Intraspecific variation in sensitivity of high yielding rice varieties towards UV-B radiation

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Abstract Effective screening of thirteen commonly cultivated rice (*Oryza sativa* L.) varieties was carried out to evaluate the varietal-specific differences in morphological, physiological and biochemical responses to various doses of UV-B irradiation (7, 14, 21 and 28 kJ $m^{-2}d^{-1}$). Determination of UV-B tolerant rice varieties would be helpful in selecting a suitable variety for the areas experiencing higher influx of UV-B radiation. Based on the initial screening of thirteen rice varieties, carried out by analyzing shoot length, fresh weight, photosynthetic pigments and the rate of lipid peroxidation under various doses of UV-B, it was found that Mangalamahsuri, Aathira, Kanchana, Jyothi and Annapoorna were tolerant lines and Neeraja, Swetha, Swarnaprabha and Aiswarya were the sensitive ones. Further screening of these nine varieties was done by analyzing primary metabolites (total protein, soluble sugar and proline content) and non enzymatic antioxidants (ascorbate and glutathione) involved in free radical scavenging mechanism to mitigate the negative effects of UV-B irradiation. Based on the cumulative stress response index (CSRI), the sum of relative individual component responses (total protein, soluble sugar, proline, ascorbate and glutathione content) at each UV-B treatment and total stress response index (TSRI), the sum of CSRI of all the four UV-B treatments for each variety, nine rice varieties selected after primary screening were classified as tolerant (Mangalamahsuri, Aathira and Kanchana), intermediate (Jyothi, Annapoorna, Neeraja and Swetha) and sensitive (Swarnaprabha and Aiswarya).

Introduction

Rapid increase in population and extreme climate changes has the potential to threaten global food security (Lesk et al. 2016). Rice is one of the world's most important staple crops feeding more than 2.7 billion people worldwide. More than 28% of the world's population lives in Asia–Pacific region, where, the current 524 million tonnes of rice produced annually will have to be increased to 700 million tonnes by the year 2025 (Papademetriou 2000). The traditional agricultural practices are not enough to produce rice according to the needs of an ever-increasing world population. It has been assumed that world rice production must be increased by 60–110% to meet the dietary needs of increasing world population by the year 2025 (Fageria 2007; Tilman et al. 2011). The shrinkage of arable land, scarcity of irrigation water and the increasing environmental stress due to climatic changes cause serious threats to global rice production. In the wake of changing climate, rice growth and productivity and consequently the food security has been seriously affected by abiotic stresses such as drought, salt, flood, ultraviolet radiation and heat in recent years (Korres et al. 2017).

Ultraviolet B (UV-B) radiation (280–315 nm) is a minor component of the solar spectrum, but it is the most energetic one that reaches the Earth's surface and is strongly absorbed by stratospheric ozone (O_3). However, the depletion of stratospheric O_3 due to anthropogenic

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activities causing production of substances that lead to ozone depletion has resulted in an increasing amount of UV-B radiation at the Earth's surface. The exposure of living organisms to UV-B cause harmful effects and due to obligatory requirement of sunlight, the exposure of plants to UV-B can be potentially detrimental for them (León-Chan et al. 2017). The tropical regions where rice is grown as the major food crop experience high level of UV-B radiation than the temperate regions. Previous studies from the last three decades suggest that nearly 50% of crop plants of India are affected by elevated levels of solar UV-B as it is geographically located in low ozone belt which recieves high UV-B radiation in comparison to temperate regions with higher latitudes (Mitra 1991; Madronich et al. 1995).

In plants, UV-B irradiation can increase the level of reactive oxygen species (ROS) (Frohnmeyer and Staiger 2003). They subsequently oxidize the biomolecules in the cells and negatively influence the functionality and integrity of proteins and cell membranes. UV-B induced oxidative stress in plants results in alterations of plant growth and developmental processes (Chaudhary and Agrawal 2015; Dotto and Casati 2017), impaired photosynthetic pigments and processes (Kataria et al. 2014; Kataria 2017), changes in secondary metabolites (Escobar-Bravo et al. 2017), alteration in anatomical characters and changes in cuticular wax deposition (Nascimento et al. 2015; Willick et al. 2018) and damage to the photosystem PSI and PSII proteins and electron transport activities (Zhang et al. 2016; Rai et al. 2018).

The sensitivity to UV-B irradiation varies widely among rice varieties. Asian rice varieties showed relatively larger magnitude of difference in their responses to elevated UV-B in terms of growth and physiological processes (Hidema and Kumagai 2006). Selection of tolerant rice genotypes is one of the primary tasks for exploiting the stress-tolerant rice varieties under environmental stress conditions. For effective selection of tolerant rice varieties towards UV-B conditions, it is necessary to do a proper screening, distinguishing UV-B susceptible lines from UV-B tolerant varieties. Previous studies have ratified the various morphological, physiological and biochemical parameters such as plant height, fresh weight, chlorophyll content and yield in plants as an index of the ability of the plants to withstand the abiotic stress and can be very well adopted for screening tolerant and sensitive varieties (Jisha and Puthur 2014; Choudhary and Agrawal 2014, 2015). The present study is therefore, conducted with the objective of effective screening of thirteen high yielding rice varieties to evaluate the varietal specific differences in morphological, physiological and biochemical responses to various doses of UV-B irradiation. Determination of the best UV-B tolerant rice varieties would be helpful in selecting a suitable variety for the areas experiencing high influx of UV-B radiation and also for the future UV-B tolerance breeding program in this crop. The specific objective was to classify the selected rice varieties based on their degree of tolerance to the high doses of UV-B irradiation.

Materials and methods

Plant material

A total of 13 high yielding rice varieties collected from Regional Agricultural Research Station (RARS), Pattambi, Kerala, India were tested for tolerance to various levels of UV-B dosage. The seedlings were raised in modified half strength Hoagland medium after the seeds were surface sterilized with 0.1% $HgCl_2$ solution for 5 min. The seedlings were grown in a plant growth chamber set at a temperature (24 ± 2 °C), light intensity (300 μmol $m^{-2}s^{-1}$) and relative humidity (55 ± 5%) with a 14/10 h photoperiod.

Experimental design for UV-B treatments

The rice seedlings were irradiated with Philips TL 20W/01 RS narrowband UV-B tubes in the presence of continuous white fluorescent illumination of 300 μmol $m^{-2}s^{-1}$ held in height-adjustable frames over the plants. For imparting UV-B stress, rice seedlings were exposed to UV-B radiation for 1 week after 4 days of germination, i.e. 4–10 days of seedling growth period. Four different accumulated doses of UV-B exposure were 7, 14, 21 and 28 kJ $m^{-2}d^{-1}$. The intensity of UV-B at the top of the plant was measured with the PMA2106 UV-B sensor (Solar Light Co., USA) connected to the PMA 2200 radiometer (Solar Light Co., USA). The distance between the UV-B tubes and the tip of the seedlings was maintained at 20 ± 2 cm with the help of adjustable frames, to which the tubes were mounted. The UV-B tubes were covered with 0.13 mm thick cellulose diacetate filters to avoid transmission of wavelength below 290 nm and the cellulose diacetate filters were changed in every week. Various morphological, physiological and biochemical parameters were analyzed in rice seedlings after irradiating with the four mentioned doses of UV-B on 10 days after germination.

Morphological parameters

Standard procedures were followed for recording the shoot length (cm) from collar region to the tip of the shoot and fresh weight (mg) of seedlings. After uprooting from nutrient solution, the seedlings were washed with distilled water and blotted using tissue paper before reading the morphological parameters.

Estimation of photosynthetic pigments

The chlorophyll and carotenoid pigments in the leaves of rice seedlings were estimated following the method of Arnon (1949) by using 80% acetone as the extracting medium. To estimate pigment contents, 200 mg of fresh leaf sample was weighed, homogenized and extracted in 10 mL of 80% acetone (v/v). The optical density of extract was read at 663, 646, 750 and 470 nm against the solvent blank (80% acetone) using a UV–VIS spectrophotometer (Systronics 2201, India). The concentration of the total chlorophyll (Chl $a + b$) and carotenoids contents were expressed in mg chlorophyll/carotenoids g^{-1} dry weight of leaf sample.

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

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

where,

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

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

Estimation of malondialdehyde content

Malondialdehyde (MDA) content was estimated according to the method of Heath and Packer (1968). Two hundred milligrams of fresh plant tissue was weighed in triplicate and homogenized in 5 mL of 5% trichloroacetic acid solution. The homogenate was centrifuged at 12,000 rpm for 15 min. Two millilitre of the supernatant was mixed with an equal aliquot of 0.5% of thiobarbituric acid in 20% TCA and the solution was heated at 95 °C for 24 min, cooled and then centrifuged at 3000 rpm for 2 min. The absorbance of the supernatant was measured at 532 and 600 nm against reagent blank using UV–VIS spectrophotometer (Systronics 2201, India). The absorbance value at 532 nm was corrected for non-specific turbidity by subtracting absorbance value at 600 nm. Then, the MDA content was calculated using its extinction coefficient of 155 mM^{-1}cm^{-1}.

Estimation of metabolites

Total protein content of the plant material was estimated using Folin–Ciocalteau reagent according the method of Lowry et al. (1951). Two hundred milligrams of fresh plant tissue was homogenized in 5 mL of phosphate buffer (pH 7). A known volume of the homogenate was mixed with an equal volume of 10% trichloroacetic acid and this mixture was kept for 1 h in a refrigerator (4 °C) for flocculation. The protein precipitate was collected by centrifugation at 5000 rpm for 10 min at 4 °C. The supernatant was decanted off. The residue was washed twice with 2% TCA followed by washing separately with 30% perchloric acid, diethyl ether and 80% acetone in order to remove the starch, lipids and pigments, respectively. The pellet obtained after centrifugation was dried and later digested in 5 mL 0.1 N NaOH by heating in a water bath for 10 min. Known volume of aliquot was pipetted and made up to 1 mL with distilled water. To the aliquots, 5 mL of alkaline copper reagent was added and shaken well. After 10 min, 0.5 mL of 1 N Folin-Ciocalteu's phenol reagent was added and shaken well immediately. The tubes were kept for 30 min for colour development. The optical density of the solution was read at 700 nm using a UV–VIS spectrophotometer (Systronics 2201, India). BSA fraction V powder was used as standard.

Total soluble sugar content was estimated by the method of Dubois et al. (1956). Two hundred milligrams of fresh plant tissue was homogenized in 80% ethanol and the homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C, the supernatant was collected. From the supernatant, a known volume of aliquot was taken in the test tube and made up to 1 mL with distilled water. To this, 0.1 mL of 5% (v/v) phenol was added and mixed well. 5 mL of concentrated sulphuric acid was added to the tube quickly from a burette. After cooling, the optical density of the resultant solution was measured at 490 nm using a spectrophotometer. D-glucose was used as the standard.

Proline content in rice seedlings was estimated as described by Bates et al. (1973) using 3% sulphosalicylic acid as extraction medium and L-proline as standard. Two hundred milligrams of fresh plant tissue were homogenized in 10 mL of 3% (w/v) aqueous sulfosalicylic acid, the homogenate was centrifuged for 10 min at 10,000 rpm and the supernatant was collected. Two millilitre of supernatant was taken in test tubes in triplicate and equal volume of glacial acetic acid and 2.5% acid ninhydrin (1.25 g of ninhydrin dissolved in a mixture of 30 mL of glacial acetic acid and 20 mL of 6 M ortho phosphoric acid) were added to it. The tubes were then heated in a boiling water bath for 1 h and then the reaction was terminated by placing the tubes in ice bath. Four millilitre of toluene was added to the reaction mixture and stirred well using a vortex mixer. The

chromophore-toluene layer was separated carefully and the optical density of the separated solution was measured at a wavelength of 520 nm using spectrophotometer (Genesis 20). L-proline was used as the standard.

Determination of antioxidants

For the estimation of reduced ascorbate and glutathione content, the method of Chen and Wang (2002) was adopted and calculated from a standard curve prepared using different concentrations of ascorbate and glutathione respectively. For estimation of reduced ascorbate content, two hundred mg of fresh plant tissue was homogenized with 5 mL 5% (w/v) TCA and the homogenate was centrifuged at 12,000 rpm for 15 min at 4 °C. An aliquot of 0.1 mL of the supernatant was mixed well with 0.3 mL of 200 mM NaH_2PO_4. To this mixture, 0.5 mL of 10% (v/v) TCA, 0.4 mL of 42% (v/v) H_3PO_4, 0.4 mL of 4% (w/v) bipyridyl (dissolved in 70% alcohol) and 0.2 mL of 3% $FeCl_3$ (w/v) was added. The mixture was incubated at 42 °C for 15 min and the absorbance was measured immediately after incubation at 524 nm.

For estimation of reduced glutathione content, 200 mg of fresh plant tissue was homogenized in 5 mL of 5% TCA (w/v). The homogenate was filtered through a filter paper and centrifuged at 12,000 rpm for 15 min at 4 °C. The supernatant was collected and used for the estimation of reduced glutathione content. To an aliquot of 0.5 mL of the supernatant, 2.6 mL of 150 mM NaH_2PO_4 buffer (pH 6.8) and 0.18 mL of 3 mM 5,5-dithio-bis(2nitrobenzoic acid) (DTNB) were added (DTNB was dissolved in 100 mM phosphate buffer, pH 6.8) and kept for 5 min. Then, the absorbance was read at 412 nm.

Cumulative stress response index (CSRI) and total stress response index (TSRI)

The cumulative stress response index (CSRI) was calculated as the sum of relative individual component responses (total protein, soluble sugar, proline, ascorbate and glutathione content) at each UV-B treatment. The CSRI was calculated according to Koti et al. (2007).

$$CSRI = \left(\frac{PRTt - PRTc}{PRTt} + \frac{PRLt - PRLc}{PRLt} \right.$$
$$+ \frac{SGRt - SGRc}{SGRt} + \frac{ASCt - ASCc}{ASCt}$$
$$\left. + \frac{GLUt - GLUc}{GLUt} \right) \times 100$$

where CSRI is the cumulative stress response index, PRT the total protein content, PRL the proline content, SGR the soluble sugar content, ASC the ascorbate content, GLU the

glutathione content under t (treatment) and c (control). Nine varieties selected after initial screening were classified into tolerant, intermediate and sensitive based on total stress response index (TSRI), the sum of CSRI over all the four UV-B treatments.

Statistical analysis

Statistical analysis of physiological and biochemical parameters were carried out according to Tukey's studentized range (HSD) test at 5% probability level. One-way ANOVA was applied using the SPSS software (Version 16.0, SPSS Inc., Chicago, IL, USA) to evaluate the significant difference in the traits among rice varieties. Pearson's correlation analysis was performed to evaluate the relationships between the most important variables obtained in rice varieties exposed to UV-B stress. The data represent mean ± standard error (SE) and the values is an average of recordings from three independent experiments, each with three replicates (i.e. n = 9).

Results

Initial screening for UV-B sensitive and tolerant rice varieties

The primary screening was done on the basis of their performance after exposure to various doses of UV-B irradiation (7, 14, 21, and 28 kJ $m^{-2}d^{-1}$) for 1 week (4–10 days after germination) by analyzing shoot length, fresh weight, photosynthetic pigments and the rate of lipid peroxidation.

Various doses of UV-B treatments resulted in a decrease of shoot length in all rice varieties studied, but the lower dose of UV-B (7 kJ $m^{-2}d^{-1}$) exhibited a negligible increase in shoot length of Aathira, Mangalamahsuri, Kanchana, Jyothi and Annapoorna. Among the thirteen *O. sativa* varieties studied, Neeraja, Swetha, Aiswarya and Swarnaprabha showed maximum reduction in the shoot length of seedlings when subjected to various doses of UV-B radiation (> 40% reduction in respect to the control plants) with maximum decrease recorded at 21 kJ $m^{-2}d^{-1}$ and no further decrease was observed thereafter at 28 kJ $m^{-2}d^{-1}$. The percentage of reduction in shoot length of remaining varieties such as Mattatriveni, Karuna, Harsha and Varsha was in between 15 and 25% in respect to the untreated control plants (Table 1). As a result of the treatment with various doses of UV-B, the fresh weight in seedlings of all thirteen rice varieties declined and the least reduction was found after 21–28 kJ $m^{-2}d^{-1}$ of UV-B exposure in Aathira, Mangalamahsuri, Jyothi, Kanchana and Annapoorna (11–19%) with respect to the control

Table 1 Shoot length and fresh weight of thirteen rice varieties after exposure to various doses of UV-B (7, 14, 21 and 28 kJ m^{-2}d^{-1})

UV-B (kJ m^{-2}d^{-1})	Aathira	Mangalamahsuri	Kanchana	Jyothi	Annapoorna	Mattatriveni	Karuna
Shoot length/plant (cm)							
Control	20.53 ± 0.81^{ab}	22.10 ± 0.30^b	20.40 ± 0.30^a	19.20 ± 0.70^a	17.28 ± 0.28^{ab}	23.80 ± 0.20^a	21.97 ± 0.58^a
7	21.47 ± 0.81^a	23.07 ± 0.51^a	20.83 ± 0.15^a	19.22 ± 0.92^a	18.24 ± 0.86^a	23.20 ± 0.79^{ab}	21.00 ± 1.13^a
14	20.60 ± 0.46^{ab}	21.10 ± 0.53^c	19.20 ± 0.35^b	18.20 ± 0.78^b	16.70 ± 0.67^{bc}	22.84 ± 0.80^b	18.66 ± 0.60^b
21	19.93 ± 0.32^b	20.20 ± 0.72^d	19.00 ± 0.20^{bc}	18.00 ± 0.50^b	15.62 ± 0.54^d	20.50 ± 0.67^b	20.47 ± 0.92^a
28	19.53 ± 0.50^b	20.10 ± 0.26^d	18.60 ± 0.46^c	17.7 ± 0.32^b	15.76 ± 0.01^{cd}	20.13 ± 0.23^c	17.67 ± 0.70^b
Fresh weight/plant (g)							
Control	0.121 ± 0.01^a	0.153 ± 0.00^a	0.122 ± 0.01^a	0.159 ± 0.01^a	0.096 ± 0.01^a	0.122 ± 0.01^a	0.141 ± 0.01^a
7	0.114 ± 0.01^{ab}	0.143 ± 0.00^b	0.11 ± 0.01^{ab}	0.15 ± 0.00^b	0.094 ± 0.00^a	0.114 ± 0.00^{ab}	0.142 ± 0.01^a
14	0.112 ± 0.00^{ab}	0.142 ± 0.00^b	0.116 ± 0.01^{ab}	0.142 ± 0.00^c	0.085 ± 0.00^b	0.102 ± 0.00^{bc}	0.121 ± 0.01^b
21	0.108 ± 0.01^b	0.135 ± 0.01^c	0.102 ± 0.01^c	0.136 ± 0.00^c	0.079 ± 0.00^b	0.088 ± 0.01^{cd}	0.099 ± 0.01^c
28	0.107 ± 0.00^b	0.124 ± 0.00^d	0.109 ± 0.01^{bc}	0.129 ± 0.00^d	0.083 ± 0.00^b	0.075 ± 0.01^d	0.064 ± 0.01^d

UV-B (kJ m^{-2}d^{-1})	Harsha	Varsha	Neeraja	Swetha	Aiswarya	Swarnaprabha
Shoot length/plant (cm)						
Control	24.03 ± 0.45^a	17.30 ± 0.26^a	23.67 ± 0.83^a	24.50 ± 0.81^a	23.10 ± 0.75^a	24.50 ± 0.70^a
7	22.87 ± 0.12^b	16.23 ± 0.40^b	21.77 ± 0.32^b	22.40 ± 0.29^b	19.87 ± 0.75^b	20.60 ± 0.58^b
14	23.13 ± 0.61^b	15.03 ± 0.55^c	19.77 ± 0.25^c	17.80 ± 0.72^c	17.17 ± 0.21^c	19.20 ± 0.58^c
21	21.00 ± 0.53^c	13.73 ± 0.95^d	13.47 ± 0.45^d	13.70 ± 0.45^d	11.87 ± 0.61^e	12.20 ± 0.29^e
28	20.57 ± 0.38^c	12.87 ± 0.50^d	14.80 ± 0.70^e	16.80 ± 0.70^c	13.47 ± 0.50^d	13.90 ± 0.38^d
Fresh weight/plant (g)						
Control	0.177 ± 0.01^a	0.141 ± 0.010^a	0.088 ± 0.01^a	0.164 ± 0.00^a	0.125 ± 0.01^a	0.148 ± 0.00^a
7	0.163 ± 0.01^b	0.123 ± 0.00^b	0.072 ± 0.00^b	0.139 ± 0.01^b	0.107 ± 0.00^b	0.121 ± 0.01^a
14	0.133 ± 0.00^c	0.106 ± 0.00^c	0.045 ± 0.00^c	0.077 ± 0.00^c	0.085 ± 0.01^c	0.066 ± 0.05^b
21	0.116 ± 0.01^d	0.09 ± 0.01^d	0.034 ± 0.00^d	0.043 ± 0.01^e	0.032 ± 0.01^e	0.051 ± 0.01^b
28	0.097 ± 0.00^e	0.083 ± 0.00^e	0.033 ± 0.00^d	0.054 ± 0.00^d	0.047 ± 0.01^d	0.066 ± 0.01^b

The data represent mean ± standard error (SE) and the values is an average of recordings from three independent experiments, each with three replicates (i.e. n = 9). Different alphabetical letters indicate statistically significant differences at $p \leq 0.05$. Values followed by the same letter in a column indicate no significant difference between treatments following Tukey's studentized range (HSD) test

plants. Among thirteen rice varieties, the decrease in fresh weight was highest (> 60%) in Neeraja, Swetha, Aiswarya and Swarnaprabha and showed maximum reduction at 21 kJ m^{-2}d^{-1} in respect to the control plants. Mattatriveni, Karuna, Harsha and Varsha were showed a reduction of 38–55% as compared to the control plants after exposure to 28 kJ m^{-2}d^{-1} (Table 1).

Total chlorophyll content increased significantly at 7 kJ m^{-2}d^{-1} of UV-B exposure in Kanchana, Jyothi and Annapoorna (Table 2). Higher doses of UV-B resulted in a gradual decrease (to the extent of 20%) of total chlorophyll content. Unlike these three varieties, the other ten rice varieties showed a decreasing pattern at all UV-B treatment. The rate of reduction was much more in Neeraja, Swetha, Aiswarya and Swarnaprabha (76–90%

respectively) after exposure to 28 kJ m^{-2}d^{-1} of UV-B exposure. In most of the rice varieties treated with UV-B, carotenoid content increased with the UV-B dosage when compared to the control plants. The maximum accumulation was noticed in Aathira, Annapoorna, Mangalamahsuri, Kanchana and Jyothi (an increase of 168–508%) after exposure to 28 kJ m^{-2}d^{-1}, over the control plants. In seedlings of Neeraja, Swetha, Aiswarya and Swarnaprabha, minimum carotenoid accumulation (< 10%) was recorded on exposure to 14 kJ m^{-2}d^{-1} of UV-B, beyond which it decreased significantly. Similarly, the carotenoid accumulation was also noticed in remaining four varieties, Mattatriveni, Karuna, Harsha and Varsha at 28 kJ m^{-2}d^{-1} UV-B and it was in the range of 25–50% over the untreated plants (Table 2).

Table 2 Total chlorophyll and carotenoid content in thirteen rice varieties after exposure to various doses of UV-B (7, 14, 21 and 28 kJ m^{-2}d^{-1})

UV-B (kJ m^{-2}d^{-1})	Aathira	Mangalamahsuri	Kanchana	Jyothi	Annapoorna	Mattatriveni	Karuna
Total chlorophyll content (mg/g DW)							
Control	14.24 ± 1.67[a]	7.24 ± 1.04[a]	12.77 ± 0.37[b]	5.99 ± 0.31[ab]	4.82 ± 0.31[b]	10.79 ± 0.63[c]	7.61 ± 0.18[a]
7	12.85 ± 2.19[ab]	5.36 ± 0.85[ab]	14.09 ± 1.15[a]	6.30 ± 0.15[a]	5.85 ± 0.27[a]	9.71 ± 0.30[b]	6.30 ± 0.14[b]
14	11.74 ± 0.52[ab]	6.01 ± 0.86[ab]	12.60 ± 0.69[b]	5.41 ± 0.46[b]	4.81 ± 0.18[b]	8.91 ± 0.55[b]	5.35 ± 0.08[c]
21	10.69 ± 1.76[b]	6.02 ± 0.29[b]	11.22 ± 0.54[c]	5.41 ± 0.48[b]	4.63 ± 0.22[b]	7.25 ± 0.61[a]	4.95 ± 0.53[cd]
28	11.05 ± 0.53[b]	5.76 ± 0.18[b]	10.23 ± 0.52[c]	5.28 ± 0.43[b]	4.61 ± 0.71[b]	7.57 ± 0.30[a]	4.62 ± 0.43[d]
Total carotenoid content (mg/g DW)							
Control	0.65 ± 0.04[e]	0.40 ± 0.05[c]	0.77 ± 0.03[d]	0.47 ± 0.04[c]	0.53 ± 0.02[d]	0.41 ± 0.08[ab]	2.37 ± 0.06[c]
7	0.95 ± 0.05[d]	0.52 ± 0.06[c]	1.20 ± 0.14[c]	0.48 ± 0.07[c]	0.54 ± 0.01[d]	0.40 ± 0.11[b]	2.64 ± 0.13[b]
14	1.37 ± 0.25[c]	1.13 ± 0.07[b]	1.12 ± 0.09[c]	0.87 ± 0.12[b]	0.85 ± 0.06[c]	0.52 ± 0.04[ab]	2.55 ± 0.01[b]
21	2.58 ± 0.16[b]	2.10 ± 0.58[a]	1.45 ± 0.14[b]	1.40 ± 0.11[a]	1.15 ± 0.07[b]	0.52 ± 0.06[ab]	2.94 ± 0.04[a]
28	2.95 ± 0.07[a]	2.43 ± 0.39[a]	2.06 ± 0.16[a]	0.80 ± 0.16[b]	2.48 ± 0.06[a]	0.30 ± 0.01[a]	3.07 ± 0.13[a]

UV-B (kJ m^{-2}d^{-1})	Harsha	Varsha	Neeraja	Swetha	Aiswarya	Swarnaprabha
Total chlorophyll content (mg/g DW)						
Control	10.69 ± 0.72[a]	6.75 ± 0.60[a]	9.23 ± 0.89[a]	12.75 ± 1.07[a]	13.51 ± 1.17[a]	19.80 ± 0.56[a]
7	10.69 ± 1.50[a]	4.66 ± 0.29[b]	7.35 ± 0.63[b]	7.60 ± 0.86[b]	7.50 ± 0.77[b]	15.29 ± 1.18[b]
14	8.05 ± 0.34[b]	2.78 ± 0.23[c]	5.46 ± 0.29[c]	5.43 ± 0.91[c]	4.75 ± 0.20[c]	7.77 ± 0.58[c]
21	4.68 ± 0.31[c]	2.75 ± 0.72[c]	1.75 ± 0.15[e]	3.04 ± 0.56[d]	1.42 ± 0.47[d]	7.10 ± 0.58[c]
28	4.19 ± 0.18[c]	2.53 ± 0.44[c]	3.68 ± 0.37[d]	6.39 ± 0.40[bc]	1.48 ± 0.32[d]	4.04 ± 0.70[d]
Total carotenoid content (mg/g DW)						
Control	4.44 ± 0.19[c]	0.82 ± 0.07[b]	1.40 ± 0.07[c]	2.41 ± 0.10[a]	2.31 ± 0.28[ab]	1.20 ± 0.15[a]
7	4.42 ± 0.13[c]	1.18 ± 0.08[a]	1.43 ± 0.04[c]	2.55 ± 0.19[a]	2.54 ± 0.00[a]	1.26 ± 0.05[a]
14	4.66 ± 0.01[bc]	1.22 ± 0.12[a]	1.51 ± 0.03[ab]	2.62 ± 0.56[a]	2.54 ± 0.00[a]	1.33 ± 0.03[a]
21	4.96 ± 0.07[b]	1.10 ± 0.11[a]	1.55 ± 0.04[a]	1.55 ± 0.10[b]	2.24 ± 0.10[ab]	0.83 ± 0.13[b]
28	6.44 ± 0.48[a]	1.24 ± 0.07[a]	1.44 ± 0.01[bc]	1.70 ± 0.16[b]	1.99 ± 0.25[b]	0.41 ± 0.12[c]

The data represent mean ± standard error (SE) and the values is an average of recordings from three independent experiments, each with three replicates (i.e. n = 9). Different alphabetical letters indicate statistically significant differences at $p \leq 0.05$. Values followed by the same letter in a column indicate no significant difference between treatments following Tukey's studentized range (HSD) test

The increase of MDA content was lower in Aathira, Mangalamahsuri, Kanchana, Jyothi and Annapoorna (< 25%) as compared to the control plants and the maximum increase was recorded after exposure to 28 kJ m^{-2}d^{-1} of UV-B. Significant increase in MDA content was recorded in rice seedlings of Neeraja, Swetha, Aiswarya and Swarnaprabha exposed to low dose (7 kJ m^{-2}d^{-1}) of UV-B treatments and maximum MDA content was recorded after 21 kJ m^{-2}d^{-1} UV-B treatment (an increase of 199, 164, 450 and 229%, respectively) over the control plants, beyond which no further increase was recorded. In other four rice varieties (Mattatriveni, Karuna, Harsha and Varsha), the MDA content also increased with increase in UV-B dose and the increase was found maximum at 21 kJ m^{-2}d^{-1} of treatment in Mattatriveni, Karuna and Harsha (80–95% over the control seedlings) and in Varsha, maximum MDA content was recorded after 28 kJ m^{-2}d^{-1}

of UV-B treatment (98%) over the control seedlings (Table 3).

Final screening for most sensitive and tolerant rice varieties

The five tolerant (Aathira, Mangalamahsuri, Kanchana, Jyothi and Annapoorna) and four sensitive (Neeraja, Swetha, Aiswarya and Swarnaprabha) rice varieties selected through primary screening were further studied by analyzing the accumulation level of various primary metabolites (total protein, soluble sugar and proline content) and non enzymatic antioxidants (ascorbate and glutathione) after exposure to all doses of UV-B irradiation. Significant increase in the total protein content was recorded in all nine rice varieties on being subjected to all doses of UV-B. In Aathira, Mangalamahsuri and Kanchana, a sharp increase in the total protein content was recorded

Table 3 Malondialdehyde content (µmol/g DW) in thirteen rice varieties after exposure to various doses of UV-B (7, 14, 21 and 28 kJ m^{-2}d^{-1})

UV-B (kJ m^{-2}d^{-1})	Aathira	Mangalamahsuri	Kanchana	Jyothi	Annapoorna	Mattatriveni	Karuna
Control	8.06 ± 1.41[b]	22.62 ± 2.45[b]	33.91 ± 0.79[a]	38.29 ± 1.02[a]	19.69 ± 0.29[b]	23.47 ± 1.56[c]	6.65 ± 1.16[c]
7	6.10 ± 0.57[c]	16.92 ± 2.24[c]	36.85 ± 1.44[b]	43.10 ± 1.39[a]	19.77 ± 0.20[b]	32.03 ± 1.46[b]	6.77 ± 0.58[c]
14	8.37 ± 0.06[ab]	25.92 ± 4.14[ab]	36.93 ± 2.09[b]	46.01 ± 0.73[a]	22.60 ± 0.72[ab]	35.00 ± 1.15[b]	8.48 ± 0.06[b]
21	8.07 ± 0.74[b]	28.25 ± 1.58[a]	40.46 ± 1.02[a]	40.80 ± 7.77[a]	23.07 ± 0.64[ab]	45.66 ± 2.31[a]	12.06 ± 1.16[a]
28	9.78 ± 0.58[a]	26.58 ± 0.95[ab]	39.61 ± 074[ab]	47.84 ± 7.83[a]	24.14 ± 4.32[a]	42.63 ± 3.15[a]	10.74 ± 0.51[a]

UV-B (kJ m^{-2}d^{-1})	Harsha	Varsha	Neeraja	Swetha	Aiswarya	Swarnaprabha
Control	6.29 ± 0.55[c]	20.14 ± 2.72[d]	15.21 ± 1.52[d]	5.64 ± 0.99[c]	5.60 ± 0.23[c]	11.56 ± 0.99[b]
7	6.57 ± 0.15[c]	28.72 ± 1.60[c]	19.47 ± 2.45[cd]	12.88 ± 0.59[b]	24.88 ± 1.50[b]	36.81 ± 1.40[a]
14	6.48 ± 0.17[c]	33.66 ± 2.68[b]	23.40 ± 2.29[c]	13.06 ± 0.73[b]	27.48 ± 1.18[ab]	37.93 ± 0.71[a]
21	11.70 ± 0.64[a]	36.64 ± 0.77[ab]	45.50 ± 3.73[a]	14.88 ± 0.60[a]	30.81 ± 5.18[a]	38.03 ± 0.61[a]
28	9.44 ± 0.18[b]	39.84 ± 1.57[a]	36.68 ± 3.25[b]	14.66 ± 0.92[a]	30.43 ± 1.77[a]	37.70 ± 0.55[a]

The data represent mean ± standard error (SE) and the values is an average of recordings from three independent experiments, each with three replicates (i.e. n = 9). Different alphabetical letters indicate statistically significant differences at $p \leq 0.05$. Values followed by the same letter in a column indicate no significant difference between treatments following Tukey's studentized range (HSD) test

from 7 kJ m^{-2}d^{-1} (108, 80 and 26%, respectively) up to 28 kJ m^{-2}d^{-1} (172, 132 and 128% respectively) as compared to the control plants. Although, an increase in total protein content was recorded in Annapoorna, Neeraja, Swetha, Jyothi, Aiswarya and Swarnaprabha seedlings, the rate of increase was not significant like that in Aathira, Mangalamahsuri and Kanchana (Fig. 1a).

The percentage of increase in the soluble sugar content was more prominent in Aathira, Mangalamahsuri and Kanchana (230–288%) as compared to other varieties on exposure to 21 kJ m^{-2}d^{-1} of UV-B. However, soluble sugar content of Aiswarya and Swarnaprabha did not vary considerably on being subjected to all doses of UV-B. Neeraja, Swetha and Jyothi exhibited an intermediate level of increase up to 21 kJ m^{-2}d^{-1} (180, 175 and 36% in Neeraja, Jyothi and Swetha respectively), beyond which the increase was not significant as compared to the control plants. However, Annapoorna showed a significant decrease at the initial two doses (7 and 14 kJ m^{-2}d^{-1}) of UV-B followed by a gradual increase over the control plants up to 28 kJ m^{-2}d^{-1} (144%) (Fig. 1b). Proline content of all nine rice varieties was enhanced significantly with the UV-B dose and the increase was most prominent in Aathira, Mangalamahsuri and Kanchana after 28 kJ m^{-2}d^{-1} of UV-B treatment (212, 105 and 116%) as compared to other rice varieties. In rice varieties Aiswarya and Swarnaprabha, proline accumulation was less as compared to other varieties and it was found that maximum increase was recorded on exposure to 21 kJ m^{-2}d^{-1} of UV-B (< 20%). Moreover, a sharp increase in the accumulation of proline was recorded in Annapoorna, Neeraja, Swetha and Jyothi on exposure to 7 kJ m^{-2}d^{-1} and it

reached maximum at 21 kJ m^{-2}d^{-1} in Jyothi, Annapoorna and Neeraja and at 28 kJ m^{-2}d^{-1} of UV-B exposure in Swetha seedlings (Fig. 1c).

Significant increase in ascorbate content was recorded in Aathira, Mangalamahsuri and Kanchana even at the lowest dose (7 kJ m^{-2}d^{-1}) of UV-B treatment and maximum accumulation was recorded at 28 kJ m^{-2}d^{-1} (an increase of 11, 19 and 14 folds, respectively) over the control plants. Exposure to various doses of UV-B resulted in an enhancement in ascorbate content in all other rice varieties also although it was only about 3–5 fold increase over their control plants (Fig. 2a). Glutathione content in rice seedlings also increased gradually with increase in UV-B and the accumulation was higher in Aathira, Mangalamahsuri and Kanchana as compared to other six rice varieties; the increase was maximum upon exposure to 28 kJ m^{-2}d^{-1} (6.3–8.5 fold increase respectively). Compared to other rice varieties, glutathione accumulation in Aiswarya and Swarnaprabha (1.2 and 1.3 fold increase respectively) was much lower at 21 kJ m^{-2}d^{-1} UV-B. Treatment with UV-B in Jyothi, Annapoorna, Neeraja and Swetha resulted in significant increase in the glutathione content even at low dose (7 kJ m^{-2}d^{-1}) (1.1–1.3 folds) and the maximum increase ranged between 1.7 and 2 folds over the control seedlings (Fig. 2b).

The cumulative stress response index (CSRI) is an integration of the effect on total protein, soluble sugar, proline, ascorbate and glutathione content, which could reflect the overall sensitivity of rice varieties to various doses of UV-B irradiation. The results showed that all nine rice varieties selected after primary screening had a positive cumulative stress response index (Table 4).

Fig. 1 Total protein (**a**), sugar (**b**) and proline (**c**) content of nine rice varieties (10 days old seedlings) after exposure to various doses of UV-B (7, 14, 21 and 28 kJ m^{-2}d^{-1}) for 1 week after 4 days of germination. The data represent mean ± standard error (SE) and the values is an average of recordings from three independent experiments each with three replicates (i.e. n = 9). Different alphabetical letters indicate statistically significant differences at $p \leq 0.05$. Values followed by the same letter in a column indicate no significant difference between treatments following Tukey's studentized range (HSD) test
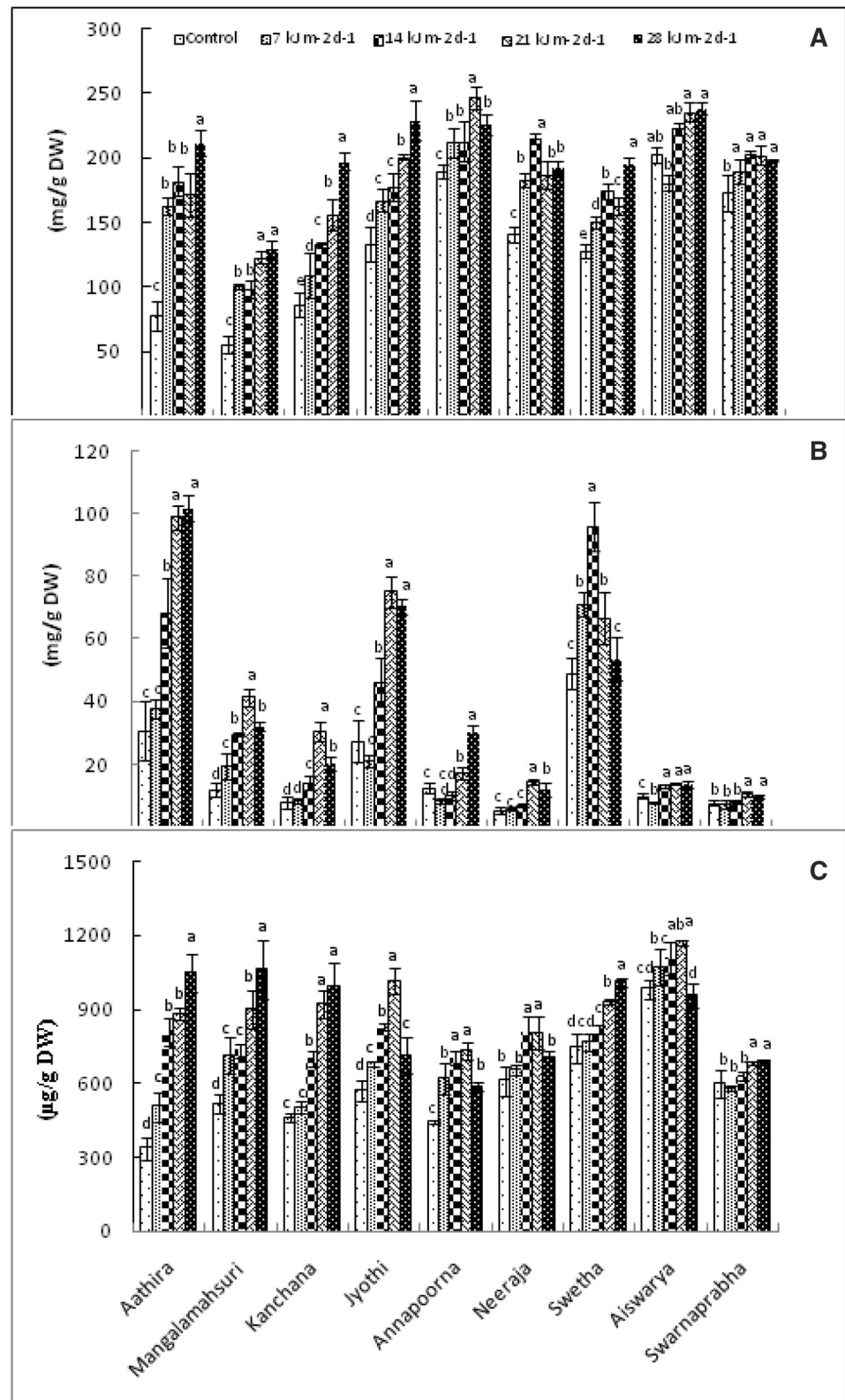
Swarnaprabha and Aiswarya were most adversely affected varieties (low index) while Mangalamahsuri, Aathira and Kanchana showed the highest cumulative stress response index (high index) on being subjected to all doses of UV-B (7, 14, 21 and 28 kJ m^{-2}d^{-1}). Total stress response index (TSRI) is the sum of cumulative stress response index over

Fig. 2 Ascorbate (**a**) and glutathione content (**b**) of nine rice varieties after exposure to various doses of UV-B (7, 14, 21 and 28 kJ m^{-2}d^{-1}) for 1 week after 4 days of germination. The data represent mean ± standard error (SE) and the values is an average of recordings from three independent experiments, each with three replicates (i.e. n = 9). Different alphabetical letters indicate statistically significant differences at $p \leq 0.05$. Values followed by the same letter in a column indicate no significant difference between treatments following Tukey's studentized range (HSD) test
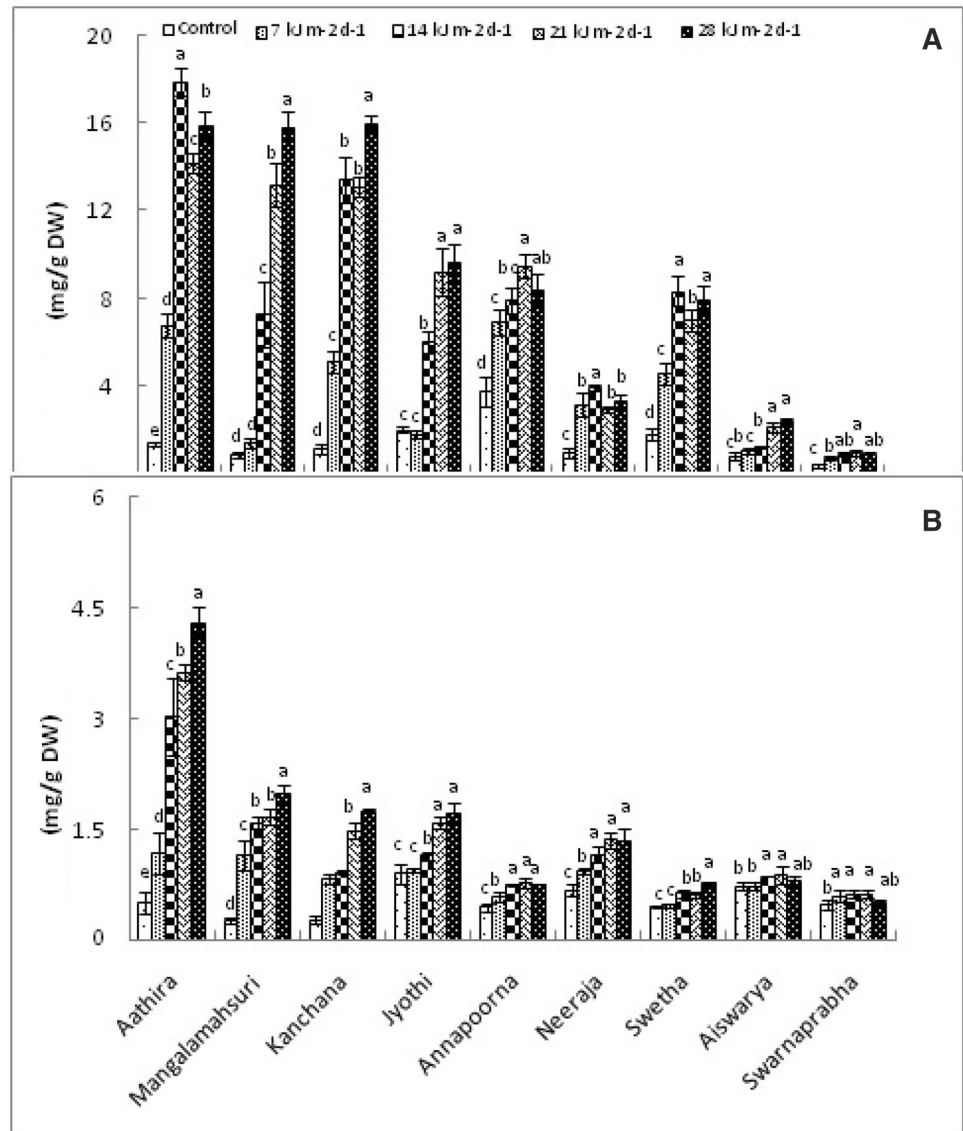

Table 4 Cumulative stress response index (CSRI), sum of relative individual component responses (total protein, soluble sugar, proline, ascorbate and glutathione content) at a given UV-B dose; and total stress response index (TSRI), pooled over all the treatments of UV-B irradiation (7, 14, 21 and 28 kJ m^{-2}d^{-1}) in nine rice varieties

Treatments	Varieties								
	Mangalamahsuri	Aathira	Kanchana	Jyothi	Annapoorna	Neeraja	Swetha	Swarnaprabha	Aiswarya
Cumulative stress response index (CSRI)									
7 kJ m^{-2} d^{-1}	597.4	702.2	584.4	316.7	219.2	225.9	233.7	121.4	3.5
14 kJ m^{-2} d^{-1}	1554.2	2088.2	1490.9	496.4	375.1	551.4	329.6	195.3	128.9
21 kJ m^{-2} d^{-1}	2472.8	2043.9	1950.1	549.7	541.7	419.7	457.3	259.0	260.8
28 kJ m^{-2} d^{-1}	2876.3	2412.0	2223.7	521.9	730.2	516.1	489.8	191.8	257.4
Total stress response index (TSRI)									
	7500.7 (t)	7246.3 (t)	6249.1 (t)	1884.7 (In)	1866.2 (In)	1713.1 (In)	1510.6 (s)	767.5 (s)	650.6 (s)

The letters in parenthesis indicate the tolerance/sensitivity of rice varieties given in accordance to their performance upon UV-B irradiation

t tolerant, *In* intermediate, *s* sensitive

Table 5 Pearson's correlation coefficients among various parameters from 9 rice varieties exposed to control and high dose of UV-B (28 kJ m^{-2}d^{-1})

Control	MDA*PRL	MDA*PRT	MDA*SGR	MDA*ASC	MDA*GLU
Aathira	0.111	− 0.765*	0.301	− 0.690	0.999**
Mangalamahsuri	− 0.135	− 0.834**	− 0.791*	− 0.104	0.920**
Kanchana	0.839**	− 0.410	0.580	− 0.801*	− 0.811*
Jyothi	− 0.653	0.879**	− 0.641	0.796*	0.882**
Annapoorna	− 0.521	− 0.638	− 0.782*	0.766*	0.200
Neeraja	− 0.002	0.268	− 0.372	0.022	− 0.245
Swetha	− 0.767*	− 0.825**	0.125	0.586	0.891**
Aiswarya	− 0.872**	− 0.601	0.834**	− 0.728*	0.258
Swarnaprabha	0.912**	0.652	0.501	0.448	− 0.978**
28 kJ m^{-2}d^{-1}	MDA*PRL	MDA*PRT	MDA*SGR	MDA*ASC	MDA*GLU
Aathira	− 0.725*	− 0.430	0.966**	0.980**	0.038
Mangalamahsuri	0.798*	0.990**	− 0.973**	− 0.827**	0.519*
Kanchana	− 0.403	− 0.098	− 0.862**	− 0.415	0.249
Jyothi	0.202	0.822**	− 0.254	0.771*	1.000**
Annapoorna	0.892**	0.531*	− 0.747*	− 0.128	0.987**
Neeraja	0.087	0.915**	0.735*	0.724*	− 0.934**
Swetha	0.626*	0.708*	0.850**	− 0.299	0.470
Aiswarya	0.134	− 0.573*	0.276	0.939**	− 0.039
Swarnaprabha	0.636*	0.092	− 0.297	− 0.736*	0.577*

MDA malondialdehyde, *PRL* proline, *PRT* protein, *SGR* sugar, *ASC* ascorbate, *GLU* glutathione

*Correlation was significant at the $p \leq 0.05$. **Correlation was significant at the $p \leq 0.01$

all the treatments of UV-B irradiation (7, 14, 21 and 28 kJ m^{-2}d^{-1}). TSRI was higher in Mangalamahsuri, Aathira and Kanchana (> 6000) as compared to Jyothi, Annapoorna, Neeraja and Swetha. Compared to all other rice varieties, TSRI was much lower in Swarnaprabha and Aiswarya (< 1000) (Table 4).

Correlation coefficients between MDA and stress related parameters were analyzed by Pearson's correlation. In control seedlings, MDA content showed a significant correlation to protein ($p < 0.05$) and glutathione content ($p < 0.01$) in Aathira and Swetha, to sugar and ascorbate content in Annapoorna ($p < 0.05$), and to ascorbate ($p < 0.05$), protein and glutathione content ($p < 0.01$) in Jyothi. After exposure to high dose of UV-B (28 kJ m^{-2}d^{-1}), MDA content had significantly positive correlation with proline, glutathione ($p < 0.05$) and protein content ($p < 0.01$) in Mangalamahsuri, sugar and ascorbate ($p < 0.01$) in Aathira, glutathione in Kanchana, protein, ascorbate and glutathione ($p < 0.01$) in Jyothi, proline, protein and glutathione content in Annapoorna, protein, sugar and ascorbate ($p < 0.05$) in Neeraja, proline, protein and sugar content in Swetha, ascorbate content in Aiswarya, proline and glutathione content ($p < 0.05$) in Swarnaprabha. Contrarily, MDA was negatively correlated with sugar and ascorbate content ($p < 0.01$) in Mangalamahsuri, protein in Aathira ($p < 0.05$), ascorbate and

glutathione in Kanchana, sugar content ($p < 0.01$) in Annapoorna ($p < 0.05$), proline, protein in Swetha and glutathione content in Swarnaprabha (Table 5).

Discussion

In this study, the morphological, physiological and biochemical features in thirteen commonly cultivated high yielding rice varieties was analyzed to show the intraspecific variation in sensitivity towards UV-B radiation. The identification of UV-B tolerant rice varieties during seedling growth is important for ensuring successful production of rice in regions with high levels of UV-B radiation (Bhattacharya et al. 2012). The oxidative stress effects induced by UV-B in plants varies between varieties to varieties and in thirteen rice varieties, the tolerance level of UV-B was found to be 28 kJ m^{-2}d^{-1} and above this dosage, nearly immediate, visually observable stress symptoms were observed. Therefore in the present study, the various doses of UV-B irradiation imparted was fixed as 0, 7, 14, 21 and 28 kJ m^{-2}d^{-1} for 1 week. The dosage of UV-B applied in the present study coincides with some previous studies in rice seedlings. Dai et al. (2006) studied the effect of UV-B radiation on 10 days old rice seedlings of two varieties and oxidative stress effects were evaluated

after irradiation with 6 and 13 kJ m^{-2}d^{-1} for 4 weeks and in this study, although dosage was less, the period of exposure was higher. Likewise, Du et al. (2011) fixed the UV-B doses at 14.4, 28.7 and 57.5 kJ m^{-2}, for irradiating rice seedlings and they noticed the appearance of light brown patches on leaves after exposure to highest dose of UV-B.

The initial screening was done on the basis of their performance after exposure to various doses of UV-B irradiation by analyzing shoot length, fresh weight, photosynthetic pigments and the rate of lipid peroxidation. Plant growth scores in the form of shoot length and fresh weight were found to be reduced with increasing UV-B dosage, implying that UV-B irradiation represses the growth of rice varieties. Similar findings were also reported earlier in plants when exposed to UV-B radiation (Shen et al. 2010; Dotto and Casati 2017; Yong-Mei et al. 2017; Faseela and Puthur 2018). Surprisingly, lower dose of UV-B (7 kJ m^{-2}d^{-1}) exhibited an increase in shoot length and chlorophyll content of tolerant rice varieties. The priming of seedlings of crop plants to counter abiotic stresses by exposure to low levels of UV is an emerging concept to make the crops hardy (Thomas and Puthur 2017). UV-B treatment enhanced the content of photosynthetic pigments and photoinhibition tolerance plants (Xu and Qiu 2007; Wargent et al. 2011; Sztatelman et al. 2015). It was clear that Aathira, Mangalamahsuri, Kanchana, Jyothi and Annapoorna showed UV-B tolerant features and Neeraja, Swetha, Aiswarya and Swarnaprabha showed sensitive features on the basis of shoot length, fresh weight, photosynthetic pigments and the rate of lipid peroxidation. The remaining four varieties (Mattatriveni, Karuna, Harsha and Varsha) showed intermediate characters with regard to UV-B tolerance and they were eliminated after the primary screening for selection of highly tolerant and sensitive types.

The chlorophyll content and carotenoid accumulation was less in sensitive varieties Aiswarya and Swarnaprabha. However, the chlorophyll content and carotenoid accumulation was higher in tolerant varieties Athira, Mangalamahsuri and Kanchana with increasing UV-B dosage. It was earlier established that rice plants on exposure to UV-B radiation showed damages to the photosynthetic machinery and degradation of photosynthetic pigments (Lidon et al. 2012; Faseela and Puthur 2018). Choudhary and Agrawal (2014) reported that *Vigna radiata* cultivar HUM 12 (tolerant) showed increased carotenoids contents as compared to HUM 1 (sensitive) under UV-B stress. Carotenoids play a significant role as efficient quenchers of UV-B induced ROS and reducing photooxidative damage. Salama et al. (2011) found that the chlorophyll contents were reduced, but carotenoids were increased under enhanced UV radiation in desert plants. Moreover, UV-B

radiation not only disturbs chlorophyll synthesis, but also affects photosynthetic efficiency of PSII (Ma et al. 2016).

In the present investigation, MDA content significantly increased in all thirteen rice varieties with increase in UV-B. This could be due to the non utilization of energy received by antenna complex and the excess energy being directed to O$_2$, resulting in generation of ROS (Foyer et al. 2017). Excessive generation of ROS causes lipid peroxidation measured as MDA content, which is the final product of lipid peroxidation (Sharma et al. 2012). MDA is a well-known marker of oxidative damage and thus it is correlated with the other parameters in rice seedlings after exposure to UV-B (28 kJ m^{-2}d^{-1}). As shown by Mishra et al. (2008) in cowpea and as confirmed in our study, the close correlation between MDA versus proline, sugar, protein, ascorbate and glutathione reinforces the involvement of primary metabolites and non enzymatic antioxidants accumulation in UV-B tolerance mechanisms. Dwivedi et al. (2015) experimentally proved that *Vigna acontifolia* is comparatively resistant to UV-B as compared to *Vigna mungo* by analyzing MDA and various enzymatic and non enzymatic antioxidants. In contrast, no dramatic changes were observed in MDA content in *Deschampsia antarctica* when exposed to UV-B stress and this could be attributed to its ability to activate both enzymatic (superoxide dismutase and total peroxidases) and non-enzymatic (total phenolics) antioxidant systems (Köhler et al. 2017).

Based on cumulative stress response index (CSRI) and total stress response index (TSRI), the rice varieties were classified as tolerant (Mangalamahsuri, Aathira and Kanchana), intermediate (Jyothi, Annapoorna, Neeraja and Swetha) and sensitive ones (Swarnaprabha and Aiswarya) towards various doses of UV-B irradiation, calculated from various primary metabolites and non enzymatic antioxidants. Previously, it was shown that measurements of CSRI and TSRI have the potential to be used for screening UV-B tolerance in different crop varieties (Zu et al. 2004; Koti et al. 2005, 2007). Similar works for the screening and fine mapping of commonly cultivated wheat varieties towards UV-B stress (Li et al. 2010) and rice varieties towards drought (Mishra and Panda 2017; Swapna and Shylaraj 2017), salinity (Kibria et al. 2017), aluminium (Guo et al. 2012) and cadmium toxicity (Bai et al. 2011) by using various physiological and biochemical parameters are reported. Li et al. (2010) determined the intraspecific variation in sensitivity of ten wheat cultivars (four tolerant, two moderately sensitive and four sensitive) to UV-B radiation and found that the responses were cultivar-specific.

Recently Fahad et al. (2017) proposed that plant cells exhibit decreased rates of total protein levels when exposed to various oxidative stress-inducing agents. In this study, we observed an increase in total protein content of all rice

varieties studied under UV-B irradiation, as previously reported in soybean (Shen et al. 2010), cucumber (Peng et al. 2017) and rice (Du et al. 2011). These proteins might be related to defense, enzymes required in plants for defending against UV-B stress, which are the potential candidates for improvement of stress tolerance in plants (Lloret et al. 2017). Such proteins might have played a role in tolerance of black gram resistant variety 'U-Taung-2' as compared to sensitive variety 'Mut Pe Khaing To' by inducing adaptation to salt stress (Win and Oo 2017). In addition, our results indicated that soluble sugar content was significantly enhanced in Aathira, Mangalamahsuri and Kanchana than other varieties in response to UV-B stress and could help plants to cope up with the UV-B irradiation since this metabolite was involved in increasing plant adaption to oxidative stress through biochemical adjustment. Most recent studies also have reported that UV-B radiation can induce soluble sugar content in plant tissues (Bacelar et al. 2015; Peng et al. 2017). Upon comparing significant critical differences ($p < 0.05$) in UV-B stressed seedlings of all rice varieties, the higher degree of total protein and soluble sugar was recorded in varieties Aathira, Mangalamahsuri and Kanchana, revealing their high tolerance potential towards various doses of UV-B.

On the basis of the accumulation of non enzymatic antioxidants, some varieties of rice were identified as UV-B tolerant types. As reported by Szabados and Savoure (2009), proline, an imino acid, is involved in tolerance mechanisms against oxidative stress in plants, and is the main strategy to avoid the negative effects of UV-B in plants. Likewise, highly increased proline accumulation was recorded in rice varieties Aathira, Mangalamahsuri and Kanchana exposed to 28 kJ m^{-2}d^{-1} of UV-B. The induction of antioxidants in plants is a natural adaptation strategy to overcome the oxidative stress (Sharma et al. 2012), which is evident by the significant increases of total ascorbate and glutathione content in rice varieties. Changes in ascorbate and glutathione contents are critical stress indicators determining the survival ability of plants during various oxidative stresses (Faseela et al. 2018; Lou et al. 2018; Nahar et al. 2018). The multifold enhancement in ascorbate and glutathione content of Aathira, Mangalamahsuri and Kanchana even after the initial dose (7 kJ m^{-2}d^{-1}) of UV-B treatments and maximum accumulation recorded upon 28 kJ m^{-2}d^{-1} of UV-B irradiation, suggests that ascorbate and glutathione function as major and prominent soluble antioxidant metabolites in rice seedlings subjected to UV-B irradiation. Previous studies reported that UV-B exposure resulted in accumulation of glutathione in *Prunella vulgaris* (Zhang et al. 2017). Our findings are in agreement with Dwivedi et al. (2015), who reported that UV-B treatment caused higher proline and ascorbate content in tolerant variety

Vigna acontifolia in comparison with susceptible one, *Vigna mungo* and they suggested this tolerant variety for cultivation in areas with high UV-B irradiation.

Conclusion

UV radiation reaching Earth's surface has increased markedly over the last decades and the determination of UV-B tolerant varieties would be helpful for rice cultivation in the areas experiencing higher influx of UV-B radiation. The morphological, physiological and biochemical parameters of thirteen high-yielding rice varieties were evaluated after exposure to various doses of UV-B irradiation. The UV-B tolerance of rice varieties was found to have a positive correlation towards carotenoids, total protein, soluble sugar, proline, ascorbate and glutathione contents under the stress. Cumulative stress response index (CSRI) and total stress response index (TSRI) was employed to classify them as highly tolerant (Mangalamahsuri, Aathira and Kanchana), intermediate (Jyothi, Annapoorna, Neeraja and Swetha) and highly sensitive lines (Swarnaprabha and Aiswarya) towards UV-B irradiation. The outcome of this study proves that the varieties Mangalamahsuri, Aathira and Kanchana can be recommended as the most UV-B tolerant genotypes and can also be used as the donors for further crop improvement programs for the development of more UV-B tolerant varieties.

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