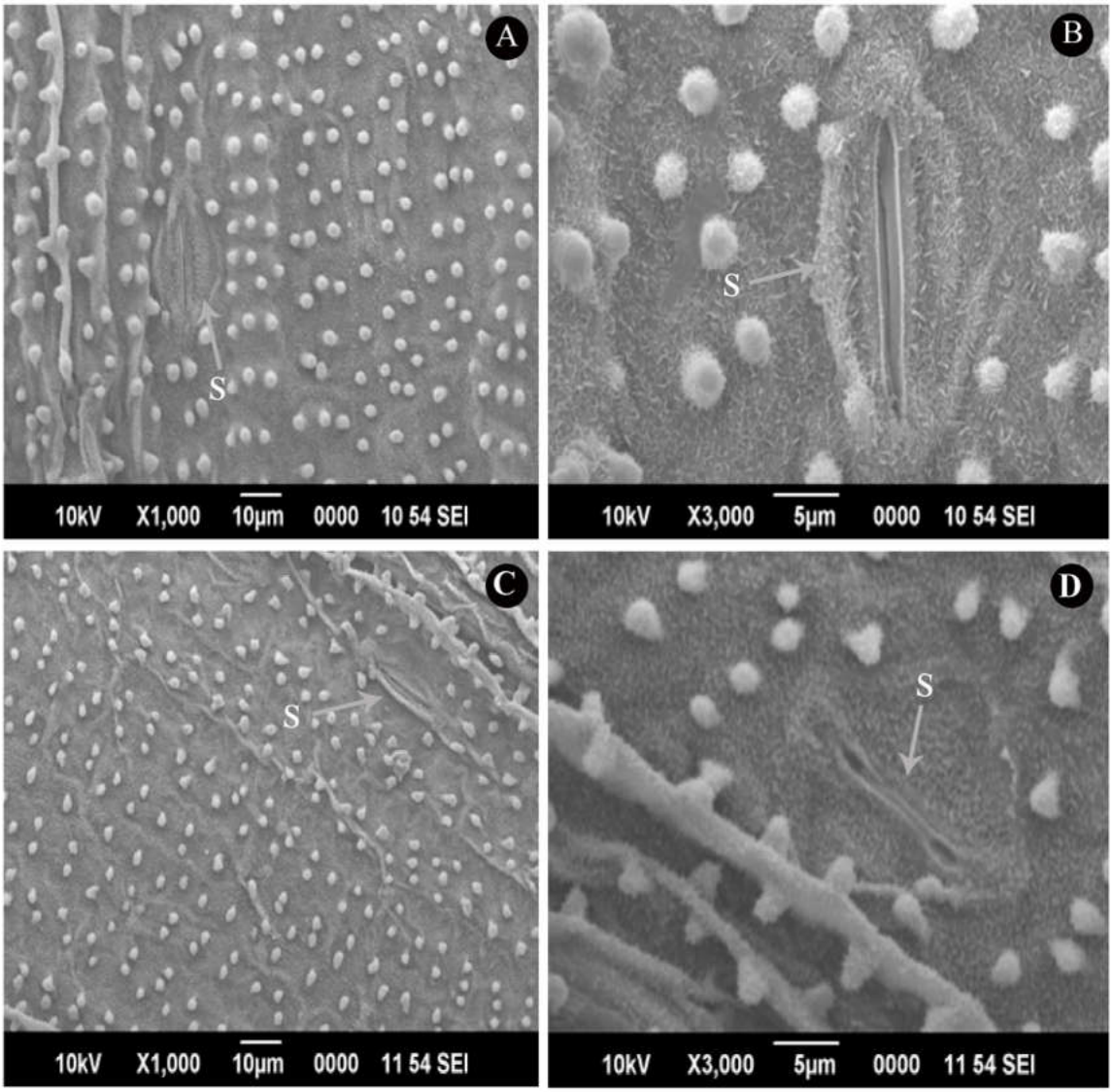

Figure 21: Scanning electron micrograph of the structural changes in the stomata of abaxial epidermis in *O. sativa* var. Aathira leaves after exposure to 0 (A&B) and 8 h (C&D) of HL stress (2000 µmolm^{-2}s^{-1}).

S-stomata

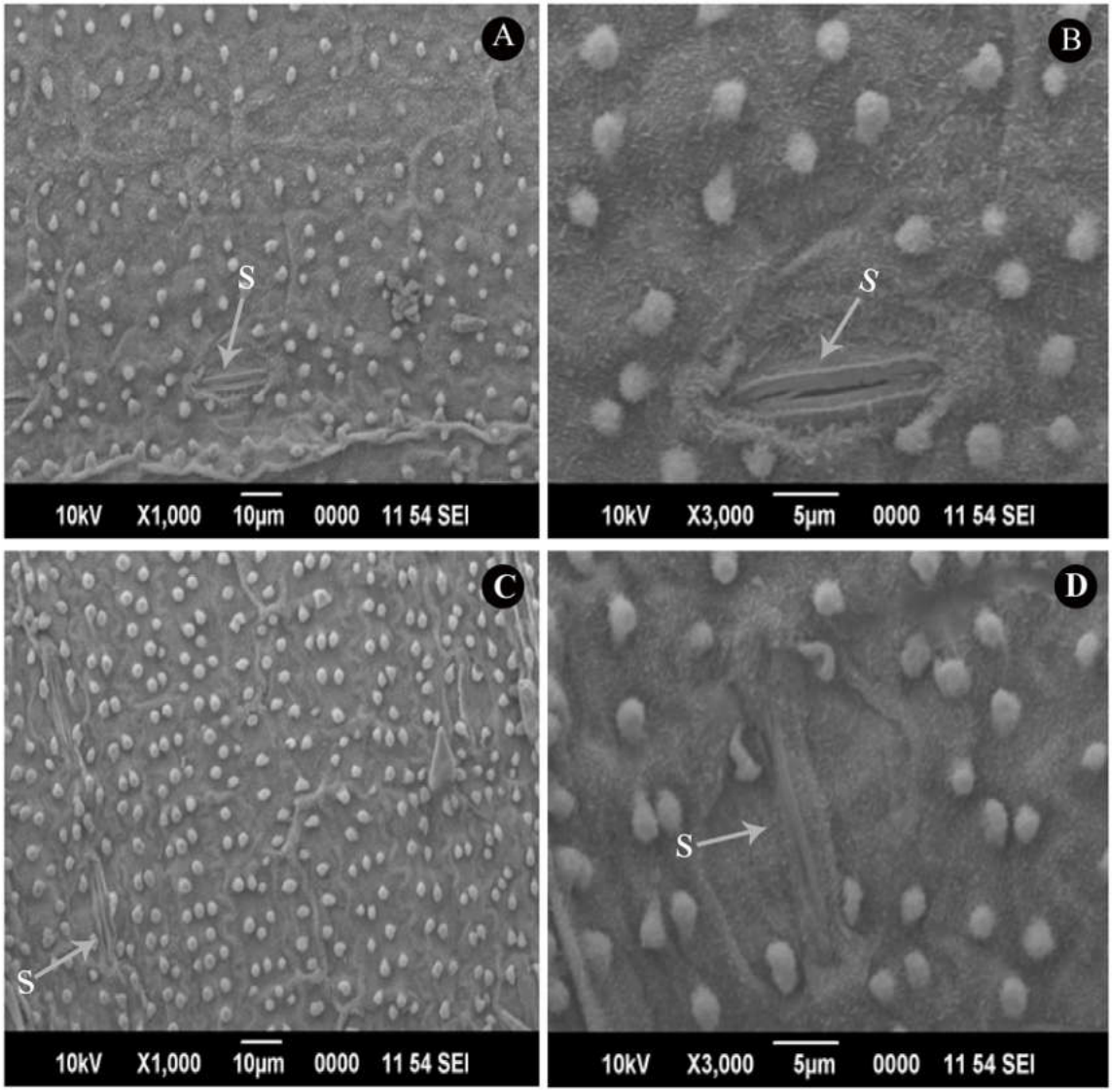

Figure 22: Scanning electron micrograph of the abaxial epidermis of *O. sativa* var. Swarnaprabha leaves after exposure to 0 (A&B) and 8 h (C&D) of HL stress (2000 µmolm^{-2}s^{-1}).

S-stomata

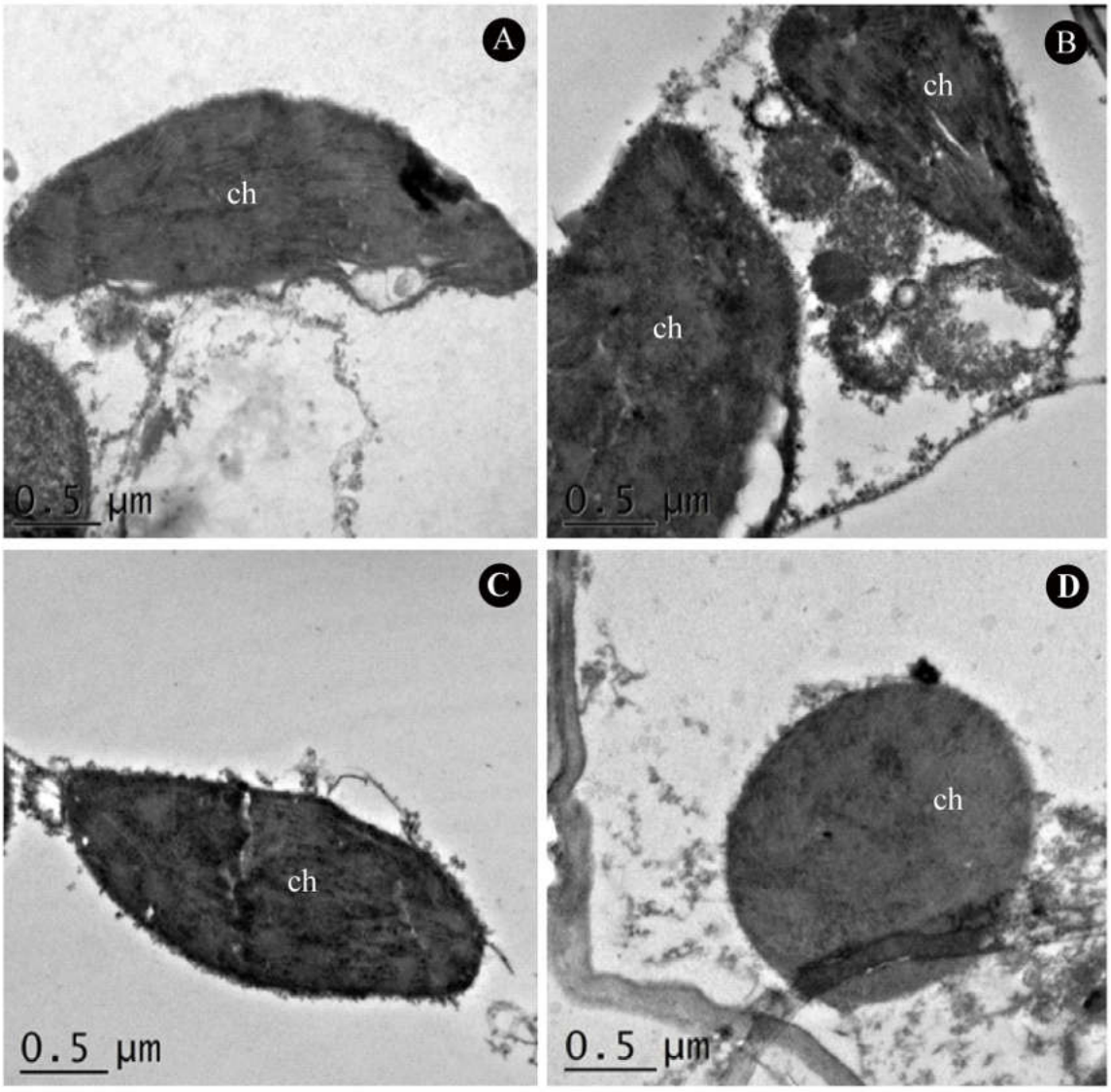

Figure 23: Transmission electron micrographs of chloroplasts from *O. sativa* var. Aathira (A&B) and *O. sativa* var. Swarnaprabha leaves (C&D) after exposure to 0 and 8 h of HL stress (2000 µmolm^{-2}s^{-1}).

A&C-Control; B&D-8 h; ch-choroplast

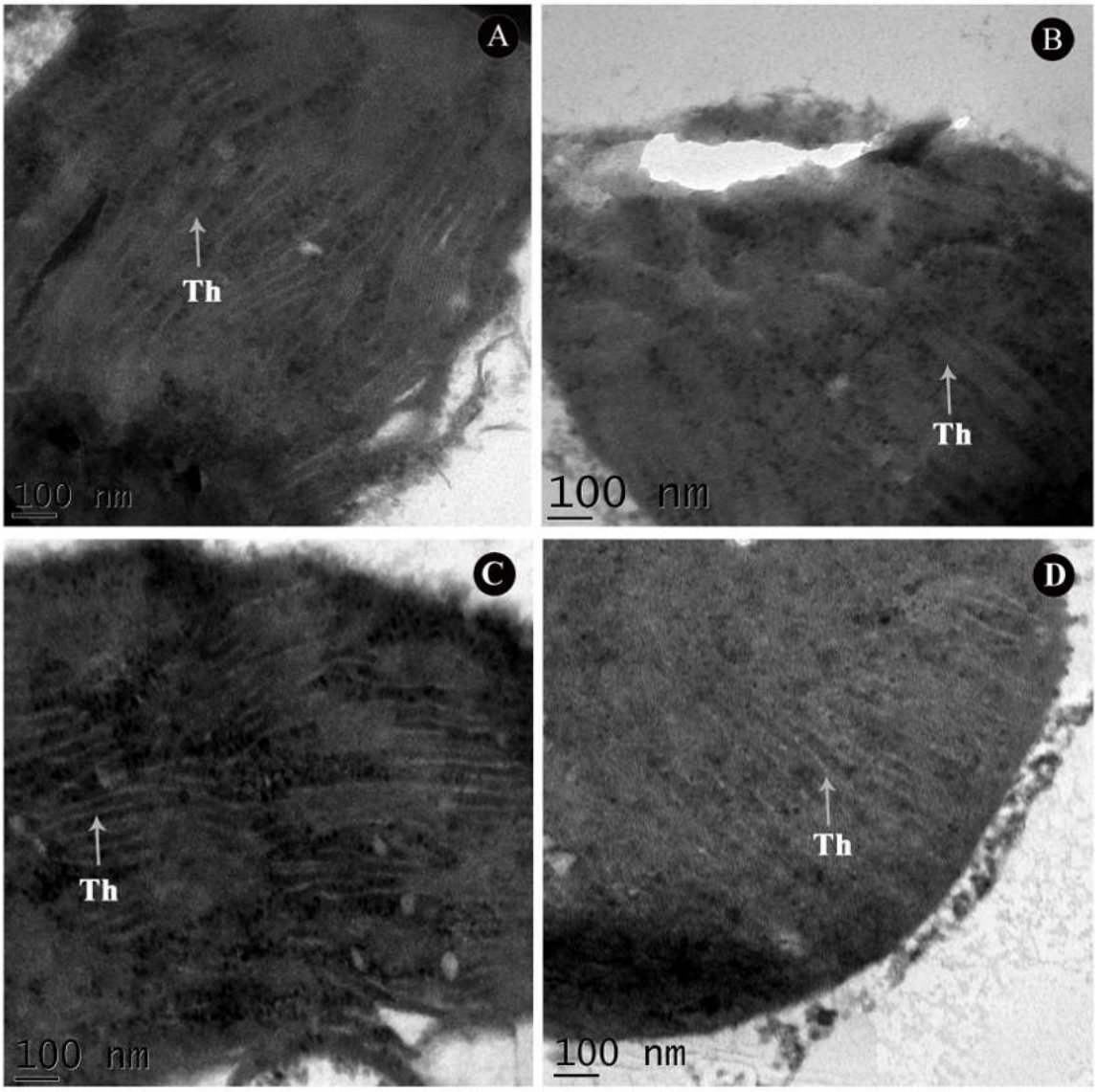

Figure 24: Ultrastructure of chloroplasts from *O. sativa* var. Aathira (A&B) and *O. sativa* var. Swarnaprabha leaves (C&D) after exposure to 0 and 8 h of HL stress (2000 µmolm^{-2}s^{-1}).

A&C-Control; B&D-8 h; Th-thylakoids

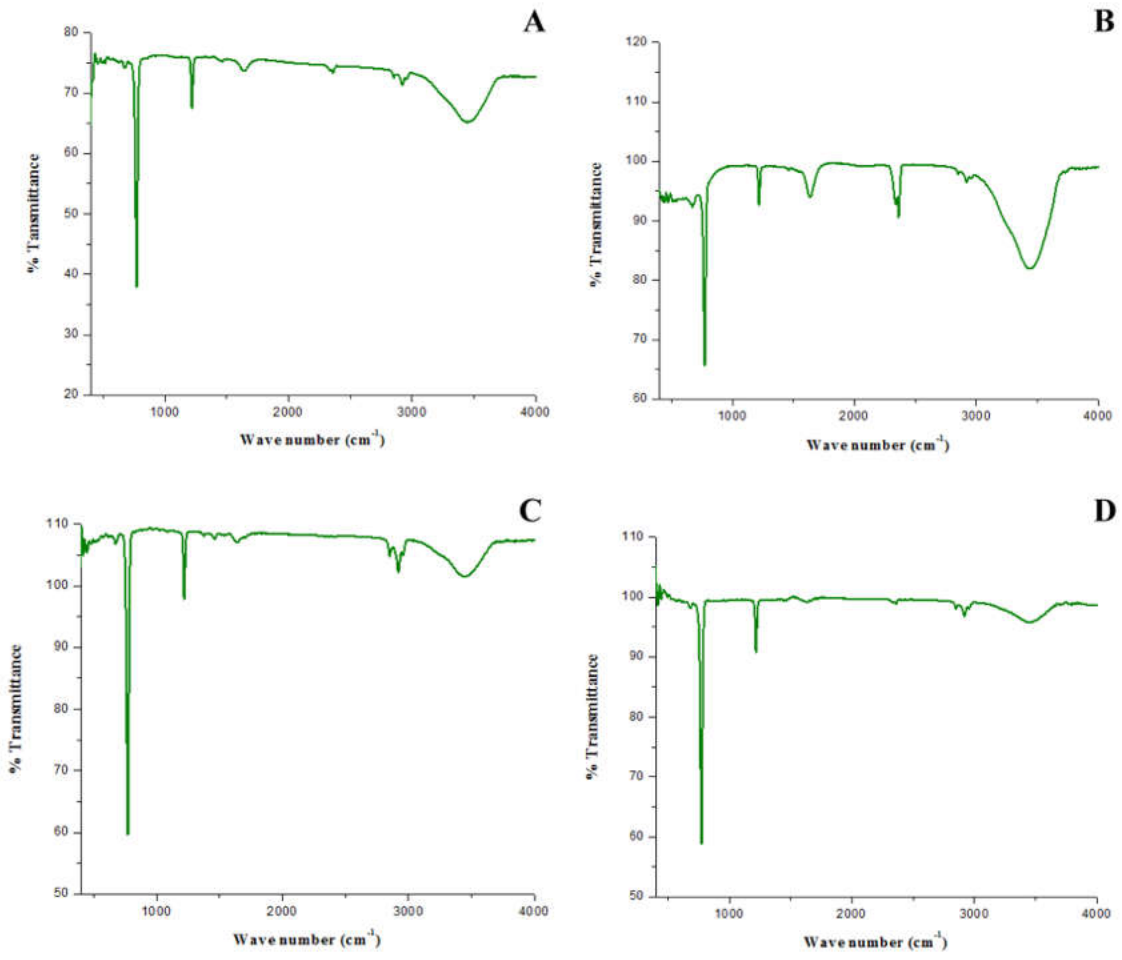

Figure 25: FT-IR spectra of epicuticular wax extracted from *O. sativa* var. Aathira (A&B) and *O. sativa* var. Swarnaprabha leaves (C&D) after exposure to 0 and 8 h of HL stress (2000 µmolm^{-2}s^{-1}).

A&C-Control; B&D-8 h

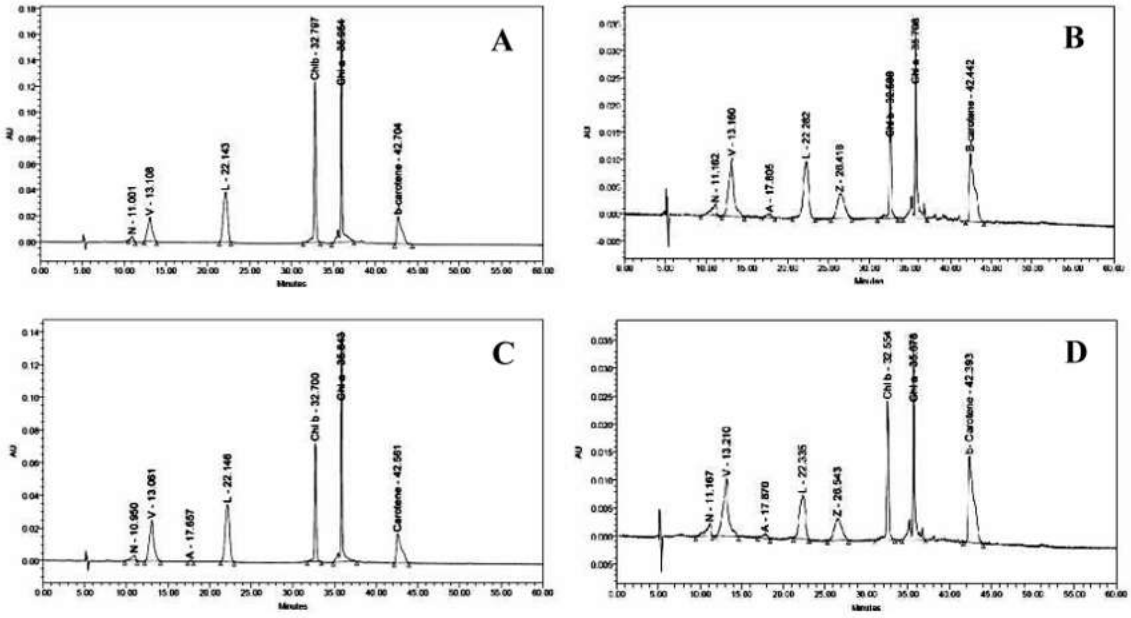

Figure 26: The HPLC profile of photosynthetic pigments of *O. sativa* var. Aathira (A&B) and *O. sativa* var. Swarnaprabha leaves (C&D) after exposure to 0 and 8 h of HL stress (2000 µmolm^{-2}s^{-1}).

A&C-0 h; B&D-8 h; N-neoxanthin, V-violaxanthin, A-antheraxanthin, L-lutein, Z-zeaxanthin, a-C- α-carotene and b-C- β-carotene

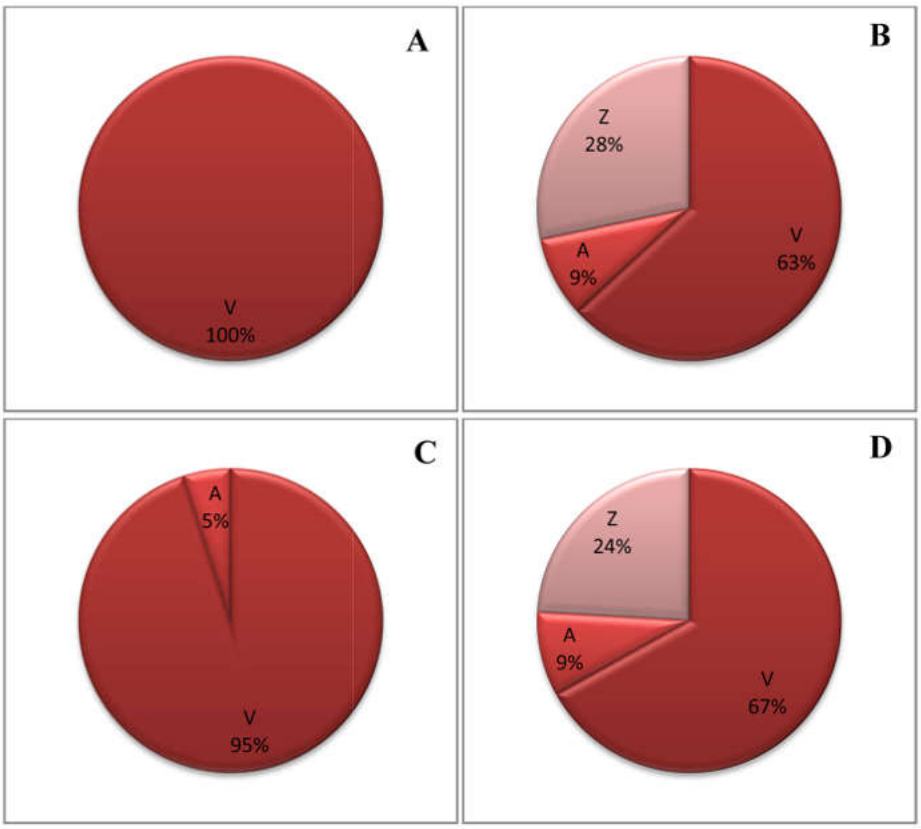

Figure 27: Composition of xanthophyll cycle pigments in *O. sativa* var. Aathira (A&B) and *O. sativa* var. Swarnaprabha (C&D) after exposure to 0 and 8 h of HL stress (2000 µmolm^{-2}s^{-1}).

A&C-0 h; B&D-8 h; A-antheraxanthin, Z-zeaxanthin, V-violaxanthin

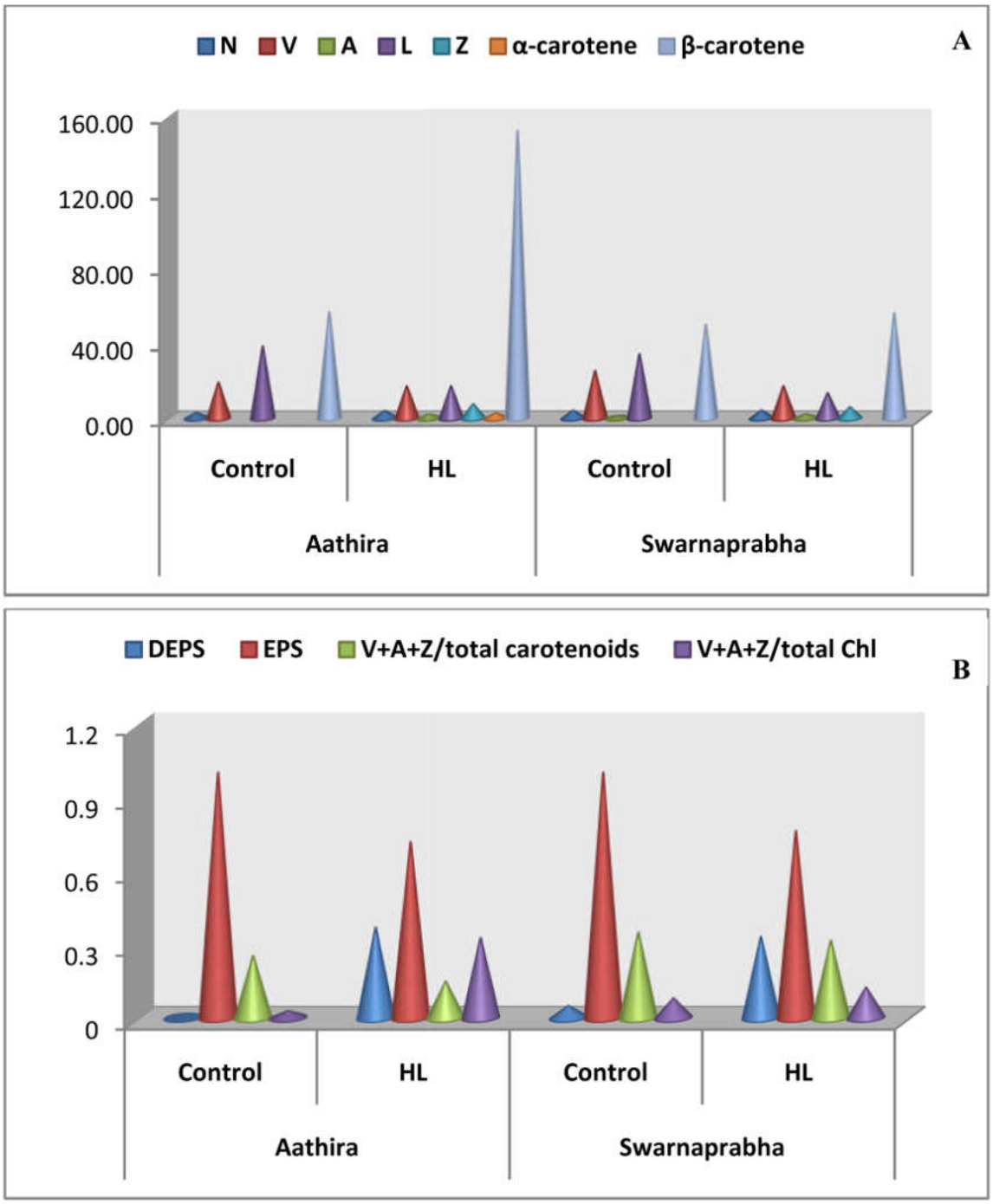

Figure 28: HPLC elution components of the photosynthetic xanthophyll pigments (A), changes in de-epoxidation (DEPS) and epoxidation (EPS) state of xanthophyll cycle pigments, the proportion of the xanthophyll cycle pool (V+A+Z) to total Chl and carotenoids (B) in *O. sativa* var. Aathira and *O. sativa* var. Swarnaprabha after exposure to 0 and 8 h of HL stress (2000 µmolm^{-2}s^{-1}).

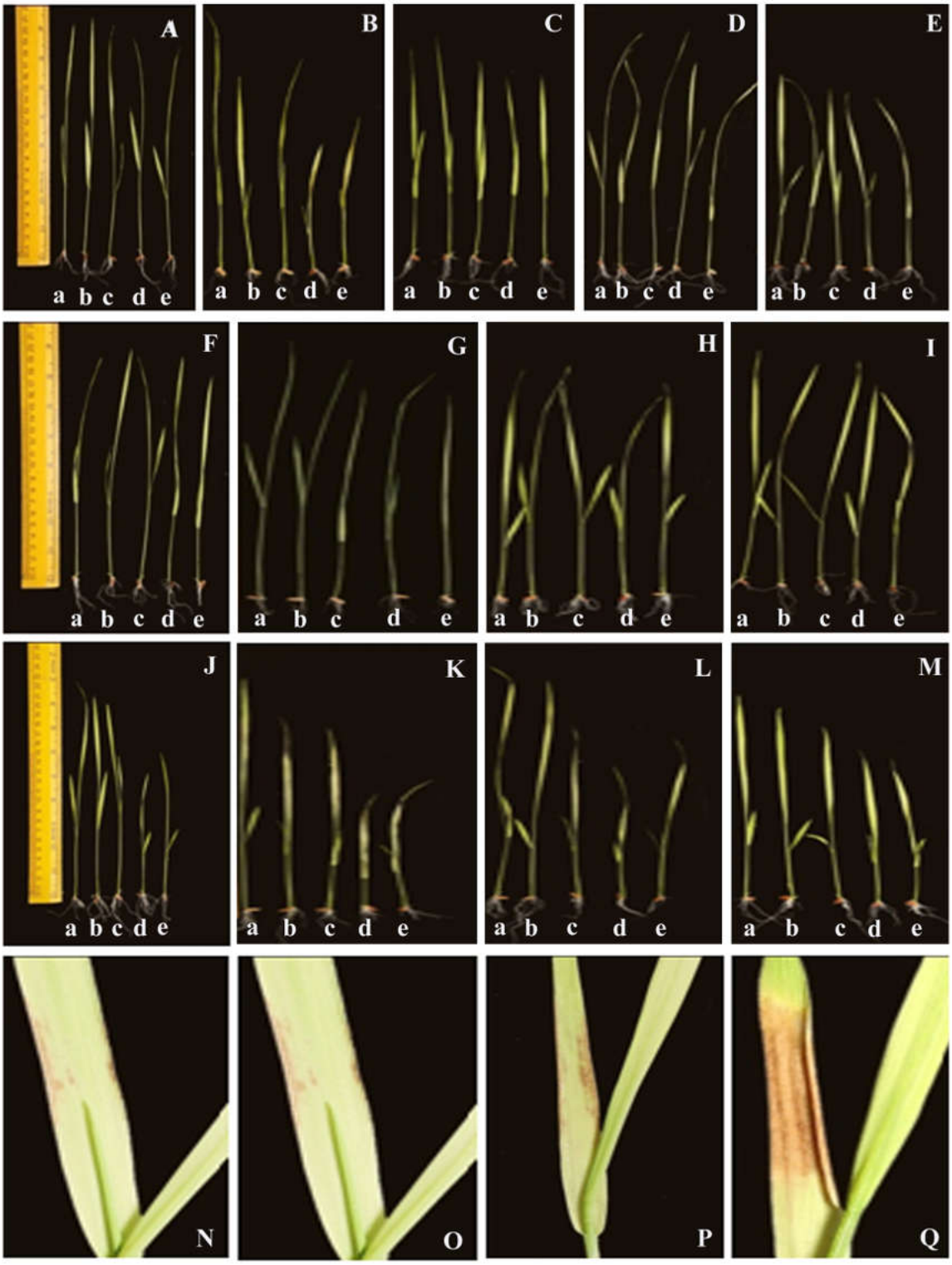

Figure 29: Shoot length of thirteen O. sativa varieties after exposure to various doses of UV-B irradiation [0 (a), 7 (b), 14 (c), 21 (d) and 28 $kJm^{-2}d^{-1}$ (e)]. Yellow patches observed after 21 and 28 $kJm^{-2}d^{-1}$ of UV-B treatment in Aiswarya (N&O, respectively) and Swarnaprabha (P&Q, respectively) leaves.

A-Aathira, B-Aiswarya, C-Annapoorna, D-Harsha, E-Jyothi, F-Kanchana, G-Karuna, H-Mangalamahsuri, I-Mattatriveni, J-Neeraja, K-Swarnaprabha, L-Swetha, M-Varsha.

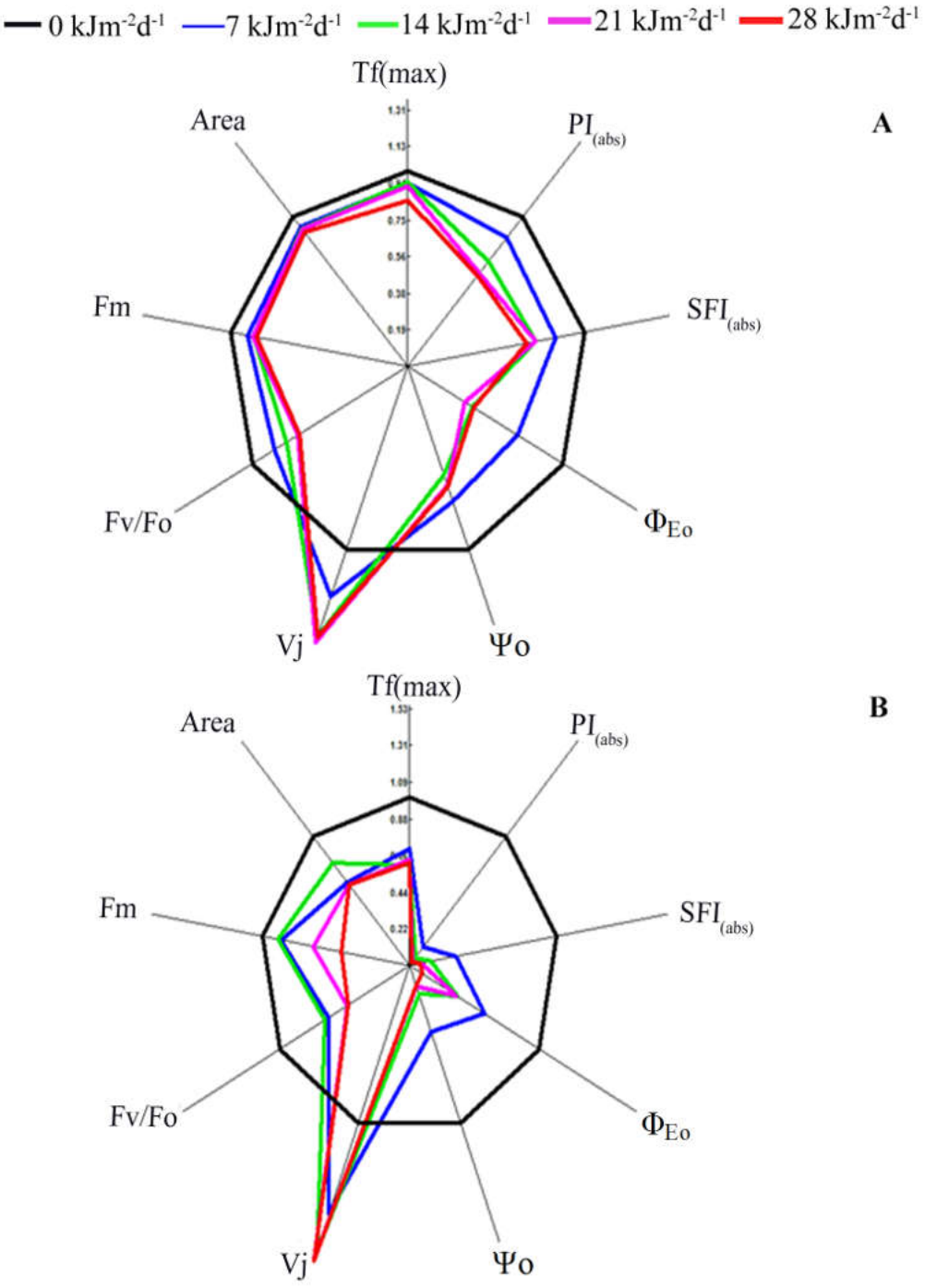

Figure 43: Radar plot of selected Chl *a* fluorescence parameters recorded in *O. sativa* var. Aathira (A) and *O. sativa* var. Swarnaprabha leaves (B) after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 kJm^{-2}d^{-1}).

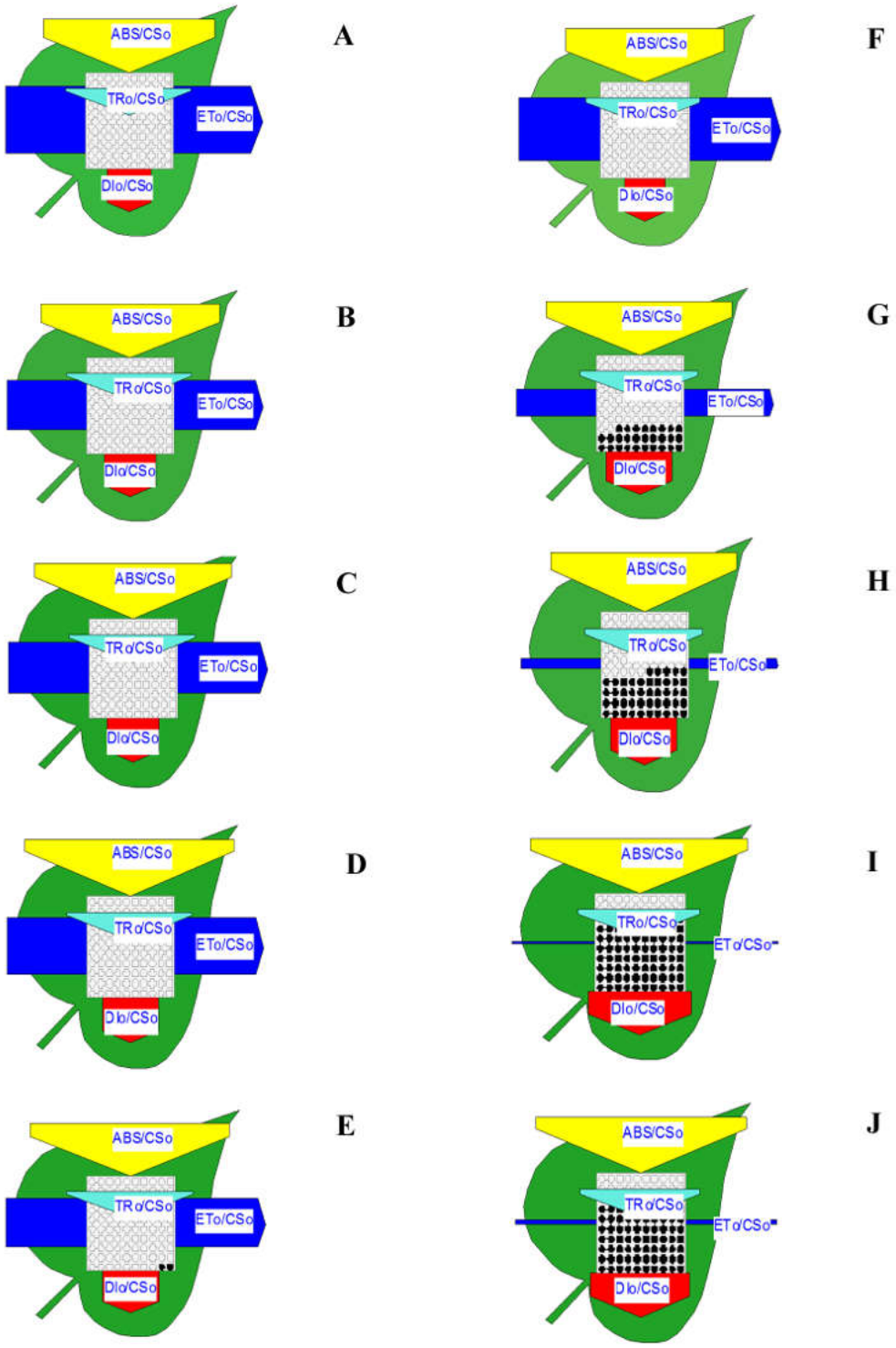

Figure 44: Energy pipeline leaf model of phenomenological energy fluxes per cross section (CSo) in *O. sativa* var. Aathira (A, B, C, D & E) and *O. sativa* var. Swarnaprabha leaves (F, G, H, I & J) after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$). The value of each parameter can be seen in relative changes in width of each arrow.

A&F-0 $kJm^{-2}d^{-1}$; B&G-7 $kJm^{-2}d^{-1}$; C&H-14 $kJm^{-2}d^{-1}$; D&I-21 $kJm^{-2}d^{-1}$; E&J-28 $kJm^{-2}d^{-1}$

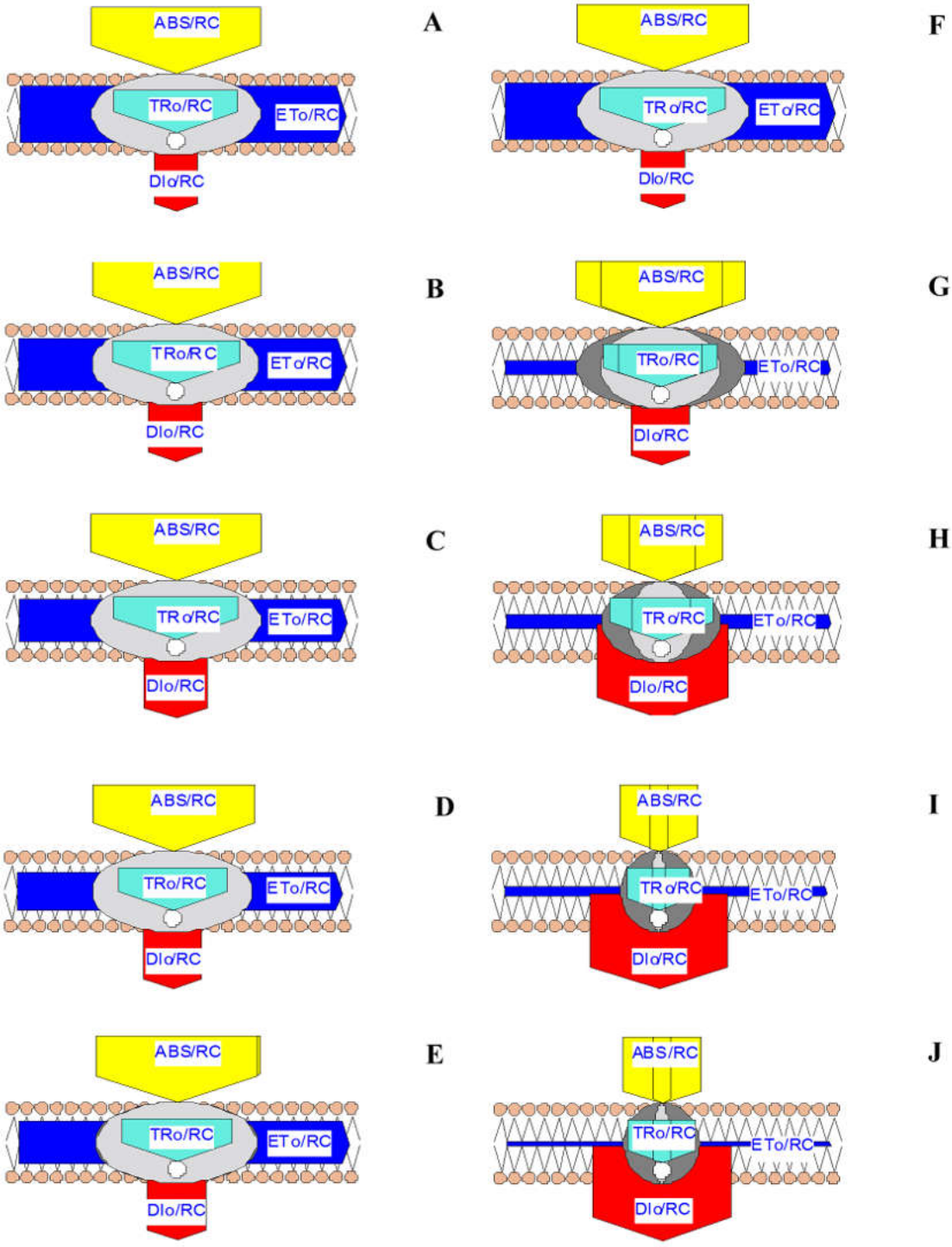

Figure 45: Specific membrane model of energy fluxes per reaction centre (RC) in *O.sativa* var. Aathira (A, B, C, D & E) and *O. sativa* var. Swarnaprabha leaves (F, G, H, I & J) after exposure to various doses of UV-B irradiation (0, 7, 14, 21 and 28 $kJm^{-2}d^{-1}$). The value of each parameter can be seen in relative changes in width of each arrow.

A&F-0 $kJm^{-2}d^{-1}$; B&G-7 $kJm^{-2}d^{-1}$; C&H-14 $kJm^{-2}d^{-1}$; D&I-21 $kJm^{-2}d^{-1}$; E&J-28 $kJm^{-2}d^{-1}$

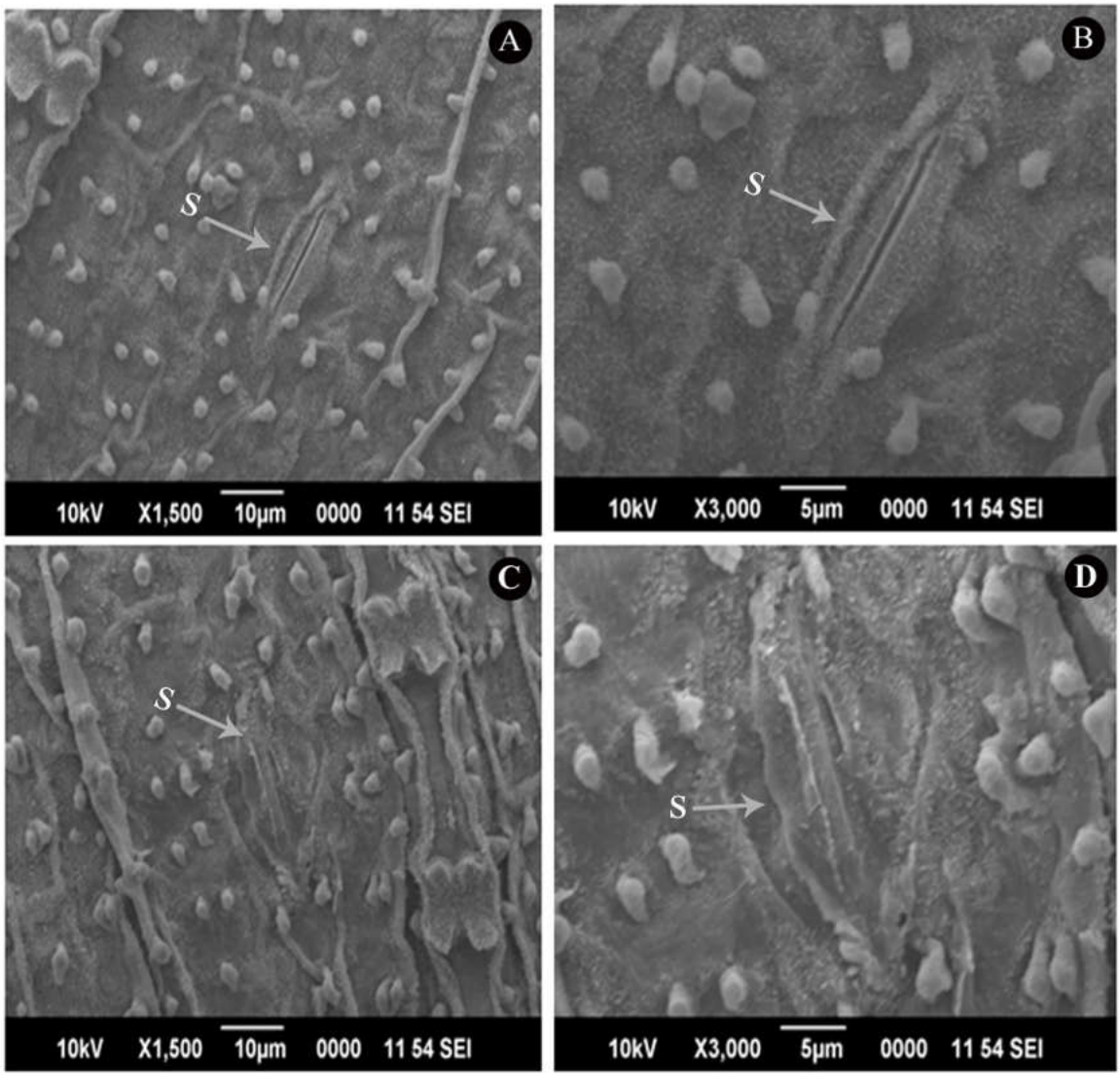

Figure 48: Scanning electron micrograph of the adaxial epidermis of *O. sativa* var. Aathira leaves after exposure to 0 (A&B) and 28 $kJm^{-2}d^{-1}$ (C&D) of UV-B irradiation.

S-stomata

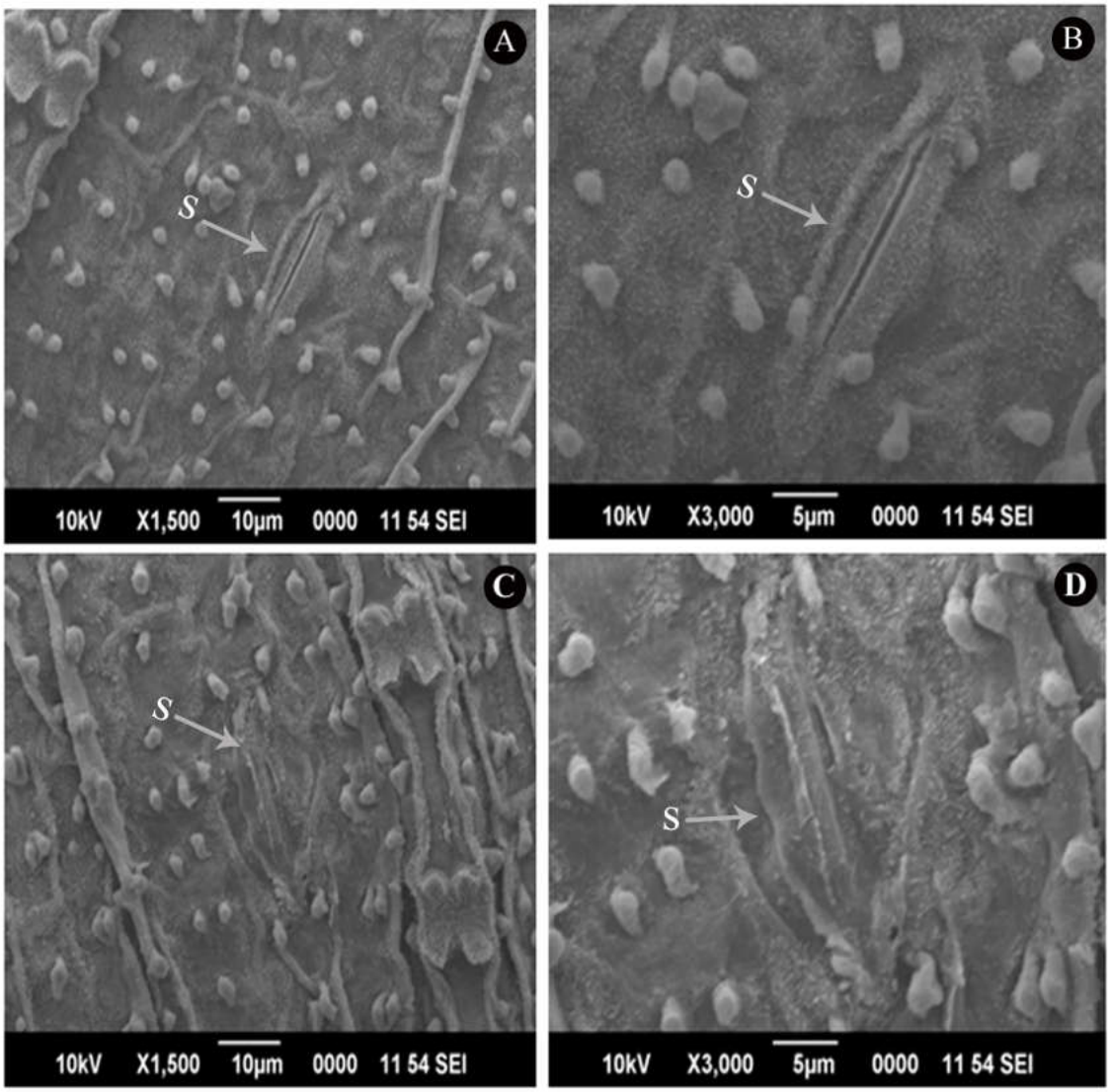

Figure 48: Scanning electron micrograph of the adaxial epidermis of *O. sativa* var. Aathira leaves after exposure to 0 (A&B) and 28 $kJm^{-2}d^{-1}$ (C&D) of UV-B irradiation.

S-stomata

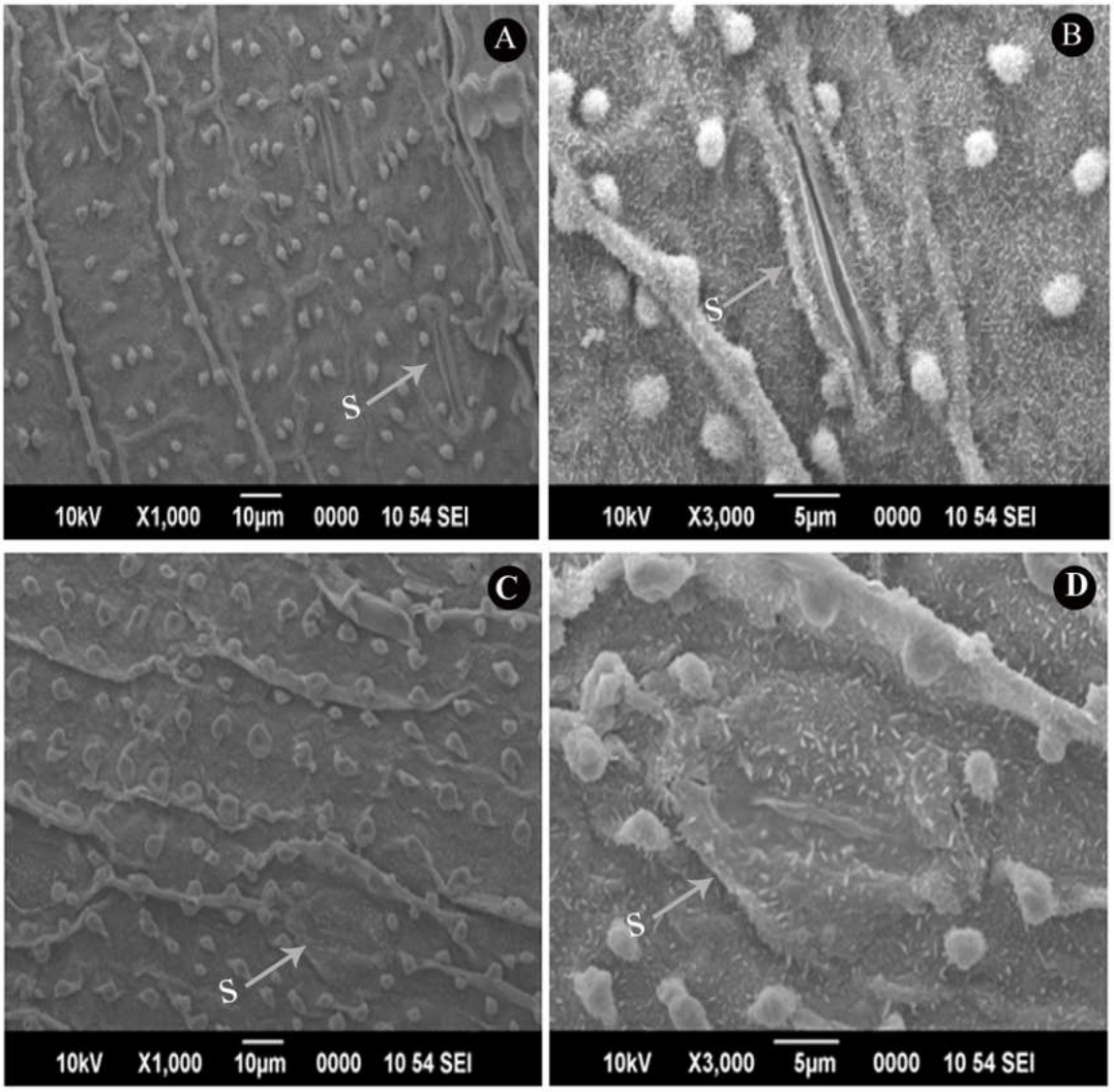

Figure 49: Scanning electron micrograph of the adaxial epidermis of *O. sativa* var. Swarnaprabha leaves after exposure to 0 (A&B) and 28 $kJm^{-2}d^{-1}$ (C&D) of UV-B irradiation.

S-stomata

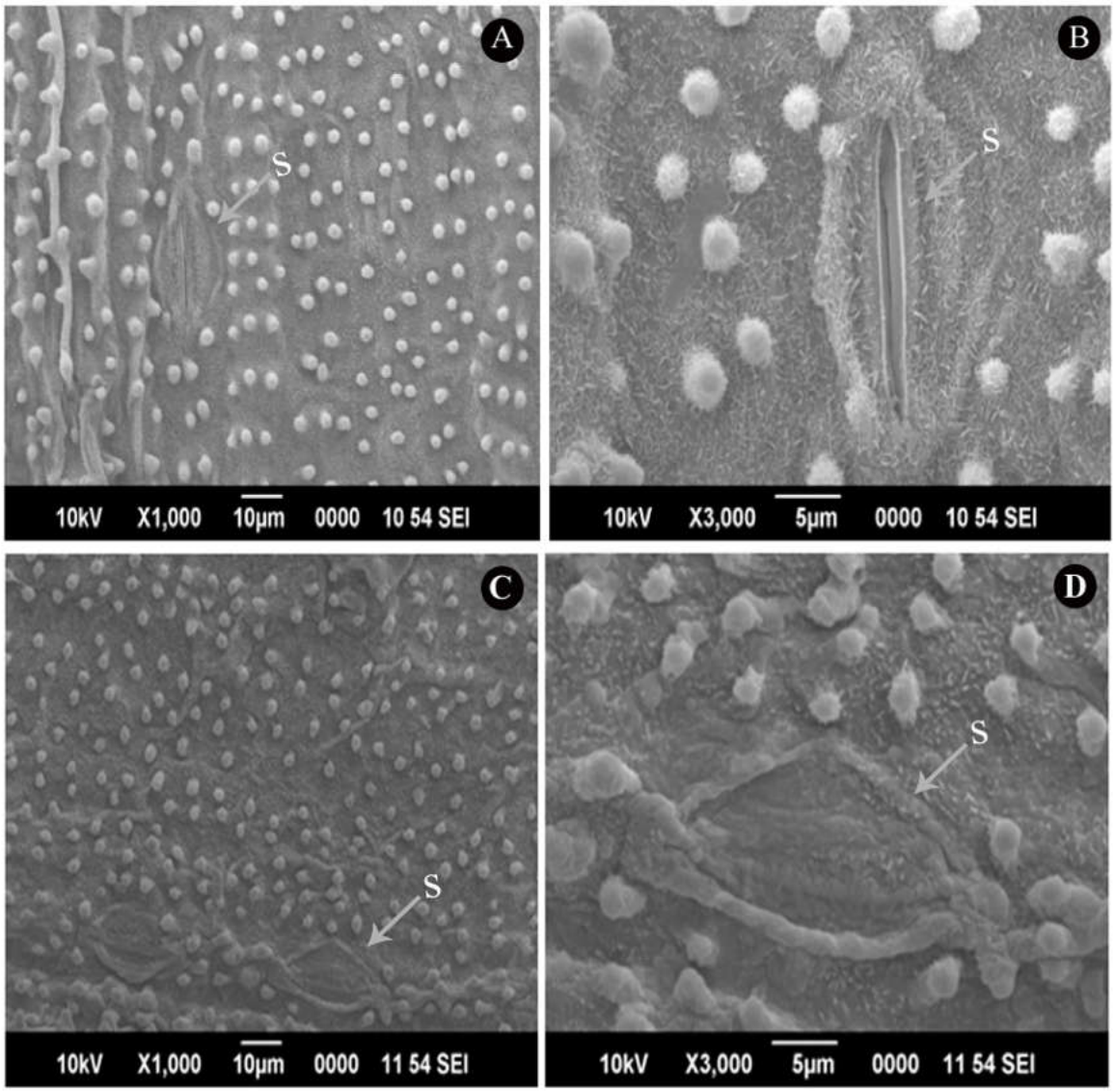

Figure 50: Scanning electron micrograph of the abaxial epidermis of *O. sativa* var. Aathira leaves after exposure to 0 (A&B) and 28 $kJm^{-2}d^{-1}$ (C&D) of UV-B irradiation.

S-stomata

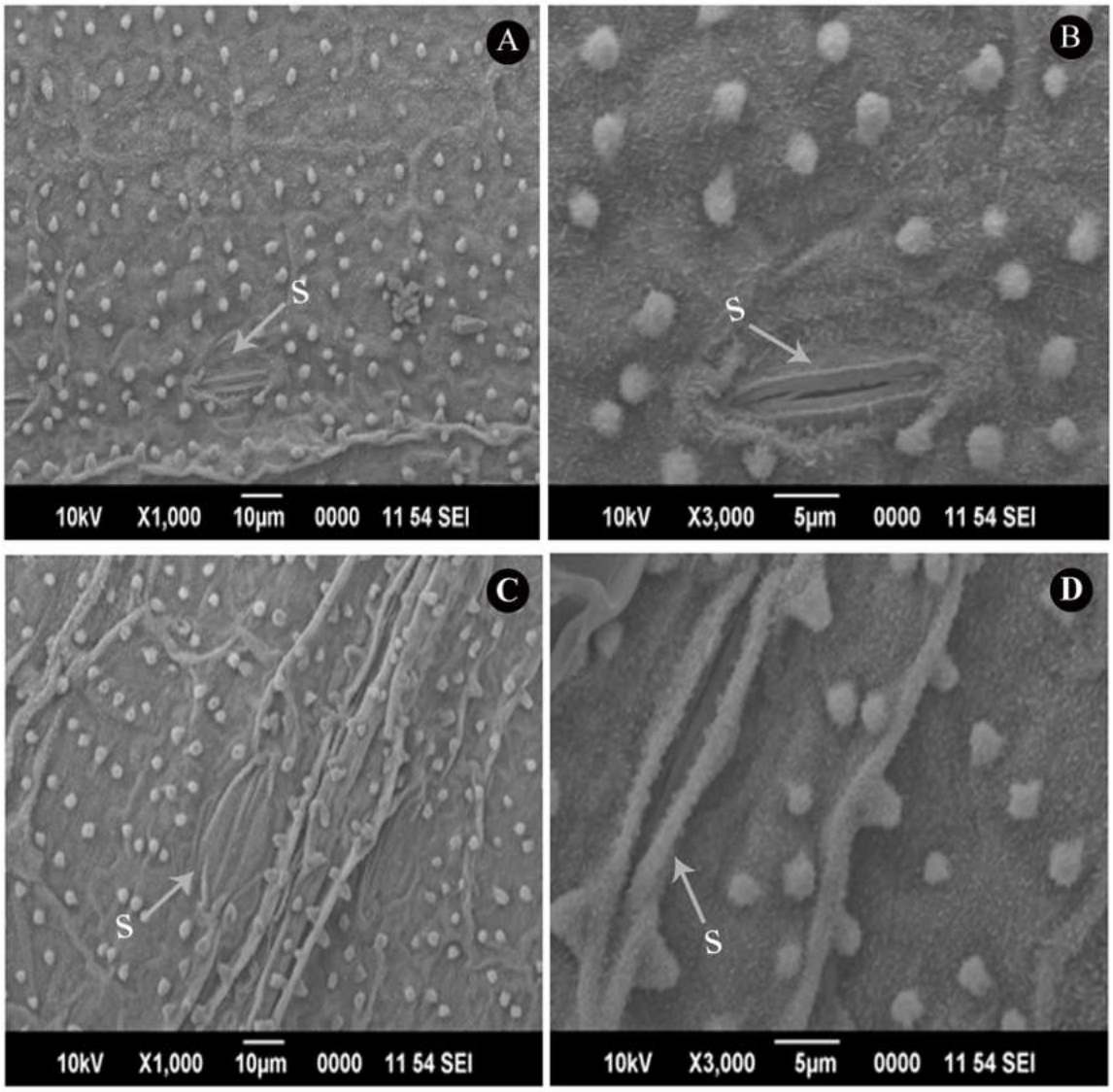

Figure 51: Scanning electron micrograph of the abaxial epidermis of *O. sativa* var. Swarnaprabha leaves after exposure to 0 (A&B) and 28 $kJm^{-2}d^{-1}$ (C&D) of UV-B irradiation.

S-stomata

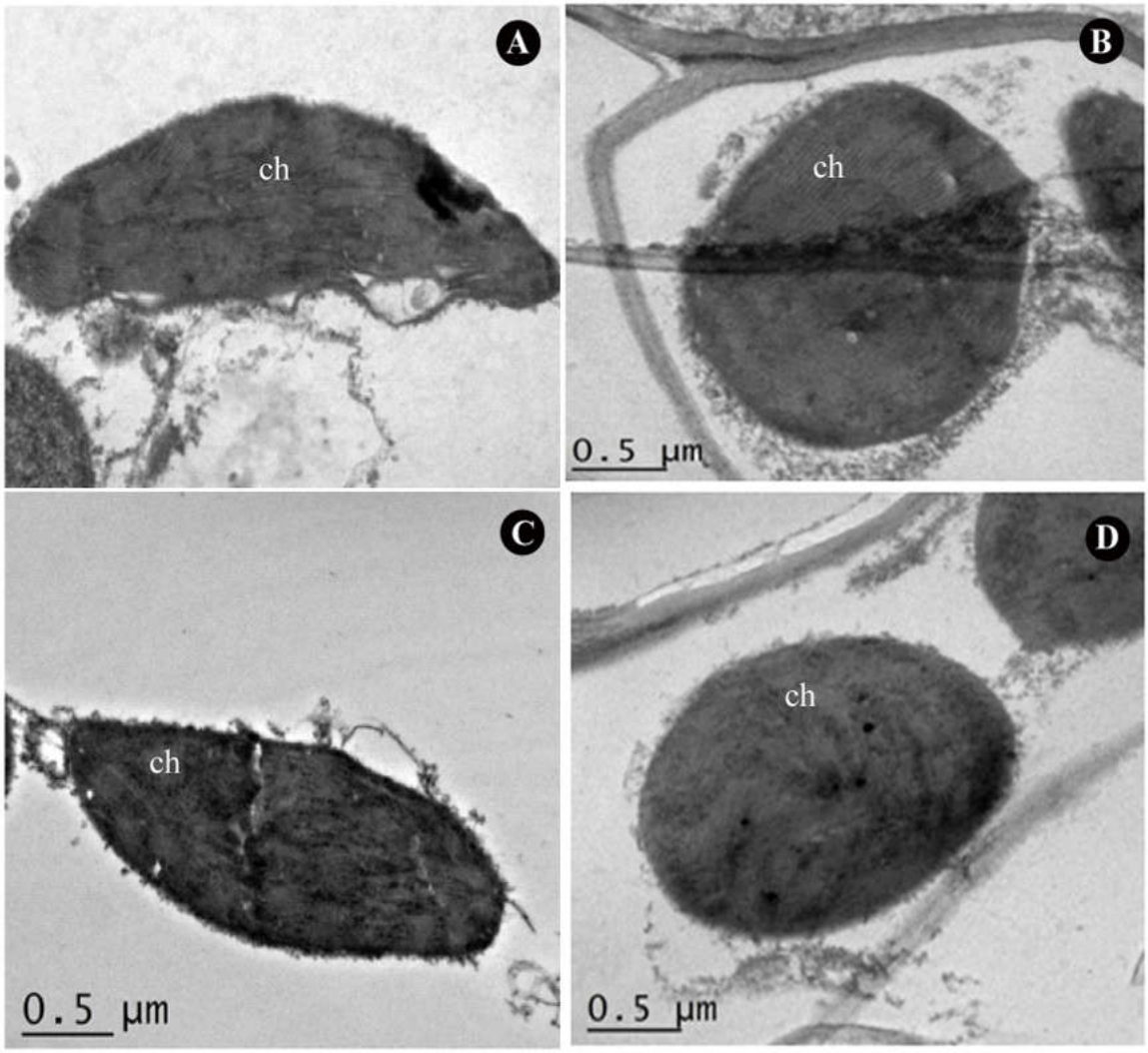

Figure 52: Transmission electron micrographs of chloroplasts from *O. sativa* var. Aathira (A&B) and *O. sativa* var. Swarnaprabha leaves (C&D) after exposure to 0 and 28 $kJm^{-2}d^{-1}$ of UV-B irradiation.

A&C-0 $kJm^{-2}d^{-1}$; B&D-28 $kJm^{-2}d^{-1}$; ch-chloroplast

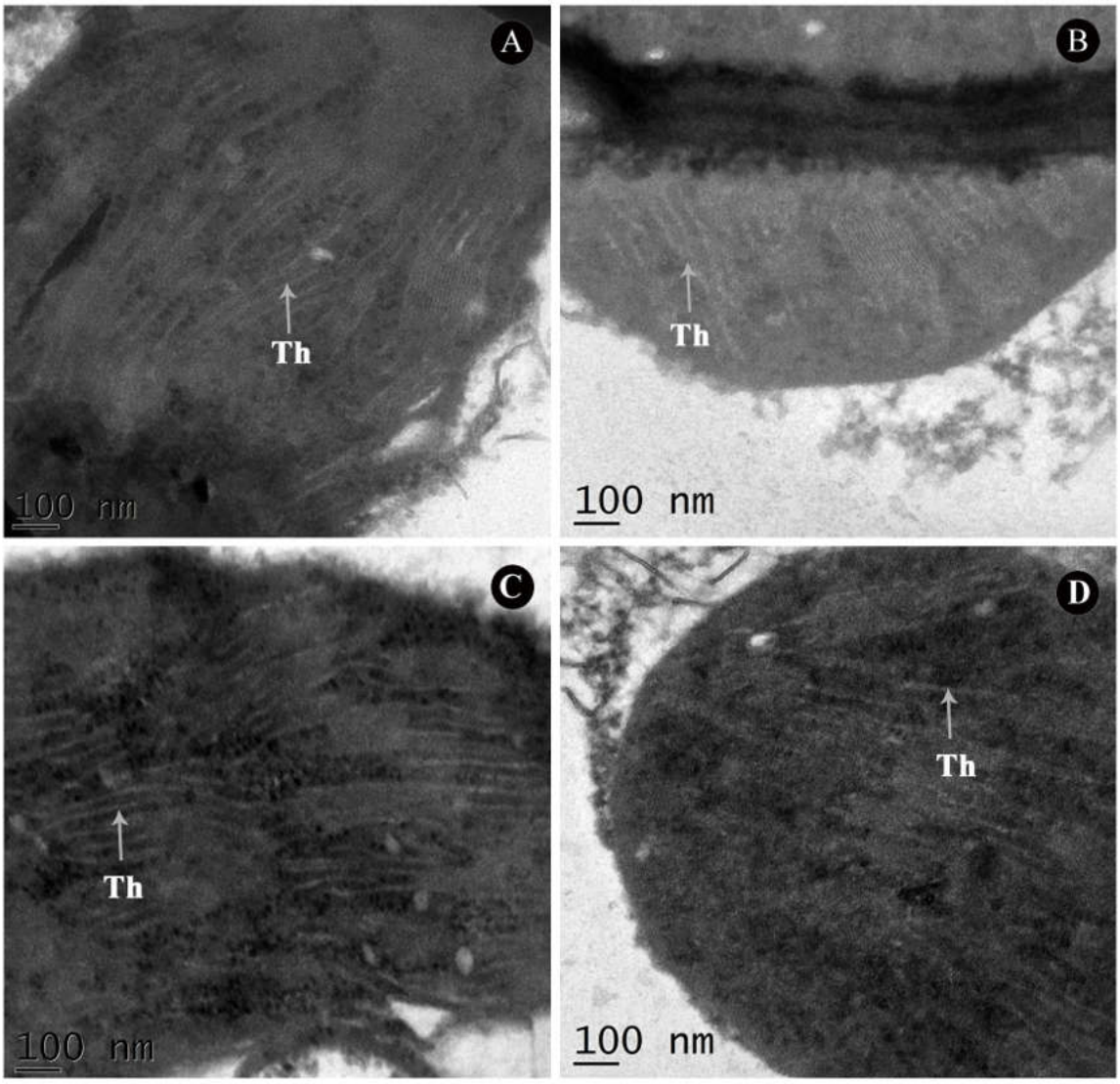

Figure 53: Ultrastructure of chloroplasts from *O. sativa* var. Aathira (A&B) and *O. sativa* var. Swarnaprabha leaves (C&D) after exposure to 0 and 28 $kJm^{-2}d^{-1}$ of UV-B irradiation.

A&C-0 $kJm^{-2}d^{-1}$; B&D-28 $kJm^{-2}d^{-1}$; Th-thylakoids

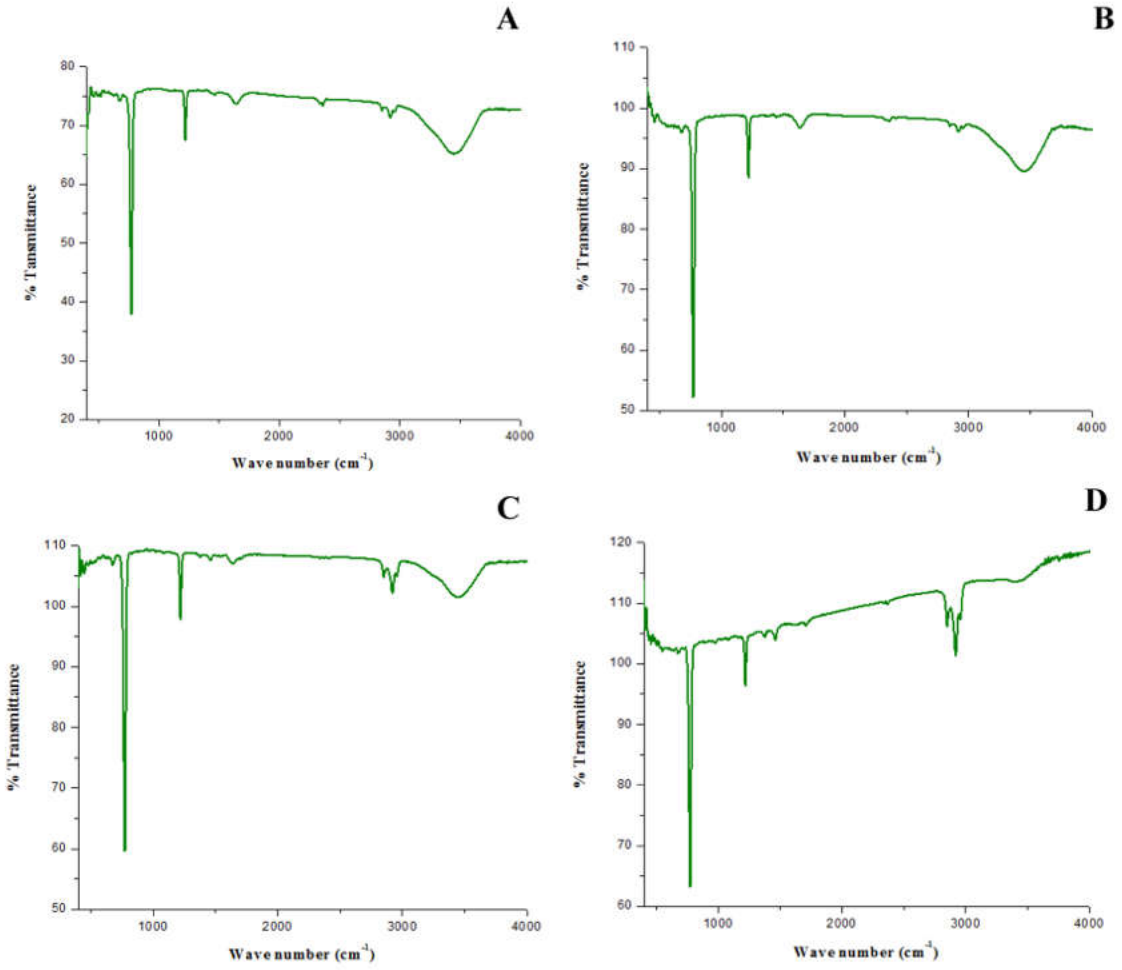

Figure 54: FT-IR spectra of epicuticular wax extracted from *O. sativa* var. Aathira (A&B) and *O. sativa* var. Swarnaprabha leaves (C&D) after exposure to 0 and 28 kJm^{-2}d^{-1} of UV-B irradiation.

A&C-0 kJm^{-2}d^{-1}; B&D-28 kJm^{-2}d^{-1}

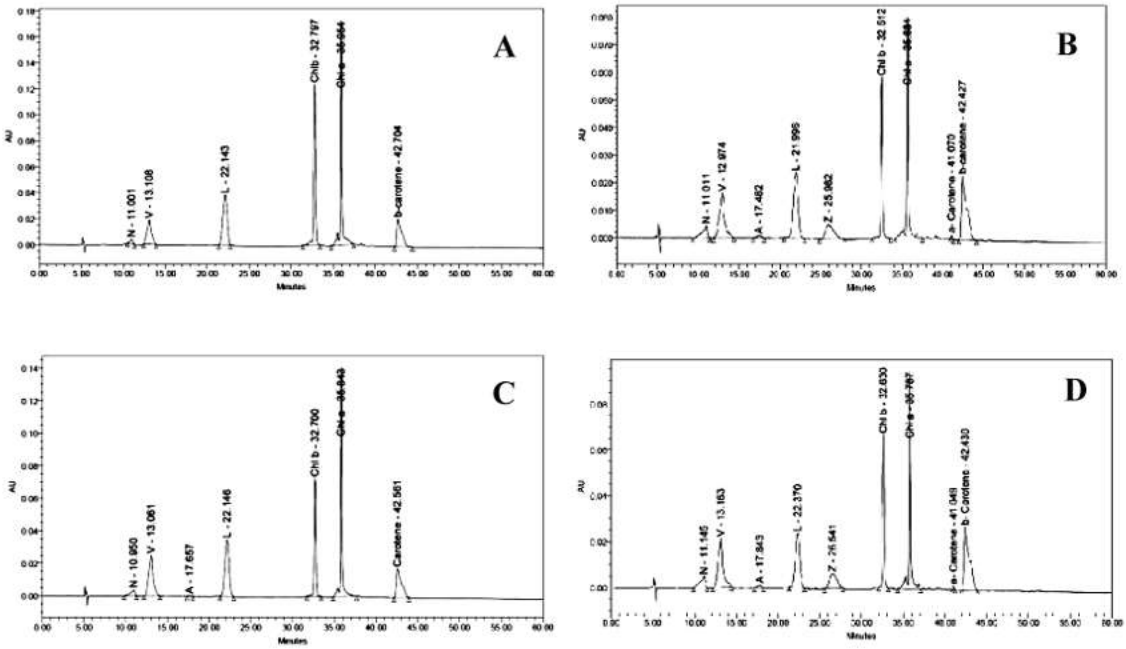

Figure 55: The HPLC profile of photosynthetic pigments of *O. sativa* var. Aathira (A&B) and *O. sativa* var. Swarnaprabha leaves (C&D) after exposure to 0 and 28 kJm^{-2}d^{-1} of UV-B irradiation.

A&C-0 kJm^{-2}d^{-1}; B&D-28 kJm^{-2}d^{-1}; N-neoxanthin, V-violaxanthin, A-antheraxanthin, L-lutein, Z-zeaxanthin, a-C- α-carotene and b-C- β-carotene

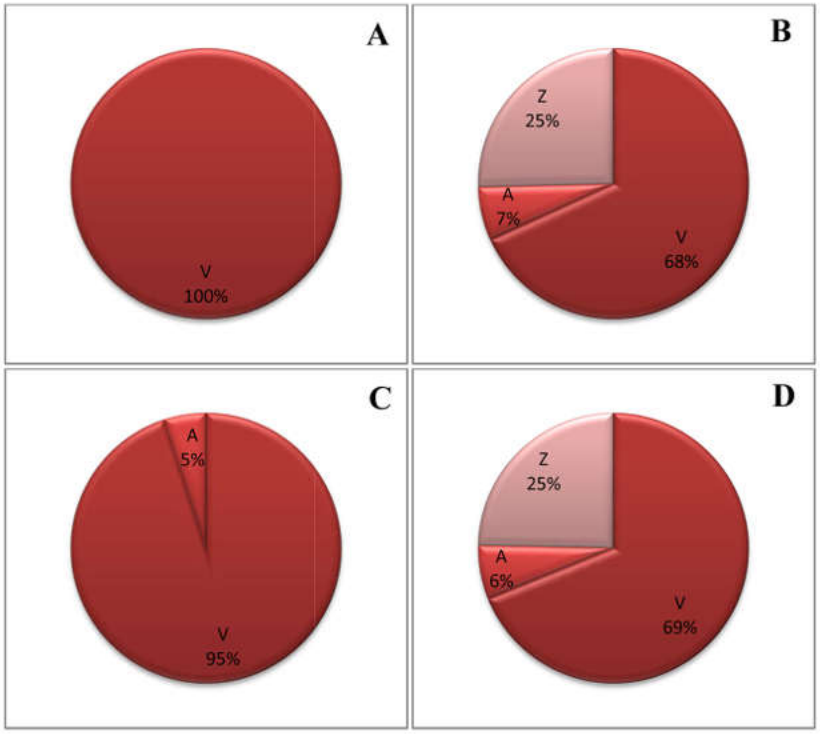

Figure 56: Composition of xanthophyll cycle pigments in *O. sativa* var. Aathira (A&B) and *O. sativa* var. Swarnaprabha (C&D) after exposure to 0 and 28 kJm^{-2}d^{-1} of UV-B irradiation.

A&C-0 kJm^{-2}d^{-1}; B&D-28 kJm^{-2}d^{-1}; A-antheraxanthin, Z-zeaxanthin, V-violaxanthin

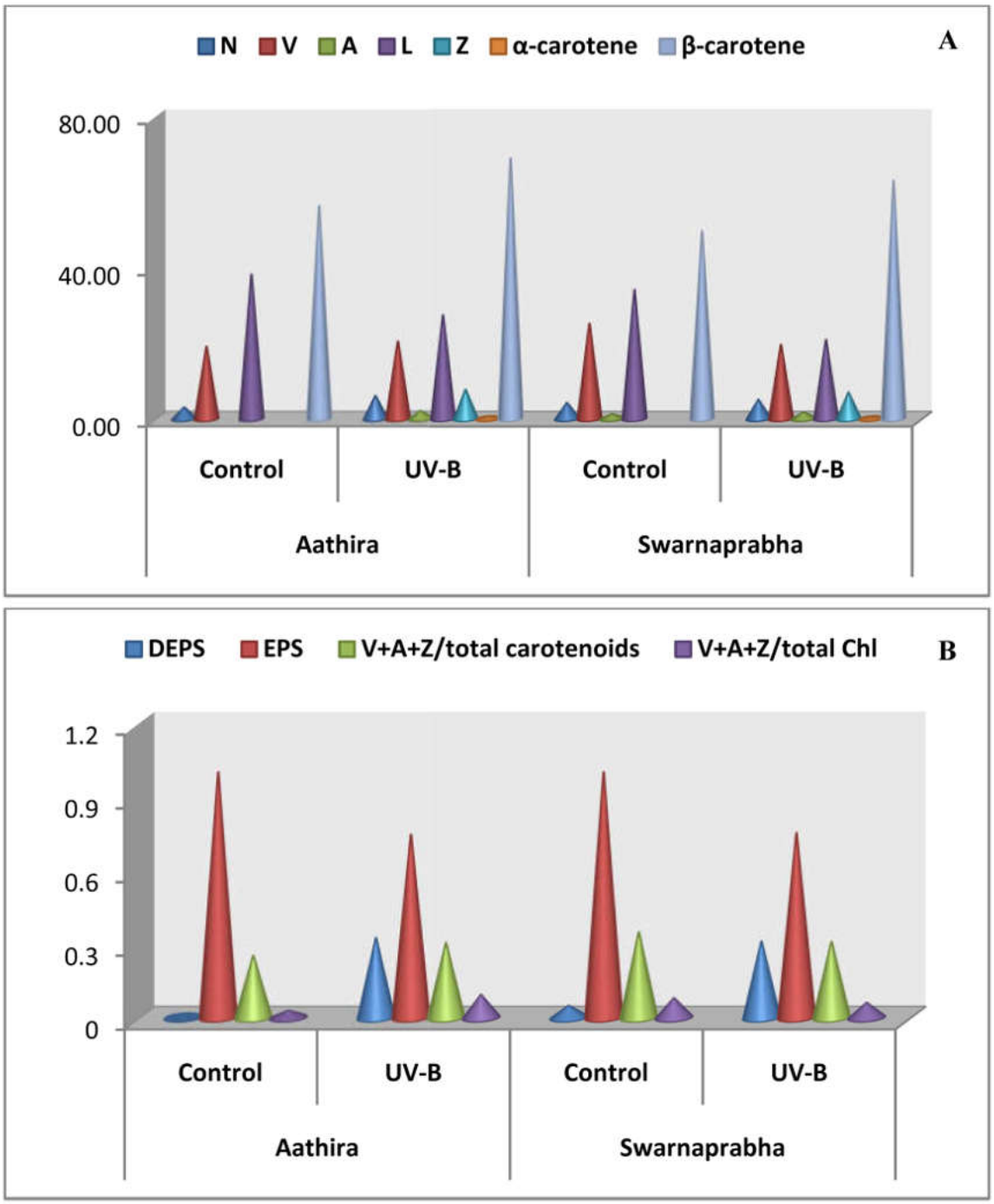

Figure 57: HPLC elution components of the photosynthetic xanthophyll pigments (A), changes in de-epoxidation (DEPS) and epoxidation (EPS) state of xanthophyll cycle pigments, the proportion of the xanthophyll cycle pool (V+A+Z) to total Chl and carotenoids (B) in *O. sativa* var. Aathira and *O. sativa* var. Swarnaprabha after exposure to 0 and 28 $kJm^{-2}d^{-1}$ of UV-B irradiation.

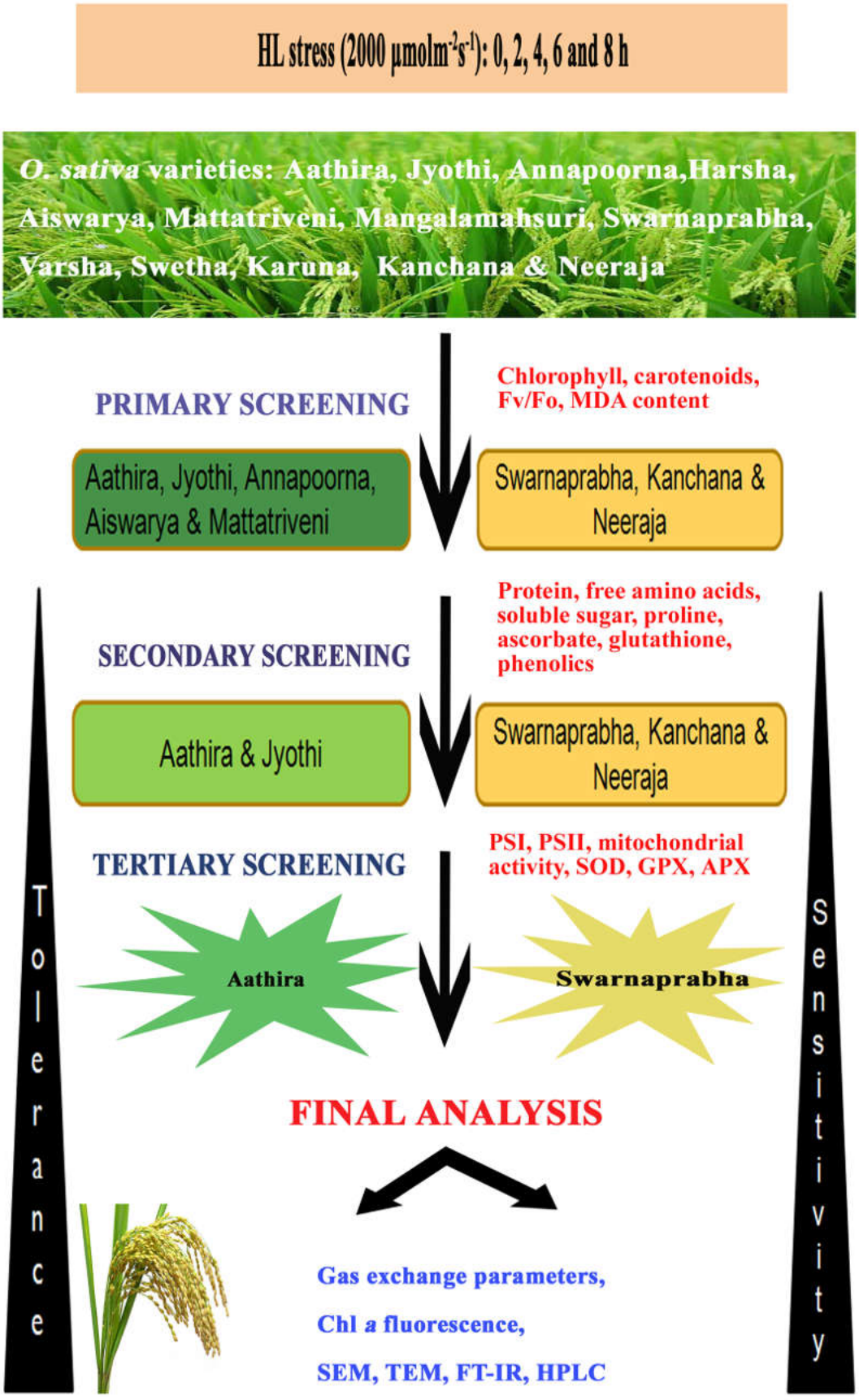

Chart 1: Experimental design of the work on imparting HL stress to thirteen varities of *O. sativa* and diffrent stages of screening with the parameters analysed at each stage.