Enhancing abiotic stress tolerance of *Oryza sativa* L. by priming seeds and seedlings with UV-B radiation

Thesis submitted to
the University of Calicut in partial fulfillment of
the requirements for the Degree of

DOCTOR OF PHILOSOPHY IN BOTANY

By

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SEPETEMBER 2020

CONTENTS

ABBREVIATIONS

ABS/CSm	-	Absorption flux per cross section
ABS/RC	-	Absorption flux per reaction center
APX	-	Ascorbate peroxidase
AsA	-	Ascorbate
CAT	-	Catalase
Ci	-	Intercellular CO_2 concentration
Cu/Zn SOD	-	Copper/Zinc superoxide dismutase
DCMU	-	3 (3,4-dichlorophenyl) -1, 1-dimethyl urea
DCPIP	-	2,6-dicholrophenolindophenol
DHA	-	Dehydroascorbate
DHAR	-	Dehydroascorbate reductase
DI_o/CSm	-	Dissipated energy flux per cross section
DI_o/RC	-	Dissipated energy flux per reaction center
DTNB	-	5-dithio-bis-2-nitrobenzoic acid
DW	-	Dry weight
DW%	-	Dry weight percentage
EDTA	-	Ethylene diaminetetra acetic acid
EL%	-	Electrolyte leakage percentage
ET_o/CSm	-	Electron transport flux per cross section
ET_o/RC	-	Electron transport flux per reaction center
Fe SOD	-	Iron superoxide dismutase
F_m	-	Maximum Chl *a* fluorescence
F_o	-	Initial Chl *a* fluorescence
F_v	-	Variable Chl *a* fluorescence
F_v/F_m	-	Potential or maximum quantum yield of PSII
F_v/F_o	-	Ratio between variable fluorescence to minimal fluorescence
GPOX	-	Guaiacol peroxidase
GR	-	Glutathione reductase

GS	-	Glutathione synthetase
GSH	-	Glutathione
GPX	-	Glutathione peroxidase
gs	-	Stomatal conductance
GSSG	-	Oxidized glutathione
H_2O_2	-	Hydrogen peroxide
HEPES	-	[N-(2-Hydroxyethyl) piperazine-N-(2-Ethanesulphonic acid)
HSPs	-	Heat-shock proteins
LEA	-	Late Embryogenesis Abundant
MDA	-	Malondialdehyde
MDHA	-	Monodehydroascorbate
MDHAR	-	Monodehydroascorbate reductase
Mn SOD	-	Manganese superoxide dismutase
MSI	-	Membrane stability index
MV	-	Methyl viologen
NaCl	-	Sodiun chloride
NADH	-	Nicotinamide adenine dinucleotide
NaN_3	-	Sodium azide
NBT	-	Nitroblue tetrazolium chloride
NP_s	-	Non-primed seeds
NP_{sl}	-	Non-primed seedlings
OP	-	Osmotic potential
PAL	-	Phenyl alanine ammonia Lyase
pBQ	-	Parabenzoquinone
PEG	-	Polyethylene glycol
$PI_{(abs)}$	-	Performance index on absorption basis
Pn	-	Net photosynthetic rate
P_s	-	Seed priming
PSI	-	Photosystem I
PSII	-	Photosystem II

P_{sl}	-	Seedling priming
RC/CSm	-	Reaction center per cross section
ROS	-	Reactive oxygen species
SOD	-	Super oxide dismutase
TBA	-	Thiobarbituric acid
TCA	-	Trichloroacetic acid
T_{fm}	-	Time to reach the maximum fluorescence
TR_o/CSm	-	Trapping flux per cross section
TR_o/RC	-	Trapping flux per reaction center
UV	-	Ultraviolet
V_j	-	Variable fluorescence intensity at the J_step

1. INTRODUCTION

Plants are facing diverse abiotic stress factors throughout the course of their growth and development. Abiotic stresses are major factors for crop failure over worldwide, dropping the average yields of major crops by more than 50%. Abiotic stress factors include extreme temperatures (heat, cold and freezing), too high or too low light irradiations, water logging, drought, inadequate mineral nutrients in the soil and excessive soil salinity (Slama et al. 2015; Zhu 2016). The crop plants are critically affected by various abiotic stress factors which have a negative influence on plant development and productivity, leading to agricultural yield losses (Golldack et al. 2014; Kazan 2015; Wani et al. 2016). Mild effects of these stress factors have the potential to up-regulate various genes and accumulate proteins and metabolites which are directly or indirectly involved in alleviating the deleterious effects of stress by adjustment of the cellular mechanism and plant tolerance (Hasanuzzaman et al. 2017; Martinez et al. 2018).

Drought, excess salinity and UV-B are some of the main abiotic constraints affecting the crop yields worldwide, which have many negative impacts on normal plant functions. These stresses cause various biochemical, physiological, metabolic and molecular changes leading to oxidative stress and negatively influence plant growth and metabolism (Negrão et al. 2017). Among various stress conditions, drought cause a severe stress on plants, which seriously affects plant growth, membrane integrity, osmotic adjustments, pigment content and photosynthetic activity (Sanghera et al. 2011; Pathak et al. 2019). Salinity is another major stress on plants, which causes ionic imbalance, critically influencing the photosynthetic apparatus, cause oxidative damages to membrane lipids, proteins and nucleic acids (AbdElgawad et al. 2016).

The UV spectrum is usually divided into three regions: UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (200-280 nm). The depletion of stratospheric ozone over the Antarctic and Arctic has been observed since 1974 and 1990, respectively. The principal consequence of stratospheric ozone depletion is the increase in UV-B radiation reaching the Earth's surface. The destruction of the ozone layer has been more intense at high latitudes, particularly in Antarctica, where ozone concentrations have decreased by 40-50% as compared to the status of 1980 and minor changes have occurred in the area of Ecuador (3-6%) (35-60° N and 35-60° S), where UV radiation is intense in nature (UNEP, 2002). Accordingly, from 1980 to present, the flux of UVB, mainly within the range of 290-315 nm, has increased in the troposphere on an average of 6-14% (Kakani et al. 2003). Therefore, since the discovery of so-called "hole" in ozone at Antarctic, the main interest in studying the effects of UV-B radiations on plants has increased considerably (Costa et al. 2012).

UV-B radiation is one of the major abiotic stress that reach to the earth causing major deleterious effects in plants. UV-B radiation causes reduction in plants biomass, loss of membrane integrity and enzyme degradation, damage the photosystem (PSI and PSII) proteins, increases the production of ROS (Kataria et al. 2014; Robson et al. 2015; Kohler et al. 2017; Faseela and Puthur 2018). UV-B radiations have significant impacts on growth, development, biomass accumulation, yield and metabolism of plants. Moreover, such situation can adversely influence DNA, membranes, photosynthesis, phytohormones and morphogenesis. Plants have inbuilt mechanisms to counter the UV-B stress situation of which the production of secondary metabolites (e.g. flavonoids, anthocyanin and epicuticular wax) is considered as a prominent one (Qaderi et al. 2010). UV-B can induce several stress responses, which include physiological and photomorphogenic responses. Mechanisms to counter UV-B irradiations in plants are increased

leaf thickness, alterations in cuticle thickness and increased production of UV-B protective pigments (Shaukat et al. 2013).

Crop plants encounter various environmental stresses such as drought, salinity, UV, high and low temperatures etc. at different instances of their life span. Among these abiotic stresses salinity, drought and UV-B radiations are important plant stressors which affect development, productivity and accounts for heavy agricultural losses (Anjum et al. 2015; Czarnocka and Karipnski 2018). These abiotic stresses are most destructive for plants and can have huge impact on world's food security. Under stress conditions plants are adopted to various survival mechanisms, which includes different metabolic and structural changes. Various adaptations and mitigation mechanisms operate in plants to cope up with drought, salinity and UV-B stresses (Vurukonda et al. 2016; Tripathi et al. 2017).

Reactive oxygen species (ROS) is an outcome of different metabolic reactions which accumulated in various regions of the plant cell. ROS like singlet oxygen, superoxide radicals, hydrogen peroxide and hydroxyl radicals are reactive forms of molecular oxygen. Low levels of ROS are essential for plant growth and it act as necessary secondary messengers for cell metabolism, but the overproduction of ROS causes oxidative stress which leads to protein denatuation, lipids peroxidation, and nucleotides degradation, thereby resulting in cellular damage and ultimately leading to cell death (Anjum et al. 2015; Choudhury et al. 2017). ROS accumulation is injurious to plant cells and at the same time which plays a key role in signaling pathways that regulate acclamatory and defense mechanisms in crop plants. ROS production results in chlorophyll degradation and membrane lipid peroxidation, reduces membrane fluidity and selectivity. Over produced ROS cause oxidative damages and destruction of plant macromolecules, cell structures and also disturb the redox homeostasis which leads to induced cell

death and ultimately leads to yield reduction (Hossain et al. 2015; Czarnocka and Karipnski 2018).

Plants have a highly sophisticated and efficient antioxidant defense mechanism to overcome the excessive production of ROS. The perturbation of cellular homeostasis and stress induced damages are assuaged by the action of various enzymatic and non-enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), ascorbate, glutathione etc. by scavenging ROS and thereby enhancing the tolerance towards various stresses (Chen et al. 2016; Li et al. 2017; Raja et al. 2017; Rossatto et al. 2017). Of the ROS-scavenging enzymes, the most important enzyme is SOD which plays a major role in the prime cellular defense against abiotic stresses and superoxide anion to hydrogen peroxides. Other antioxidative enzymes such as CAT and APX alter the H_2O_2 level by converting it into water. SODs along with the other antioxidative enzymes retain the intercellular ROS homeostasis and endow the required level of tolerance to plants against abiotic stresses. Overexpression of genes synthesizing SOD, CAT and APX bestow the plants with required level of tolerance towards abiotic stresses (Li et al. 2017; Rossatto et al. 2017).

Rice constitutes a major crop in the world and is one of the main staple food of the world population and it plays a crucial role in national food supply of India (Jisha and Puthur 2016b). Rice is one of the most important food crops that feed over half of the world population. Major areas of Asia, Africa and South America use rice and its derived products as their chief food source. In order to meet the demand of the increasing population, rice production has to be increased by at least 70% by 2050. Rice production is facing various environmental challenges, like water scarcity, radically declining arable lands and land degradation, dramatic climate change leading

to global warming (Chunthaburee et al. 2016; Bahuguna et al. 2018; Bergman 2019). Rice is vulnerable to various abiotic stress factors such as salinity, drought and UV radiations which lead to reduction in rice production all over the world (Kang et al. 2017). The abiotic stresses have severely hampered the rice production, with strong social and economic impact. The impact of abiotic stresses on rice results in significant yield reduction worldwide (Bergman 2019). World population is rapidly increasing day by day, so it is essential to increase the production of food crops so as to meet the demand. In order to achieve this, the crop plants have to be equipped with abiotic stress tolerance, which will aid in enhancing the crop production. The production of rice has improved enormously during the postgreen revolution period mainly because of the advanced technological interventions and implication of improved rice varieties (Bahuguna et al. 2018). Researchers are adopting different methods to attain high yield and yield stability in rice production and developing tolerance towards various stresses is one of the prime ways.

Different methodologies were adopted from time to time to achieve tolerance towards stresses. These includes, conventional breeding methods like selection, hybridization and modern methods like mutation breeding, polyploidy breeding, genetic engineering, etc. Limitations of conventional breeding techniques are the requirements of large man power, energy, etc. Generating plant varieties with better abiotic stress tolerance have proved mostly unsuccessful because of the well recognized complexity or multigenic nature of abiotic stress-tolerance traits (Cushman and Bohnert 2000; Flowers et al. 2000). Transgenic plants production for abiotic stress tolerance has proved to be successful against different types of stresses. Although stress tolerance potential achieved through genetic engineering is fast and predictable, its constrain was the targeted introduction of individual, heterologous traits into elite crop lines (Gust et al. 2010). Because of the effects of pleiotropy and gene silencing, it is not feasible to continue smoothly

through the genetic engineering process of plant improvement (Flowers et al. 1997). Moreover, these methods are also costly, cumbersome and there exist biosafety rules and regulations which hamper the introduction of transgenics into the field. Because of these limitations, it has become crucial to look out for other solutions to impart tolerance in plants against various stresses. The alternative solution should be simple, cost effective and can be adopted by the farmers without any complication and at the same time it should be effective in tolerance (Jisha et al. 2013).

Nowadays different approaches are made to generate plants that can withstand different abiotic stresses. In recent years, seed priming has been developed as one of the most effective method to induce tolerance against various abiotic stresses. Seed priming is one of the most acceptable techniques developed by the researchers for enhancing stress tolerance in plants (Paparella et al. 2015; Thomas and Puthur 2017). It consists of initial exposure to an eliciting factor or condition making plants more tolerant to stress exposure. The priming process enhances various physiological and biochemical mechanisms for defense and thus empower the seeds/seedlings to overcome the different environmental stress conditions (Ibrahim 2016). Priming treatment causes rapid and uniform germination of seeds and successful crop establishment under stress conditions (Sharma et al. 2014). It is an easy, low cost and low risk method to overcome various abiotic stress conditions in agricultural lands. The priming mediated metabolic processes in seed/seedlings neutralize the adverse effects of diverse abiotic stress conditions in crop plants (Maiti and Pramanik 2013; Jisha and Puthur 2016 a,b).

Priming influences ‘pre- germinative metabolism’, which involves seed repair such as activation of DNA repair pathways, repair damaged proteins and mitochondria, antioxidant mechanisms, thereby ensuring

successful germination and seedling development. It increases the seed vigour, germination potential, enhanced stress tolerance and also ensures the seed quality and improved activities of antioxidant machinery for scavenging ROS and ensures appropriate seedling development and tolerance towards stress conditions (Paparella et al. 2015; Ghezal et al. 2016; Hussain et al. 2016; Ali et al. 2017). In primed seeds, the seed germination rate was augmented and strengthened, which can enhance biotic/abiotic stress tolerance at further growth stages and ultimately increase crop yields. Priming makes the seedlings adaptable to wider range of germination temperatures and also improves the capacity to compete with weeds and pathogens (Ali et al. 2017; Hussain et al. 2018).

Seed priming techniques such as hydopriming, osmopriming, chemical priming and priming with low dose of UV-B are known to strategically improve abiotic stress tolerance in crop plants (Abid et al. 2018; Dillon et al. 2018; Fang et al. 2018; Irani and Todd 2018; Noorhosseini et al. 2018). Normally UV-B radiations cause stress in plants but researchers have reported the role of UV radiation (in low dose) in bringing about positive effects in enhancing stress tolerance potential (Dillon et al. 2018; Xu et al. 2019). Low dose of UV-B radiation has numerous positive effects with regard to growth and plant productivity via effects on developing plant hardiness, enhanced plant resistance and improved quality of agricultural products with subsequent implications on food security. The UV-B priming induces stress adaptation in rice plants, which involve various morphological, physiological and biochemical changes, that positively contributes to abiotic stress tolerance (Thomas and Puthur 2017, 2019, 2020). UV-B seed priming cause alterations even at genomic level and aid the plants to attain tolerance against various abiotic stresses (Arunkumar et al. 2019). There are few earlier reports stating that low doses of UV-B impart priming effects and enhances abiotic stress tolerance in different crops. This study focus on the physiological and

biochemical and molecular changes in primed (low dose of UV-B) and non-primed seeds/seedlings of rice subjected to different stresses (NaCl, PEG and high dose of UV-B stress) and condition without any stresses.

Although there are very few reports on the positive effects of UV-B priming for enhancing tolerance towards various stresses, few works have been done to analyze the comparative performance of seedlings emerging from primed seeds of rice and explore the priming induced stress tolerance mechanisms (Thomas and Puthur 2017). Priming at seed stage is the familiar practice and most reported and at the same time plants are also primed at seedling stage. Reports on the application of priming in the field are less (Capanoglu, 2010). When priming is done at seedling stage, the most favoured form of application is soil drenching (Cohen et al. 2007) or foliar spraying (Jeun et al. 2004). Priming with seeds is one of the most adaptable and effective strategy to augment uniform germination, high seedling vigor and better yields in crop plants under various environmental stress conditions.

There are no earlier reports of UV-B priming effects on seed/seedlings of rice for enhancing abiotic stress tolerance. Therefore, it was felt important to study the variation in physio-biochemical features and molecular mechanisms of rice seedlings emerged from UV-B primed seeds as well as directly primed with UV-B in imparting tolerance towards NaCl, PEG and UV-B stresses. Prominent high yielding rice varieties cultivated in Kerala with varied in built potentials such as Aiswarya, Jyothi, Kanchana, Neeraja, Samyuktha and Swetha were selected for this study. From these six varieties, two were selected for detailed study to analyze various physiological, biochemical and molecular changes related to stresses (NaCl, PEG and UV-B) tolerance mechanisms in seedlings either directly primed with UV-B or emerged from UV-B primed seeds. The present study was designed with the following objectives.

1. To analyze the dosage of UV-B which can specifically prime rice seeds as well as seedlings for enhancing tolerance towards NaCl, PEG and UV-B stresses.

2. To study the effect of priming rice seeds and seedling with UV-B in imparting tolerance towards abiotic stresses such as NaCl, PEG and UV-B by analyzing,

    a. Photosynthetic performance

    b. Metabolite accumulation

    c. Oxidative stress and antioxidation mechanism

3. To analyze the expression of genes encoding antioxidant enzymes in UV-B primed and non-primed seedlings exposed to three different stresses.

4. To analyze the expression of stress regulated genes such as Late Embryogenesis Abundant (LEA) proteins and Heat Shock Proteins (HSP) in UV-B primed and non-primed seedlings subjected to three different stresses.

5. To analyze the UV-B specific parameters such as anthocyanin, flavonoids content, phenylalanine ammonia lyase (PAL) activity and culticular wax accumulation and their functional group analysis.

2. REVIEW OF LITERATURE

2.1. Abiotic stress

Plants grow in changing environmental conditions which sometimes turn unfavourable or stressful for plant growth. These stressful situations includes sub or supra optimal conditions of cold, heat, heavy metal, nutrient deficiency, salinity and drought which adversely affects biomass production and yield and can generate food insecurity throughout the world (Ashraf et al. 2018). Abiotic stresses are posing rigorous threat to agriculture, which finally reduce the crop yield (He et al. 2018). In plants, stress is an external condition which badly affects its growth, development and productivity. Various stresses prompt different plant responses such as distorted growth rate, abnormal cellular metabolism, different gene expression, reduced crop yields etc. Plants are exposed to different stress conditions which make them to acclimatize to specific stress conditions in a time dependent manner (Gull et al. 2019). Stress damages the cells and cause abnormal metabolism, which disturbs the plants equilibrium leads to the variations in physiological parameters as well as changes the chemical and physiological characters (Fathi et al. 2016). Abiotic stresses are the principal motive for the reductions in plant growth and worldwide food production, decreasing the average yield of crops by 65 to 87 % (León-Chan et al. 2017).

World's agriculture faces serious challenges to meet food demand, consist of increased consumption, allocation of land for other uses and the use of chemical products with implications for health safety. At present, food security depends on the augmented production of principally three cereals: wheat, rice and maize (Caverzan et al. 2016). World population is increasing day by day and may reach over 9.8 billion in 2050 (Anjum et al. 2017; United

Nations 2017). To fulfil the food requirements of a growing population, the agriculture food production must be increased up to 70%. In order to secure future generations from the upcoming crisis, it is important to develop technologies and policies to counter various abiotic stresses (Shivakrishna et al. 2018).

Nowaday, one of the biggest challenges faced by the world is feeding the larger number of people with increasingly restrained resources (Davies et al. 2009; Labanowska et al. 2016). Current day global relative yield losses are estimated to range between 7 and 12% for wheat, 6 and 16% for soybean, 3 and 4% for rice and 3 and 5% for maize (Van Dingenen et al. 2009). The data from Asia shows that the yield losses for wheat, rice and legumes are towards higher extend. The yield loss of cereals and soybean varies from 13 and 23% as well as 16 and 35%, respectively (Emberson et al. 2009).

Any variation from optimal external conditions, excess or deficiency in the chemical or physical environment is considered as abiotic stress which adversely affects the plant growth, development and/or productivity (Koyro et al. 2012). Abiotic stresses threaten the sustainability of agricultural industry. With the effect of these stress factors, up-regulation and down regulation of various genes occur, which can alleviate the deleterious effects of stresses and lead to adjustment in cellular processes leading to plant stress tolerance. In response to the stress signals that cross talk with each other, plants have developed diverse pathways which act in cooperation to mitigate stress (Mahajan and Tuteja 2005).

Drought and salinity stresses cause various biochemical, physiological and metabolic changes in plants leading to oxidative stress and affect plant growth and metabolism. Salinity and osmotic stresses delays the seed germination and seedling establishment. Other abiotic stress factors also adversely affect the crop production (Jisha et al. 2013). Drought and salt

stresses are prime environmental factors which affect the geographical distribution of plants in nature and threaten plant productivity. These stresses cause oxidative stresses in plants, which lead to damage of cellular components like membrane lipids, proteins and nucleic acids and metabolic dysfunction (Zhu 2016). Abiotic stresses induce the generation of reactive oxygen species (ROS) in plants. Antioxidants reduce the deleterious effects of ROS, which in turn reduces the stress effects (Ashraf et al. 2018). Lowest levels of ROS can also act as an agent for signal transduction and can reduce the negative impact on photosynthesis and cellular damage caused by stress conditions. Various studies have reported that different parameters are monitored to investigate the effects of abiotic stresses in different plants, such as NaCl stress in wheat (Mehta et al. 2010) and barley (Kalaji et al. 2011), drought stress in wheat, ryegrass (Živcák et al. 2008b; Dabrowski et al. 2019) and maize (Lepeduš et al. 2012).

2.1.1. Salinity stress in plants

Salinity or salt stress is one of the major problems faced by semiarid and arid regions which may cause yield reduction of crops. Nearly one third of the arable land is affected by salinity. Salinity affected lands over the entire world have been more than 9 billion ha and this area is still increasing at the rate of about 2 million ha (approximately 1%) yearly. An increased salinization may lead to 50% loss in the productivity of the land by 2050. World's growing population will reach about ten billion by the year 2050 and in that case about two times of current agricultural productivity is needed to satisfy the food demand (United Nations, 2015, Kumar et al. 2017). World agriculture faces a major challenge for the production of 70% more food crop by 2050 worldwide. In worldwide cultivated land, more than 20% is affected by salinity and the area is increasing day by day. On the basis of adaptive evolution of plants against salinity, they can be classified into two: the

halophytes (that can withstand salinity) and the glycophytes (that cannot withstand salinity).

Salt stress imposes two types of effects on plants such as osmotic stress and ion toxicity. High salinity lowers water potential creating a situation wherein water and nutrients are inaccessible to plants. The accumulation of Na^+ and Cl^- cause ion toxicity and ion imbalance that reduces the other nutrients uptake. Excess Na^+ accumulation in roots causes osmotic stress and also increases ionic stress due to imbalance in essential nutrients. Higher Na^+ reduces the influx of K^+ that affects enzymatic activity, osmotic balance and stomatal functions and this also affects physiological attributes such as fruiting, flowering, seed growth and seed germination. Ion toxicity also increases the ROS generation in cells (Konuskan et al. 2017; Ashraf et al. 2018; Kanwal et al. 2018; Khoshbakht et al. 2018). When plants are under salinity stress, osmoticum in plant cells were less than the osmoticum in soil solution, so that the absorption ability of water and minerals like K^+ and Ca^{2+} gets reduced. This may cause some secondary effects such as reduced cell expansion, membrane function and decreased cytosolic metabolism (Gull et al. 2019).

Salinity stress triggers various physiological and metabolic processes for developing tolerance depending on severity and duration of the stress. Primarily soil salinity suppresses the plant growth and it was largely affected by osmotic stress, followed by ion toxicity. In the first phase of salt stress, water absorption capacity of root systems gets reduced but water loss from leaves continues uninterrupted. This may cause damage of membranes, damage the ability to detoxify ROS, cause nutrient imbalance, diminishes photosynthetic activity and decrease in stomatal aperture. Plants develop diverse physiological and biochemical mechanisms in order to tolerate salinity. This includes ion homeostasis and compartmentalization,

biosynthesis of osmoprotectants and compatible solutes, ion uptake and transport, synthesis of polyamines, activation of antioxidant enzyme, synthesis of antioxidant compounds, hormone modulation and generation of nitric oxide (NO) (Gupta and Huang 2014). Osmotic stress evokes closure of stomata, reduce CO_2 fixation, cause over-reduction of mitochondrial electron transport components and stimulation of photorespiration, which can accumulate ROS (Rejeb et al. 2015; Hossain and Dietz 2016; Singh and Thakur 2018). Wheat exposed to salinity stress reduced the stomatal conductance, leaf water potential, photosynthetic rate, root length and shoot biomass (Kanwal et al. 2018).

Majority of the plants cannot survive above 200 mM NaCl concentration, because the increased concentration of salinity interrupts the plant life cycle such as seed germination, seedling establishment, vegetative growth and flower fertility etc. (He et al. 2018). Under saline stress condition, chlorophyll *a*, *b*, total chlorophyll and carotenoids content gets reduced in rice (Amirjani 2011; Chutipaijit et al. 2011) and *Vigna* (Saha et al. 2010). In cucumber total leaf chlorophyll contents significantly decreased with increasing NaCl levels (Khan et al. 2013).

Salt stress induces degradation of chloroplast structure and cause abnormalities in thylakoid membranes and thereby decreases net photosynthetic rate in *Cucumis sativus* (Shu et al. 2012). Salinity inhibits the activity of enzymes involved in photosynthesis; deteriorate the proteins/enzymes of both the light and dark reactions. It decreases activity of PSII and Rubisco thereby affecting photosynthesis (Ashraf and Harris 2013; Singh and Thakur 2018). Aldesuquy et al. (2014) reported reduction of the photosynthetic pigments, light reaction, photosynthesis and leaf area in wheat under salinity stress condition. Moreover, Mittal et al. (2012) reported that salt stress deteriorate growth of *Brassica juncea* by altering photosystem II

(PSII) structure and function and also the electron transport rates. Under salinity stress the photosynthetic rate and CO_2 assimilation rate was reduced in *Arabidopsis* (Stepien and Johnson 2009).

In plants chlorophyll molecules absorb the light energy which can be used for photosynthesis, dissipated as heat or re-emitted as fluorescence. Chlorophyll *a* fluorescence parameters were analyzed to study the function and performance of photosynthetic machinery in various plants and also assess the physiological responses of plants under stress condition (Kalaji et al. 2017, 2018). Chlorophyll *a* fluorescence parameters such as minimal fluorescence (F_o), maximal fluorescence (F_m), area above the fluorescence curve (area) and performance index on absorption basis [$PI_{(abs)}$], energy absorbed per excited cross section (ABS/CSm), non-photochemical quenching per CSm (DI_o/CSm), trapped energy flux per CSm (TR_o/CSm), electron transport flux per CS (ET_o/CSm) etc. were analyzed. ET_o/CSm was decreased in soybean under salinity stress (Baghel et al. 2016). The ABS/RC can be taken as a calculated average amount of chlorophyll which channels excitation energy into reaction centers (Strasser et al. 2004). Similarly the other Chl *a* fluorescence parameters could also assessed on the basis of a single reaction centre. $PI_{(abs)}$ was reduced in *Brassica napus* under salt stress (Bacarin et al. 2011). Augmentation in ABS/RC during salinity stress is usually accompanied by increased TR_o/RC. $PI_{(abs)}$ was decreased due to lower number of active RCs per PSII antenna chlorophyll (Galić et al. 2019). The ABS/RC, DI_o/RC and TR_o/RC were increased while the ET_o/CSm was reduced under NaCl treatment in maize. F_o increased and $PI_{(abs)}$ and F_m were reduced in maize plants under NaCl stress condition (Galić et al. 2019). V_j, F_o, ABS/RC, TR_o/RC, ET_o/RC, DI_o/RC were found to be increased and at the same time F_m, F_v/F_o, RC/CSm, ABS/CSm, TR_o/CSm, ET_o/CSm, DI_o/CSm were decreased in sunflower under salinity stress (Noreen et al. 2017).

Salinity stress induced ROS formation may lead to oxidative damages in proteins, lipids and DNA, interrupting critical cellular functions of plants. Exposure of plants to salinity stress increases the generation of ROS, as by-products, which in turn damage the cellular components (Taïbi et al. 2016). Under salt stress conditions, reduced growth and increased hydrogen peroxide, lipid peroxidation and accumulation of osmolytes was observed in rice, ground nut and maize (Theerakulpisut and Gunnula 2012; Kaya et al. 2013). Increased lipid peroxidation and hydrogen peroxide level were seen with increased salinity in *Brassica napus* (Hasanuzzaman and Fujita 2011a) and rapeseed (Hasanuzzaman and Fujita 2011b).

According to Wu et al. (2013), increased salt stress concentrations elevate the levels of several compatible solutes such as proline, glycine, alanine, mannitol, inositol, raffinose etc. in barley. In tobacco plant subjected to NaCl stress, the protein content was increased (Çelik and Atak 2012). Total free amino acids and total protein content were increased in peanut under salt stress condition (Parida and Jha 2013). Faba beans under saline stress condition significantly increased the total soluble sugars, free amino acids and proline (Sadak and Abdelhamid 2015). Total free amino acids were increased under saline conditions in wheat cultivars (Perveen et al. 2012). Kholova et al. (2010) observed that higher activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) lowers superoxides and hydrogen peroxide content in maize genotypes under different salinity levels.

2.1.2. Drought stress in plants

Drought is caused due to prolonged duration of substantial lack of rainfall. Under drought conditions the existing water levels gets reduced and loss of water occurs through evaporation or transpiration. Water scarcity causes drought, which is the most dangerous hazard to food security all over

the world. The severity of drought stress is unpredictable because it is due to various circumstances like moisture quality of soil, rainfall distribution and its occurrence. Drought stress directly affects the plant growth and threatens the economy through the reduction of crop production (Martinez et al. 2018). Drought stress decreases the growth and produces osmo-protective molecules in *Ailanthus altissima*, apple and cotton (Li et al. 2011; Filippou et al. 2014). Qiu et al. (2008) observed that lipid peroxidation was higher in young wheat seedlings under drought. Drought stress deteriorates the plant growth and developmental processes, such as germination, plant height, stem diameter, number of leaves, leaf size and area, flower and fruit production and maturity in rice, marigold and maize (Manikavelu et al. 2006; Farooq et al. 2009; Asrar and Elhindi 2011; Anjum et al. 2017). Amino acids and proteins content were increased in wheat exposed to drought stress (Saeedipour and Moradi 2012), in maize (Bano et al. 2013) and cassava (Shan et al. 2015).

The first effect of drought in plants is reduced germination and plant growth. It reduces the photosynthetic activity by negatively affecting the photosynthetic machinery and leaf expansion that reduces the food production. It disturbs the photosynthetic machinery which in turn reduces the production of photosynthetic components and pigments and decrease the crop yield by damaging functions of Calvin cycle. Under drought stress condition plants undergo changes in different physiological, biochemical (antioxidant defence, cell membrane stability, plant growth regulators, osmotic adjustment) and molecular mechanisms (aquaporins, stress protein) (Mathobo et al. 2017; Ashraf et al. 2018; Dias et al. 2018). Under drought stress photosynthetic machinery turns out to be the main physiological target. It reduces the chlorophyll contents and photosynthetic process and also alters the key enzymes activity involved in carbon metabolism and antioxidant processes (Hussain et al. 2019).

Polyethylene glycol (PEG-6000) induces drought stress in plants, but at the same time this compound will not have any toxic effects in plants (Shivakrishna et al. 2018). PEG induces osmotic stress which in turn reduces photosynthetic rate, chlorophyll *a* and chlorophyll *b* contents and negatively affects other pigments, photosystems, electron transport system, CO_2 reduction pathways and thus finally reduce photosynthesis (Shivakrishna et al. 2018). In rice, drought stress decreases the growth, photosynthesis and transpiration (Pandey and Shukla 2015). Drought can also induce oxidative stress (Mathobo et al. 2017; Dias et al. 2018). Plants under the drought stress condition showed reduced total chlorophyll content which lowered the light harvesting capacity. Since the photosynthetic apparatus absorb excess energy that drives the production of reactive oxygen species, it degrades the light absorbing pigments (Fathi et al. 2016).

Drought stress reduces the yield due to the lower photosynthetic output. The drought induced photosynthetic reduction in plants is resulted by the diminishing of CO_2 conductance through stomata and mesophyll cells. Under drought stress, leaf CO_2 transport rate was decreased which leads to reduction in CO_2 concentration in chloroplasts that weakens photosynthesis. Inside cells the CO_2 level was reduced which deactivates Rubisco and also lower sucrose phosphate synthase and nitrate reductase activity (Perdomo et al. 2017). Under drought stress condition the electron transport and energy transformation were negatively influenced, causing damage to PSII RCs.

Various JIP parameters were used to identify the damages on the acceptor side of PSII, such as energy absorption, energy trapping and electron transport. The data from these parameters shows that some RCs were inactive and at the same time the efficiency of an individual RC was increased. Increased level of absorbed and trapped energy could not result in the increase of electron transport energy ET_o/RC, but cause a sharp enhancement of

DI_o/RC, which is the dissipation of energy in the form of heat. It's a self-protection mechanism of plant leaves under drought conditions (Meng et al. 2016). ABS/RC was increased which indicated the inhibition of electron transport from Q_A^- to Q_B and transformation of RCs to 'silent' RCs. Increased DI_o/RC shows the change in RC functionality, some of the RCs have transformed to 'heat sinks' to dissipate excess energy. This was usually accompanied by diminishing of Q_A^- reducing RCs per PSII antenna chlorophyll (RC/ABS). Augmentation in ABS/RC during drought stress is usually accompanied by increased trapped energy flux (TR_o /RC), but does not properly get utilized for effective electron transport (Galić et al. 2019). The light energy absorption per unit reaction center (ABS/RC) was increased, which was not the indication for the increase in efficiency of RC but indicated that the number of reaction centers was greatly reduced and therefore the absorption rate per RC seems to be increased. Drought stress also diminishes the utilization of absorbed light energy for electron transfer, as the proportion of energy dissipation was augmented, that was an important reason for the reduction of photochemical activity in leaves (Zhang et al. 2018). The relative variable fluorescence of point J at 2 ms (V_j) was increased indicating that the drought-induced decrease of PSII photochemical activity was related to the obstruction of electron transfer from Q_A to Q_B on the acceptor side of PSII.

Performance index [$PI_{(abs)}$] is a complex parameter which was related to the ratio of reaction center per (light) absorption flux, maximal quantum yield for primary photochemistry and quantum yield for electron transport (Baghel et al. 2016). Performance index on absorption basis [$PI_{(abs)}$] reflects the performance of the overall energy flow and it gets reduced in alfalfa under drought stress condition (Zhang et al. 2018). Various stress conditions inhibits the activity of the oxygen-evolving complex on the donor side of the PSII reaction center in leaves (Zhang et al. 2018). The value of F_v/F_o indicated the activity of the water-splitting complex on the donor side of the PSII. The

changes of V_j were in accordance with the changes of F_v/F_o and $PI_{(abs)}$ that indicated the drought stress induced decreases of photochemical activity of PSII.

Drought stress accumulates ROS through various pathways. Electron transport chain reduction generates singlet oxygen in PSII. Reduced CO_2 concentration stimulates photorespiration and that is one of the sources of hydrogen peroxides. These ROS deteriorate the photosynthetic rate significantly under drought stress by triggering the damage to photosynthetic machinery (D1 and D2 proteins of the PSII complex), thylakoid membranes, chlorophyll pigments and inhibits the translation of new D1, D2 and other cell proteins (Anjum et al. 2011; Singh and Thakur 2018). Drought stress induces the ROS production causing lipid peroxidation and membrane deterioration in plants (Fathi et al. 2016).

2.1.3. UV-B stress in plants

Sun light provides the necessary energy for plant growth and development via photosynthesis. But high light and ultraviolet (UV) light causes stress to plants, potentially causing serious damage to DNA, proteins and other cellular components (Muller et al. 2014). UV radiations (100- 400 nm) is a fraction of the electromagnetic spectrum emitted by the sun. There are three types of UV radiations, UV-A rays (400- 315 nm), UV-B rays (315-280 nm) and UV-C rays (280-100 nm). UV-C and a part of UV-B rays are absorbed by the stratospheric ozone layer. UV-A and 2-5% of UV-B rays reach the Earth's surface. As the emission of chlorofluorocarbons (CFC's) increases, the ozone layer is increasingly getting depleted, which permits the entry of more and more UV-B rays to the Earth's surface (Russo et al. 2010; McKenzie et al. 2011; Mohammed and Tarpley 2019). UV-B radiations at the tropical and subtropical latitudes is more than that in the temperate zones because the ozone layer is less thick in tropical and subtropical regions. After

introducing of Montreal Protocol, that mandate the reduction of the substances which deteriorate the ozone layer; the ozone depletion has been partially halted (Dwivedi et al. 2015; León-Chan et al. 2017).

Ozone is the major constituent in the earth's atmosphere, which absorbs 97-99% of sun's UV light. It is mainly concentrated at the lower portion of the stratosphere, which varies from approximately 13 to 40 km above Earths surface and its thickness varies seasonally and geographically (Nishanth et al. 2013; Srivastava et al. 2015). Chlorofluoro carbons (CFCs) emitted from refrigerators and their subsequent discharge into the atmosphere cause stratospheric ozone depletion and this depletion increases influx of the solar UV-B radiations on the earth's surface affecting living organisms. The human-made CFC's and the halons are influential ozone depletors, which are released through aerol sprays, light industry and from refrigerators (Godlee 1991). Main reasons for the widespread concern about the ozone depletion is the increased UV radiations reaching to the surface of the earth and the adverse effects of it on all living forms. It has been observed that ozone depletion occurs during spring time in every year above Antarctica, and to a lesser extent in the Arctic region (Sivasakthivel and Reddy 2011). Alvarez-Madrigal and Perez-Peraza (2005), reported an increase in ozone hole size from 1982-2003 during the period September-November and was found to be rapid in September. In Antarctic, the ozone hole was reported at altitudes from 12 to 24 km under extreme cold conditions (Solomon et al. 2007). Similarly, in South America and South Georgia, the hole grew to reach an area of around $24x10^6$ km^2 by mid-September, but had declined to $12x10^6$ km^2 by mid-November. According to Jeffrey Masters, in Southern Hemisphere, on September 25, 2010, the hole reached its maximum size of $22x10^6$ km^2, which was slightly smaller than that seen in North America, which is 25 $x10^6$ km^2 (Sivasakthivel and Reddy 2011).

UV-B reaching the earth's surface can cause various deleterious effects on living organisms, especially on plants which are more prone as being directly exposed to UV-B radiations (Reyes and Cisneros- Zevallos 2007; Zhao et al. 2007). UV-B radiations cause serious threat for plant physiological, biochemical and molecular processes and inhibits photosynthetic CO_2 assimilation, reduces plant height, leaf area, biomass accumulation and synthesis of Rubisco (Tripathi et al. 2017; Mohammed and Tarpley 2019). The ultimate deleterious effect of UV-B could be the decrease in plant growth and reduction in crop productivity. Various forms of other abiotic and biotic stresses also secondarily affects the UV-B stressed plants resulting in reduction of yield in different crops. The morphological changes due to UV-B stress are reduction in plant height, fresh mass, shortening of internodes, over secretion of wax, leaf thickening, reduced branching and fewer leaves per shoot (Day et al. 2001; Xiong and Day 2001; Alexieva et al. 2003; Shafi et al. 2009). Also alterations in the physiological and biochemical properties, such as reduced CO_2 uptake, rubisco activity, photosynthetic electron transport, dark respiration, stomatal behaviour, pigment content, endogenous level of phytohormones, enzyme activities and levels of several key photosynthesis proteins were reported (Dooslin et al. 2010).

Enhanced UV-B radiations also lead to decrease in pollen production, viability and germination and also negatively affects leaf photosynthetic rate, thereby reducing yield (Koti et al. 2005; Mohammed and Tarpley 2013). UV-B exposure induces changes in dry matter production and crop yield and these phenomena may vary with species. *Pisum sativum*, *Hordeum vulgare* and *Brassica juncea* shows severe reduction in yield; whereas *Vigna unguiculata*, millets and tobacco show less or no yield reduction (Reddy and Prasad 2010). Chlorophyll content was radically decreased and relative proportions of photoprotective carotenoids, especially ß-carotene and zeaxanthin, was found to be increased by the influence of UV radiations (Robinson et al. 2005). In

soyabean F_v/F_o was reduced due to UV-B radiations (Li and Zhang 2012) and also increased the F_o and decreased the F_m and F_v/F_o in cucumber plant (Skórska 2011; Skórska and Murkowski 2012).

UV-B radiations damages the photosystems, reduces the leaf photosynthetic rate (*Pn*), stomatal conductance, ribulose-1,5-bisphosphate carboxylase/oxygenase content, chlorophyll content, protein content, nitrogen concentration, chlorophyll fluorescence and alter photosynthesis-related gene expression (Kataria et al. 2019). Plant morphology, growth and development, pollen viability and germination, fertilization processes, seed set, yield and quality are deteriorated by elevated level of UV-B radiations (Chen et al. 2020). UV-B levels reaching earth would increase with ozone depletion in future, potentially reducing global rice productivity. As a result of elevated UV-B radiations various morphological, physiological and biochemical changes occurs in plants such as damage to DNA, proteins and plant membranes (Mohammed and Tarpley 2019). It also impede photosynthetic activities, produce reactive oxygen species (ROS) and impair pathogen resistance and results in drastic changes in cellular processes and thus disturbs plant growth (Jenkins 2009; Hideg et al. 2013; Li et al. 2013; Dobrikova et al. 2013; Czegeny et al. 2016). Rather than the physiological changes, UV irradiations also affect the molecular level changes, which include damages to the DNA, decline in total RNA content (Dooslin et al. 2010). UV-B radiations can increase the ROS generation which inhibit photosynthesis, damage DNA, proteins, photosystems (PSI and PSII) and the light harvesting complexes (LHCs) (Koti et al. 2005; Mohammed and Tarpley 2013).

Plants mitigate the elevated UV-B through increasing epicuticular wax, carotenoids and secondary metabolites such as phenolics and flavonoids. Exposure to UV radiations increases the allocation of newly assimilated carbon to polyphenols and in particular, flavonoid compounds, indicative of

an energy shift in order to acclimatize towards stress conditions (Ballare et al. 2011; Guidi et al. 2011). UV-B radiations activates defence mechanism by the biosynthesis of secondary metabolites, such as phenolic acids and flavonoids. Flavonoids are mainly found in the epidermis of plant tissues, which are related to absorption of UV-B radiations and also quench the ROS produced during stress. Each flavonoid has different capacity for UV-B absorbance and antioxidant capacity (Dwivedi et al. 2015; León-Chan et al. 2017). Moreover, UV-B absorbing phenolic compounds, flavonoids and sinapate esters gets synthesized and accumulated in the vacuole of plant epidermal cells subjected to UV-B stress (Jenkins 2009; Stracke et al. 2010).

UV-B radiations induces the generation of ROS and the accumulation of ROS causes lipid peroxidation, which ultimately lead to plant cell death. Plants have the enzymatic antioxidant system functioning in order to take care of the ROS generated. This includes SOD, APX, CAT, guaiacol peroxidase **(**GPOX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and GR etc. and non-enzymatic antioxidants such as ascorbate, glutathione, phenolics and flavonoids also scavenge the ROS and thus avoids its deleterious effects (Esringu et al. 2016; Mohammed and Tarpley 2019; Hassan et al. 2020).

Various approaches are being tried out to develop abiotic stress tolerance in plants. Seed priming, is one of the strategies for inducing tolerance in plants against various abiotic stresses. Seeds are treated with synthetic or natural compounds which initiate germination and specific physiological state in plants. Plants arising from primed seeds can quickly respond to abiotic stresses through various metabolic events (Ashraf et al. 2018; Abdelhamid et al. 2019)

Although many of the literatures cite the deleterious effect of UV radiations, the positive effects of the same with regard to plant growth was

discussed in few reports. UV radiations have a role in regulation of plant morphological, physiological, biochemical and genetic processes and are also important in animal and plant signalling. The enhanced level of UV-B radiations has beneficial effects in plant productivity via effects on plant hardiness, enhanced plant resistance to herbivores and pathogens, and improved quality of agricultural products with subsequent implications on food security (Bornman et al. 2015). Low dose of UV-B radiations can stimulate alterations in antioxidant status, such as regulation of glutathione pathways, phenylpropanoids, cinnamates, flavonoids pathways and pyridoxine biosynthesis pathways (Hideg et al. 2013). Low doses of UV-B and UV-C activates the enzymatic and non-enzymatic protective systems of plants to surmount different stresses. Moreover, the low dose of UV-B radiations affects several vital processes of plants such as seed germination, growth pattern, harvesting index, shelling percentage, nitrogen and protein content, chlorophyll development, various enzyme activities in imbibed seeds and even anatomy of various plant parts (Katerova and Todorova 2011; Rai et al. 2011).

2.2. Seed priming

Various methodologies were adopted to achieve stress tolerance in plants. Of the different methodologies, seed priming was found to be most adaptive and cost effective one. Through priming a ‘pre- germinative metabolism’, was activatedsuch as activation of DNA repair pathways and antioxidant mechanisms, ensuring appropriate germination and seedling development. It increases the seed vigour, germination potential and enhanced stress tolerance and also ensure the seed quality. In primed seeds the germination rate was augmented that results in elevation of biotic/abiotic stress resistance and crop yields. Priming reduces the photo-and thermo-

dormancy, facilitates wider range of germination temperatures and improves the capacity to compete with weeds and pathogens (Paparella et al. 2015).

UV radiations and climate variables can be usefully exploited for inducing high yield in agricultural crops (Bornman et al. 2015). The exposure of seeds to low doses of UV radiations is a safe method to improve plant productivity and yield as well as to protect the plants from various diseases (Delibaltova and Ivanova 2006; Aladjadjiyan 2007). Plants have been found to activate enzymatic and non-enzymatic protective systems to overcome different stresses by treating with low doses of UV-B and UV-C. This technique of treating the plants initially with the biotic and abiotic agents are generally known as priming (Katerova and Todorova 2011; Rai et al. 2011).

In recent years, seed priming has been developed as an indispensable method to produce stress tolerant plants. The seed priming method was found to be effective, with low risk and low cost method (Jisha et al. 2013). The stimulation of a particular physiological state in plants by the treatment of natural and synthetic compounds to the seeds before germination is known as seed priming. In plant defence mechanism, seed priming is defined as a physiological process by which a plant prepares to respond to imminent abiotic stresses more quickly or aggressively. Moreover, plants from primed seeds showed strong and quick cellular defence responses against abiotic stresses. Due to the seed-priming treatments, plants exhibited enhanced overall growth (Jisha et al. 2013; Moulick et al. 2016). The benefits of seed priming technologies are: enhanced, rapid and uniform emergence of seedlings with high vigor which will reflect in better yields (Garcia-Cristobal et al. 2015; Ibrahim 2016).

The enhanced abiotic stress tolerance of plants by priming is due to the activation of different stress related metabolic processes. The effectiveness of various priming agents varies under different stresses and with different crop

species (Iqbal and Ashraf 2005). The different seed priming methods include hydropriming, osmopriming, chemical priming, hormonal priming, biological priming, redox priming, solid matrix priming etc. (Mohammadi 2009; Zhu et al. 2010; Yari et al. 2011, 2012; Zhang 2015; Espanany et al. 2016). In cabbage (*Brassica oleracea* L.) seeds were treated with low UV-C (3.6 kJm^{-2}) which aid to resist the black rot disease. This technique also improved the quality and growth of cabbage (Brown et al. 2001). Results of our own research group have shown that abiotic stress tolerance can be imparted to various crops by seed priming, like halopriming and hydropriming in *Oryza sativa* (Jisha and Puthur 2014b); β-amino butyric acid (BABA) priming in *O. sativa* and *Vigna radiata* (Jisha and Puthur 2016 a,b); γ-amino-butyric acid (GABA) priming in *Piper nigrum* (Vijayakumari and Puthur 2016).

Although priming at seed stage is the common practice; plants are also primed at seedling stage (Capanoglu 2010). For priming at seedling stage, the priming agent is applied as soil drenching (Cohen et al. 2007) or foliar spraying (Jeun et al. 2004). Priming at seedling stage has also resulted in improvement of initial plant growth, increase of shoot and root length and total plant weight, pest resistance and disease resistance (Demir and Mavi 2004; Cohen et al. 2007; Benincasa et al. 2013). BABA applications to foliage or roots of lettuce have shown that it effectively controls downy mildew development (Cohen et al. 2007). Gibberellic acid-3 (GA_3) priming of rape-seed seedlings improved initial plant growth under salt conditions (Benincasa et al. 2013). Priming of seed and seedlings with UV was found to result in various positive effects in different crop plants, like *Vigna mungo* and *V. acontifolia* (Dwivedi et al. 2015); different species of *Phaseolus vulgaris* (Kacharava et al. 2009); sugar beet, red cabbage and fenugreek (El-Shora et al. 2015); *V. unguiculata* (Mishra et al. 2008) and green bean (Fotouh et al. 2014) etc.

2.2.1. UV priming (seed/seedling)

The UV induced acclimatization involve the effective scavenging of reactive oxygen species and morphological, physiological and biochemical changes, that positively contributes to drought and high temperature tolerance, and brings about positive plant-insect interactions (Mazza et al. 2013; Bussotti et al. 2014). Various scientists have reported the beneficial effects of UV seed priming in different crop species. For example, UV (460-760 $\mu W/cm^2$) was exposed for 30, 60 and 90 min to two varieties of kidney bean (*Phaseolus vulgaris* var. oratus and var. ellipticus), cabbage (*Brassica oleracea* var. capitata and var. capitatarubra) and beet (*Beta vulgaris*, var. saccharifera and ssp. *esculenta*, var. rubra) seeds. It was found that irradiations of UV lead to changes in ascorbic acid, tocopherol and different pigments etc. in these crop species (Kacharava et al. 2009). Similarly, the seeds of different wheat varieties exposed for 3 h to UV-A, B, and C radiations at dosages of 1.19, 1.3 and 1.84 mW/cm^2 respectively and further treated with acid spraying exhibited varied responses depending on the UV to which it was exposed. The varieties of wheat used for the study was soft wheat (*Triticum aestivum* var. ferrugineum); durum wheat (*T. durum*); macha wheat (*T. macha*); and three varieties of the dika wheat [(*T. persicum*)*; T. persicum* var. stramineum (white dika), *T. persicum* var. fuliginosum (black dika), and *T. persicum* var. rubiginosum (red dika)]. The treatment enhanced the chlorophyll synthesis, photosynthetic rate, protein content etc. (Badridze et al. 2015, 2016), (*detailed in later paragraphs*). The earlier studies showed that the treatment of seeds with UV radiations is an ecologically safe method to improve plant productivity and yield as well as to protect plants from various diseases (Delibaltova and Ivanova 2006; Aladjadjiyan 2007; Badridze et al. 2016).

Besides priming the seeds with UV-B, priming was also done at the seedling stage in *V. mun*go and *V. acontifolia*, (Dwivedi et al. 2015); cowpea (*V. unguiculata*) (Mishra et al. 2008); bitter gourd (Mishra et al. 2009) and UV-C priming in green bean (Fotouh et al. 2014) etc. Various dosages of UV-B ranging from 1.2 to 7.2 kJm^{-2} were provided to the seedlings of *V. mungo* and *V. acontifolia* (Dwivedi et al. 2015). The pollen grains were also exposed with UV radiations (Wang et al. 2010). The UV exposure increased the metabolite production including ascorbic acid, glutathione, tocopherol etc. along with the changes in morphology, pigment accumulation etc. The modulating effects of UV-B radiations were found to strengthen crop production, increase the level of photoprotection, antioxidative response, enhanced production of secondary metabolites and alter the resistance capacity against pests and disease attack (Wargent and Jordan 2013).

2.2.1.1. Beneficial effects of seed/seedling priming by UV radiation

2.2.1.1.1. Growth parameters

Seed germination rate was found to be positively influenced by UV-B radiations. The seeds of *V. mungo* exposed to UV-B radiations exhibited 33% increase in seed germination (Shaukat et al. 2013). The exposure of *Phaseolus vulgaris* (black bean) seeds to UV-C also increased the germination rate. Low level of UV (460 $\mu W/cm^2$ for 60 min) exposure for longer periods enhanced the growth and biomass accumulation in kidney bean. However, biennial crops responded positively to shorter period of irradiations. The exposure of kidney bean to UV radiations leads to increase in the plant height by 11-39% (Kacharava et al. 2009). Similarly the seed coat thickness increased by 28% in soft, 25-36% in macha and 66-77% in red dika varieties of wheat on being exposed to UV radiations for 3h at an irradiance of 1.3 mW/cm^2. Of the three varieties of wheat, the red dika wheat showed the highest seed coat thickness as compared to other varieties (Badridze et al. 2016). Mung bean (*Vigna*

radiata) seeds exposed with UV-A, stimulated the germination rate and the seedling performance in terms of leaf area, root and shoot length and dry weight (Hamid and Jawaid 2011).

The seeds of mung bean (*Vigna radiata*) and ground nut (*Arachis hypogaea*) treated with UV-C (280 nm) for different time intervals (5-60 min), increased the germination rate and seedling vigour. The seed germination was maximum in mung bean (100%) at 30 min and in ground nut (87%) at 60 min of UV-C exposure. Maximum shoot length and shoot weight was attained in mung bean on 15 min exposure to UV-C, but root length, root weight was maximum on 30 min exposure. But in the case of ground nut, all the above parameters attained a maximum on 60 min UV exposure (Siddiqui et al. 2011). When the ground nut seeds were exposed to UV-C for periods varying from 5-60 min, the seedling vigour and flower production increased as compared to controls. Similarly, the pod biomass also increased in all UV-C treatments (Neelamegam and Sutha 2015). When buck wheat seeds were irradiated with UV-B radiations in field condition, with filtered UV fluorescent lamps (275-380 nm), it was shown to positively affect the height and yield of the plant (Yao et al. 2007). Similarly, enhanced vegetative growth and increased number of peltate glandular trichomes per leaf was observed in peppermint by the effect of combined treatment of UV-B and Photosynthetically Active Radiations *(*PAR*)* (Behn et al. 2010).

The UV-C primed green bean seedlings showed enhanced tolerance to salinity stress and resulted in an enhancement of fresh and dry weights of shoots and roots as compared to non-primed ones. The dosage of UV priming also had an influential role on the growth of roots and shoots in plants under saline conditions and this increase was at its best when the UV-C radiations exposure to seedlings was for 60 min. The increase in shoot and root weight of seedlings primed with UV-C (60 min) was 83 and 94%, respectively as

compared to non-primed ones (Fotouh et al. 2014). UV-C radiations induces rapid germination of wheat cultivars (Abu-Elsaoud and Hassan 2016; Rupiasih and Vidyasagar 2016), common bean (Guajardo-Flores et al. 2014), mung bean (Hamid and Jawaid 2011; Siddiqui et al. 2011), peanut (Siddiqui et al. 2011), and UV-B in mash bean (Shaukat et al. 2013). Thus, numerous studies have clearly shown that the UV priming in different crops positively altered the different morphological and growth characters, which play a major role in enhancing the yield of different crops.

2.2.1.1.2. Physiological effects

The enhancement in photosynthetic pigments is an important physiological response observed as a result of UV priming. On exposure of seeds to UV-B (760 μW/cm^2 for 90 min), the plastid pigments content enhanced about 35% in kidney bean varieties and in cabbage and beet the increase was 45%. Carotenoid synthesis was stimulated in cabbage and beet with both high and low level of UV irradiations (760 and 460 μW/cm^2 for 30 min) and the increase was to the extent of 62% in red cabbage, 20% in white cabbage, 40% in red beet, and 28% in white beet. Carotenoids possess diverse functions including the light harvesting and protection from oxidative damage caused by abiotic stresses. Thus, the increased content of carotenoids indicates an important stress tolerance strategy adopted by the plants (Jaleel et al. 2009). Exposure of seeds to UV stimulated the synthesis of carotenoids in kidney bean, white beet, cabbage, red beet and sugar beet (Kacharava et al. 2009). The exposure of UV radiations alone enhanced the chlorophyll synthesis in red dika wheat (Badridze et al. 2016). When mung bean and groundnut seeds were exposed with UV-C radiations for different time intervals, chlorophyll content was enhanced in the former on 20 min exposure and in the latter 15 min exposure enhanced the chlorophyll content (Siddiqui et al. 2011). The pre-sowing treatment of wheat seeds with UV resulted in

significant increase of photosynthesis in all three varieties studied (macha, black dika and durum) (Badridze et al. 2016). Under field conditions, when the wheat seeds were exposed to ambient UV-B (5 kJm^{-2} supplementary) radiations the stomatal conductance, transpiration rate and intercellular CO_2 concentration were significantly increased (Li et al. 2010). When seedlings of cowpea (*V. unguiculata*) were treated with UV-B (0.4 Wm^{-2}) irradiations for 15, 30, 45 and 60 min along with dimethoate (an insecticide used to control the insect population), it was found that the level of caroteniods was enhanced by the treatment with UV-B alone (19%) and in combination with 50 ppm dimethoate (25%) (Mishra et al. 2008). When the rice seedlings in the field were irradiated with the supplemented ambient UV-B radiations (9.7 kJm^{-2}), it upregulated the photosynthetic activity and increased the chlorophyll concentration by 35% as compared with the control. These UV-B irradiated rice seedlings also showed enhanced tolerance to photoinhibition (Xu and Qiu 2007).

UV radiations has been reported to improve total chlorophyll content and carotenoid content in mung bean (Hamid and Jawaid 2011; Siddiqui et al. 2011), peanut (Siddiqui et al. 2011), mash bean (Shaukat et al. 2013). Total chlorophyll and carotenoids were significantly increased with UV-B exposure of barley and oats (Singh et al. 2019). The seed primed with UV-B (4 kJm^{-2}) reduced the PSI and PSII damages and also the mitochondrial damages in tolerant (Vaisakh) and sensitive (Neeraja) rice varieties when exposed to NaCl and PEG stress conditions (Sen et al. 2020). According to Singh et al. (2019), the phenomenological energy flux ratios of chlorophyll *a* fluorescence parameters, such as RC/CSm, ABS/CSm, TR/CSm and ET/CSm were enhanced and at the same time DI/CSm was seen decreased in UV-B primed barley and oats, not subjected to any stress conditions as compared to control. *Pn*, *gs* and *Ci* were also increased in barley and oats seeds subjected to UV-B priming (Singh et al. 2019). NaCl and PEG stresses reduces the mitochondrial

activity in rice seedlings, while the rice seedlings emerged from UV-B primed seeds reduces the mitochondrial damages when subjected to NaCl and PEG stress conditions (Sen et al. 2020).

2.2.1.1.3. Metabolites

UV radiations induces several metabolic responses in plants which are reflected in the increased primary metabolites like carbohydrates, proteins, amino acids. Proteins are direct effectors of abiotic stress response and have stress acclimatization functions leading to changes in plasma membrane, cell cytoplasm, cytoskeleton as well as intracellular compartment composition (Kosova et al. 2011). Compatible solutes are small organic compounds with high solubility, low toxicity and electrical neutrality. It consists of sugars, amino acids and their derivatives such as raffinose, trehalose, inositol, mannitol, proline and glycine betaine (GB). Under stressful conditions, these metabolites act as osmoprotectants against dehydration, scavengers of ROS, stabilize proteins and membranes. Proline was able to buffer cellular redox potential and induce gene expression (He et al. 2018). Proline is one of the osmolyte and a potent antioxidant, also a strong inhibitor of programmed cell death (Hussain et al. 2019).

The UV exposure to seeds stimulated the protein synthesis in two different varieties of kidney bean leaves (Kacharava et al. 2009). Also stimulated the starch accumulation in seeds of soft wheat and increased protein content in seeds of soft wheat and red dika wheat on UV exposure (Badridze et al. 2016). Macha wheat, soft wheat, white and red varieties of dika wheat leaves exhibited elevated content of protein under combined action of two different stresses (UV irradiations and acid spraying) (Badridze et al. 2015). In another study, ten different wheat cultivars were irradiated with UV $B_{BE\ (biologically\ effective)}$ radiations (5 kJm^{-2} supplementary) at field conditions, the cultivars showed significant increases of proteins, total sugars,

total amino acids content and grain quality index (Zu et al. 2004). When the rice plants were irradiated with ambient UV-B radiations (1 Wm^{-2} supplementary), total grain nitrogen content and grain storage protein (glutelin) significantly increased (Hidema et al. 2005). Also the amylose concentration significantly increased due to the irradiations of rice seedlings with UV-B radiations (9.7 kJm^{-2}) (Xu and Qiu 2007). Proline, total sugars and free amino acids content were enhanced in rice seedlings emerged from UV-B primed seeds (Sen et al. 2020; Sen and Puthur 2020). Thus, by UV-B irradiations, the protein, amino acids and sugar content were increased in different crops, which will ultimately aid in improving the growth of the plants as well as nutritional quality.

2.2.1.1.4. ROS accumulation and membrane damage

There is a disturbance in metabolic activities during abiotic stress condition which results in the overproduction of ROS in plants. ROS includes hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), singlet oxygen ($^1O_2$), superoxide radical ($O_2^{\cdot -}$**)** etc. which induces lipid peroxidation in plants. Also low level of ROS regulate the plants responses to different biotic and abiotic stress factors. It is generated as by-product of aerobic metabolism in various intracellular compartments involving chloroplasts, mitochondria and peroxisomes. ROS have dual function in plants; it acts as toxic compounds and also as signalling molecules for distinct physiological process (Dwivedi et al. 2015; He et al. 2018). Hydrogen peroxide is highly reactive toxic compounds which affect the plants and its toxicity is dependent on the site of production, concentration, stage of occurrence and the stress to which plant is exposed (Petrov and Breusegem 2012). ROS at lower level act as signalling molecules, which in turn influence several physiological processes such as stress perception, cell cycle, programmed cell death, gene regulation, hypersensitive response and also senescence. Hydrogen peroxide production

plays a key role in the up-regulation of heme-oxygeneases through enhancing mRNA level and protein expression, which leads to adaptation towards oxidative stress (Pathak et al. 2019).

ROS cause degradation of chlorophylls and membrane lipid peroxidation, reducing membrane fluidity and selectivity. In response to salinity, plants activate enzymatic (e.g. catalase, glutathione reductase and several peroxidases) and non-enzymatic (ascorbate, carotenoids, flavonoids and other phenolic compounds etc.) detoxification systems to encounter ROS and protect cells from oxidative damage. Over accumulation of ROS causes oxidative stress which leads to protein denaturation, lipids peroxidation and nucleotides degradation, that results in cellular damage and ultimately cell death (Raja et al. 2017). Under stress conditions ROS may cause cellular damage, toxicity and photosynthesis inhibition. ROS production negatively affects the normal plant growth and homeostasis that inhibits various cellular processes. To detoxify ROS plants activate different pathways, such as the Asada-Foyer-Halliwell pathway, that consume energy in the form of NADPH. Higher production of ROS occurs during photorespiration and photosynthesis in peroxisomes and chloroplasts respectively (Hussain et al. 2019). UV-B priming (4 kJm^{-2}) significantly reduced the superoxide, hydrogen peroxide contents and lipid peroxidation in tolerant (Vaisakh) and sensitive (Neeraja) rice varieties under NaCl and PEG stresses as compared to control (Sen et al. 2020; Sen and Puthur 2020). Barley and oats treated with UV-B (3.2 kJm^{-2}) reduces the increased malondialdehyde (MDA) content (Singh et al. 2019). UV-C seed priming (0.6 kJm^{-2}) reduces the ROS accumulation in strawberry than non-primed control (Xu et al. 2019).

Stress induces the production of ROS in seedlings resulting in the peroxidation of lipids, which ultimately leads to membrane leakage; disrupt the metabolic activity, non-specific damage to DNA, proteins etc. (Lidon et

al. 2012; Hideg et al. 2013). Compared with non-primed plants exposed to stress condition, the MDA content and electrolyte leakage was reduced in primed plants exposed to stress condition but at the same time it was increased as compared with the control. Reduced electrolyte leakage and increased membrane stability index was seen in rice seedlings emerged from UV-B primed seeds exposed to NaCl and PEG stress conditions (Sen and Puthur 2020). UV-B priming (4 kJm^{-2}) reduces the leaf osmolality in Vaisakh and Neeraja rice varieties under NaCl and PEG stress conditions (Sen et al. 2020).

2.2.1.1.5. Antioxidation influenced by UV-B priming

Plants have developed a sophisticated mechanism for ROS scavenging which includes enzymatic and non-enzymatic antioxidant mechanisms. Different abiotic stresses leads to enhanced activities of enzymatic antioxidants like superoxide dismutase, catalase, peroxidase and accumulation of non-ezymatic antioxidants like ascorbic acid, α-tocopherol, glutathione, proline which are actively involved in scavenging of free radicals (Gill and Tuteja 2010; Miller et al. 2010; Das and Roychoudhury 2014). Low level of UV-B radiations can stimulate alterations in antioxidant status, such as regulation of glutathione pathways, phenylpropanoids, cinnamates, flavonoids pathways and pyridoxine biosynthesis pathways (Hideg et al. 2013). The exposure of UV-B to cucumber seeds results in accumulation of non-enzymatic radical scavengers (eg. glutathione and ascorbate) and enhanced activities of enzymatic antioxidants (eg. SOD) (Takeuchi et al. 1996).

In different varieties of kidney bean, the total content of major antioxidants such as ascorbic acid, glutathione and tocopherol was increased by treating them with UV irradiations. The exposure to low level of UV irradiations enhances the ascorbic acid content in leaves of kidney bean varieties (32-35%). Another major antioxidant tocopherol was found to

increase several folds on UV exposure, i.e. 2-4 and 5-9 folds higher in kidney bean and white beet, respectively as compared to control plants. Two varieties of kidney bean (*Phaseolus vulgaris* var. oratusand var. ellipticus) showed an increase in vitamin C (32%) and E (79%) content on being primed with UV-B. Similarly, in sugar beet subjected to 90 min UV-B irradiations enhanced vitamin C content (74%) was observed. The low levels of UV radiations stimulated the synthesis of antioxidants and in turn enhanced the nutritional value of some major food crops such as kidney bean, cabbage and beet. It was further shown that enhanced synthesis of antioxidants could increase the tolerance potential of these food crops towards various environmental factors (Kacharava et al. 2009). In three varieties of dika wheat, the proline content increased by the combined effect of UV irradiations and acid spraying (Badridze et al. 2016). Likewise under the influence of same stresses, ascorbate content increased in macha wheat, durum wheat and red dika wheat leaves, and the proline content enhanced by 29-33% in winter wheat (*T. macha*, *T. durum* and *T. aestivum*). The combination of two stresses, UV and acid spraying stimulated the enzymatic antioxidant system (catalase and peroxidase) and increased phenolic content (16-58%) in winter wheat (Badridze et al. 2015). The total phenol (TP's) content was increased up to 403%, in UV-C treated plants as compared to the non-treated, when exposed to NaCl stress condition (Ouhibi et al. 2014). The UV irradiations enhances the synthesis/activity of antioxidants at varying levels in different plants. In kidney bean, enhancement of the ascorbic acid, tocopherol and vitamins contents gets enhanced on UV exposure (Kacharava et al. 2009). Whereas, in winter wheat, in response to UV irradiations there was higher increase of proline and phenolic content get increased (Badridze et al. 2015) and in *V. mungo* flavonoid content was increased (Dwivedi et al. 2015).

Ascorbic acid is a water-soluble and potential antioxidant seen abundantly in meristems and in photosynthetic cells reducing the ROS

damages. Glutathione (GSH) is a major metabolite that scavenges ROS to avoid oxidative damages. It is abundantly seen in all plant tissues and protects the photosynthetic apparatus against ROS-induced oxidative damage (Hussain et al. 2019). Total phenolics, asorbate and glutathione content were increased during barley and oats seeds treated with UV-B (Singh et al. 2019). Ascorbate (AsA) is a multifunctional non-enzymatic antioxidant which stands second to tripeptide glutathione and major function in plants is the role played as antioxidant metabolite, redox buffer in plant cells, and also has a key role in plant growth, metabolism, development and stress responses. Ascorbate have a major role in maintaining equilibrium between the production and elimination of ROS and reducing metabolic disorders and oxidative burst caused in cells. In AsA-GSH pathway, ascorbate directly interacts with ROS or it gets consumed by APX which reduce hydrogen peroxide to water and leads to the generation of MDHA, which can be subsequently converted into DHA and back to ascorbate in chloroplasts or other cells by the ascorbate - regenerating system consisting of DHAR and MDHAR (Anjum et al. 2014).

Another major role of ascorbate is the regeneration of α-tocopherol and zeaxanthin and also modulates the PSII activity. It is also needed in the transition from G1 to S phase in the cell cycle, control of cell cycle progression, cell proliferation of meristematic root cells and in root growth and development. In addition ascorbate is also involved in the synthesis of various growth regulators, including ethylene, abscisic acids and gibberellins and also act as a co-factor of dioxygenases (Rizhsky et al. 2002; Yoon et al. 2004). The level of non-enzymatic antioxidants such as ascorbate, glutathione and total phenolics was higher in tolerant and sensitive rice varieties treated with UV-B and then exposed to NaCl and PEG stresses (Sen et al. 2020; Sen and Puthur 2020). Phenolic compounds have multiple roles in plants; act as structural components of cell walls, regulate the growth and developmental

processes, also involved in defence mechanisms against abiotic stresses (Taïbi et al. 2016, Pathak et al. 2019).

When *V. mungo* and *V. acontifolia* seedlings were treated with UV-B, it resulted in enhanced synthesis of non-enzymatic and enzymatic antioxidants. The non-enzymatic antioxidants like proline, ascorbic acid, total phenols (TP's) and total flavonoids (TF's) increased in the leaves of *V. acontifolia* on exposure to UV-B and the accumulation of these secondary compounds were found to be higher in the epidermal layer. The increasing intensities of UV-B (1.2 to 7.2 kJm^{-2}) also caused progressive increase in ascorbic acid and proline contents in *V. mungo*. The enhanced level of these metabolites has the capacity to scavenge the ROS. The increase in TP's and TF's were higher in *V. accontifolia* than that recorded in *V. mungo* (Dwivedi et al. 2015).

SOD converts O_2^- to H_2O_2 which was further reduced to water by CAT and PODs. APX is a key enzyme for scavenging hydrogen peroxideto water (Dwivedi et al. 2015; He et al. 2018). In plants three isozymes of SOD such as copper/zinc SOD (Cu/Zn-SOD), manganese SOD (Mn-SOD) and iron SOD (Fe-SOD) are reported. MnSOD is seen in mitochondria, Fe-SOD is seen in chloroplasts and Cu/Zn-SOD is localized in different organelles i.e. cytosol, chloroplasts, peroxisomes and mitochondria (Sharma et al. 2012). CAT enzymes contain tetrameric heme, converting hydrogen peroxide into water and molecular oxygen. CATs are highly active enzymes which detoxifies ROS under stress conditions. In 1 min, one CAT molecule can convert about 6 million hydrogen peroxide molecules into water and molecular oxygen. GR is a key enzyme in the ascorbic acid-glutathione cycle that detoxifies the superoxides (Hussain et al. 2019). APX conducts hydrogen peroxide scavenging through the ascorbate-glutathione cycle and the water–water cycle (Hussain et al. 2019). *V. mungo* and *V. accontifolia* also exhibited an

enhancement in SOD and GPX activities after the treatment of seedlings with UV-B. At the same time higher CAT activity was recorded in *V. acontifolia* than in *V. mungo* (Dwivedi et al. 2015). The GPX activity enhanced in both species, increased progressively in *V. mungo* whereas maximum activity of this enzyme was recorded in ambient UV-B (4.8 kJm^{-2}) treated leaves of *V. acontifolia*. The potential of *V. acontifolia* to exhibit faster and greater increase in the accumulation of various antioxidants, aid this species in exhibiting enhanced tolerance to UV-B induced damages than *V. mungo* (Dwivedi et al. 2015).

When the bitter gourd seedlings was exposed to UV-B (30 min) and dimethoate (200 ppm), the SOD, CAT and POD activities were increased by the combined action of both dimethoate and UV-B (0.4 Wm^{-2}). When the seedlings were exposed to UV-B alone, they exhibited increase in the activity of SOD, CAT and POD by a range of 25-48% over the control (Mishra et al. 2009). The maize seedlings (21 d) exposed to UV-B radiations for 10 min for first day and then exposure was increased every day by 10 min and on analyzing the activity of antioxidant enzymes on the final day (8 d), it was seen that the activities of APX and GPX increased in roots and leaves and the increase in roots were higher. APX activity enhanced 3 folds and GPX activity 2 folds in roots as compared with the leaves (Javadmanesh et al. 2012). Different plants when exposed to UV irradiations exhibited differential responses in terms of antioxidant enzyme activities. The SOD, CAT and GPOX activities was higher in bitter gourd, but in the case of maize, the APX and GPX activities was higher (Mishra et al. 2009).

When the germinating green bean seeds were exposed to UV-C (254 nm) for different time intervals ranging from 15-60 min and then subjected to salinity stress, it was found that the seedlings was less affected by salinity stress as compared to non-treated ones. In saline stressed condition, the leaves

and roots of seedlings raised from UV-C treated seeds accumulated higher proline concentration. The maximum proline content (48%) was recorded in seedlings subjected to UV-C treatment for 15 min. Antioxidant enzymes (SOD, CAT, GPOX and APX) also showed higher activities in leaves and roots after the treatment with UV-C. The SOD activity (21%) and CAT activity (160%) enhanced after 15 min UV-C exposure. The guaiacol peroxidases (GPOX) activity also increased in leaves (59%) and roots (100%) on 60 min UV-C exposure. The APX activity was higher in roots (73%) than in leaves (8%) after 60 min UV-C treatment as compared to non-UV treated plants. When the UV-C exposed seedlings of green bean were further subjected to saline condition, seedlings could develop tolerance to saline stress by the prior activation of antioxidant system (Fotouh et al. 2014).

Exposure of wheat seeds to UV-A and UV-B promote the activity of antioxidants such as peroxidase, catalase and SOD (Abu-Elsaoud and Hassan 2016). Activities of SOD, CAT, APX and GR were increased in strawberry primed with UV-C (Xu et al. 2019). Two stress treatments such as NaCl and PEG increased the antioxidative enzymatic activities of the UV-B primed rice seeds. The enzymatic antioxidants, SOD, CAT and APX and their gene expression such as *Cu/ZnSOD*, *CatA* and *APx1* were higher in UV-B primed seeds subjected to stress conditions (Sen et al. 2020).

When fenugreek was treated with 15 and 30 W of UV irradiations at different time intervals (1-4 h), the antioxidant enzymes like SOD, GPX, GR and APX as well as accumulation of non-enzymatic antioxidant compound like reduced glutathione were found to get enhanced and maximum enhancement was observed on exposure to 30 W at 4 h treatment (El-Shora et al. 2015). The UV-B (350 $\mu W/cm^2$) irradiated maize plants, increased the phenol content and up-regulated the activity of CAT, APX and glutathione S transferase (GST). Of the three antioxidant enzymes highest enhancement in

gene expression level was recorded in the case of GST (>62%) (Rudnoy et al. 2015). Out of the twenty cultivars of soyabean, the SOD activity was significantly increased in ten cultivars treated with UV-B irradiations (Yanqun et al. 2003). At field conditions, when two rice varieties (Baijiaolaojing and Yuelianggu) were exposed to UV-B radiations (2.5, 5.0 and 7.5 kJm^{-2} supplementary), the SOD, CAT and POD activities were found to get up-regulated considerably. When blueberry (*Vaccinium corymbosum* cv. Brigitta and Bluegold) plants were irradiated with UV-B_{BE} radiations (0.07, 0.12 and 0.19 Wm^{-2} at 0 -72 h), the SOD activity was up-regulated and six fold increment occurred in the case of APX gene expression. On exposure to UV-B irradiations (0.12 Wm^{2}) showed higher expression of genes encoding for antioxidant enzymes such as APX (VcAPX), GST (VcGST) and aldehyde dehydrogenases (ALDH) (VcALDH) respectively occurred at *V. corymbosum* at 6, 24 and 48 h (Inostroza-Blancheteau et al. 2016).

APX, MDHAR, DHAR and GR were involved in ascorbate-glutathione (ASA-GSH) cycle (He et al. 2018). Hydrogen peroxide priming regulates AsA-GSH cycle and also increases transcript levels and activities of SOD, APX, GR, DHAR and MDHAR in wheat under drought stress (Pathak et al. 2019). Ascorbate and glutathione are involved in scavenging hydrogen peroxide and MDHAR as well as GR, are involved in regenerating ascorbate (Taïbi et al. 2016; Pathak et al. 2019).

DHAR is a monomeric thiol enzyme which is physiologically important reducing enzyme in the AsA-GSH recycling reaction in higher plants. MDHAR is a flavin adenine dinucleotide monomeric enzyme of the AsA-GSH cycle and is seen as chloroplastic and cytosolic isozymes (Rizhsky et al. 2002; Yoon et al. 2004). MDHAR activity differs in different stages of cell growth and plant tissues, which lead to different AsA/MDHA ratios. In meristematic cells, MDHAR activity was higher and therefore, a large amount

of MDHA was reduced to ascorbate; in expanding cells, the MDHAR activity was relatively lower and consequently, the accumulated MDHA was converted to DHA. MDHAR is the only one enzyme having carbon-based radical as its substrate and is also highly specific for MDHA as the electron acceptor and which prefer NADH rather than NADPH as the electron donor for ascorbate regeneration in plants. Owing to its involvement in ascorbate regeneration, MDHAR have a role in maintaining the antioxidant properties of ascorbate. APX use ascorbate as an electron donor, which is a heme-containing homodimeric protein. Compared with catalase, APX has higher affinity for hydrogen peroxide. This isozyme scavenge hydrogen peroxide at the expense of ascorbate and protect plant cells against potential deteriorating effects of hydrogen peroxide (Shin et al. 2013; Anjum et al. 2014; Singh and Bhardwaj 2016).

The 10 day seedlings of cucumber (*Cucumis sativus*) were exposed for 6 days with 3.4, 5.5 or 10.6 kJm^{-2} of ambient UV-B irradiations. The low and medium level of UV-B treated plants were later exposed to heat stress (46^{o}C, 1 h), the survival rate of the seedlings improved by 112 and 82% and growth in height increased by 35 and 40%, respectively (Teklemariam and Blake 2003). The UV irradiations up-regulated the antioxidant mechanisms in different plants, which indicate that the plants had the capacity to scavenge ROS, protect form photoinhibition, DNA damage, lipid peroxidation and these features ultimately lead to enhancement in the stress tolerance of the plant.

2.2.1.1.6. Enhanced stress responsive protein synthesis

Various abiotic stresses such as low temperature, osmotic stress, salinity, desiccation, high intensity irradiations, wounding and heavy metals stresses induce the production of a group of proteins called heat-shock proteins (HSPs) or other stress-induced proteins (Al-Whaibi 2011). Abiotic

stresses can prompt certain mechanisms of defence like the gene expression of some specific genes which was not expressed under ‘‘normal’’ conditions. This type of stress responses seen in genotypic expression results in an increase in the synthesis of certain proteins. These proteins are called as ‘‘heat-shock proteins’’ (HSPs), ‘‘Stress-induced proteins’’ or ‘‘Stress proteins’. Main role of HSPs is molecular chaperonins, which regulate the folding of proteins in plants. Other than acting as molecular chaperons, Hsp90 have a role in signalling protein function and trafficking, regulating the cellular signals such as the regulation of glucocorticoid receptor (GR) activity (Al-Whaibi 2011). In several species priming induces HSP production, which stabilizes protein and membrane structures (Chen and Arora 2013). Heat shock proteins (HSPs) act as a molecular chaperone, which gets induced or constitutively expressed to promote protein folding, assembly, transport and degradation. Five conserved HSP classes are seen in plants namely HSP100/Clp, HSP90, HSP70/DnaK, HSP60/Chaperonin and small HSP (smHSP) in accordance with molecular weight (He et al. 2018). Heat shock proteins are well known molecular chaperones which is responsible for protein synthesis, folding, targeting assembly, translocation and degradation in many normal cellular processes and are involved in membrane stabilization and also assist in protein refolding under stress (Ali et al. 2017).

Abiotic stresses such as drought, salinity, osmotic stress, cold and freezing temperatures produce cellular water deficit, which leads to the accumulation of a group of highly hydrophilic proteins, called Late Embryogenesis Abundant (LEA) proteins (Battaglia and Covarrubias 2013). LEA proteins reduce the damage and protect the cells from various stresses. Abiotic stress protection strategies of LEA proteins include hydration buffering, metal ion binding, antioxidant activity, membrane stabilization, DNA and RNA interactions (Zeng et al. 2018). LEA proteins also can stabilize cell structure, preventing inactivation and aggregation of proteins

and the loss of membrane integrity (Lutts et al. 2016). LEA proteins were mainly seen in seeds and also are present in vegetative tissues. LEA gene is induced through environmental stresses such as, salinity and drought. LEA is related with dehydration tolerance and resistance to drought, salt and cold stresses. It acts as water holding molecules and has the ability for membrane and protein stabilization (Lim and Kim 2013). Different seed priming treatments alter the expression/ accumulation of LEA transcript/protein in association with stress tolerance mechanisms (Chen et al. 2012; Kubala et al. 2015). Gene expression at mRNA level was significantly enhanced in the case of stress responsive proteins such as HSP (*Hsp90*) and LEA (group 3 *LEA*) in UV-B primed rice seedlings exposed to NaCl and PEG stress conditions. However, remarkable enhancement in gene expression level was recorded in UV-B primed seedlings subjected to PEG stress condition (Sen et al. 2020).

2.2.1.1.7. Enhanced epicuticular wax and secondary metabolites

Plants were more acclimatized to the UV irradiations by the increased production of UV absorbing compounds (phenolic substance) and various morphological changes that depends on the species and are also region specific (Ballare et al. 2011; Rizzini et al. 2011; Williamson et al. 2014). Radiations absorbing pigments such as anthocyanins and flavonoids tend to accumulate in response to UV-B irradiations (Shaukat et al. 2013). Water-soluble pigments derived from flavonoids via shikimic acid pathway are anthocyanin, which provides a protective role in plants under stress conditions. The anthocyanins are a group of flavonoids that impart pink to purple colours in leaves and other organs. The production of anthocyanins in plants is affected by various stresses. These stresses often affect anthocyanin content through the inhibitory action on the synthesis process of transcription factors involved in anthocyanin synthesis (Shaki et al. 2018).

Plants usually boost up the de novo synthesis of flavonoids as antioxidant substances under increasing UV-B radiations, due to the enhanced synthesis of key enzymes of the phenylpropanoid pathway (Manaf et al. 2016). Flavonoids play significant roles in plants by acting as signalling molecules, phytoalexins and detoxifying agents. It also plays a significant role in seed germination; act as UV filters, temperature acclimation, drought resistance, pollinator attractants and as allelochemical agents. Flavonoids are secondary metabolites that are synthesized through the phenylpropanoid pathway and act as antioxidant agents by scavenging ROS and have a key role in encountering stress situations (Hussain et al. 2019). Flavonoids are the most complex subgroup of polyphenols with a wide array of biological functions including inhibition of lipid peroxidation (Taïbi et al. 2016; Pathak et al. 2019). Exposure to UV radiations increases the allocation of newly assimilated carbon to polyphenols and in particular, flavonoid compounds, indicative of an energy shift in order to cope up with the stress conditions (Ballare et al. 2011; Guidi et al. 2011). The flavonoid content is an effective free radical scavenger and also acts as a 'sunscreen' in plants (Rice-Evans et al. 1997; Larson 1988). Flavonoids take a vital role in pigmentation of seeds, flowers and fruit; pathogen defence mechanisms; protects from ultraviolet light; and germination of pollen and plant fertility. When two varieties of rape- seed (*Brassica napus* L. cvs Paroll and Stallion) seeds were exposed to 13 $kJm^{-2}d^{-1}$ UV-B_{BE} radiations, the flavonoid content was increased in two varieties. In Paroll variety of rape seed, the total soluble flavonoids content enhanced by 150% and in Stallion variety the increase was 70% (Olsson et al. 1998). When *V. mungo* was treated with UV-B radiations (40 min), about 4 fold enhancement in flavonoid and soluble phenols contents were observed (Shaukat et al. 2013). The flavonoid content also got enhanced up to two fold in seed coat of black bean on exposure to UV-C for 10 h (Guajardo-Flores et al. 2014). When twenty cultivars of soyabean were treated with supplemental

UV-B_{BE} radiations (10 kJm^{-2}) at field condition, it was found that in the case of seven cultivars the flavonoid content was increased, decreased in five and there was no effect in eight cultivars (Yanqun et al. 2003).

An enhanced phenylalanine ammonia lyase activity (PAL) is symptomatic of plant tissues subjected to various kinds of stresses such as UV-B radiations, heavy metals, disease wounding, heat shock etc. This enzyme convert their substrates (phenylalanine) to phenolic acids that are modified through phenylpropanoid metabolism to precursors of secondary metabolites including lignin, flavonoids and phytoalexins and these compounds provide protection against various forms of stresses (Shaukat et al. 2013). PAL is an important enzyme in regulating flavonoid biosynthesis and transcriptionally induced by UV radiations. Increased PAL activity stimulates the synthesis of flavonoid and anthocyanin (Ravindran et al. 2010). PAL is a key enzyme in phenylpropanoid pathway, which is involved in the defence response of plant cells. Increased PAL activity could be a response to the cellular damage provoked by various stresses (Shaki et al. 2018). Therefore, PAL has been recognized as a prominent bio-marker of environmental stresses encountered by some group of plant species. Improved activity of PAL was observed in common bean (Guajardo-Flores et al. 2014) and strawberry (Xu et al. 2019) exposed to UV-C and in mash bean (Shaukat et al. 2013) exposed to UV-B. Flavonoid content was enhanced due to the UV-C seed priming treatment in common bean (Guajardo-Flores et al. 2014) and UV-B seed priming treatment in barley and oats (Singh et al. 2019).

UV-B radiations generally stimulates protective responses in plants, such as accumulation of wax on the leaf surface. The epicuticular waxes are known to contain unbound flavonoids and flavones. Hence, the well-developed wax layer on leaf surface can prevent UV-B penetration (Zu et al. 2011). Cuticular waxes is a complex mixture of homologous series of very-

long-chain fatty acids (VLCFAs), primary n-alcohols, secondary n-alcohols, n-aldehydes, n-alkanes, n-alkylesters, and cyclic organic compounds like penta cyclic triterpenoids, flavonoids, tocopherols and hydroxyl cinnamic acids derivatives. In wax biosynthesis, the first step is the elongation of C_{16} and C_{18} fatty acids in the endoplasmic reticulum (ER) into VLCFAs. Following elongation, VLCFAs are modified into various wax products via the distinct alcohol-forming and alkane-forming pathways (Li et al. 2019). Higher levels of non-polar, long chain aliphatic wax compounds of cuticular wax such as hydrophobic alcohols, n-alkanes, and aldehydes tend to prevent the cuticular water loss (Hasanuzzaman et al. 2017).

Wax content, composition and homologue distribution patterns vary with plant species and environmental factors such as UV-B radiations. Epicuticular wax on the surface of leaves contribute as a first line of defence mechanism by acting as a barrier between leaf internal structures and the environment. The wax coating function in attenuating the harmful effects of UV-B by increasing reflectance and scattering of light from leafy surfaces (Kumari and Agrawal 2010). Cuticular wax is the outermost hydrophobic layer of the aerial plant tissues and plays an important role in protecting plants against abiotic stresses and acts as a barrier to excessive non-stomatal transpiration. The major functions of cuticular waxes include maintaining equilibrium between the transpirational water loss and root water uptake by transpiration control, reducing water retention on plant surfaces by controlling surface wettability, controlling loss and uptake of polar solutes and regulating the exchange of gases and vapour. The plants having a thicker cuticle or a cuticle containing larger amount of waxes are more efficient in reducing non-stomatal transpiration and thus better adapted to water stress conditions (Hasanuzzaman et al. 2017; Xue et al. 2017).

3. METHODOLOGY

3.1. Plant materials

Rice (*Oryza sativa* L.) belongs to the family poaceae. The seeds of 6 high yielding rice varieties such as Aiswarya, Jyothi, Kanchana, Neeraja, Samyuktha and Swetha were collected from Regional Rice Research station, Kerala Agriculture University, Pattambi, Kerala, India. The study was conducted mainly in three stages. In the first stage, stress imparting concentrations/dosages of NaCl, PEG-6000 and UV-B was selected from six different concentrations/dosages. For this purpose six different rice varieties were imparted with different concentrations/dosages of these three stresses and stress tolerance potential of these varieties were analyzed on the basis of various morphological, physiological and biochemical parameters. For the second stage of the study two stress tolerant varieties viz. Kanchana and Swetha as well as two stress sensitive varieties viz. Samyuktha and Neeraja were selected based on the analysis carried out in the first stage of the study and imparted with different dosages of UV-B radiations to select the UV-B seed priming dosage on the basis of morpho-physio-biochemical parameters. One tolerant variety viz. Kanchana and one sensitive variety viz. Aiswarya were selected for third stage of the study, wherein detailed analysis were carried out.

3.2. Methods

3.2.1. Selection of stress imparting concentrations of NaCl, PEG and high UV-B radiation as well as UV-B priming concentrations

For the identification of stress imparting concentration of NaCl and PEG, surface sterilized rice seeds of six varieties (*detailed in section 3.2.2.*)

were treated with six different concentrations of NaCl (0, 25, 50, 75, 100 and 125 mM), PEG 6000 (0, 5, 10, 15, 20 and 25%), by incubation in the respective solutions. For identification of stress imparting UV-B irradiation dosage, surface sterilized seeds were incubated in distilled water and further exposed to varying dosages of UV-B irradiation (0, 7, 14, 21, 28 and 35 $kJm^{-2}d^{-1}$) by placing under UV-B tubes (*detailed in section 3.2.2.*). Based on the morphological, physiological and biochemical analysis NaCl concentration of 75/100 mM, PEG concentration of 15/20% and UV-B dosage of 21/28 $kJm^{-2}d^{-1}$ were selected for further studies. For the selection of optimal dosage for UV-B priming, 0, 2, 4, 6 and 8 kJm^{-2} of UV-B irradiations were imparted to the seeds/seedlings. The UV-B primed seeds were further allowed to germinate on stress imparting concentrations of NaCl (75/100 mM) and PEG (15/20%). For imparting UV-B stress, UV-B primed seeds were incubated in distilled water and irradiated with UV-B dosage of 21/28 $kJm^{-2}d^{-1}$. For the identification of stress imparting concentrations/dosage of NaCl, PEG and UV-B as well as the optimal dose of UV-B to impart seed priming and seedling priming, parameters such as shoot length and photosynthetic pigments were analyzed on 9 d of seedling growth. Based on this analysis UV-B priming dosage of 4/6 kJm^{-2} was selected for further studies.

3.2.2. Seed as well as seedling priming techniques

For the surface sterilization, the seeds were treated with 0.1% $HgCl_2$ solution for 5 min and further washed thoroughly with distilled water. After washing, the seeds were separated into two sets, one set of seeds was exposed to low dose of UV-B (4/6 kJm^{-2}) for seed priming (P_s) treatment and another set was germinated in Petri-dishes layered with germination paper and wetted with distilled water for seedling priming (P_{sl}). For seedling priming, the 4 d old seedlings were exposed to low dose of UV-B (4/6 kJm^{-2}). The primed and non-primed seeds as well as seedlings were transferred to plastic bottles

(22x12 cm) containing cotton soaked with distilled water, NaCl (100 mM for tolerant and 75 mM for sensitive) and PEG-6000 (20% for tolerant and 15% for sensitive) solutions separately. Non-primed seeds and seedlings incubated in distilled water were taken as the control. The bottles were kept in a growth chamber (INLABCO, India) under a 14/10 h light-dark cycles at 300 $\mu molm^{-2}s^{-1}$, 25±3^{0}C and RH 55±5%. For UV-B stress, UV-B irradiation (28 $kJm^{-2}d^{-1}$ for tolerant & 21 $kJm^{-2}d^{-1}$ for sensitive) in addition to continuous white fluorescent illumination of 300 $\mu mol\ m^{-2}\ s^{-1}$ was given with the aid of mobile adjustable frames over the plants. The biologically effective UV-B ($UV\text{-}B_{BE}$) was gained through normalization at 300 nm; and the calculation of the dosage was based on the intensity and the time of exposure to UV-B irradiation (Caldwell 1971). The UV-B intensity was measured using UV-B irradiation meter (RM-12, Opsytec Dr. Grobel, Ettlingen, Germany). The UV-B tubes (Philips TL 20W/01, Amsterdam, Netherlands) were covered with 0.13 mm thick cellulose diacetate filters to avoid transmission of wave lengths below 280 nm. Further physiological, biochemical and molecular analysis were done in rice seedlings emerging from UV-B primed seed (P_s) and non-primed seeds (NP_s) as well as UV-B primed seedlings (P_{sl}) and non-primed seedlings (NP_{sl}) after 9 d of growth.

3.3. Growth parameters

Shoot length of rice seedlings was measured using graduated student scale. For dry weight measurements, the pre-weighed seedlings were kept in hot air oven for 1 h at 100^{o}C and then transferred into an oven maintained at 60^{o}C for 24 h. The samples were further cooled in desiccators with vacuum and then weighed. Drying and weighing of samples were repeatedly done at regular intervals (24 h) till the values became constant. The percentage of dry weight was calculated by using the following formula:

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

3.4. Physiological parameters

3.4.1. Photosynthetic pigment analysis

Chlorophyll and carotenoid pigments were analyzed in rice seedlings leaves by Arnon (1949) method, using 80% acetone as extracting medium. Two hundred milligram of fresh leaf sample was used for analyzing pigment contents, which was homogenized and extracted in 80% acetone (v/v). It was centrifuged at 5000 rpm for 10 min at 4°C and the supernatant was collected. The pellet was re-extracted with the same extracting medium and then centrifuged at 5000 rpm for 10 min. This was repeatedly done till the pellet turn out colourless. The final volume of the acetone extract was used and the optical density was read at 663, 646, 750 and 470 nm using a UV-VIS spectrophotometer (Systronics 2201, Gujarat, India). The total chlorophyll (Chl *a*+*b*) and carotenoids contents were expressed in mg chlorophyll/ carotenoids g^{-1} dry weight of leaf sample.

$$\text{Chlorophyll } a + b = \frac{20.12\,(A_{646} - A_{750}) + 8.02\,(A_{663} - A_{750})}{\text{Fresh weight of the sample}} \times \text{volume}$$

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

Where,

$$\text{Chlorophyll } a = \frac{12.69\,(A_{663} - A_{750}) - 2.69\,(A_{646} - A_{750})}{\text{Fresh weight of the sample}}) \times \text{volume}$$

$$\text{Chlorophyll } b = \frac{22.9\,(A_{646} - A_{750}) - 4.68\,(A_{663} - A\,750)\ )}{\text{Fresh weight of the sample}} \times \text{volume}$$

3.4.2. Assessment of photosynthetic electron transport activities

Oxygen electrode system (Oxygraph Plus, Hansatech, Norfolk, UK) was used to analyze the photosynthetic electron transport activities. Polarographic oxygen electrode disc was mounted within a DW1/AD electrode chamber and linked to the Oxygraph Plus electrode control unit (OXYG1, Hansatech). Sample and electrode disc temperature was controlled through linking the water jacket of the DW1/AD to a thermoregulated circulating water bath. Thylakoids from rice leaves were isolated at 4^{o}C and photosystem I (PSI) (O_2 uptake) and photosystem II (PSII) (O_2 evolution) activities were evaluated according to Puthur (2000). The light dependent O_2 uptake/evolution was assessed through irradiating the thylakoid suspension with white light (1800 µmol$m^{-2}s^{-1}$) provided by a 100W halogen lamp (LS2, Hansatech). The PSI and PSII activities was represented in µmol of O_2 consumed (PSI)/evolved (PSII) $min^{-1}mg^{-1}$ chlorophyll.

3.4.2.1. Preparation of thylakoid membranes

Isolation of the thylakoids membranes from leaves of rice seedlings was done according to Puthur (2000). The fresh leaves tissue of 100 mg was homogenized with ice-cold mortar and pestle in a chilled isolation buffer consisting of 400 mM sucrose, 10 mM NaCl and 20 mM tricine (pH 7.8). Using six layers of Mira cloth the homogenate was filtered to remove debris and the filtrate solution was centrifuged at 5000 rpm for 6 min at 4^{o}C. The supernatant was discarded and the thylakoid pellets was suspended in 500 µl suspension buffer (pH 7.5) containing 2 mM $MgCl_2$, 10 mM NaCl, 100 mM sucrose and 20 mM HEPES [N-(2-Hydroxyethyl) piperazine-N (2-Ethanesulphonic acid)] and then it was transferred to a clean ice-cold tube and kept at 4^{o}C for the assays to be done.

3.4.2.2. Estimation of the total chlorophyll concentration in the thylakoid suspension

The total chlorophyll content of the thylakoid samples was estimated according to the method of Arnon (1949). Thylakoid suspension of 20 µl was made up to 3 mL with 80% acetone. Using parafilm the tube was covered and the mixture was thoroughly mixed by using a vortex mixer to dissolve the chlorophyll in the solvent. It was centrifuged at 5000 rpm for 5 min and the supernatant was collected. The absorbance of the supernatant was measured at 645, 663 and 750 nm. 80% acetone was taken as blank. The concentration of total chlorophyll was calculated from the following equation:

$$Total\ chl = 20.12\ (A_{646}\text{-}A_{750}) + 8.02\ (A_{663}\text{-}A_{750}) \times Dilution\ factor$$

3.4.2.3. Assay of photosystem I and II activities

Using oxygen electrode system the PSI and PSII activities were analyzed in accordance with the procedure followed by Puthur (2000). The activity of PSI was measured by estimating oxygen consumption after blocking PSII activity by adding DCMU (3-(3,4-dichlorophenyl)-1,1-dimethyl urea) to the medium. The reaction mixture contain reaction buffer, reduced 2,6 dichlorophenolindophenol (DCPIP) (0.1 mM), ascorbate (600 µM), MV (methyl viologen) (500 µM), NaN_3 (sodium azide) (1 mM) and DCMU (5 µM). 20 µg chlorophyll equivalent thylakoid suspension was added into the electrode chamber and it was made up to 1 mL with reaction buffer. Ascorbate functioned as reductant via donating electrons to DCPIP. The electrons provided by reduced DCPIP to plastocyanin gets transferred to PSI. The electrons from PSI moves to MV, which is an artificial electron acceptor in the reaction mixture as a substitute of FeS centre. Reduced MV reacts with oxygen molecules in the medium and produce hydrogen peroxides. NaN_3 in the reaction mixture can arrest the catalase action in the plant cells so that

hydrogen peroxide does not break down to release oxygen. In oxygen electrode system the oxygen consumption through the PSI activity was measured by estimating the oxygen content consumed.

PSII activity was measured based on oxygen evolution and for this para-benzo quinone (pBQ) was used as artificial electron acceptor, which scavenge the electrons from plastoquinone. On transfer of the electrons from water splitting complex to PSII, oxygen molecules evolution taken place in the medium, which was measured. The reaction mixture (1 mL) in DW1/AD electrode chamber contains isolated thylakoid suspension equivalent to 20 µg chlorophyll, reaction buffer and pBQ (500 µM).

3.4.3. Chlorophyll *a* fluorescence parameters

Using Plant Efficiency Analyzer (Handy PEA; Hansatech Ltd., Norflok, UK) Chl *a* fluorescence parameters were analyzed. Handy PEA 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 adaption for a period of 20 min using the leaf exclusion clips and then they were illuminated with continuous red light of high intensity (3000 $\mu molm^{-1}s^{-1}$). 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.

Various Chl *a* fluorescence parameters such as minimal fluorescence (F_o), maximum fluorescence (F_m), activity of the water-splitting complex on the donor side of PSII (F_v/F_o), performance index on absorption basis [$PI_{(abs)}$], relative variable fluorescence at J step (V_j), time taken to achieve maximum fluorescence value (T_{fm}) and area over the fluorescence curve were measured. The energy pipeline leaf model of the photosynthetic apparatus was used to visualize the phenomenological energy fluxes per cross section of PSII and the density of active reaction centers (RC/CSm), specific energy fluxes for

absorption per cross section (ABS/CSm), energy trapping per cross section (TR_o/CSm), electron transport per cross section (ET_o/CSm) and the ratio of total dissipation per cross section (DI_o/CSm) were also analyzed. PSII energy fluxes per reaction center (RC) [flux of absorption per reaction center (ABS/RC), trapping per reaction center (TR_o/RC), electron transport flux (ET_o/RC) and dissipated energy per reaction center (DI_o/RC)] were also deduced. The data were recorded and analysed with the help of energy pipeline model, prepared using the software Biolyzer HP 3 (Bioenergetics Laboratory, University of Geneva, Switzerland).

3.4.4. Leaf gas exchange parameters

Leaf gas exchange parameters were analyzed using LI-6400 portable photosynthesis system (Infra-red gas analyzer, LI-COR, Lincoln, Nebraska, USA). For gas exchange measurements cleaned leaf was enclosed in leaf chamber. All measurements were done in first formed fully expanded rice leaves and readings were recorded between 9.00 to 10.00 am at growth temperature (28-32ºC) at ambient CO_2 conditions. An intensity of 1500 $\mu molm^{-2}s^{-1}$ was set as internal light source in LI-6400 to ensure a constant and uniform light for all measurements. The different leaf gas exchange parameters, net photosynthetic rate, *Pn* ($\mu molm^{-2}s^{-1}$), stomatal conductance, *gs* ($\mu molm^{-2}s^{-1}$) and intercellular CO_2, *Ci* ($\mu molmol^{-1}$) was measured in leaves of rice seedlings. The *Pn*, *gs* and *Ci* were calculated according to von Caemmerer and Farquhar (1981) equations.

3.4.5. Assay of mitochondrial activity

3.4.5.1. Isolation of mitochondria

The mitochondria were isolated from the leaves of rice seedlings in accordance with Kollöffel (1967) method. Gently homogenize plant materials in an ice-cold mortar and pestle at 4ºC after adding chilled 0.05 M phosphate

buffer (isolation buffer, pH 7.2) consisting of 0.4 M sucrose and 5 mM ethylene diamine tetraacetic acid (EDTA). Filter the homogenate by four layers of Mira cloth and centrifuge the filtrate at 5000 rpm for 10 min. Again centrifuge the supernatant at 20,000 rpm for 15 min and collect the pellet. The pellet consists of mitochondria, which 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 assesed by the method of Bradford (1976).

3.4.5.2. Determination of mitochondrial electron transport activity

Mitochondrial oxygen consumption was measured at 25^{o}C by oxygen electrode system according to the method of Schmitt and Dizengremel (1989) protocol. Reaction medium consist of 935 µl of assay 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 at last, because oxygen uptake starts immediately. The NADH oxidation rate was calculated in terms of µmol O_2 consumed $min^{-1}mg^{-1}$ protein.

3.4.6. Leaf osmolality

Osmolality of leaf sap was determined as per the Hura et al. (2007) protocol, in a vapor pressure osmometer (Wescor, 5520, USA). Osmometer chamber calibration was done by 100, 290 and 1000 $mmolkg^{-1}$ standard solutions. Cell sap from leaves was collected by freeze thawing method.

Weighed 200 mg of seedling leaves which was wrapped in aluminium foil and transferred to liquid nitrogen and was then kept in a deep freezer (-80°C) for 30 min. For the determination of osmolality, the leaf samples were thawed at room temperature and the sap extruding from the leaf

discs was collected with a 10 µL pipette and transferred quickly to the disc chamber of the osmometer and readings were recorded.

3.5. Biochemical parameters

3.5.1. Primary metabolites

3.5.1.1. Total protein

Total protein content of the rice seedlings tissues was estimated using Folin-Ciocalteau reagent in accordance with Lowry et al. (1951).

Extraction*:* Five hundred mg of rice seedlings tissues was homogenized in 5 mL of phosphate buffer. Pipette a known volume of homogenate in to a centrifuge tube and add equal volume of 10% TCA. For flocculation this mixture was kept in a refrigerator (4^{o}C) for 1 h. After that the mixture was centrifuged at 5000 rpm for 10 min at 4^{o}C and the pellet was collected after decanting the supernatant. The pellet was washed twice with ice-cold 2% TCA followed by washing with 30% perchloric acid to remove starch. Lipids were extracted by using diethyl ether and 80% acetone was used to remove the pigments.

Estimation*:* The pellet obtained from centrifugation was dried and then digested in 5 mL 0.1 N sodium hydroxide by heating in a water bath for 10 min. After cooling, the suspension was purified by centrifugation (5000 rpm for 10 min at 4^{o}C) and the supernatant was collected. Pipette known volume of aliquot and made up to 1 mL with distilled water. To this aliquot, add 5 mL of alkaline copper reagent and shake well. After 10 min, add 0.5 mL of 1 N Folin-Ciocalteu's phenol reagent and was shaken well immediately. The tubes were kept for 30 min for colour development. The optical density of the solution was read at 700 nm in a UV-VIS spectrophotometer. BSA fraction V

was used as standard. Total protein was expressed in terms of mg of protein g^{-1} dry weight of rice seedlings.

3.5.1.2. Total soluble sugars

The total soluble sugar was determined by Dubois et al. (1956) protocol.

Extraction: Five hundred mg of rice seedlings tissues was homogenized with 80% ethyl alcohol. It was centrifuged for 10 min at 10000 rpm at 4°C and the supernatant was collected. The pellet was re-extracted using 80% alcohol. The total soluble sugar content was estimated in the collected supernatant.

Estimation: Known volumes of supernatant aliquot was taken in the test tube and made up to 1 mL with distilled water and add 0.1 mL of 5% (v/v) phenol and mixed well. From burette quickly add 5 mL of concentrated sulphuric acid to the tube. After cooling, the optical density of the solution was recorded at 490 nm using a spectrophotometer. D-glucose was taken as the standard.

3.5.1.3. Total free amino acids

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

Extraction: Five hundred mg of rice seedlings tissues was homogenized with 80% (v/v) ethanol. The extract was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was made up to 10 mL with 80% ethanol.

Estimation: One milllilitre of supernatant was added with 1 mL of ninhydrin reagent in a test tube. Tubes were incubated in boiling water bath for 20 min and then add 5 mL of diluent (equal volume of water and n-propanol) into it. This mixture was incubated at room temperature for 15 min and absorbance

was recorded at 570 nm using a UV-VIS spectrophotometer against a reagent blank and the results were expressed as mg g^{-1} sample. Standard curve was plotted with leucine in 0.1 M citrate buffer at pH 5.

***Preparation of reagent*:** The reagent solution was prepared by dissolving 20 g of ninhydrin and 3 g of hydrindantin in 750 mL of methyl cellosolve. Add 250 mL of sodium acetate buffer (pH 5.5) into this solution and the resultant reddish reagent solution was immediately transferred to a 1 L dark glass bottle. The reagent was used freshly without storage.

3.5.1.4. Proline

Proline content was estimated in rice seedlings tissues according to Bates et al. (1973) method.

Extraction: Two hundred mg rice seedlings tissue was homogenized in 10 mL of 3% (w/v) aqueous sulfosalicylic acid. Then it was centrifuged at 10,000 rpm for 10 min and the supernatant was collected. The proline content was estimated using acid ninhydrin.

Estimation: Two millilitres of supernatant was taken in triplicate and add 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) in test tube. Test tubes were incubated at 100^{o}C in a boiling water bath for 1 h and after that tubes were placed in ice bath for stopping the reaction. 4 mL of toluene was added into the reaction mixture and stirred well using a vortex mixer. The chromophore-toluene layer was carefully separated and the optical density was recorded at a wavelength of 520 nm using spectrophotometer. L-proline was taken as the standard.

3.5.2. ROS types

3.5.2.1. Superoxide ($O_2^{\cdot-}$) content

Superoxide content was determined as per the method of Doke (1983).

***Extraction*:** Two hundred mg of rice seedling tissue was weighed and cut into 1×1mm size and immersed in 0.01 M potassium phosphate buffer (pH 7.8) consisting 0.05% nitroblue tetrazolium chloride (NBT) and 10 mM sodium azide (NaN_3).

***Estimation*:** The mixture was incubated in water bath (85°C) for 15 min. After incubation, the mixture was transferred quickly to ice bath for lowering the temperature. After cooling, the absorbance of the mixture was measured using spectrophotometer at 580 nm. Sodium nitrate ($NaNO_2$) was used as the standard.

3.5.2.2. Hydrogen peroxide content

Hydrogen peroxide content was determined according to Junglee et al. (2014) protocol.

***Extraction*:** Two hyndred mg of rice seedling tissue was weighed in duplicate and homogenized with 5 mL of 0.1% ice cold trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000 rpm for 15 min. The supernatant collected was used for the determination of hydrogen peroxide.

***Estimation*:** Five hundred millilitres of supernatant was mixed with 0.5 mL of potassium phosphate buffer (pH 7), into this 1mL of 1M potassium iodide was added. The absorbance of the mixture was recorded at 390 nm. Hydrogen peroxide was used as the standard.

3.5.2.3. Malondialdehyde (MDA) content

According to the protocol of Heath and Packer (1968) the malondialdehyde content (MDA) was estimated.

Extraction: Two hundred mg of rice seedlings tissues was weighed in triplicate and was homogenized with 5 mL of 5% TCA solution. It was centrifuged at 12,000 rpm for 15 min and the supernatant was used for MDA estimation.

Estimation: The MDA content in rice seedlings tissues was estimated in accordance with Heath and Packer (1968) method. 2 mL of the supernatant was added with an equal aliquot of 0.5% of thiobarbituric acid (TBA) in 20% TCA and the mixture was heated at 95^{o}C for 24 min, then cooled and centrifuged at 3000 rpm for 2 min. Using UV-VIS spectrophotometer the absorbance of supernatant was measured at 532 and 600 nm. MDA content was calculated using its extinction coefficient of 155 $mM^{-1}cm^{-1}$.

3.5.2.4. Membrane stability index (MSI)

Membrane stability index (MSI) was estimated according to Sairam et al. (1997). Two hundred mg of rice seedlings tissues was cut into 10 mm^2 sized segments and placed in tubes consisting of 5 mL distilled water in two sets. One set was kept for 30 min at 40°C and electric conductivity (C_1) was determined by conductivity meter (Eutech, Cyberscan 600, Vernon Hills, USA). Another set was kept for 15 min in boiling water bath (100°C) and its electric conductivity (C_2) was also determined. The MSI was calculated as,

$$MSI = [1- (C_1/C_2)] \times 100$$

3.5.2.5. Electrolyte leakage (EL%)

Electrolyte leakage (EL%) was determined as per Lutts et al. (1996) with some modifications. 200 mg of rice seedlings tissues was cut into 10 mm^2 sized pieces and then placed in tubes consisting of 25 mL of distilled water and it was incubated at 4°C for 24 h and then brought to the room temperature and electrical conductivity was determined (EC_1). Further the tissue was autoclavedat 120°C for 15 min and electrical conductivity (EC_2) was determined again by conductivity meter. The EL% was calculated as,

$$EL\% = (EC_1/EC_2) \times 100$$

3.6. Antioxidant defence mechanism

3.6.1. Non-enzymatic antioxidants

3.6.1.1. Ascorbate (AsA) content

Chen and Wang (2002) protocol was adopted for the estimation of AsA content in rice seedlings tissues.

***Extraction**:* Five hundred mg of rice seedlings tissue was homogenized in 5 mL of 5% (w/v) TCA. The homogenate was transferred into centrifuge tubes and centrifuged at 12,000 rpm for 15 min at 4ºC. The supernatant was collected and used for the estimation of AsA content.

***Estimation**:* From the supernatant 0.1 mL of aliquot was taken and mixed with 0.3 mL of 200 mM NaH_2PO_4. To this reaction 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) were added and the mixture was incubated at 42ºC for 15 min. After the incubation, the absorbance was measured immediately at 524 nm and AsA content was calculated with a standard curve prepared using varying concentrations of AsA.

3.6.1.2. Glutathione (GSH) content

The GSH content was estimated in rice seedlings tissues according to Chen and Wang (2002) method.

Extraction: Five hundred mg of rice seedlings tissues was weighed and homogenized with 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^{o}C. The supernatant was collected and it was used for the estimation of reduced glutathione content.

Estimation: From the supernatant, 0.5 mL of aliquot was taken and mixed with 2.6 mL of 150 mM NaH_2PO_4 buffer (pH 6.8) and 0.18 mL of 3 mM 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) (DTNB was dissolved in 100 mM phosphate buffer, pH 6.8) and incubated for 5 min. The absorbance was recorded at 412 nm and GSH content was calculated with a standard curve using different concentrations of reduced glutathione.

3.6.1.3. Total phenolics

Total phenolics was estimated using Folin-Denis reagent in accordance with Folin and Denis (1915) method.

Extraction: Five hundred mg of rice seedling tissue was weighed and homogenized with 80% ethanol (v/v). The homogenate was centrifuged at 10,000 rpm for 20 min and the supernatant was collected. The pellet was re-extracted in 80% ethanol and then the homogenate was again centrifuged. The supernatant was pooled. Pooled supernatant was dried in an oven and the residue was dissolved in 5 mL of distilled water.

Estimation: Aliquots of 50 µl of supernatant in triplicate were pipetted out and made up to 2 mL with distilled water. Added equal volume of Folin-Denis reagent in to it. The mixture was thoroughly mixed and after 3 min,

added 2 mL of 1N sodium carbonate. After thorough mixing this mixture was incubated for 1 h for colour development. The optical density of the solution was read at 700 nm and total phenolics content in the rice seedlings tissues was calculated using tannic acid as standard.

3.6.2. Enzymatic antioxidant system assay

3.6.2.1. Superoxide dismutase (SOD, EC 1.15.1.1)

SOD activity in the rice seedling tissue was estimated according to Giannopolitis and Ries (1977) protocol with minor modification.

Extraction*:* Five hundred mg of plant tissue was weighed and homogenized gently with 50 mM phosphate buffer of pH 7.8 in ice-cold mortar and pestle. It was centrifuged at 16,000 rpm for 15 min in refrigerated centrifuge (Thermo scientific X1R, Osterode am Harz, Germany) at 4ºC. The supernatant was collected and it was used for enzyme assay.

Enzyme assay*:* The activity of SOD was measured by monitoring the ability of SOD to inhibit the photochemical reduction of nitro blue tetrazolium chloride (NBT). The reaction mixture contained 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 enzyme extract. By using phosphate buffer (50 mM, pH 7.8) the reaction mixture was made up to 3 mL. For the assay of SOD activity, different assay systems were set, such asdark-control, light-control and test samples. Test tubes consisting of only assay mixture without enzyme extract were illuminated under fluorescent lamp for 30 min (light-controls). Test samples (test tubes containing assay mixtures with enzyme extract) were also illuminated and another set (test tubes containing assay mixtures with enzyme extract) was put in dark (dark-control). The accumulation of formazan in different tubes was measured using UV-VIS spectrophotometer by reading the absorbance of the

developed blue colour at 560 nm against the blank (reaction mixture without NBT). Result was expressed in units SOD $mg^{-1}protein^{-1}$. One unit of SOD was the enzyme activity that inhibited the photo reduction of NBT to blue formazan by 50%.

3.6.2.2. Catalase (CAT, EC 1.11.1.6)

CAT activity in the fresh samples was measured according to the protocol of Kar and Mishra (1976).

Extraction: Five hundred mg of plant tissue was weighed and homogenized in an ice cold glass mortar and pestle with a medium containing 50 mM phosphate buffer (pH7.0) and 200 mg of polypyrrolidone as phenolic binder. Using two layered muslin cloth the homogenate was filtered and it was made upto 10 mL by phosphate buffer. The filtrate was centrifuged at 16,000 rpm for 15 min at 4ºC in refrigerated centrifuge. The collected supernatant was used for the enzyme assay.

Enzyme assay: Enzyme assay mixture contained 1 mL of 50 mM phosphate buffer (pH 7.0), 2 mL enzyme extract and 1 mL of 30 mM hydrogen peroxide. The enzyme assay mixture contained 2.4 mL of 50 mM phosphate buffer (pH 7.0), 0.3 mL enzyme extract and 0.3 mL of 30 mM hydrogen peroxide. The enzyme extract and phosphate buffer was pipetted out and mixed well in a test tube. Hydrogen peroxide was added into this mixture which initiated the enzyme activity. After the addition of hydrogen peroxide immediately the enzyme activity was measured at 240 nm for 90 seconds. Readings were recorded at 15 seconds interval. The activity of CAT was determined in terms of µmol hydrogen peroxide oxidised per min per gram fresh weight. The CAT activity was expressed in terms of decrease in absorbance at 240 nm for 1 min following the decomposition of hydrogen

peroxide. One unit of the enzyme was defined as µmoles hydrogen peroxide decomposed per min per mg protein.

3.6.2.3. Ascorbate peroxidase (APX, EC 1.11.1.11)

APX activity was assayed in the rice seedlings tissues in accordance with the method of Nakano and Asada (1981).

Extraction: Enzyme extraction from rice seedling tissues for the assay was prepared according to Zhang and Kirkham (1996). 500 mg of tissues from seedlings was homogenized gently with 10 mL of extraction medium in ice cold mortar and pestle. The extraction buffer contained 50 mM sodium phosphate buffer (pH 7.0), 0.33 M sorbitol, 1 mM $MgCl_2$, 2 mM EDTA, 10 mM NaCl, 0.5 mM KH_2PO_4 and 1 mM ascorbate. Using two layers of cheese cloth the homogenate was filtered and centrifuged at 4^oC for 4 min at 2000 rpm. The pellet was discarded and the supernatant was centrifuged at 5000 rpm for 15 min at 4^oC. Again the supernatant was centrifuged at 15000 rpm for 15 min at 4^oC and the final supernatant was used for APX assay.

Enzyme assay: The APX activity was assayed by monitoring the decreases in absorbance at 290 nm due to AsA oxidation. 3 mL assay mixture contained 0.5 mM AsA, 0.1 mM EDTA in 50 mM sodium phosphate buffer (pH 7.0). 20 µL of enzyme extract was added into the buffer and the enzyme reaction was initiated by adding 10 µL of 100 mM hydrogen peroxide to reach a concentration of 0.1 mM hydrogen peroxide in the final reaction mixture. The hydrogen peroxide dependent oxidation of AsA (ε=2.8 $mM^{-1}cm^{-1}$) seen as the decrease in absorbance at 290 nm was monitored. One unit of APX activity was defined as the amount of enzyme that oxidized one µmol of AsA min^{-1} at room temperature under the above conditions.

3.6.2.4. Guaiacol peroxidase (GPOX, EC 1.11.1.7)

GPOX activity in the fresh rice seedlings tissues was estimated as per the protocol of Gaspar et al. (1975).

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

Enzyme assay: The GPOX activity was measured following the hydrogen peroxide dependent oxidation of guaiacol (extinction coefficient 26.6 $mM^{-1}cm^{-1}$) at 420 nm. 3 mL of assay mixture contained 2.86 mL 100 mM phosphate buffer (pH 7.8), 30 µL 1% guaiacol and 100 µl enzyme extract. Blank reaction mixture contained 50 mM Tris-HCl (pH 7.5) instead of enzyme extract. All the components were mixed well and 12 µL of hydrogen peroxide was added to initiate the enzyme activity. Immediately after the addition of hydrogen peroxide, the increase in absorbance due to oxidation of guaiacol was recorded at 420 m using a UV-VIS spectrophotometer 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.

3.6.2.5. Glutathione reductase (GR, EC 1.6.4.2)

Glutathione reductase (GR) activity was determined as per the method of Carlberg and Mannervik (1975).

Extraction: Five hundred mg fresh tissue of rice seedlings was homogenized in 5 mL of 50 mM sodium phosphate buffer (pH 7) containing 0.2 mM EDTA in an ice cold mortar and pestle. The homogenate was centrifuged at 4°C for 20 min at 14,000 rpm and the supernatant was used for measuring the GR activity.

Enzyme assay: GR activity was measured by determining the oxidation of NADPH at 340 nm. The 3 mL reaction mixture consisted of 3 mM EDTA, 0.1 mM NADPH, and 0.1 mM oxidized glutathione (GSSG) in 1.8 mL phosphate buffer (pH 7.6), into this, 0.3 mL enzyme extract was added. The GR activity was determined by observing the reduction in absorbance per minute. One unit of the enzyme activity was defined as the amount of enzyme required to oxidize 1 μmol of NADPH per min.

3.6.2.6. Monodehydroascorbate reductase (MDHAR, EC 1.1.5.4)

Activity of monodehydroascorbate reductase (MDHAR) was assayed by following the method of Hossain et al. (1984).

Extraction: Five hundred mg freshtissue of rice seedlings was homogenized in 5 mL of 50 mM potassium phosphate buffer (pH 7) consisting of 0.2 mM EDTA in a ice-cold mortar and pestle. The homogenate was centrifuged at 14,000 rpm for 20 min at 4°C and the supernatant was used for determining the MDHAR activity.

Enzyme assay: The reaction mixture consisted of 0.1 mM EDTA, 0.25% of triton X 100, 3 mM NADH, 30 mM ascorbate, 0.25 units ascorbate oxidase with 2.7 mL of phosphate buffer (150 mM). 3 μl of enzyme extract was transferred to test tube and the MDHAR activity was measured by determining the oxidation of NADH at 340 nm in UV-VIS spectrophotometer. One unit of the enzyme activity was defined as the amount of enzyme required to oxidize 1μmol of NADH.

3.6.2.7. Dehydroascorbate reductase (DHAR, EC 1.8.5.1)

Dehydroascorbate reductase (DHAR) activity was determined in accordance with Dalton et al. (1986).

Extraction: Fresh tissue of rice seedlings 500 mg was homogenized in 5 mL of 50 mM sodium phosphate buffer (pH 7) consisting of 0.2 mM EDTA in an ice-cold mortar and pestle. The homogenate was centrifuged at 4°C for 20 min at 14,000 rpm and the supernatant was used for determining the DHAR activity.

Enzyme assay: The enzyme assay mixture contained 1 mM EDTA, 15 mM reduced glutathione, 2 mM dehydroascorbate in 1.5 mL phosphate buffer (pH 7). 400 µl of enzyme extract was added into this mixture and the increase in absorbance was read at 265 nm using UV-VIS spectrophotometer. One unit of the enzyme activity was defined as the amount of enzyme required to catalyze the formation of 1 µmol of ascorbate per min.

3.7. Gene expression analysis of enzymatic antioxidants and stress responsive proteins

Total RNA was extracted from the UV-B primed and non-primed rice seedlings tissues subjected to three different stresses using the protocol of Valenzuela-Avendano et al. (2005). The RNA concentration and integrity was checked by Nano Drop spectrophotometer (Jenway, Genova Nano Staffordshire, United Kingdom) and by agarose gel electrophoresis. For RT-PCR analysis 2µg of total RNAs were treated with 10 U RNase-free DNase I (Sigma) in 10 µl reaction media containing 1X DNase I buffer (20 mM Tris/HCl pH 8.4; 50 mM KCl and 2 mM $MgCl_2$) at 37°C for 5 min followed by the addition of EDTA to deactivate DNase I. First-strand cDNA synthesis was performed from total RNA using iScript cDNA synthesis kit (Bio-Rad, California, United States). Primers for SOD (*Cu/Zn SOD*), CAT (*CatA*) and

APX (*APx1*), HSP (*Hsp90*), LEA (group 3 *LEA*) and actin (*ACTIN*) (internal control) were designed using the primer- 3 software [Table 1] and the RT-PCR was performed using Thermo cycler (Bio-Rad C1000, California, United States). Differential expression of the genes was analyzed by running the PCR products in 1% agarose gel followed by measuring the band intensities using ImageJ program. The signal intensity value of each sample and for a specific gene was divided by the value obtained for *ACTIN* gene for the same sample. The resulting ratios of at least three gels were plotted for each sample.

Table 1: The details of forward and reverse primers for actin, *Cu/Zn SOD*, *CatA*, *APx1*, *Hsp90* and group 3 *LEA*.

Sl No	Gene	Forward primer	Reverse primer	Gen Bank Id
1	Actin	TGGACTCTGGTGATGGTGTC	AAGGTGCTAAGGGAGGCAAG	AY212324.1
2	*Cu/Zn SOD*	GTGTCAAGGGCACCATCTTT	GCAAACTGCACACTGGTCAT	D01000.1
3	*CatA*	TCAACACCTACACGCTCGTC	TAGCGGGATGGGAAGTAGTC	AB020502.1
4	*APx1*	GAGCCCATCAAGGAGGAGAT	CACGGGCAATGTACTAGCAG	D45423.1
5	*Hsp90*	CACCGTTCCTTGAGAGGCTA	CACCGACCTGTAGATGCTGA	AB037681.1
6	Group3 *LEA*	AAGGGGCAGGATACCAAGGA	TCACCACACCCGTCAGAATC	EF444535.1

3.8. UV-B specific compounds

3.8.1. Estimation of UV-B absorbing compounds

UV-B absorbing compounds such as anthocyanin and flavonoids were estimated in leaf material of rice seedlings tissues spectrophotometrically.

Anthocyanin content was assessed according to the method of Mancinelli et al. (1975) with minor modifications. Fresh rice leaf samples (0.2 g) were homogenized and extracted in 5 mL of acidified methanol (1:99, HCl:methanol, v/v). The extract was incubated at 4°C for 24 h and the content was made up to 10 mL. The anthocyanin content was assessed from the

absorbance at 530 nm using UV-VIS spectrophotometer. It was expressed as μmolg^{-1}DW and the concentration of anthocyanin was calculated using its extinction coefficient of 33 mM^{-1}cm^{-1}.

Flavonoids were extracted and estimated according to the method of Mirecki and Teramura (1984). Two hundred mg of fresh leaf samples was homogenized with 5 mL of solvent containing acidified methanol:HCl:H_2O (79:1:20). The homogenate was incubated for 24 h at room temperature. The flavonoid content was estimated from the absorbance of the supernatant at 315 nm using UV-VIS spectrophotometer. Flavonoid content was expressed as μmolg^{-1}DW and the concentration of flavonoids was calculated using its extinction coefficient of 33 mM^{-1}cm^{-1}.

3.8.2. Phenylalanine ammonia lyase activity (PAL, EC 4.3.1.24)

PAL activity was estimated in accordance with the methodology of Zucker (1965).

***Extraction*:** Fresh leaves 200 mg was homogenised in 3 mL borate buffer (pH 8.8) containing 23 μl of mercaptoethanol at 2°C. The homogenate was centrifuged at 8,500 rpm for 20 min at 4°C in refrigerated centrifuge.

Enzyme assay: The PAL assay system consisted of 1 mL of the supernatant, 1mL of buffer, 1 mL of 0.05 M L-phenylalanine as substrate and it was incubated at 37°C for 1 h. After 1 h the reaction was stopped by adding 3 mL 30% trichloroacetic acid and absorbance of the trans-cinnamic acid formed was measured at 290 nm wavelength. 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 estimated by the method of Bradford (1976).

3.8.3. Functional group analysis of epicuticular wax deposition

Fourier transform infrared spectrometry (FT-IR) analysis was used to determine the major functional groups in chloroform extract of cuticular wax deposition with support of IR spectrometer.

3.8.3.1. 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 followed with some modifications. For the extraction fresh leaf samples (0.5 g) were immersed two times repetitively for 30 s each in a test tube with 25 mL chloroform at room temperature. During each extraction, the solvent was shaken for 30 s by pumping air into it with a Pasteur pipette.

3.8.3.2. Infra-red analysis of the epicuticular wax deposition: The waxy deposition was accurately weighed and it was mixed with 0.5 mL chloroform which was placed in light beam path to measure the liquid state spectrum in a Fourier transform infrared spectrometry (FT-IR- JASCO-4100, Easton, USA). Infra-red analysis of the wax extracts were recorded in the 400 to 4000 cm^{-1} range with 2 cm^{-1} resolution.

3.9. Statistical analysis

Statistical analysis of the data was done according to Duncan's test ($P \leq 0.05$). The data was average of three separate experimental observations with three triplicates (i.e. n=9). The data denote mean ± standard error (SE). One-way ANOVA was applied using the SPSS software (Version 16.0, SPSS Inc., Chicago, USA) to examine the effect of UV-B primed rice seeds as well as seedlings exposed to various stress conditions.

3.10. Chemicals

Analytical reagent (AR) or guaranteed reagent (GR) chemicals were used and was purchased from Merck, SRL, Qualigens, BDH, GMBH, Spectrochem and Himedia companies. Riboflavin, glutaraldehyde, bovine serum albumin (BSA), methyl viologen, 3- (3, 4-dichlorophenyl)-1,1-dimethyl urea (DCMU), sodium azide and L-ascorbate were purchased from Sigma chemicals, USA.

4. RESULTS

4.1. First stage of the study: Screening for identifying tolerant and sensitive varieties

In this study seeds of six different rice varieties such as Aiswarya, Jyothi, Kanchana, Neeraja, Samyuktha and Swetha were germinated in different concentrations/dosage of NaCl (0, 25, 50, 75, 100 and 125 mM), PEG (0, 5, 10, 15, 20 and 25%) and UV-B (0, 7, 14, 21, 28 and 35 $kJm^{-2}d^{-1}$). Preliminary screening was held to identify the stress imparting concentration of NaCl and PEG and UV-B dosages. Stress imparting concentrations/dosage of NaCl, PEG and UV-B, specific to each variety was determined through the analysis of morphological and physiological characters such as shoot length, total chlorophyll and carotenoid content.

4.1.1. Shoot length

On the basis of analyzing shoot length and photosynthetic pigment content, the stress imparting concentrations/dosages of NaCl, PEG and UV-B radiations were selected from six different concentrations/dosages of the above stresses. Out of the various concentrations of NaCl (0, 25, 50, 75, 100 and 125 mM) and PEG (0, 5, 10, 20 and 25%), 100 mM NaCl and 20% PEG imparted, ~50% reduction in shoot length for varieties such as Jyothi, Swetha and Kanchana and in the case of varieties such as Aiswarya, Samyuktha and Neeraja ~50% reduction in shoot length occurred at 75 mM NaCl and 15% PEG as compared to control (0 mM). Although various intensities of UV-B radiations (14, 21, 28 and 35 $kJm^{-2}d^{-1}$) reduced the shoot length, 50% reduction occurred in seedlings exposed to 28 $kJm^{-2}d^{-1}$ UV-B in some varieties (Neeraja, Swetha and Kanchana) and 21 $kJm^{-2}d^{-1}$ for other varieties (Aiswarya, Samyuktha and Jyothi). The shoot length was either less or more

than 50% in seedlings/exposed to other concentrations/dosages of NaCl, PEG and UV-B as compared to the control. However, in 25 mM NaCl, 5% PEG and 7 $kJm^{-2}d^{-1}$ UV-B, the shoot length was negligibly increased than the control (Table 2, 3,4).

4.1.2. Photosynthetic pigments

One hundred mM NaCl and 20% PEG treatments brought about ~50% reduction in leaf total chlorophyll content in rice seedlings of Jyothi, Swetha and Kanchana varieties whereas in the case of Aiswarya, Samyuktha and Neeraja varieties, 50% reduction occurred when seedlings were exposed to 75 mM NaCl and 15% PEG as compared to control. In the case of UV-B radiation, ~50% reduction in chlorophyll content was observed on imparting 28 $kJm^{-2}d^{-1}$ in Neeraja, Swetha and Kanchana rice varieties whereas same extent of reduction occurred in Aiswarya, Samyuktha and Jyothi on imparting 21 $kJm^{-2}d^{-1}$ UV-B radiation. In the case of other NaCl, PEG and UV-B concentrations/dosages, the total chlorophyll content were either lower or higher than 50% as compared to control. However, in 25 mM NaCl, 5% PEG and 7 $kJm^{-2}d^{-1}$ UV-B treatments, the total chlorophyll content was negligibly increased (Table 2, 3, 4).

Under the influence of 100 mM NaCl and 20% PEG concentrations, carotenoid content was reduced to ~50% in leaves of Jyothi, Swetha and Kanchana seedlings and under the influence of 75 mM NaCl and 15% PEG, same extent of decrease in carotenoid content occurred in Aiswarya, Samyuktha and Neeraja as compared to control. Under the influence of 28 $kJm^{-2}d^{-1}$ UV-B radiations, ~50% reduction in leaf carotenoid content was noticed in Neeraja, Swetha and Kanchana and in Aiswarya, Samyuktha and Jyothi, ~50% reduction occurred on exposure to 21 $kJm^{-2}d^{-1}$. The percentage reduction of carotenoid content under the influence of other concentrations of NaCl, PEG and UV-B irradiation was either less or higher than 50%. When

25 mM NaCl, 5% PEG and 7 $kJm^{-2}d^{-1}$ UV-B irradiation was imparted, there was negligible increment in carotenoid content (Table 2, 3, 4).

Table 2: Shoot length, total chlorophyll and carotenoid content in rice seedlings subjected to different concentrations of NaCl

Shoot length plant^{-1} (cms)						
Concentrations	**Ais**	**Sam**	**Nee**	**Jyo**	**Swe**	**Kan**
0 mM	8.92±0.12^{b}	9.04±0.07^{b}	9.28±0.10^{b}	9.32±0.12^{b}	9.68±0.10^{b}	10.72±0.09^{b}
25 mM	9.00±0.12^{a}	9.12±0.10^{a}	9.52±0.08^{a}	9.52±0.14^{a}	9.76±0.21^{a}	11.08±0.12^{a}
50 mM	7.64±0.13^{c}	7.72±0.04^{c}	7.60±0.09^{c}	8.76±0.11^{c}	7.52±0.08^{c}	8.56±0.07^{c}
75 mM	4.56±0.08^{d}	4.76±0.12^{d}	4.76±0.10^{d}	6.04±0.15^{d}	5.92±0.08^{d}	7.64±0.10^{d}
100 mM	3.52±0.09^{e}	3.68±0.14^{e}	3.56±0.07^{e}	4.52±0.18^{e}	5.00±0.09^{e}	5.56±0.11^{e}
125 mM	2.68±0.10^{f}	3.32±0.15^{f}	2.64±0.08^{f}	3.80±0.14^{f}	3.76±0.13^{f}	4.32±0.17^{f}
Total chlorophyll content (mg g^{-1} DW)						
Concentrations	**Ais**	**Sam**	**Nee**	**Jyo**	**Swe**	**Kan**
0 mM	4.34±0.08^{b}	3.83±0.08^{b}	4.23±0.11^{b}	4.53±0.07^{b}	5.75±0.03^{b}	6.92±0.07^{b}
25 mM	4.51±0.09^{a}	3.91±0.04^{a}	4.35±0.14^{a}	4.78±0.07^{a}	5.86±0.05^{a}	7.46±0.08^{a}
50 mM	2.83±0.06^{c}	2.82±0.01^{c}	3.04±0.12^{c}	4.43±0.08^{c}	5.12±0.06^{c}	5.32±0.09^{c}
75 mM	2.24±0.01^{d}	1.96±0.08^{d}	2.24±0.15^{d}	3.77±0.02^{d}	4.69±0.04^{d}	4.76±0.07^{d}
100 mM	1.66±0.03^{e}	1.56±0.09^{e}	1.52±0.18^{e}	2.31±0.12^{e}	2.81±0.05^{e}	3.43±0.07^{e}
125 mM	1.15±0.10^{b}	1.43±0.11^{f}	1.37±0.14^{f}	1.83±0.11^{f}	2.13±0.10^{f}	2.83±0.03^{f}
Carotenoid content (mg g-1 DW)						
Concentrations	**Ais**	**Sam**	**Nee**	**Jyo**	**Swe**	**Kan**
0 mM	2.58±0.03^{b}	2.26±0.06^{b}	2.27±0.03^{b}	2.87±0.01^{b}	3.23±0.03^{b}	3.74±0.06^{b}
25 mM	2.80±0.04^{b}	2.90±0.04^{a}	2.41±0.05^{a}	2.92±0.02^{a}	3.35±0.06^{a}	3.85±0.05^{a}
50 mM	1.84±0.01^{b}	2.68±0.05^{c}	1.76±0.04^{c}	2.40±0.01^{c}	2.69±0.08^{c}	3.17±0.06^{c}
75 mM	1.38±0.01^{b}	1.09±0.09^{d}	1.16±0.05^{d}	1.92±0.02^{d}	1.98±0.08^{d}	2.65±0.08^{d}
100 mM	1.01±0.02^{b}	0.81±0.05^{e}	0.83±0.06^{e}	1.37±0.05^{e}	1.64±0.06^{e}	1.81±0.10^{e}
125 mM	0.71±0.02^{b}	0.57±0.11^{f}	0.68±0.07^{f}	1.15±0.03^{f}	1.06±0.13^{f}	1.37±0.05^{f}

Table 3: Shoot length, total chlorophyll and carotenoid content in rice seedlings subjected to different concentrations of PEG

Shoot length plant^{-1} (cms)						
Concentrations	**Ais**	**Sam**	**Nee**	**Jyo**	**Swe**	**Kan**
0%	8.76±0.07^{b}	9.16±0.16^{b}	9.12±0.13^{b}	9.36±0.07^{b}	9.6±0.10^{b}	10.6±0.10^{b}
5%	8.8±0.11^{a}	10.2±0.10^{a}	9.28±0.14^{a}	9.48±0.10^{a}	9.72±0.14^{a}	10.92±0.12^{a}
10%	7.48±0.10^{c}	7.84±0.21^{c}	7.88±0.26^{c}	8.32±0.08^{c}	8.48±0.08^{c}	8.4±0.12^{c}
15%	4.6±0.06^{d}	4.84±0.16^{d}	4.6±0.16^{d}	6.52±0.11^{d}	6.4±0.11^{d}	7.44±0.11^{d}
20%	3.48±0.10^{e}	3.64±0.12^{e}	3.68±0.13^{e}	4.72±0.13^{e}	4.8±0.07^{e}	5.36±0.08^{e}
25%	1.96±0.11^{f}	3.56±0.11^{f}	3.32±0.08^{f}	3.36±0.07^{f}	3.68±0.08^{f}	4.08±0.10^{f}
Total chlorophyll content (mg g^{-1} DW)						
Concentrations	**Ais**	**Sam**	**Nee**	**Jyo**	**Swe**	**Kan**
0%	4.26±0.02^{b}	3.82±0.02^{a}	4.16±0.06^{b}	4.50±0.14^{b}	5.68±0.08^{b}	6.88±0.01^{b}
5%	4.37±0.03^{a}	3.47±0.03^{b}	4.21±0.09^{a}	4.68±0.18^{a}	5.81±0.04^{a}	7.13±0.09^{a}
10%	2.81±0.07^{c}	3.18±0.05^{c}	3.28±0.07^{c}	3.50±0.07^{c}	4.93±0.05^{c}	5.22±0.03^{c}
15%	1.87±0.02^{d}	1.83±0.06^{d}	2.01±0.08^{d}	2.96±0.10^{d}	4.37±0.07^{d}	4.24±0.05^{d}
20%	1.25±0.01^{e}	1.29±0.04^{e}	1.38±0.03^{e}	2.30±0.08^{e}	2.79±0.08^{e}	3.28±0.06^{e}
25%	0.88±0.03^{f}	0.78±0.03^{f}	0.91±0.04^{f}	1.59±0.17^{f}	2.09±0.13^{f}	2.61±0.07^{f}
Carotenoid content (mg g^{-1} DW)						
Concentrations	**Ais**	**Sam**	**Nee**	**Jyo**	**Swe**	**Kan**
0%	2.23±0.10^{b}	2.20±0.06^{a}	2.31±0.01^{b}	2.85±0.08^{b}	3.19±0.09^{a}	3.69±0.07^{b}
5%	2.35±0.03^{a}	2.15±0.05^{b}	2.50±0.05^{a}	2.90±0.04^{a}	3.15±0.01^{b}	3.75±0.06^{a}
10%	1.30±0.05^{c}	1.74±0.02^{c}	1.63±0.13^{c}	2.54±0.09^{c}	2.71±0.06^{c}	3.10±0.03^{c}
15%	1.16±0.07^{d}	1.14±0.06^{d}	1.17±0.05^{d}	2.20±0.10^{d}	2.07±0.08^{d}	2.44±0.06^{d}
20%	0.56±0.02^{e}	0.85±0.04^{e}	0.91±0.03^{e}	1.44±0.13^{e}	1.61±0.07^{e}	1.71±0.06^{e}
25%	0.51±0.04^{f}	0.52±0.05^{f}	0.78±0.08^{f}	1.07±0.04^{f}	1.16±0.04^{f}	1.26±0.05^{f}

Table 4: Shoot length, total chlorophyll and carotenoid content in rice seedlings subjected to different dosages of UV-B irradiations

Shoot length plant^{-1} (cms)						
Dosages	**Ais**	**Sam**	**Nee**	**Jyo**	**Swe**	**Kan**
0 kJm^{-2}d^{-1}	8.84±0.11^{b}	9.20±0.14^{a}	9.20±0.74^{a}	9.40±0.09^{b}	9.60±0.12^{a}	10.68±0.20^{b}
7 kJm^{-2}d^{-1}	8.92±0.8^{a}	8.60±0.10^{b}	9.32±0.48^{b}	9.52±0.35^{a}	9.52±0.14^{b}	11.08±0.08^{a}
14 kJm^{-2}d^{-1}	7.56±0.10^{c}	8.48±0.67^{c}	8.36±0.83^{c}	7.96±0.68^{c}	8.56±0.11^{c}	8.92±0.12^{c}
21 kJm^{-2}d^{-1}	4.68±0.74^{d}	5.2±0.41^{d}	6.36±0.24^{d}	4.92±0.14^{d}	7.44±0.07^{d}	7.56±0.11^{d}
28 kJm^{-2}d^{-1}	3.52±0.89^{e}	3.80±0.20^{e}	4.84±0.14^{e}	3.76±0.11^{e}	4.76±0.48^{a}	5.44±0.07^{e}
35 kJm^{-2}d^{-1}	2.48±0.13^{f}	3.20±0.12^{f}	3.68±0.54^{f}	3.44±0.10^{f}	3.80±0.14^{f}	4.20±0.14^{f}
Total chlorophyll content (mg g^{-1} DW)						
Dosages	**Ais**	**Sam**	**Nee**	**Jyo**	**Swe**	**Kan**
0 kJm^{-2}d^{-1}	4.25±0.32^{b}	3.76±0.11^{b}	4.19±0.05^{b}	4.52±0.07^{b}	5.72±0.05^{b}	6.97±0.06^{b}
7 kJm^{-2}d^{-1}	4.39±0.53^{a}	3.84±0.69^{a}	4.44±0.14^{a}	4.75±0.04^{a}	5.81±0.05^{a}	7.25±0.04^{a}
14 kJm^{-2}d^{-1}	2.54±0.15^{c}	2.03±0.25^{c}	3.45±0.02^{c}	4.03±0.10^{c}	4.72±0.07^{c}	5.35±0.05^{c}
21 kJm^{-2}d^{-1}	1.98±0.50^{d}	1.62±0.49^{d}	3.05±0.07^{d}	2.25±0.04^{d}	3.24±0.05^{d}	4.33±0.08^{d}
28 kJm^{-2}d^{-1}	1.33±0.22^{e}	1.10±0.21^{e}	2.05±0.07^{e}	1.81±0.05^{e}	2.82±0.02^{e}	3.35±0.02^{e}
35 kJm^{-2}d^{-1}	0.99±0.76^{f}	0.96±0.56^{f}	1.44±0.07^{f}	1.55±0.13^{f}	2.18±0.01^{f}	2.76±0.03^{f}
Carotenoid content (mgg^{-1} DW)						
Dosages	**Ais**	**Sam**	**Nee**	**Jyo**	**Swe**	**Kan**
0 kJm^{-2}d^{-1}	2.31±0.07^{b}	2.13±0.07^{b}	2.29±0.05^{b}	2.84±0.05^{b}	3.22±0.09^{b}	3.73±0.05^{b}
7 kJm^{-2}d^{-1}	2.41±0.06^{a}	2.17±0.05^{a}	2.54±0.08^{a}	2.92±0.06^{a}	3.32±0.01^{a}	3.87±0.05^{a}
14 kJm^{-2}d^{-1}	1.29±0.01^{c}	1.42±0.02^{c}	1.84±0.06^{c}	2.34±0.08^{c}	2.58±0.01^{c}	3.13±0.07^{c}
21 kJm^{-2}d^{-1}	1.22±0.12^{d}	1.14±0.05^{d}	1.43±0.06^{d}	1.42±0.05^{d}	2.16±0.05^{d}	2.54±0.09^{d}
28 kJm^{-2}d^{-1}	0.73±0.04^{e}	0.72±0.05^{e}	1.17±0.05^{e}	1.19±0.09^{e}	1.54±0.08^{e}	1.79±0.03^{e}
35 kJm^{-2}d^{-1}	0.61±0.05^{f}	0.47±0.04^{f}	0.92±0.08^{f}	0.99±0.14^{f}	1.21±0.05^{f}	1.31±0.05^{f}

4.2. Second stage of the study: Screening for UV-B priming dosage

Based on the results of first stage of the study, two sensitive varieties (Aiswarya and Samyuktha) and two tolerant varieties (Swetha and Kanchana) of rice were selected for the second stage of study wherein plants were imparted with different low dosages of UV-B irradiations for the selection of effective UV-B priming dosage. The seeds of four varieties were treated with different low doses of UV-B (0, 2, 4, 6 and 8 kJm^{-2}) and then subjected to stress imparting concentration of NaCl (75/100 mM), PEG (15/20%) and UV-B (21/28 $kJm^{-2}d^{-1}$). The tolerant rice varieties were raised in higher concentrations and sensitive varieties in lower concentrations of each stress.

4.2.1. Shoot length

UV-B primed seeds were germinated in three different stress conditions viz. NaCl, PEG and UV-B. In all the above stress conditions, shoot length of the seedlings were increased in the case of seedlings emerging from seeds primed with 2 to 6 kJm^{-2}. In Aiswarya and Samyuktha maximum increase in shoot length was seen upon imparting the seedling with UV-B dosage of 4 kJm^{-2} whereas in Swetha and Kanchana maximum increase in shoot length occurred on imparting 6 kJm^{-2} as compared to control. On exposure to concentrations above 4 and 6 kJm^{-2}, there was a reduction in shoot length in seedlings emerging from primed seeds and exposed to stress conditions as compared to 4 and 6 kJm^{-2} (Fig. 1, 3, 5).

4.2.2. Dry weight percentage

On exposure to all the stress conditions, dry weight of the rice seedlings were increased in the case of Aiswarya and Samyuktha seedlings emerging from seeds primed with 2 and 4 kJm^{-2} of UV-B and the increase was to the extent of 45-50% as compared to control. Beyond it i.e., 6 and 8 kJm^{-2}, a reduction was observed in dry weight of seedlings exposed to stress

conditions. In Swetha and Kanchana seedlings emerging from seeds primed with 2, 4 and 6 kJm^{-2} UV-B, increment of dry weight was maximum at 6 kJm^{-2} and beyond that the dry weight percentage was reduced (Fig. 1, 3, 5).

4.2.3. Photosynthetic pigments

Total chlorophyll content was found to be increased in Aiswarya, Samyuktha, Swetha and Kanchana seedlings emerged from primed seeds. The maximum increase of total chlorophyll content was observed on imparting 4 kJm^{-2} UV-B for Aiswarya and Samyuktha andon imparting 6 kJm^{-2} UV-B for Swetha and Kanchana varieties of rice seedlings. Among the four varieties of UV-B primed seeds subjected to three different stress conditions, the prominent increase in photosynthetic pigment content was noticed in Kanchana but it was lower in Aiswarya. Beyond these dosages, a decreasing trend of total chlorophyll content was registered in four varieties under three different stress conditions viz. NaCl, PEG and UV-B.

Similar tendency was observed in carotenoid content of four rice varieties. Carotenoid content was found to be increased in Aiswarya and Samyuktha on imparting UV-B dosages up to 4 kJm^{-2} and then subjected to three stress conditions, wheras in Swetha and Kanchana the increase occurred on exposure to 6 kJm^{-2}. Beyond it, the carotenoid content was decreased in four varieties. Of the four varieties the highest carotenoid content was noticed in Kanchana and lowest in Aiswarya (Fig. 2, 4, 6).

4.2.4. MDA content

A decreasing trend of MDA content was noticed in UV-B primed Aiswarya, Samyuktha, Swetha and Kanchana seeds subjected to three stress conditions as compared with respective control with an exception of 8 kJm^{-2}. The decreasing pattern of MDA content was maximum in Aiswarya and Samyuktha imparted with 4 kJm^{-2} UV-B whereas in Swetha and Kanchana

maximum reduction occurred at 6 kJm^{-2}. Out of the four varieties, Kanchana showed higher reduction of MDA content and lower reduction was seen in Aiswarya. Beyond these concentrations MDA content was increasing in seedlings (Fig. 2, 4, 6).

4.3. Third stage of the study

In the third stage of the study, for detailed analysis only the most tolerant variety Kanchana and sensitive variety Aiswarya rice was selected. The seed priming concentrations selected was 6 kJm^{-2} UV-B radiations for tolerant variety Kanchana and 4 kJm^{-2} for sensitive Aiswarya variety. In the third stage of the study, both seed and seedlings were primed and priming dosage for seedlings was also same as that of seeds. The seedlings emerged from primed and non-primed seeds (P_s) or directly primed seedlings (P_{sl}) of both varieties subjected to three stresses [NaCl (75/100 mM), PEG (15/20%) and UV-B (21/28 $kJm^{-2}d^{-1}$)] conditions were used for further analysis. Non-primed seedlings of both varieties, not subjected to any stress conditions were taken as the control.

4.3.1. Physiological parameters

Various physiological parameters such as chlorophyll *a* fluorescence, PSI and PSII activities, leaf gas exchange and mitochondrial activity were analyzed in primed and non-primed Aiswarya and Kanchana rice seedlings exposed to different stress conditions.

4.3.1.1. Photosystem activities

4.3.1.1.1. PSI activity

The activities of PSI increased in leaves of seedlings emerged from primed seeds (P_s) and primed seedlings (P_{sl}) subjected to NaCl, PEG and UV-B stresses as compared to non-primed seedlings (NP_s & NP_{sl}). The PSI

activity was increased to the maximum in Kanchana P_s & P_{sl} not exposed to any stress and it was to the extent of 44%. In the case of Aiswarya P_s & P_{sl} without any stress, the rate of increase in PSI activity was only ≤25%. In primed seedlings of Aiswarya and Kanchana (P_s & P_{sl}) subjected to stress conditions (NaCl, PEG and UV-B), the PSI activity was increased up to 33%. However, there was a reduction observed in PSI activity in non-primed seedlings (NP_s & NP_{sl}) subjected to stress conditions as compared to control (Fig. 7).

4.3.1.1.2. PSII activity

PSII activity was appreciably improved in Aiswarya and Kanchana rice seedlings with the effect of UV-B priming. An increasing trend in PSII activity was found in primed seedlings (P_s & P_{sl}) not subjected to any stress conditions.Maximum increase was observed in Kanchana P_s & P_{sl} not exposed to any stress i.e., an increase of 44% over the control. In the case of primed seedlings (P_s & P_{sl}) subjected to stress conditions showed an increasing trend in PSII activity. Among various stressors, highest increase was recorded in the case of primed Aiswarya and Kanchana rice seedlings subjected to NaCl (P_s & P_{sl}+N) condition followed by UV-B (P_s & P_{sl}+U) and PEG (P_s & P_{sl}+P). However, on exposure of non-primed rice seedlings of both varieties (NP_s & NP_{sl}) to NaCl, UV-B and PEG stresses, PSII activity was reduced dramatically and the reduction was highest in Aiswarya exposed to PEG stress, followed by UV-B and NaCl stresses (Fig. 7).

4.3.1.1.3. Chlorophyll *a* fluorescence

Various Chl *a* fluorescence parameters were analyzed in the leaves of Aiswarya and Kanchana rice varieties to study the effect of priming and stress conditions on the PSII photochemistry. Pronounced changes were observed in the Chl *a* fluorescence parameters of both varieties compared to the control.

Primed (P_s & P_{sl}) and non-primed (NP_s & NP_{sl}) seedlings of both varieties subjected to stress conditions showed a reduction in plant vitality as assessed by performance index calculated on absorption basis [$PI_{(abs)}$]. The rate of decrease was more prominent in non-primed seedlings (NP_s & NP_{sl}) subjected to NaCl, PEG and UV-B stresses. Among these three stresses the reduction was highest in NP_s & NP_{sl}+P of both varieties and it was to the extent of 42-79%, of which Aiswarya NP_s+P showed maximum reduction. Although $PI_{(abs)}$ reduced in both varieties of primed seedlings (P_s & P_{sl}) subjected to stress conditions, the rate of decrease was moderate in primed seedlings of Kanchana rice variety (P_s & P_{sl}) subjected to NaCl, PEG and UV-B stresses and the reduction was in the range of 17-31%. However, in Aiswarya the reduction was much higher than Kanchana i.e. 34-55%.

In non-primed state of both varieties (NP_s & NP_{sl}) subjected to NaCl, PEG and UV-B stresses a noticeable enhancement of minimal fluorescence (F_o) was recorded and the percentage of increase was 21-55%. In the case of primed seedlings of Aiswarya and Kanchana (P_s & P_{sl}) subjected to different stress conditions also F_o was enhanced but comparatively lesser than non-primed seedlings i.e. 12-46%. A prominent reduction of maximum fluorescence (F_m) was recorded in non-primed seedlings of both varieties (NP_s & NP_{sl}) subjected to NaCl, PEG and UV-B stresses and the rate of reduction was 22-62%. Whereas in primed seedlings (P_s & P_{sl}) subjected to stress conditions the F_m was reduced but the reduction was not significant as that of non-primed stress condition in both varieties and it was in the range of 14-50%. Highest reduction was shown by Aiswarya P_s+P than primed seedlings exposed to other stress conditions. In primed seedlings (P_s & P_{sl}) not subjected to any stress conditions, the reduction in F_m was insignificant.

A significant increase in the relative variable fluorescence at J step (V_j) was noticed in primed seedlings (P_s & P_{sl}) subjected to stress conditions. A

remarkable increase of V_j was recorded in non-primed seedlings of Aiswarya rice variety (NP_s & NP_{sl}) subjected to NaCl, PEG and UV-B stresses (35-68%) than Kanchana (20-39%). Whereas, in primed seedlings of both varieties (P_s & P_{sl}) subjected to NaCl, PEG and UV-B stress conditions a moderate enhancement of V_j was noticed, except P_s & P_{sl}+P of Aiswarya, where in a higher enhancement (55 and 52%) was recorded. Negligible change in V_j was seen in primed seedlings (P_s & P_{sl}) but not subjected to any stress conditions.

The time taken to achieve maximum fluorescence value (T_{fm}) was noticeably enhanced in non-primed seedlings (NP_s & NP_{sl}) subjected to stress conditions. The enhancement was maximum in NP_s & NP_{sl}+P (38-54%) of both varieties. T_{fm} was also enhanced in primed seedlings of both varieties (P_s & P_{sl})subjected to stress conditions (18-40%) but the enhancement was not to the extent recorded in non-primed seedlings (NP_s & NP_{sl}) subjected to stress conditions. As compared to control, negligible variation of T_{fm} was observed in primed seedlings (P_s & P_{sl}) but not subjected to any stress conditions. Area over the fluorescence curve was significantly decreased in non-primed seedlings of both varieties (NP_s & NP_{sl}) subjected to different stresses and the rate of decrease was 20-47%. In primed seedlings ofboth varieties (P_s & P_{sl}) subjected to stresses the area was also declined to the extent of 13-40% than the control. Insignificant variation in area was noticed in primed seedlings of both varieties (P_s & P_{sl}) not exposedtoany stress conditions. The activity of the water-splitting complex on the donor side of PSII (F_v/F_o) was reduced in non-primed seedlings of both varieties (NP_s & NP_{sl}) subjected to NaCl, PEG and UV-B stresses than the control and it was to the extent of 20-54% with an exception NP_s+P, where drastic reduction was recorded (73%) than other stress conditions. Although F_v/F_o was reduced in primed seedlings of Aiswarya and Kanchana (P_s & P_{sl}) subjected to stress conditions, the reduction

was only ≤31% leaving alone Aiswarya P_s+P, which showed a radical reduction (64%) than control (Fig. 8, 9, 10, 11).

The energy pipeline leaf model of the photosynthetic apparatus was used to visualize the phenomenological energy fluxes per cross section of PSII in leaves of primed, non-primed seedlings of both varieties subjected to different stresses and without any stress condition. The density of active reaction centers (RC/CSm) was reduced in both varieties of seedlings emerged from primed and non-primed seeds (P_s & NP_s) subjected to NaCl, PEG and UV-B stress conditions and reduction was to the extent of 7-34%. Whereas in primed and non-primed seedlings of both varieties (P_{sl} & NP_{sl}) subjected to stress conditions the RC/CSm was enhanced by 27-59% than control. Primed (P_s & P_{sl}) and non-primed (NP_s & NP_{sl}) seedlings of both varieties subjected to stress conditions showed a similar pattern of reduction in specific energy fluxes for ABS/CSm and the reduction was 14-59%. Seedlings which were primed (P_s & P_{sl}) and not subjected to any stress conditions also showed a reduction in ABS/CSm than the control. Similarly, energy trapping per cross section (TR_o/CSm), electron transport per cross section (ET_o/CSm) and the ratio of total dissipation per cross section (DI_o/CSm) decreased in primed (P_s & P_{sl}) and non-primed (NP_s & NP_{sl}) seedlings of both varieties subjected to stress conditions. The decrease was in the range of 6-25% for TR_o/CSm, 4-21% for ETo/CSm and 3-22% for DIo/CSm in primed rice seedlings and 28-49% for TR_o/CSm, 25-50% for ETo/CSm and 32-60% for DIo/CSm in non-primed rice seedlings than the control. Except NP_s+U of Kanchana showed insignificant increases in DIo/CSm. In the case of primed seedlings of both varieties (P_s & P_{sl}) not subjected to any stress conditions TR_o/CSm, ET_o/CSm and DI_o/CSm were also reduced than control but the reduction was insignificant (Fig. 12, 13, 14, 15).

An alteration of PSII energy fluxes per reaction center (RC) of PSII in response to UV-B priming in both rice varieties (P_s & P_{sl}) subjected to stress conditions was visualized by specific membrane models of photosynthetic apparatus. It was found that the flux of absorption (ABS/RC), trapping per reaction center (TR_o/RC) of PSII, electron transport flux (ET_o/RC) and dissipated energy (DI_o/RC) was increased in primed (P_s & P_{sl})and non-primed (NP_s & NP_{sl}) seedlings of both varieties subjected to stress conditions and the extent of increases was up to 40% for ABS/RC, 50% for TR_o/RC, 42% for ET_o/RC and 30% for DI_o/RC than their control. These parameters were also enhanced in primed (P_s & P_{sl}) seedlings of both varieties not subjected to any stress conditions but to a lower extent (Fig. 16, 17, 18, 19).

4.3.1.2. Leaf gas exchange parameters

4.3.1.2.1. Net photosynthetic rate (*Pn*)

The leaf gas exchange parameters such as *Pn*, *gs* and *Ci* were significantly augmented in rice seedlings subjected to both modes of priming. The maximum *Pn* was recorded in primed seedlings i.e., 110 and 112% in Kanchana P_s & P_{sl} over the control. The primed seedlings of Aiswarya and Kanchana (P_s & P_{sl}) subjected to NaCl, UV-B and PEG stresses exhibited enhanced *Pn* and the rate of enhancement was higher in P_s & P_{sl}+N than P_s & P_{sl}+U and P_s & P_{sl}+P of Aiswarya and Kanchana. In the case of non-primed seedlings of both varieties (NP_s & NP_{sl}) subjected to three stress conditions, *Pn* was significantly reduced. Superlative reduction was observed in primed Aiswarya seedlings (P_s & P_{sl}) exposed to PEG stress i.e., 76 and 73% and in Kanchana seedlings the reduction was 70 and 69% in P_s & P_{sl} respectively as compared to control. In seedlings (NP_s & NP_{sl}) exposed to NaCl and UV-B stresses, the reduction of *Pn* was lesser than that observed in the case of PEG stress (Fig. 20).

4.3.1.2.2. Stomatal conductance (*gs*)

Stomatal conductance (*gs*) was significantly augmented in seedlings subjected to both modes of priming. The highest *gs* was noticed in primed Kanchana seedlings (P_{sl}), not exposed to any stress conditions i.e., 218% increase over the control. Aiswarya and Kanchana primed seedlings (P_s & P_{sl}) subjected to NaCl, UV-B and PEG stresses exhibited enhanced *gs* and the rate of enhancement was maximum in P_s & P_{sl}+N than P_s & P_{sl}+U and P_s & P_{sl}+P of both varieties. In the case of Aiswarya and Kanchana non-primed seedlings (NP_s & NP_{sl}) subjected to three stress conditions *gs* was significantly reduced. Drastic reduction was observed in Aiswarya and Kanchana primed seedlings (P_s & P_{sl}) subjected to PEG stress condition and the extent of reduction was 73 and 62% in Aiswarya and 61 and 60% in Kanchana seedlings respectively as compared to control. In the case of primed seedlings (P_s & P_{sl}) subjected to NaCl and UV-B stresses, the reduction of *gs* was negligible in comparison to PEG stress (Fig. 20).

4.3.1.2.3. Intercellular CO_2 concentration (*Ci*)

Intercellular CO_2concentration (*Ci*) was significantly increased in primed seedlings (P_s & P_{sl}). The maximum *Ci* was reported in primed Aiswarya and Kanchana seedlings (P_s & P_{sl}) and not exposed to any stress conditions i.e., 59% increases over the control. The primed seedlings of Aiswarya and Kanchana (P_s & P_{sl}) subjected to NaCl, UV-B and PEG stresses exhibited enhanced *Ci* and the rate of enhancement was higher in P_s & P_{sl}+N than P_s & P_{sl}+U and P_s & P_{sl}+P. In the case of Aiswarya and Kanchana non-primed seedlings (NP_s & NP_{sl}) subjected to three stress conditions, *Ci* was significantly reduced. Maximum reduction was observed in primed seedlings of both varieties (P_s& P_{sl}) exposed to PEG stress compared to control. In seedlings exposed to NaCl and UV-B stresses, the reduction of *Ci* was negligible in comparison to PEG stress (Fig. 20).

4.3.1.3. Mitochondrial activity

The mitochondrial activity was reduced in Aiswarya and Kanchana rice seedlings subjected to different stress conditions, whether primed or non-primed. While in the primed seedlings of Aiswarya and Kanchana (P_s & P_{sl}) and not subjected to any stress conditions, the mitochondrial activity was increased up to 17% as compared to control. In the case of non-primed seedlings of Aiswarya and Kanchana (NP_s & NP_{sl}) subjected to three different stress conditions, a major reduction of mitochondrial activity was recorded. Even though primed Aiswarya and Kanchana seedlings (P_s & P_{sl}), showed a decrease of mitochondrial activity in primed and stress state, it was very negligible (Fig. 21).

4.3.1.4. Leaf osmolality

Primed Aiswarya and Kanchana seedlings exposed to stress conditions showed an enhancement in leaf osmolality. Prominent increase in osmolality was seen in seedlings of P_s & P_{sl}+N condition in both varieties and it was in the range of 76-78%. Primed seedlings of both varieties subjected to PEG stress (P_s & P_{sl}+P) also showed enhanced osmolality to the extent of 55-61%. But in P_s & P_{sl}+U of Aiswarya and Kanchana, the osmolality was moderately enhanced than their control. Similar pattern of increased leaf osmolality but to a lower extend was observed in Aiswarya and Kanchana non-primed seedlings (NP_s & NP_{sl}) subjected to different stresses (NaCl, PEG and UV-B). Primed seedlingsof both varieties (P_s & P_{sl}) not exposed to any stress conditions showed increased osmolality but it was insignificant (Fig. 21).

4.3.2. Biochemical parameters

4.3.2.1. Primary metabolites

Different primary metabolites such as total protein, total soluble sugar and total free amino acids were analyzed in primed and non-primed Aiswarya

and Kanchana rice seedlings subjected to NaCl, PEG and UV-B stress conditions. These metabolite accumulations was enhanced significantly in UV-B primed rice seedlings subjected to three stress conditions. The increases were higher in UV-B primed Kanchana rice seedlings subjected to NaCl stress conditions than other stress conditions.

4.3.2.1.1. Total protein content

Protein content in UV-B primed rice seedlings of both rice varieties (P_s & P_{sl}) showed significant variations under the influence of three stresses. Compared with control, the protein content of primed rice seedlings exposed to NaCl (P_s & P_{sl}+N) was found to be higher by 151 and 159% in Kanchana and by 137 and 143% in Aiswarya rice varieties respectively. The protein content in primed seedlings of both varieties (P_s & P_{sl}) subjected to other stress conditions such as P_s & P_{sl}+U and P_s & P_{sl}+P recorded an increase of 102-147%. However, the protein content in non-primed seedlings (NP_s & NP_{sl}) subjected to stress conditions was reduced than primed seedlings exposed to stress conditions. But in comparison to the control the non-primed seedlings exposed to stress conditions had higher protein content. In non-primed seedlings of both varieties (NP_s & NP_{sl}) subjected to stress conditions lowest protein content was registered in seedlings exposed to PEG stress conditions. The seedlings of Aiswarya showed least increase in protein content i.e., 47% in NP_s+P and 55% in NP_{sl}+P than the control. In the case of seedlings of both varieties subjected to both priming (P_s & P_{sl}) modes and not exposed to any stress conditions showed a slight increases of protein content than the control (Fig. 22).

4.3.2.1.2. Total soluble sugar content

` A significant increase of sugar content was recorded in primed (P_s & P_{sl}) and non-primed (NP_s & NP_{sl}) rice seedlings grown under three different

stress conditions. The highest accumulation of this metabolite was observed in primed seedlings subjected to NaCl stress (P_s & P_{sl}+N of Kanchana and Aiswarya i.e., 321-328% increase), followed by P_s & P_{sl}+U and P_s & P_{sl}+P conditions of Aiswarya and Kanchana as compared to control. However, sugar content accumulation was also enhanced in non-primed seedlings of Aiswarya and Kanchana (NP_s & NP_{sl}) exposed to NaCl stress, followed by UV-B and PEG stresses but to a lesser extent than observed in the case of primed seedlings (P_s & P_{sl}) exposed to these stress conditions. In the case of primed seedlings (P_s & P_{sl}) not exposed to any stress conditions this metabolite accumulated negligibly as compared to the control (Fig. 23).

4.3.2.1.3. Total free amino acids

Total free amino acids content was enhanced significantly in primed seedlings of both Kanchana and Aiswarya (P_s & P_{sl}) under stress conditions. Highest rate of enhancement was recorded in Aiswarya P_{sl}+N (140%), which was followed by P_s+N (132%) and 130% of increase in P_s+N and 120% of increases in P_{sl}+N of Kanchana than the respective controls. 99-121% increase in total free amino acids content was recorded in P_s & P_{sl}+U and 83-101% increase in P_s & P_{sl}+P of both varieties. Likewise in non-primed seedlings exposed to stress conditions, total free amino acids content was enhanced in NP_s & NP_{sl}+N and NP_s & NP_{sl}+U of Kanchana and Aiswarya seedlings. Although the total free amino acids content was also increased in non-primed seedlings subjected to PEG stress (NP_s & NP_{sl}+P) but the increase was not prominent as that of other non-primed stress conditions. In P_s & P_{sl} of Kanchana and P_s of Aiswarya not subjected to any stress conditions the total free amino acids content was enhanced significantly, but the increase was insignificant in Aiswarya (P_{sl}) (Fig. 22).

4.3.2.1.4. Proline

A significant increase of proline content was recorded in primed (P_s& P_{sl}) and non-primed (NP_s & NP_{sl}) rice seedlings grown under three different stress conditions. The highest accumulation of this imino acid was observed in primed Aiswarya and Kanchana seedlings subjected to NaCl stress (104% in P_s & P_{sl}+N of Aiswarya and 94% in P_s & P_{sl}+N of Kanchana respectively) as compared to control. Although in primed seedlings of Aiswarya and Kanchana varieties (P_s & P_{sl}) subjected to other stresses (UV-B & PEG) conditions the accumulation rate of this metabolite was appreciably enhanced, the rate of enhancement was only about 69-84% than their control. However, proline content accumulation was enhanced in non-primed seedlings of Aiswarya and Kanchana (NP_s & NP_{sl}) subjected to all three stress conditions, but it was lower than the primed seedlings exposed to three different stresses. In the case of primed (P_s & P_{sl}) seedlings not exposed to any stress condition, this metabolite accumulated negligibly as compared to control (Fig. 23).

4.3.3. ROS production

ROS such as superoxide and hydrogen peroxide content was increased in non-primed rice seedlings subjected to three stresses. UV-B primed rice seedlings subjected to stress conditions showed enhanced ROS production but the enhancement was less than non-primed ones. However, UV-B primed rice seedlings not subjected to any stress conditions showed reduced ROS production than the control.

4.3.3.1. Superoxide content

Areduced accumulation of superoxide was noticed in primed seedlings of both varieties (P_s& P_{sl}) not subjected to any stress conditions as compared to control plants. On the other hand, in non-primed seedlings (NP_s) subjected to three different stresses the rate of superoxide accumulation was

tremendously increased. The highest increase of superoxide was observed in non-primed seedlings subjected to PEG stress (NP_s & NP_{sl}+P) i.e. 92 and 81% increase in Aiswarya and 70 and 59% increase in Kanchana respectively as compared with control. Similarly, in non-primed seedlings subjected to UV-B stress (NP_s & NP_{sl}+U), the superoxide content increased to 46-64% whereas in NaCl stress (NP_s & NP_{sl}+N) condition, the increase was found to be only 42-56% respectively. In primed seedlings (P_s & P_{sl}) subjected to stress conditions the accumulation of superoxide content increased as compared to control but the rate of increase was very less as compared to non-primed seedlings (NP_s & NP_{sl}) subjected to different stress conditions. The primed seedlings subjected to NaCl stress (P_s & P_{sl}+N) of Aiswarya and Kanchana showed minimal increase (≤23%) of superoxide as compared with other treatments (Fig. 24).

4.3.3.2. Hydrogen peroxide content

Exposure of non-primed Aiswarya and Kanchana rice seedlings (NP_s & NP_{sl}) to NaCl, UV-B and PEG stresses significantly increased the hydrogen peroxide content in them. The increase of hydrogen peroxide was maximum in non-primed seedlings of Aiswarya subjected to PEG stress i.e. 224% increase over the control. In non-primed seedlings of Aiswarya and Kanchana subjected to other stress conditions, the hydrogen peroxide content was increased in the range of 156-196%. However, the priming treatment of Aiswarya and Kanchana rice seedlings with UV-B (P_s & P_{sl}), significantly assuaged the damaging effects of all three stress conditions. Compared with control plant, the hydrogen peroxide content was increased in primed seedlings (P_s & P_{sl}) subjected to stress conditions but the increases was not as high as in non-primed seedlings (NP_s & NP_{sl}) subjected to stress. The least increment of hydrogen peroxide content (≤30%) was observed in UV-B primed seedlings of Aiswarya and Kanchana subjected to NaCl stress (P_s &

P_{sl}+N). Even the control plants had a noticeable level of hydrogen peroxide content and in primed Aiswarya and Kanchana rice seedlings (P_s & P_{sl}) not subjected to any stress conditions, the level of ROS species was reduced as compared to the control (Fig. 24).

4.3.4. ROS induced membrane damage

ROS induced membrane damages were analyzed by assessing the electrolyte leakage and membrane stability index. Electrolyte leakage was increased in non-primed stress conditions than UV-B primed rice seedlings subjected to stress condition. Reduction of membrane stability index was lesser in UV-B primed rice seedlings subjected to stress conditions and higher in non-primed rice seedlings subjected to stress conditions.

4.3.4.1. Electrolyte leakage (EL%)

In non-primed seedlings (NP_s & NP_{sl}) subjected to different stresses state, electrolyte leakage was significantly increased and that was highest in seedlings subjected to PEG stress condition i.e. 32% for Aiswarya and 36% for Kanchana respectively as compared to control. Compared with non-primed seedlings (NP_s & NP_{sl}) subjected to stress conditions, the electrolyte leakage was reduced in primed seedlings (P_s & P_{sl}) subjected to same stress conditions. The increment of electrolyte leakage was recorded least in primed seedlings of Aiswarya and Kanchana subjected to NaCl stress (P_s & P_{sl}+N). As compared with control, reduced electrolyte leakage was observed in primed seedlings (P_s & P_{sl}) not subjected to any stress conditions (Fig. 25).

4.3.4.2. Membrane stability index (MSI)

The three stress conditions were found to hamper the membrane stability in Aiswarya and Kanchana rice seedlings. Compared with control, the membrane stability index (MSI) in non-primed Aiswarya and Kanchana

seedlings (NP_s & NP_{sl}) subjected to NaCl, UV-B and PEG stresses were reduced in the range of 14-35% respectively. An insignificant reduction of MSI was observed in all primed seedlings of Aiswarya and Kanchana (P_s & P_{sl}) subjected to stress conditions as compared to control. However, in primed seedlings of Aiswarya and Kanchana (P_s & P_{sl}) not subjected any stress there was slight increase in MSI with respect to control (Fig. 25).

4.3.5. Free radical scavenging mechanism

Non-enzymatic antioxidants such as ascorbate, glutathione and total phenolics and enzymatic antioxidants such as SOD, CAT, APX, POD, GR, MDHAR and DHAR were analyzed to study the free radical scavenging process. The accumulation of non-enzymatic antioxidants and activities of enzymatic antioxidants were significantly enhanced in UV-B rice seedlings subjected to NaCl, PEG and UV-B stress conditions, of which it was highest in NaCl stress conditions.

4.3.5.1. Non-enzymatic antioxidants

4.3.5.1.1. Ascorbate

The UV-B primed (P_s & P_{sl}) and non-primed (NP_s & NP_{sl}) seedlings of Aiswarya and Kanchana rice varieties exposed to three different stress conditions, showed significantly enhanced ascorbate content. Ascorbate content was increased progressively up to 289 and 357% in Aiswarya and 202 and 224% in Kanchana, respectively in primed seedlings exposed to NaCl (P_s & P_{sl}+N). Likewise, in other primed seedlings (P_s & P_{sl}) subjected to other stress conditions also the ascorbate content got enhanced to the extent of 137-329% in P_s & P_{sl}+U and P_s & P_{sl}+P of Aiswarya and Kanchana as compared to control. However, in the case of non-primed seedlings of Aiswarya and Kanchana (NP_s & NP_{sl}) subjected to stress conditions, the ascorbate content was increased only to the extent of 112-259% in NaCl, 83-177 % in UV-B

and 65-111% in PEG stress, respectively. In primed seedlings of Aiswarya and Kanchana (P_s & P_{sl}) not subjected to any stress conditions the ascorbate content was enhanced up to 44-70% as compared to the control (Fig. 26).

4.3.5.1.2. Glutathione

In comparison with control, primed seedlings (P_s& P_{sl}) subjected to NaCl stress showed significant increase in glutathione content and it was to the extent of 485 and 461% in seedlings from primed seeds of Aiswarya and Kanchanaand 416 and 349% in directly primed seedlings of Aiswarya and Kanchanarespectively. In primed seedlings of Aiswarya and Kanchana subjected to other stress conditions (UV-B and PEG), this non-enzymatic antioxidant content was enhanced moderately but was lesser than that observed in P_s & P_{sl}+N condition. However, primed seedlings subjected to UV-B stress (P_s & P_{sl}+U) showed higher accumulation of this non-enzymatic antioxidant as compared to PEG stress (P_s & P_{sl}+P) condition in both varieties. In the case of non-primed Aiswarya and Kanchana seedlings (NP_s & NP_{sl}) subjected to NaCl stress, glutathione content was enhanced to the extent of 275 and 255% in NP_s+N and 257 and 221% in NP_{sl}+N of Aiswarya and Kanchana respectively and the accumulation of the glutathione was still lower in non-primed Aiswarya and Kanchana seedlings subjected to UV-B stress (NP_s & NP_{sl}+U), followed by PEG stress (NP_s & NP_{sl}+P). The increase in glutathione content in seedlings from primed seeds (P_s) subjected to different stress conditions was less than that observed in the case of Aiswarya and Kanchana seedlings directly primed with UV-B (P_{sl}). Even in the case of Aiswarya and Kanchana primed seedlings (P_s & P_{sl}) not subjected to any stress, a moderate increase in accumulation of glutathione was observed and it was not higher than 65% as compared with the control (Fig. 26).

4.3.5.1.3. Total phenolics content

Total phenolics content was increased in primed seedlings (P_s & P_{sl}) subjected to three different stresses. The effects of UV-B priming with respect to total phenolics accumulation was most effectively observed in primed seedlings subjected to NaCl stress (P_s & P_{sl}+N) and it was 288-350% higher in Aiswarya and Kanchana over the control. There was increased accumulation of total phenolics in non-primed seedlings of Aiswarya and Kanchana seedlings (NP_s & NP_{sl}) subjected to different stresses than the control but was at a reduced rate than in the primed seedlings (P_s & P_{sl}) subjected to stress conditions. The increase of total phenolics was least in seedlings which were non-primed and exposed to PEG stress (NP_s & NP_{sl}+P) and there was ~120% increase in Aiswarya and Kanchana respectively over the control. In the case of primedseedlings (P_s & P_{sl}) not exposed to any stress, negligible increase of total phenolics content was observed as compared to control (Fig. 26).

4.3.5.2. Enzymatic antioxidants

4.3.5.2.1. Superoxide dismutase (SOD, EC1.15.1.1)

The activity of SOD significantly enhanced in primed seedlings (P_s & P_{sl}) subjected to stress conditions than non-primed seedlings (NP_s & NP_{sl}) subjected to stress conditions. The activity of SOD was found to be highest in P_s & P_{sl}+N condition i.e., 825 and 819% increase in Aiswarya as well as 913 and 896% increase in Kanchana, over that of the control seedlings. Similarly, in the other primed stress conditions, SOD activity was significantly enhanced in Aiswarya (689 and 618%) and Kanchana (717 and 715%) exposed to UV-B stress (P_s & P_{sl}+U) and 501 and 500% increase in Aiswarya and 665 and 648% increase in Kanchana exposed to PEG stress (P_s & P_{sl}+P) respectively. In non-primed Aiswarya and Kanchanaseedlings (NP_s & NP_{sl}) under stress conditions the activity of SOD was also found to be increased in NaCl, UV-B

and PEG stresses but was at lower rate than the primed seedlings subjected to stress. However, in the primed seedlings (P_s & P_{sl}) not subjected to any stress, the activity of SOD was slightly increased with respect to the control seedlings (Fig. 27).

4.3.5.2.2. Catalase (CAT, EC1.11.1.6)

The primed seedlings (P_s & P_{sl}) subjected to NaCl, PEG and UV-B stresses resulted in enhanced activities of CAT. The higher activity of CAT was seen in P_s & P_{sl}+N state and the increase was 409 and 408% in Aiswarya and 415 and 380% in Kanchana respectively over the control. Non-primed seedlings subjected to PEG stress (NP_s & NP_{sl}+P) showed lowest increment of CAT activity (143 and 132% for Aiswaraya as well as 173 and 158% for Kanchana respectively) than control. Very less increment in CAT activity was observed in primed seedlings (P_s & P_{sl}) not exposed to any of the stresses as compared to the control (Fig. 27).

4.3.5.2.3. Ascorbate peroxidase (APX, EC 1.11.1.11)

Significant enhancement of APX activity was observed in primed seedlings (P_s & P_{sl}) subjected to stress conditions as compared to control. About 303-355% increase of APX activity was observed in P_s & P_{sl}+N condition of Aiswarya and Kanchana. Likewise, in P_s & P_{sl}+U, the activity of APX was enhanced to the extent of 253-302%. While in P_s & P_{sl}+P condition, 164-245% increase of APX activity was recorded. On the other hand, the increases of APX activity in primed seedlings (P_s & P_{sl}) not subjected to any stress condition were only ≤81%. However, in non-primed seedlings (NP_s & NP_{sl}) exposed to stress conditions, the APX activity enhanced in Aiswarya and Kanchana seedlings exposed to NaCl and it was in the range of 149-190% and in UV-B increase was 253-302% and in PEG increase was 70-121%, respectively as compared to the control seedlings (Fig. 27).

4.3.5.2.4. Guaiacol peroxidase (GPOX, EC1.11.1.7)

The activity of GPOX showed an increasing trend in primed seedlings of Aiswarya and Kanchana rice varieties (P_s & P_{sl}) subjected to stress conditions. Increased GPOX activity was observed in primed seedlings of Aiswarya and Kanchana rice seedlings (P_s & P_{sl}) subjected to stress conditions. Highest activity of GPOX was shown by Kanchana P_s+N (865%) and P_{sl}+N (835%) followed by Aiswarya P_s+N (788%) and P_{sl}+N (768%) than their respective controls. Similarly, enhanced activity of GPOX was also recorded in other primed stress conditions. There was enhanced GPOX activity in Kanchana P_s+U (773%) and P_{sl}+U (745%) as well as Aiswarya P_s+U (686%) and P_{sl}+U (667%) over their respective control. While in primed seedlings subjected to PEG stress, the GPOX activity was increased in Kanchana P_s+P (701%) and P_{sl}+P (671%) followed by Aiswarya P_s+P (601%) and P_{sl}+P (538%). In non-primed seedlings of both rice varieties (NP_s & NP_{sl}) subjected to stress conditions the GPOX activity was also enhanced but it was at a lower level than that observed in primed seedlings. Highest rate of GPOX activity was recorded in Kanchana and it was to the extent of 540, 504 and 401% in NP_s+N, NP_s+U and NP_s+P and 507, 461 and 369% of increase was recorded in NP_{sl}+N, NP_{sl}+U and NP_{sl}+P respectively. While in Aiswarya NP_s+N, NP_s+U and NP_s+P the increased rate was only 506, 438 and 356% and NP_{sl}+N, NP_{sl}+U and NP_{sl}+P was 499, 414 and 321% respectively. Even in primed seedlings of Aiswarya and Kanchana (P_s & P_{sl}), not exposed to any stress conditions the GPOX activity was found to be increased in the range of 104-145% than the control (Fig. 28).

4.3.5.2.5. Glutathione reductase (GR) (EC 1.6.4.2)

In Aiswarya and Kanchana primed seedlings (P_s & P_{sl}) subjected to stress conditions, GR activity was found to be upregulated. The highest GR activity was recorded in P_s+N (497%) and P_{sl}+N (461%) of Kanchana, followed by P_s+N (455%) and P_{sl}+N (435%) of Aiswarya respectively. In the case of primed seedlings of Aiswarya and Kanchana exposed to UV-B stress (P_s & P_{sl}+U) the activity of GR was increased in the range of 326-428%, and in P_s & P_{sl}+P of Aiswarya and Kanchana rate of increase was 276-376%. In non-primed seedlings of both rice varieties (NP_s & NP_{sl}) subjected to stress conditions, the GR activity was moderately enhanced. The moderate enhancement of GR activity was in the range of 125-282% in non-primed seedlings of Aiswarya and Kanchana (NP_s & NP_{sl}) subjected to NaCl, UV-B and PEG stresses. Least enhancement of GR activity was found in NP_s & NP_{sl}+P of Aiswarya which were 142 and 125% only. In the case of primed seedlings of Aiswarya and Kanchana (P_s & P_{sl}) without any stress, the rate of increase in GR activity was only in the range of 82-137% than their respective control (Fig. 29).

4.3.5.2.6. Monodehydroascorbate reductase activity (MDHAR, EC 1.6.5.4)

MDHAR activity was remarkably enhanced by the influence of UV-B priming in seeds/seedlings (P_s& P_{sl}) subjected to stress conditions. The highest MDHAR activity was recorded in P_s+N of Kanchana (466%) followed by Kanchana P_{sl}+N (455%), Aiswarya P_s+N (453%) and P_{sl}+N (426%) than the respective controls. In other primed stress conditions of Kanchana and Aiswarya, the increase in activity of MDHAR was comparatively lower and was in the range of 362-401% in P_s & P_{sl}+U and 288-338% in P_s & P_{sl}+P conditions. Whereas in non-primed seedlings of both varieties (NP_s & NP_{sl}) subjected to stress conditions the MDHAR activity was

enhanced only by a moderate level. In Kanchana and Aiswarya NP_s & NP_{sl}+N and NP_s & NP_{sl}+U condition the rate of enhancement in MDHAR activity was 145-223%. But in NP_s & NP_{sl}+P of both varieties only a slight increase of MDHAR activity was noticed. An insignificant increase in MDHAR activity was seen in primed seedlings of both varieties (P_s & P_{sl}) not subjected to any stress conditions (Fig. 29).

4.3.5.2.7. Dehydroascorbate reductase activity (DHAR, EC 1.8.5.1)

The activity of DHAR was significantly augmented in primed seedlings of both varieties (P_s & P_{sl}) subjected to stress conditions. The rate of enhancement of DHAR activity was higher in P_s & P_{sl}+N of both varieties, it was 502-524% higher than their control. In P_s & P_{sl}+U the rate of increase in DHAR activity was 461-494% and in P_s & P_{sl}+P the increase was 425-452% in both Kanchana and Aiswarya. However, in non-primed seedlings of Kanchana and Aiswarya (NP_s & NP_{sl}) the activity of DHAR was only reasonably enhanced. Only about 142-206% increase of activity was noticed in NP_s & NP_{sl}+N and NP_s & NP_{sl}+U of both varieties. But, only very less increase of DHAR activity was seen in NP_s & NP_{sl}+P of both varieties. Slight increase in activity of DHAR was recorded in both varieties of primed seedlings (P_s & P_{sl}) not exposed to any stress conditions (Fig. 29).

4.3.6. Gene expression analysis

Gene expression of major antioxidant enzymes such as SOD (*Cu/Zn SOD*), CAT **(***CatA***)** and APX (*APx1*) and stress responsive proteins like HSP (*HSP90*) and LEA (group 3) were increased. UV-B primed rice seedlings subjected to NaCl stress showed higher gene expression of antioxidant enzymes. *HSP90*was higher in UV- B primed rice seedlings subjected to UV-B.Group 3 *LEA* was higher in UV- B primed rice seedlings subjected to PEG stress.

4.3.6.1. Enzymatic antioxidants

4.3.6.1.1. Superoxide dismutase (*Cu/Zn SOD*)

In rice seedlings under the influence of both types of UV-B priming, a drastic increase in expression of *Cu/Zn SOD* was observed in both P_s & P_{sl}+N condition and it was to the tune of 99 and 114% in seedlings from primed seeds (P_s) of Aiswarya and Kanchana and 104 and 107% in primed seedlings (P_{sl}) of Aiswarya and Kanchana respectively over the control plant. In primed seedlings of Aiswarya and Kanchana subjected to other stress conditions like P_s & P_{sl}+U and P_s & P_{sl}+P, the expression of *Cu/Zn SOD* was found to be increased notably in seedlings subjected to both modes of priming but to a lesser extent than that recorded in P_s & P_{sl}+N. The augmentation in expression of *Cu/Zn SOD* in seedlings from Aiswarya and Kanchana was 77 and 101% for P_s+U and 84 and 86% for P_{sl}+U. In the case of P_s & P_{sl}+P, the increase in gene expression levels of enzyme was still lower. The gene expression levels in non-primed Aiswarya and Kanchana seedlings (NP_s & NP_{sl}) subjected to different stress conditions were also increased but at a reduced level than that of primed seedlings (P_s & P_{sl}) subjected to stress conditions. However, the seedlings subjected to both modes of priming (P_s & P_{sl}) and which was not subjected to any stresses showed slight increase in expression of *Cu/Zn SOD* and the increase was less than 27% (Fig. 30, 32, 33).

4.3.6.1.2. Catalase (*CatA*)

Expression of *CatA* was found to be significantly enhanced in primed seedlings (P_s& P_{sl}) subjected to stress conditions. Superlative increase in expression of *CatA* was noticed in Kanchana P_s+N (452%) than their control. In Kanchana P_{sl}+N, Aiswarya P_s & P_{sl}+N the rate of enhancement in expression of *CatA* was in the range of 371-390%. Similarly, in P_s & P_{sl}+U and P_s & P_{sl}+P of Kanchana, the expression of *CatA* was also enhanced in the range of 182-284%. In primed seedlings of Aiswarya subjected to UV-B (P_s & P_{sl}+U) and PEG (P_s & P_{sl}+P) stresses as well as the non-primed seedlings of

both varieties (NP_s & NP_{sl}) subjected to all three stresses, the expression of *CatA* was moderately increased. An insignificant enhancement in expression of *CatA* was noticed in primed Aiswarya and Kanchana rice seedlings (P_s & P_{sl}), not exposed to any stress conditions (Fig. 30, 32, 33).

4.3.6.1.3. Ascorbate peroxidase (*APx1*)

Significant enhancement in expression of *APx1* was noticed in primed seedlings (P_s& P_{sl}) subjected to stress conditions. The expression level was higher in P_s & P_{sl}+N condition of Aiswarya and Kanchana seedlings and the rate of enhancement was 404-443%. In primed seedlings of Aiswarya and Kanchana exposed to UV-B stress (P_s & P_{sl}+U) the expression of *APx1* was also augmented by 229-287% at the same time the increase was only 194-244% in P_s & P_{sl}+P. However in Aiswarya and Kanchana primed rice seedlings (P_s & P_{sl}) but not exposed to any stress conditions slight increase in expression of *APx1* was noticed. Whereas in non-primed seedlings of both varieties (NP_s & NP_{sl}) subjected to stress conditions the enhancement in expression of *APx1* was in the range of 92-230% (Fig. 30, 32, 33).

4.3.6.2. Stress responsive proteins

4.3.6.2.1. Heat Shock Proteins (*HSP90*)

Expression of *HSP90* was significantly enhanced in the case of stress responsive proteins such as HSP and LEA proteins in primed seedlings (P_s & P_{sl}) subjected to different stress conditions. Remarkable enhancement in expression of *HSP90* was recorded in UV-B primed Aiswarya and Kanchana seedlings subjected to UV-B stress (P_s & P_{sl}+U) (337-413%) and the increase in P_s & P_{sl}+N and P_s & P_{sl}+P was only 140-203%. Only moderate enhancement in expression of *HSP90* was noticed in non-primed seedlings subjected to NaCl (NP_s & NP_{sl}+N) and PEG (NP_s & NP_{sl}+P) stresses but in those exposed to UV-B stress (NP_s & NP_{sl}+U) an increase of 232-275% was recorded. An insignificant enhancement of expression *HSP90* was noted in

non-primed seedlings of both varieties (NP_s & NP_{sl}) subjected to stress conditions than the control (Fig. 31, 32, 33).

4.3.6.2.2. Late Embryogenesis Abundant proteins (LEA – group 3)

Expression of group 3 *LEA* has been significantly influenced in primed and non-primed seedlings of both Kanchana and Aiswarya varieties subjected to PEG stress (P_s & P_{sl}+P and P_s & P_{sl}+P) conditions. Higher level of expression of group 3 *LEA* was recorded in Aiswarya P_{sl}+P (532%) followed by Kanchana P_{sl}+P (530%), P_s+P (521%) and Aiswarya P_s+P (508%). In NP_s & NP_{sl}+P of both varieties the expression level of this gene was also enhanced and the enhancement was 392-432%. Although in other primed seedlings (P_s & P_{sl}) subjected to NaCl and UV-B stresses the expression of group 3 *LEA* was increased, the increase was not to the extent as observed in P_s & NP_s+P, and it was only 202-325% of increase in P_s & P_{sl}+N and P_s & P_{sl}+U of both rice varieties. While in NP_s & NP_{sl}+N and NP_s & NP_{sl}+U of both varieties a moderate level of increase in expression of group 3 *LEA* was noticed. An insignificant increase of expression of group 3 *LEA* was seen in Kanchana and Aiswarya primed seedlings (P_s & P_{sl}) not exposed to any stress conditions (Fig. 31, 32, 33).

4.3.7. UV-B specific compounds

UV-B specific compounds such as anthocyanin, flavonoids and cuticular wax content was higher in UV-B primed and non-primed rice seedlings subjected to UV-B stress than other stress conditions.

4.3.7.1. Anthocyanin content

The rice seedlings of both sensitive and tolerant varieties subjected to UV-B priming (P_s & P_{sl}) and exposed to different stress conditions, positively influenced the accumulation of anthocyanin content. A radical increase of accumulation in anthocyanin content was observed in P_s & P_{sl}+U of Aiswarya (383 and 367%) and Kanchana (386 and 384% respectively). However, in

other primed seedlings (P_s & P_{sl}) subjected to NaCl and PEG stresses, the anthocyanin content was moderately increased, i.e., 55-62% of increase in P_s & P_{sl}+P and 37-60% of increase in P_s & P_{sl}+N of Aiswarya and Kanchana respectively than their control. In primed seedlings of Aiswarya and Kanchana (P_s & P_{sl}) not subjected to any stress conditions, the accumulation of anthocyanin was only 27-42% more than the control. In the case of non-primed seedlings exposed to stress conditions, a drastic enhancement in anthocyanin accumulation was observed in both varieties subjected to UV-B stress (NP_s & NP_{sl}+U) only, which was to the extent of 255-284%. However, least increment of anthocyanin content was recorded in seedlings from non-primed seeds (NP_s) and seedlings (NP_{sl}) of both varieties subjected to NaCl and PEG stresses (Fig. 34).

4.3.7.2. Flavonoid content

Flavonoid content was found to be significantly enhanced in seedlings which were primed (P_s & P_{sl}) and subjected to stress conditions. A drastic increase in flavonoid content was noticed in P_s, P_{sl}, NP_s and NP_{sl} of Aiswarya and Kanchana subjected to UV-B stress. In P_s & P_{sl}+U of the variety Aiswarya, the increase in flavonoid content was 271 and 268%, whereas in Kanchana it was 342 and 337% respectively. Similarly, flavonoid content was enhanced upto 217% in NP_s & NP_{sl} of Aiswarya and Kanchana subjected to UV-B stress. Whereas, in the case of seedlings primed with UV-B (P_s & P_{sl}) and exposed to NaCl and PEG stress conditions, the flavonoid content was moderately enhanced. The enhancement of flavonoid content was in the range of 97-122% in P_s & P_{sl}+N and P_s & P_{sl}+P of Aiswarya and Kanchana. In non-primed seedlings of Aiswarya and Kanchana (NP_s & NP_{sl}) subjected to NaCl and PEG stresses, the flavonoid content was mildly increased and it was to the extent of 68-95% only. Similarly, in primed seedlings of Aiswarya and

Kanchana (P_s & P_{sl}) not subjected to any stress conditions, the flavonoid content was insignificantly increased than the respective controls (Fig. 34).

4.3.7.3. Phenylalanine ammonia lyase (PAL, EC 4.3.1.24)

PAL activity was significantly influenced by UV-B priming (P_s & P_{sl}) and stress conditions to which Aiswarya and Kanchana rice seedlings were exposed. The PAL activity was prominent in P_s, P_{sl}, NP_s and NP_{sl} exposed to UV-B stress and it was 210 and 116% higher in Kanchana P_s+U and NP_s+U and 202 and 118% higher in P_{sl}+U and NP_{sl}+U than their respective controls. Likewise, in Aiswarya the PAL activity was higher by 171 and 117% in P_s+U and NP_s+U and 164 and 115% higher in P_{sl}+U and NP_{sl}+U over the respective controls. However, in other UV-B primed seedlings (P_s & P_{sl}) subjected to NaCl and PEG stresses as well as non-primed seedlings exposed to stress conditions, the activity of PAL was moderately enhanced. In primed seedlings of Aiswarya and Kanchana (P_s & P_{sl}) subjected to NaCl and PEG stresses, the enhancement in PAL activity was to the extent of 74-107% only. Similarly in the non-primed seedlings of Aiswarya and Kanchana (NP_s & NP_{sl}) subjected to NaCl and PEG stress conditions, the PAL activity was increased by 39-63% only. However, in primed seedlings (P_s & P_{sl}) the PAL activity was slightly increased in both rice varieties even when not exposed to any of the stresses (Fig. 34).

4.3.7.4. Cuticular wax content and functional group analysis of the epicuticular wax deposition

Due to the effect of UV-B stress, epicuticular wax accumulation was higher in both rice varieties. In the case of primed seedlings (P_s & P_{sl}) exposed to UV-B stress, superlative increase in accumulation of epicuticular wax was seen in P_{sl}+U (402%) and 397% increase in P_s+U of Kanchana, 389% in P_{sl}+U and 383% in P_s+U of Aiswarya. In primed seedlings (P_s & P_{sl}) subjected to NaCl and PEG stresses, the rate of accumulation of epicuticular

wax content was moderately enhanced which was in the range of 176-242% in P_s & P_{sl}+N and P_s & P_{sl}+P of both rice varieties. Whereas in non-primed seedlings subjected to UV-B stress (NP_s & NP_{sl}+U), the epicuticular wax deposition was increased and the accumulation was to the extent of 301-354% in both varieties. In NP_s & NP_{sl}+N and NP_s & NP_{sl}+P, a moderate accumulation of epicuticular wax content (78-146%) was recorded. In UV-B primed seedlings (P_s & P_{sl}) but not exposed to any stress conditions, a slight increase of epicuticular wax accumulation was seen in both varieties as compared to control (Fig. 35).

In FT-IR spectra of the epicuticular wax in leaves of rice seedlings subjected to UV-B priming (P_s & P_{sl}) and exposed to different stress conditions, major and minor peaks representing various functional groups were observed. In the case of Aiswarya and Kanchana control, primed (P_s & P_{sl}) and non-primed (NP_s & NP_{sl}) ones subjected to three different stress conditions and without any stress conditions, the cuticular wax showed different absorption peaks at 850-550 cm^{-1} range. This was common in all including control seedlings and it represents C-Cl of alkyl halides (Fig. 38, 39; Table 5).

When compared with the control, additional absorption peaks were recorded mainly in seedlings of primed seedlings subjected to PEG and NaCl stresses (P_s & P_{sl}+P and P_s+N) and non-primed seedlings subjected to UV-B stress (NP_s & NP_{sl}+U). Four prominent peaks were seen in epicuticular wax from P_s+P and this was in the range of 3000-2850 cm^{-1} (specifically at 2914 cm^{-1}), 3300-2500 cm^{-1}range (specifically at 2848 cm^{-1}), 1250-1020 cm^{-1} range (specifically at 1099cm^{-1}) and in the range of 1000-650 cm^{-1} (specifically at 951cm^{-1}), representing C-H alkanes, O-H carboxylic acid, C-N aliphatic amines and C-H of alkenes respectively. Prominent peaks in the range of 3300-2500 cm^{-1} (specifically at 3017 cm^{-1}) and in the range of 1250-1020 cm^{-1} (specifically at 1214 and 1096 cm^{-1}) were observed in P_{sl}+P which stands for O-H carboxylic acids and C-N aliphatic amines. However, in seedlings of

Aiswaraya (P_{sl}+N) extra peaks were observed in the range of 3000-2850 cm^{-1} (specifically at 2956 and 2953 cm^{-1}), 1250-1020 cm^{-1} range (specifically at 1215 cm^{-1}) and in the range of 1000-650 cm^{-1}(specifically at 956 cm^{-1}), corresponding to C-H of alkanes, C-N of aliphatic amines and C-H alkenes (Fig. 38, 39; Table 5).

In P_{sl}+U extra absorption peaks were noticed in the range of 3300-2500 cm^{-1} (specifically at 3018 cm^{-1}) and in the range of 1250-1020 cm^{-1} (specifically at 1214 and 1097 cm^{-1}) which represents O-H of carboxylic acid and C-N of aliphatic amines respectively. Peak at the range of 1250-1020 cm^{-1} (specifically at 1214 and 1095 cm^{-1}) seen in NP_{sl}+U stands for C-N of aliphatic amines. In NP_s+U condition, two extra peaks at the range of 1250-1020 cm^{-1} (specifically at 1214 and 1095 cm^{-1}), which corresponds to C-N of aliphatic amines was observed. In the case of seedlings of Aiswarya emerging from UV-B primed seeds and not subjected to any stress conditions, four additional absorption peaks in the range of 3000-2850 cm^{-1} (specifically at 2953 and 2914 cm^{-1}), 3300-2500 cm^{-1} range (specifically at 2848 cm^{-1}) and the range of 1740-1720 cm^{-1}(specifically at 1733 cm^{-1}) representing C-H of alkanes, O-H of carboxylic acid and C=O of aldehyde saturated aliphatics were observed (Fig. 38, 39; Table 5).

In the variety Kanchana, extra peaks were recorded only in UV-B primed seedlings (P_s) without any stress and P_{sl}+P condition and the peaks were in the range of 3000-2850 cm^{-1} (specifically at 2954 and 2915 cm^{-1}), 3300-2500 cm^{-1} range (specifically at 2848 cm^{-1}), 1740-1720 cm^{-1} range (specifically at 1733 cm^{-1}) and at 1376 cm^{-1}, which corresponds to C-H alkanes, O-H carboxylic acid, C=O aldehyde saturated aliphatics and CH_3 of 4-methyl indole. Additional absorption peaks were observed in the range of 3300-2500 cm^{-1} (specifically at 3018 cm^{-1}) and 1250-1020 cm^{-1} (specifically at 1096 cm^{-1}) representing O-H carboxylic acid and C-N aliphatic amines when primed seedlings (P_{sl})was exposed to UV-B stress (Fig. 36, 37; Table 5).

Table 5: Functional group analysis of epicuticular wax in UV-B primed and non-primed rice seedlings subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(Ps & Psl)+N- Primed+NaCl; P(Ps & Psl)+P- Primed+PEG; P(Ps & Psl)+U- Primed+UV-B).

Treatments	Wavelength	Functional Group
Kanchana seed priming		
Control	1218.79, 772.35 cm^{-1}	Aliphatic amines, alkyl halides
P+C	2954.41, 2848.35, 1733.69,1376.93, 1214.93 cm^{-1}	Alkanes, aldehydes, 4-methyl indole, aliphatic amines
NaCl	742.46 cm^{-1}	Alkyl halides
P+N	742.46, 667.25 cm^{-1}	Alkyl halides
PEG	742.46 cm^{-1}	Alkyl halides
P+P	743.42,667.25 cm^{-1}	Alkyl halides
UV-B	743.42, 667.25 cm^{-1}	Alkyl halides
P+U	743.42 cm^{-1}	Alkyl halides
Kanchana seedling priming		
Control	744.36 cm^{-1}	Alkyl halides
P+C	743.42 cm^{-1}	Alkyl halides
NaCl	742.46 cm^{-1}	Alkyl halides
P+N	746.31 cm^{-1}	Alkyl halides
PEG	743.42,667.25 cm^{-1}	Alkyl halides
P+P	2952.48,2914.88, 2848.35, 1734.66, 1376.93 cm^{-1}	Alkanes, aldehyde, 4- methyl indole, alkyl halides
UV-B	743.42 cm^{-1}	Alkyl halides
P+U	3018.05,743.42,667.25 cm^{-1}	Carboxylic acids, Alkyl halides
Aiswarya seed priming		
Control	744.38,667.25 cm^{-1}	Alkyl halides
P+C	2953.45,2914.88,2848.35,1733.69,759.81 cm^{-1}	Alkanes, carboxylic acids, aldehyde, alkyl halides
NaCl	746.32 cm^{-1}	Alkyl halides
P+N	742.46 cm^{-1}	Alkyl halides
PEG	743.42 cm^{-1}	Alkyl halides
P+P	2914.88,2848.35,1099.23,951.69,845.63,763.67 cm^{-1}	Alkanes, carboxylic acids, aliphatic amines, alkenes, aldehyde Alkyl halides
UV-B	1214.93,1095.37,744.388,367.25 cm^{-1}	Aliphatic amines, alkyl halides
P+U	744.38,667.25 cm^{-1}	Alkyl halides
Aiswarya seedling priming		
Control	667.25 cm^{-1}	Alkyl halides
P+C	743.42 cm^{-1}	Alkyl halides
NaCl	750.17 cm^{-1}	Alkyl halides
P+N	2953.45,2926.45,1215.9,963.26,757.88 cm^{-1}	Alkanes, aliphatic amines, alkenes, alkyl halides
PEG	2909.09,750.17 cm^{-1}	Alkanes, alkyl halides
P+P	3017.09,1214.93,1096.33,744.38,667.25 cm^{-1}	Carboxylic acid, aliphatic amines, alkyl halides
UV-B	1214.93,1095.37,743.42,667.25 cm^{-1}	Aliphatic amines, alkyl halides
P+U	3018.05,1214.93,1097.3,746.31,667.25 cm^{-1}	Carboxylic acid, Aliphatic amines, alkyl halides

5. DISCUSSION

Plants encounter various environmental stresses in their life cycle which reduces the productivity of agricultural crops. The abiotic stresses such as harmful level of radiations, salinity, drought, floods, extremes in temperature, heavy metals, etc. are antagonistic to plant growth and development, which causes great crop yield penalty worldwide. It is becoming essential to equip crop plants with multi-stress tolerance to alleviate the pressure of environmental changes so as to achieve maximum productivity (He et al. 2018; Gull et al. 2019). Salinity is one of the major abiotic stresses which affect more than 7% of land area in the entire world, which is further increasing because of soil salinization by ground water. Salinity is the main obstacle to increase the production of rice throughout the world. Among the various stress condition, drought also constitutes severe stress on plants that seriously affects plant growth. Drought affects the rice production in rain fed systems, affecting 10 million hectares of upland rice and over 13 million hectares of rain fed lowland rice in Asia alone (Jisha 2014). Another major abiotic stress, UV-B radiation reaching the earth, has major deleterious effects on plants. The dose of UV-B radiation is relatively higher in rice grown areas which badly affects the production of rice (Thomas and Puthur 2019).

5.1. Determination of stress imparting concentrations of NaCl, PEG and dosage of UV-B

In the present study six varieties of *O. sativa* were used, which differed in their abiotic stress tolerance nature. The NaCl, PEG and UV-B stress tolerance potential of all these varieties were studied to identify a particular concentration/dosage which imparted ~50% growth retardation and it was found to vary for each variety. Seedling growth difference of rice varieties under each stress conditions depends on the tolerance potential of the

varieties. The selection of stress imparting concentration of NaCl, PEG and UV-B was done through the analysis of photosynthetic pigments and seedling growth (shoot length) of rice seedlings exposed to three different stresses. From six different concentrations of NaCl, PEG and six different dosages of UV-B, around 50% reduction in the above parameters was observed in some varieties of rice seedlings exposed to NaCl (100 mM), PEG (20%) and UV-B irradiation (28 $kJm^{-2}d^{-1}$). And in other varieties of rice seedlings 50% reduction occurred on exposure to NaCl (75 mM), PEG (15%) and UV-B irradiation (21 $kJm^{-2}d^{-1}$). Among six rice varieties stress imparting dosage/concentrations of three stress conditions were not the same because the stress tolerance potential of each variety was different. From the studies of Jisha and Puthur (2016b) and Sen and Puthur (2020), it was clear that the stress imparting concentrations of NaCl and PEG for three rice varieties such as Neeraja (75 mM NaCl and 15% PEG), Vaisakh (75 mM NaCl and 20% PEG) and Vyttila-6 (100 mM NaCl and 20% PEG) were different.

When these six rice varieties were grown in higher concentrations/ dosages of NaCl, PEG and UV-B stress conditions, seedlings showed reduction in shoot length. The reduction of shoot length was due to a hindrance of cell division as well as suppression of cell expansion and cell growth owing to the low turgor pressure (Jaleel et al. 2009; Taïbi et al. 2016). Moreover, plants under stress conditions are prone to growth reduction due to the altered level of plant hormones (Hasanuzzaman et al. 2013). Physiological and biochemical changes due to salinity can bring about a decrease in shoot length in maize (Shtereva et al. 2015; Konuşkan et al. 2017), legumes (Taïbi et al. 2016) and wheat (Kanwal et al. 2018). The reduction in vegetative growth was generally due to the effect of changed metabolic processes, restricted cell division and accumulation of different osmolytes and enhanced activities of metabolic enzymes that play a crucial role in salinity tolerance (Kanwal et al. 2018). UV-B stress reduces the shoot length in tomato plant

because of the reduced photosynthetic activity (Bano et al. 2017). Rice varieties such as Jyothi, Swetha and Kanchana showed 50% reduction in shoot length on exposure to 100 mM NaCl, 20% PEG and Neeraja, Swetha and Kanchana at 28 $kJm^{-2}d^{-1}$ of UV-B i.e. beyond these concentrations/dosage the shoot length reduced drastically. Rice sensitive varieties such as Aiswaraya, Samyuktha and Neeraja showed the same effect of 50% reduction in shoot length at lower concentrations/dosages and it was at 75 mM NaCl, 15% PEG and Aiswaraya, Samyuktha and Jyothi at 21 $kJm^{-2}d^{-1}$ of UV-B. In the present study Neeraja and Jyothi showed differences in the stress tolerance potential towards NaCl, PEG and UV-B. As the rice varieties respond to different environmental conditions differently and the tolerance mechanisms to individual stress factors are also different, it can be concluded that the different rice varieties elicits different response towards stress conditions. Earlier findings of Faseela and Puthur (2018), have shown that Kanchana rice variety have differentially modulated response towards UV-B and high light exposure, with more tolerance in UV-B and less tolerance in the high light and this supports the findings of the present study.

NaCl, PEG and UV-B stressors leads to the reduction of total chlorophyll content in all varieties of rice seedlings. The probable reasons for the reduction of chlorophyll content could be due to the inhibition of enzymes involved in chlorophyll biosynthetic pathways, and/or faster degradation of pigment molecules. Chlorophyll content was reduced in osmotic as well as other stressors as a result of inhibition of enzymes involved in chlorophyll synthesizing pathway (Jisha and Puthur 2014b). Under osmotic stress the chloroplast destruction and pigment protein complex instability were reported by El-Samad et al. (2011). The reduction of chlorophyll content was also due to the production of reactive oxygen species which lead to lipid peroxidation of membranes and consequently leads to chlorophyll destruction (Shivakrishna et al. 2018). The decrease in chlorophyll levels in plants was

indicated as a typical symptom of oxidative stress and was attributed to the inhibition of chlorophyll synthesis, together with the activation of its degradation by the enzyme chlorophyllase. Reduction of chlorophyll contents either due to slow synthesis or fast breakdown could be a part of photoprotection mechanism by which light absorbance was reduced by decreasing chlorophyll contents (Taïbi et al. 2016). The reduction of chlorophyll content in salinity stress condition was either due to the accumulation of Cl^- and Na^+ or due to the changes in the lipid protein ratio of pigment-protein complexes, increased chlorophyllase activity, and degradation and inhibition in synthesis of photosynthetic pigments (Khoshbakht et al. 2018). The reduction of chlorophyll content in wheat plants under salinity could be also attributed to the decreased nitrogen uptake of plants (Kanwal et al. 2018).

Drought stress induced chlorophyll content reduction has been reported in maize (Mohammadkhani and Heidari 2007), chickpea (Mafakheri et al. 2010), soybean (Makbul et al. 2011), wheat (Talebi 2011), rice (Chutia and Borah, 2012) and bean (Mathobo et al. 2017). In peanut plant PEG stress causes a reduction in total chlorophyll and carotenoid content. Drought stress causes the production of reactive oxygen species (ROS) such as O_2^- and H_2O_2, which consequently results in chlorophyll destruction (Mathobo et al. 2017). The UV-B radiation causes the reduction of chlorophyll in lettuce (Esringu et al. 2016), bell pepper (León-Chan et al. 2017), wheat (Tripathi et al. 2017) and rice (Faseela and Puthur 2018). UV-B radiations directly cause damage to the photosystems and light harvesting systems in plants and to add to this, UV-B induced ROS generation may also lead to the chlorophyll degradation.

Carotenoids act as antioxidants; have the potential to detoxify the cells from the effects of reactive oxygen species. Carotenoids are known to function as accessory pigment harvesting light energy for photosynthesis and

also as quenchers of triplet chlorophyll and oxygen. Moreover, they dissipate excess energy via the xanthophyll cycle and can act as powerful chloroplast membrane stabilizers that gets partitioned between light-harvesting complexes (LHCs) and the lipid phase of thylakoid membranes, reducing membrane fluidity and susceptibility to lipid peroxidation (Taïbi et al. 2016). Carotenoids also act as photoprotector of chlorophyll molecules by neutralizing singlet oxygen ($^1O_2$) produced in photosystems (León-Chan et al. 2017). Salinity induces a reduction in carotenoid content in bean plants (Taïbi et al. 2016). Lettuce seedlings exposed with UV-B radiations exhibited a significant reduction in carotenoid content (Esringu et al. 2016). Similarly, NaCl, PEG and UV-B stressors negatively influenced the photosynthetic pigments in Jyothi, Swetha and Kanchana rice varieties and 50% reduction of the pigments in these varieties occurred at100 mM NaCl, 20% PEG and in Neeraja, Swetha and Kanchana at 28 $kJm^{-2}d^{-1}$ UV-B. In the case of Aiswaraya, Samyuktha and Neeraja 50% reduction of photosynthetic pigments occurred on exposure to 75 mM NaCl, 15% PEG and in Aiswaraya, Samyuktha and Jyothi at 21 $kJm^{-2}d^{-1}$ UV-B.

Rice varieties showed 40-50% of growth retardation when grown in 100 mM NaCl and 20% PEG for tolerant varieties and 75 mM NaCl and 15% PEG for sensitive varieties on 9^{th} day of germination (Jisha and Puthur 2014a). According to Faseela and Puthur (2018), UV-B tolerant rice variety 'Kanchana' showed tolerance towards UV-B at a dosage of 28 $kJm^{-2}d^{-1}$ on 9^{th} day of germination. From the analysis of shoot length and photosynthetic pigments in rice varieties, it was clear that the varieties Swetha and Kanchana showed more tolerance potential than Aiswarya and Samyuktha. Neeraja was sensitive towards NaCl and PEG stressors but tolerant towards UV-B stress. Similarly the variety Jyothi showed tolerance towards NaCl and PEG stressors but was sensitive towards UV-B stressor. For further studies four

varieties were selected, of which Kanchana and Swetha were designated as tolerant varieties and Aiswarya and Samyuktha as sensitive varieties.

5.2. Preliminary screening for selecting UV-B priming concentration

5.2.1. Growth parameters

Growth reduction occurred in the seedlings of rice which were subjected to NaCl, PEG and UV-B stressors but interestingly, due to the influence of UV-B seed priming treatments, this reduction in growth characters such as shoot length and dry weight of UV-B primed seedlings were lesser than non-primed ones. The reduction of shoot length in all the above mentioned cases was due to the inhibition of cell division and also due to inhibition in cell expansion and cell growth because of low turgor pressure (Dash et al. 2017). Plants under stress conditions trigger the physiological and biochemical features which will finally enable them to cope up with the stress situations but with reduced growth.

Four rice varieties such as Kanchana, Swetha, Samyuktha and Aiswarya responded differentially to the UV-B seed priming. Generally, the seedlings raised from UV-B primed seeds of tolerant rice variety showed more enhancements in the seedling growth parameters when the seedlings were grown under stress (NaCl, PEG and UV-B) conditions. In UV-B primed rice seedlings, increased respiration rate and higher ATP production could be supporting the growth of seedlings under NaCl, PEG and UV-B stresses (*detailed in section 5.3.1.2*.). Comparing among four rice varieties, maximum growth parameters was noticed in UV-B primed Kanchana rice seedlings subjected to NaCl, PEG and UV-B stress conditions and lowest in Aiswarya rice seedlings. This indicated that Kanchana rice variety was more tolerant and Aiswarya variety was more sensitive towards NaCl, PEG and UV-B stressors.

The increase in growth parameters of the rice seedlings emerged from UV-B primed seeds was the result of increased cell division, cell elongation, cell expansion and increase of the seedling vigour. Seed priming decreases the resistance of the endosperm envelope, which is an impediment for the expansive growth of the embryo (Luttus et al. 2016). This condition permits the turgor threshold for germination to be attained at a faster rate in primed seeds than in non-primed seeds, thereby helping the seedlings from primed seeds to achieve greater root and shoot length. The enhanced shoot and root length in plants emerged from primed seeds also owes to the alterations in germination events such as reducing the lag time between imbibition and radical emergence (Jisha 2014). All these events support UV-B priming mediated increase in shoot length and dry weight in rice seedlings under different stresses.

Various researchers have already reported various positive effects of seed priming in stress tolerance potential of different crops. Salinity stress imparted to tomato plant reduce the dry weight, shoot length as compared to control plant but on subjecting the tomato seeds to NaCl/osmo priming the dry weight/shoot length of tomato plant was increased as compared to that without priming (Zhang et al. 2012; İşeri et al. 2014). Similarly in alfalfa the shoot length and dry weight was reduced during salinity stress but NaCl priming, osmopriming and hydro-priming of seeds leads to the increases of shoot length and dry weight (Amooaghaie 2011).

Positive effects of salicylic acid priming was seen on increased shoot growth and dry weight in maize (ur Rehman et al. 2012), in rice (Pouramir-Dashtmian et al. 2014) and in safflower plant (Mohammadi et al. 2017). Similar effects were noticed in this study, the negative effects of stress condition were partially mitigated with UV-B seed priming treatments in four rice varieties. The shoot length and dry weight were increased in seedlings

emerged from UV-B primed Kanchana followed by Swetha, Samyuktha and Aiswarya under NaCl, PEG and UV-B stress conditions. The increased shoot length and dry weight of Kanchana and Swetha seedlings was observed at a dosage of UV-B 6kJm^{-2} and for Samyuktha and Aiswarya at 4kJm^{-2}. Beyond these priming concentrations the shoot length and dry weight was reduced probably because higher dosages acted as stress.

5.2.2. Photosynthesis

As compared to control condition, the photosynthetic pigments were increased in UV-B primed rice seedlings under stress conditions. The priming of seeds with low dose of UV-B would have accelerated the synthesis processes of photosynthetic pigments. Similar effects of UV-B seed priming was found in wheat (Li et al. 2010), maize (Wang et al. 2010) and rice (Inostroza-Blancheteau et al. 2016), wherein it was observed that the UV-B seed priming enhances the leaf chlorophyll content in these crops. Total chlorophyll and carotenoids was increased in leaves of wheat emerged from seeds primed with spermine and further subjected to drought stress condition (Hassan et al. 2020). Total chlorophyll and carotenoid contents were decreased in leaves of salt treated tomato plants but it was enhanced by NaCl priming (İşeri et al. 2014). Silicon priming of seedlings increases total chlorophyll and carotenoids in wheat under drought stress condition (Maghsoudi et al. 2016).

Seed priming has a role in the synthesis of chlorophyll pigments by triggering some signalling pathways like that of cytokinins. The cytokinins are already known to enhance the photosynthetic efficiency of plants under adverse conditions by triggering signalling pathways (Jisha 2014). Similarly, the UV-B seed priming significantly improved the activation of carotenogenesis and the resulting carotenoids synthesized have the capacity to neutralize the reactive oxygen species and thus, protect the photosynthetic

apparatus and the pigments associated with it from oxidative reactions. Carotenoids act as photoprotectors which protect the chlorophyll molecules from singlet oxygen and excess energy harvested by chlorophylls (Badridze et al. 2016). The enhancement of carotenoid content in the rice seedlings emerged from UV-B primed seeds, subjected to stress conditions facilitate the seedlings to cope with the adverse effects of stresses to which they were subjected. Protective role of carotenoids was already well defined in terms of quenching triplet chlorophyll and reducing the peroxidation of chloroplast membrane.

Among all the varieties of rice, maximum enhancement of chlorophyll pigments and carotenoid content due to UV-B seed priming treatments was observed in Kanchana rice seedlings which were grown under stress (NaCl, PEG and UV-B) conditions and least in Aiswarya. This indicated that the UV-B seed priming effects in Kanchana rice seedlings was more prominent and effective under stress conditions. It is clear that, the UV-B seed priming enhances the photosynthetic pigment content in three stress conditions as compared to non-primed seedlings and it could be partially on account of the reduced chlorophyll degradation seen in the former as compared to later and also uninterrupted fresh synthesis of photosynthetic pigments would be ensuring the reduced loss of pigments in leaves of seedlings emerging from UV-B primed seeds.

Of the four varieties the effective UV-B seed priming dosage was found to vary depending upon the stress tolerance potential of the rice varieties. In Kanchana and Swetha, which was found to be with higher stress tolerance potential towards three different stressors was found to accumulate high level of pigment content when imparted with a dosage of $6kJm^{-2}$. The highest increases of photosynthetic pigment as a result of UV-B seed priming in Aiswarya and Samyuktha occurred on priming with a lower dose of $4kJm^{-2}$.

5.2.3. Lipid peroxidation

Primed rice seedlings raised from UV-B primed seeds exhibited reduced rate of lipid peroxidation, especially under stress conditions. The MDA produced from the peroxidation of unsaturated fatty acids in phospholipids indicated cell damage by free radicals under stress condition. The lipid peroxidation disturbs different metabolic activities by altering physiochemical properties of cell membranes via interruption of lipid bilayers which cause leakage of solutes leading to cell death. MDA act as an indicator of stress in plants and it changes the cell membrane properties, such as fluidity, ion transport and enzyme activity (Faseela and Puthur 2018). Researchers have reported that the lipid peroxidation was significantly reduced in maize (Javadmanesh et al. 2012), in sorgum (Zhang et al. 2015), in rice (Zheng et al. 2016) and in alfalfa (Mouradi et al. 2016) under drought conditions as a result of seed priming (hydro-priming, KNO_3 and PEG priming). Seed priming with UV-B reduced lipid peroxidation and electrolyte leakage (*detailed in section 5.3.4.*) and thus enhanced the stability of the cell membrane, resulting in decreased stress effects.

In the present study, rice seedlings on exposure to NaCl, PEG and UV-B stressors showed increased MDA content in the seedlings, because these stressors cause lipid peroxidation of biomembranes in the rice seedlings and UV-B seed priming decreases the MDA content in the seedlings of all rice varieties exposed to stress conditions. One of the major reasons for the reduction of MDA content in primed seedlings subjected to stress condition was the decreased ROS production by the effect of UV-B seed priming (*detailed in section 5.3.4.*). Moreover, UV-B seed priming effectively enhances the antioxidation potential in primed rice seedlings than non-primed rice seedlings subjected to stress conditions. This was achieved by an effective antioxidant mechanism, scavenging the ROS and thus reducing the

MDA content in UV-B primed rice seedlings exposed to stress conditions (*detailed in section 5.3.4.*). Another reason for the reduction of MDA content achieved through seed priming would be by initiating the repair of the damaged membranes and ensuring uninterrupted functioning of the metabolic processes (Vijayakumari 2015). In primed seedlings subjected to stress conditions, the MDA content was reduced in tolerant rice varieties such as Kanchana and Swetha at 6 kJm^{-2} and in sensitive varieties such as Samyuktha and Aiswarya it was at 4 kJm^{-2}.

5.3. Detailed analysis of most tolerant and sensitive varieties towards NaCl, PEG and UV-B

After screening of four rice varieties, (Kanchana, Swetha, Samyuktha and Aiswarya), Kanchana (tolerant variety) and Aiswarya (sensitive variety) was selected for further detailed analysis. Based on the above screening analysis, UV-B priming dosage of tolerant variety was fixed as 6 kJm^{-2} and for sensitive variety as 4 kJm^{-2}. The same dosage of UV-B was provided for directly priming the seedlings, so that the priming dosage was same for priming at two different stages of growth. For stress sensitive variety the UV-B priming dose as well as stress dosage of UV-B, concentration of NaCl and PEG imparted as stress was less than Kanchana rice variety. The stress dosage of UV-B for Aiswarya was 21 $kJm^{-2}d^{-1}$ and for Kanchana it was 28 $kJm^{-2}d^{-1}$. In the case of NaCl stress concentration, it was 75 mM for Aiswarya and 100 mM for Kanchana, PEG stress was imparted at 15% for Aiswarya and 20% for Kanchana. This makes it very clear that the priming dosage of UV-B varies with the stress tolerance potential of the variety. Even the stress imparting dosage/concentrations of various stressors (UV-B, NaCl and PEG) varies depending upon the stress tolerance potential of the variety. As priming dosage of UV-B varies for each variety of rice, standardization studies to identify the optimal dosage becomes more important because anything more

than the optimal can turn out to be stress and anything less would not bring about the priming effects.

5.3.1. Physiological parameters

5.3.1.1. Photosynthesis

In rice seedlings, the decrease of photosynthesis was analyzed in terms of PSI and PSII activities and significant changes in various Chl *a* fluorescence related parameters during NaCl, PEG and UV-B stresses. The reduction of these parameters indicated that a reduction in photochemistry occurred in seedlings subjected to these stresses. The effects of these stresses in rice seedlings was noticed as reduction in photosynthetic oxygen evolution and performance index and this was the whole some of the decreases in various other photosynthetic parameters **(**total chlorophyll and carotenoid content, area above fluorescence curve, maximal fluorescence [F_m], ratio between variable fluorescence to minimal fluorescence [F_v/F_o], density of reaction centres, light energy absorbed, rates of light energy trapped and electron transport per cross-section**)** or increases in certain other parameters **(**like minimal fluorescence [F_o], variable fluorescence intensity at the J_step (V_j), time to reach the maximum fluorescence (T_{fm}) and the light energy dissipated (DI) directly related to photosynthesis.

Various abiotic stresses may cause a serious damage to photosynthetic machinery, of which PSII is the most vulnerable component that bears the major brunt of abiotic stresses. Over produced ROS damages the photosynthetic apparatus especially PSII which results in photoinhibition by an imbalance in photosynthetic redox status and the inhibition of PSII repair (Sasi et al. 2018). The degradation of chlorophyll content, decrease in chlorophyll synthesis and partial breakdown of thylakoid membrane causes the reduction of PSII activity during stress conditions in rice seedlings. In rice

seedlings subjected to NaCl, PEG and UV-B stressors, the activity of PSII was more reduced because the reduction of oxygen evolution in PSII was related with the extensive accumulation of ROS in thylakoid membranes, which hinders the oxygen evolution at the oxygen evolving complex (OEC) of PSII. The excessive peroxidation and de-esterification of thylakoid membrane lipids was caused by ROS. ROS production also leads to the protein denaturation and disrupt the functionality of various proteins of thylakoid membrane system and enzymes essential for photosynthetic machinery (Gill and Tuteja 2010). Under stress conditions lipid peroxidation was increased which radically reduces the photosynthetic pigment content (*detailed in section 5.2.3*.). Plants have various stress countering measures to prevent the damages of photosynthetic machinery during stress conditions and it comprises of early closing of stomata, increased synthesis of carotenoids and prevention of chlorophyll degradation etc. Compared to PSII activity the stress effects on PSI activity was less in rice seedlings, due to the capacity of PSI to withstand the stress more effectively (Thomas and Puthur 2019).

In previous studies, it was shown that the NaCl treatment inactivates the PSII centers in wheat and results in decline of PSII activities (Singh-Tomar et al. 2012). In plants under UV-B stress condition, PSII was more prone to degradation due to the vulnerability of D1 and D2 proteins, wherein changes occur in between the double bonds of amino acids. Moreover, the oxygen evolving complex, reaction centre proteins D1/D2 and various components in the acceptor and donor side of PSII were mainly affected by UV-B. The enzymatic activity of the water-splitting manganese complex on the PSII electron donor side was inhibited partially or completely under strong light which causes incomplete oxidation of water to hydrogen peroxide. ROS generation suppress the synthesis of proteins which are involved in the repair mechanism of photodamaged PSII. Plants under drought stress had enhanced production of superoxides on acceptor side of

PSI, which finally reduced the activity of PSI (Oukarroum et al. 2009). But in contrast PSI activity was highly increased in rice seedlings under UV-B stress, the increased PSI activity was related to the increased demand for ATP through cyclic photophosphorylation so as to cope up with UV-B stress condition (Faseela and Puthur 2018).

In UV-B primed rice seedlings exposed to NaCl, PEG and UV-B stress conditions, the reduction of PSI and PSII activities were comparatively lesser than non-primed stress condition. This was due to the lesser oxidative damages in chloroplasts of UV-B primed leaves which diminishes chlorophyll degradation and the ROS induced injury on thylakoid membranes and other protein complexes related to photosystem functioning. In UV-B primed Aiswarya and Kanchana rice seedlings subjected to stress conditions, the PSI and PSII activities were higher in Kanchana rice seedlings. As a result of seed priming the PSI and PSII activities were significantly enhanced by the virtue of increase in number of photosystem reaction centers and/or by the enhancement of efficiency of existing reaction centers (Jisha and Puthur 2016a,b). The PSI activity was more efficiently enhanced due to UV-B priming treatment, so that the ATP generated out of the cyclic phosphorylation would aid the seedlings to overcome the stress condition. Plants activate the cyclic electron flow in order to generate additional ATP required for sufficing energy requirement for stress related metabolism (Roach and Krieger-Liszkay 2014).

In this study, lesser reduction of PSI and PSII activities was recorded in Aiswaraya and Kanchana rice seedlings emerged from seeds subjected to UV-B priming and exposed to three stress conditions, because of the efficient activity of antioxidant machineries, which reduces the photodamages of reaction centers.

Rice seedlings encountered with NaCl, PEG and UV-B stresses showed a decrease of Chl *a* fluorescence parameters such as F_m, F_v/F_o, area, $PI_{(abs)}$, ABS/CSm, TR_o/CSm, DI_o/CSm and ET/CSm. The decreased F_m was due to the inhibition of electron transfer rates from reaction centers to quinone, resulting in excess of excitation energy which gets dissipated as heat (Faseela et al. 2019). In dry bean the F_o was increased under drought stress condition, because of PSII inactivation and the resulting increase in reduced plastoquinone acceptor, which was unable to be oxidized completely because of the electron flow retardation through PSII (Mathobo et al. 2017). F_o increases may also be due to the detachment of light harvesting complex from PSII. Increase of F_o and decreases of F_m indicate a block in the electron transport to Q_A (Hassannejad and Ghafarbi 2018). In general F_o increased and F_m decreased in plants under environmental stresses reflects the destruction of PSII reaction centers, or disruption of electron transport (Zhu et al. 2010).

Decreased F_v/F_o and increased minimum fluorescence (F_o) were observed due to enhanced UV-B intensities in grapevine (Schoedl et al. 2013) and in cucumber (Skórska 2011; Skórska and Murkowski 2012). The increase of F_o points towards the photoinhibition correlating RCs damage at the acceptor side of PSII. Decrease of the F_v/F_o parameter can result from increase of F_o and concomitant decrease of F_m. Exposure to NaCl, PEG and UV-B stresses causes a severe damage to PSII through the primary inactivation of oxygen-evolving complex (OEC) activity inhibiting the electron transfer at the donor side. The F_v/F_o ratio suggests the efficiency of the water-splitting complex on the donor side of PSII and photosynthetic electron transport at acceptor side of PSII, which was decreased in these three stress conditions. The decrease in F_v/F_o also suggests a decrease in the ratio between the rate constants of photochemical and non-photochemical deactivation of excited Chl molecules.

The area over the fluorescence induction curve (between F_o and F_m) is relative to the pool size of the electron acceptor Q_A on the reducing side of PSII and considerable decreases in area denoted the decrease in electron transfer from reaction center to quinone pool (Mehta et al. 2010). So the reduction in F_m, F_v/F_o and area above fluorescence curve in rice seedlings subjected to stress was either owing to the inhibition of electron transport at the donor side of the PSII or owing to a reduction in the pool size of Q_A. In UV-B primed rice seedlings, the decrease in F_v/F_o and area above fluorescence curve caused by NaCl, PEG and UV-B stressors was significantly lesser. This indicates that UV-B priming positively regulated the inhibition of electron transport at donor side and/or acceptor side of PSII in rice seedlings subjected to NaCl, PEG and UV-B stresses.

Under UV-B stress conditions F_m was decreased which was accompanied by an enhancement of relative variable fluorescence intensity at the J-step (V_j). The increased V_j denoted the increases in the proportion of closed PSII RCs and the proportion of reduced Q_A at J step. V_j increases in salt stressed canola plant because of the reduction of total Q_A and the lower value of reoxidation of the Q_A (Jafarinia and Shariati 2012). The decreased F_m indicated an increase in the proportion of the closed PSII RCs, not participating in electron transport. In UV-B primed rice seedlings, the increases of V_j and decreases of F_m caused by NaCl, PEG and UV-B stressors was significantly lesser. This was due to the increases of active reaction centres seen in PSII, which was successively enhances the photosynthetic efficiency in primed rice seedlings.

Performance index [$PI_{(abs)}$] decreased and the functional antenna size (ABS/RC) increased during UV-B stress condition. These results indicate that even though the absorption per RC increased it could not turn out into effective photochemistry. The increased F_o in rice seedlings under UV-B

stress was due to the decreased efficiency of energy transfer from the antenna chlorophyll *a* to the RCs and/or the inactivation of PSII RCs. A significant increase of ABS/RC, ET_o/RC, TR_o/RC and DI_o/RC was seen in UV-B stress conditions. The increases in DI_o/RC recommend that PSII RCs are transformed into dissipative sinks for excitation energy under UV-B stress. ET_o/RC is related to the reoxidation of reduced Q_A via electron transport in an active RC (Pan et al. 2011). The increased ET_o/RC is the representation of just a single reaction centre. Therefore, it need not indicate overall enhancement in electron transport because major proportion of RC's are turned to be inactive.

$PI_{(abs)}$ reveals the three steps of photosynthetic activity which occur in PSII RC, such as absorption and trapping of excitation energy and transfer of it through electron transport and thus the $PI_{(abs)}$ is regarded as a multi-parametric expression for monitoring and evaluating overall photosynthesis (Strasser et al. 2000). In Aiswarya and Kanchana rice leaves under NaCl, PEG and UV-B stressors significant decrease occurs in $PI_{(abs)}$ on absorption basis which can be linked to the plant vitality. The reduction is due to inactive reaction centers and reduction in the electron transfer from Q_A^-. Reduction in $PI_{(abs)}$ act as a major indicator of drought and salt stresses. In wheat plants the $PI_{(abs)}$ was decreased due to drought and salt stresses. These stresses may causes stomatal closure which eventually leads to reduction in dark (biochemical) reaction of photosynthesis and initiates photoinhibition and photodamage of light reaction centres and membranes (Maswada et al. 2020). $PI_{(abs)}$ was decreased in sesame plant under drought stress (Boureima et al. 2012). The time of the induction curve to reach the maximum fluorescence (T_{fm}) was increased in lettuce under drought stress (Cocetta et al. 2016). While in the case of UV-B primed rice seedlings subjected to stress conditions, the reduction of $PI_{(abs)}$ and increase of T_{fm} was lesser because of the increased efficiency of active reaction centres. Similar to this result $PI_{(abs)}$ decreased and T_{fm} increased in mung bean under salt stress but the salicylic

acid priming altered the stress effect in mung bean by enhancing $PI_{(abs)}$ and decreasing T_{fm} (Ghassemi-Golezani and Lotfi 2015).

Chl *a* fluorescence parameters revealed that seedlings of Aiswarya and Kanchana varieties subjected to stress conditions had negative impact on the photochemistry similar to that of photoinhibition. It has been already explained that photoinhibition caused by unfavourable conditions activates photoprotection mechanisms in plants. In such a situation the cyclic electron flow (CEF) between photosystems can be activated by stress and UV-B priming treatment can lead to proper reduction and oxidation of the PSI acceptor side. The stress conditions also activate other photoprotection mechanisms like non-photochemical quenching (NPQ, xanthophylls cycle) and water-water cycle (WWC) (Maswada et al. 2020). The activation of photoprotection mechanisms can be elevated in rice seedlings subjected to UV-B priming treatment.

The UV-B seed priming significantly increased the distribution of reaction centers in a unit area and also enhanced the activity of existing reaction centers in the leaves of the rice seedlings. The increased count of open reaction centers enhanced the efficiency of absorption, trapping, and transport of electrons per cross-section. But in stress condition the trapped energy was highly dissipated due to the increase in number of closed reaction centers. On the other hand, the UV-B priming maximized the potential of reaction centers by increased trapping and utilization of energy in seedlings subjected to stress condition and thus reducing the dissipation energy. Similar positive effects of seed priming with increased count of active RCs and with increased efficiency of RCs were reported in rice (Li and Zhang 2012) and *Vigna radiata* (Jisha and Puthur 2015).

The leaf model of Chl *a* fluorescence related parameters revealed that under normal condition (without any stress and any priming), the density of

active reaction centers was higher in the tolerant variety than in the sensitive variety. The density of active reaction centers was reduced by the closing of various active reaction centers during NaCl, PEG and UV-B stresses in both rice varieties. During stress condition reduction in RC/CS ratio reflects that few of the efficient RCs are converted into inactive RCs and inactivation or 'silencing' of RCs is a protective mechanism in plants during stress conditions to prevent the photooxidative damage in thylakoids (Strasser et al. 2000). The density of inactive RCs in rice leaves was relatively lesser in UV-B primed rice seedlings exposed to NaCl, PEG and UV-B stresses. In supportive to this it was found that just with UV-B priming the density of active/open reaction centers were high as compared to control.

In energy 'leaf model' which demonstrated phenomenological energy fluxes, the ABS/CSm denoted the total absorption of light energy by total number of RC of an excited cross-section of leaf and TR_o/CSm was related to the total energy trapped by the whole RCs of a leaf cross-section. In energy membrane model that portray specific energy fluxes, the ABS/RC established the total absorption of light energy by PSII antenna chlorophyll molecules of a single RC and TR_o/RC was related to the maximal trapped energy by a RC causing the reduction of Q_A.

The specific activities per reaction centre (RC); ABS/RC, ET_o/RC, DI_o/RC and TR_o/RC were increased and phenomenological fluxes per cross section (CSm); ABS/CSm, TR_o/CSm, DI_o/CSm and ET_o/CSm were decreased respectively in rice under drought stress condition (Wang et al. 2016). The phenomenological energy fluxes of absorption and trapping per leaf cross-section [(ABS/CSm) and (TR_o/CSm) respectively] decreased during NaCl, PEG and UV-B stresses in rice seedlings. While the energy fluxes of absorption and trapping per reaction centers [(ABS/RC) and (TR_o/RC)] increased during NaCl, PEG and UV-B stresses in rice seedlings. Because of

the closing of most of the RCs in rice seedlings density of active RCs in an excited cross-section of rice leaves decreases which was reflected in reduced total absorption and trapping flux per cross-section of leaves. Since the total numbers of active RCs were fewer in excited leaf area, each of the active RCs exhibited increased rate of absorption and trapping that resulted in the increase of ABS/RC and TR_o/RC. The inactivation of some RCs reduces the total number of active RCs and the functional antenna size of remaining active RCs was augmented causing an increase in ABS/RC and TR_o/RC (Gururani et al. 2015). Wheat leaves under salt stress condition, the flux ratios ABS/RC and TR_o/RC were found to be increased and it may be due to inactivation of few active RCs (Mehta et al. 2010; Mathur et al. 2011).

Due to inefficiency in energy trapping some of the absorbed energy was non-utilized which resulted in the increase of dissipated energy per CSm and dissipated energy per RC ((DI_o/CS and DI_o/RC, respectively) in rice leaves under NaCl, PEG and UV-B stresses. ET_o/RC reduced in rice seedlings under NaCl, PEG and UV-B stresses, which in turn inhibited the re-oxidation of reduced Q_A^- via electron transport in an active RC owing to damages at donor/acceptor side of PS II. Enhancement in the TR_o/RC and reduction in the ET_o/RC in rice seedlings during NaCl, PEG and UV-B stresses denoted that the trapped energy was not utilized efficiently for electron transport. OEC of PSII was damaged owing to ROS attack, reducing the flow of electrons to the RCs of PSII and thus declining the concentration of oxidized Q_A molecules which was the major reason for reduced electron transport in reaction centers during stress conditions (Mathur et al. 2011).

In UV-B primed rice seedlings, the stress-induced increase of ABS/RC, TR_o/RC and DI_o/RC were relatively lesser. The reduction of ABS/RC, TR_o/RC and DI_o/RC in plants due to the regulation of the light captured by RCs is a means of tolerance in stress tolerant cultivars (Strasser et

al. 2004). The stress-induced reduction in energy fluxes of ABS/CSm, TR_o/CSm and DI_o/CSm were relatively lesser in UV-B primed rice seedlings and this shows efficient protection of photosynthetic apparatus in primed states of rice seedlings on encountering with NaCl, PEG and UV-B stresses. Due to the effect of UV-B priming, the stress-induced reduction of ET_o/RC and ET_o/CSm was reduced in both rice varieties and this resulted in enhanced photochemical quenching, which reflected in relatively higher performance index [$PI_{(abs)}$] in primed rice seedlings when compared to non-primed rice seedlings.

The priming-induced responses in UV-B primed rice seedlings shows regulation at different steps of photosynthesis which was established through different fluorescence and phenomenological energy flux linked parameters measured in leaves of rice seedlings. The reduction in stress-induced absorption and trapping energy fluxes and significantly enhanced electron transport per excited cross-section in leaves of primed rice seedlings clearly denoted that under NaCl, PEG and UV-B stresses, UV-B primed seedlings utilize photosynthetic energy more effectively and with minor negative impacts on photosynthetic performance.

The photosynthetic efficiency in crop plants is highly influenced by different stresses. Although *Pn*, *gs* and *Ci* was reduced in seedlings subjected to three stress conditions, the major reduction was observed in seedlings subjected to PEG stress. The highly reduced *gs* influence the *Pn* and *Ci* and accordingly both gets reduced (Vincent et al. 2015; Wang et al. 2016). The reduction of stomatal aperture and increased stomatal resistance results in lowered *gs* and lowering of CO_2 levels inside leaf tissues. Salinity may cause the closure of stomata, thus decreasing the partial CO_2 pressure and *C*i and consequently resulting in a decreased *Pn* (Khoshbakht et al. 2018). Not only stomatal factors but non-stomatal factors also cause the reduction in

photosynthetic rates. Reduction of photosynthetic rates due to stomatal closure has been reported in bean under drought stress (Mathobo et al. 2017). The decrease in *Ci* points out the dominance of stomatal limitations for effective photosynthesis, with intensification of drought stress in plants (Mathobo et al. 2017). Moreover, the reduction in *gs* prevents CO_2 from entering the leaf and thus favours photorespiration (Mathobo et al. 2017). Although net photosynthesis rate (*Pn*), transpiration rate (*E*) and stomatal conductance (*gs*) were significantly decreased under drought conditions, these parameters were increased in wheat plants subjected to silicon priming (Maghsoudi et al. 2016). As a result of UV-B priming *Pn*, *gs* and *Ci* were increased in rice seedlings confirming that UV-B priming could reduce the inhibition on stomatal/non-stomatal factors imposed due to stresses. In Aiswarya and Kanchana rice seedlings subjected to both modes of priming, the stomatal conductance was optimized to ensure ambient intercellular CO_2 concentrations as well as the photosynthetic rate. UV-B priming effectively enhances the photosynthesis and accelerates the PSII photochemistry (*detailed in above paragraphs of this section*) in rice seedlings subjected to stresses. The same priming also could increase stomatal conductance, enhancing the gaseous exchange in seedlings which ultimately results in enhanced carbon fixation in UV-B primed rice seedlings subjected to NaCl, PEG and UV-B stresses.

5.3.1.2. Respiratory activity

Normally abiotic stresses are known to trigger mitochondrial functions in terms of enhanced respiratory electron transport. According to Atkin and Macherel (2009), mitochondria have a role in maintaining energy and redox balance of the cell. In the present study, the mitochondrial activity was reduced significantly in rice seedlings subjected to NaCl, PEG and UV-B stresses. The production of ROS (*detailed in section 5.3.4.*) damages the

membranes and mitochondrial proteins and thus the mitochondrial activity gets reduced in non-primed rice seedlings subjected to NaCl, PEG and UV-B stresses. Mitochondria are important sites of ROS production so the mitochondrial membranes are easily prone to ROS attack (Sharma et al. 2012).

During stress condition, stomatal closure takes place which narrows down the CO_2 entry finally resulting in the reduction of photosynthate synthesis. This affects the rate of mitochondrial activity due to decreased availability of respiratory substrate. The derailing of respiratory process will result in the over production of ROS in mitochondria causing disturbance in electron transport activities and ultimately leads to lipid peroxidation (Chen et al. 2009). Such a condition was observed in stress condition and reduction of mitochondrial activity was maximum in seedlings subjected to PEG stress. UV-B priming enhances the mitochondrial activity by maintaining the intactness of the membrane through the reduction of lipid peroxidation (*detailed in section 5.2.3.*) and increased rate of mitochondrial development. There are two types of mitochondrial developments, such as repair and reactivation of pre-existing mitochondria as well as biogenesis of new ones (Chen and Arora, 2013). Priming induces efficient development of mitochondria and thus facilitates energy metabolism, essential for high stress tolerance potential.

UV-B priming of rice varieties decreases the impact of NaCl, PEG and UV-B stresses on mitochondrial activity; therefore rate of mitochondrial oxygen consumption was enhanced as compared to non-primed seedlings at stress conditions. The enhanced production of ascorbate content (*detailed in section 5.3.4.*) in primed seedlings also denoted the relatively enhanced efficiency of mitochondrial functioning in withstanding the NaCl, PEG and UV-B stresses. Normal mitochondrial functioning is important for cellular

antioxidant defence, because the final step of ascorbate synthesis takes place in inner mitochondrial membrane (Bartoli et al. 2000). Moreover, in priming, mitochondria were protected by the accumulated LEA and HSP. Also the repair and reactivation of mitochondria and biogenesis of new ones contributes towards enhanced activity. Also the maintenance of integrity of inner and outer membranes of mitochondria was retained by priming (Chen and Arrora 2013).

UV-B priming effectively enhances the antioxidant mechanism so as to scavenge ROS and thereby reduces the lipid peroxidation in rice seedlings. The low availability of redox equivalents in mitochondria under stress conditions causes the decrease of mitochondrial functioning in non-primed rice seedlings. In the case of primed seedlings the enhanced photosynthetic efficiency (*detailed in section 5.3.1.1*.) assured the availability of redox equivalents resulting in enhanced mitochondrial activity. Different seed priming treatments (hydro, halo, chemical priming) augments the mitochondrial functions which was earlier reported in pea (Benamar et al. 2003) and *Vigna* and rice (Jisha 2014; Jisha and Puthur, 2014a, 2014b, 2015,).

The UV-B priming efficiently enhances the ATP production for accomplishing the energy requirements of post-germination stages especially during stress conditions. This is being ascertained by the work of Chen and Arora (2013), wherein they established that the priming had the potential for up-regulating the genes of key enzymes involved in glycolysis, Krebs cycle and anaerobic respiration (Chen and Arora 2013). In response to stress conditions, the mitochondria act as a key hub for the signalling to other parts of the cell (Huang et al. 2016).

5.3.2. Leaf osmolality

Increase in osmolality may be due to the water loss in plants and simultaneous synthesis of osmo-solutes (Müller et al. 2012). Increased leaf osmolality resulted in better osmotic adjustment (Abdolahpour and Lotfi 2014). Osmolality takes a major role in the inward and outward movement of water from plant cells (Mohammed and Ibrahim 2017). The biophysical properties of the cell plasma membrane are affected by high osmolality not accompanied with water intake. This can induce cell shrinking, causing a decrease in cell membrane tension. Osmolality was enhanced by drought stress condition in various crop plants such as rape seed (Müller et al. 2012) and rice (Li et al. 2015). Salinity also enhances the osmolality in wheat (Cuin et al. 2010), barley (Vysotskaya et al. 2010), pea (Pandolfi et al. 2012), quinoa (Shabala et al. 2013), barley and wheat (Puniran-Hartley et al. 2014), maize (Kaya et al. 2015) and rice (Mohammed and Ibrahim 2017). Osmolality increases by an increase in the solutes content and/or by decrease in the amount of water uptake into the cell (Fathali et al. 2017). In mustard plant the osmolality was increased due to the increased exposure to UV-B (Pandey et al. 2012). To overcome the UV-B stress conditions different osmolytes were accumulated in plant cells that result in the enhancement of the leaf osmolality.

Various researchers have reported the influential role of priming in bringing about osmotic adjustment in different crops. Different priming techniques such as hydro, KNO_3 and silicon seed priming enhance the osmolality in rice (Ming et al. 2012), chickpea (Abdolahpour and Lotfi 2014) and wheat (Azeem et al. 2015) respectively under salt stress. Hydro, halo, osmo and solid matrix priming significantly influence the osmolality in okra plant (Sharma et al. 2014). Similarly UV-B priming plays a major role in increasing the leaf osmolality in both varieties under NaCl, PEG and UV-B

stress conditions. Such a situation paves way for the increased uptake of water and hydration of the cell.

When the stressors PEG and NaCl were provided the accumulation of osmo-solute was at high level as compared to seedlings exposed to UV-B stress. It is a well-known fact that both PEG and NaCl impart osmotic stress and to counter this situation plants synthesize osmo-solutes to a higher extent which will result in increase of leaf osmolality (Mohammed and Ibrahim 2017). Osmolality is generally built up by the accumulation of various osmo-solutes comprising of various soluble sugars and amino acids like proline. UV-B priming induces the synthesis of these metabolites, which may have primarily the task in antioxidative function (*detailed in section 5.3.3*.) and the accumulation of these osmo-solutes will simultaneously add up to the leaf osmolality.

5.3.3. Primary metabolites

Total proteins, sugars, amino acids and proline content were significantly enhanced in primed rice varieties under stress conditions. Sugars, amino acids and proteins act as compatible osmolytes in plants. The primed rice seedlings subjected to NaCl, PEG and UV-B stresses showed increased proteins content contributed by increased accumulation of some stress responsive proteins such as LEA and HSP (*detailed in section 5.3.5*.) and also the proteins/enzymes involved in photosynthetic reactions, mitochondrial respiration and synthesis of secondary metabolites in defence reactions. Protein content was increased in rice under NaCl stress up to 100 mM NaCl concentration (Hakim et al. 2014). In rice under stress condition several osmo-responsive proteins were accumulated to mitigate the stress effects. The accumulated proteins under stress conditions may act as a storage form of nitrogen which gets re-utilized in post-stress recovery and also has a key role in osmotic adjustment (Sunita et al. 2011; Parida and Jha 2013). The

proteins, amino acids and sugars act as a osmolytes under stress condition, which protect the cellular macromolecules and also protects subcellular structures by regulating cellular osmotic potential and/or by scavenging reactive oxygen species (ROS) (Deivanai et al. 2011; García-Morales et al. 2014). Multiple functions of proteins involve osmotic pressure regulation, protection of membrane integrity, stabilization of enzymes/proteins, retaining appropriate $NADP^+$/NADPH ratios and scavenging free radicals (Khoshbakht et al. 2018).

UV-B priming in rice seedlings showed an increased accumulation of protein content than non-primed seedlings. Because of UV-B priming, the accumulation of inactive defence metabolite-conjugates occurs and these precursors undergo a secondary post translational level modification when sensing the stresses; which assist the faster action/response of primed plants during the stress with minor wastage of metabolic energy (Pastor et al. 2013). This may cause the increased accumulation of proteins in UV-B primed rice seedlings subjected to NaCl, PEG and UV-B stresses. The enhanced content of proteins in UV-B primed seedlings subjected to stresses denoted that the priming can influence the protein composition in the cells of rice seedlings by enhancing the absorption of growth nutrients for protein synthesis, signalling the post translational modification of inactive proteins and reducing the rate of ROS stimulated damages to proteins and protein synthesizing machinery. UV-B priming enhances the protein accumulation and reduces the rate of protein degradation probably by the action of accumulated HSP in rice seedlings.

Seed priming with spermine increases the protein content in wheat under drought stress condition (Hassan et al. 2020). Total protein content was also enhanced in rice varieties under NaCl and PEG stress conditions on BABA priming. Additional protein synthesis may occur in plants under

abiotic stress condition to mitigate the stress effects. These additional proteins synthesized include heat-shock proteins, molecular chaperones and late embryogenesis abundant proteins, which have the potential to induce tolerance in plants (Jisha and Puthur 2016b). Protein regulates the osmolytic action and osmotic pressure (García et al. 2012). BABA seed priming enhanced the accumulation of total protein content in *Vigna radiata* under NaCl and PEG stress condition. Increased protein content in primed plants subjected to stress indicated the accumulation of stress proteins and transcription factors specifically involved in synthesis of proteins, having role in stress tolerance (Jisha and Puthur 2016a,b). UV-B priming of Aiswarya and Kanchana subjected to stresses prominently enhances the total protein content because of the enhanced production of stress responsive proteins, proteins involved in photosynthesis reactions and mitochondrial respiration.

Sugars have a role in carbon storage, osmoprotection, osmotic homeostasis and free radicals scavenging. Sugar accumulation also activates the antioxidant enzymes such as SOD, APX and CAT (Sami et al. 2016). In different stress conditions sugars contribute towards osmotic adjustment, protection to membranes and ROS scavenging (Keunen et al. 2013; Singh et al. 2015). In plants, sugars and phenolic compounds interaction had a role in integrated redox systems, ROS scavenging and increasing tolerance against various stresses (Keunen et al. 2013). The synergistic interaction of sugars and phenolic compounds act as an integrated redox system in plants, scavenging of ROS, enhancing the tolerance mechanisms against stress (Faseela and Puthur 2018). In line with this study, previous studies have also reported that seed priming significantly activated the total soluble sugar content in rice seedlings (Nawaz et al. 2013; Jisha and Puthur 2014a; Zheng et al. 2016). UV-B primed rice seedlings subjected to stress accumulated more sugar content than non-primed seedlings subjected to stresses. The increase of sugar accumulation in rice seedlings subjected to stresses was due to the

degradation of starch. Total soluble sugar content was increased significantly as a result of starch degradation in black pepper plants under non-primed PEG stress condition (Vijayakumari 2015). The increased content of sugar was due to the breakdown of larger carbohydrate molecules that retain the turgidity of the cell (Kanwal et al. 2018). UV-B priming may directly or indirectly involve in sugar metabolism of rice seedlings. UV-B priming would activate the signalling molecules regulating the gene expression and metabolism of various stress related molecules for the faster and timely accumulation of sugars to encounter the stresses.

Accumulation of amino acids is an important feature in plants enabling to overcome environmental stresses. Amino acids also act as compatible osmolytes and reduce the osmotic potential of the cell so that turgor pressure and turgor-related processes may be maintained during abiotic stress conditions (Gomes et al. 2010). The increased accumulation of amino acids protects the cellular macromolecules maintaining the osmotic balance and scavenges the free radicals (Paul and Roychoudhury 2016). Increased concentration of proline and amino acids maintains stabilization and osmoregulation of proteins and other macromolecules (Sneha et al. 2013). Moreover amino acids are considered as a source of nitrogen in plants and are major components for the process of protein synthesis. Still another major role of amino acids is their participation in the biosynthesis of a large variety of non-protein nitrogenous materials, i.e. pigments, vitamins, co-enzymes, purine and pyrimidine bases (Sadak and Abdelhamid 2015). Another major role of amino acids is to regulate ion transport and stomatal opening, and affect the synthesis and activity of enzymes, gene expression, redox homeostasis and also help to tolerate harmful effects of osmotic stress (Kovács et al. 2012). During stress conditions the amino acids biosynthesis was increased which are not only acting as building blocks of proteins but also for the other metabolic activities (Ali et al. 2019).

Free amino acids and proline are compatible solutes which get increased in *Panicum sumatrense* subjected to drought stress (Ajithkumar and Panneerselvam 2014). The increased accumulation of amino acids and proline plays a vital role in osmotic balance of plants imparting tolerance towards salinity and drought stress (Azooz et al. 2013; Ajithkumar and Panneerselvam 2014). Various seed priming treatments have been found to enhance the amino acids content in different crop plants. Zn priming increases the free amino acids and total soluble sugars in rice under salt stress (Ashraf et al. 2014). Seed priming with spermine enhances the amino acids and proline accumulation in rice under salt stress (Paul and Roychoudhury 2016). In present study, the amino acids accumulation was increased as a result of UV-B priming of rice seedlings subjected to different stresses and this could aid in osmotic adjustment. UV-B priming induced higher accumulations of amino acids could be mainly from the increased biosynthesis of various amino acids under stress condition and not due to the protein degradation. Because it was observed that the protein content was increased in UV-B primed rice seedlings exposed to stress conditions. This enhanced amino acids accumulation contributes towards protein biosynthesis and also acts as an osmolyte as well as nitrogen and carbon reserve.

The proline content increased in UV-B primed and non-primed rice seedlings subjected to various stress conditions. The proline accumulation was enhanced by the synthesis of proline from glutamic acids and/or preventing the proline oxidation to other compounds (Salama et al. 2011). Proline has strong potential for free radical scavenging mechanism in plants under stress condition. Accumulation of proline is a well-known strategy adopted by plants to cope with drought or salinity stresses. It also takes a key role in protecting the sub-cellular structures and arbitrating osmotic adjustment in stressed conditions. In addition, it plays an adaptive role like protection of cellular functions by scavenging ROS, acting as storage form of

carbon to supply energy required during recovery from stress and also acts as a signal molecule regulating reproductive development (Chunthaburee et al. 2016). Moreover, under osmotic stress conditions proline act as osmolyte maintaining membrane stability and protein machinery (Pandey and Shukla 2015; Hussain et al. 2016).

Various studies reported that the seed priming enhances the proline content in different plants subjected to different stresses (Jisha and Puthur 2014 a, b, 2016). In earlier studies, the proline content was enhanced by UV priming of seeds in *Vigna mungo* (Das and Roychoudhury 2014) and wheat (Badridze et al. 2015, 2016). Higher accumulation of proline was reported in rape seeds during osmopriming and post-priming germination (Kubala et al. 2015). In this study the UV-B priming enhances the proline accumulation in Aiswarya and Kanchana rice seedlings, but more enhancement was noticed in seedlings emerged from seed priming, especially in tolerant Kanchana rice variety. UV-B priming induced proline accumulation takes a role in mitigation of stress effects in rice seedlings. Increased accumulation of proline act as an osmoregulator as well as a free radicals scavenger in rice seedlings. ROS scavenging capacity of proline getting accumulated in UV-B primed seedlings reduces the lipid peroxidation under stress conditions. Thereby proline maintains membrane stability and protein synthesis machinery in UV-B primed seedlings.

Various metabolites discussed above gets highly accumulated in plants in response to stress tolerance/adaptive mechanism. UV-B priming significantly enhances the total proteins, sugars amino acids and proline content so as to cope up with various stresses. Higher accumulation of these metabolites was noted in UV-B primed rice seedlings than non-primed seedlings exposed to three different stresses. Similar, to this observation, enhanced accumulation of total proteins, sugar, amino acids and proline was

reported in seedlings of various plants as a result of different seed priming techniques (Nouman et al. 2012; Espanany et al. 2016; Khaliq et al. 2015; Zhang et al. 2015).

5.3.4. Oxidative stress and antioxidative mechanism

Reactive oxygen species are partially reduced or activated forms of oxygen. The increased concentration of ROS may cause oxidative damage to membranes (lipid peroxidation), proteins, RNA and DNA molecules (Choudhury et al. 2017; McGrann and Brown 2018). ROS production is a common process in plant metabolism under normal physiological condition. The accumulation of ROS in different sites of plant cell (viz chloroplast, mitochondria, plasma membrane, peroxisomes, apoplast, endoplasmic reticulum and cell wall) increases during NaCl, PEG and UV-B stresses. Enhanced ROS generation in plants during stress conditions owes to the closing of stomata and reduced rate of carbon fixation which cause reduction of $NADP^+$ via Calvin cycle (Vijayakumari 2015). $NADP^+$ scarcity cause decline in the electron transport chain and leakage of electrons to O_2 and this can culminate in the formation of ROS. Accumulation of ROS (viz. $O_2^{\cdot -}$, H_2O_2 and •OH) occurs when the oxygen molecules accept leaked electrons from the overloaded PSI. It causes the production of superoxide radicals or singlet oxygen that reacts with metals ions and other cellular compounds (Choudhury et al. 2017).

The present study revealed that exposure of primed and non-primed rice seedlings exposed to different stresses led to higher accumulation of ROS. NaCl, PEG and UV-B stresses will result in over accumulation ROS in seedling tissues, owing to ion imbalance and hyperosmotic stresses. Over production of ROS is a major indicator of the oxidative stress in rice seedlings. The phenomenon of oxidative injury modifies the structures of the membranes by the lipid peroxidation resulting in electrolyte leakage and

ultimately results in loss of cell viability (Espanany et al. 2016; Chunthaburee et al. 2016; Choudhury et al. 2017). Lipid peroxidation is an ascribed symptom to oxidative damage and it is used as a marker of oxidative stress (Taïbi et al. 2016). MDA is the main cytotoxic product of lipid peroxidation and is a major indicator to assess the level of free radical production (Espanany et al. 2016). Although hydrogen peroxide is comparatively stable ROS, its higher levels causes oxidative stress causing modifications in membrane permeability (Khoshbakht et al. 2018).

In rice seedlings subjected to UV-B priming and exposed to different stress conditions, it was observed that the production of ROS content was significantly reduced as compared with seedlings subjected to non-primed stressed conditions, which denotes that oxidative stress and seedling damage were effectively assuaged after priming both at seed or seedling stage. The over produced ROS in stress conditions resulted in damaging the cell components whereas in the case of seedlings subjected to UV-B priming either at seed or seedling stage, the content of ROS produced was in limits and could even serve as a positive regulator of signalling cascade. A minor quantity of ROS could initiate a signalling cascade ultimately equipping the plant to effectively counter the stress. Researchers have reported that the moderate accumulation of ROS can act as signalling molecule for plant growth and development and various stress tolerance mechanisms (Hossain et al. 2015). Compared with seedling priming, seedlings emerged from UV-B primed seeds showed lesser production of ROS and MDA and therefore the positive regulation of ROS would be higher in the latter case. Similar results were obtained in maize (Javadmanesh et al. 2012) and rice (Zheng et al. 2016), where the accumulation of ROS was diminished significantly by the effect of treatment with low dose of UV-B.

In primed rice seedlings subjected to stress conditions, the hydrogen peroxide and superoxide contents were reduced which was directly proportionate to the reduced MDA content (*detailed in section 5.2.3.*) and electrolyte leakage in rice seedlings. These reductions points out that the stress induced oxidative damages were effectively alleviated by priming of the rice seedlings with UV-B. Also the efficient photosynthetic machinery in UV-B primed seedlings ensures smooth electron flow and will control the production of ROS. Previous researchers have concluded that the positive effects of various seed priming treatments under various abiotic stresses occur presumably due to the protection against oxidative damages (Abid et al. 2018; Dillon et al. 2018; Fang et al. 2018; Irani and Todd 2018; Noorhosseini et al. 2018). Similar positive effects were reported as a result of seed priming with low dose of UV-B radiation, hydro-priming, KNO_3 and PEG and the positive effects include averting lipid peroxidation, electrolyte leakage and ROS production as noted in maize (Javadmanesh et al. 2012), black cumin (Espanany et al. 2016) and rice (Hussain et al. 2016; Zheng et al. 2016).

Retention of membrane integrity and stability under abiotic stress is a major component of stress tolerance in plants. The membrane stability index (MSI) is a physiological index that has been widely used to evaluate stress tolerance. Disruption of membrane integrity due to oxidative damages causes increased ion conductivity (İşeri et al. 2014; Khoshbakht et al. 2018). Various stress conditions affect membrane stability through lipid peroxidation, leading to the production of peroxide ions and malondialdehyde (MDA). Thus, changes in the concentration of MDA and electrolyte leakage are good indicators of membrane structural integrity under stress conditions (Li et al. 2011; Sanchez-Reinoso et al. 2014). Drought stress negatively affects the membrane integrity and electrolyte leakage in borage leaves and durum wheat (Ahmadizadeh 2011; Dastborhan and Ghassemi-Golezani 2015). NaCl stress increases the electrolyte leakage and lipid peroxidation, and decreases the

membrane stability index (MSI) in *Vigna radiata* (Hayat et al. 2010), rice (Farhoudi et al. 2012) and cucumber (Khan et al. 2013). Elevated UV-B radiation increases oxidative stress leading to the destruction of phospho- and glycolipids in the cell membrane, thereby resulting in electrolyte leakage of membranes, and thus decreases membrane stability (Mohammed and Tarpley 2010). UV-B priming primarily reduced the ROS generation and thus enhanced the membrane stability index and lowered electrolyte leakage in rice seedlings.

Various researchers have studied the significant influence of seed priming on MSI and electrolyte leakage. Although drought stress elevated the electrolyte leakage of membranes in wheat leaves, seed priming with spermine was found to be effective in reducing electrolyte leakage (Hassan et al. 2020). Similarly, increasing salinity damaged the cell membrane stability and electrolyte leakage in alfalfa and the osmo- and hydropriming of seeds retained the cell membrane stability and thus reduces the electrolyte leakage (Amooaghaie 2011). Abiotic stresses leads to various malfunctions at cellular level that damage the membrane, one of which is increased membrane permeability, which was reduced by salicylic acid priming in rice (Pouramir-Dashtmian et al. 2014). These previous reports supported the results of this study wherein seed/seedling priming with UV-B positively influenced to reduce the electrolyte leakage. Electrolyte leakage was decreased during seed/seedling priming of Kanchana and Aiswarya rice varieties under NaCl, PEG and UV-B stress conditions and this was due to the retention of membrane integrity in stress conditions. Although priming does not have a direct influence on membrane integrity it may be averting the damage of ROS on membrane due to reduced rate of ROS synthesis.

Plant's antioxidant defence mechanism aids the plants to overcome the cascades of uncontrolled oxidation and protect the cells from ROS induced

oxidative damage (Anjum et al. 2015; Chen et al. 2016). In plants, non-enzymatic antioxidants play a key role in stress tolerance and are mainly involved in many cellular processes under stress by directly detoxifing the ROS and thus contributing to non-enzymatic ROS scavenging (Gill and Tuteja 2010; Palma et al. 2015). It was observed that rice seedlings from both modes of priming with UV-B hasten the total phenolics, ascorbate and glutathione accumulation. The reduced rate of non-enzymatic antioxidants accumulation was observed in seedlings subjected to stress conditions in comparison with primed seedlings subjected to stress conditions denoting that UV-B priming by two different modes can positively influence the biosynthesis of non-enzymatic antioxidants process. The hastening of non-enzymatic antioxidants accumulation was principally elevated in seedlings from primed seeds than seedlings directly primed with UV-B.

Ascorbate is a water soluble antioxidant and act as an enigmatic component of plant defence mechanism. Ascorbate has multiple functions in plants, influencing growth and development by metabolizing ROS and its products (Anjum et al. 2014). It is a non-enzymatic antioxidant which serves as electron donor in various enzymatic and non-enzymatic reactions. Ascorbate also plays major role in plant development such as regulation of cell division, cell cycle and cell elongation. It can also act as an alternative electron donor to PSII, when the oxygen evolving complex (OEC) is damaged during stress condition (Toth et al. 2011). This enhancement in ascorbate accumulation provides an explanation of better PSII activity in UV-B irradiated seedlings (Faseela and Puthur 2018).

The increased content of ascobate and glutathione in rice seedlings raised from UV-B primed seeds shows a clear cut indication of their capacity to adapt to environmental stresses. The ascorbic acid directly scavenges the $^1O_2$ and OH^- and in addition removes the H_2O_2 through Asada-Halliwell

pathway and this antioxidant was over accumulated in *Vigna* species on exposure to UV-B radiation (7.2 kJm^{-2}) (Dwivedi et al. 2015). The increased level of antioxidants confers the ability to plants for scavenging ROS and thus to effectively withstand the NaCl, PEG and UV-B stresses. The protective role of ascorbate, glutathione and total phenolics against oxidative stress was well evident in many crop plants (Gill and Tuteja 2010). Ascorbate and glutathione were involved in scavenging H_2O_2 in combination with activities of monodehydroascorbate reductase and glutathione reductase, which regenerates ascorbate. Glutathione (GSH) is non-enzymatic antioxidant and it is involved in various cellular processes under stress and it also acts as a substrate for glutathione S-transferase and glutathione peroxidase. Moreover, it can detoxify superoxide and hydroxyl radical and also function as non-enzymatic ROS scavenger (Foyer 2018). According to Hussain et al. (2016) respiration take a major role in the synthesis of GSH. At the time of respiration, different metabolites such as glycine are produced, that could be used for the synthesis of GSH. GSH also act as a substrate of the enzyme GR, so that the augmented GSH content in UV-B primed rice seedlings can be correlated with enhanced GR activity in stress conditions.

Phenolic compounds function as strong scavenger of free radicals. UV treatment activates the phenolic biosynthetic pathways and enhances the accumulation of phenolic compounds (Bravo et al. 2012). According to Bravo et al. (2012), the UV treatment improved the accumulation of phenolic compounds in tomato plants because of the activation of phenolic biosynthetic pathway as compared with the untreated samples. Similar results was observed in lettuce plants, which shows that the UV seed priming enhanced the total phenolics content as compared with non-primed plants (Ouhibi et al. 2014). Phenolics compounds take multiple roles in plants such as structural components of cell walls, involved in the modulation of growth and developmental processes as well as in the mechanisms of defence against

abiotic stresses (Taïbi et al. 2016). Phenolic compounds protect the cells from oxidative damages, increase the cell membrane stability and mitigate the stress effects in seedlings primed with UV-B. This was predominantly observed in UV-B primed rice seedlings as compared to non-primed seedlings subjected to different stresses. In response to UV-B stress, plants accumulate phenolic compounds and antioxidants which is an attribute of PAL activation in plants (Esringu et al. 2016).

In this study, the UV-B priming induced enhanced synthesis of ascorbate, glutathione and total phenolics in Aiswarya and Kanchana rice seedlings. The enhanced accumulation of ascorbate, glutathione and total phenolics content aid in the detoxification of the ROS and thus play a major role in effectively controlling lipid peroxidation and electrolyte leakage by retaining the membrane stability.

The SOD, CAT, APX, GPOX are the most important enzymes involved in free radical scavenging process in plants. GR, MDHAR and DHAR were indirectly involved in the ROS scavenging mechanisms in plants. In earlier studies, it was well illustrated that the activities of these enzymes were enhanced, aiding in scavenging of the free radicals and thus reducing the lipid peroxidation during UV-B radiation stress (Dwivedi et al. 2015), NaCl and PEG stresses (Ma et al. 2016). The elevated gene expression of these enzymes during stress indicates that plants attained the potential to efficiently control the ROS and thus attain stress tolerance potential (He et al. 2014). SOD activity accelerated the conversion of superoxide to hydrogen peroxide, whereas CAT and APX dismutated hydrogen peroxide into water and oxygen (Wang et al. 2010; Li et al. 2017). In antioxidant enzymatic defence system APX have major role in averting oxidative membrane damage and this was due to its higher affinity to hydrogen peroxide, converting it to water by utilizing thylakoid lumen ascorbate as electron donor (Sofo et al.

2015; Vuleta et al. 2016). According to Faseela and Puthur (2018), the antioxidant enzymes such as SOD, CAT and APX efficiently got elevated in Kanchana rice variety under UV-B treatments. The UV-B radiations trigger the de novo synthesis of SOD as well as other antioxidant enzymes (Wang et al. 2015). GPOX is a heme containing enzyme and removes excess hydrogen peroxide during stress conditions. GPOX prefers guaiacol and pyragallol as electron donors. GPOX activity was higher in lettuce plants during UV-B stress condition (Esringu et al. 2016) and rice seedlings under drought stress conditions (Das and Roychoudhury 2014).

Previous studies have reported that various seed priming methods positively influenced the antioxidant mechanism in different crops under various stress conditions. The osmo-priming treatment enhanced the activities of GPOX and CAT reduced the malonyldialdehyde (MDA) content and the electrolyte leakage in alfalfa under drought conditions (Mouradi et al. 2016). Polyethylene glycol priming increases the antioxidant activities of APX, CAT, GPOX and SOD in sorghum under drought condition (Zhang et al. 2015). The UV seed priming significantly improved the SOD, CAT and APX activities in lettuce (Ouhibi et al. 2014), fenugreek (El-Shora et al. 2015), maize (Rudnóy et al. 2015), wheat (Badridze et al. 2015; 2016), rice (Inostroza-Blancheteau et al. 2016). The previous studies have reported that, during the hydropriming and hormonal priming, synthesis of new CAT isoforms were initiated in wheat under drought stress condition (Eisvand et al. 2010). In this study the APX activity was higher in UV-B primed rice seedlings subjected to various stresses than GPOX activity and has a greater role in detoxication of hydrogen peroxide. APX shows higher affinity towards hydrogen peroxide than CAT and GPOX enzymes (Ali et al. 2019).

The activities of various enzymes coming under AsA-GSH cycle was effectively influenced by UV-B priming that results in increased

accumulation of ascorbate and glutathione content which also supported the APX enzymatic activity in successive manner. Over-produced ROS was removed by the activity of these antioxidants that reduces the lipid peroxidation and electrolyte leakage in rice seedlings and thereby the membrane stability was retained. The effective functioning of antioxidant system was ensuring the protection of the photosynthetic apparatus and thereby increases the photosynthetic activities in UV-B rice seedlings under stress conditions. In present study, it was observed that the UV-B priming improved antioxidant activities in rice seedlings; the elevation was higher in primed rice seedlings subjected to NaCl stress condition followed by UV-B and PEG stresses than control. By the enhanced action of antioxidants, the MDA content was reduced in primed than that of non-primed seedlings exposed to stresses condition. The increased antioxidant activity in UV-B primed rice seedlings improves the growth, enhanced ROS scavenging capacity and better tolerance to NaCl, PEG and UV-B stresses.

APX, MDHAR, DHAR and GR are major enzymes involved in AsA-GSH cycle which controls ascorbate and glutathione regeneration. APX utilize two molecules of ascorbate to reduce H_2O_2 and generate two molecules of monodehydroascorbate (MDHA). MDHA is converted to ascorbate through the action of MDHAR consuming NADPH as the electron donor or it gets converted non-enzymatically to ascorbate and dehydroascorbate (DHA). DHA is recycled to ascorbate through the action of DHAR. GSH was regenerated from GSSG by the action of GR (Sharma et al. 2015; Sofo et al. 2015). Previous researchers have reported the enhanced activity of MDHAR, DHAR and GR under salinity stress in maize roots (AbdElgawad et al. 2016), in wheat under drought stress (Devi et al. 2012) and in rice under UV-B stress (Faseela and Puthur 2018).

The MDHAR, DHAR and GR activity was positively influenced by UV-B priming. This was also evident from the increased ascorbate and glutathione content in primed rice seedlings. Increased MDHAR, DHAR and GR activity was responsible for the higher accumulation of ascorbate and glutathione content (*detailed discussion in earlier paragraphs of this section*) and also the higher activity of APX in UV-B primed rice seedlings under stress condition. Although in non-primed seedlings subjected to stress condition, the MDHAR, DHAR and GR activity was also enhanced but it was much lower than in the UV-B primed rice seedlings under stress conditions.

The mRNA level expression of SOD, CAT and APX genes was significantly augmented in rice seedling by the influence of priming with UV-B and also on exposure to NaCl, PEG and UV-B stresses. The isoforms of SOD, CAT and APX such as *Cu/Zn SOD, CatA* and *APx1* got enhanced under the influence of oxidative, cold and drought stresses to detoxify the ROS (Li et al. 2017; Rossatto et al. 2017). The results clearly indicated that the UV-B priming has specifically influenced the expression of genes of very important antioxidant enzymes so that the rice seedlings could cope with the three different stresses.

According to Paul and Roychoudhury (2017), rice seedlings raised from seed priming showed higher expression levels of genes encoding SOD, CAT and APX under NaCl stress. Under salinity conditions, increased activities of APX, CAT and SOD as well as enhanced expression levels of APX, CAT and SOD genes have also been shown in osmoprimed *Brassica napus* seedlings and in chemical primed *Arabidopsis* plant (Kubala et al. 2013; Irani and Todd 2018). APX gene expression was increased in seedlings of *Physalis angulata* from osmoprimed seeds under salt stress condition (Souza et al. 2016). Similarly various reports have suggested that the *Cu/Zn SOD, CatA* and *APx1* have a greater role in enhancing the tolerance against

oxidative, cold and drought stresses through improving ROS scavenging capability (Li et al. 2017; Rossatto et al. 2017). According to Sen et al. (2020), UV-B seed priming activated the expression of genes *Cu/Zn SOD, CatA* and *APx1* in rice seedlings. These genes were also highly expressed in polyethylene glycol primed rice seedlings subjected to nano-ZnO stress (Salah et al. 2015). Similarly in this study, UV-B primed seedlings showed slight increase in the expression of SOD, CAT and APX without any stress conditions and this was because of the base level activation of antioxidant enzymes by the action of UV-B priming and this signifies the importance of priming for initiation of enhanced expression of genes encoding antioxidant enzymes.

In the present study *Cu/Zn SOD, CatA* and *APx1* were highly expressed in UV-B primed rice seedlings subjected to NaCl stress than PEG and UV-B. In the case of SOD, *Cu/ZnSOD* and other isozymes such as *MnSOD* and *FeSOD* are co-expressed so that the activity of SOD was sum total of all these and it was higher in UV-B primed rice seedlings subjected to stress condition. But the gene expression studies were restricted to *Cu/ZnSOD* and therefore the SOD activity analyzed based on gene expression would be less than the activity studied. The results discussed above clearly indicated that the UV-B priming beneficially influenced at genetic level modification of rice seedlings. These highly expressed genes contributed to higher accumulation of antioxidant enzymes such as SOD, CAT and APX (*detailed in section 5.3.4.*) in rice seedlings. Thereby in UV-B primed seedlings subjected to stress conditions the antioxidant activity was efficient so as to take care of the stress to which the rice seedling was exposed to.

5.3.5. Stress responsive proteins

UV-B priming also significantly altered the mRNA level expression of stress responsive proteins such as HSP and LEA in rice seedlings. HSPs act as

molecular chaperones, also a key factor contributing to cellular homeostasis in cells under both normal and adverse growth conditions. HSPs and LEA are known stress proteins and priming with UV-B involves a mild dose of stress triggering the enhancement of gene expression of these two prominent stress proteins. According to Al-Whaibi (2011), when plants were exposed to UV-B, the gene for HSPs was highly expressed in their aerial tissues (shoot). In rice plants the HSP genes were over expressed as a response towards tolerance mechanisms against UV-B stress (Xu et al. 2011). The role of HSPs extends beyond their chaperone activity, by reducing the damage that result from ROS accumulation. In rice, salinity (NaCl), desiccation (PEG), high pH and high temperature stresses induces the expression of Hsp90 gene as part of a stress tolerance mechanism (Al-Whaibi 2011). Liu et al. (2006) reported that expression of gene for Hsp90 was significantly increased in rice under salt stress. HSPs stabilize protein and membrane structures, and are induced during priming in various plants (Catusse et al. 2011; Chen and Arora 2013). In tomato plants the HSP gene activation was induced by osmopriming of seeds (Gupta et al. 2008). The HSP gene was highly expressed in both primed and non-primed rice seedlings of Kanchana variety under UV-B stress than those subjected to other stress conditions. The accumulated HSP would contribute towards the increased protein accumulation in seedlings subjected to UV-B priming. Moreover the HSP would also prevent the degradation of protein under stress and therefore there is increased protein accumulation in primed seedlings subjected to stress. Heat-shock proteins prevent UV-B related protein damage and directly inhibit the DNA damage and/or improve DNA repair by interacting with repair enzymes (Jantschitsch and Trautinger 2003).

LEA proteins have been implicated in various stress response of plants. High level accumulation of LEA proteins were reported in drought, osmotic, salt and cold stresses (Lutts et al. 2016). LEA proteins retains cell structure

and macromolecules upon cell dehydration as a result of preventing inactivation and aggregation of proteins and the loss of membrane integrity. In rice, a group 3 LEA protein gene was over-expressed under drought, salt and abscisic acid (Xiao 2007). The enhanced expression of LEA transcript in rice seedlings by UV-B priming improves the stress tolerance potential of seedlings. Various seed priming techniques were reported to induce the expression/accumulation of LEA transcript/protein in association with improved stress tolerance of primed seedlings (Chen et al. 2012; Kubala et al. 2015). It was found that due to seed osmopriming in *Spinacia oleracea* (Chen et al. 2012) and *Brassica napus* (Kubala et al. 2015), the expression/ accumulation of LEA transcript/protein were significantly altered under drought stress. LEA retains cell structure and macromolecules upon cell dehydration as a result of preventing inactivation and aggregation of proteins and the loss of membranes integrity.

Both primed and non-primed rice seedlings subjected to PEG stress radically induced the expression of LEA proteins than NaCl and UV-B. This observation can be justified as LEA proteins are well known to be involved in stress tolerance process associated with drought/osmotic stress tolerance. Various reports have conclusively showed that drought stress significantly alters the expression/accumulation of LEA transcript/protein in different plants (Wojtyla et al. 2016).

5.3.6. Synthesis of UV-B absorbing compounds

Seed as well as seedling priming with UV-B in both rice varieties significantly enhances the PAL activity, flavonoid and anthocyanin content in rice seedlings predominantly under UV-B stress condition. UV-B is well known to have the potential to enhance these features to counter the UV-B specific stress encountered by the plants. Priming with UV-B have further enhanced this potential and all these features were seen to enhance further

making the plant still more tolerant towards UV-B stress. Dual function of flavonoids and anthocyanins as UV-B protector and ROS scavenger remarkably enhances the stress tolerance potential more intensively in both varieties of UV-B primed rice seedlings subjected to UV-B stress, followed by NaCl and PEG stresses.

Various other cases of priming inducing the activities of PAL and accumulation of flavanoids and anthocyanins enabling the plants to encounter various types of stresses have been reported earlier. Biopriming enhances the flavonoid content in pea plants under salt stress condition (Ghezal et al. 2016). Anthocyanin and flavanoids content was increased due to the hydro and osmo-priming effects in rice under salt stress (Chunthaburee et al. 2016; Paul and Roychoudhury 2016). Yücel and Heybet (2016) reported that the salicylic acid and calcium seed priming enhances the PAL activity and flavonoid content in wheat under high salinity. Similar results were also reported in salicylic acid priming of safflower and *Vigna mungo* wherein PAL activity, flavanoid and anthocyanin accumulation was increased as a result of salinity stress and these secondary metabolites act as a free radical scavengers (Shaukat et al. 2013; Shaki et al. 2018). UV-B radiation exposure causes flavonoid accumulation in bell pepper. Anthocyanin accumulation in plants, imparts protection against high salt concentration through the osmo-protective responses. NaCl priming enhances the anthocyanin content in tomato plants under saline stress condition (İşeri et al. 2014).

PAL acts as a key enzyme in phenylpropanoid pathway, which is involved in the synthesis of secondary compounds such as flavonoids (Shaki et al. 2018). Flavonoids are secondary metabolites that are synthesized through the phenylpropanoid pathway which act as antioxidant agents by scavenging ROS and have a key role in stress protection. Water-soluble pigments derived from flavonoids via the shikimic acid pathway are

anthocyanins and have a protective role in plants under stress conditions (Shaki et al. 2018).

Flavonoids and anthocyanins acts as powerful antioxidants and antiradical agents. UV-B irradiation can hasten the biosynthesis of flavonoids and anthocyanins, which serve to protect the cells from UV-B radiations (Shaukat et al. 2013). Increased flavonoid content can reduce the UV-B penetration and protect the photosynthetic apparatus. Stress-responsive flavonoids have the potential to inhibit the generation of ROS and reduce the levels of ROS through its antioxidation capacity. Flavonoids have indeed the capacity to absorb UV-B radiation, inhibit the generation of ROS and once they are formed it quench the ROS (Ravindran et al. 2010).

Cuticular wax is a complex mixture of a homolog series of very-long chain aliphatic lipids, triterpenoids and minor secondary metabolites, such as sterols and flavonoids. Cuticular waxes protect the aerial parts of plant from uncontrolled water loss and UV radiations (Bourdenx et al. 2011). Cuticular waxes act as a protective sunscreen in plants which provides a shielding ability in leaves for attenuation of UV-B radiation. Increased wax on leaf surfaces reduces the penetration of UV due to increased reflection from the surfaces (Kumari and Agrawal 2010).

In this study the epicuticular wax accumulation was highest in UV-B stress condition than other stress conditions. These deposited wax content attenuates the harmful effects of UV-B stress in rice seedlings. Wax deposition was higher in tolerant Kanchana rice variety and this could impart enhanced tolerance against UV-B. UV-B induced wax deposition was remarkably reported by various researchers in different plants. Epicuticular wax deposition was increased in *Cymbopogon citrates* under UV-B stress condition (Kumari and Agrawal 2010). The epicuticular wax accumulation

was positively altered in seedlings from primed seeds as well as directly primed seedlings exposed to UV-B stress.

In Kanchana and Aiswarya rice varieties the epicuticular wax deposition was also enhanced under PEG stress condition. This wax accumulation was to assist in preventing the excess water loss from rice seedlings, due to the presence of alkanes in wax content. Cuticular wax contains alkanes which are important for plant water status control and water stress response (Bourdenx et al. 2011). Wax accumulated in drought stress condition has a connection with tolerance mechanisms of plants (He et al. 2018). Kanchana variety being a tolerant variety showed a higher accumulation of wax content than Aiswarya rice seedlings exposed to PEG stress and this would enable the former variety to counter water loss more effectively. This result was supported by earlier reports, wherein enhanced deposition of epicuticular wax on the surface of leaves of several plants has been reported to shield them from excess water loss through transpiration and act as an effective constituent of drought resistance (Wijewardana et al. 2016). PEG induced drought stress enhances the leaf epicuticular wax content in *Phoenix dactylifera* (Al-Mayahi 2016). Besides UV-B, priming with silicon was reported to enhance the epicuticular wax content in wheat subjected to drought (Ahmed et al. 2016).

The present study clearly revealed the efficacy of UV-B priming in imparting abiotic stress tolerance in tolerant and sensitive rice varieties under three stress conditions. The UV specific characters such as UV absorbing compounds and cuticular wax deposition have been prominent in UV-B primed seed as well as seedlings subjected to UV-B stress conditions than NaCl and PEG stresses.

5.3.7. UV-B priming bring about tolerance towards multiple stresses also shows cross tolerance and stress memory

The findings of this study also gave information on two very important aspects such as the stress memory or priming imprints as well as the cross-tolerance mechanisms operational in rice seedlings which were directly exposed to UV-B priming and those that emerged from primed seeds. The treatment with low dose of UV-B act as key factor for priming induced stress memory or developing priming imprints in rice seedlings. This imprints gets hoarded and later rejuvenates when the seedlings were exposed to stress conditions and thus triggers the stress tolerance mechanisms. In this study, although the priming was carried out with UV-B, the imprints get activated and functional not only in UV-B stress but also in NaCl and PEG stress and thus exhibits cross tolerance. The priming imprints developed as a result of UV-B priming, has also the potential to induce cross tolerance mechanisms in rice seedlings. It was reported in previous studies that NaCl priming induces the stress tolerance towards drought stress in sugarcane and heat tolerance in barley, H_2O_2 priming induces the tolerance towards salt and heat in wheat and maize respectively; hydro, $CaCl_2$ and ABA priming helps to tolerate salt and PEG stresses in Indian mustard (Wahid et al. 2007, 2008; Mei and Song 2008; Patade at al 2009; Srivastava et al. 2010). Initial mild stress exposure during UV-B priming helps the plants to acquire stress tolerance capacity which gets stored as priming memory. This priming memory induces quick stress responses when seedlings are encountered with stress conditions at anytime of its life span.

Bandurska and Cieslak (2013) reported that the cross tolerance effect of UV-B pre-treatments in barley results in stress tolerance particularly under drought conditions. Same scenario was observed in this study, the UV-B pre-treatments with low dose prominently enhanced the stress tolerance in NaCl

stress rather than UV-B and PEG stresses. Photosynthesis and respiration rates were prominent in UV-B primed seed and seedlings subjected to NaCl stress condition by the effect of augmented activities of antioxidative machineries, which act as a shield for photosynthetic and respiratory machineries from ROS toxicity and lipid peroxidations. The study indicated that the UV-B priming imprints and cross tolerance mechanisms was more prominent and more efficient in NaCl stress conditions rather than UV-B and PEG stress.

Priming memories of one stress make plant respond to another stressor (Hilker et al. 2016). UV-B primed rice seedlings subjected to NaCl stress in rice varieties showed more efficient PSI and PSII functioning as well as the increase in *gs, Pn* and *Ci* was higher than seedlings exposed to other stressed conditions due to the priming induced cross-tolerance mechanisms becoming more successful in NaCl stress conditions. UV-B primed Kanchana rice seedlings subjected to NaCl stress showed increased metabolite accumulation. These metabolites also help the rice seedlings to undergo osmotic adjusment under three stress conditions especially under NaCl stress.

The effective and efficient action of enzymatic and non-enzymatic antioxidants was observed in UV-B primed Kanchana rice seedlings exposed to NaCl stress than in the case of UV-B and PEG stresses. Under the influence of UV-B priming of rice seedlings, the antioxidant machinery was activated right in the seed stage itself. In Aiswarya and Kanchana rice seedlings raised from primed seeds on exposure to stress condition, the antioxidant machinery was seen to be robust due to the retention of 'stress memory' induced as a result of priming. This stress memory plays a key role in priming induced cross-tolerance (Chen and Arora 2013).

UV-B priming activates stress-responsive system in rice seedlings to resist NaCl, PEG and UV-B stresses as a result of effective stress imprints.

Molecular alterations such as gene expression changes by chromosomal modification like DNA methylation and histone alterations imparts stress memory in plants (Chen and Arora 2013). These modifications are inheritable that mediate trans-generation memory in seedlings. The priming gives stress memory in plants, this past event memory shape the response to future environmental stimuli. Priming efficiently alters the future plant performance when the plant was exposed again to stress conditions. While experiencing mild stress, plants are warned of future stress, which was reviewed as a priming stimulus that strengthens plant's response to forthcoming stresses. Priming equips the organism for an enhanced response to an imminent future stress.

5.3.8. Cost effectiveness of UV-B priming towards three different stresses

UV-B priming triggers a set of metabolic adaptation leading to stress memory and allowing plants to adapt more efficiently to stress conditions. Antioxidation related innate tolerance potential was kept in an alert mode in 'primed' seedlings, rather than the continuous accumulation of antioxidants (Thomas et al. 2019, 2020).

In rice seedlings primed with UV-B but not subjected to any stress, the activities of both enzymatic and non-enzymatic antioxidants were marginally increased, while the rate of ROS production and lipid peroxidation was slightly reduced in comparison with the control. The slight increase in antioxidants even when the ROS production is less than control shows the alertness of the system in primed state with the synthesis process of the antioxidants becoming already functional even when the ROS production was moderate and unharmful. It does not have any additional energy cost for the plants until and unless a stress is initiated. This ensures that a cost effective mechanism of stress tolerance is operational in plants subjected to UV-B priming.

The cost effective stress tolerance mechanism is one of the biggest advantage of priming induced abiotic stress tolerance (Chen and Arora 2013; Wojtyla et al. 2016). The term cost effectiveness is not in the economic aspects, but in the aspects of energy utilization. Priming initiates stress tolerance mechanisms especially antioxidation process and permits the processes to continue in an alert mode, without exerting any additional energy cost for the plants until and unless a stress is initiated.

Priming enables future plant fitness with desirably lesser investment of resources. Therefore, priming is likely to diminish the costs of responding to future stressful situations. In a primed plant response towards stress stimulus gets displayed in a quick mode than non-primed plants. Thereby, the biosynthesis rate of defensive compounds is elevated, and efficient levels are reached faster. It was found that defence machinery gets activated in a cost effective manner i.e. the defence process gets activated in tune with the intensity of the stress. Such a strategy would ensure that the plant does not allocate more reserves for stress tolerance process and thus the productivity does not gets compromised to a higher extend when plants are exposed to stress.

Priming with low dose of UV-B has the potential to equip the plant, by over synthesizing various antioxidants depending on the intensity of the stress encountered by it. Priming is basically alerting the existing defence machinery of a plant and the utilization of the same depends on the stress encountered by the plants. Therefore, mere priming would in no way tax extra to the plants, which is cited as one of the greatest advantages of priming by many authors (Jisha and Puthur 2015; Badridze et al. 2015, 2016; Inostroza-Blancheteau et al. 2016; Vijayakumari and Puthur 2016).

UV-B priming has got the capacity to amplify and tune the stress tolerance mechanism in Aiswarya and Kanchana rice seedlings based on the

stress to which it is exposed to. That could be the reason why after providing a low dosage of UV-B for priming, the stress tolerance mechanisms gets activated in rice seedlings of var. Kanchana and Aiswarya and performs in a big way based on the stress to which it is exposed to. This finding corroborates with the earlier outcome of GABA/BABA priming in rice, *Vigna* and black pepper exposed to NaCl or PEG stresses (Jisha and Puthur 2014b, 2015; Vijayakumari and Puthur 2016).

5.3.9. Seed priming *vs* seedling priming

The antioxidative potential and photosynthetic activities were effective in seed priming rather than seedling priming. Accordingly, the priming imprints and cross tolerance was efficiently expressed in seed priming than seedling priming. In seed priming, the specific transcription factors or protective metabolites as well as epigenetic modifications are accumulated at seed stage (Chen and Arora 2013). These pre-germinative mechanisms efficiently accelerate stress tolerance when seeds were later subjected to stress conditions. Whereas, in the seedling priming, stress tolerance mechanisms gets activated at a later stage and the resources would be shared between other processes required for the establishment of the seedling (Bruce et al. 2007). From this study it can be concluded that the cross-tolerance and priming imprints were more prominent in seed priming than seedling priming.

Compared with seedling priming the effective priming imprints due to seed priming leads to the increased accumulation of enzymatic and non-enzymatic antioxidants which imparts an enhanced protection to the photosynthetic machinery, which is reflected in increased PSI and PSII activities in seedlings raised from primed seeds. Studies have reported that enhanced activities of antioxidants during priming, assisted rice seedlings to overcome the stress-induced challenges (Khaliq et al. 2015; Zheng et al. 2015; Hussain et al. 2016). When the seedlings subjected to priming were exposed to stress condition, the priming imposed a rapid and efficient response towards the stress through activation of antioxidant machineries by

taking advantage of stress memory and hastily acclimatizing to the subsequent episodes of stress and this was predominant in seedlings from UV-B primed seeds than seedlings directly primed with UV-B.

Compared to the seedling priming, seed priming resulted in significant enhancement of enzymatic and non-enzymatic antioxidants activities in seedlings, which results in the reduction of ROS and MDA content. UV-B priming accelerated the antioxidant activities of SOD, CAT, APX and GPOX as well as upholds the production of proline, soluble sugars, free amino acids and proteins contents. In seedling priming, there are greater chances for the priming imprints to be frequently interrupted and negotiated by the active plant growth. However in the seed priming, the preparative stage for antioxidants synthesis becomes active in seed stage itself before the initiation of germination and this would ensure that priming imprints and the associated functions would progress uninterrupted into the seedlings. Superior growth features as well as antioxidant machinery were observed in seedlings emerged from primed seeds over seedlings growing after seedling priming and this could be possible because of efficient germination process and unfaded priming imprints in the former. The seedlings emerged from primed seeds tackle the abiotic stresses by vigorous head-start or/and by exhibiting cross tolerance (Chen and Arora 2013).

UV-B primed rice seedlings shows enhanced stress tolerance potential through the over-expression of defence related components including osmolytes (proline and sugars), heat shock proteins (HSPs) and late embryogenesis abundant (LEA) proteins for encountering unpredictable negative effects of stress conditions on growth and development of seedlings. It can be considered that UV-B priming minimized the negative effects through timely management of osmotic balance inside cells. Like in the case of seed priming, seedling priming also stimulated the expression of LEA and HSP genes and also enhances the accumulation of osmolytes in rice seedlings.

UV-B priming not only influences seeds but also the whole plant system itself. It equips the plants to reacts more quickly and efficiently to a stress. An efficient stress tolerance adaptation of primed seedlings could be related to a direct impact of pre-treatment on cell cycle regulation and cell elongation processes occurring right at the embryo development stage. Seed priming mediated stress tolerance could be noted in the seedling vigour which was developmentally more advanced than seedling priming.

5.3.10. Tolerant variety vs sensitive variety

Tolerant Kanchana rice seedlings showed more efficiency in photosynthesis than sensitive Aiswarya rice seedlings in both modes of priming on exposure to stress conditions. In Kanchana rice variety UV-B priming could generate efficient antioxidation process for scavenging ROS and thereby reduces the lipid peroxidation and electrolyte leakage retaining membrane stability and photosynthetic efficiency when rice seedlings were subjected to stress conditions. The respiratory activity was more prominent in seedlings from primed seeds and of the two varieties and it was prominent in Kanchana. The higher influence of priming in ensuring optimal respiratory activity was observed in primed seedlings subjected to NaCl stress as compared to other stresses. When compared to Aiswarya rice variety the priming effect was more prominent in Kanchana rice variety, because it has an innate tolerance potential, which gets accelerated further by UV-B priming.

HSP and LEA gene expression was higher in tolerant rice variety Kanchana than Aiswarya variety. The HSP gene expression was higher in UV-B primed Kanchana rice seedlings subjected to UV-B stress condition of Kanchana while the LEA gene expression was more in UV-B primed Kanchana rice seedlings subjected to PEG stress. This is a clear indication that enhancement of flavanoid and anthocyanin content is more responsible for countering UV-B stress than NaCl and PEG stresses. On the basis of PAL activity, flavanoid and anthocyanin content the stress tolerance effect of

priming was mainly noticed in Kanchana than Aiswarya. The genetic potential of Kanchana, a tolerant variety would be responding to UV-B stress in a better way than Aiswarya, which is a well-known stress sensitive variety. Although UV-B stress is known to induce more PAL activity as well as flavanoid and anthocyanin accumulation, the other stresses like NaCl and PEG too could induce these factors but at a lower rate than UV-B. This could be primarily because these features listed above impart greater tolerance potential under UV-B stress than any other stresses.

This clearly indicates that the seed priming effects in the tolerant varieties are more prominent when they are encountered with different stress conditions. Compared with seed and seedling priming, seed priming was more effectively influencing the rate of enhancement of these parameters mainly in tolerant Kanchana rice seedlings than Aiswarya sensitive variety. Priming is more effective in rejuvenating the less expressed stree tolerance features and that is what precisely occurring in a tolerant variety.

6. SUMMARY AND CONCLUSIONS

Studies on six commonly cultivated high yielding rice varieties (Aiswarya, Jyothi, Kanchana, Neeraja, Samyuktha and Swetha) was carried out for analyzing the stress tolerance potential towards NaCl, PEG and UV-B stresses. Each variety of rice seeds were imparted with six different concentrations of NaCl (25, 50, 75, 100 and 125 mM) and PEG (5, 10, 15, 20 and 25%) and six different dosages of UV-B irradiations (7, 14, 21, 28 and 35 $kJm^{-2}d^{-1}$). From this study the stress imparting concentrations/dosages of NaCl, PEG and UV-B were identified and also the two tolerant and sensitive varieties were identified based on their NaCl, PEG and UV-B stress tolerance potential. The two stress tolerant (Swetha and Kanchana) and two stress sensitive (Aiswarya and Samyuktha) varieties were imparted with four different dosages of UV-B priming such as 2, 4, 6 and 8 kJm^{-2} to find out the effective priming dosage. UV-B primed seeds as well as seedlings were imparted with NaCl, PEG and UV-B stresses and the effect of UV-B priming on various morphological, physiological, biochemical and molecular processes in rice varieties was studied.

The major conclusions derived from the present study are summarized below:

- Based on the analysis of various morphological and physiological parameters, Aiswarya and Samyuktha were identified as stress sensitive varieties and Swetha and Kanchana as stress tolerant rice varieties towards NaCl, PEG and UV-B stresses. The stress imparting concentration of NaCl (75/100 mM), PEG (15/20%) and dosage of UV-B irradiations (21/28 $kJm^{-2}d^{-1}$) were also identified. Stress sensitive varieties had lower concentrations/dosages as the stress imparting concentration (75 mM NaCl, 15% PEG and 21$kJm^{-2}d^{-1}$ UV-

B). Stress tolerant varieties had higher concentrations/dosages as the stress imparting concentration (100 mM NaCl, 20% PEG and 28 $kJm^{-2}d^{-1}$ UV-B)

- The four varieties selected were imparted with different UV-B dosages to identify the priming dosages. Based on the morphological and physiological parameters most tolerant rice variety Kanchana and sensitive variety Aiswarya was selected. The selected seed priming concentrations was 6 kJm^{-2} UV-B radiations for tolerant Kanchana and 4 kJm^{-2} for sensitive Aiswarya variety. The priming dosage for seedlings was also same as that of seeds.

- Photosynthetic efficiency was assessed in terms of PSI, PSII activities, Chlorophyll *a* fluorescence analysis and gas exchange parameters in UV-B primed and non-primed rice seedlings subjected to stress and without stress conditions. Photosynthetic efficiency was higher in UV-B primed rice seedlings not subjected to any stress conditions as compared to non-primed ones. Even though photosynthetic efficiency decreased in both UV-B primed and non-primed rice seedlings on being exposed to different stresses, the decrease recorded in primed seedlings was comparatively less.

- In Aiswarya and Kanchana rice seedlings subjected to both modes of priming (seed/seedling priming), but not exposed to any stress conditions, the mitochondrial activity was slightly increased and this could also alert various systems involved in stress tolerance.

- UV-B primed rice seedlings subjected to stress conditions recorded higher accumulation of various metabolites such as total protein, total free amino acids, total sugar and proline content.

- Enhanced high tolerance of UV-B primed rice seedlings toward three stresses was also due to the enhanced activities of enzymatic (SOD, CAT, APX, GPOX, GR, MDHAR and DHAR) and non-enzymatic (ascorbate, glutathione and total phenolic content) antioxidants.
- The mRNA level expression of SOD, CAT and APX genes was enhanced in UV-B primed rice seedling under NaCl, PEG and UV-B stresses.
- Highly efficient antioxidant machinery scavenge the over accumulated ROS (superoxide and hydrogen peroxide) and thereby protect the photosynthetic and mitochondrial machineries in rice seedlings under stress exposure.
- Enhanced antioxidant mechanism retains the membrane stability and thereby reduces the electrolyte leakage in UV-B primed rice seedlings.
- UV-B absorbing compounds such as anthocyanin and flavonoids as well as phenylalanine ammonia lyase activity and cuticular wax deposition and its functional group modifications (revealed by FT-IR spectrometry) were highly significant in UV-B primed rice seedlings exposed to three stress conditions.
- The enhanced accumulation and greater modifications in functional groups of epicuticular wax in leaves of UV-B primed rice seedlings protected the rice seedlings from UV-B irradiation.
- The enhanced epicuticular wax deposition in UV-B primed rice seedlings subjected to PEG stress could assist in preventing excess water loss.
- The mRNA level expression of stress responsive protein HSP was greatly enhanced in UV-B primed rice seedlings subjected to UV-B

stress conditions. The accumulated HSP would prevent the protein degradation in UV-B primed rice seedlings subjected to stresses.

- Increased expression of genes for LEA protein synthesis was seen to be higher in UV-B primed rice seedlings subjected to PEG stress, followed by other stresses. LEA proteins retain cell structure and macromolecules upon cell dehydration in rice seedlings under stress condition.

- The enhanced gene expression of HSP and LEA is an indication that UV-B priming can influence at the genetic level and this priming imprint could carried over from seeds to the seedlings.

- UV-B primed Kanchana rice seedlings showed enhanced stress tolerance potential towards NaCl, PEG and UV-B stresses than Aiswarya rice seedlings. UV-B priming is technique which can transform a tolerant variety more tolerant and a sensitive variety to a tolerant one.

- On comparison between seed and seedling priming, seed priming with UV-B efficiently enhances the stress tolerance potential of both Kanchana and Aiswarya rice seedlings towards three stress conditions. In rice seedlings the stress memory/priming imprints were successively retained in seed priming than seedling priming.

- In the case of three stress conditions, the priming effects were predominantly showed up in rice seedlings subjected to NaCl stress rather than PEG and UV-B as a result of priming induced cross tolerance mechanisms. Also the stress memory was successively recollected in UV-B primed rice seedlings subjected to NaCl stress condition than UV-B and PEG stresses.

- UV-B priming only alerts the defence mechanism towards various stresses and swings into complete action only when encountered with stress and therefore no energy drain occurs unnecessarily for the activation of defence mechanisms in controlled conditions. This is referred to as cost effectiveness of UV-B priming technique for inducing abiotic stress tolerance.

- Rice variety Kanchana is a known tolerant variety, till now there are no studies carried out to make it more tolerant. Earlier studies revealed Kanchana as a tolerant variety yet its maximum tolerance potential was not tapped. And through this study, UV-B priming could scale up the tolerance potential of Kanchana variety to a maximum level.

Thus from the above facts it can be concluded that UV-B priming of seeds is an effective technique for enhancing abiotic stress tolerance in rice seedlings subjected to different stresses.

REFERENCES

AbdElgawad H, Zinta G, Hegab MM, Pandey R, Asard H, Abuelsoud W. High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. Frontiers in Plant Science. 2016; 7:276-287.

Abdelhamid MT, El-Masry RR, Darwish DS, Abdalla MM, Oba S, Ragab R, Sabagh AE, El Kholy MH, Omer E. Mechanisms of seed priming involved in salt stress amelioration. In: Priming and Pretreatment of Seeds and Seedlings. 2019; 219-251. Springer, Singapore.

Abdolahpour M, Lotfi R. Seed priming affected physiology and grain yield of chickpea under salt stress. Journal of Biodiversity and Environmental Sciences 2014; 5:442-446.

Abid M, Hakeem A, Shao Y, Liu Y, Zahoor R, Fan Y, Suyu J, Ata-Ul-Karim ST, Tian Z, Jiang D, Snider JL. Seed osmopriming invokes stress memory against post-germinative drought stress in wheat (*Triticum aestivum* L.). Environmental and Experimental Botany. 2018; 145:12-20.

Abu-Elsaoud AM, Hassan HM Effect of UVA+B on germination consequences, oxidative stress and antioxidant defence mechanisms of wheat (*Triticum aestivum* L.). Journal of Ecology, Health and Environment. 2016; 4:75-86.

Ahmadizadeh M, Valizadeh M, Zaefizadeh M, Shahbazi H. Antioxidative protection and electrolyte leakage in durum wheat under drought stress condition. Journal of Applied Sciences Research. 2011; 7:236-246.

Ahmed M, Qadeer U, Ahmed ZI, Hassan FU. Improvement of wheat (*Triticum aestivum*) drought tolerance by seed priming with silicon. Archives of Agronomy and Soil Science. 2016; 62:299-315.

Ajithkumar IP, Panneerselvam R. ROS scavenging system, osmotic maintenance, pigment and growth status of *Panicum sumatrense* Roth. under drought stress. Cell Biochemistry and Biophysics. 2014; 68:587-595.

Aladjadjiyan A. The use of physical methods for plant growing stimulation in Bulgaria. Journal of Central European Agriculture. 2007; 8:369-380.

Aldesuquy H, Baka Z, Mickky B. Kinetin and spermine mediated induction of salt tolerance in wheat plants: Leaf area, photosynthesis and chloroplast ultrastructure of flag leaf at ear emergence. Egyptian Journal of Basic and Applied Sciences. 2014; 1:77-87.

Alexieva V, Ivanov S, Sergiev I, Karanov E. Interaction between stresses. Bulgarian Journal of Plant Physiology. 2003; 29:1-7.

Ali F, Bano A, Fazal A. Recent methods of drought stress tolerance in plants. Plant Growth Regulation. 2017; 82:363-375.

Ali Q, Daud MK, Haider MZ, Ali S, Rizwan M, Aslam N, Noman A, Iqbal N, Shahzad F, Deeba F, Ali I. Seed priming by sodium nitroprusside improves salt tolerance in wheat (*Triticum aestivum* L.) by enhancing physiological and biochemical parameters. Plant Physiology and Biochemistry. 2017; 119:50-58.

Ali Q, Habib-ur-Rehman Athar MZ, Haider SS, Aslam N, Shehzad F, Naseem J, Ashraf R, Ali A, Hussain SM. Role of amino acids in improving abiotic stress tolerance to plants. In: Plant Tolerance to Environmental Stress: Role of Phytoprotectants. 2019; 10-41.

Al-Mayahi AM. Effect of silicon (Si) application on *Phoenix dactylifera* L. growth under drought stress induced by polyethylene glycol (PEG) in vitro. American Journal of Plant Sciences. 2016; 7:1711-28.

Alvarez-Madrigal M, Pérez-Peraza J. Analysis of the evolution of the Antarctic ozone hole size. Journal of Geophysical Research: Atmospheres. 2005; 27:110-125.

Al-Whaibi MH. Plant heat-shock proteins: a mini review. Journal of King Saud University Science. 2011; 23:139-150.

Amirjani MR. Effect of salinity stress on growth, sugar content, pigments and enzyme activity of rice. International Journal of Botany. 2011; 7:73-81.

Amooaghaie R. The effect of hydro and osmopriming on alfalfa seed germination and antioxidant defenses under salt stress. African Journal of Biotechnology. 2011; 10:6269-6275.

Anjum NA, Gill SS, Gill R, Hasanuzzaman M, Duarte AC, Pereira E, Ahmad I, Tuteja R, Tuteja N. Metal/metalloid stress tolerance in plants: role of ascorbate, its redox couple, and associated enzymes. Protoplasma. 2014; 251:1265-1283.

Anjum SA, Ashraf U, Zohaib A, Tanveer M, Naeem M, Ali I, Tabassum T, Nazir U. Growth and development responses of crop plants under drought stress: a review. Zemdirbyste. 2017; 104:267-276.

Anjum SA, Tanveer M, Hussain S, Bao M, Wang L, Khan I, Ullah E, Tung SA, Samad RA, Shahzad B. Cadmium toxicity in maize (*Zea mays* L.): consequences on antioxidative systems, reactive oxygen species and cadmium accumulation. Environmental Science and Pollution Research. 2015; 22:17022-17030.

Anjum SA, Wang LC, Farooq M, Hussain M, Xue LL, Zou CM. Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. Journal of Agronomy and Crop Science. 2011; 197:177-185.

Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiology. 1949; 24:1-13.

Arunkumar K, Jegadeeswari V, Ushamalini C. Seed priming technology in spice crops: A review. Journal of Phytology. 2019: 21-34.

Ashraf MA, Akbar A, Askari SH, Iqbal M, Rasheed R, Hussain I. Recent advances in abiotic stress tolerance of plants through chemical priming: an overview. In: Advances in Seed Priming. 2018; 51-79. Springer, Singapore.

Ashraf MH, Harris PJ. Photosynthesis under stressful environments: an overview. Photosynthetica. 2013; 51:163-190.

Ashraf MY, Iqbal N, Ashraf M, Akhter J. Modulation of physiological and biochemical metabolites in salt stressed rice by foliar application of zinc. Journal of Plant Nutrition. 2014; 37:447-457.

Asrar AW, Elhindi KM. Alleviation of drought stress of marigold (*Tagetes erecta*) plants by using arbuscular mycorrhizal fungi. Saudi Journal of Biological Sciences. 2011; 18:93-98.

Atkin OK, Macherel D. The crucial role of plant mitochondria in orchestrating drought tolerance. Annals of Botany. 2009; 103:581-597.

Azeem M, Iqbal N, Kausar S, Javed MT, Akram MS, Sajid MA. Efficacy of silicon priming and fertigation to modulate seedling's vigor and ion homeostasis of wheat (*Triticum aestivum* L.) under saline environment.

Environmental Science and Pollution Research. 2015; 22:14367-14371.

Azooz MM, Alzahrani AM, Youssef MM. The potential role of seed priming with ascorbic acid and nicotinamide and their interactions to enhance salt tolerance in broad bean (*Vicia faba* L.). Australian Journal of Crop Science. 2013; 7:2091-2113.

Bacarin MA, Deuner S, Silva FS, Cassol D, Silva DM. Chlorophyll *a* fluorescence as indicative of the salt stress on *Brassica napus* L. Brazilian Journal of Plant Physiology. 2011; 23:245-253.

Badridze G, Kacharava N, Chkhubianishvili E, Rapava L, Kikvidze M, Chanishvili S, Shakarishvili N, Mazanishvili L, Chigladze L. Effect of UV radiation and artificial acid rain on productivity of wheat. Russian Journal of Ecology. 2016; 47:158-166.

Badridze G, Kacharava N, Chkhubianishvili E, Rapava L, Kikvidze M, Chanishvili SH, Chigladze L. Influence of ultraviolet irradiation and acid precipitations on the content of antioxidants in wheat leaves. Applied Ecology and Environmental Research. 2015; 13:993-1013.

Baghel L, Kataria S, Guruprasad KN. Static magnetic field treatment of seeds improves carbon and nitrogen metabolism under salinity stress in soybean. Bioelectromagnetics. 2016; 37:455-470.

Bahuguna RN, Gupta P, Bagri J, Singh D, Dewi AK, Tao L, Islam M, Sarsu F, Singla-Pareek SL, Pareek A. Forward and reverse genetics approaches for combined stress tolerance in rice. Indian Journal of Plant Physiology. 2018; 23:630-646.

Ballare CL, Caldwell MM, Flint SD, Robinson SA, Bornman JF. Effects of solar ultraviolet radiation on terrestrial ecosystems. Patterns,

mechanisms, and interactions with climate change. Photochemical & Photobiological Sciences. 2011; 10:226-241.

Bandurska H, Cieślak M. The interactive effect of water deficit and UV-B radiation on salicylic acid accumulation in barley roots and leaves. Environmental and Experimental Botany. 2013; 94:9-18.

Bano C, Amist N, Singh NB. UV-B radiation escalate allelopathic effect of benzoic acid on *Solanum lycopersicum* L. Scientia Horticulturae. 2017; 220:199-205.

Bano QU, Ilyas N, Bano A, Zafar NA, Akram AB, Hassan F. Effect of Azospirillum inoculation on maize (*Zea mays* L.) under drought stress. Pakistan Journal of Botany2013; 45:13-20.

Bartoli CG, Pastori GM, Foyer CH. Ascorbate biosynthesis in mitochondria is linked to the electron transport chain between complexes III and IV. Plant Physiology. 2000; 123:335-344.

Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and Soil. 1973; 39:205-217.

Battaglia M, Covarrubias AA. Late embryogenesis abundant (LEA) proteins in legumes. Frontiers in Plant Science. 2013; 4:190-205.

Behn H, Albert A, Marx F, Noga G, Ulbrich A. Ultraviolet-B and photosynthetically active radiation interactively affect yield and pattern of monoterpenes in leaves of peppermint (*Mentha piperita* L.). Journal of Agricultural and Food Chemistry. 2010; 58:7361-7367.

Benamar A, Tallon C, Macherel D. Membrane integrity and oxidative properties of mitochondria isolated from imbibing pea seeds after priming or accelerated ageing. Seed Science Research. 2003; 13:35-49.

Benincasa P, Pace R, Quinet M, Lutts S. Effect of salinity and priming on seedling growth in rapeseed (*Brassica napus* var oleifera Del.). Acta Scientiarum Agronomy. 2013; 35:479-86.

Bergman CJ. Rice end-use quality analysis. In Rice 2019; 273-337. AACC International Press.

Bornman JF, Barnes PW, Robinson SA, Ballare CL, Flint SD, Caldwell MM. Solar ultraviolet radiation and ozone depletion-driven climate change: effects on terrestrial ecosystems. Photochemical and Photobiological Sciences. 2015; 14:88-107.

Bourdenx B, Bernard A, Domergue F, Pascal S, Léger A, Roby D, Pervent M, Vile D, Haslam RP, Napier JA, Lessire R. Overexpression of *Arabidopsis ECERIFERUM1* promotes wax very-long-chain alkane biosynthesis and influences plant response to biotic and abiotic stresses. Plant Physiology. 2011; 156:29-45.

Boureima S, Oukarroum A, Diouf M, Cisse N, Van Damme P. Screening for drought tolerance in mutant germplasm of sesame (*Sesamum indicum*) probing by chlorophyll *a* fluorescence. Environmental and Experimental Botany. 2012; 81:37-43.

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976; 72:248-254.

Bravo S, García-Alonso J, Martín-Pozuelo G, Gómez V, Santaella M, Navarro-González I, Periago MJ. The influence of post-harvest UV-C hormesis on lycopene, β-carotene, and phenolic content and antioxidant activity of breaker tomatoes. Food Research International. 2012; 49:296-302.

Brown JE, Lu TY, Stevens C, Khan VA, Lu JY, Wilson CL, Collins DJ, Wilson MA, Igwegbe EC, Chalutz E, Droby S. The effect of low dose ultraviolet light-C seed treatment on induced resistance in cabbage to black rot (*Xanthomonas campestris* pv. campestris). Crop Protection. 2001; 20:873-883.

Bruce TJ, Matthes MC, Napier JA, Pickett JA. Stressful "memories" of plants: evidence and possible mechanisms. Plant Science. 2007; 173:603-618.

Bussotti F, Ferrini F, Pollastrini M, Fini A. The challenge of Mediterranean sclerophyllous vegetation under climate change: from acclimation to adaptation. Environmental and Experimental Botany. 2014; 103:80-98.

Caldwell MM. Solar UV irradiation and the growth and development of higher plants. Photophysiology. 1971; 6:131-177.

Capanoglu E. The potential of priming in food production. Trends in Food Science and Technology. 2010; 21:399-407.

Carlberg IN, Mannervik BE. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. Journal of Biological Chemistry. 1975; 250:5475-5480.

Catusse J, Meinhard J, Job C, Strub JM, Fischer U, Pestsova E, Westhoff P, Van Dorsselaer A, Job D. Proteomics reveals potential biomarkers of seed vigor in sugarbeet. Proteomics. 2011; 11:1569-1580.

Caverzan A, Casassola A, Brammer SP. Antioxidant responses of wheat plants under stress. Genetics and Molecular Biology. 2016; 39:1-6.

Çelik Ö, Atak C. The effect of salt stress on antioxidative enzymes and proline content of two Turkish tobacco varieties. Turkish Journal of Biology. 2012; 36:339-356.

Chen JX, Wang XF. Guide to plant physiological experiments. South China University of Technology Press. 2002:123-127. Guangzhou.

Chen K, Arora R. Priming memory invokes seed stress-tolerance. Environmental and Experimental Botany. 2013; 94:33-45.

Chen K, Fessehaie A, Arora R. Dehydrin metabolism is altered during seed osmopriming and subsequent germination under chilling and desiccation in *Spinacia oleracea* L. cv. Bloomsdale: possible role in stress tolerance. Plant Science. 2012; 183:27-36.

Chen W, Guo C, Hussain S, Zhu B, Deng F, Xue Y, Geng M, Wu L. Role of xylo-oligosaccharides in protection against salinity-induced adversities in Chinese cabbage. Environmental Science and Pollution Research. 2016; 23:1254-1264.

Chen X, Wang Y, Li J, Jiang A, Cheng Y, Zhang W. Mitochondrial proteome during salt stress-induced programmed cell death in rice. Plant Physiology and Biochemistry. 2009; 47:407-415.

Chen Z, Gao W, Reddy KR, Chen M, Taduri S, Meyers SL, Shankle MW. Ultraviolet (UV) B effects on growth and yield of three contrasting sweet potato cultivars. Photosynthetica. 2020; 58:37-44.

Choudhury FK, Rivero RM, Blumwald E, Mittler R. Reactive oxygen species, abiotic stress and stress combination. The Plant Journal. 2017; 90:856-867.

Chunthaburee S, Dongsansuk A, Sanitchon J, Pattanagul W, Theerakulpisut P. Physiological and biochemical parameters for evaluation and clustering of rice cultivars differing in salt tolerance at seedling stage. Saudi Journal of Biological Sciences. 2016; 23:467-477.

Chutipaijit S, Cha-um S, Sompornpailin K. High contents of proline and anthocyanin increase protective response to salinity in '*Oryza sativa*' L. spp. 'indica'. Australian Journal of Crop Science. 2011; 5:1191-1214.

Cocetta G, Ferrante A, Trivellini A, Francini A. Effect of washing treatments on chlorophyll *a* fluorescence and vitamin C content in minimality processed lamb's lettuce during storage.Agrochimica. 2016; 60:1-4.

Cohen Y, Baider A, Gotlieb D, Rubin AE. Control of *Bremia lactucae* in field-grown lettuce by DL-3-amino-n-butanoic acid (BABA). In Improving sustainability in organic and low input food production systems. 2007; 172-186. University of Hohenheim, Germany.

Costa R, Pinheiro N, Almeida AS, Maçãs B. Influence of enhanced UV-B radiation on wheat production in relation with abiotic, biotic and socioeconomics constraints. Emirates Journal of Food and Agriculture. 2012; 24:565-575.

Cuin TA, Parsons D, Shabala S. Wheat cultivars can be screened for NaCl salinity tolerance by measuring leaf chlorophyll content and shoot sap potassium. Functional Plant Biology. 2010; 37:656-664.

Cushman JC, Bohnert HJ. Genomic approaches to plant stress tolerance. Current Opinion in Plant Biology. 2000; 3:117-124.

Czarnocka W, Karpiński S. Friend or foe? Reactive oxygen species production, scavenging and signaling in plant response to

environmental stresses. Free Radical Biology and Medicine. 2018; 122:4-20.

Czégény G, Le Martret B, Pávkovics D, Dix PJ, Hideg É. Elevated ROS-scavenging enzymes contribute to acclimation to UV-B exposure in transplastomic tobacco plants, reducing the role of plastid peroxidases. Journal of Plant Physiology. 2016; 201:95-100.

Dąbrowski P, Baczewska-Dąbrowska AH, Kalaji HM, Goltsev V, Paunov M, Rapacz M, Wójcik-Jagła M, Pawluśkiewicz B, Bąba W, Brestic M. Exploration of chlorophyll *a* fluorescence and plant gas exchange parameters as indicators of drought tolerance in perennial ryegrass. Sensors. 2019;19:2736-2749.

Dalton DA, Baird LM, Langeberg L, Taugher CY, Anyan WR, Vance CP, Sarath G. Subcellular localization of oxygen defense enzymes in soybean (*Glycine max* [L.] Merr.) root nodules. Plant Physiology. 1993; 102:481-489.

Das K, Roychoudhury A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in Environmental Science. 2014; 2:53-65.

Dash AP, De DK, Mohanty S, Lenka D. Screening of Lentil (*Lens culinaris* Medik.) genotypes and correlation analysis under PEG imposed water stress condition. International Journal of Bio-resource and Stress Management. 2017; 8:539-547.

Dastborhan S, Ghassemi-Golezani K. Influence of seed priming and water stress on selected physiological traits of borage. Folia Horticulturae. 2015; 27:151-159.

Davies B, Baulcombe D, Crute I, Dunwell J, Gale M, Jones J, Pretty J, Sutherland W, Toulmin C. Reaping the Benefits: Science and the sustainable intensification of global agriculture. 2009; 24-39. Royal Society, London.

Day TA, Ruhland CT, Xiong FS. Influence of solar ultraviolet-B radiation on Antarctic terrestrial plants: results from a 4-year field study. Journal of Photochemistry and Photobiology B: Biology. 2001; 62:78-87.

Deivanai S, Xavier R, Vinod V, Timalata K, Lim OF. Role of exogenous proline in ameliorating salt stress at early stage in two rice cultivars. Journal of Stress Physiology and Biochemistry. 2011; 7:157-174.

Delibaltova V, Ivanova R. Impact of the pre-sowing irradiation of seeds by helium-neon laser on the dynamics of development of some cotton varieties. Journal of Environmental Protection and Ecology. 2006; 7:909-917.

Demir I, Mavi K. The effect of priming on seedling emergence of differentially matured watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) seeds. Scientia Horticulturae. 2004; 102:467-473.

Devi R, Kaur N, Gupta AK. Potential of antioxidant enzymes in depicting drought tolerance of wheat (*Triticum aestivum* L.). Indian Journal of Biochemistry and Biophysics. 2012; 49:257-265

Dias KO, Gezan SA, Guimarães CT, Nazarian A, e Silva LD, Parentoni SN, de Oliveira Guimarães PE, de Oliveira Anoni C, Pádua JM, de Oliveira Pinto M, Noda RW. Improving accuracies of genomic predictions for drought tolerance in maize by joint modeling of additive and dominance effects in multi-environment trials. Heredity. 2018; 121:24-37.

Dillon FM, Tejedor MD, Ilina N, Chludil HD, Mithöfer A, Pagano EA, Zavala JA. Solar UV-B radiation and ethylene play a key role in modulating effective defenses against *Anticarsia gemmatalis* larvae in field-grown soybean. Plant, Cell and Environment. 2018; 41:383-394.

Dobrikova AG, Krasteva V, Apostolova EL. Damage and protection of the photosynthetic apparatus from UV-B radiation. I. Effect of ascorbate. Journal of Plant Physiology. 2013; 170:251-257.

Doke N. Involvement of superoxide anion generation in the hypersensitive response of potato tuber tissues to infection with an incompatible race of *Phytophthora infestans* and to the hyphal wall components. Physiological Plant Pathology. 1983; 23:345-357.

Dooslin MD, Johnson M, Gerardin J. UV-B Response of *Oryza sativa* L. var. ADT (R) 45 seedlings. International Journal of Biotechnology and Biochemistry. 2010; 6:411-418.

Dubois M, Gilles KA, Hamilton JK, Rebers PT, Smith F. Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 1956; 28:350-356.

Dwivedi R, Singh VP, Kumar J, Prasad SM. Differential physiological and biochemical responses of two *Vigna* species under enhanced UV-B radiation. Journal of Radiation Research and Applied Sciences. 2015; 8:173-181.

Eisvand HR, Tavakkol-Afshari R, Sharifzadeh F, Maddah Arefi H, Hesamzadeh Hejazi SM. Effects of hormonal priming and drought stress on activity and isozyme profiles of antioxidant enzymes in deteriorated seed of tall wheatgrass (*Agropyron elongatum* Host). Seed Science and Technology. 2010; 38:280-297.

El-Samad HMA, Shaddad MA, Barakat N. Improvement of plants salt tolerance by exogenous application of amino acids. Journal of Medicinal Plants Research. 2011; 5:5692-5699.

El-Shora HM, El-Farrash AH, Kamal H, Aya A. Enhancement of antioxidant defense system by UV-radiation in fenugreek as a medicinal plant. International Journal of Advanced Research. 2015; 3:529-535.

Emberson LD, Büker P, Ashmore MR, Mills G, Jackson LS, Agrawal M, Atikuzzaman MD, Cinderby S, Engardt M, Jamir C, Kobayashi K. A comparison of North American and Asian exposure–response data for ozone effects on crop yields. Atmospheric Environment. 2009; 43:1945-1953.

Espanany A, Fallah S, Tadayyon A. Seed priming improves seed germination and reduces oxidative stress in black cumin (*Nigella sativa*) in presence of cadmium. Industrial Crops and Products. 2016; 79:195-204.

Esringu A, Aksakal O, Tabay D, Kara AA. Effects of sodium nitroprusside (SNP) pretreatment on UV-B stress tolerance in lettuce (*Lactuca sativa* L.) seedlings. Environmental Science and Pollution Research. 2016; 23:589-597.

Fang S, Gao K, Hu W, Snider JL, Wang S, Chen B, Zhou Z. Chemical priming of seed alters cotton floral bud differentiation by inducing changes in hormones, metabolites and gene expression. Plant Physiology and Biochemistry. 2018; 130:633-640.

Farhoudi R, Hussain M, Dong-Jin L. Modulation of enzymatic antioxidants improves the salinity resistance in canola (*Brassica napus*). International Journal of Agriculture and Biology. 2012; 14: 465-468.

Farooq M, Wahid A, Kobayashi N, Fujita DB, Basra SM. Plant drought stress: effects, mechanisms and management. In: Sustainable Agriculture. 2009; 153-188. Springer, Dordrecht.

Faseela P, Puthur JT. The imprints of the high light and UV-B stresses in *Oryza sativa* L. 'Kanchana' seedlings are differentially modulated. Journal of Photochemistry and Photobiology B: Biology. 2018; 178:551-559.

Faseela P, Sinisha AK, Brestič M, Puthur JT. Chlorophyll *a* fluorescence parameters as indicators of a particular abiotic stress in rice. Photosynthetica. 2019; 57:108-115.

Fathali H, Dunevall J, Majdi S, Cans AS. Extracellular osmotic stress reduces the vesicle size while keeping a constant neurotransmitter concentration. ACS Chemical Neuroscience. 2017; 8:368-375.

Fathi A, Tari DB. Effect of drought stress and its mechanism in plants. International Journal of Life Sciences. 2016; 10:1-6.

Filippou P, Bouchagier P, Skotti E, Fotopoulos V. Proline and reactive oxygen/nitrogen species metabolism is involved in the tolerant response of the invasive plant species *Ailanthus altissima* to drought and salinity. Environmental and Experimental Botany. 2014; 97:1-12.

Flowers TJ, Garcia A, Koyama M, Yeo AR. Breeding for salt tolerance in crop plants-the role of molecular biology. Acta Physiologiae Plantarum. 1997; 19:427-433.

Flowers TJ, Koyama ML, Flowers SA, Sudhakar C, Singh KP, Yeo AR. QTL: their place in engineering tolerance of rice to salinity. Journal of Experimental Botany. 2000; 51:99-106.

Folin O, Denis W. A colorimetric method for the determination of phenols (and phenol derivatives) in urine. Journal of Biological Chemistry. 1915; 22:305-318.

Fotouh AMM, Moawad FG, El-Naggar HA, El-Din MT, Eldeen HS. Influence of seed treatment with UV-C on saline stress tolerance in green beans (*Phaseolus vulgaris* L.). Environmental Science and Technology. 2014; 9:391-414.

Foyer CH. Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. Environmental and Experimental Botany. 2018; 154:134-142.

Galić V, Mazur M, Šimić D, Zdunić Z, Franić M. Plant biomass in salt-stressed young maize plants can be modelled with photosynthetic performance. Photosynthetica. 2019; 57:9-19.

García AC, Berbara RL, Farías LP, Izquierdo FG, Hernández OL, Campos RH, Castro RN. Humic acids of vermicompost as an ecological pathway to increase resistance of rice seedlings to water stress. African Journal of Biotechnology. 2012; 11:3125-3134.

García-Cristobal J, García-Villaraco A, Ramos B, Gutierrez-Mañero J, Lucas JA. Priming of pathogenesis related-proteins and enzymes related to oxidative stress by plant growth promoting rhizobacteria on rice plants upon abiotic and biotic stress challenge. Journal of Plant Physiology. 2015; 188:72-79.

García-Morales S, Gómez-Merino FC, Trejo-Téllez LI. NAC transcription factor expression, amino acid concentration and growth of elite rice cultivars upon salt stress. Acta Physiologiae Plantarum. 2014; 36:1927-1936.

Gaspar T, Penel C, Greppin H. Peroxidase and isoperoxidase in relation to root and flower formation. Plant Biochemistry of Journal. 1975; 2:33-47.

Ghassemi-Golezani K, Lotfi R. The impact of salicylic acid and silicon on chlorophyll *a* fluorescence in mung bean under salt stress. Russian Journal of Plant Physiology. 2015; 62:611-616.

Ghezal N, Rinez I, Sbai H, Saad I, Farooq M, Rinez A, Zribi I, Haouala R. Improvement of *Pisum sativum* salt stress tolerance by bio-priming their seeds using *Typha angustifolia* leaves aqueous extract. South African Journal of Botany. 2016; 105:240-250.

Giannopolitis CN, Ries SK. Superoxide dismutases: Occurrence in higher plants. Plant Physiology. 1977; 59:309-314.

Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry. 2010; 48:909-930.

Godlee F. Dangers of ozone depletion. BMJ: British Medical Journal. 1991; 303:1326-1339.

Golldack D, Li C, Mohan H, Probst N. Tolerance to drought and salt stress in plants: unraveling the signaling networks. Frontiers in Plant Science. 2014; 5:151-161.

Gomes FP, Oliva MA, Mielke MS, Almeida AA, Aquino LA. Osmotic adjustment, proline accumulation and cell membrane stability in leaves of *Cocos nucifera* submitted to drought stress. Scientia Horticulturae. 2010; 126:379-384.

Guajardo-Flores D, Serna-Guerrero D, Serna-Saldívar SO, Jacobo-Velázquez DA. Effect of germination and UV-C radiation on the accumulation of flavonoids and saponins in black bean seed coats. Cereal Chemistry. 2014; 91:276-289.

Guidi L, Degl'Innocenti E, Remorini D, Biricolti S, Fini A, Ferrini F, Nicese FP, Tattini M. The impact of UV-radiation on the physiology and biochemistry of *Ligustrum vulgare* exposed to different visible-light irradiance. Environmental and Experimental Botany. 2011; 70:88-95.

Gull A, Lone AA, Wani NU. Biotic and Abiotic Stresses in Plants. In: Abiotic and Biotic Stress in Plants. 2019; 7-29. Intech Open.

Gupta A, Dadlani M, Arun Kumar MB, Roy M, Naseem M, Choudhary VK, Maiti RK. Seed priming: the aftermath. International Journal of Agriculture Environment and Biotechnology. 2008; 1:199-209.

Gupta B, Huang B. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. International Journal of Genomics. 2014; 2014:18-30.

Gururani MA, Venkatesh J, Tran LS. Regulation of photosynthesis during abiotic stress-induced photoinhibition. Molecular Plant. 2015; 8:1304-1320.

Gust AA, Brunner F, Nürnberger T. Biotechnological concepts for improving plant innate immunity. Current Opinion in Biotechnology. 2010; 21:204-210.

Hakim MA, Juraimi AS, Hanafi MM, Ismail MR, Selamat A, Rafii MY, Latif MA. Biochemical and anatomical changes and yield reduction in rice (*Oryza sativa* L.) under varied salinity regimes. Bio Med Research International. 2014; 2014:6-17.

Hamid N, Jawaid F. Influence of seed pre-treatment by UV-A and UV-C radiation on germination and growth of mung beans. Pakistan Journal of Chemistry. 2011; 1:164-167.

Hasanuzzaman M, Fujita M. Exogenous silicon treatment alleviates salinity-induced damage in *Brassica napus* L. seedlings by up-regulating the antioxidant defense and methylglyoxal detoxification system. American Society of Plant Biology. 2011a; 8:1061-1074.

Hasanuzzaman M, Fujita M. Selenium pretreatment upregulates the antioxidant defense and methylglyoxal detoxification system and confers enhanced tolerance to drought stress in rapeseed seedlings. Biological Trace Element Research. 2011b; 143:1758-1776.

Hasanuzzaman M, Nahar K, Alam M, Roychowdhury R, Fujita M. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. International Journal of Molecular Sciences. 2013; 14:9643-9684.

Hasanuzzaman M, Nahar K, Hossain M, Mahmud JA, Rahman A, Inafuku M, Oku H, Fujita M. Coordinated actions of glyoxalase and antioxidant defense systems in conferring abiotic stress tolerance in plants. International Journal of Molecular Sciences. 2017; 18:200-228.

Hassan N, Ebeed H, Aljaarany A. Exogenous application of spermine and putrescine mitigate adversities of drought stress in wheat by protecting membranes and chloroplast ultra-structure. Physiology and Molecular Biology of Plants. 2020; 26:233-245.

Hassannejad S, Porheidar Ghafarbi S. Assessment of some chlorophyll *a* fluorescence parameters of different corn cultivars in response to

clodinafop-propagrgyl herbicide and salicylic acid. Journal of Plant Physiology and Breeding. 2018; 8:47-57.

Hayat S, Hasan SA, Yusuf M, Hayat Q, Ahmad A. Effect of 28-homobrassinolide on photosynthesis, fluorescence and antioxidant system in the presence or absence of salinity and temperature in *Vigna radiata*. Environmental and Experimental Botany. 2010; 69:105-112.

He M, He CQ, Ding NZ. Abiotic stresses: general defenses of land plants and chances for engineering multistress tolerance. Frontiers in Plant Science. 2018; 9:1771-1786.

He Y, Zhan F, Zu Y, Liu C, Li Y. Effect of elevated UV-B radiation on the antioxidant system of two rice landraces in paddy fields on Yuanyang Terrace. International Journal of Agriculture and Biology. 2014; 16:585-590.

Heath RL, Packer L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics. 1968; 125:189-198.

Hideg É, Jansen MA, Strid Å. UV-B exposure, ROS, and stress: inseparable companions or loosely linked associates?. Trends in Plant Science. 2013; 18:107-115.

Hidema J, Teranishi M, Iwamatsu Y, Hirouchi T, Ueda T, Sato T, Burr B, Sutherland BM, Yamamoto K, Kumagai T. Spontaneously occurring mutations in the cyclobutane pyrimidine dimer photolyase gene cause different sensitivities to ultraviolet-B in rice. The Plant Journal. 2005; 43:57-67.

Hilker M, Schwachtje J, Baier M, Balazadeh S, Bäurle I, Geiselhardt S, Hincha DK, Kunze R, Mueller-Roeber B, Rillig MC, Rolff J. Priming

and memory of stress responses in organisms lacking a nervous system. Biological Reviews. 2016; 91:1118-1133.

Hossain MA, Bhattacharjee S, Armin SM, Qian P, Xin W, Li HY, Burritt DJ, Fujita M, Tran LS. Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: insights from ROS detoxification and scavenging. Frontiers in Plant Science. 2015; 6:420-432.

Hossain MA, Nakano Y, Asada K. Monodehydroascorbate reductase in spinach chloroplasts and its participation in regeneration of ascorbate for scavenging hydrogen peroxide. Plant and Cell Physiology. 1984; 25:385-395.

Hossain MS, Dietz KJ. Tuning of redox regulatory mechanisms, reactive oxygen species and redox homeostasis under salinity stress. Frontiers in Plant Science. 2016; 7:548-567.

Huang S, Van Aken O, Schwarzländer M, Belt K, Millar AH. The roles of mitochondrial reactive oxygen species in cellular signaling and stress response in plants. Plant Physiology. 2016; 171:1551-1559.

Hura T, Grzesiak S, Hura K, Thiemt E, Tokarz K, Wędzony M. Physiological and biochemical tools useful in drought-tolerance detection in genotypes of winter triticale: accumulation of ferulic acid correlates with drought tolerance. Annals of Botany. 2007; 100:767-775.

Hussain M, Farooq M, Sattar A, Ijaz M, Sher A, Ul-Allah S. Mitigating the adverse effects of drought stress through seed priming and seed quality on wheat (*Triticum aestivum* L.) productivity. Pakistan Journal of Agricultural Sciences. 2018; 55-69.

Hussain S, Khan F, Hussain HA, Nie L. Physiological and biochemical mechanisms of seed priming-induced chilling tolerance in rice cultivars. Frontiers in Plant Science. 2016; 27:116-132.

Hussain S, Rao MJ, Anjum MA, Ejaz S, Zakir I, Ali MA, Ahmad N, Ahmad S. Oxidative stress and antioxidant defense in plants under drought conditions. In: Plant abiotic stress tolerance. 2019; 207-219. Springer, Cham.

Ibrahim EA. Seed priming to alleviate salinity stress in germinating seeds. Journal of Plant Physiology. 2016; 192:38-46.

Inostroza-Blancheteau C, Acevedo P, Loyola R, Arce-Johnson P, Alberdi M, Reyes-Díaz M. Short-term UV-B radiation affects photosynthetic performance and antioxidant gene expression in highbush blueberry leaves. Plant Physiology and Biochemistry. 2016; 107:301-319.

Iqbal M, Ashraf M. Changes in growth, photosynthetic capacity and ionic relations in spring wheat (*Triticum aestivum* L.) due to pre-sowing seed treatment with polyamines. Plant Growth Regulation. 2005; 46:19-30.

Irani S, Todd CD. Exogenous allantoin increases *Arabidopsis* seedlings tolerance to NaCl stress and regulates expression of oxidative stress response genes. Journal of Plant Physiology. 2018; 221:43-50.

İşeri ÖD, Sahin FI, Haberal M. Sodium chloride priming improves salinity response of tomato at seedling stage. Journal of Plant Nutrition. 2014; 37:374-392.

Jafarinia M, Shariati M. Effects of salt stress on photosystem II of canola plant (*Barassica napus,* L.) probing by chlorophyll *a* fluorescence measurements. Iranian Journal of Science and Technology. 2012; 36:71-76.

Jaleel CA, Manivannan PA, Wahid A, Farooq M, Al-Juburi HJ, Somasundaram RA, Panneerselvam R. Drought stress in plants: a review on morphological characteristics and pigments composition. International Journal of Agriculture and Biology. 2009; 11:100-115.

Jantschitsch C, Trautinger F. Heat shock and UV-B-induced DNA damage and mutagenesis in skin. Photochemical & Photobiological Sciences. 2003; 2:899-903.

Javadmanesh S, Rahmani F, Pourakbar L. UV-B radiation, soil salinity, drought stress and their concurrent effects on some physiological parameters in mize plant. American-Eurasian Journal of Toxicological Sciences. 2012; 4:154-164.

Jenkins GI. Signal transduction in responses to UV-B radiation. Annual Review of Plant Biology. 2009; 60: 407-431.

Jeun YC, Park KS, Kim CH, Fowler WD, Kloepper JW. Cytological observations of cucumber plants during induced resistance elicited by rhizobacteria. Biological Control. 2004; 29:34-42.

Jisha KC, Puthur JT. Halopriming of seeds imparts tolerance to NaCl and PEG induced stress in *Vigna radiata* (L.) Wilczek varieties. Physiology and Molecular Biology of Plants. 2014a; 20:303-312.

Jisha KC, Puthur JT. Seed halopriming outdo hydropriming in enhancing seedling vigor and osmotic stress tolerance potential of rice varieties. Journal of Crop Science and Biotechnology. 2014b; 17:209-19.

Jisha KC, Puthur JT. Seed priming with BABA (β-amino butyric acid): a cost-effective method of abiotic stress tolerance in *Vigna radiata* (L.) Wilczek. Protoplasma. 2016a; 253:277-289.

Jisha KC, Puthur JT. Seed priming with β-amino butyric acid improves abiotic stress tolerance in rice seedlings. Rice Science. 2016b; 23:242-254.

Jisha KC, Vijayakumari K, Puthur JT. Seed priming for abiotic stress tolerance: an overview. Acta Physiologiae Plantarum. 2013; 35:1381-1396.

Jisha KC. Studies on the influence of various seed priming methods for biotic stress tolerance in *Oryza sativa* L. and *Vigna radiata* (L.) Wilczek. Ph D. Thesis, University of Calicut. 2014; 75-160.

Junglee S, Urban L, Sallanon H, Lopez-Lauri F. Optimized assay for hydrogen peroxide determination in plant tissue using potassium iodide. American Journal of Analytical Chemistry. 2014; 5:730-745.

Kacharava N, Chanishvili S, Badridze G, Chkhubianishvili E, Janukashvili N. Effect of seed irradiation on the content of antioxidants in leaves of Kidney bean, Cabbage and Beet cultivars. Australian Journal of Crop Science. 2009; 3:137-149.

Kakani VG, Reddy KR, Zhao D, Sailaja K. Field crop responses to ultraviolet-B radiation: a review. Agricultural and Forest Meteorology. 2003; 120:191-218.

Kalaji HM, Bosa K, Kościelniak J, Żuk-Gołaszewska K. Effects of salt stress on photosystem II efficiency and CO_2 assimilation of two Syrian barley landraces. Environmental and Experimental Botany. 2011; 73:64-72.

Kalaji HM, Rastogi A, Živčák M, Brestic M, Daszkowska-Golec A, Sitko K, Alsharafa KY, Lotfi R, Stypiński P, Samborska IA, Cetner MD. Prompt chlorophyll fluorescence as a tool for crop phenotyping: an

example of barley landraces exposed to various abiotic stress factors. Photosynthetica. 2018; 56:953-961.

Kalaji HM, Schansker G, Brestic M, Bussotti F, Calatayud A, Ferroni L, Goltsev V, Guidi L, Jajoo A, Li P, Losciale P. Frequently asked questions about chlorophyll fluorescence, the sequel. Photosynthesis Research. 2017; 132:13-66.

Kang JS, Singh H, Singh G. Abiotic stress and its amelioration in cereals and pulses: a review. International Journal of Current Microbiology and Applied Sciences. 2017; 6:1019-1045.

Kanwal S, Ilyas N, Shabir S, Saeed M, Gul R, Zahoor M, Batool N, Mazhar R. Application of biochar in mitigation of negative effects of salinity stress in wheat (*Triticum aestivum* L.). Journal of Plant Nutrition. 2018; 41:526-538.

Kar M, Mishra D. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. Plant Physiology. 1976; 57:315-329.

Kataria S, Jain M, Kanungo M, Sharma S. Wheat responses and tolerance to UV-B radiation: An overview. In: Wheat production in changing environments. 2019; 175-196. Springer, Singapore.

Kataria S, Jajoo A, Guruprasad KN. Impact of increasing ultraviolet-B (UV-B) radiation on photosynthetic processes. Journal of Photochemistry and Photobiology B: Biology. 2014; 137:55-66.

Katerova Z, Todorova D. Effect of enhanced UV-C irradiation on the growth, malondialdehyde, hydrogen peroxide, free proline, polyamines, IAA and IAA-oxidase activity in pea plants (*Pisum sativum* L.). Comptes Rendus de l'Académie Bulgare des Sciences. 2011; 64:1555-1562.

Kaya C, Ashraf M, Sönmez O. Promotive effect of exogenously applied thiourea on key physiological parameters and oxidative defense mechanism in salt-stressed *Zea mays* L. plants. Turkish Journal of Botany. 2015; 39:786-795.

Kaya C, Sonmez O, Aydemir S, Ashraf M, Dikilitas M. Exogenous application of mannitol and thiourea regulates plant growth and oxidative stress responses in salt-stressed maize (*Zea mays* L.). Journal of Plant Interactions. 2013; 8:234-241.

Kazan K. Diverse roles of jasmonates and ethylene in abiotic stress tolerance. Trends in Plant Science. 2015; 20:219-229.

Keunen EL, Peshev D, Vangronsveld J, Van Den Ende WI, Cuypers AN. Plant sugars are crucial players in the oxidative challenge during abiotic stress: extending the traditional concept. Plant, Cell and Environment. 2013; 36:1242-1255.

Khaliq A, Aslam F, Matloob A, Hussain S, Geng M, Wahid A, ur Rehman H. Seed priming with selenium: consequences for emergence, seedling growth, and biochemical attributes of rice. Biological Trace Element Research. 2015; 166:236-244.

Khan MM, Al-Mas'oudi RS, Al-Said F, Khan I. Salinity effects on growth, electrolyte leakage, chlorophyll content and lipid peroxidation in cucumber (*Cucumis sativus* L.). In International Conference on Food and Agricultural Sciences Malaysia. 2013; 55:28-32. IACSIT Press.

Kholova J, Sairam RK, Meena RC. Osmolytes and metal ions accumulation, oxidative stress and antioxidant enzymes activity as determinants of salinity stress tolerance in maize genotypes. Acta Physiologiae Plantarum. 2010; 32:477-486.

Khoshbakht D, Asghari MR, Haghighi M. Influence of foliar application of polyamines on growth, gas-exchange characteristics, and chlorophyll fluorescence in Bakraii citrus under saline conditions. Photosynthetica. 2018; 56:731-742.

Köhler H, Contreras RA, Pizarro M, Cortés-Antíquera R, Zúñiga GE. Antioxidant responses induced by UV-B radiation in *Deschampsia antarctica* Desv. Frontiers in Plant Science. 2017; 8:921-943.

Kollöffel C. Respiration rate and mitochondrial activity in the cotyledons of *Pisum sativum* L. during germination. Acta Botánica Neerlandica. 1967; 16:111-122.

Konuşkan Ö, Gözübenli H, Atiş İ, Atak M. Effects of salinity stress on emergence and seedling growth parameters of some maize genotypes (*Zea mays* L.). Turkish Journal of Agriculture Food Science and Technology. 2017; 5:1668-1672.

Kosová K, Vítámvás P, Prášil IT, Renaut J. Plant proteome changes under abiotic stress—contribution of proteomics studies to understanding plant stress response. Journal of Proteomics. 2011; 74:1301-1322.

Koti S, Reddy KR, Reddy VR, Kakani VG, Zhao D. Interactive effects of carbon dioxide, temperature, and ultraviolet-B radiation on soybean (*Glycine max* L.) flower and pollen morphology, pollen production, germination, and tube lengths. Journal of Experimental Botany. 2005; 56:725-736.

Kovács Z, Simon-Sarkadi L, Vashegyi I, Kocsy G. Different accumulation of free amino acids during short-and long-term osmotic stress in wheat. The Scientific World Journal. 2012; 2012:10-21.

Koyro H W, Ahmad P, Geissler N. Abiotic stress responses in plants: an overview. In Environmental adaptations and stress tolerance of plants in the era of climate change. 2012; 1-28. Springer, New York, NY.

Kubala S, Garnczarska M, Wojtyla Ł, Clippe A, Kosmala A, Żmieńko A, Lutts S, Quinet M. Deciphering priming-induced improvement of rapeseed (*Brassica napus* L.) germination through an integrated transcriptomic and proteomic approach. Plant Science. 2015; 231:94-113.

Kumar J, Singh S, Singh M, Srivastava PK, Mishra RK, Singh VP, Prasad SM. Transcriptional regulation of salinity stress in plants: A short review. Plant Gene. 2017; 11:160-169.

Kumari R, Agrawal SB. Supplemental UV-B induced changes in leaf morphology, physiology and secondary metabolites of an Indian aromatic plant *Cymbopogon citratus* (DC) Staph under natural field conditions. International Journal of Environmental Studies. 2010; 67:655-675.

Łabanowska M, Kurdziel M, Filek M. Changes of paramagnetic species in cereal grains upon short-term ozone action as a marker of oxidative stress tolerance. Journal of Plant Physiology. 2016; 190:54-66.

Larson RA. The antioxidants of higher plants. Phytochemistry. 1988; 27:969-978.

León-Chan RG, López-Meyer M, Osuna-Enciso T, Sañudo-Barajas JA, Heredia JB, León-Félix J. Low temperature and ultraviolet-B radiation affect chlorophyll content and induce the accumulation of UV-B-absorbing and antioxidant compounds in bell pepper (*Capsicum*

annuum) plants. Environmental and Experimental Botany. 2017; 139:143-151.

Lepeduš H, Brkić I, Cesar V, Jurković V, Antunović J, Jambrović A, Brkić J, Šimić D. Chlorophyll fluorescence analysis of photosynthetic performance in seven maize inbred lines under water-limited conditions. Periodicum Biologorum. 2012; 114:73-76.

Li J, Ban L, Wen H, Wang Z, Dzyubenko N, Chapurin V, Gao H, Wang X. An aquaporin protein is associated with drought stress tolerance. Biochemical and Biophysical Research Communications. 2015; 459:208-213.

Li J, Yang L, Jin D, Nezames CD, Terzaghi W, Deng XW. UV-B-induced photomorphogenesis in *Arabidopsis*. Protein and Cell. 2013; 4:485-492.

Li X, Yun J, Fan X, Xing Y, Tang Y. Effect of 1-methylcyclopropene and modified atmosphere packaging on chilling injury and antioxidative defensive mechanism of sweet pepper. African Journal of Biotechnology. 2011; 10: 6581-6589.

Li X, Zhang L. SA and PEG-induced priming for water stress tolerance in rice seedling. In: Information technology and agricultural engineering. 2012; 881-887. Springer, Berlin, Heidelberg.

Li Z, Han X, Song X, Zhang Y, Jiang J, Han Q, Liu M, Qiao G, Zhuo R. Overexpressing the *Sedum alfredii* Cu/Zn superoxide dismutase increased resistance to oxidative stress in transgenic *Arabidopsis*. Frontiers in Plant Science. 2017; 8:1010-1036.

Li Z, Lu GY, Zhang XK, Zou CS, Cheng Y, Zheng PY. Improving drought tolerance of germinating seeds by exogenous application of gibberellic

acid (GA_3) in rapeseed (*Brassica napus* L.). Seed Science and Technology. 2010; 38:432-440.

Lidon FJ, Teixeira M, Ramalho JC. Decay of the chloroplast pool of ascorbate switches on the oxidative burst in UV-B-irradiated rice. Journal of Agronomy and Crop Science. 2012; 198:130-144.

Lim JH, Kim SD. Induction of drought stress resistance by multi-functional PGPR *Bacillus licheniformis* K11 in pepper. The Plant Pathology Journal. 2013; 29:201-219.

Liu NY, Ko SS, Yeh KC, Charng YY. Isolation and characterization of tomato Hsa32 encoding a novel heat-shock protein. Plant science. 2006; 170(5):976-985.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry. 1951; 193:265-275.

Lutts S, Benincasa P, Wojtyla L, Kubala S, Pace R, Lechowska K, Quinet M, Garnczarska M. Seed priming: new comprehensive approaches for an old empirical technique. In New challenges in seed biology-basic and translational research driving seed technology. 2016; 12:1-46.

Lutts S, Kinet JM, Bouharmont J. NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Annals of Botany. 1996; 78:389-398.

Ma LJ, Wang LL, Mei YX, Zhang SW, Wei W, Wang JY, Zhang YL. Cross adaptation tolerance in rice seedlings exposed to PEG induced salinity and drought stress. International Journal of Agriculture and Biology. 2016; 18:535-541.

Mafakheri A, Siosemardeh AF, Bahramnejad B, Struik PC, Sohrabi Y. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Australian Journal of Crop Science. 2010; 4:580-594.

Maghsoudi K, Emam Y, Pessarakli M. Effect of silicon on photosynthetic gas exchange, photosynthetic pigments, cell membrane stability and relative water content of different wheat cultivars under drought stress conditions. Journal of Plant Nutrition. 2016; 39:1001-1015.

Mahajan S, Tuteja N. Cold, salinity and drought stresses: an overview. Archives of Biochemistry and Biophysics. 2005; 444:139-158.

Maiti R, Pramanik K. Vegetable seed priming: a low cost, simple and powerful techniques for farmers' livelihood. International Journal of Bio-resource and Stress Management. 2013; 4:475-481.

Makbul S, Güler NS, Durmuş N, Güven S. Changes in anatomical and physiological parameters of soybean under drought stress. Turkish Journal of Botany. 2011; 35:369-377.

Manaf HH, Rabie KA, Abd El-Aal MS. Impact of UV-B radiation on some biochemical changes and growth parameters in *Echinacea purpurea* callus and suspension culture. Annals of Agricultural Sciences. 2016; 61:207-216.

Mancinelli AL, Yang CP, Lindquist P, Anderson OR, Rabino I. Photocontrol of anthocyanin synthesis: The action of streptomycin on the synthesis of chlorophyll and anthocyanin. Plant Physiology. 1975; 55:251-257.

Manickavelu A, Nadarajan N, Ganesh SK, Gnanamalar RP, Babu RC. Drought tolerance in rice: morphological and molecular genetic consideration. Plant Growth Regulation. 2006; 50:121-138.

Martinez V, Nieves-Cordones M, Lopez-Delacalle M, Rodenas R, Mestre TC, Garcia-Sanchez F, Rubio F, Nortes PA, Mittler R, Rivero RM. Tolerance to stress combination in tomato plants: new insights in the protective role of melatonin. Molecules. 2018; 23:535-555.

Maswada HF, Sunoj VJ, Prasad PV. A comparative study on the effect of seed pre-sowing treatments with microwave radiation and salicylic acid in alleviating the drought-induced damage in wheat. Journal of Plant Growth Regulation. 2020; 3:1-9.

Mathobo R, Marais D, Steyn JM. The effect of drought stress on yield, leaf gaseous exchange and chlorophyll fluorescence of dry beans (*Phaseolus vulgaris* L.). Agricultural Water Management. 2017; 180:118-125.

Mathur S, Jajoo A, Mehta P, Bharti S. Analysis of elevated temperature-induced inhibition of photosystem II using chlorophyll *a* fluorescence induction kinetics in wheat leaves (*Triticum aestivum*). Plant Biology. 2011; 13:1-6.

Mazza CA, Giménez PI, Kantolic AG, Ballaré CL. Beneficial effects of solar UV-B radiation on soybean yield mediated by reduced insect herbivory under field conditions. Physiologia Plantarum. 2013; 147:307-315.

McGrann GR, Brown JK. The role of reactive oxygen in the development of Ramularia leaf spot disease in barley seedlings. Annals of Botany. 2018; 121:415-430.

McKenzie RL, Aucamp PJ, Bais AF, Björn LO, Ilyas M, Madronich S. Ozone depletion and climate change: impacts on UV radiation. Photochemical and Photobiological Sciences. 2011; 10:182-198.

Mehta P, Jajoo A, Mathur S, Bharti S. Chlorophyll *a* fluorescence study revealing effects of high salt stress on Photosystem II in wheat leaves. Plant Physiology and Biochemistry. 2010; 48:16-20.

Mei YQ, Song SQ. Cross-tolerance is associated with temperature and salinity stress during germination of barley seeds. Seed Science and Technology. 2008; 36:689-698.

Meng LL, Song JF, Wen J, Zhang J, Wei JH. Effects of drought stress on fluorescence characteristics of photosystem II in leaves of *Plectranthus scutellarioides*. Photosynthetica. 2016; 54:414-421.

Miller GA, Suzuki N, Ciftci-Yilmaz SU, Mittler RO. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant, Cell & Environment. 2010; 33:453-467.

Ming DF, Pei ZF, Naeem MS, Gong HJ, Zhou WJ. Silicon alleviates PEG-induced water-deficit stress in upland rice seedlings by enhancing osmotic adjustment. Journal of Agronomy and Crop Science. 2012; 198:14-26.

Mirecki RM, Teramura AH. Effects of ultraviolet-B irradiance on soybean: The dependence of plant sensitivity on the photosynthetic photon flux density during and after leaf expansion. Plant Physiology. 1984; 74:475-480.

Mishra V, Srivastava G, Prasad SM, Abraham G. Growth, photosynthetic pigments and photosynthetic activity during seedling stage of cowpea (Vigna unguiculata) in response to UV-B and dimethoate. Pesticide Biochemistry and Physiology. 2008; 92:30-37.

Mishra V, Srivastava G, Prasad SM. Antioxidant response of bitter gourd (*Momordica charantia* L.) seedlings to interactive effect of dimethoate and UV-B irradiation. Scientia Horticulturae. 2009; 120:373-378.

Mittal S, Kumari N, Sharma V. Differential response of salt stress on *Brassica juncea*: photosynthetic performance, pigment, proline, D1 and antioxidant enzymes. Plant Physiology and Biochemistry. 2012; 54:17-26.

Mohammadi GR. The influence of NaCl priming on seed germination and seedling growth of canola (*Brassica napus* L.) under salinity conditions. American-Eurasian Journal of Agricultural and Environmental Science. 2009; 5:696-700.

Mohammadi L, Shekari F, Saba J, Zangani E. Effects of priming with salicylic acid on safflower seedlings photosynthesis and related physiological parameters. Journal of Plant Physiology and Breeding. 2017; 7:1-3.

Mohammadkhani N, Heidari R. Effects of water stress on respiration, photosynthetic pigments and water content in two maize cultivars. Pakistan Journal of Biological Sciences. 2007; 10:4022-4038.

Mohammed AR, Tarpley L. Differential response of Southern US rice (*Oryza sativa* L.) cultivars to ultraviolet-B radiation. Journal of Agronomy and Crop Science. 2010; 196:286-295.

Mohammed AR, Tarpley L. Effects of enhanced ultraviolet-B (UV-B) radiation and antioxidative-type plant growth regulators on Rice (*Oryza sativa* L.) leaf photosynthetic rate, photochemistry and physiology. Journal of Agricultural Science. 2013; 5:115-121.

Mohammed AR, Tarpley L. Rice Responses and Tolerance to Ultraviolet-B (UV-B) Radiation: Plant Growth Regulators Provide a Management Option. In: Advances in Rice Research for Abiotic Stress Tolerance. 2019; 709-724. Woodhead Publishing.

Mohammed RK, Ibrahim KM. Cytological effects of mutagenic agents and nacl on mitotic division in two iraqi rice (*Oryza sativa* L.) Genotypes. Al-Nahrain Journal of Science. 2017; 20:114-119.

Moore S, Stein WH. Photometric nin-hydrin method for use in the chromatography of amino acids. Journal of Biological Chemistry. 1948;176:367-388.

Moulick D, Ghosh D, Santra SC. Evaluation of effectiveness of seed priming with selenium in rice during germination under arsenic stress. Plant Physiology and Biochemistry. 2016; 109:571-578.

Mouradi M, Bouizgaren A, Farissi M, Makoudi B, Kabbadj A, Very AA, Sentenac H, Qaddoury A, Ghoulam C. Osmopriming improves seeds germination, growth, antioxidant responses and membrane stability during early stage of *Moroccan alfalfa* populations under water deficit. Chilean Journal of Agricultural Research. 2016; 76:265-272.

Müller T, Lentzsch P, Müller ME. Carbohydrate dynamics in leaves of rapeseed (*Brassica napus*) under drought. Journal of Agronomy and Crop Science. 2012; 198:207-217.

Müller-Xing R, Xing Q, Goodrich J. Footprints of the sun: memory of UV and light stress in plants. Frontiers in Plant Science. 2014; 5:474-482.

Nahar S, Kalita J, Sahoo L, Tanti B. Morphophysiological and molecular effects of drought stress in rice. Annals of Plant Sciences. 2016; 5:1409-1416.

Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology. 1981; 22:867-880.

Nawaz F, Ashraf MY, Ahmad R, Waraich EA. Selenium (Se) seed priming induced growth and biochemical changes in wheat under water deficit conditions. Biological Trace Element Research. 2013; 151:284-293.

Neelamegam R, Sutha T. UV-C irradiation effect on seed germination, seedling growth and productivity of groundnut (*Arachis hypogaea* L.). Internatioonal Journal of Current Microbiology and Applied Science. 2015; 4:430-443.

Negrão S, Schmöckel SM, Tester M. Evaluating physiological responses of plants to salinity stress. Annals of Botany. 2017; 119:1-11.

Nishanth T, Praseed KM, Kumar MS, Valsaraj KT. Observational study of surface O_3, NOx, CH_4 and total NMHCs at Kannur, India. Aerosol and Air Quality Research. 2013; 14:1074-1088.

Noorhosseini SA, Jokar NK, Damalas CA. Improving seed germination and early growth of garden cress (*Lepidium sativum*) and basil (*Ocimum basilicum*) with hydro-priming. Journal of Plant Growth Regulation. 2018; 37:323-334.

Noreen S, Siddiq A, Hussain K, Ahmad S, Hasanuzzaman M. Foliar application of salicylic acid with salinity stress on physiological and biochemical attributes of sunflower (*Helianthus annuus* L.) crop. Acta Scientiarum Polonorum-Hortorum Cultus. 2017; 16:57-74.

Nouman W, Siddiqui MT, Basra SM, Afzal I, Rehman HU. Enhancement of emergence potential and stand establishment of *Moringa oleifera* Lam.

by seed priming. Turkish Journal of Agriculture and Forestry. 2012; 36:227-235.

Olsson LC, Veit M, Weissenböck G, Bornman JF. Differential flavonoid response to enhanced UV-B radiation in *Brassica napus*. Phytochemistry. 1998; 49:1021-1028.

Ouhibi C, Attia H, Rebah F, Msilini N, Chebbi M, Aarrouf J, Urban L, Lachaal M. Salt stress mitigation by seed priming with UV-C in lettuce plants: Growth, antioxidant activity and phenolic compounds. Plant Physiology and Biochemistry. 2014; 83:126-133.

Oukarroum A, Schansker G, Strasser RJ. Drought stress effects on photosystem I content and photosystem II thermotolerance analyzed using Chl *a* fluorescence kinetics in barley varieties differing in their drought tolerance. Physiologia Plantarum. 2009; 137:188-199.

Palma JM, Sevilla F, Jiménez A, del Río LA, Corpas FJ, Álvarez de Morales P, Camejo DM. Physiology of pepper fruit and the metabolism of antioxidants: chloroplasts, mitochondria and peroxisomes. Annals of Botany. 2015; 116:627-636.

Pan XL, Zhang DY, Li L. Responses of photosystem II of white elm to UV-B radiation monitored by OJIP fluorescence transients. Russian Journal of Plant Physiology. 2011; 58:864-879.

Pandey M, Srivastava AK, Suprasanna P, D'Souza SF. Thiourea mediates alleviation of UV-B stress-induced damage in the Indian mustard (*Brassica juncea* L.). Journal of Plant Interactions. 2012; 7:143-150.

Pandey V, Shukla A. Acclimation and tolerance strategies of rice under drought stress. Rice Science. 2015; 22:147-161.

Pandolfi C, Mancuso S, Shabala S. Physiology of acclimation to salinity stress in pea (*Pisum sativum*). Environmental and Experimental Botany. 2012; 84:44-51.

Paparella S, Araújo SS, Rossi G, Wijayasinghe M, Carbonera D, Balestrazzi A. Seed priming: state of the art and new perspectives. Plant Cell Reports. 2015; 34:1281-1293.

Parida AK, Jha B. Inductive responses of some organic metabolites for osmotic homeostasis in peanut (*Arachis hypogaea* L.) seedlings during salt stress. Acta Physiologiae Plantarum. 2013; 35:2821-2832.

Pastor V, Luna E, Mauch-Mani B, Ton J, Flors V. Primed plants do not forget. Environmental and Experimental Botany. 2013; 94:46-56.

Patade VY, Bhargava S, Suprasanna P. Halopriming imparts tolerance to salt and PEG induced drought stress in sugarcane. Agriculture, Ecosystems and Environment. 2009; 134:24-28.

Pathak K, Kataria S, Gadre R. Trending methods to enhance antioxidant activities in wheat. In wheat production in changing environments. 2019; 241-260. Springer, Singapore.

Paul S, Roychoudhury A. Seed priming with spermine ameliorates salinity stress in the germinated seedlings of two rice cultivars differing in their level of salt tolerance. Tropical Plant Research2016; 3:616-633.

Perdomo JA, Capó-Bauçà S, Carmo-Silva E, Galmés J. Rubisco and rubisco activase play an important role in the biochemical limitations of photosynthesis in rice, wheat, and maize under high temperature and water deficit. Frontiers in Plant Science. 2017; 8:490-502.

Perveen S, Shahbaz M, Ashraf M. Is pre-sowing seed treatment with triacontanol effective in improving some physiological and biochemical attributes of wheat (*Triticum aestivum* L.) under salt stress?. Journal of Applied Botany and Food Quality. 2012; 85:41-62.

Petrov VD, Van Breusegem F. Hydrogen peroxide- a central hub for information flow in plant cells. AoB Plants. 2012; 2012:14-25.

Pouramir-Dashtmian F, Khajeh-Hosseini M, Esfahani M. Improving chilling tolerance of rice seedling by seed priming with salicylic acid. Archives of Agronomy and Soil Science. 2014; 60:1291-1302.

Puniran-Hartley N, Hartley J, Shabala L, Shabala S. Salinity-induced accumulation of organic osmolytes in barley and wheat leaves correlates with increased oxidative stress tolerance: in planta evidence for cross-tolerance. Plant Physiology and Biochemistry. 2014; 83:32-39.

Puthur JT.Photosynthetic events in *Sesbania sesban* (L.) Merrill in relation to osmotic stress during different developmental stages, Ph.D. Thesis. Jamia Millia Islamia, New Delhi. 2000

Qaderi MM, Basraon NK, Chinnappa CC, Reid DM. Combined effects of temperature, ultraviolet-B radiation, and watering regime on growth and physiological processes in canola (*Brassica napus*) seedlings. International Journal of Plant Sciences. 2010; 171:466-481.

Qiu ZB, Liu X, Tian XJ, Yue M. Effects of CO_2 laser pretreatment on drought stress resistance in wheat. Journal of Photochemistry and Photobiology B: Biology. 2008; 90:17-25.

Rai R, Meena RP, Smita SS, Shukla A, Rai SK, Pandey-Rai S. UV-B and UV-C pre-treatments induce physiological changes and artemisinin

biosynthesis in *Artemisia annua* L.-An antimalarial plant. Journal of Photochemistry and Photobiology B: Biology. 2011; 105:216-225.

Raja V, Majeed U, Kang H, Andrabi KI, John R. Abiotic stress: Interplay between ROS, hormones and MAPKs. Environmental and Experimental Botany. 2017;137:142-157.

Ravindran KC, Indrajith A, Pratheesh PV, Sanjiviraja K, Balakrishnan V. Effect of ultraviolet-B radiation on biochemical and antioxidant defence system in *Indigofera tinctoria* L. seedlings. International Journal of Engineering, Science and Technology. 2010; 2:226-232.

Reddy KR, Prasad PV, Singh SK. Effects of ultraviolet-B radiation and its interactions with climate change factors on agricultural crop growth and yield. In UV radiation in global climate change. 2010; 395-436. Springer, Berlin, Heidelberg.

Rejeb KB, Benzarti M, Debez A, Bailly C, Savouré A, Abdelly C. NADPH oxidase-dependent H_2O_2 production is required for salt-induced antioxidant defense in *Arabidopsis thaliana*. Journal of Plant Physiology. 2015; 174:5-15.

Reyes LF, Cisneros-Zevallos L. Electron-beam ionizing radiation stress effects on mango fruit (*Mangifera indica* L.) antioxidant constituents before and during postharvest storage. Journal of Agricultural and Food Chemistry. 2007; 55:6132-6149.

Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenolic compounds. Trends in Plant Science. 1997; 2:152-159.

Rizhsky L, Hallak-Herr E, Van Breusegem F, Rachmilevitch S, Barr JE, Rodermel S, Inzé D, Mittler R. Double antisense plants lacking ascorbate peroxidase and catalase are less sensitive to oxidative stress

than single antisense plants lacking ascorbate peroxidase or catalase. The Plant Journal. 2002; 32:329-342.

Rizzini L, Favory JJ, Cloix C, Faggionato D, O'Hara A, Kaiserli E, Baumeister R, Schäfer E, Nagy F, Jenkins GI, Ulm R. Perception of UV-B by the *Arabidopsis* UVR8 protein. Science. 2011; 332:103-116.

Roach T, Krieger-Liszkay A. Regulation of photosynthetic electron transport and photoinhibition. Current Protein and Peptide Science. 2014; 15:351-362.

Robinson SA, Turnbull JD, Lovelock CE. Impact of changes in natural ultraviolet radiation on pigment composition, physiological and morphological characteristics of the Antarctic moss, *Grimmia antarctici*. Global Change Biology. 2005; 11:476-489.

Robson TM, Klem K, Urban O, Jansen MA. Re-interpreting plant morphological responses to UV-B radiation. Plant, Cell and Environment. 2015; 38:856-866.

Rossatto T, do Amaral MN, Benitez LC, Vighi IL, Braga EJ, de Magalhaes Júnior AM, Maia MA, da Silva Pinto L. Gene expression and activity of antioxidant enzymes in rice plants, cv. BRS AG, under saline stress. Physiology and Molecular Biology of Plants. 2017; 23:865-875.

Rudnóy S, Majláth I, Pál M, Páldi K, Rácz I, Janda T. Interactions of S-methylmethionine and UV-B can modify the defence mechanisms induced in maize. Acta Physiologiae Plantarum. 2015; 37:148-156.

Rupiasih NN, Vidyasagar PB. Effect of UV-C radiation and hypergravity on germination, growth and content of chlorophyll of wheat seedlings. In AIP Conference Proceedings 2016; 1719-1721. AIP Publishing LLC.

Russo R, Zito F, Costa C, Bonaventura R, Matranga V. Transcriptional increase and misexpression of 14-3-3 epsilon in sea urchin embryos exposed to UV-B. Cell Stress and Chaperones. 2010; 15:993-1001.

Sadak MS, Abdelhamid MT. Influence of amino acids mixture application on some biochemical aspects, antioxidant enzymes and endogenous polyamines of *Vicia faba* plant grown under seawater salinity stress. Gesunde Pflanzen. 2015; 67:119-129.

Saeedipour S, Moradi F. Stress-induced changes in the free amino acid composition of two wheat cultivars with difference in drought resistance. African Journal of Biotechnology. 2012; 11:9559-9571.

Saha P, Chatterjee P, Biswas AK. NaCl pretreatment alleviates salt stress by enhancement of antioxidant defense system and osmolyte accumulation in mungbean (*Vigna radiata* L. Wilczek). Indian Journal of Experimental Biology. 2010; 48:593-600

Sairam RK, Deshmukh PS, Shukla DS. Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. Journal of Agronomy and Crop Science. 1997; 178:171-178.

Salah SM, Yajing G, Dongdong C, Jie L, Aamir N, Qijuan H, Weimin H, Mingyu N, Jin H. Seed priming with polyethylene glycol regulating the physiological and molecular mechanism in rice (*Oryza sativa* L.) under nano-ZnO stress. Scientific Reports. 2015; 5:14278-14295.

Salama HM, Al Watban AA, Al-Fughom AT. Effect of ultraviolet radiation on chlorophyll, carotenoid, protein and proline contents of some annual desert plants. Saudi Journal of Biological Sciences. 2011; 18:79-86.

Sami F, Yusuf M, Faizan M, Faraz A, Hayat S. Role of sugars under abiotic stress. Plant Physiology and Biochemistry. 2016; 109:54-61.

Sanchez-Reinoso AD, Garces-Varon G, Restrepo-Diaz H. Biochemical and physiological characterization of three rice cultivars under different daytime temperature conditions. Chilean Journal of Agricultural Research. 2014; 74:373-379.

Sanghera GS, Wani SH, Hussain W, Singh NB. Engineering cold stress tolerance in crop plants. Current Genomics. 2011; 12:30-43.

Sasi S, Venkatesh J, Daneshi RF, Gururani MA. Photosystem II extrinsic proteins and their putative role in abiotic stress tolerance in higher plants. Plants. 2018; 7:100-111.

Schmitt N, Dizengremel P. Effect of osmotic stress on mitochondria isolated from etiolated mung bean and sorghum seedlings. Plant Physiology and Biochemistry. 1989; 27:17-26.

Schoedl K, Schuhmacher R, Forneck A. Correlating physiological parameters with biomarkers for UV-B stress indicators in leaves of grapevine cultivars Pinot noir and Riesling. The Journal of Agricultural Science. 2013; 151:189-200.

Sen A, Challabathula D, Puthur JT. UV-B priming of *Oryza sativa* seeds augments the innate tolerance potential in a tolerant variety more effectively toward NaCl and PEG stressors. Journal of Plant Growth Regulation. 2020; 30:1-5.

Sen A, Puthur JT. Influence of different seed priming techniques on oxidative and antioxidative responses during the germination of *Oryza sativa* varieties. Physiology and Molecular Biology of Plants. 2020; 7:1-5.

Shabala S, Hariadi Y, Jacobsen SE. Genotypic difference in salinity tolerance in quinoa is determined by differential control of xylem Na^+ loading and stomatal density. Journal of Plant Physiology. 2013; 170:906-914.

Shafi M, Bakht J, Hassan MJ, Raziuddin M, Zhang G. Effect of cadmium and salinity stresses on growth and antioxidant enzyme activities of wheat (*Triticum aestivum* L.). Bulletin of Environmental Contamination and Toxicology. 2009; 82:772-786.

Shaki F, Maboud HE, Niknam V. Growth enhancement and salt tolerance of Safflower (*Carthamus tinctorius* L.), by salicylic acid. Current Plant Biology. 2018; 13:16-22.

Shan C, Zhou Y, Liu M. Nitric oxide participates in the regulation of the ascorbate-glutathione cycle by exogenous jasmonic acid in the leaves of wheat seedlings under drought stress. Protoplasma. 2015; 252:1397-1405.

Sharma AD, Rathore SV, Srinivasan K, Tyagi RK. Comparison of various seed priming methods for seed germination, seedling vigour and fruit yield in okra (*Abelmoschus esculentus* L. Moench). Scientia Horticulturae. 2014; 165:75-81.

Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany. 2012; 2012:217-230.

Shaukat SS, Farooq MA, Siddiqui MF, Zaidi SA. Effect of enhanced UV-B radiation on germination, seedling growth and biochemical responses of *Vigna mungo* (L.) Hepper. Pakistan Journal of Botany. 2013; 45:779-785.

Shin SY, Kim IS, Kim YS, Lee H, Yoon HS. Ectopic expression of *Brassica rapa* L. MDHAR increased tolerance to freezing stress by enhancing antioxidant systems of host plants. South African Journal of Botany. 2013; 88:388-400.

Shivakrishna P, Reddy KA, Rao DM. Effect of PEG-6000 imposed drought stress on RNA content, relative water content (RWC), and chlorophyll content in peanut leaves and roots. Saudi Journal of Biological Sciences. 2018; 25:285-289.

Shtereva LA, Vassilevska-Ivanova RD, Karceva TV. Effect of salt stress on some sweet corn (*Zea mays* L. var. saccharata) genotypes. Archives of Biological Sciences. 2015; 67:993-1000.

Shu S, Guo SR, Sun J, Yuan LY. Effects of salt stress on the structure and function of the photosynthetic apparatus in *Cucumis sativus* and its protection by exogenous putrescine. Physiologia Plantarum. 2012; 146:285-296.

Siddiqui A, Dawar SH, Zaki MJ, Hamid N. Role of ultraviolet (UV-C) radiation in the control of root infecting fungi on groundnut and mung bean. Pakistan Journal of Botany. 2011; 43:2221-2224.

Singh J, Thakur JK. Photosynthesis and abiotic stress in plants. In Biotic and abiotic stress tolerance in plants. 2018; 27-46. Springer, Singapore.

Singh M, Kumar J, Singh S, Singh VP, Prasad SM. Roles of osmoprotectants in improving salinity and drought tolerance in plants: a review. Reviews in Environmental Science and Bio/Technology. 2015; 14:407-426.

Singh N, Bhardwaj RD. Ascorbic acid alleviates water deficit induced growth inhibition in wheat seedlings by modulating levels of endogenous antioxidants. Biologia. 2016; 71:402-413.

Singh S, Rai K, Agrawal SB, Agrawal M. Comparative assessment of UV-B priming on vegetative and reproductive stages of oat and barley. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. 2019; 25:1-9.

Singh-Tomar R, Mathur S, Allakhverdiev SI, Jajoo A. Changes in PSII heterogeneity in response to osmotic and ionic stress in wheat leaves (*Triticum aestivum*). Journal of Bioenergetics and Biomembranes. 2012; 44:411-419.

Sivasakthivel T, Reddy KK. Ozone layer depletion and its effects: a review. International Journal of Environmental Science and Development. 2011; 2:30-37.

Skórska E, Murkowski A. Comparison of susceptibility of leaves on short-term UV-B irradiation. International Agrophysics. 2012; 26:395-410.

Skórska E. Comparison of chlorophyll fluorescence parameters of *Cucumis sativus* and *Mentha piperita* leaves exposed to short-term UV-B irradiation. Acta Biologica Cracoviensia. Series Botanica. 2011; 53:16-19.

Slama I, Abdelly C, Bouchereau A, Flowers T, Savoure A. Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. Annals of Botany. 2015; 115:433-447.

Sneha S, Rishi A, Dadhich A, Chandra S. Effect of salinity on seed germination, accumulation of proline and free amino acid in

Pennisetum glaucum (L.) R. Br. Pakistan Journal of Biological Sciences. 2013; 16:877-881.

Sofo A, Scopa A, Nuzzaci M, Vitti A. Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. International Journal of Molecular Sciences. 2015; 16:13561-13578.

Solomon S, Portmann RW, Thompson DW. Contrasts between Antarctic and Arctic ozone depletion. Proceedings of the National Academy of Sciences. 2007; 104:445-449.

Souza MO, Pelacani CR, Willems LA, Castro RD, Hilhorst HW, Ligterink W. Effect of osmopriming on germination and initial growth of *Physalis angulata* L. under salt stress and on expression of associated genes. Anais da Academia Brasileira de Ciências. 2016; 88:503-516.

Srivastava AK, Lokhande VH, Patade VY, Suprasanna P, Sjahril R, D'Souza SF. Comparative evaluation of hydro-, chemo- and hormonal-priming methods for imparting salt and PEG stress tolerance in Indian mustard (*Brassica juncea* L.). Acta Physiologiae Plantarum. 2010; 32:1135-1144.

Srivastava RK, Sarkar S, Beig G. Brief Review: The study of ozone and its precursors gases. International Journal of Environmental Research and Public Health. 2015; 1:166-173

Stepien P, Johnson GN. Contrasting responses of photosynthesis to salt stress in the glycophyte *Arabidopsis* and the halophyte *Thellungiella:* role of the plastid terminal oxidase as an alternative electron sink. Plant Physiology. 2009; 149:1154-1165.

Stracke R, Favory JJ, Gruber H, Bartelniewoehner L, Bartels S, Binkert M, Funk M, Weisshaar B, Ulm R. The *Arabidopsis* bZIP transcription factor HY5 regulates expression of the PFG1/MYB12 gene in response to light and ultraviolet-B radiation. Plant, Cell and Environment. 2010; 33:88-103.

Strasser RJ, Srivastava A, Tsimilli-Michael M. The fluorescence transient as a tool to characterize and screen photosynthetic samples. Probing photosynthesis: mechanisms, regulation and adaptation. 2000; 445-483.

Strasser RJ, Tsimilli-Michael M, Srivastava A. Analysis of the chlorophyll *a* fluorescence transient. In Chlorophyll *a* fluorescence. 2004; 321-362. Springer, Dordrecht.

Sunita DT, Vinay K, Varsha S. Differential response of two scented indica rice (*Oryza sativa*) cultivars under salt stress. Journal of Stress Physiology and Biochemistry. 2011; 7: 387-397.

Tahira T, Riaz A, Muhammad F, Basra SM. Improving the drought tolerance in barley by osmopriming and biopriming. International Journal of Agriculture and Biology. 2018; 20:1597-1606.

Taïbi K, Taïbi F, Abderrahim LA, Ennajah A, Belkhodja M, Mulet JM. Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in *Phaseolus vulgaris* L. South African Journal of Botany. 2016; 105:306-312.

Takeuchi Y, Kubo H, Kasahara H, Sakaki T. Adaptive alterations in the activities of scavengers of active oxygen in cucumber cotyledons irradiated with UV-B. Journal of Plant Physiology. 1996; 147:589-592.

Talebi R. Evaluation of chlorophyll content and canopy temperature as indicators for drought tolerance in durum wheat (*Triticum durum* Desf.). Australian Journal of Basic and Applied Sciences. 2011; 5:1457-1462.

Teklemariam T, Blake TJ. Effects of UV-B preconditioning on heat tolerance of cucumber (*Cucumis sativus* L.). Environmental and Experimental Botany. 2003; 50:169-182.

Theerakulpisut P, Gunnula W. Exogenous sorbitol and trehalose mitigated salt stress damage in salt-sensitive but not salt-tolerant rice seedlings. Asian Journal of Crop Science. 2012; 4:165-170.

Thomas DT, Challabathula D, Puthur JT. UV-B priming of *Oryza sativa* var. Kanchana seedlings augments its antioxidative potential and gene expression of stress-response proteins under various abiotic stresses. 3 Biotech. 2019; 9:375-389.

Thomas DT, Puthur JT. Amplification of abiotic stress tolerance potential in rice seedlings with a low dose of UV-B seed priming. Functional Plant Biology. 2019; 46:455-466.

Thomas DT, Puthur JT. UV radiation priming: A means of amplifying the inherent potential for abiotic stress tolerance in crop plants. Environmental and Experimental Botany. 2017; 138:57-66.

Thomas TD, Dinakar C, Puthur JT. Effect of UV-B priming on the abiotic stress tolerance of stress-sensitive rice seedlings: Priming imprints and cross-tolerance. Plant Physiology and Biochemistry. 2020; 147:21-30.

Thomas TT, Puthur JT. UV-B priming enhances specific secondary metabolites in *Oryza sativa* (L.) empowering to encounter diverse abiotic stresses. Plant Growth Regulation. 2020; 20:153-167.

Tóth SZ, Nagy V, Puthur JT, Kovács L, Garab G. The physiological role of ascorbate as photosystem II electron donor: protection against photoinactivation in heat-stressed leaves. Plant Physiology. 2011; 156:382-392.

Tripathi DK, Singh S, Singh VP, Prasad SM, Dubey NK, Chauhan DK. Silicon nanoparticles more effectively alleviated UV-B stress than silicon in wheat (*Triticum aestivum*) seedlings. Plant Physiology and Biochemistry. 2017; 110:70-81.

UNEP. Environmental effects of ozone depletion 2002 assessment: high level ozone. 2002; 65-84.New York, USA.

United Nations, Department of Economic and Social Affairs, Population Division. World population prospects: The 2015 revision: key findings and advance tables. 2015; 6-19. New York, USA.

United Nations. World population prospects: the 2017 revision, key findings and advance tables. Department of Economics and Social Affairs. 2017. New York: United Nations.

ur Rehman H, Afzal I, Farooq M, Aziz T, Ahmad SM. Improving temperature stress resistance in spring maize by seed priming. In: Third International Conference-Frontiers in Agriculture. 2012; 28-32.

Valenzuela-Avendaño JP, Mota IA, Uc GL, Perera RS, Valenzuela-Soto EM, Aguilar JJ. Use of a simple method to isolate intact RNA from partially hydrated *Selaginella lepidophylla* plants. Plant Molecular Biology Reporter. 2005; 23:199-200.

Van Dingenen R, Dentener FJ, Raes F, Krol MC, Emberson L, Cofala J. The global impact of ozone on agricultural crop yields under current and

future air quality legislation. Atmospheric Environment. 2009; 43:604-618.

Vijayakumari K, Puthur JT. γ-aminobutyric acid (GABA) priming enhances the osmotic stress tolerance in *Piper nigrum* Linn. plants subjected to PEG-induced stress. Plant Growth Regulation. 2016; 78:57-67.

Vijayakumari K. Effect of γ-aminobutyric acid (GABA)/β-aminobutyric acid (BABA)-priming on drought tolerance potential of pepper (*Piper nigrum* L.): A process with less defense investment.Ph D. Thesis, University of Calicut. 2015; 99-134.

Vincent C, Rowland DL, Schaffer B. The potential for primed acclimation in papaya (*Carica papaya* L.): determination of critical water deficit thresholds and physiological response variables. Scientia Horticulturae. 2015; 194:344-352.

Von Caemmerer SV, Farquhar GD. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta. 1981; 153:376-387.

Vuleta A, Jovanović SM, Tucić B. Adaptive flexibility of enzymatic antioxidants SOD, APX and CAT to high light stress: The clonal perennial monocot *Iris pumila* as a study case. Plant Physiology and Biochemistry. 2016; 100:166-173.

Vurukonda SS, Vardharajula S, Shrivastava M, SkZ A. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiological Research. 2016; 184:13-24.

Vysotskaya L, Hedley PE, Sharipova G, Veselov D, Kudoyarova G, Morris J, Jones HG. Effect of salinity on water relations of wild barley plants differing in salt tolerance. AoB Plants. 2010; 2010: 6-15.

Wahid A, Perveen M, Gelani S, Basra SM. Pretreatment of seed with H_2O_2 improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. Journal of Plant Physiology. 2007; 164:283-294.

Wahid A, Sehar S, Perveen M, Gelani S, Basra SM, Farooq M. Seed pretreatment with hydrogen peroxide improves heat tolerance in maize at germination and seedling growth stages. Seed Science and Technology. 2008; 36:633-645.

Walton TJ. Waxes, cutin and suberin. Methods in Plant Biochemistry. 1990; 4:5-158.

Wang S, Xie B, Yin L, Duan L, Li Z, Egrinya Eneji A, Tsuji W, Tsunekawa A. Increased UV-B radiation affects the viability, reactive oxygen species accumulation and antioxidant enzyme activities in maize (*Zea mays* L.) pollen. Photochemistry and Photobiology. 2010; 86:110-116.

Wang Y, Yu G, Li K, Wu M, Ma J, Xu J, Chen G. Responses of photosynthetic properties and antioxidant enzymes in high-yield rice flag leaves to supplemental UV-B radiation during senescence stage. Environmental Science and Pollution Research. 2015; 22:4695-4705.

Wang YW, Xu C, Lv CF, Wu M, Cai XJ, Liu ZT, Song XM, Chen GX, Lv CG. Chlorophyll *a* fluorescence analysis of high-yield rice (*Oryza sativa* L.) LYPJ during leaf senescence. Photosynthetica. 2016; 54:422-429.

Wang Z, Li H, Li X, Xin C, Si J, Li S, Li Y, Zheng X, Li H, Wei X, Zhang Z. Nano-ZnO priming induces salt tolerance by promoting photosynthetic carbon assimilation in wheat. Archives of Agronomy and Soil Science. 2019; 8:1-5.

Wani SH, Kumar V, Shriram V, Sah SK. Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. The Crop Journal. 2016; 4:162-176.

Wargent JJ, Jordan BR. From ozone depletion to agriculture: understanding the role of UV radiation in sustainable crop production. New Phytologist. 2013; 197:1058-1076.

Wijewardana C, Henry WB, Gao W, Reddy KR. Interactive effects on CO_2, drought, and ultraviolet-B radiation on maize growth and development. Journal of Photochemistry and Photobiology B: Biology. 2016; 160:198-209.

Williamson CE, Zepp RG, Lucas RM, Madronich S, Austin AT, Ballaré CL, Norval M, Sulzberger B, Bais AF, McKenzie RL, Robinson SA. Solar ultraviolet radiation in a changing climate. Nature Climate Change. 2014; 4:434-441.

Wojtyla Ł, Lechowska K, Kubala S, Garnczarska M. Molecular processes induced in primed seeds-increasing the potential to stabilize crop yields under drought conditions. Journal of Plant Physiology. 2016; 203:116-126.

Wu D, Cai S, Chen M, Ye L, Chen Z, Zhang H, Dai F, Wu F, Zhang G. Tissue metabolic responses to salt stress in wild and cultivated barley. PLoS One. 2013; 8:55431-5551.

Xiao B, Huang Y, Tang N, Xiong L. Over-expression of a LEA gene in rice improves drought resistance under the field conditions. Theoretical and Applied Genetics. 2007; 115:35-46.

Xiong FS, Day TA. Effect of solar ultraviolet-B radiation during spring time ozone depletion on photosynthesis and biomass production of Antarctic vascular plants. Plant Physiology. 2001; 125:738-751.

Xu K, Qiu BS. Responses of superhigh-yield hybrid rice Liangyoupeijiu to enhancement of ultraviolet-B radiation. Plant Science. 2007; 172:139-149.

Xu Y, Charles MT, Luo Z, Mimee B, Tong Z, Véronneau PY, Roussel D, Rolland D. Ultraviolet-C priming of strawberry leaves against subsequent *Mycosphaerella fragariae* infection involves the action of reactive oxygen species, plant hormones, and terpenes. Plant, Cell and Environment. 2019; 42:815-831.

Xu Y, Zhan C, Huang B. Heat shock proteins in association with heat tolerance in grasses. International Journal of Proteomics. 2011; 2011:41-60.

Xue D, Zhang X, Lu X, Chen G, Chen ZH. Molecular and evolutionary mechanisms of cuticular wax for plant drought tolerance. Frontiers in Plant Science. 2017; 8:621-634.

Yanqun Z, Yuan L, Haiyan C, Jianjun C. Intraspecific differences in physiological response of 20 soybean cultivars to enhanced ultraviolet-B radiation under field conditions. Environmental and Experimental Botany. 2003; 50:87-97.

Yao Y, Xuan Z, He Y, Lutts S, Korpelainen H, Li C. Principal component analysis of intraspecific responses of tartary buckwheat to UV-B radiation under field conditions. Environmental and Experimental Botany. 2007; 61:237-245.

Yari L, Khazaei F, Sadeghi H, Sheidaei S. Effect of seed priming on grain yield and yield components of bread wheat (*Triticum aestivum* L.). Journal of Agricultural and Biological Science. 2011; 6:1-5.

Yari L, Zareyan A, Hasani F, Sadeghi H, Sheidaie S. Germination and seedling growth as affected by presowing PEG seed treatments in (*Oryza sativa*L.). Technical Journal of Engineering and Applied Sciences. 2012; 2:425-429.

Yoon HS, Lee H, Lee IA, Kim KY, Jo J. Molecular cloning of the monodehydroascorbate reductase gene from *Brassica campestris* and analysis of its mRNA level in response to oxidative stress. Biochimica et Biophysica Acta (BBA)-Bioenergetics. 2004; 1658:181-186.

Yücel NC, Heybet E. Salicylic acid and calcium treatments improves wheat vigor, lipids and phenolics under high salinity. Acta Chimica Slovenica. 2016; 63:738-746.

Zeng X, Ling H, Yang J, Li Y, Guo S. LEA proteins from *Gastrodia elata* enhance tolerance to low temperature stress in Escherichia coli. Gene. 2018; 646:136-142.

Zhang F, Yu J, Johnston CR, Wang Y, Zhu K, Lu F, Zhang Z, Zou J. Seed priming with polyethylene glycol induces physiological changes in sorghum (*Sorghum bicolor* L. Moench) seedlings under suboptimal soil moisture environments. PLoS One. 2015; 10:10-25.

Zhang H. Nitric oxide alleviates the inhibition of salinity stress on seed germination and seedling growth of *Cynanchum bungei* Decne (Asclepiadaceae). HortScience. 2015; 50:119-122.

Zhang HH, Xu N, Wu XY, Wang JR, Ma SL, Li X, Sun GY. Effects of 4 kinds of sodium salt stress on plant growth, PSII and PSI function in leaves of *Sorghum*. Journal of Plant Interactions. 2018; 13:506-513.

Zhang J, Kirkham MB. Antioxidant responses to drought in sunflower and sorghum seedlings. New phytologist. 1996; 132:361-373.

Zhang M, Wang Z, Yuan L, Yin C, Cheng J, Wang L, Huang J, Zhang H. Osmopriming improves tomato seed vigor under aging and salinity stress. African Journal of Biotechnology. 2012; 11:6305-6311

Zhao TJ, Liu Y, Yan YB, Feng F, Liu WQ, Zhou HM. Identification of the amino acids crucial for the activities of drought responsive element binding factors (DREBs) of *Brassica napus*. FEBS Letters. 2007; 581:3044-3050.

Zheng M, Tao Y, Hussain S, Jiang Q, Peng S, Huang J, Cui K, Nie L. Seed priming in dry direct-seeded rice: consequences for emergence, seedling growth and associated metabolic events under drought stress. Plant Growth Regulation. 2016; 78:167-178.

Zhu JK. Abiotic stress signaling and responses in plants. Cell. 2016; 167:313-324.

Zhu SY, Hong DL, Yao J, Zhang XL, Luo TK. Improving germination, seedling establishment and biochemical characters of aged hybrid rice seed by priming with KNO_3^+ PVA. African Journal of Agricultural Research. 2010; 5:78-83.

Zhu XC, Song FB, Xu HW. Arbuscular mycorrhizae improves low temperature stress in maize via alterations in host water status and photosynthesis. Plant and Soil. 2010; 331:129-137.

Živčák M, Brestič M, Olšovská K, Slamka P. Performance index as a sensitive indicator of water stress in *Triticum aestivum* L. Plant Soil and Environment. 2008; 54:133-139.

Zu Y, Li Y, Chen J, Chen H. Intraspecific responses in grain quality of 10 wheat cultivars to enhanced UV-B radiation under field conditions.

Journal of Photochemistry and Photobiology B: Biology. 2004; 74:95-100.

Zu YG, Wei XX, Yu JH, Li DW, Pang HH, Tong L. Responses in the physiology and biochemistry of Korean pine (*Pinus koraiensis*) under supplementary UV-B radiation. Photosynthetica. 2011; 49:448-459.

Zucker M. Induction of phenylalanine deaminase by light and its relation to chlorogenic acid synthesis in potato tuber tissue. Plant Physiology. 1965; 40:779-798.

LIST OF PUBLICATIONS AND PRESENTATIONS

Papers in Journals

1. **Dhanya Thomas T T** and Jos T Puthur (2017). UV radiation priming: A means of amplifying the inherent potential for abiotic stress tolerance in crop plants. *Environmental and Experimental Botany*, 138, 57–66 (IF- 4.027)

2. **Dhanya Thomas T T** and Jos T Puthur (2019). Amplification of abiotic stress tolerance potential in rice seedlings with a low dose of UV-B seed priming. *Functional Plant Biology*, 46, 455–466 (IF-2.491)

3. **Dhanya Thomas T T**, Dinakar C, Jos T Puthur (2019). UV-B priming of *Oryza sativa* var. Kanchana seedlings augments its antioxidative potential and gene expression of stress response proteins under various abiotic stresses. *3Biotech*, 9:375 (IF-2.29)

4. **Dhanya Thomas T T**, Dinakar C, Jos T Puthur (2020). Effect of UV-B priming on the abiotic stress tolerance of stress-sensitive rice seedlings: priming imprints and cross-tolerance. *Plant Physiology and Biochemistry*, 147:21-30 (IF-3.72)

5. **Dhanya Thomas T T** and Jos T Puthur (2020). UV-B priming enhances specific secondary metabolites in *Oryza sativa* (L.) empowering to encounter diverse abiotic stresses. *Plant Growth Regulation*, 20 (IF-2.38)

Papers in book chapter

1. **Dhanya Thomas T T**, Jos T Puthur (2017). Low NaCl concentration enhances primephysiological features in O*ryza sativa* cv. Jyothi. In: Modern Trends in Conservation, Utilizationand Improvement of Plant Genetic Resources. Proceedings of Gregor Mendel Foundation Seminar. Gregor Mendel Foundation.

2. Parammal Faseela, Asari Kandi Sinisha, **Dhanya Thomas T T**, Jos T Puthur 2019. In: Metabolic adaptations in Plants during abiotic stress Oxidative stress and its management in plants during abiotic stress. Eds. Akula Ramkrishna and Sarvajeet Sing Gill. Taylor & Francis group, CRC press, London, New York. 111-126

Presentations in National / International seminars and conferences

1. **Dhanya Thomas T T** and Jos T Puthur. "UV-B priming imparts NaCl and PEG stress tolerance potential to rice seedlings". 8th International conference on photosynthesis and hydrogen energy research for sustainability- 2017.

2. **Dhanya Thomas T T** and Jos T Puthur. "Augmentation of UV-B stress tolerance mechanism in rice seedlings with low dose of UV-B priming". International conference on photochemistry and its application- 2017.

3. **Dhanya Thomas T T** and Jos T Puthur. "Low NaCl concentration enhances prime physiological features in *Oryza sativa* cv. Jyothi". National seminar on modern trends in conservation, utilization and improvement of plant genetic resources- 2017.

4. **Dhanya Thomas T T** and Jos T Puthur. "Low dose of UV-B seed priming intensify the UV-B stress tolerance mechanism in rice

seedlings". International conference on Recent Scenario in Plant Science Research– climate change and its Associated variations (ICRSPSR- 2018)

5. **Dhanya Thomas T T** and Jos T Puthur. "UV-B radiation: Act as a factor for stress induction and priming agent for stress tolerance". National seminar on Plant Sciences: Current challenges & perspectives - 2019

6. **Dhanya Thomas T T** and Jos T Puthur. "Dual role of UV-B in stress induction and alleviation is dosage dependent in rice"**.** International Conference on Plant Functional Biology- 2020.

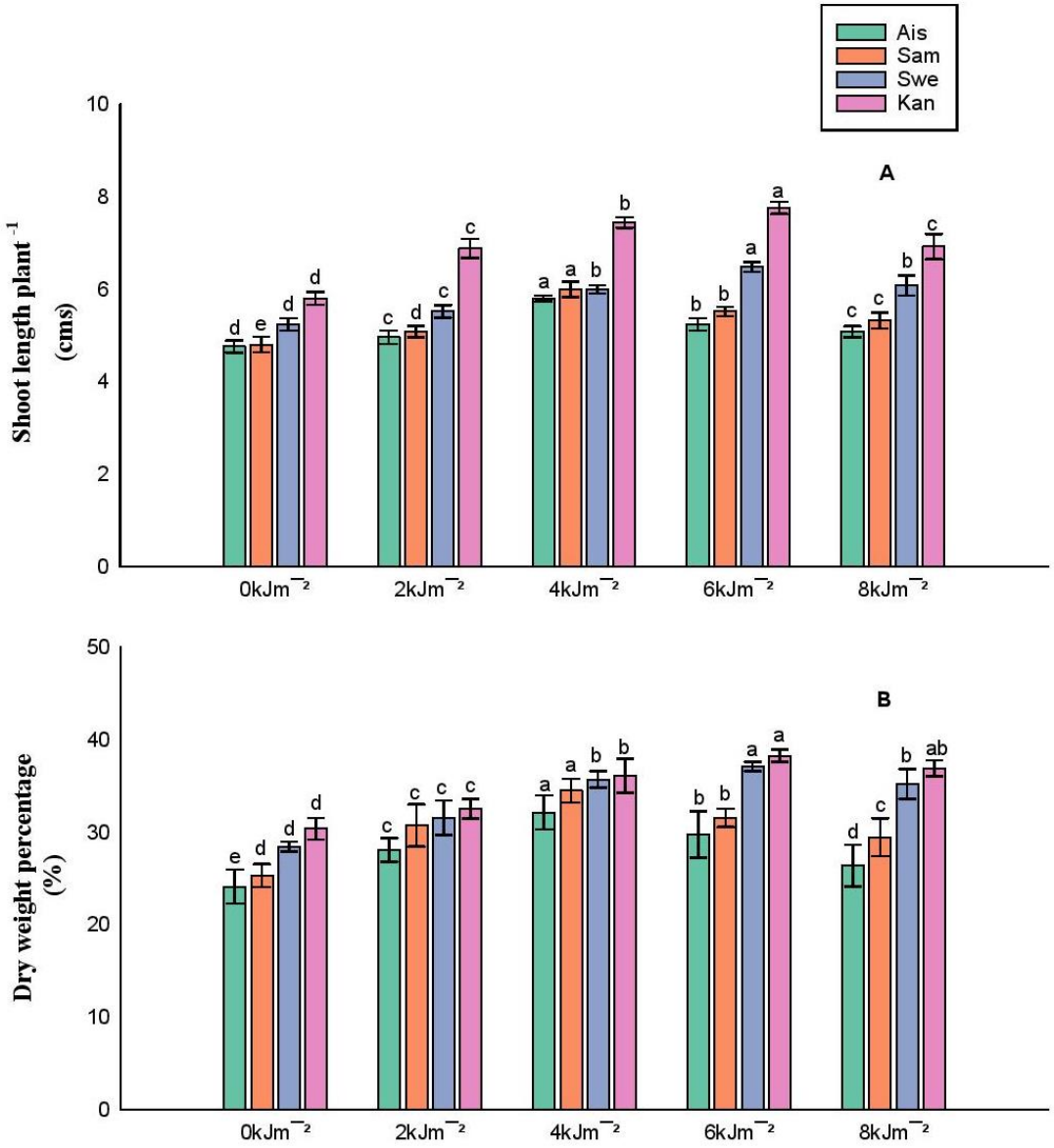

Fig. 1: Shoot length (A) and dry weight percentage (B) of rice seedlings raised from UV-B primed (different doses) rice seeds and subjected to NaCl stress condition.

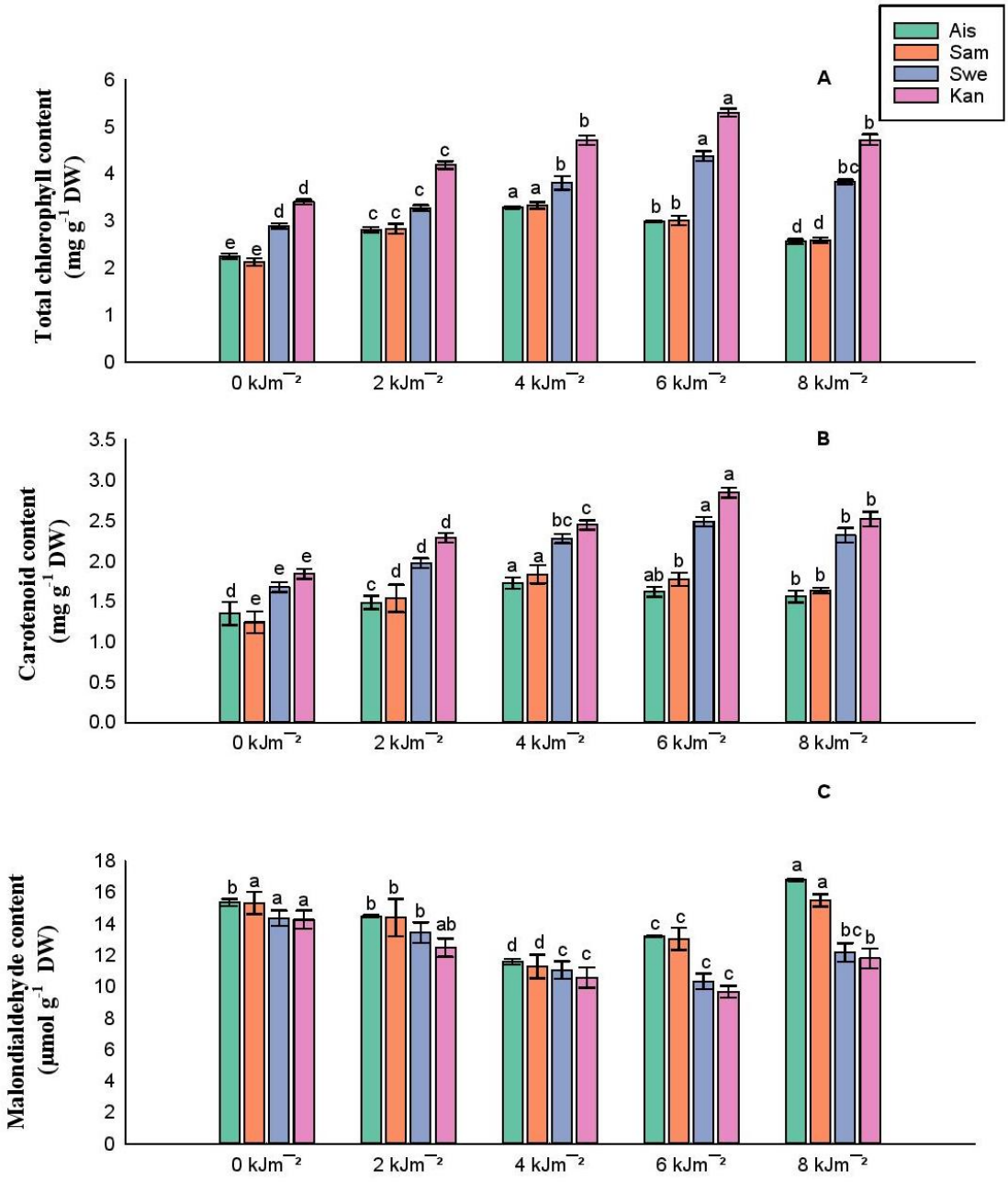

Fig. 2: Total chlorophyll (A), carotenoid (B) and malondialdehyde (C) content of rice seedlings raised from UV-B primed (different doses) rice seeds and subjected to NaCl stress condition.

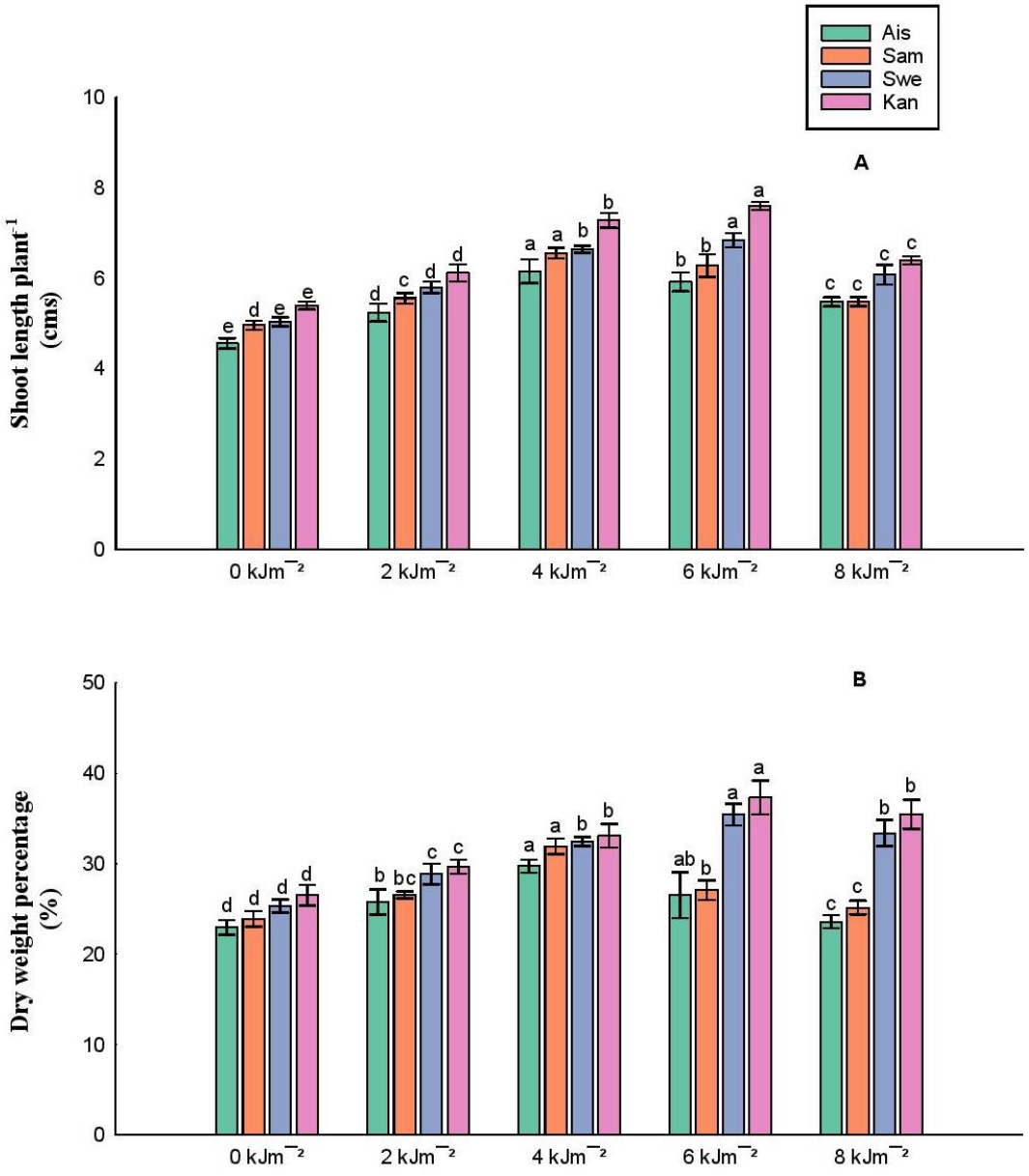

Fig. 3: Shoot length (A) and dry weight percentage (B) of rice seedlings raised from UV-B primed (different doses) rice seeds subjected to PEG stress condition.

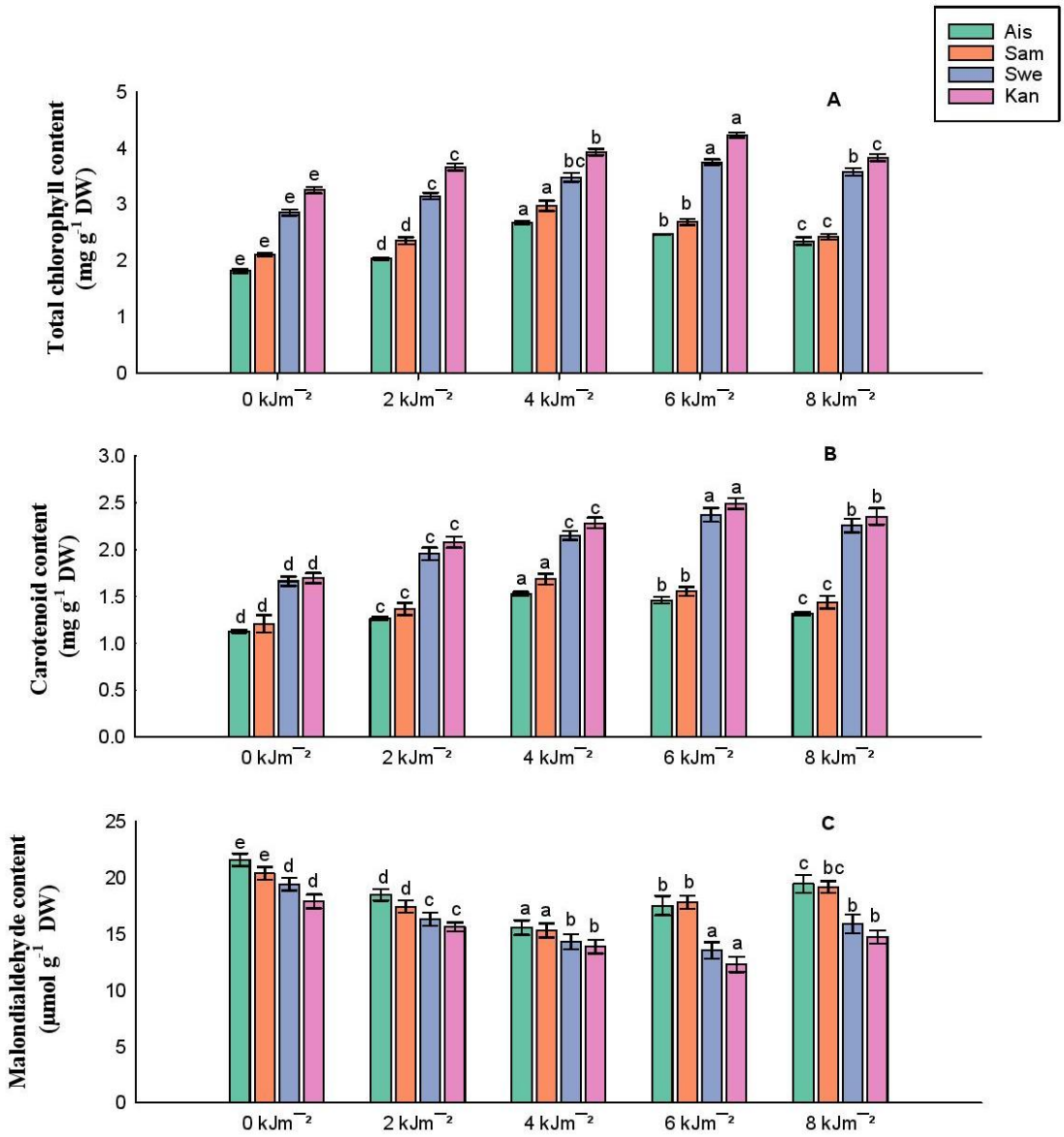

Fig. 4: Total chlorophyll (A), carotenoid (B) and malondialdehyde (C) content of rice seedlings raised from UV-B primed (different doses) rice seeds subjected to PEG stress condition.

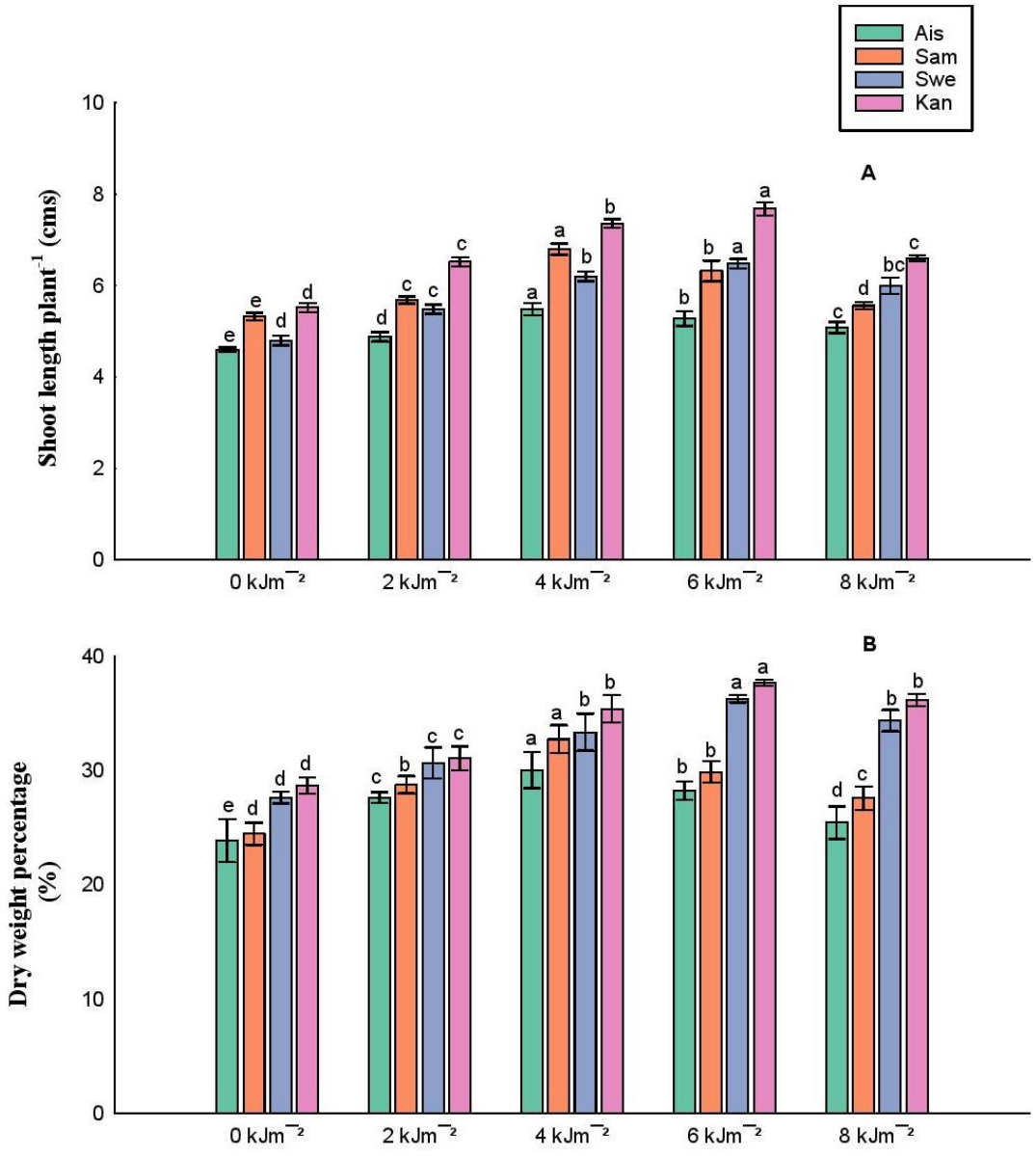

Fig. 5: Shoot length (A) and dry weight percentage (B) of rice seedlings raised from UV-B primed (different doses) rice seeds and subjected to UV-B stress condition.

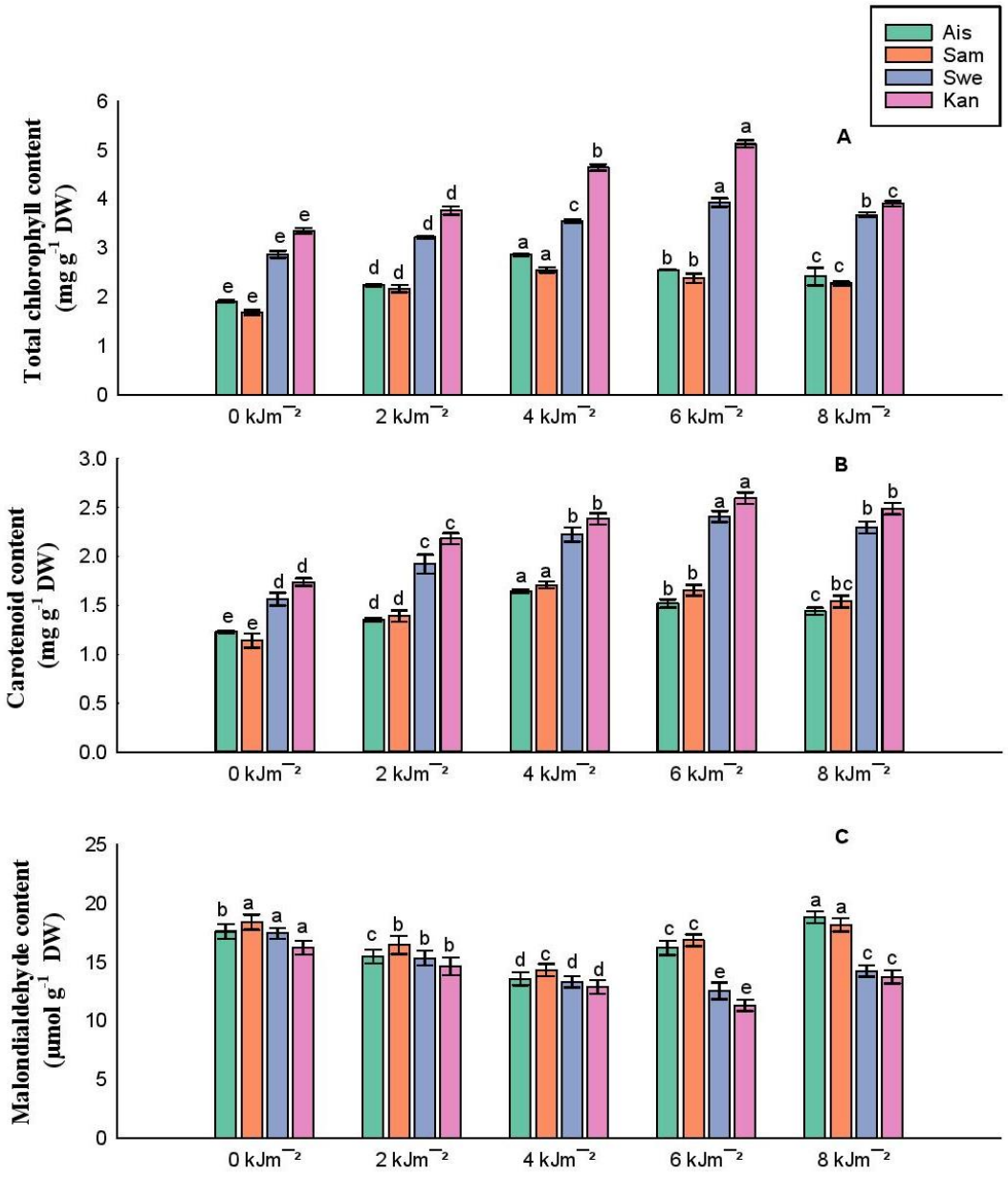

Fig. 6: Total chlorophyll (A), carotenoid (B) and malondialdehyde (C) content of rice seedlings raised from UV-B primed (different doses) rice seeds and subjected to UV-B stress condition.

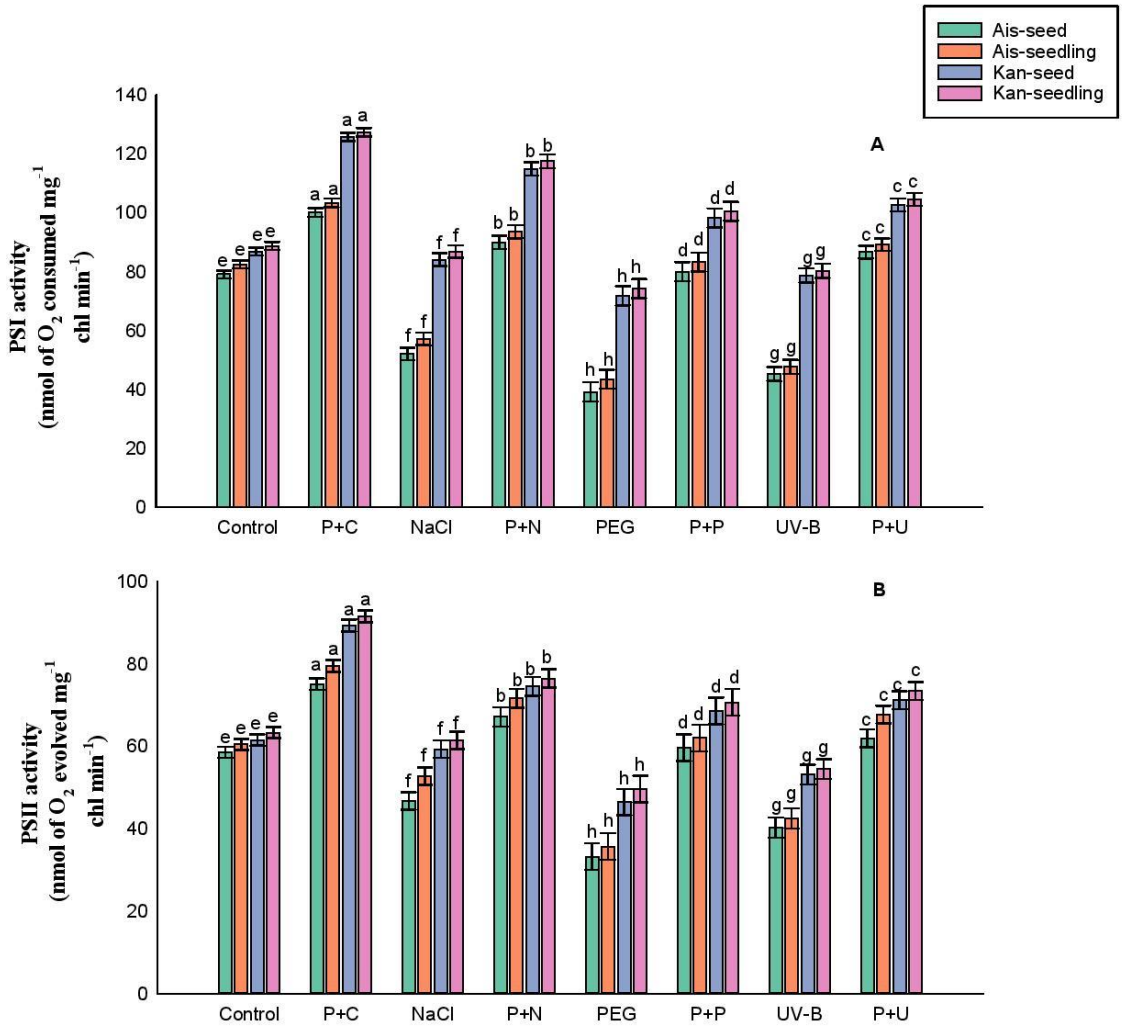

Fig. 7: PSI (A) and PSII (B) activities in leaves of rice seedlings from UV-B primed and non-primed seeds (P_s) as well as seedlings (P_{sl}) directly primed with UV-B and subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

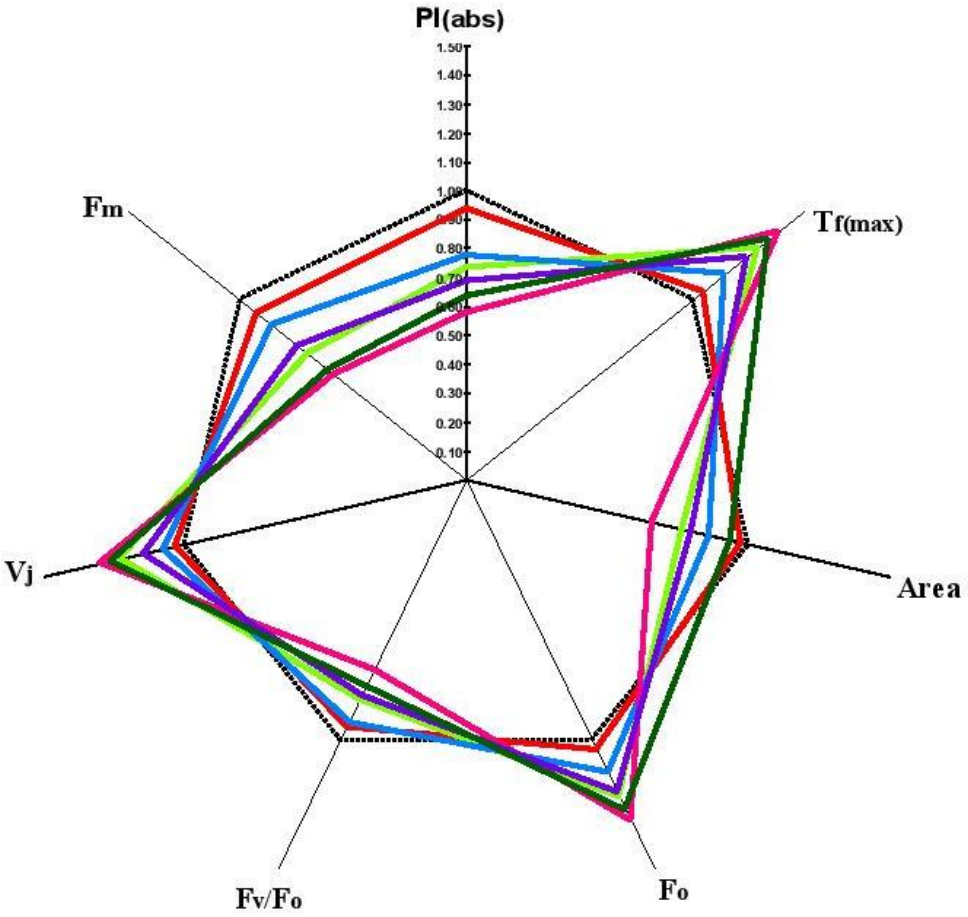

Fig. 8: Radar plot of selected Chl *a* fluorescence parameters recorded in leaves of rice seedlings from UV-B primed Kanchana seeds subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

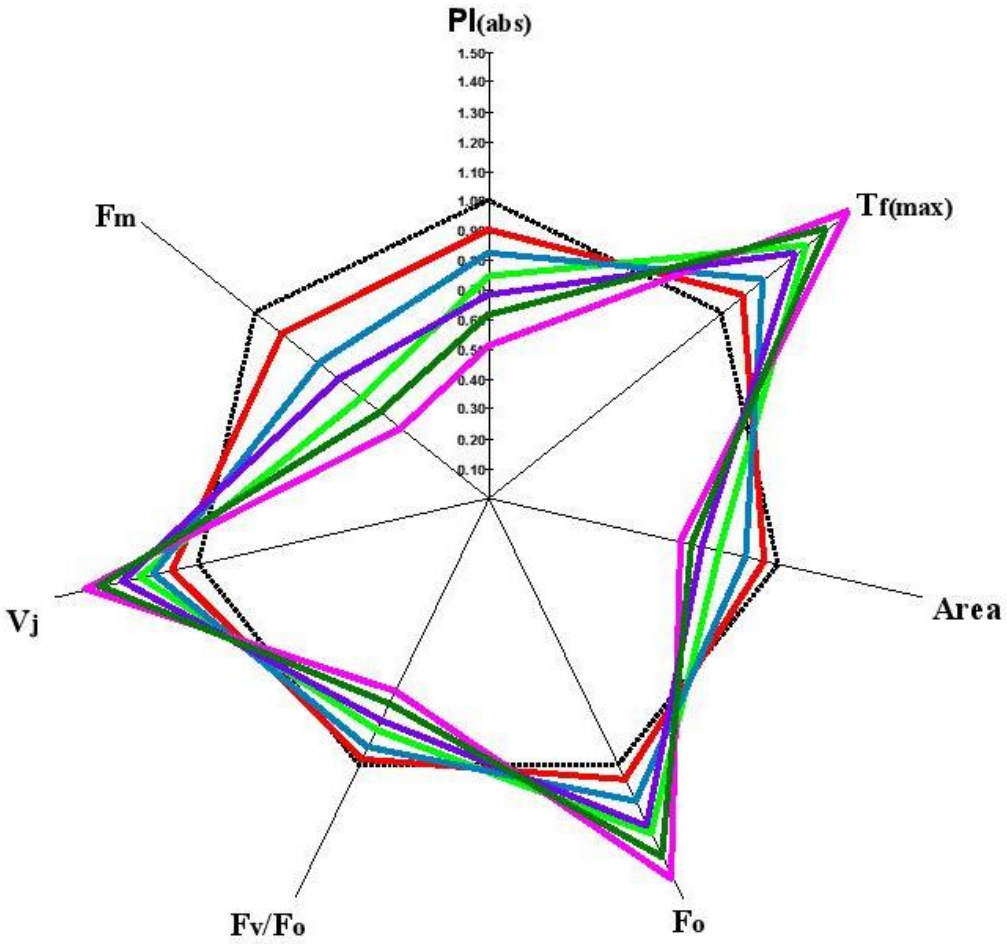

Fig. 9: Radar plot of selected Chl *a* fluorescence parameters recorded in leaves of rice seedlings from UV-B primed Kanchana seedlings subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

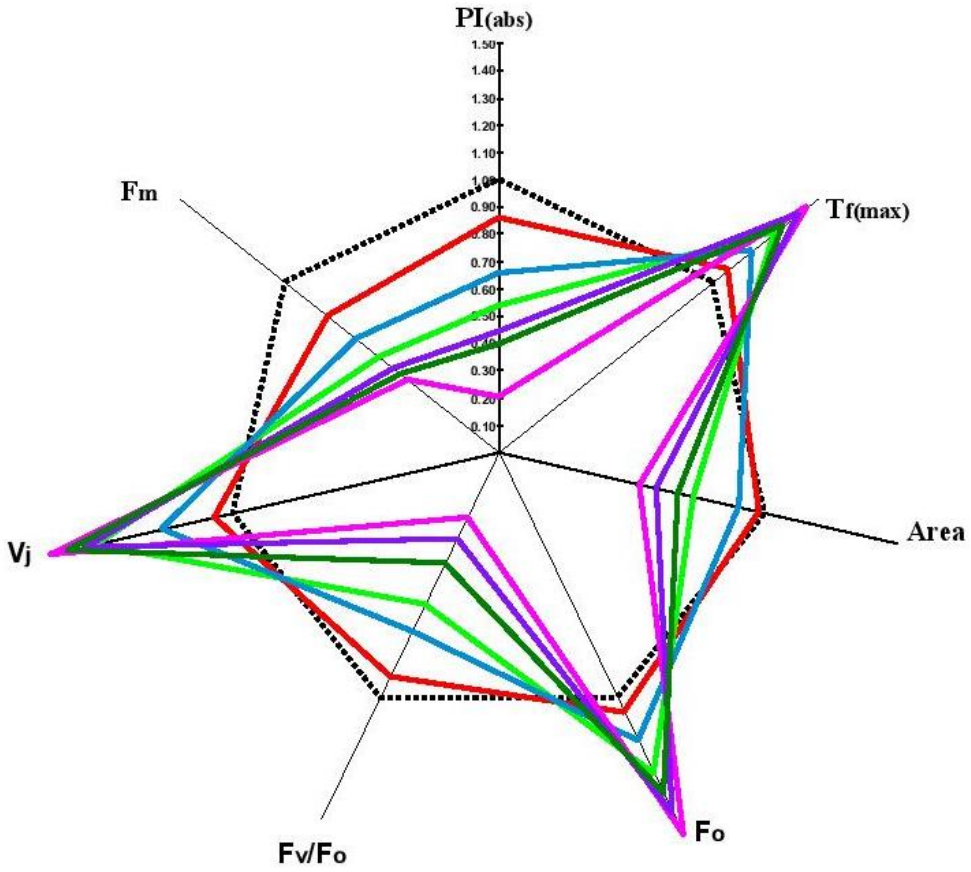

Fig. 10: Radar plot of selected Chl *a* fluorescence parameters recorded in leaves of rice seedlings from UV-B primed Aiswarya seeds subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

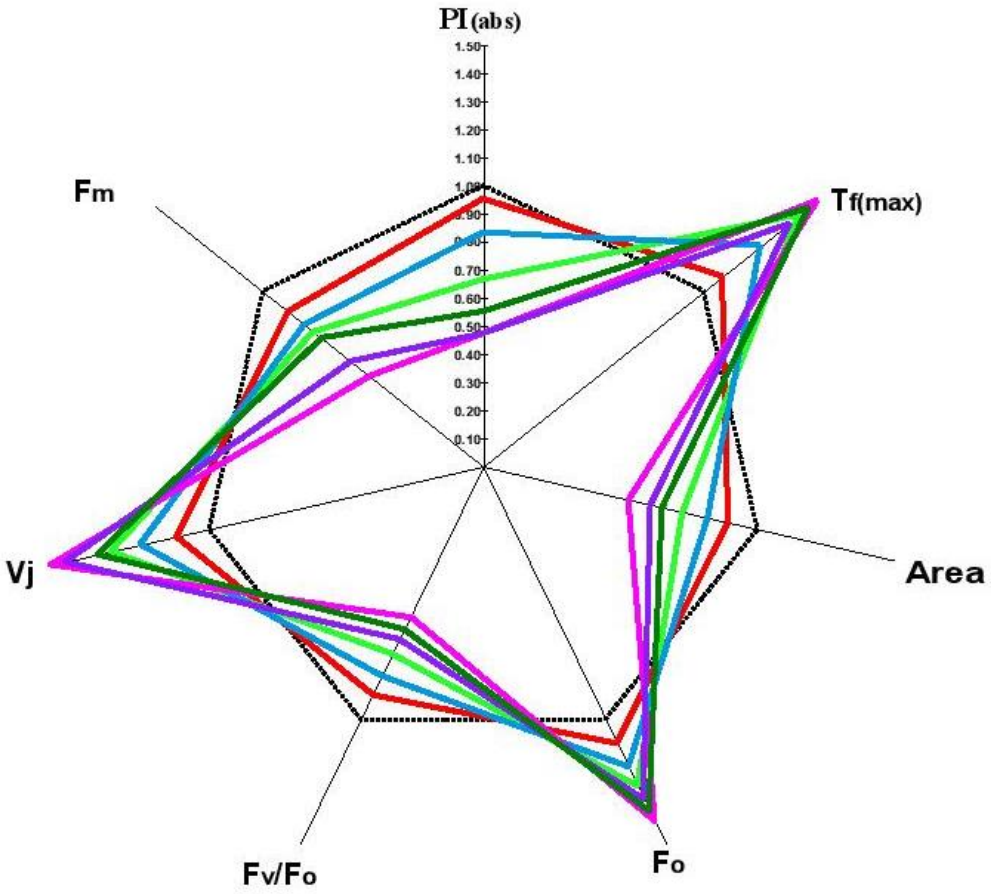

Fig. 11: Radar plot of selected Chl *a* fluorescence parameters recorded in leaves of rice seedlings from UV-B primed Aiswarya seedlings subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N-Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U-Primed+UV-B).

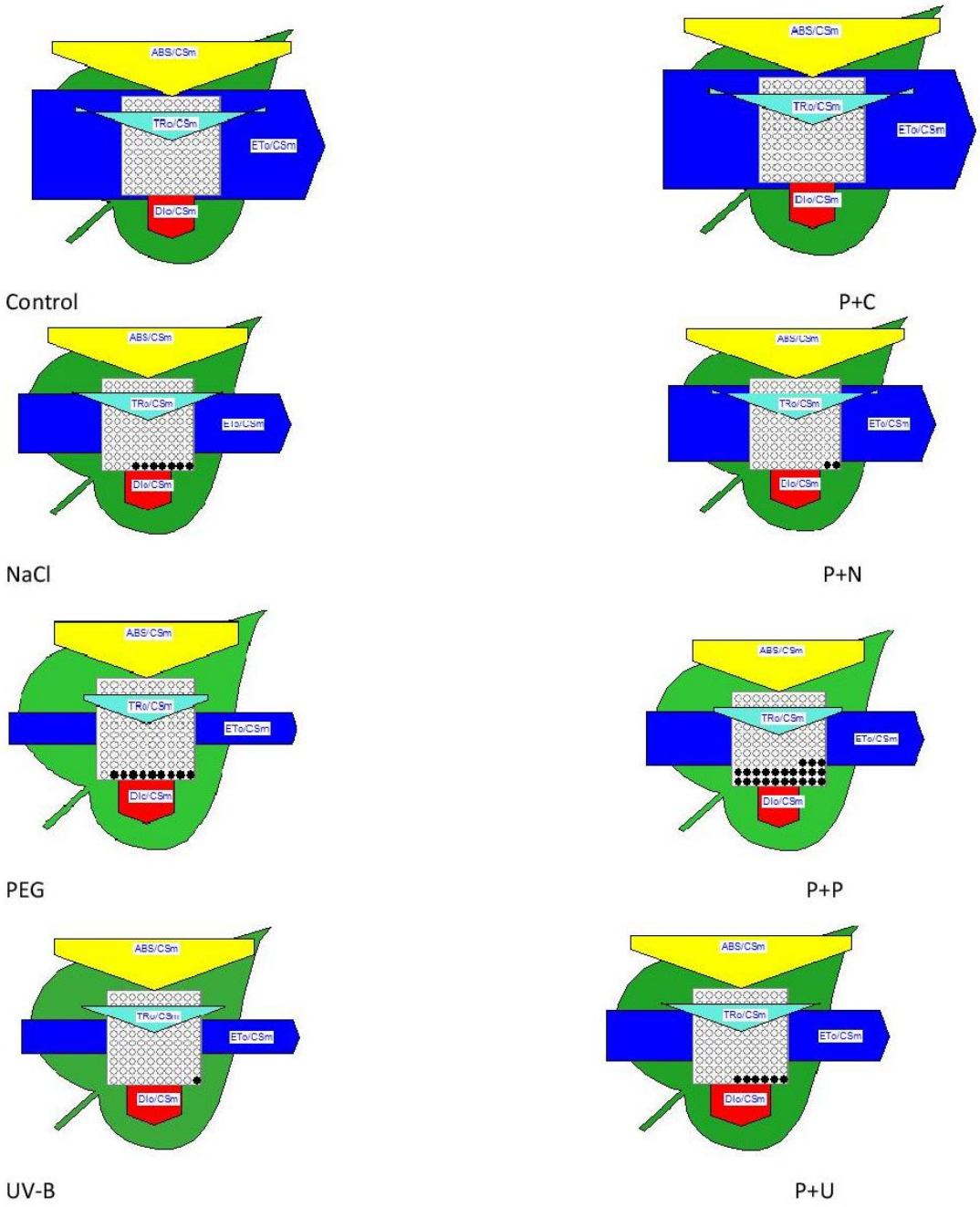

Fig. 12: Energy pipeline leaf model of phenomenological energy fluxes per cross section (CS_m) in leaves of rice seedlings from UV-B primed Kanchana seeds subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+ Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

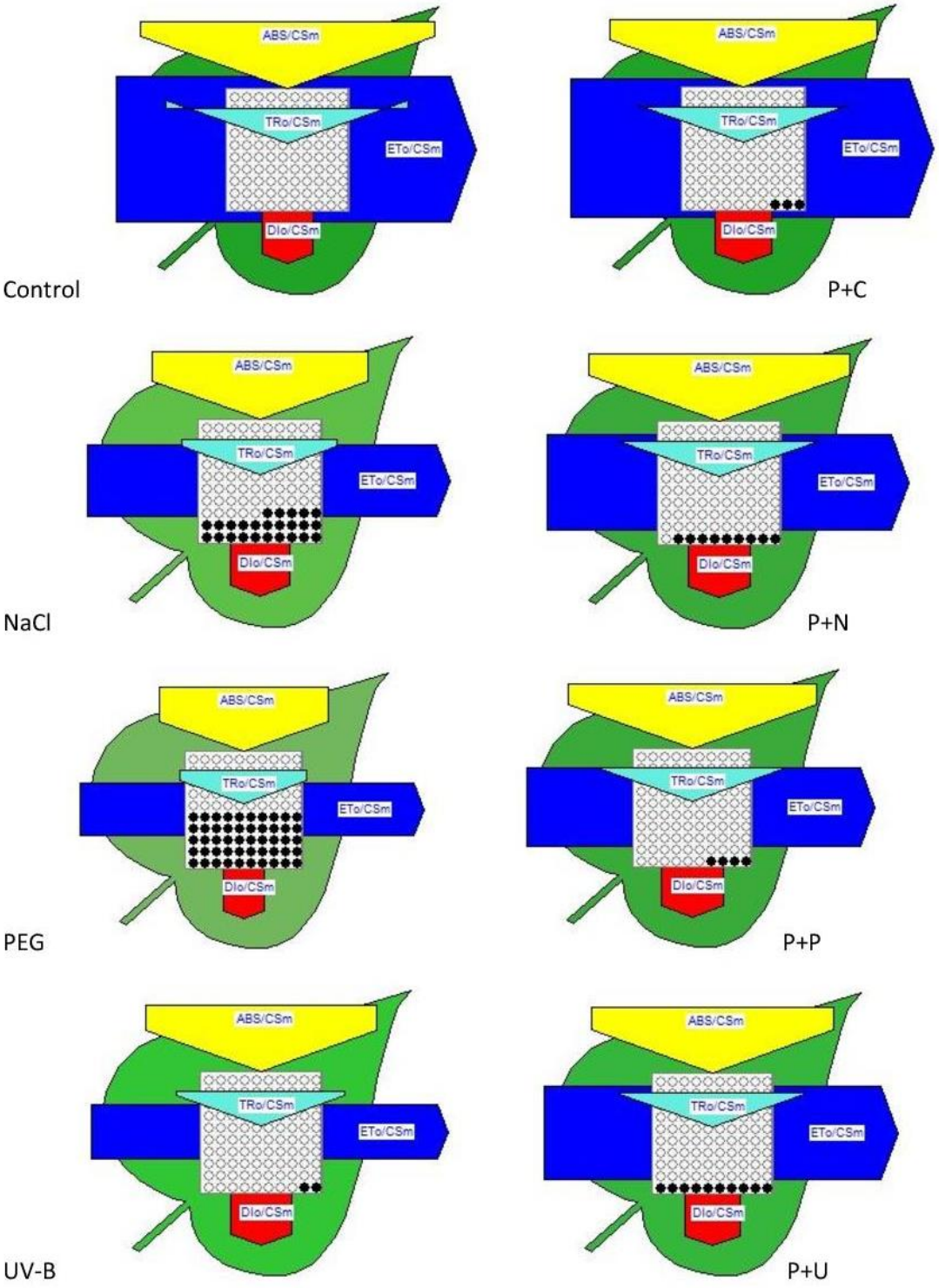

Fig. 13: Energy pipeline leaf model of phenomenological energy fluxes per cross section (CS_m) in leves of rice seedlings from UV-B primed Kanchana seedlings subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+ Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

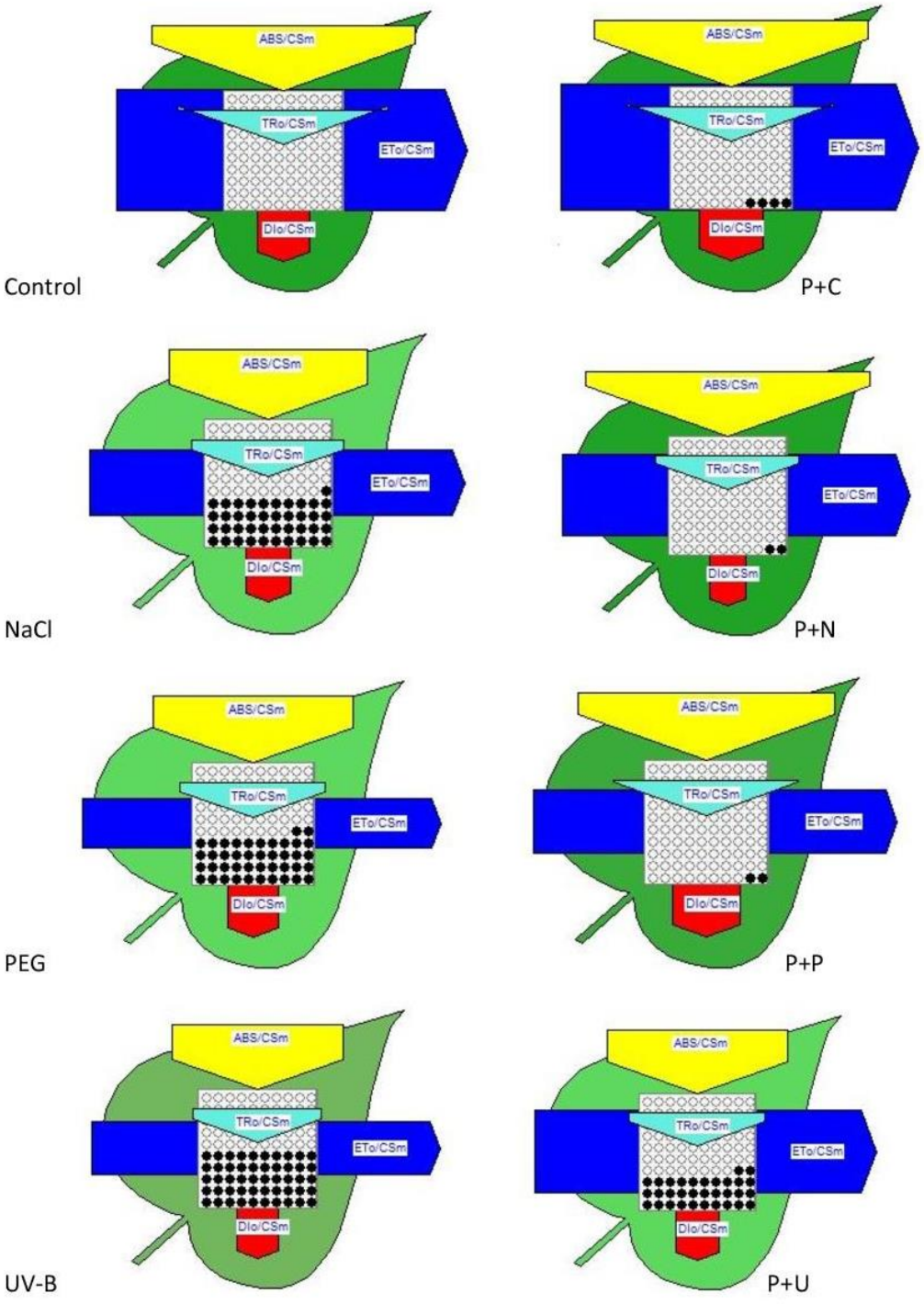

Fig. 14: Energy pipeline leaf model of phenomenological energy fluxes per cross section (CS_m) in leaves of rice seedlings from UV-B primed Aiswarya seeds subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

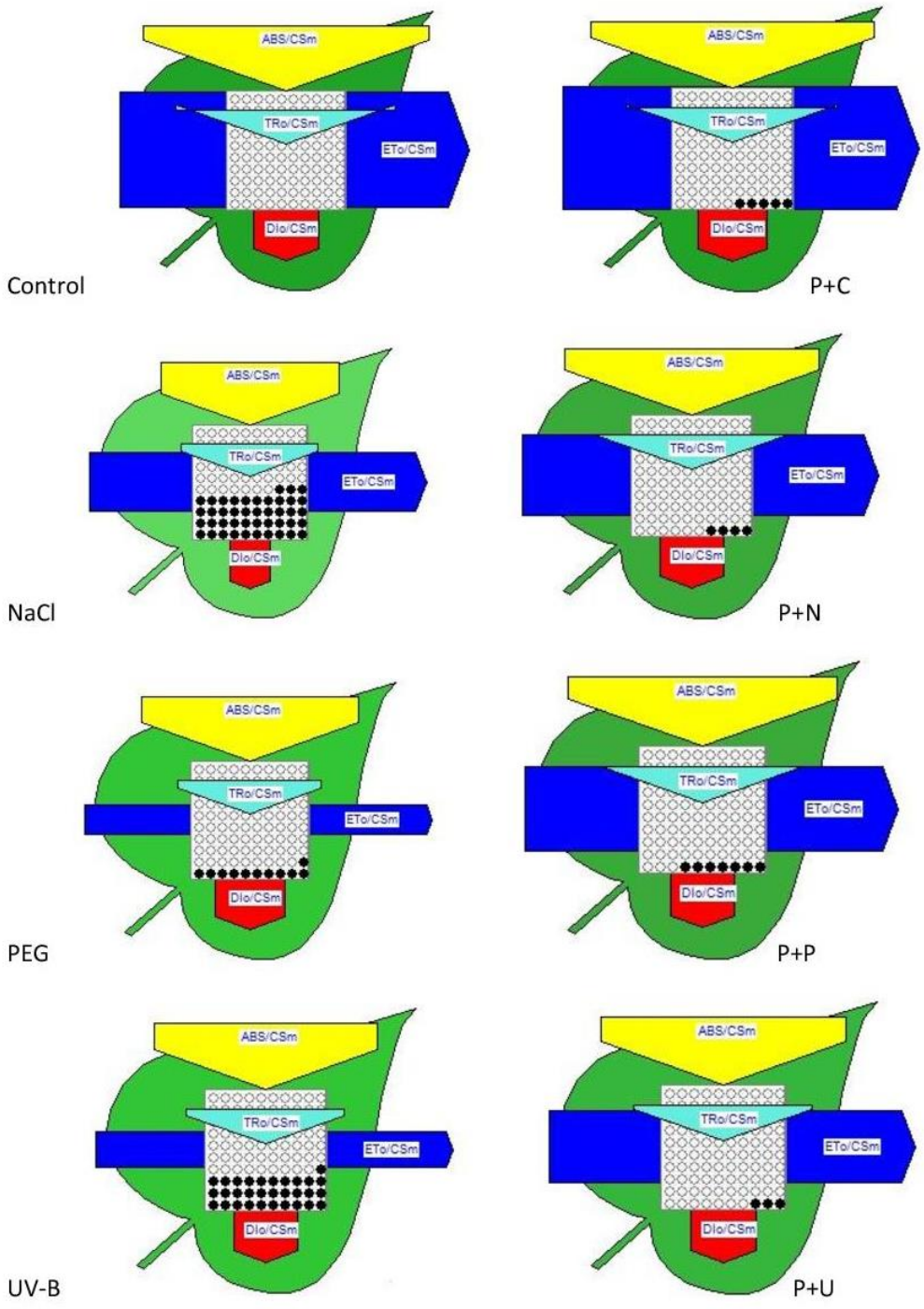

Fig. 15: Energy pipeline leaf model of phenomenological energy fluxes per cross section (CS_m) in leaves of rice seedlings from UV-B primed Aiswarya seedlings subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+ Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

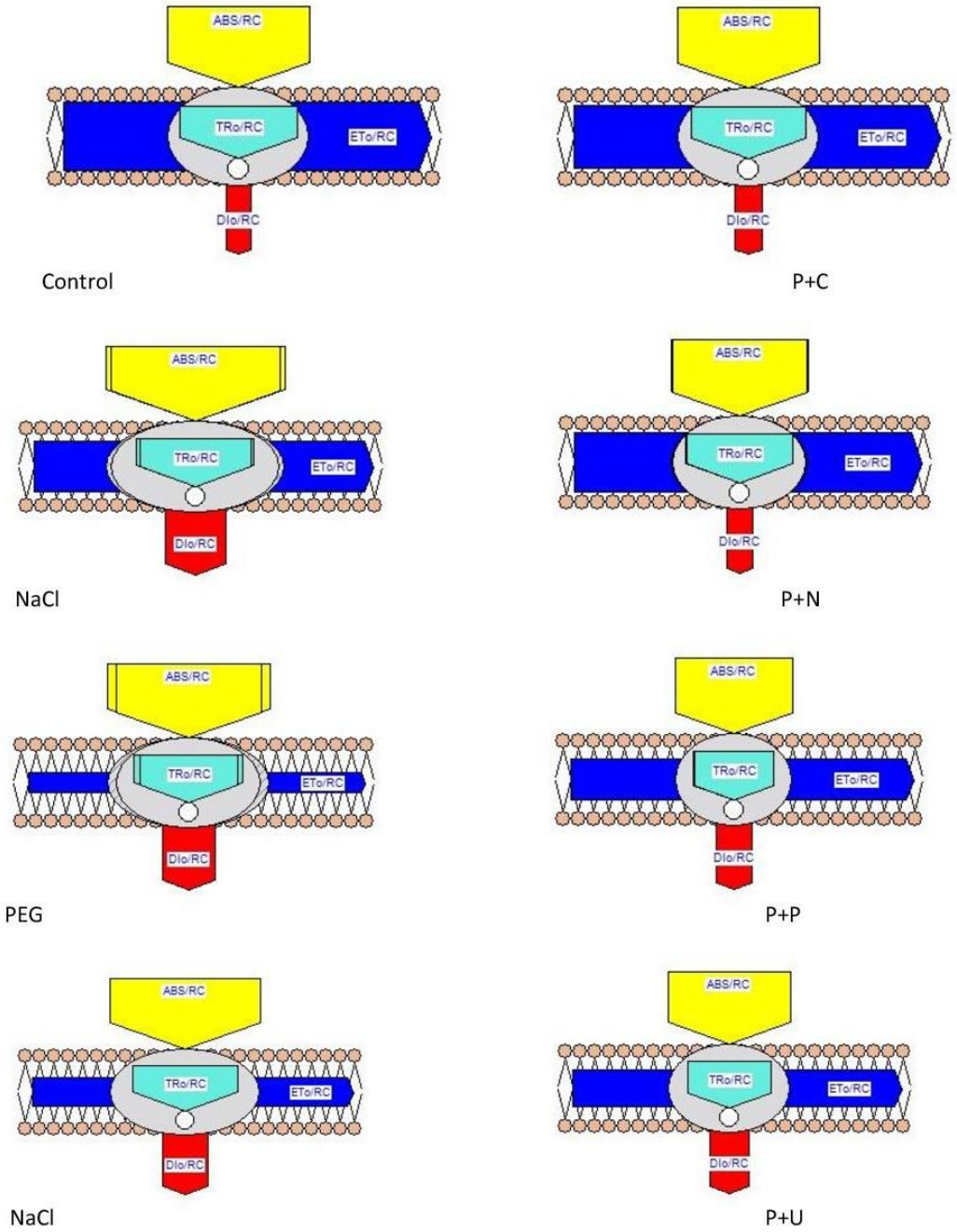

Fig. 16: Specific membrane model energy fluxes per reaction centre (RC) in leaves of rice seedlings from UV- B primed Kanchana seeds subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

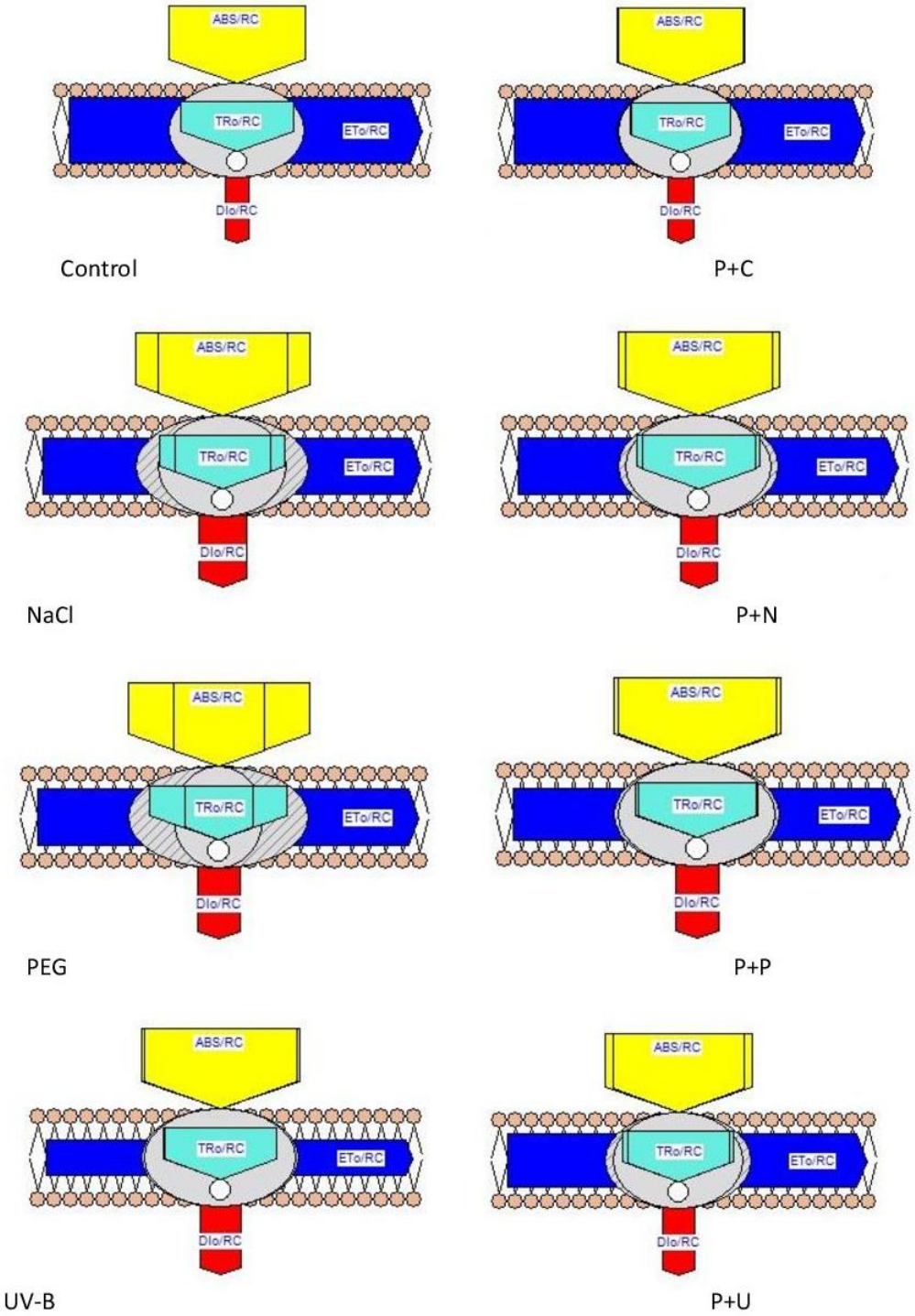

Fig. 17: Specific membrane model energy fluxes per reaction centre (RC) in leaves of rice seedlings from UV- B primed Kanchana seedlings subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

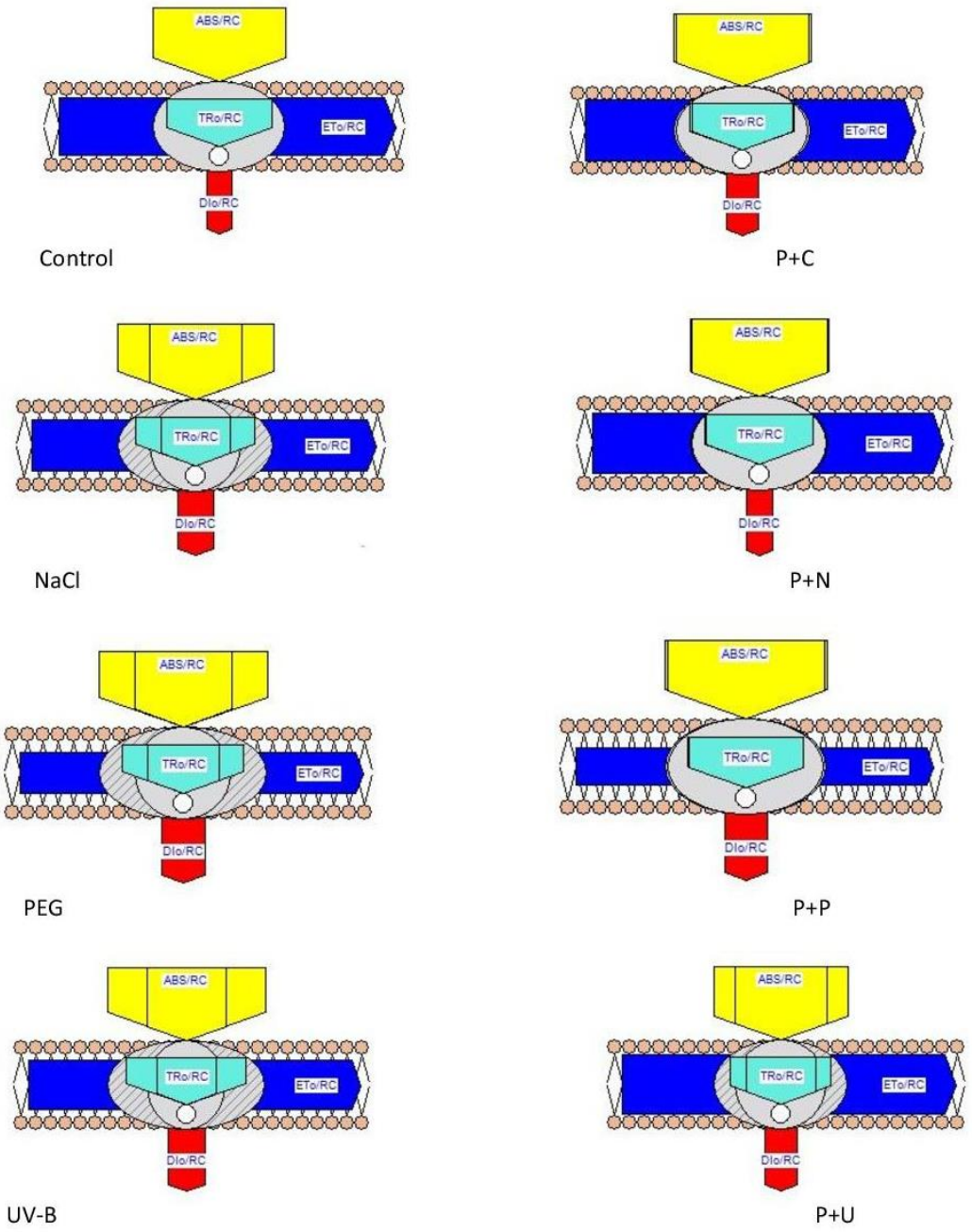

Fig. 18: Specific membrane model energy fluxes per reaction centre (RC) in leaves of rice seedlings from UV- B primed Aiswarya seeds subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

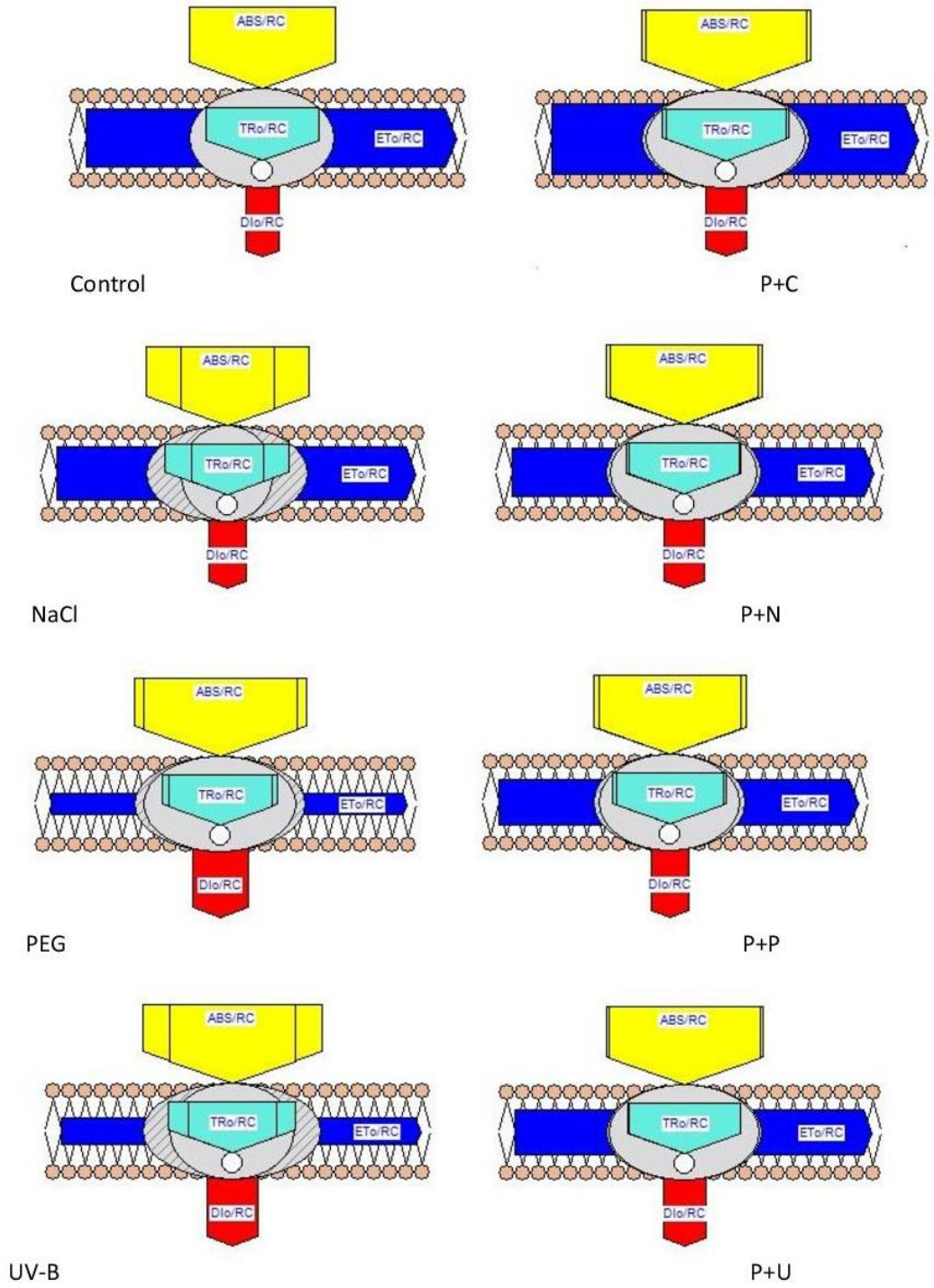

Fig. 19: Specific membrane model energy fluxes per reaction centre (RC) in leaves of rice seedlings from UV- B primed Aiswarya seedlings subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

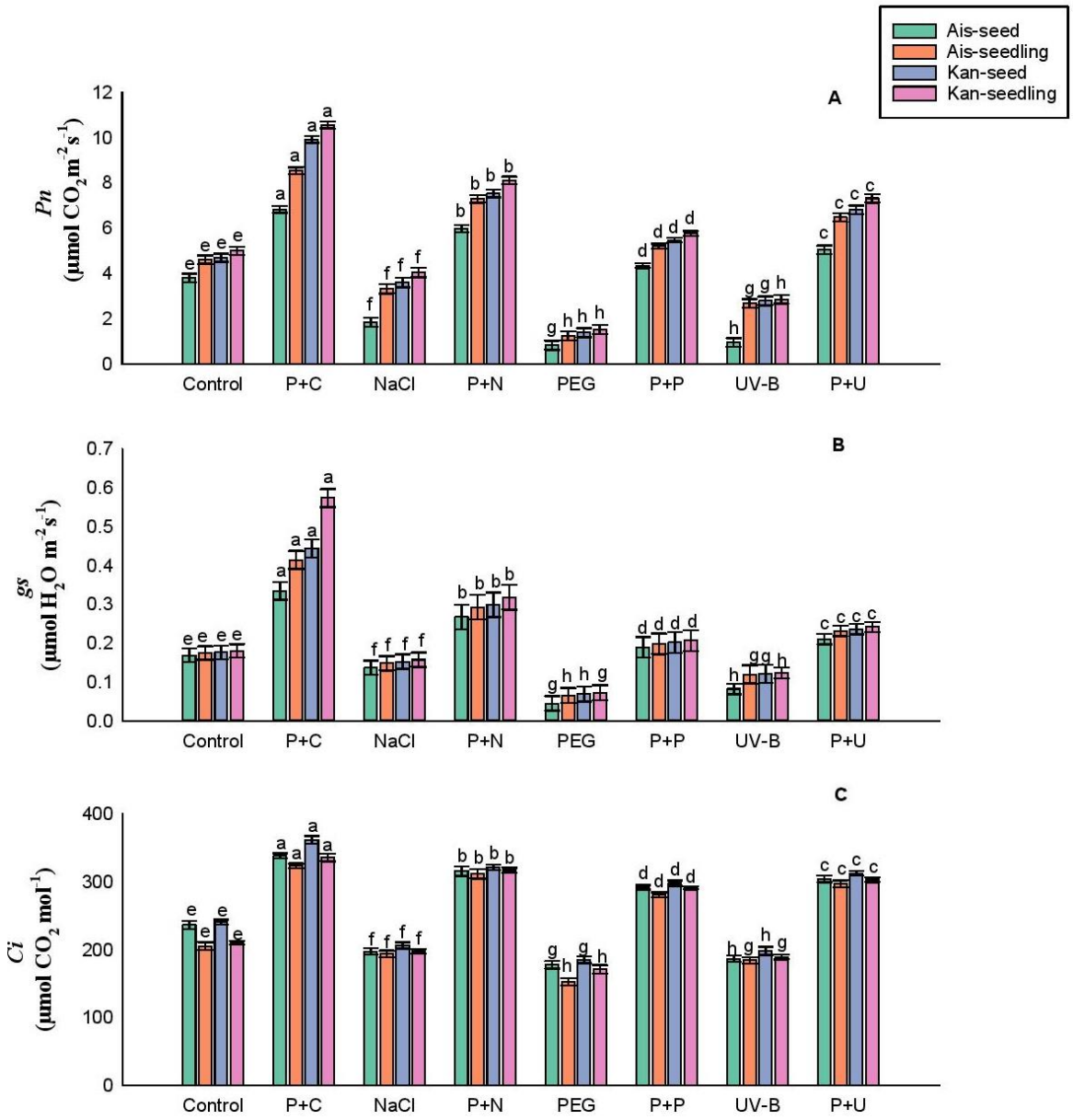

Fig. 20: *Pn* (A), *gs* (B) and *Ci* (C) in leves of rice seedlings from UV-B primed and non-primed seeds (P_s) as well as seedlings (P_{sl}) directly primed with UV-B and subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+ Control; P (P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

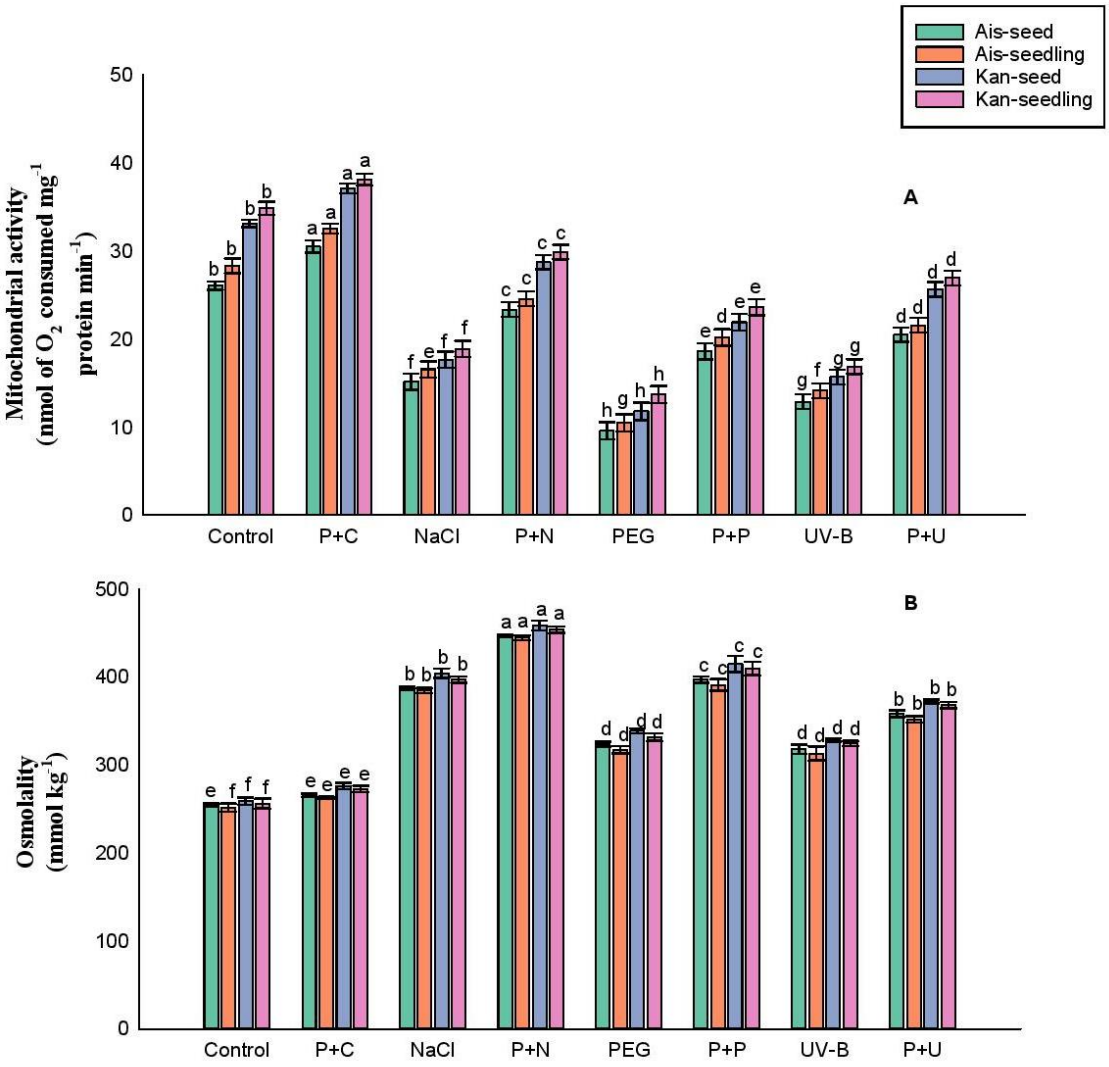

Fig. 21: Mitochondrial activity (A) and osmolality (B) in leaves of rice seedlings from UV-B primed and non-primed seeds (P_s) as well as seedlings (P_{sl}) directly primed with UV-B and subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

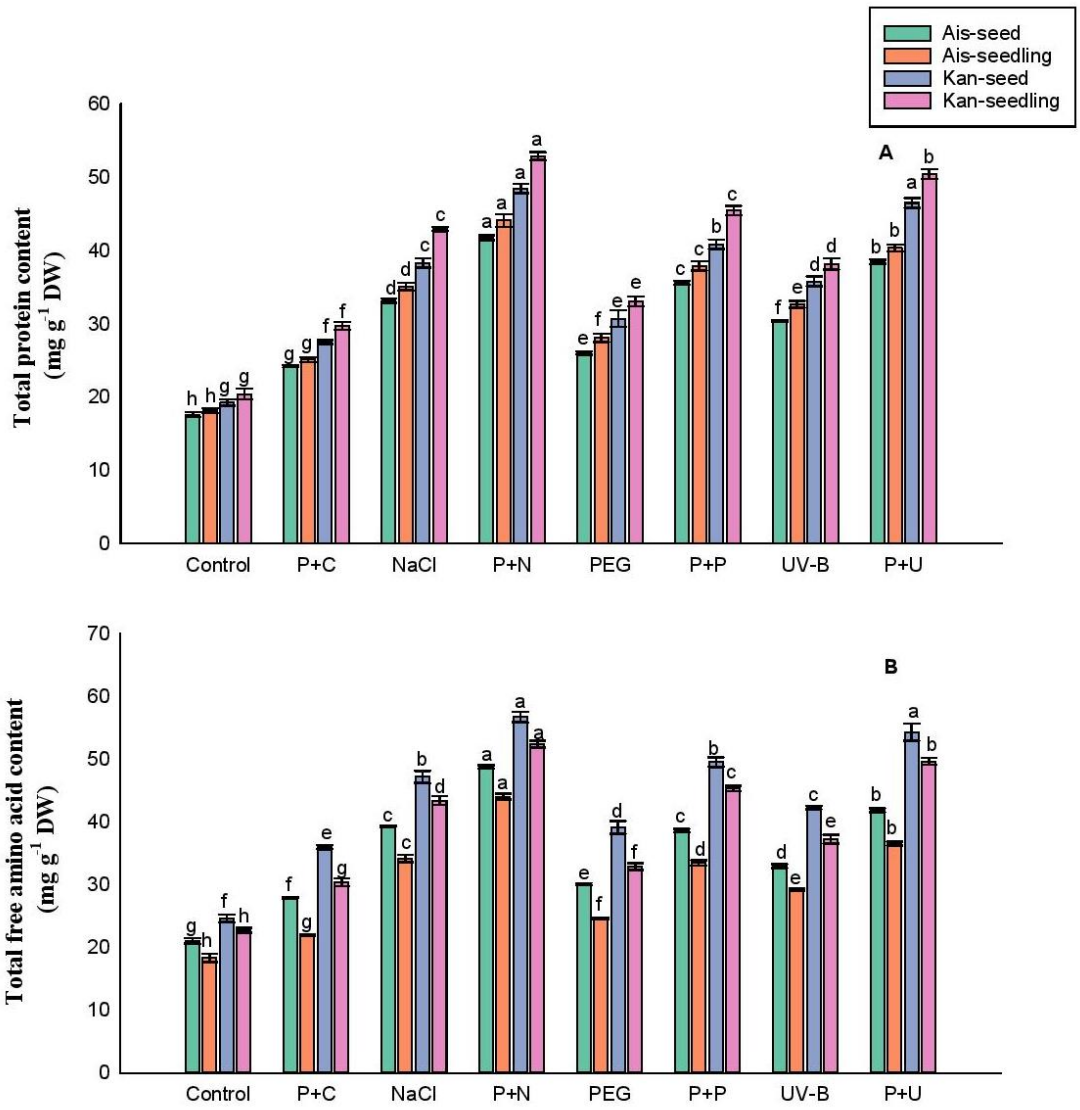

Fig. 22: Total protein (A) and total free amino acids (B) in rice seedlings from UV-B primed and non-primed seeds (P_s) as well as seedlings (P_{sl}) directly primed with UV-B and subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

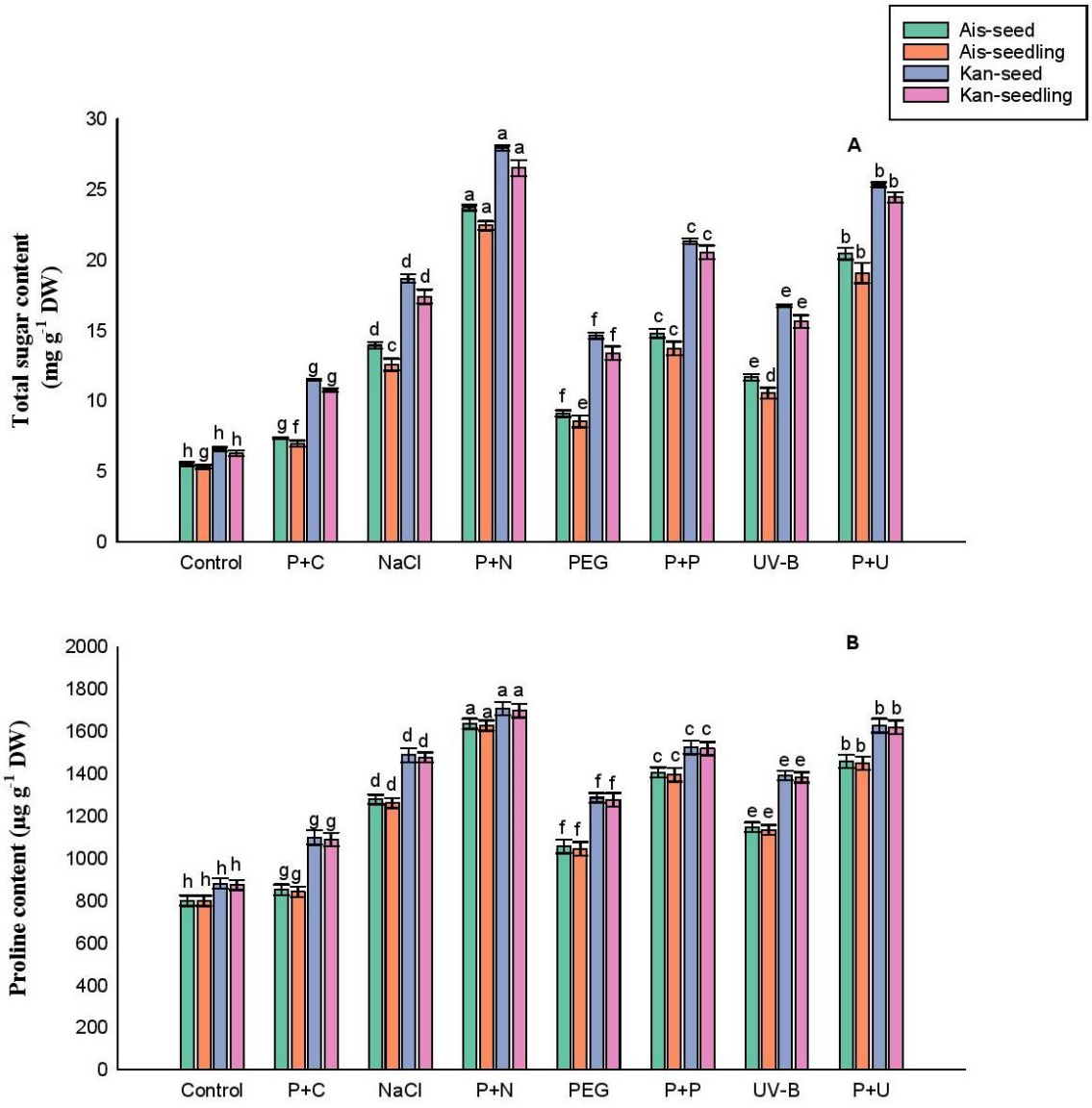

Fig. 23: Total sugar (A) and proline (B) content in rice seedlings from UV-B primed and non-primed seeds (P_s) as well as seedlings (P_{sl}) directly primed with UV-B and subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+ Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

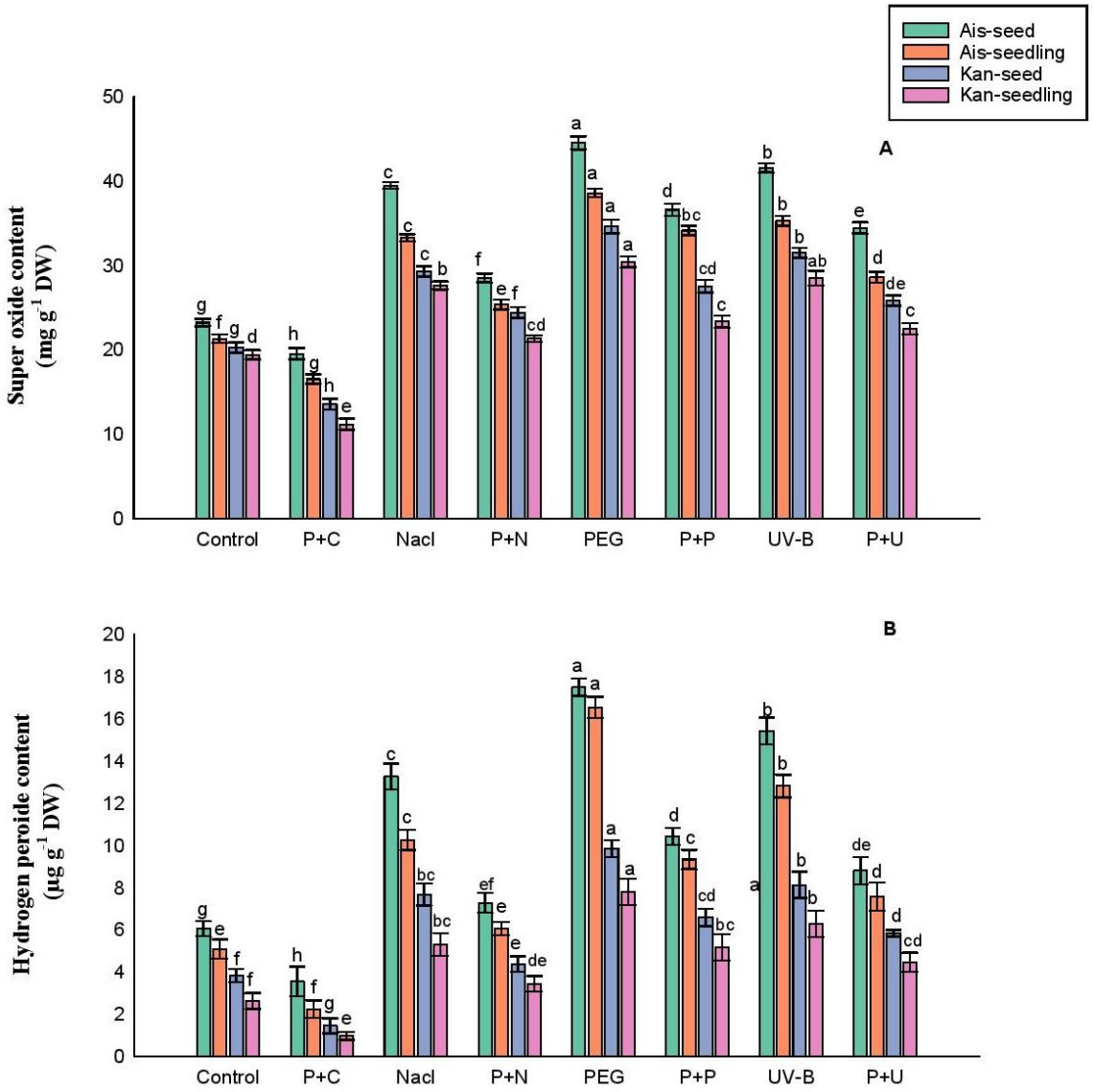

Fig. 24: Super oxide (A) and hydrogen peroxide (B) content in rice seedlings from UV-B primed and non-primed seeds (P_s) as well as seedlings (P_{sl}) directly primed with UV-B and subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P-Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

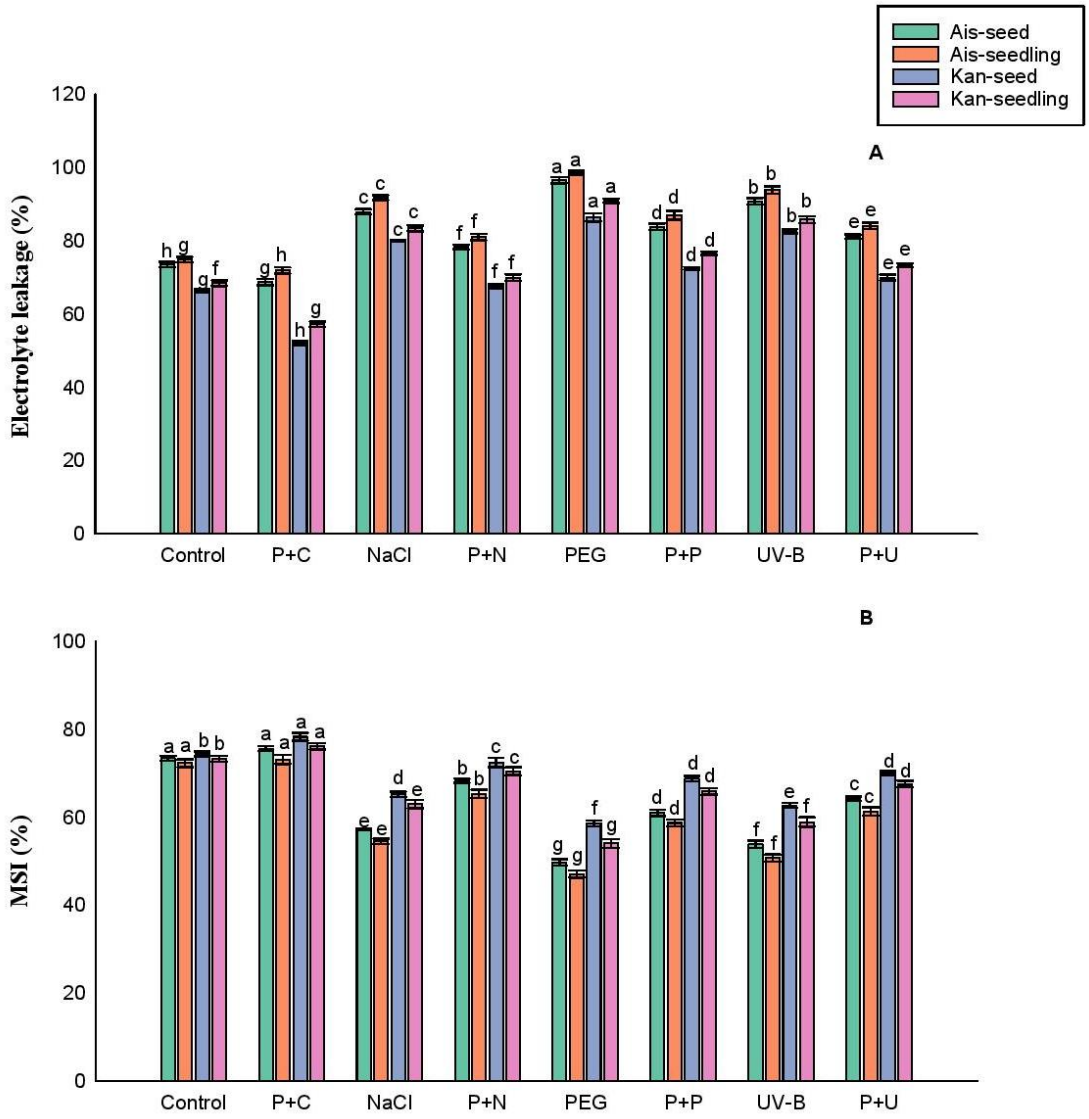

Fig. 25: Electrolyte leakage (A) and membrane stability (B) in rice seedlings from UV-B primed and non-primed seeds (P_s) as well as seedlings (P_{sl}) directly primed with UV-B and subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

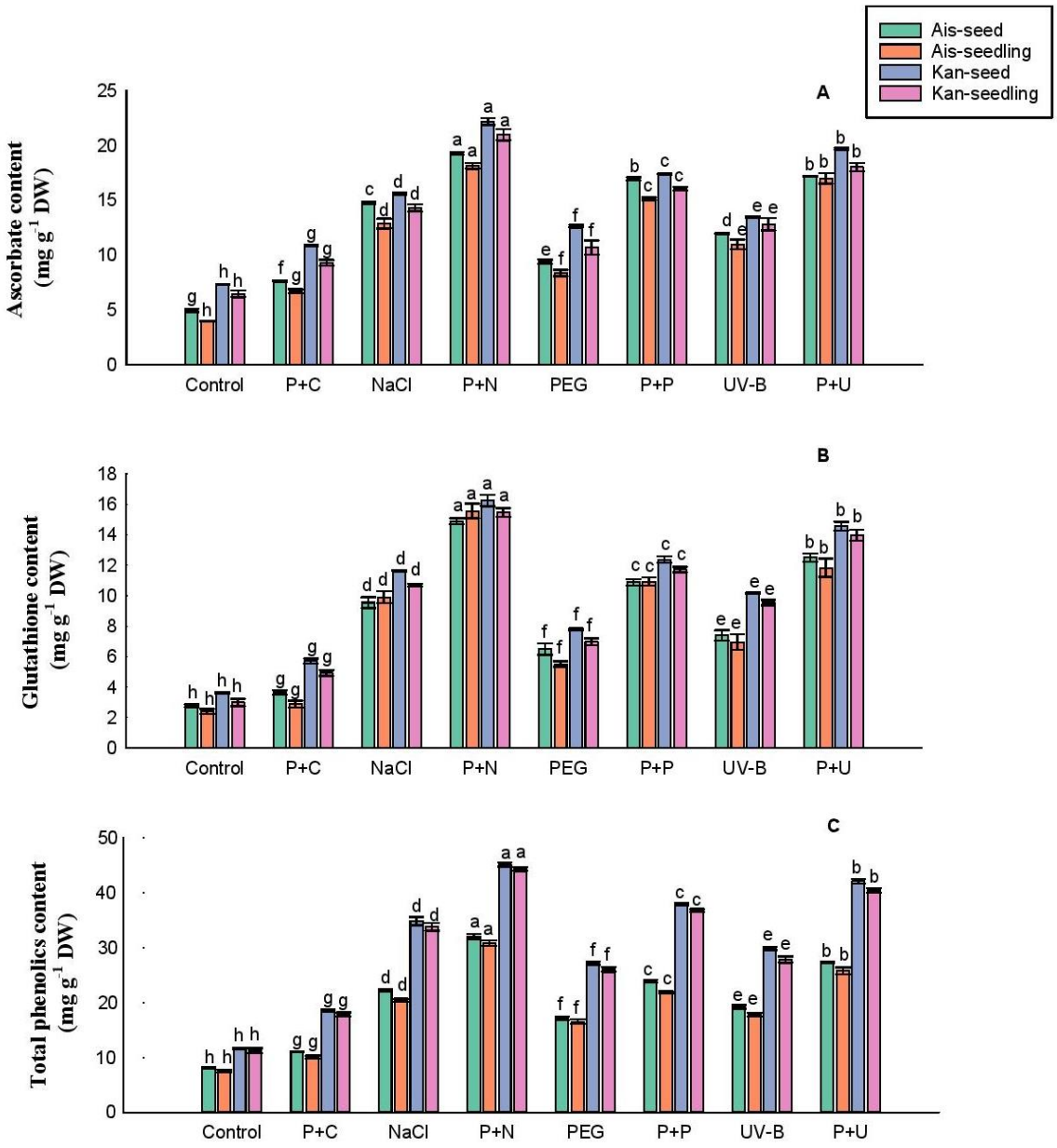

Fig. 26: Ascorbate (A), Glutathione (B) and phenolics (C) content in rice seedlings from UV-B primed and non-primed seeds (P_s) as well as seedlings (P_{sl}) directly primed with UV-B and subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+ PEG; P(P_s & P_{sl})+U- Primed + UV-B).

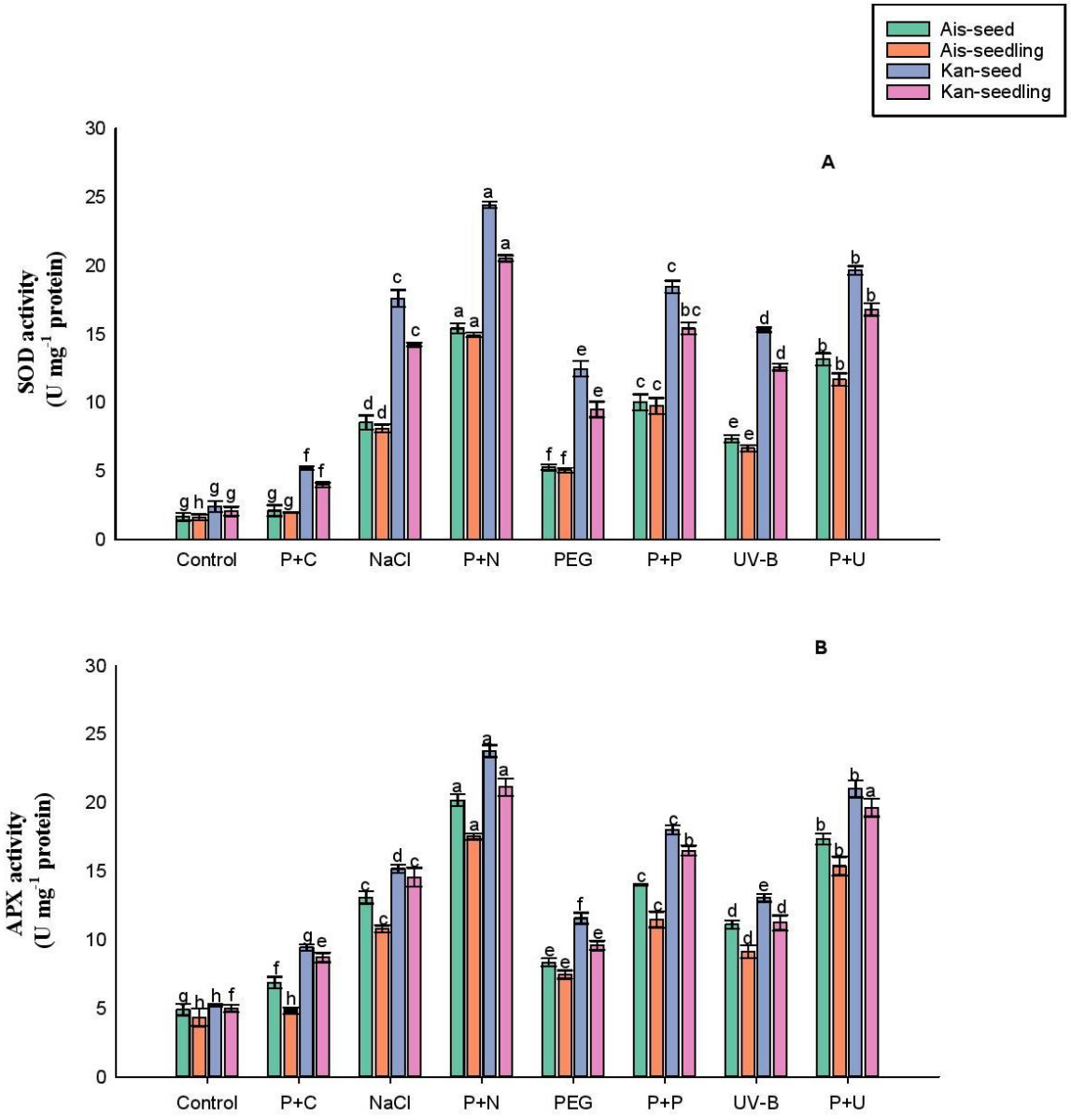

Fig. 27: SOD (A) and APX (B) activity in rice seedlings from UV-B primed and non-primed seeds (P_s) as well as seedlings (P_{sl}) directly primed with UV-B and subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed + UV-B).

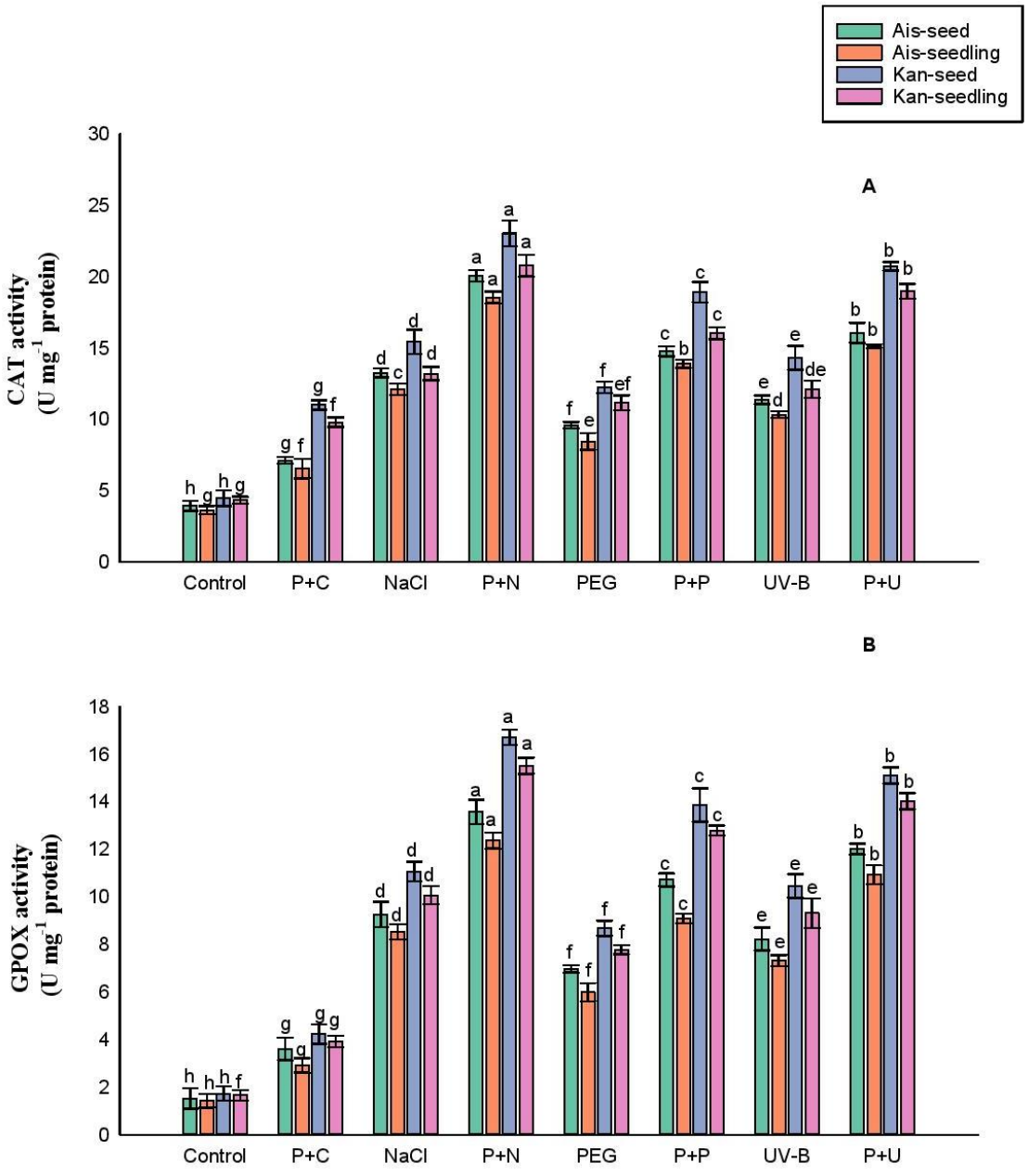

Fig. 28: CAT (A) and GPOX (B) activity in rice seedlings from UV-B primed and non-primed seeds (P_s) as well as seedlings (P_{sl}) directly primed with UV-B and subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+ Control; P(P_s & P_{sl})+N-Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U-Primed+UV-B).

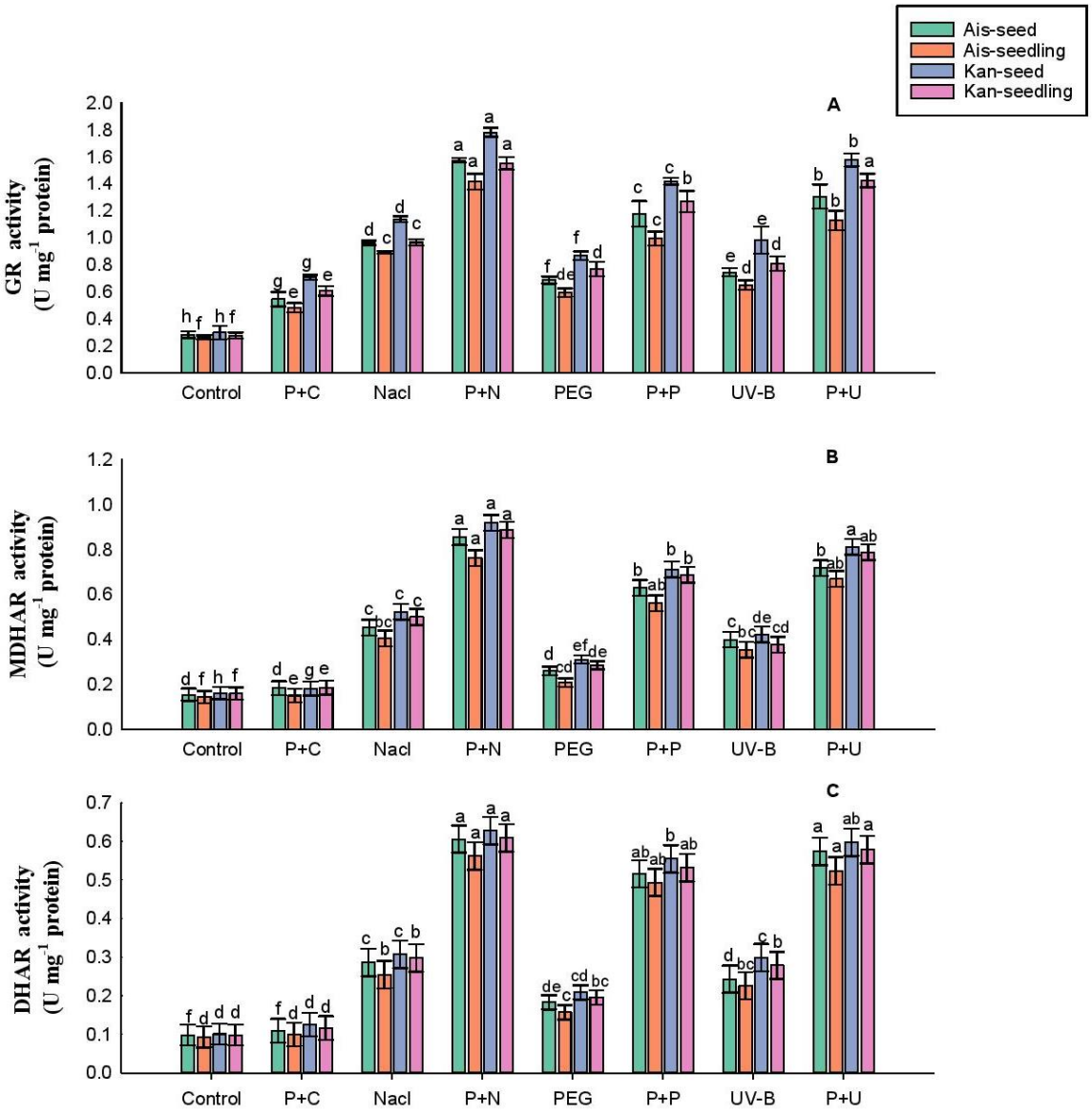

Fig. 29: GR (A), MDHAR (B) and DHAR (C) activity in rice seedlings from UV-B primed and non-primed seeds (P_s) as well as seedlings (P_{sl}) directly primed with UV-B and subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

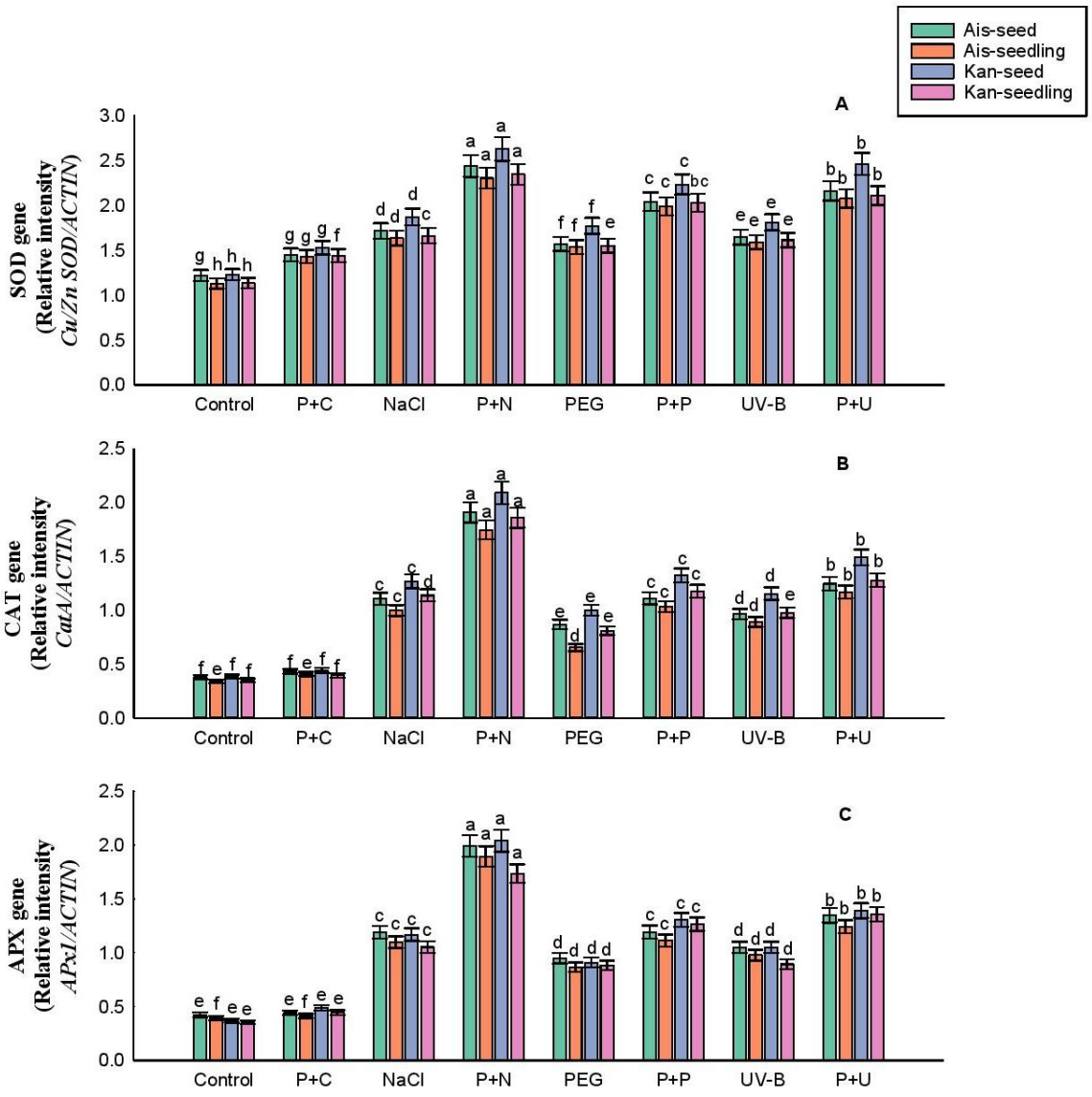

Fig. 30: mRNA level expression of SOD (*Cu/Zn SOD*) (A), CAT (*CatA*) (B) and APX (*APx1*) (C) gene in rice seedlings from UV-B primed and non-primed seeds (P_s) as well as seedlings (P_{sl}) directly primed with UV-B and subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+ NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

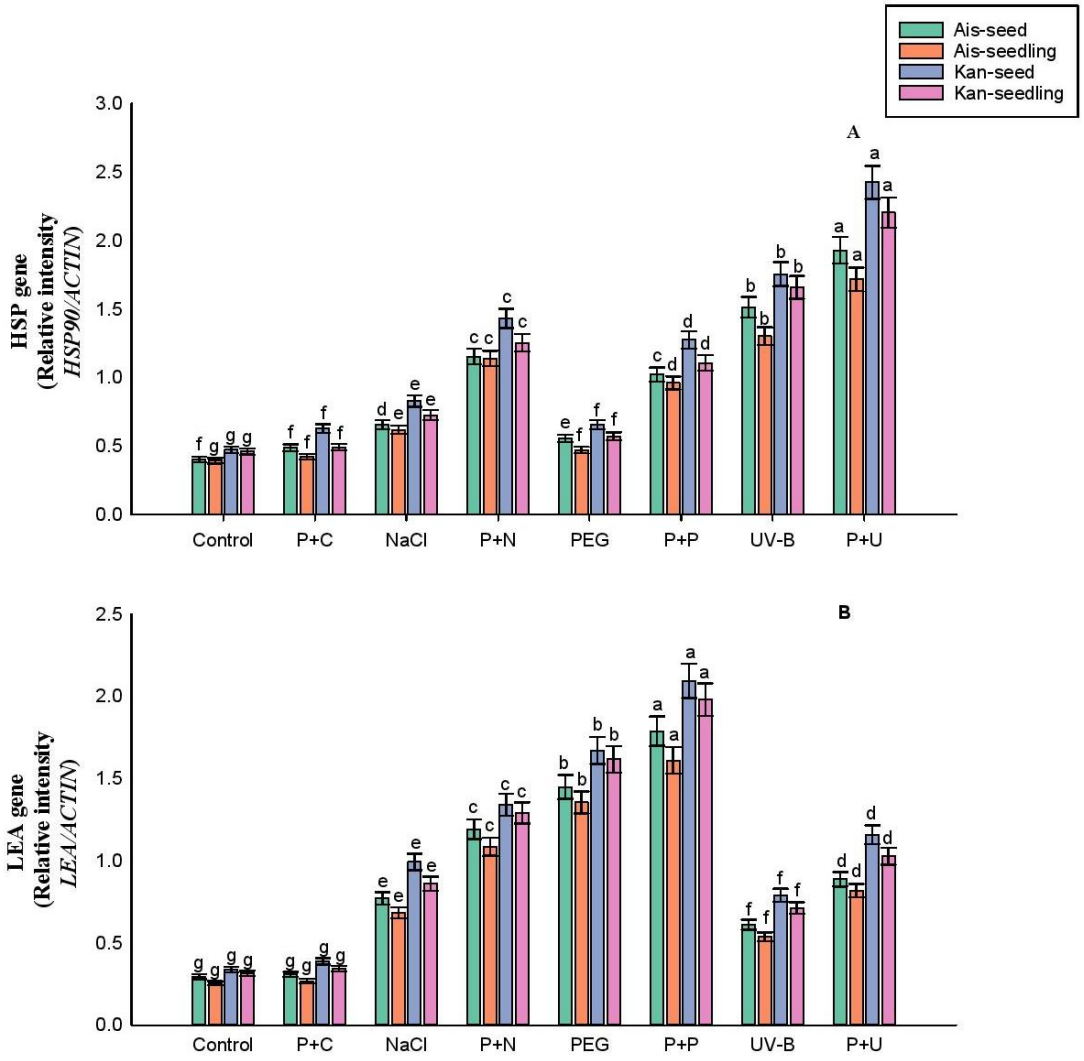

Fig. 31: mRNA level expression of HSP (*HSP90*) (A) and LEA (*LEA*) (B) gene in rice seedlings from UV-B primed and non-primed seeds (P_s) as well as seedlings (P_{sl}) directly primed with UV-B and subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

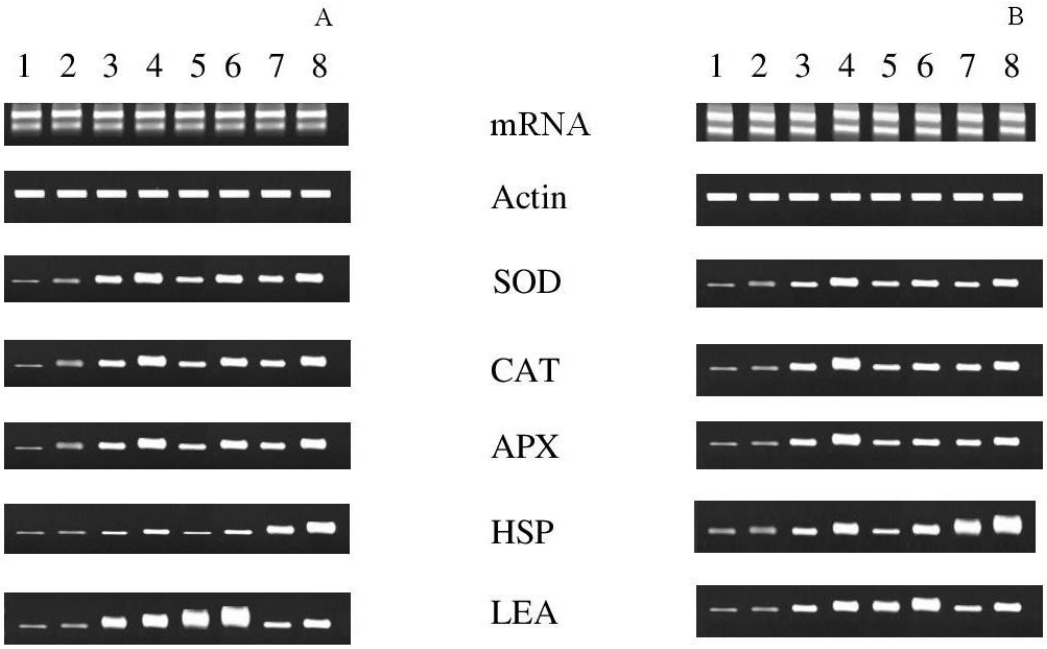

Fig. 32: mRNA level expression of Actin (*ACTIN*), SOD (*Cu/Zn SOD*), CAT (*CatA*), APX (*APx1*), HSP (*HSP90)* and LEA (group *3 LEA*) in Kanchana and Aiswarya rice seedlings from UV-B primed and non-primed seeds (P_s) subjected to various stress conditions (NaCl, PEG and UV-B). (1- Control, 2- Primed+Control; 3- Non-primed+NaCl; 4- Primed+NaCl; 5- Non-primed+PEG; 6- Primed+PEG; 7- Non-primed+UV-B; 8- Primed+UV-B).

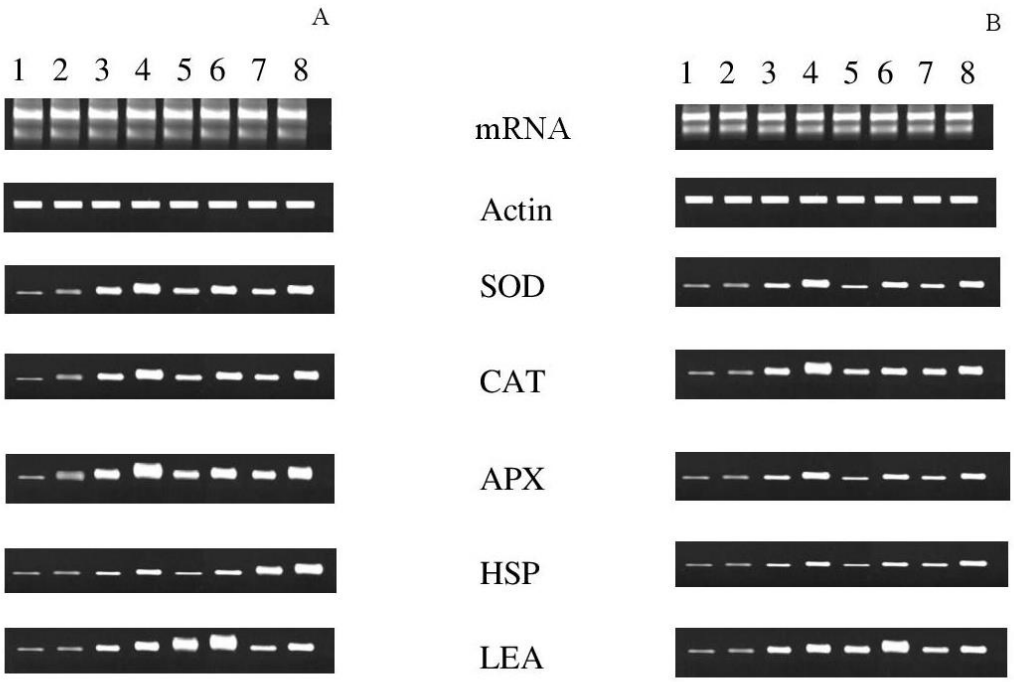

Fig. 33: mRNA level expression of Actin (*ACTIN*), SOD (*Cu/Zn SOD*), CAT (*CatA*), APX (*APx1*), HSP (*HSP90)* and LEA(group *3 LEA*) in Kanchana (A) and Aiswarya (B) rice seedlings from seedlings (P_{sl}) directly primed with UV-B subjected to various stress conditions (NaCl, PEG and UV-B). (1- Control, 2- Primed+Control; 3- Non-primed+NaCl; 4- Primed+NaCl; 5- Non-primed+PEG; 6-Primed+PEG; 7- Non-primed+UV-B; 8- Primed+UV-B).

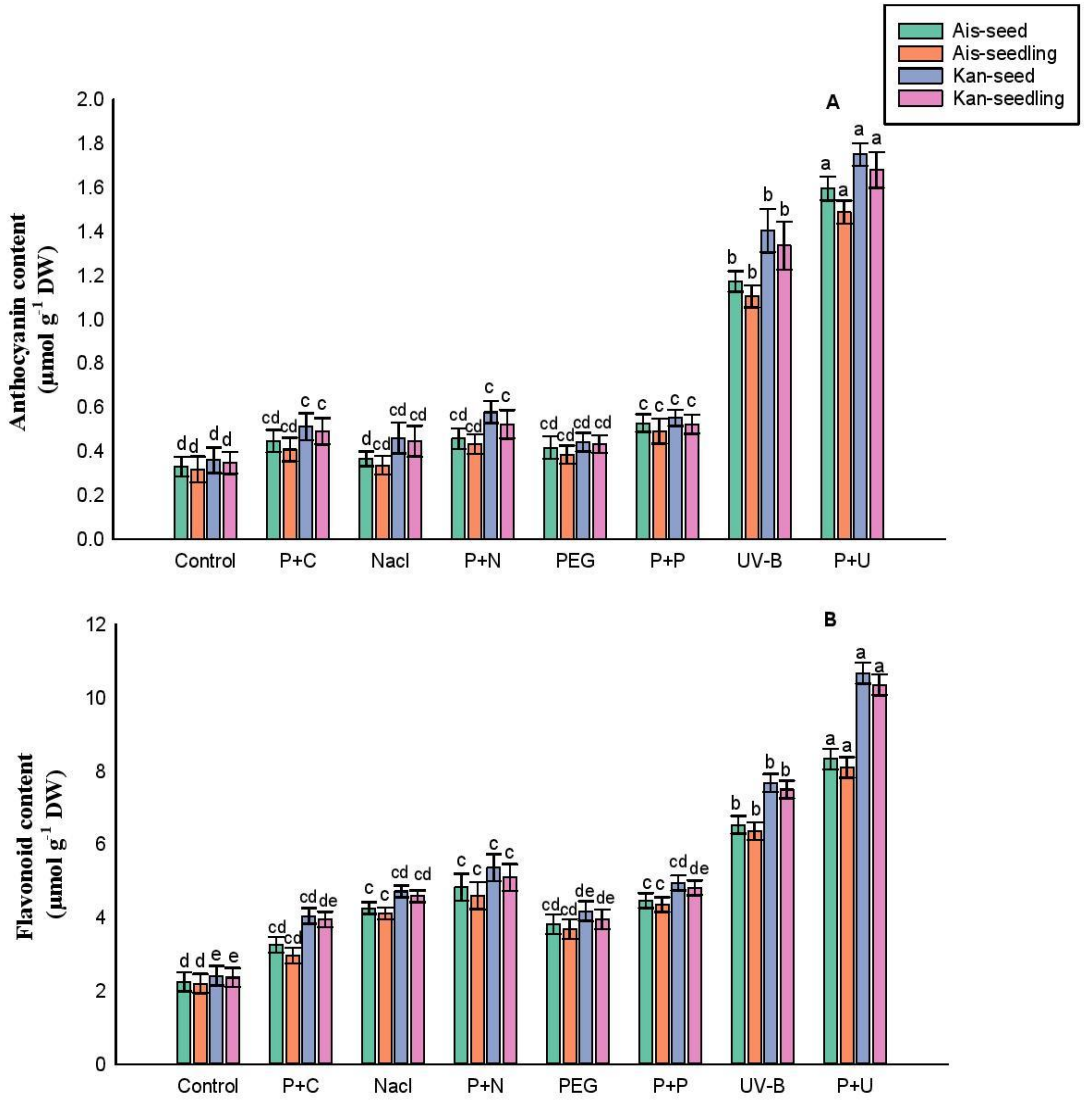

Fig. 34: Anthocyanin (A) and flavonoid (B) content in seedlings from UV-B primed and non-primed seeds (P_s) as well as seedlings (P_{sl}) directly primed with UV-B and subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+ Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

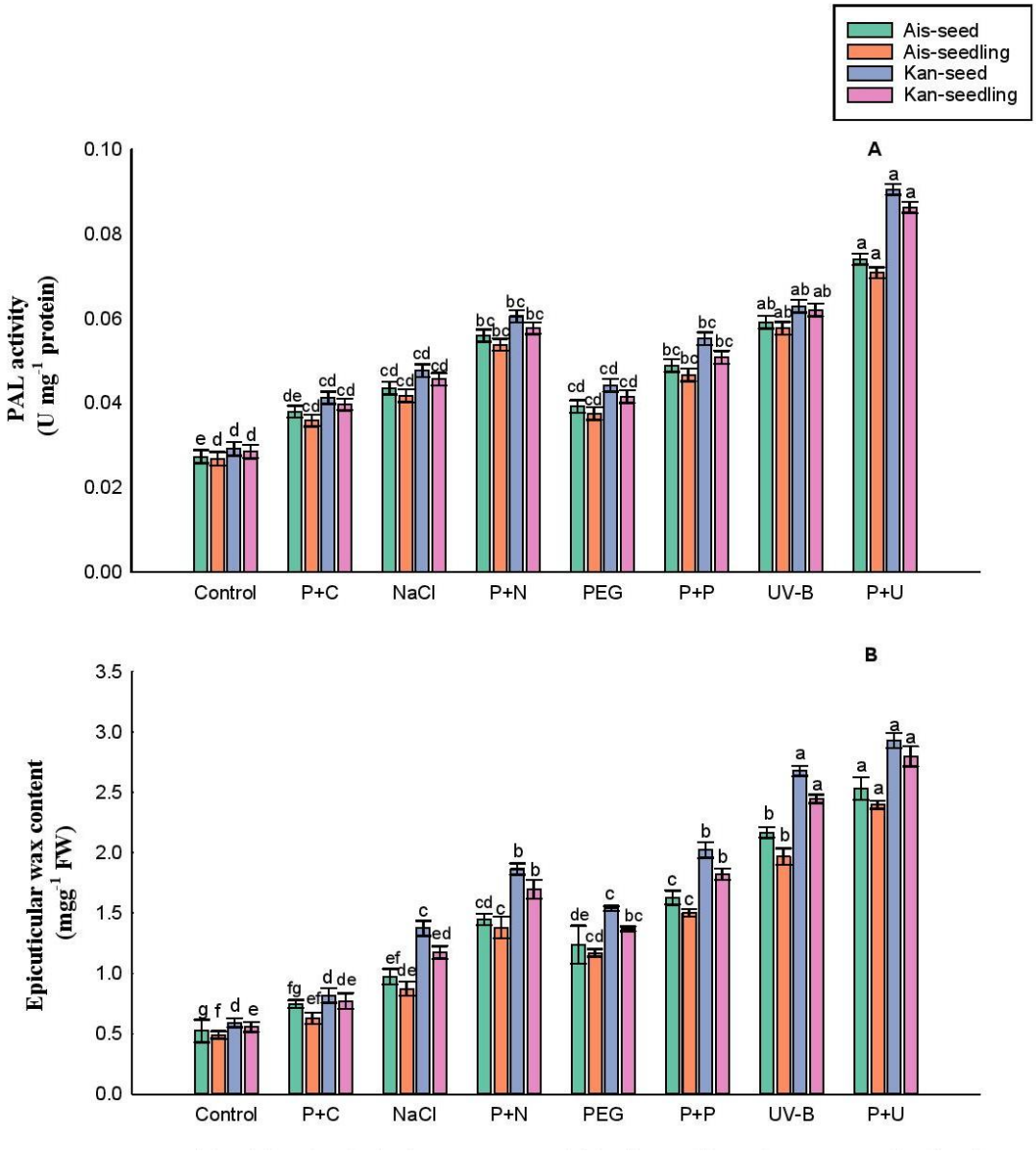

Fig. 35: PAL activity (A) and epicuticular wax content (B) in rice seedlings from UV-B primed and non-primed seeds (P_s) as well as seedlings (P_{sl}) directly primed with UV-B and subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

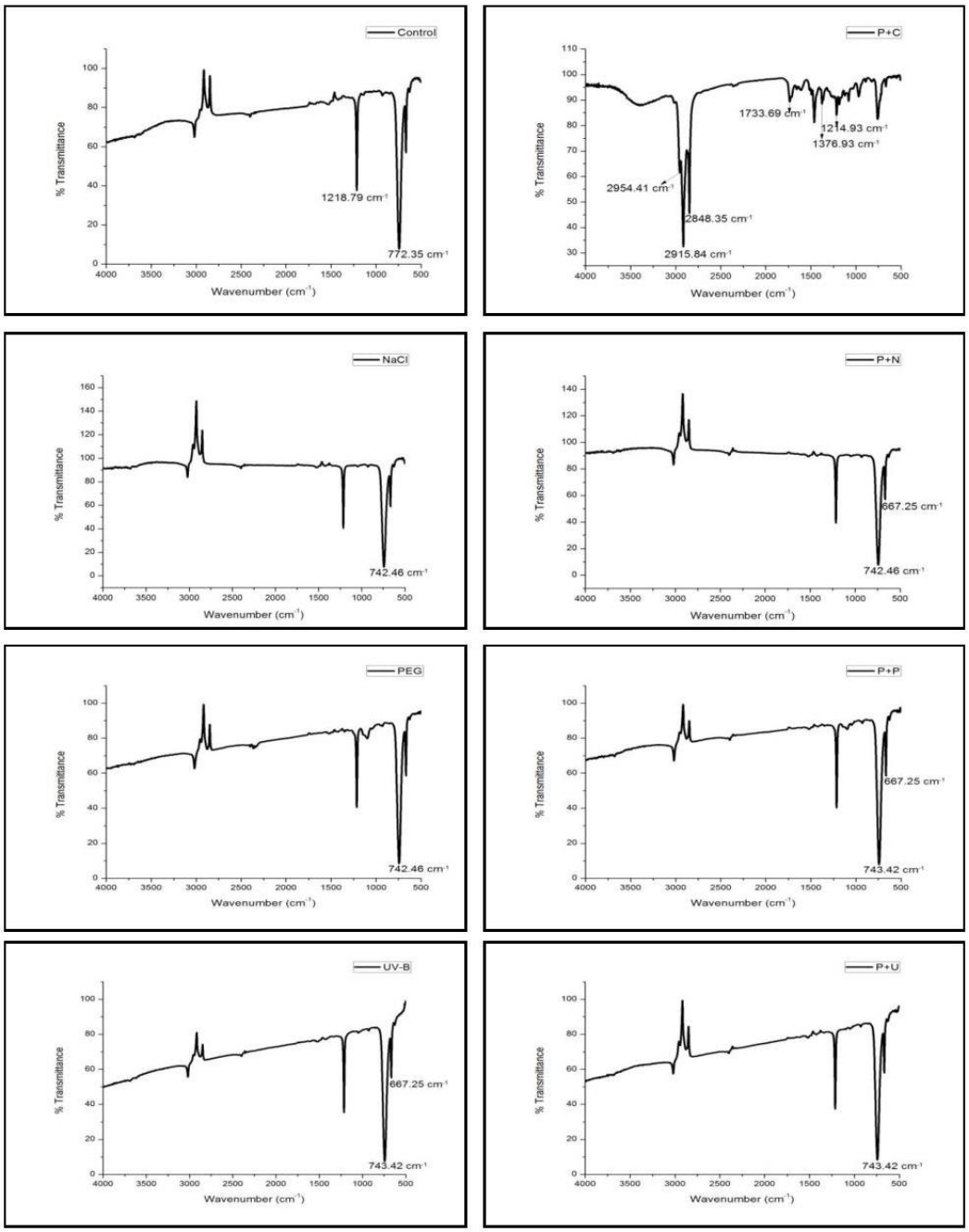

Fig. 36: FT-IR spectra of epicuticular wax in rice seedlings from UV-B primed and non-primed Kanchana seeds subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

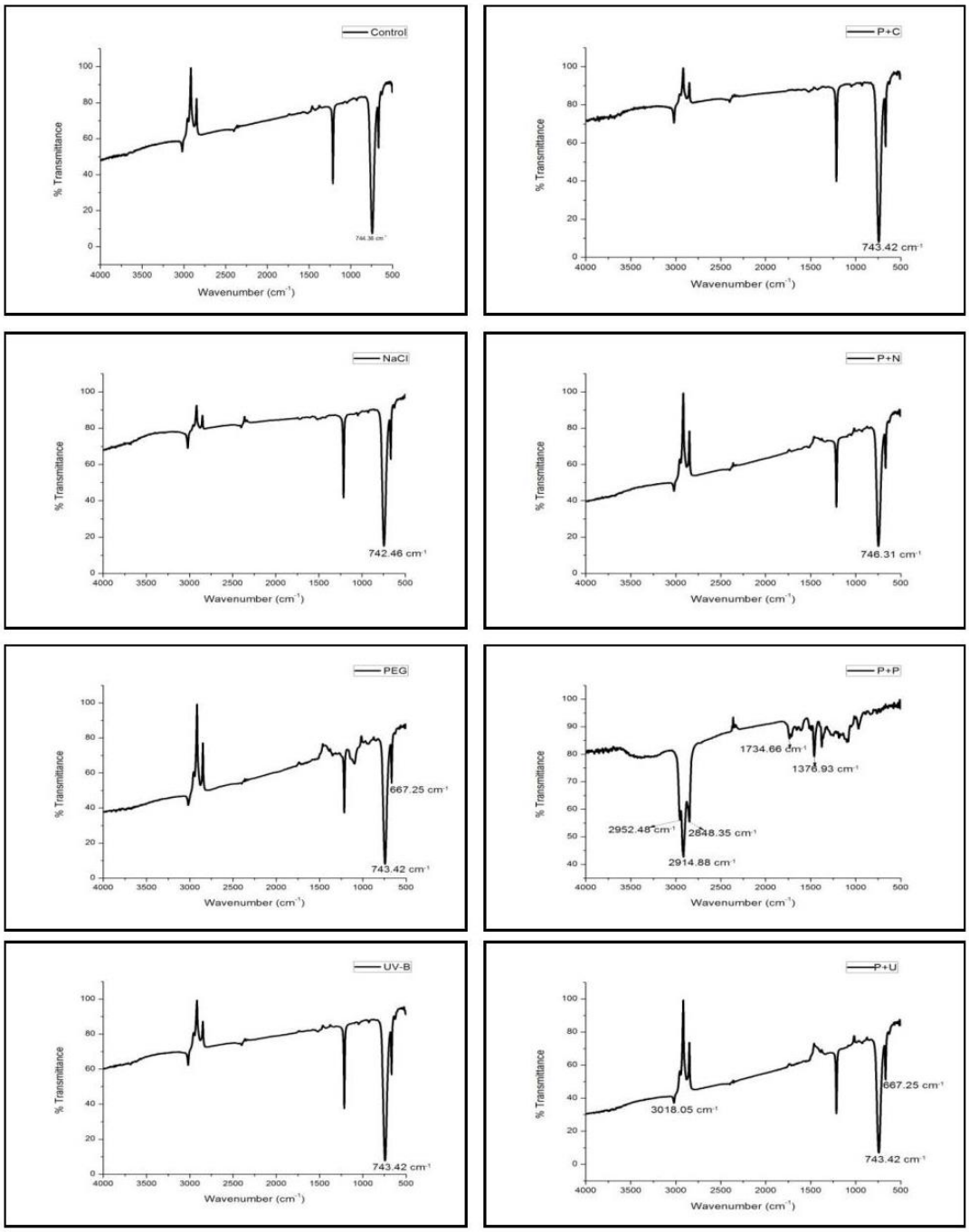

Fig. 37: FT-IR spectra of epicuticular wax in rice seedlings from UV-B primed and non-primed Kanchana seedlings subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

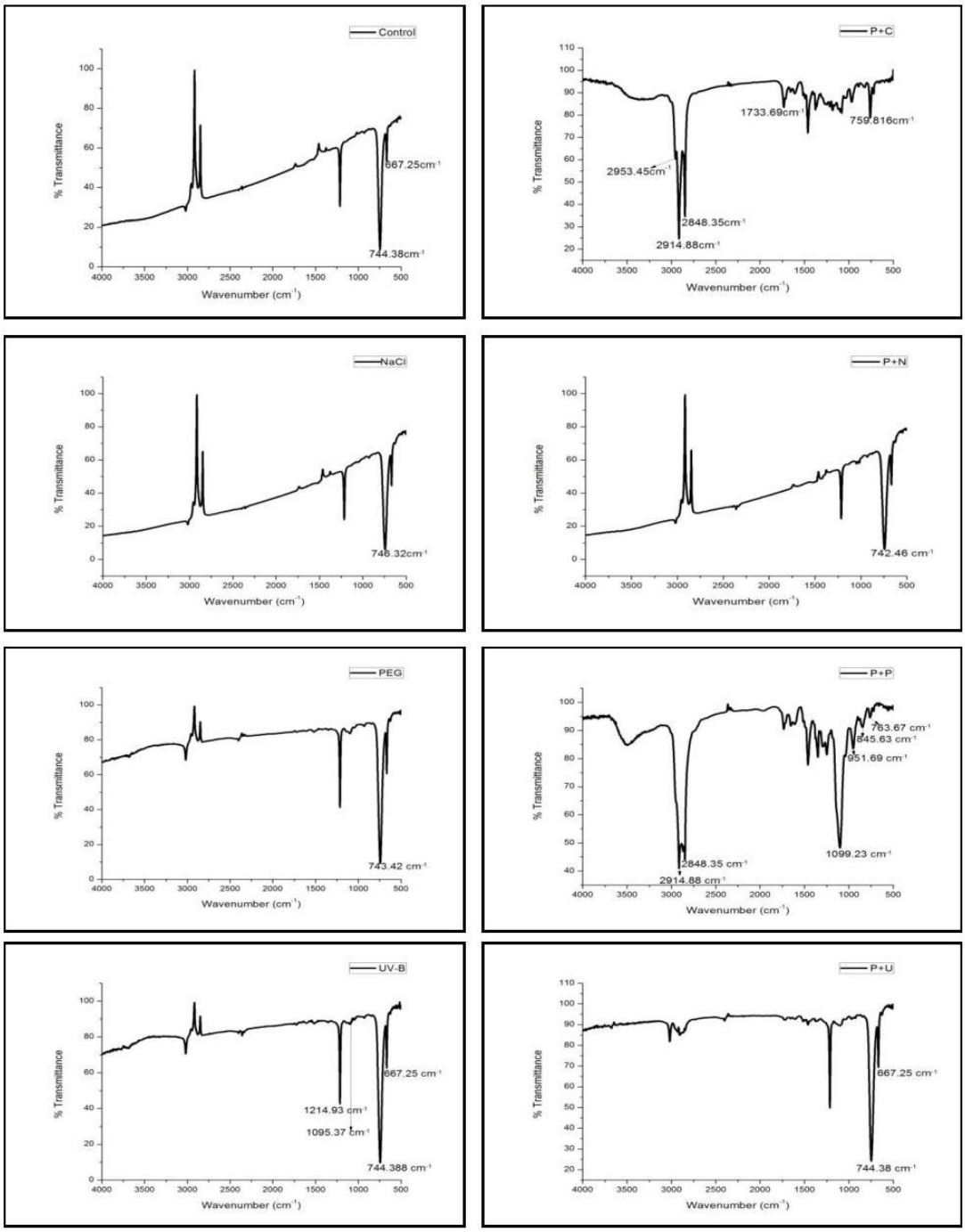

Fig. 38: FT-IR spectra of epicuticular wax in rice seedlings from UV-B primed and non-primed Aiswarya seed subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & P_{sl})+U- Primed+UV-B).

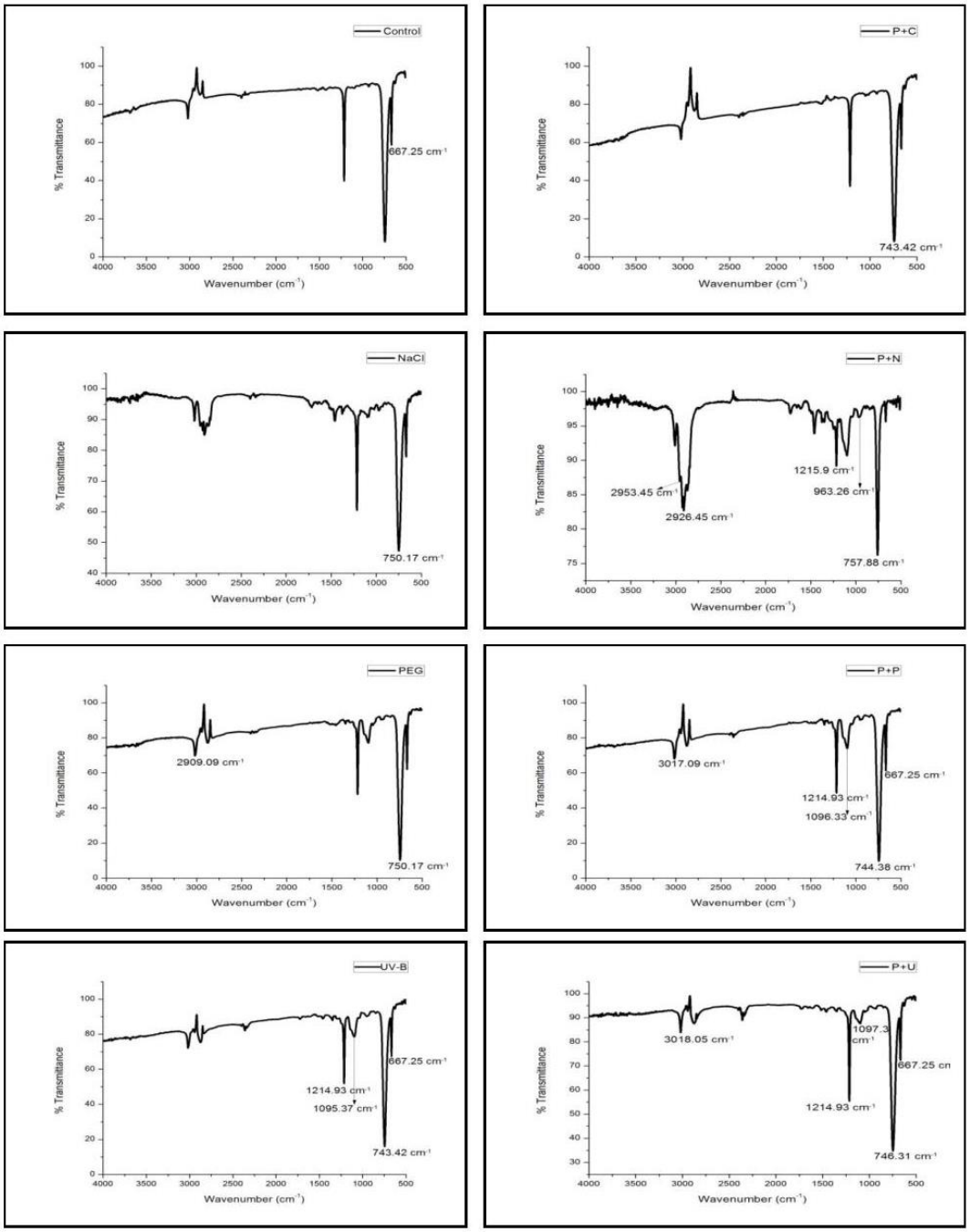

Fig. 39: FT-IR spectra of epicuticular wax in rice seedlings from UV-B primed and non-primed Aiswarya seedlings subjected to various stress conditions (NaCl, PEG and UV-B). (P+C- Primed+Control; P(P_s & P_{sl})+N- Primed+NaCl; P(P_s & P_{sl})+P- Primed+PEG; P(P_s & Psl)+U- Primed+UV-B).