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SHORT COMMUNICATION

HETEROSIS FOR ANTIOXIDANT ENZYMES IN RICE HYBRID UNDER ABIOTIC STRESS CONDITIONS

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Activities of Super oxide dismutase (SOD), Glutathione reductase (GR), Catalase (CAT) were analyzed in rice hybrid DRRH-2 and its respective male (DR 714-1-2R) and female (IR 68897A) parents under water, salinity and low temperature stresses. The Superoxide dismutase (SOD) activity was significantly increased in all the three stresses and in all the three genotypes, while highest increase was observed under WS. In case of glutathione reductase (GR) activity the hybrid did not show significant change with the type of stress, where as male parent exhibited decrease and female parent exhibited increase in the enzyme activity under water stress and salt stresses. The Catalase (CAT) activity decreased in all the three types of stresses in hybrid and both parents, while the reduction was relatively higher in male parent followed by hybrid. The hybrid DRRH-2 showed distinct heterosis under all the stresses for the activities of SOD and GR as indicated by their heterosis over the better of parents (HOBP) and heterosis over the mean of parents (HOMP) values, while for CAT activity these heterosis values were non-significant. According to the results, the heterotic vigor for the antioxidant enzymes (SOD, GR) in this study suggests the improvement of stress tolerance level in the hybrid than the parental lines, which could be an important biochemical basis towards understanding the heterosis under abiotic stress conditions.

***Key words*: Abiotic stress, catalase, glutathione reductase, heterosis, rice, superoxide dismutase**

Rice (*Oryza sativa* L.) is one of the most important crops in the world, and the foremost staple food in Asia, providing 35 to 60% of the dietary calories consumed by more than 3 billion people. Hybrid rice is a readily adaptable feasible option to enhance rice yields over about 15-20%. Considerable amounts of rice yield are lost due to various abiotic stresses such as drought, salinity and temperature. Reactive oxygen species (ROS) are formed in biological systems as part of normal metabolism. Adverse environmental factors like drought stress result in increased levels of ROS that are detrimental to the plant. Increased oxidative stress leads to the production of reactive oxygen species (ROS). Enzymes such as superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase play an important role in lowering the ROS levels and helping avoid oxidative stress.

Heterosis breeding is an important genetic tool to facilitate yield enhancement and help enrich many other desirable quantitative and qualitative traits in crops. The performance of hybrids in general was better as compared to parents for yield and yield enhancing traits. Appreciable amounts of heterosis have been detected and exploited for agronomical and physiological traits (Sarial and Singh 2006), yet studies describing heterosis for antioxidant enzymes are very few (Singh *et al.* 2010). This study focuses on the heterotic behavior of hybrids

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over its parents by comparing the antioxidant enzyme activities under different abiotic stresses such as low temperature, drought and salt stress of hybrids and its parents and the contribution from each of the parent for these characters.

Seeds of rice hybrid DRRH-2 and its parents *viz.* female cytoplasmic A line IR 68897A, and male restorer line DR714-1-2R were obtained from Hybrid Rice Section, DRR, Hyderabad. Seeds were surface sterilized with 0.1% (w/v) $HgCl_2$ for 20 min and were allowed to germinate on water soaked filter paper in petridishes at 37°C in dark. The germinated seedlings were grown in the half strength Hoagland solution in large glass containers up to two weeks (Hoagland 1950). The nutrient solutions were changed for once in two days. Two week old seedlings were transferred to 10% PEG (PEG 6000) solution (W.P: -0.54 MPa: pF: 3.75) or to 200 mM NaCl solution (W.P: -1.26 MPa: pF: 4.11) so as the seedlings are exposed to PEG induced water stress (WS) and NaCl induced salinity (SS) stresses respectively. For low temperature stress (LS) treatment seedlings were kept in an incubator at 8-10°C±0.2°C where as control treatment (CN) was maintained at room temperature with adequate amount of distilled water. Treatment duration lasted for 3 days for all the stress treatment. One gram leaf sample was extracted in 10 ml of 0.1 M, pH 7.5 M phosphate buffer containing 1 mM EDTA, 5% sorbitol, 0.1% Triton X-100 and then centrifuged at 12000 rpm for 20 min. The supernatant was used as the enzyme source. All the operations were carried at 4°C. Super oxide dismutase (E.C.1.15.1) activity was measured following Dhindsa *et al.* (1981). The sample tubes were illuminated under 15W fluorescent lamp for 10 min. The tubes lacking enzyme extract but containing the assay mixture were also illuminated and served as control. A non irradiated complete reaction mixture served as blank. Absorbance was recorded at 560 nm, and one unit of enzyme activity was taken as the quantity of enzyme which reduced the absorbency reading to 50% in comparison with the control. The activity was expressed as units of SOD per minute per gram fresh weight. Glutathione reductase (EC 1.6.4.2) was assayed as per the method of Smith *et al.* (1988). The increase in absorbance at 412 nm was recorded spectro-photometrically. The increase in absorbance at 412 nm was recorded spectro-photometrically and the activity of enzyme was expressed as change in absorbance per mg protein per minute. Catalase (E.C. 1.11.1.6) was measured according the method of Aebi (1984) in a 3.0 ml reaction mixture containing 1.5 ml of 0.1 M phosphate buffer (pH

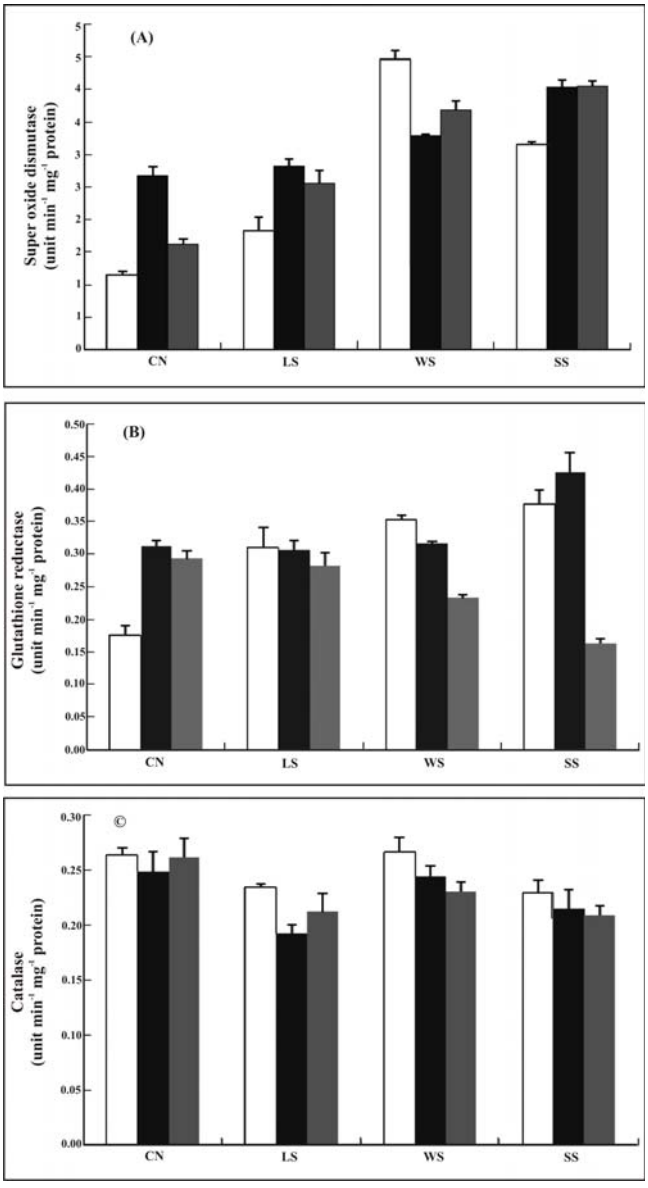

Fig. 1. Comparison on (A) Super oxide dismutase (SOD), (B) Glutathione reductase (GR) and (C) catalase (CAT) activity under different abiotic stress conditions between hybrid and parents. Each point represents the mean of 3 replication.
CN = Control, LS = Low Temperature Stress, WS = PEG Induced Water Stress, SS = Salt Stress

7.0), 0.5 ml of 75 mM H_2O_2, 0.2 ml crude enzyme extract and 0.8 ml water. Enzyme activity was calculated on the basis of the amount of H_2O_2 decomposed and the activity was expressed in units of decrease in absorbance per mg protein per minute. The statistical analysis was carried out by one way ANOVA.

In the present investigation antioxidant enzymes such as super oxide dismutase (SOD), catalase and glutathione reductase activities in female parent (IR 68897A) and male parent (DR 714-1-2R) and their corresponding hybrid (DRRH2) were studied. The SOD activity was significantly increased in all the three stresses and in all the three genotypes. The increase in SOD activity was higher in female parent under WS than the male parent and hybrid. The increase was highest under WS. Increase in SOD activity under various stresses was largely attributed to the membrane damage (Foyer and Noctor 2003). The over expression of SOD if accompanied by other H_2O_2 scavenging enzymes like CAT, GR and POD enzyme activity has been considered as an important anti-drought mechanism to cope with oxidative stress during water deficit condition (McKersie *et al.* 1999).

Glutathione reductase activity varied significantly in the genotypes and also varied with the type of stress imposed. Though the hybrid did not any show significant change in GR activity, parents responded independently *viz*., male parent exhibited decrease and female parent exhibited increase in the enzyme activity under water stress and salt stresses. The average increase in GR activity across the genotypes was higher under cold stress (33%) than the other two stresses. Female parent responded similarly to the three stress situations with an increase in GR activity while the male parent exhibited varied response with the type of stress *viz.*, increase under cold stress while decrease water and salt stresses. Similar results in case of chlorophyll and proline content were reported in *Brassica* (Voleti *et al.* 1998).

The catalase activity was more in control conditions than the stresses in the hybrid and both the parents, while the female parent recorded higher activity than the male parent and hybrid. The CAT activity decreased in all the genotypes under three types of stresses, while the reduction was relatively higher in male parent (34%) followed by hybrid (31%). The reduction in enzyme activity was more or less similar in all three genotypes under low temperature and PEG induced water stress, respectively. The decline in catalase activity is regarded as general response to many stresses (Gunes *et al.* 2008, Liu *et al.* 2008). The reduction of CAT activity showed that catalase might be also a limiting factor in the antioxidative mechanism in rice (Nguyen *et al.* 2005). The hybrid DRRH-2 showed distinct heterosis under all the stresses for the activities of SOD and GR as indicated by their HOBP and HOMP values, while for CAT activity these heterosis values were non-significant (Table 1). Significantly positive HOBP and HOBP values were recorded for SOD activity under cold and salt treatments, while for GR activity positive heterosis values were recorded under cold stress only. The increased activity of SOD and GR under stress conditions as indicated by the HOBP and HOMP values shows the contribution of heterotic vigor to regulate the antioxidant defense system in order to cope with different stress conditions. Both HOMP and HOBP for catalase activity

Table 1. Comparison between hybrid and its parents on enzyme activities of SOD, GR and CAT

Treatment	HOBP (%)			HOMP (%)		
	SOD	GR	CAT	SOD	GR	CAT
Control	79.84	16.49	-8.32	99.64	39.61	-1.91
Low temperature	14.49*	2.58*	-9.88	33.23*	7.67*	-6.05
Water	-26.03*	-4.91*	0.42	-17.09*	15.17*	4.40
Salt	3.81*	-0.12*	-12.26	7.88	45.45	-2.98

*Significant difference between hybrid and better parent, the mean of both parents is significant at 95% probability level.
HOBP: Heterosis over the better of parents (BP) and calculated by (F1-BP)/BP x 100%
HOMP: Heterosis over the mean of parents (MP) and calculated by (F1-MP)/MP x 100%

were negative under all the stresses except under WS treatment, where positive heterosis was recorded. Previous studies on hybrids indicated the high initial growth vigor and high metabolic activity in the hybrids (Jing *et al.* 2006). The heterotic vigor for the antioxidant enzymes (SOD, GR) in hybrid observed in this study could form a biochemical basis towards understanding the heterosis phenomena for stress tolerance.

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