Original Research Article

Variation in Antioxidant Responses in Rice (*Oryza sativa* L.) Genotypes Differing in Sensitivity to Heat Stress

K.P. Sharma[1*], N. Sharma[2] and R. Kaur[2]

[1]Department of Biochemistry, Punjab Agricultural University, Ludhiana, Punjab, India 141004
[2]Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India 141004

Corresponding author

A B S T R A C T

A field experiment was conducted to study the response of antioxidant enzymes to oxidative damage caused by heat stress induced by changing dates of transplanting in two rice (*Oryza sativa* L.) genotypes viz., 'N22' (heat tolerant) and 'IR8' (heat susceptible). During grain filling period, constitutive level of antioxidative enzymes viz., superoxide dismutase, catalase, ascorbate peroxidase, was found to be 35.7, 88.8 and 84.1% higher respectively, while the content of non-enzymatic antioxidants viz., ascorbate and proline was 78.5 and 81.8% higher respectively, in 'N22' than in 'IR8' under heat stress encountered during early transplanting. In contrast, the hydrogen peroxide and malondialdehyde content were 42.1 and 28.4% higher in 'IR8' during early transplanting. Heat stress resulted in significant reduction of chlorophyll content during early transplanting in both the genotypes though the reduction was less in 'N22' (27.6%) as compared to 'IR8' (45.2%). The increased and timely response of antioxidant enzymes along with the non enzymatic antioxidants is suggestive of their significant role in development of tolerance exhibited by the cultivar 'N22' under heat stress conditions.

Introduction

Heat stress is one of the major factors affecting rice (*Oryza sativa* L.) productivity in many areas and it occurs more frequently and severely because of the global warming. Global climatic predictions indicate increased frequency of heat spikes and warmer nights which exert additional challenges to achieve higher crop yields (IPCC, 2013). Taking global harvested area in consideration, climate model predicts that 16% of the rice growing area would be exposed to at least 5°C of temperature above the critical threshold during the reproductive period, by 2030, with a constant increase to 27% of rice growing area by 2050 (Gourdji *et al.,* 2013). High temperature stress during grain filling can diminish seed set by accelerating senescence thereby reducing crop yields ((Wahid *et al.,*

2007; Hasanuzzaman *et al.,* 2013; Zafar *et al.,* 2017 and Siddique *et al.,* 1999).

Under prolonged stress exposure, photosynthetic activity is further inhibited by excessive accumulation of reactive oxygen species (ROS), causing damage to the membranes, proteins and chlorophyll molecules of the photosynthetic apparatus (Allakhverdiev *et al.,* 2008; Silva et al2010; Redondo-Gómez, 2013; Awasthi *et al.,* 2014; Das *et al.,* 2016; Tricker *et al.,* 2018). Reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide ($O_2{}^-$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\cdot}$) produced during aerobic metabolism in the intracellular organelles of plant cells are readily scavenged by plant antioxidant defense systems which are chiefly antioxidant enzymes and non-enzymatic antioxidants. Plants use a complex antioxidants system to regulate ROS levels and avoid toxicity, but changes in redox status are also perceived by plants as a signature of a specific stress that will result in a corresponding acclimation response (Foyer and Noctor, 2005; Choudhury *et al.,* 2017). Various environmental stresses such as high temperature, salt stress, drought stress, disrupt photosystem energy balance, functions of plasma membrane enzymes and glyoxisomal photorespiration, which disturb biochemical reactions in chloroplasts and mitochondria. These changes result in accumulation of ROS beyond the antioxidant capacity of plants (Karuppanapandian *et al.,* 2013). Genotypes vary in their response to various stresses because of variation in their antioxidant systems.

Rice is tolerant to high temperature, but excessively high temperature impairs its growth and reproduction, especially impacts on the upper three functional leaves, which are the main photosynthetic organs during the later growth period and are important for grain filling. Significant variation exists among rice germplasm in response to temperature stress. Booting and flowering stages are the most sensitive to high temperature, and may sometimes lead to complete sterility (Shah *et al.,* 2011). Heat stress at this time could cause a serious reduction in grain yield due to pollen sterility, empty or unfilled grains, low grain weight and poor seed setting (Tao *et al.,* 2007). High temperature during grain filling stage of rice can decrease starch content, grain plumpness and protein accumulation, affecting both quality and yield of rice (Li *et al.,* 2002). Therefore, the present investigation was carried out to study the oxidative status of rice genotypes during their reproductive phase of growth when transplanted at different times.

Materials and Methods

Plant material and Experimental conditions

Two rice genotypes viz. heat tolerant 'N22' and heat susceptible 'IR8' were employed in this study. The rice genotypes were sown during the second week of March (early) and in the third week of May (normal) to expose the crops to different temperature regimes. The seedlings of rice cultivars were raised in the wooden trays (50 x 40 x 10 cm) in the glasshouse. Thirty- day old seedlings were transplanted in the field in complete randomized block design with three replications.

The recommended agronomic practices were followed. The temperatures under the two sowings were recorded by the field meterological laboratory and then the activities of the enzymes were studied in the flag leaf during anthesis and dough stage of grain development. All enzymatic and other estimations like malondialdehyde, ascorbate, chlorophyll and proline content were estimated in the mid- region of the flag leaf.

Extraction and estimation of antioxidant enzymes

For enzyme extracts and assays, leaf tissue from each treatment was homogenized in ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM ethylene diamine tetraacetic acid (EDTA) and 1% polyvinyl pyrrolidone (PVP). The homogenate was centrifuged at 10,000 x g for 20 min at 4°C, and the supernatant was used for activity measurement.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured by monitoring its ability to inhibit the auto-oxidation of pyrogallol. The assay was carried out in 100 mM Tris HCl buffer (pH 8.2), 3 µM EDTA, 6 µM pyrogallol solution to which enzyme extract was added. The change in absorbance was recorded for 3 minutes at 420 nm at regular intervals (Marklund and Marklund, 1974). One unit of SOD activity has been defined as the amount of enzyme required to cause 50% inhibition of auto-oxidation of pyrogallol under the experimental conditions. The specific activity of SOD is expressed as units mg^{-1} protein.

Peroxidase (POX, EC 1.11.1.7) activity was determined by following the appearance of brown colouration resulting from guaiacol oxidation to tetraguaiacol in the presence of H_2O_2. To 150 µM guaiacol prepared in 100 mM potassium phosphate buffer (pH 6.5), To 0.1 ml of enzyme extract, 0.08 mM H_2O_2 was added and the change in absorbance was recorded at 470 nm (Shannon *et al.,* 1966). The unit of enzyme activity has been defined as the change in absorbance at 470 nm min^{-1} g^{-1} fresh weight and min^{-1} mg^{-1} protein.

Glutathione reductase (GR, EC 1.6.4.2) was estimated by the method of Esterbauer and Grill (1978). The reaction was started by adding 0.2 M potassium phosphate buffer (pH

7.5), 0.04 µM EDTA, 0.3 µM MgCl$_2$, 0.001 µM of NADPH and 0.8 ml of enzyme extract followed by 0.1 µM of oxidized glutathione. The enzyme activity was estimated as decrease of absorbance at 340 nm after an interval of 30 seconds upto 3 minutes. The molar extinction coefficient for NADPH is 6.22 mM^{-1} cm^{-1}. The specific activity of GR was expressed as nmoles of NADP$^+$ formed min^{-1} mg^{-1} protein.

Catalase (CAT, EC 1.11.1.6) activity was assayed following Chance and Maehly (1955). The assay was carried out in 50 mM sodium phosphate buffer (pH 7.5) to which 0.1 ml of enzyme extract and 0.039 mM H_2O_2 was added. The utilization of H_2O_2 was recorded for 3 minutes by measuring the decrease in absorbance at 240 nm. Extinction coefficient for hydrogen peroxide has the value of 0.0394 mM^{-1}cm^{-1}. CAT activity was expressed as µmoles of H_2O_2 decomposed min^{-1} mg^{-1} protein.

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was measured according to the method of Nakano and Asada (1987) by estimating the rate of ascorbate oxidation. The reaction mixture consisted of 50 mM sodium phosphate buffer (pH 7.5), 0.4 µM ascorbic acid, 0.2 ml of enzyme extract and 0.039 mM H_2O_2 solution. Decrease in absorbance was measured at 290 nm for 3 minutes at an interval of 30 seconds (ε = 2.8 mM^{-1}cm^{-1}). APX was expressed as nmoles of monodehydroascorbate formed min^{-1} mg^{-1} protein. The protein amount was determined by the Lowry method (1951) using bovine serum albumin (BSA) as a standard.

Estimation of hydrogen peroxide, ascorbic acid, malondialdehyde and proline content

Hydrogen peroxide (H_2O_2) content was measured according to Sinha (1971). Leaf tissue was homogenized with 10 mM

potassium phosphate buffer (pH 7.0). The homogenate was centrifuged at 10,000 x g for 20 minutes. An aliquot of 2 ml was mixed with equal volume of 5% potassium dichromate and glacial acetic acid (1:3 v/v) and the absorbance was read at 570 nm against the reagent blank. H_2O_2 content was calculated from the standard curve prepared using H_2O_2 concentrations ranging from 20 to 100 µmoles. The H_2O_2 content was expressed as mmoles g^{-1} FW.

Ascorbic acid (Asc) content was evaluated according to method of Law *et al.,* (1983). The leaf tissue was extracted with 5% ice cold metaphosphoric acid. An aliquot measuring 0.4 ml was added to reaction mixture containing 0.002 mM EDTA, 1.7% TCA, 0.013mM $FeCl_3$ prepared in 0.1 M potassium phosphate buffer (pH 7.5), 7.6% *o*-phosphoric acid and 0.035 mM bipyridyl. After an incubation of 40 min at 40ºC the absorbance was measured at 525 nm. A standard curve in the range of 10-40 nmoles of ascorbic acid was used for determination of ascorbic acid content. Asc concentration was expressed as nmoles g^{-1} FW.

For the extraction of malondialdehyde (MDA) assay, leaf tissue was homogenized with 5% trichloroacetic acid (TCA) and centrifuged at 10,000 x g for 15 minutes at 25°C. Supernatant was taken for estimation of MDA (Ohkhawa *et al.,* 1979). The supernatant was mixed with an equal volume of 20% (w/v) TCA containing 0.5% thiobarbituric acid (TBA). The mixture was heated to 96ºC for 30 minutes and immediately cooled in ice and centrifuged at 10,000 x g for 10 minutes at 4ºC. The absorbance was recorded at 532 nm. Correction for non– specific absorbance was made by subtracting the absorbance value taken at 600 nm. The results were calculated by using extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nmoles g^{-1} FW.

The powdered dried tissue was extracted with 3% aqueous sulfosalicylic acid (w/v) and the extract was filtered through Whatman filter paper no.1. The filterate was used for proline (Pro) estimation (Bates *et al.,* 1973). An aliquot of 2 ml was mixed with 2 ml of acid ninhydrin solution and 2 ml of glacial acetic acid.

The reaction mixture was incubated in boiling water for 1 hour. The reaction was terminated by immersing the tubes in ice-cold water for 5 min. The reaction mixture was extracted with 4 ml toluene, mixed vigorously and absorbance of the chromophore containing upper toluene layer was read at 520 nm using toluene as blank. The concentration of proline was calculated from standard curve containing 0.02 to 0.2 µmoles of proline run simultaneously.

Chlorophyll content was estimated by following the method of Anderson and Boardman (1964). The leaf tissue was homogenized with 80% ice cold acetone and centrifuged at 5,000 x g for 5 minutes at 4°C.

The residue was recentrifuged with 80% acetone at 5,000 x g for 5 minutes. This process was repeated twice to completely extract chlorophyll. Supernatants were pooled and the absorbance of the supernatant was recorded at 645 nm and 663 nm. The chlorophyll content was expressed as mg g^{-1} FW.

Statistical analysis

Data were reported as mean ± standard deviation for triplicates determinations of each sample. Analysis of variance and Tukey Test were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA) to identify differences between values. Statistical significance was defined at level of $\leq 0.05\%$ unless specified otherwise.

Results and Discussion

During our study, the mean maximum temperature encountered during anthesis by 'N22' and 'IR8' was $3.7^{o}C$ and $3.6^{o}C$ higher respectively in early transplanting (ET) compared to normal transplanting (NT). During dough stage, the temperature rise observed was $4.4^{o}C$ and $4^{o}C$ in 'N22' and 'IR8' respectively at ET compared to NT.

The maximum temperature recorded during anthesis stage was $26.4^{o}C$ and $32^{o}C$, during NT while it was observed to be $33.6^{o}C$ and $36^{o}C$ during ET for, N22 and IR8 respectively. The minimum temperature was $25.6^{o}C$ and $25.3^{o}C$ during NT, while during ET, it was $25.8^{o}C$ and $28.4^{o}C$ for N22 and IR8 respectively. The mean temperature was $26^{o}C$ and $28.65^{o}C$ and $29.7^{o}C$ and $32.2^{o}C$ for N22 and IR8, during NT and ET, respectively. The maximum temperature during dough stage was observed to be $31.2^{o}C$ and $27.8^{o}C$ during NT and $37^{o}C$ and $32.2^{o}C$ during ET, for N22 and IR8 respectively. The minimum temperature was $26.4^{o}C$ and $23.4^{o}C$ during NT, and $28.4^{o}C$ and $27^{o}C$ during ET respectively. The mean temperature was $28.3^{o}C$ and $25.6^{o}C$ during NT, and $32.7^{o}C$ and $29.6^{o}C$ during ET, for N22 and IR8 respectively.

Under NT conditions, oxidative damage to cellular constituents is limited because of efficient scavenging of ROS by rapidly responsive antioxidant system composed of antioxidant enzymes and redox metabolites. However, under ET, when the crop is exposed to high temperature during grain filling stage, cellular damage may occur due to an imbalance in the formation and scavenging of ROS. The present study has shown that there were significant changes in the activity of the antioxidant enzymes viz., SOD, GR, POX, CAT and APX in response to early transplanting. The co-ordinated function of following antioxidant enzymes help in the processing of ROS and regeneration of reduced ascorbate and glutathione metabolites.

Effect of high temperature on antioxidant enzyme activities

SOD has been proposed to provide the first line of defence against ROS by catalyzing the dismutation of O_2^{-} to H_2O_2 and O_2. In our study, both cultivars showed higher SOD activity when the crop encountered heat stress during grain development period (Fig. 1a). The highest SOD activity was found in 'N22' at the anthesis stage (18.21 units mg^{-1} protein) during ET followed by the dough stage while the lowest activity (1.66 units mg^{-1} protein) was found at anthesis in 'IR8' during normal transplanting (NT). The overall mean activity irrespective of the grain filling stage, was 1.6 fold higher in 'N22' compared to 'IR8' ($p < 0.01$ level). The upregulation of SOD is implicated in combating oxidative stress caused due to biotic and abiotic stress and has a critical role in the survival of plants under heat stress (Gur *et al.,* 2010). The genotype 'N22' exhibited significantly higher constitutive and induced SOD activity compared to 'IR8' during both anthesis and dough stage, indicating that it has better scavenging capacity and higher tolerance to heat stress than 'IR8'.

A much lower activity was observed during NT compared to ET in both the genotypes. During NT, CAT activity was higher in 'IR8' compared to 'N22' while the reverse was true during ET at both anthesis and dough stages. The mean CAT activity was found to be 8.9 fold higher during ET in 'N22' compared to 'IR8' (Fig. 1b). CATs have the potential to directly dismutate H_2O_2 into H_2O and O_2 and is indispensable for the detoxification of ROS during stress generated in peroxisomes by oxidases involved in ß-oxidation of fatty

acids, photorespiration and purine catabolism (Gill and Tuteja, 2010). CAT activity in our study was found to increase significantly (p < 0.01 level) in both the cultivars, at ET as compared to NT but this increase was 104 - fold and 4.2- fold in 'N22' and 'IR8', respectively. This shows an enhanced ability of 'N22' to tolerate high temperature.

Significant increase in APX activity was observed during ET at both anthesis and dough stages in 'N22' compared to NT (p < 0.01 level) (Fig. 1c). In contrast, the APX activity in 'IR8' was not significantly affected at the two transplanting dates at both anthesis and dough stages during grain filling. The highest APX activity was observed in 'N22' during ET at the dough stage (64.73 nmoles of monohydroascorbic acid formed min^{-1} mg^{-1} protein) while the lowest activity was also observed in 'IR8' at anthesis stage (6.62 nmoles of monohydroascorbic acid formed min^{-1} mg^{-1} protein). APX is thought to play the most essential role in scavenging ROS and protecting cells in higher plants. APX is involved in scavenging of H_2O_2 in water-water and ASH-GSH cycles and utilizes ascorbate (ASH) as the electron donor. APX has a higher affinity for H_2O_2 (µM range) than CAT and PO (mM range) and it may have a more crucial role in the management of ROS during abiotic stress (Gill and Tuteja, 2010). In the present study, higher induction of APX was observed at ET as compared to NT in 'N22' and may be a contributing factor in conferring tolerance to heat stress.

A low GR activity was observed in our study but followed the same pattern as that of other ROS scavenging enzymes (Fig. 1d). Higher GR activity was observed during ET in both genotypes with the highest being observed in 'N22' at anthesis (7.06 nmoles $NADP^+$ formed min^{-1} g^{-1} fresh weight) and the lowest in dough stage of 'IR8' (0.61 nmoles $NADP^+$ formed min^{-1} g^{-1} fresh weight) (Fig. 1d) (p < 0.01 level). GR activity was reported to

increase during the presence of Cd during drought stress in *O. sativa* seedlings (Sharma and Dubey, 2005).

POXs are known as stress ameliorating enzymes that actively respond to damages in plant metabolism and act as early and sensitive indicators of heat stress. As compared to other antioxidant enzymes, a low activity of peroxidase (POX) in both the genotypes was recorded. However, at ET compared to NT; it was significantly higher i.e; 3 and 8 folds in 'N22' and 'IR8' respectively (p < 0.01 level). The heat tolerant genotype 'N22' possessed a higher constitutive level of POX compared to the heat susceptible 'IR8' genotype at both transplanting dates. The POX activity was higher at the anthesis stage in both 'N22' and 'IR8' compared to the dough stage during both transplanting dates except 'N22' during NT when it exhibited similar activity at both anthesis and dough stage. Increase in the activity of POX in response to heat stress in cotton (*Gossypium hirsutum* L.) has been reported (Gur *et al.,* 2010) where the POX activity increased as a response to increase in temperatures to 38°C and 45°C. Similarly, though the contribution of POX towards manipulating the redox status in 'N22' and 'IR8' was very less in our study, it was appreciably higher during ET in 'N22' compared to 'IR8'. During NT, almost negligible POX activity was detected in both cultivars.

It is clear from our data that there was a significant increase in the activity of the antioxidant enzymes viz., SOD, CAT and APX in response to ET in both the genotypes except during anthesis in IR, APX activity was lower than NT. This increase was significantly higher in 'N22' compared to 'IR8' and may contribute towards the tolerance exhibited by it to high temperature encountered during ET (Fig. 1a- c).

Non enzymatic antioxidants

Hydrogen peroxide (H_2O_2) regulates the expression of various genes, including those encoding antioxidative enzymes (Geisler *et al.,* 2006). It is the most stable of the ROS and therefore plays a crucial role as a signalling molecule in various physiological processes. H_2O_2 acts as a second messenger for signals generated by means of ROS because of its relatively long life and high permeability across membranes (Quan *et al.,* 2002). H_2O_2 content was found to be significantly higher during ET compared to NT in both the genotypes (Fig. 1e). However, there was a more drastic increase in 'IR8' (12.24 fold) as compared to 'N22' (1.36 fold) during ET. H_2O_2 content was higher in the dough stage in both the genotypes at both transplanting dates. The highest content was present at the dough stage of 'IR8' (264.33 mmoles g^{-1} FW) at ET. It was interesting to note that during NT, 'N22' possessed 5.19 fold higher H_2O_2 content as compared to 'IR8' while during ET, 'IR8' possessed 1.73 times higher H_2O_2 content (p < 0.01 level). Higher SOD activity results in higher production of H_2O_2. In 'IR8', the higher H_2O_2 content during ET could be a consequence of low APX and CAT activity. Suriyasak *et al.,* (2017) reported H_2O_2 content to be significantly higher under heat stress (30°C) than under control (25°C) conditions.

Ascorbate (Asc) content decreased with ET in both the genotypes though the decrease was much higher than NT for 'IR8' (10.9 fold) as compared to 'N22' (1.8 fold) (p < 0.01 level). One of the reason for 'N22' being thermotolerant may be the presence of higher Asc content even at high temperature which helps to prevent or minimize the damaged caused by ROS. Ascorbic acid (ASH) is one of the most abundant, powerful and water soluble antioxidant which acts to prevent or minimize the damage caused by ROS in plants. It occurs in all plant tissues, usually being higher in photosynthetic cells and meristems (and some fruits). Higher ascorbate content along with APX activity in 'N22' demonstrates its superior tolerance mechanism in terms of H_2O_2 scavenging over 'IR8' (Fig. 1f). Yang *et al.,* (2008) have reported the importance of ascorbate in the amelioration of oxidative stress under abiotic stress in *Picea asperata*.

Malondialdehyde (MDA) content was significantly higher during ET in both the genotypes compared to NT (Fig. 2a). Maximum content was present in 'IR8' (49.10 μmoles FW) during ET at the dough stage while the minimum content was recorded in 'N22' at the anthesis stage (9.74 μmoles g^{-1} FW) (p < 0.01 level). MDA, a decomposition product of polyunsaturated fatty acid hydroperoxides, has been utilized as a suitable biomarker for the evaluation of lipid peroxidation or damage to plasmalemma and organelle membranes that increases with environmental stress. Lipid peroxidation is linked to the activity of antioxidant enzymes since with the increase of SOD, APX, GPX, CAT, etc oxidative stress tolerance is enhanced while MDA is decreased. Wang *et al.,* (2011) reported that high temperature results in increase content of MDA, free proline and soluble sugars in the function leaves, while the chlorophyll content and photosynthetic rate of leaves was decreased. We observed that heat stress encountered during the grain filling stage of ET rice genotypes increased the MDA content in both the genotypes with 'N22' having much lower MDA content than 'IR8' at both anthesis and dough stage of grain filling. This indicates that 'N22' is less susceptible to lipid peroxidation compared to 'IR8'.

Proline (Pro) content was much higher in 'N22' both during ET and NT than 'IR8'. During ET, a much lower Pro content was estimated in 'IR8' (5.5 fold) than 'N22'. We

found that the Pro content was highest at ET in 'N22' whereas, in 'IR8' it did not vary significantly with date of transplanting (p < 0.01 level) (Fig. 2b). Pro is accumulated as osmoprotectant of cellular structure in response to osmotic stress but may also be strongly correlated with the capacity of plants to sense heat stress (Gosavi *et al.,* 2014). The role of Pro accumulation towards greater heat tolerance reported in maize and rice by Kumar *et al.,* (2012) and in cotton by Sekmen *et al.,* (2014) also supports our study.

Fig.1 Changes in the activities of (a) Superoxide dismutase, (b) Catalase, (c) Ascorbate peroxidase, (d) Glutathione reductase and (e) H_2O_2 content, (f) Ascorbate content, in rice genotypes at anthesis and dough stage under normal and early transplanting. Genotypes indicated by the same letters, do not differ statistically. Data in the diagram represents mean ± standard deviation
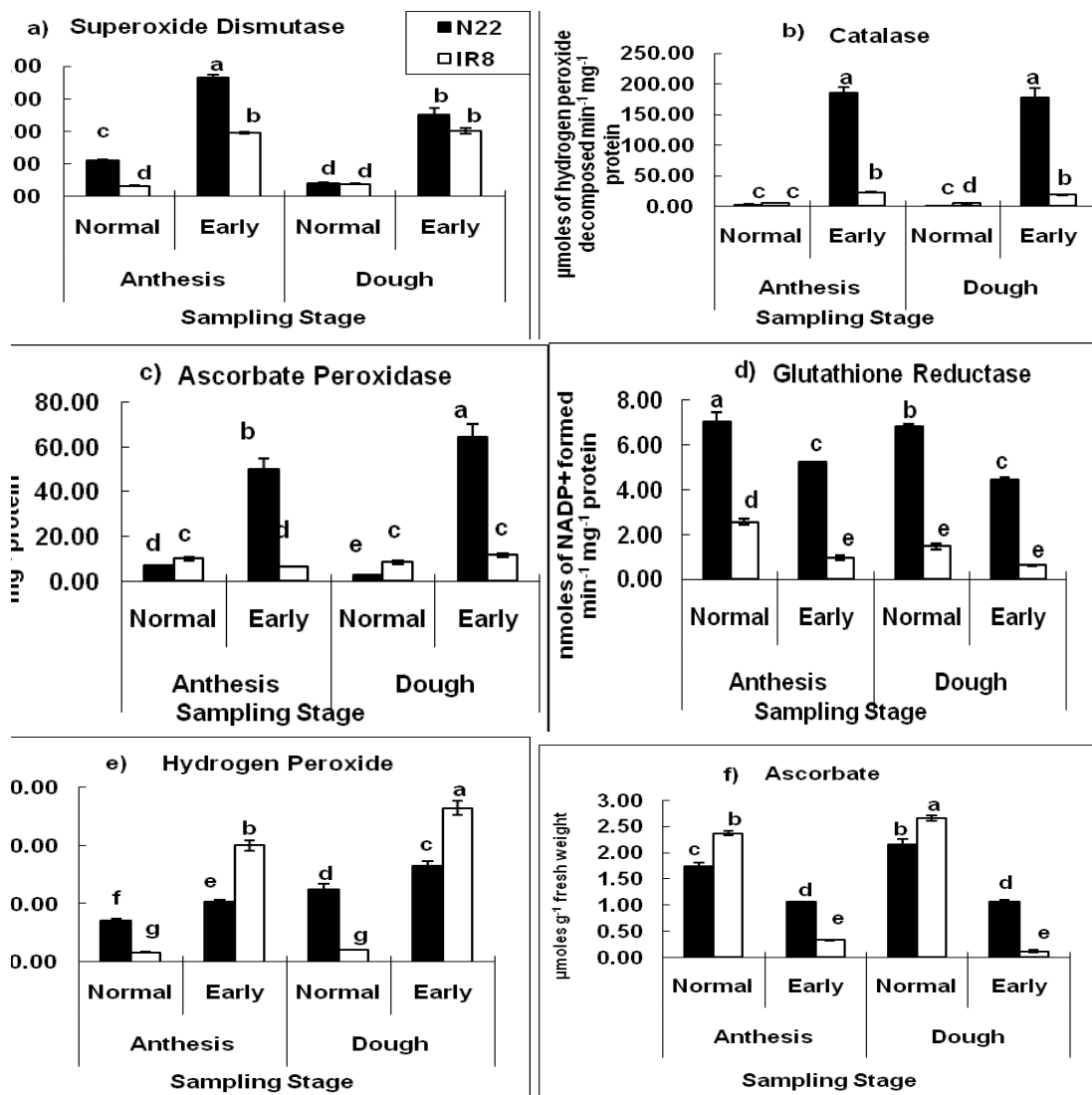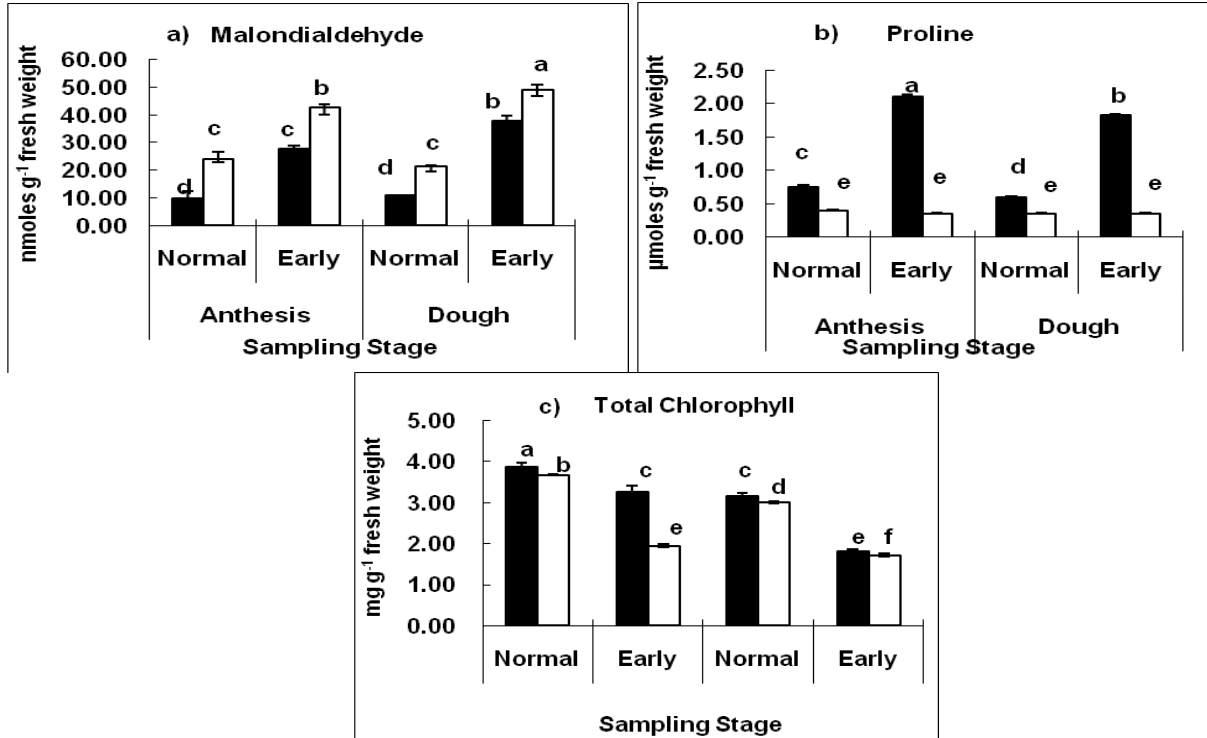
Fig.2 Changes in the (a) Malondialdehyde, (b) Proline, and (c) Total chlorophyll in rice genotypes at anthesis and dough stage under normal and early transplanting. Genotypes indicated by the same letters, do not differ statistically. Data in the diagram represents mean ± standard deviation

Chlorophyll content in plants is an important trait to assess photosynthesis efficiency under stress conditions. Chlorophyll synthesis is sensitive to heat stress and plants exposed to chill or heat stress have impaired chlorophyll biosynthesis due to downregulation of gene expression and protein abundance of several enzymes involved in tetrapyrrole metabolism (Mohanty *et al.,* 2006). Total chlorophyll content decreased during ET in both the genotypes. A higher reduction (15.13%) was found in 'IR8' compared to 'N22', indicating its role in the amelioration of heat stress (p < 0.01 level) (Fig. 2c). Reynolds *et al.,* (1994) gave physiological evidence indicating that loss of chlorophyll during grain filling was associated with reduced yield in wheat. Both chlorophyll a and chlorophyll b content were significantly higher in 'N22' compared to 'IR8', during both NT and ET.

Our study revealed that high temperature tolerant genotype 'N22' showed increase in SOD, APX and CAT (ROS scavenging enzymes) under ET and also maintained a greater activity of GR and POX, though the total activity of POX was very low as compared to other antioxidant enzymes. 'N22', the heat tolerant genotype, had lower H_2O_2 (a potent oxidant), MDA (lipid peroxidation product) and higher ascorbate (potent antioxidant) and Pro content (osmoprotectant and potent antioxidant) during ET than the temperature sensitive genotype 'IR8'.

Higher activities of SOD and CAT in the scavenging of H_2O_2 in both 'N22' and 'IR8' are indicated, suggesting that a major amount of H_2O_2 was being scavenged by the CAT route. It may be surmised that the temperature

tolerance in rice is associated with the timely and co- ordinated response of the antioxidative machinery which includes up-regulation of antioxidants and activation of antioxidant enzymes.

List of Abbreviations

APX: Ascorbate peroxidase; Asc: Ascorbic acid; CAT: Catalase; Chl: Chlorophyll; ET: Early transplanting; GR: Glutathione reductase; H_2O_2: Hydrogen peroxide; MDA: Malondialdehyde; NT: Normal transplanting; $^1O_2$: Singlet oxygen; O_2^-: Superoxide; $OH^.$: Hydroxyl radical; POX: Peroxidase; Pro: Proline; ROS: Reactive oxygen species; SOD: Superoxide dismutase; TCA: Trichloroacetic acid

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