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Research Article

Effect of Chilling stress on Scavenging Enzymes Activity in Boro Rice (*Oryza sativa* L.) at Seedling Stage

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ABSTRACT

An experiment was conducted with six rice genotypes to study the scavenging enzymes activities in the rice seedlings grown under low temperature stress condition. Results showed that with the increase in duration of chilling and seedling age, peroxidase activity decreased by 14.33-25.55 percent, catalase activity decrease by 14.38- 30.11 percent and IAA oxidase activity decreased by 26.49-37.55 percent at 70 DAS(days after sowing) stage of various rice genotypes in respect of 40 DAS seedling. Relatively higher activity of catalase, peroxidase and IAA oxidase in tolerant genotypes provided defense against the free radical damage. On the other hand, catalase, peroxidase and IAA oxidase showed decreased activity in susceptible genotype and affected severely the integrity of membranous systems for metabolic functions.

Key words: *Boro rice, Cold stress, Catalase, IAA oxidase, Peroxidase.*

INTRODUCTION

Temperature is one of the vital factor for growth and development of plants. Beyond a certain limit of temperature, all the physiological and biochemical processes of plant may be directly or indirectly affected. A key adaptive mechanism in many plants grown under abiotic stresses, including salinity, water and temperatures, is accumulation of certain organic compounds of low molecular mass, generally referred to as compatible osmolytes[12]. Accumulation of such solutes may contribute to enhanced stress tolerance of plants. The success of boro rice in low land areas taking advantages of the residual water in the field after harvest of kharif paddy, longer moisture retentivity of the soil and surface water stored in the nearby ditches have encouraged the farmers in eastern states to increase the boro rice area to supplement poor kharif harvest.

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On the other hand in plants, reactive oxygen species such as O_2 and H_2O_2 are produced during photosynthesis, photorespiration, flowering and other reactions of cellular metabolism[3]. Plant possesses a protective system composed of antioxidant such as peroxidase and catalase. Catalase is primary H_2O_2 scavenger in the peroxisomes and the mitochondria[1]. An increase in peroxidase activity has been reported as an early response to different stresses and may provide cells with resistance against formation of H_2O_2 which is formed when plants are exposed to stress factor. Also peroxidase is involved in a large number of biochemical and physiological processes and may change quantitatively and qualitatively during growth and development[15]. Indeed accumulation of H_2O_2 may cause change in plant metabolism[19]. H_2O_2 production appears to be the key factor in determining the rate of lignin biosynthesis in plants[7]. It has become clear that peroxidase plays a key role in biosynthesis of lignin due to its special catalytic properties for oxidizing cinnamyl alcohols[10] and anionic peroxidase isozyme involved in lignin biosynthesis[17]. Also activity of the different peroxidase isoenzyme depend on season, temperature and many types of stress parameters like flowering, leaf fall etc[2].

The objective of this study is to examine the impact of low temperature stress on scavenging enzymes activities during seedling growth stage in boro rice.

MATERIAL AND METHODS

Twenty six genotypes of rice (Table-1.) were subjected to preliminary screening under Boro season. Genotypes were selected on the basis of their differential ability to tolerate cold condition at seedling stage. Cold tolerance was determined on the basis of yellow, chlorosis of seedlings survival on 1-9 scale by simple eyes observation. For further study six genotypes viz V_1- Gautam, V_2-Richharia, V_3-Dhanlaxmi, from cold tolerant group and V_4 -Turanta, V_5 - Jaya and V_6 –Heera from cold susceptible group were selected. The experiment was conducted in rabi season and was repeated two years at Rajendra Agricultural University, Pusa campus. The maximum and minimum temperature during the experiment period ranged between 27.74-11.60 in 1st year and 23.53 to 11.88 in 2nd year. Sowing was done on 8th Nov. and the data was recorded at three periods of fifteen days intervali.e.T_1-40days after sowing (DAS), T_2-55DAS, T_3-70DAS.Analysis of enzymes viz. peroxidase, catalase and IAA oxidase were done in fresh plant samples. Enzymes extraction was done by grinding the samples in a chilled mortar using 0.5M phosphate buffer (Ph6.8). The slurry was centrifuged at 10,000Xg for 20 minutes. All the operations, like extraction of samples, centrifugation and other extraction processes were conducted at 4^OC. The activity of peroxides was determined by the method of Palmiano and Juliano[9]. Assay of catalase was done by the method of Kar and Mishra[6]. The activity of IAA oxidase was determined by method described by Hare[4].

Table1. Planting material of rice used and their cold tolerance(CT) score:

Name of Genotypes	**Cold tolerance score**
RAU441-65-88-1	5.00
RAU448-46-47-1	4.33
RAU520-34-8	5.00
RAU1344-4-1	6.67
RAU494-624	6.67
RAU1346-4-1	5.00
RAU1344-3-2(Dhanlaxmi)	4.00
PSBR-150	7.00
IR59471-213-20-2-1	7.67
IR55275-8-8-1-1-1-3	4.67
IR56383-77-1-1-1	5.33

IR53970-100-3-3-2	7.00
RAU8772	7.33
PUSA835-203-2-13-102	6.67
CN925-KGR-88-2-52	4.33
RAU1345-2(Richharia)	4.67
RAU461-55-1	4.00
RAU1345-12-1	5.33
PSRM1-15-3b-13	7.67
CN881-5-1-2	8.00
PSRM16-4B-11(Gautam)	3.00
Heera	8.67
IR25374-3-7-3-3-3	7.67
Turanta	8.00
Pusa2-21	7.00
Jaya	7.67
S.Em± C.D.(P=0.05)	0.585 1.662

RESULTS AND DISCUSSION

The results showed that with the increase in duration of chilling and seedling age, peroxidase activity decreased by 14.33-25.55 percent, catalase and IAA oxidase activity decreased by 14.38- 30.11 percent and 26.49-37.55 percent at 70 DAS (days after sowing) stage of various rice genotypes in respect of 40 DAS seedling (Table-2& Fig.-1). In consonance with these findings, the estimation of relative activity of peroxidase and catalase determined in rice seedlings during the cold period, revealed the decline in the activity of enzymes with the duration of stress. Such a trend is indicative of a severe disruption in the equilibrium between oxidative and anti-oxidative metabolism. Maruyama *et al*., observed specific inhibition of catalase during growth at low temperature and has suggested that the synthesis of intracellular components in particular of key proteins required for photosynthesis, is specifically susceptible to low temperature stress during development of rice seedling. The mean range of peroxidase activity 842.41-772.69 (units/mg protein) in tolerant genotypes and 736.44-631.75(units/mg protein) in susceptible genotypes. However, among the genotypes studied, the decrease of peroxidase activity of tolerant group (V_1,V_2 & V_3) by 3.97-9.88 percent during T_1-T_2 period and by 10.78-16.81 percent during T_1-T_3 period depicted relatively low decline in activity with seedling age. The decrease in susceptible genotypes (V_4,V_5 & V_6) was faster, ranging between 8.67-34.44 percent and 27.09-44.89percent, respectively (Table-2 & Fig-1). The analysis of data indicated that V_1 was significantly superior over the mean value while other genotypes were statistically inferior except V_2 which was statistically at par. Among the genotypes, the decrease in catalase activity of tolerant genotypes by 2.62-5.66 percent during T_1-T_2 and 6.14-16.45 percent during T_1-T_3 period was observed. The decrease in susceptible genotypes was relatively more ranging between 23.52-27.26 percent and 31.83 – 63.40 percent, respectively. The data further revealed that interaction of genotypes and the sampling stage of seedlings were highly significant in affecting the catalase activity of seedlings . The IAA oxidase activity during T_1-T_3 period, on an average, depicted a consistent decrease from 98.40-61.48 units/mg protein (Table-2 & Fig.-1). However among the genotypes IAA oxidase activity during T_1-T_2 decreased by 17.65-23.59 per cent in V_1, V_2, and V_3 while the decrease in V_4,V_5 and V_6 was in the range of 33.35-38.12 per cent. The data further revealed that the mean value of V_3 showed maximum average of 86.85 units/mg protein while in V_1, V_2,V_5 and V_4 the values were relatively less being 85.39, 79.22,74.19 and 72.26 units/mg

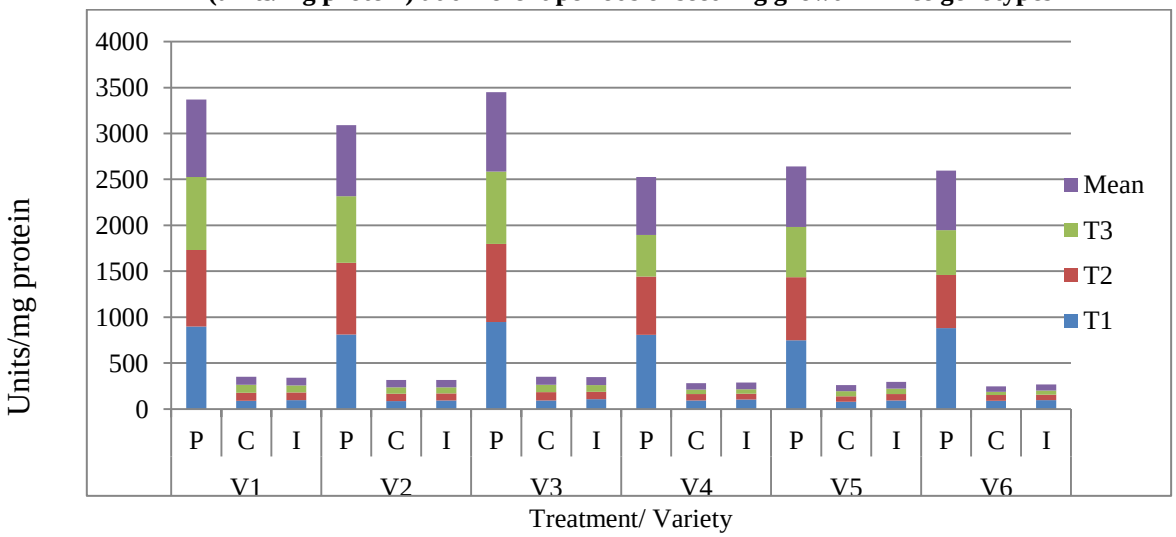
protein. IAA oxidase activity of 66.89 units/mg protein in V_6 was recorded to be minimum among the genotypes. Statistical data revealed that V_3 was significantly superior over the mean value whereas rest of the genotypes were statistically at par except V_6 which was statistically inferior. The interaction effect of the genotypes and their sampling time were highly significant in altering the IAA oxidase activity. Enzymes catalyse the reactions involving change in carbohydrates, protein, amino acids and other organic compounds. The alteration in the metabolites status, in comparison to those of control plants, is a reflection of the sensitivity of enzymes and enzyme activity to the stress.

Table 2. Effect of low temperature on specific activity of peroxidase(P) , catalase C and IAAoxidase(I) (units/mg protein) at different periods of seedling growth in rice genotypes

Variety/Treatment	V_1			V_2			V_3			V_4			V_5			V_6		
	P	C	I	P	C	I	P	C	I	P	C	I	P	C	I	P	C	I
T_1	897	90.3	98.5	813	85.6	92.9	946	94.2	107	808	92	103	750	79.9	93.5	882	89.1	95.8
T_2	836	88	80.9	780	80.8	76.5	853	90.6	82	633	70.4	63.9	685	60.2	71.6	579	64.8	59.3
	-6.84	-2.62	-17.8	-3.97	-5.66	-17.65	-9.88	-3.79	-23.59	-21.67	-23.5	-37.79	-8.67	-24.61	-23.35	-34.44	-27.26	-38.12
T_3	795	84.8	76.7	725	71.5	68.3	787	79.7	70.5	454	48.2	50.2	547	54.5	57.5	486	32.6	45.6
	-11.42	-6.14	-22.16	-10.78	-16.45	-26.43	-16.81	-15.39	-34.30	-43.76	-47.67	-51.12	-27.08	-31.83	-38.50	-44.89	-63.41	-52.34
Mean	842	87.7	85.4	773	79.3	79.2	862	88.2	86.7	632	70.2	72.3	661	64.9	74.2	649	62.2	66.9
	V			T			V X T											
S. Em.(+)	31.790	1.744.92	2.998.49	22.463.6	1.223.48	2.116.00	54.8156	3.008.53	5.18	V_1,V_2 & V_3-Tolerant Group V_4,V_5 & V_6-Susceptable Group								
CD(P=0.05)									14.7									

Figures in parentheses indicate percent decrease (-) over T_1

Fig. 1. Effect of low temperature on specific activity of peroxoidase (P), catalase (C) and IAA oxidase (I) (units/mg protein) at different periods of seedling growth in rice genotypes



More activities of antioxidant enzymes in stress condition compared with of normal condition has been reported by Janda *et al*.[5], and Sagisaka[11] that were due to chilling effect on levels of active oxygen species[8] and role of catalase and other antioxidant enzymes in alleviating chilling – induced oxidative stress[1]. More number of cathodic peroxidase isozyme bands in high and low temperature could be related as a protective against frost. Peroxidase

and catalase activity decreased simultaneous with increase of air temperature[14]. The result of the present study, however, indicated that the activity of antioxidative enzymes were severely affected during cold. A decrease in the activity of peroxidase on exposure to chilling temperature has been reported by several investigators[18]. Similarly, a decline in the activity of catalase under low temperature exposure of plants has also been recorded[16]. Under the conditions of loss of activity of antioxidant enzymes, the ability to scavenge oxygen free radicals would be weakened and the free radical levels would increase promoting membrane lipid peroxidation and polymerization of membrane protein resulting in injury of the seedlings.

Exposure to low temperature depicted drastic effect on scavenging enzymes. Peroxidase, catalase and IAA oxidase activity of seedlings at different growth stages showed a gradual decrease in activity with the increase in duration of chilling. Peroxidase, catalase and IAA oxidase activity remained high in Gautam, Richharia and Dhanlaxmi and low in Turanta, Jaya and Heera, on the other hand, showed decreased activity affecting severely the integrity of membranous systems for metabolic functions.

REFERENCES

1. Anderson, D., Prasad, K. and Stewart, R., Changes in isozyme Profiles of catalase, peroxidase and Glutathione reductase during acclimation to chilling in mesocotyls of maize seedlings. *Plant physiology*, **109:** 1247-1257 (1995).
2. Ebermann, R., Stich, K. and Korori, S.A.A., Wood peroxidase activity dependence on environmental conditions. Conference on Peroxidase Biotechnology and Application, Moscow (1995).
3. Foyer, C., and Harbinson, J., Oxygen metabolism and regulation of photosynthetic electron transport. In: C. Foyer and P. Mullineaux, eds.,Photooxidative stresses in plants: causes and amelioration.P.2-42, CRC Press, Inc., Boca Raton. (1994).
4. Hare, R. C., IAA oxidase, *Bot. Rev.*, **30:** 129–165 (1964).
5. Janda, T., Szalai, G., Rios-Gonzalez, K., Veisz, O. and Paldi, E., Correlation between frost tolerance and antioxidative activities in cereals. *Acta Biologica Szege diensis*, **46(3/4):** 67-69 (2002).
6. Kar, M. and Mishra, D., Catalase, Peroxidase and polyphenol oxidase activities during rice leaf senescence. *Plant Physiol*., **57:** 315-319 (1976).
7. Musel, G., Schindler, T., Bergfeld, R., Ruel, K., Jacquet, G., Lapierre, C., Speth, V. and Schopfer, P., Structure and distribution of lignin in primary and secondary cell walls of maize coleoptiles analysed by chemical and immunological probes. *Planta* **201:** 146-159 (1997).
8. Omran, R.G., Peroxidase levels and activities of Catalase, Peroxidase and Indoleacetic acid oxidase during and after chilling cucumber seedlings. *Plant Physiol.*, **65:** 407–408 (1980).
9. Palmiano, E. P. and Juliano, B.D., Changes in the activity of some hydrolase, peroxidases and catalase in rice seed during germination. *Plant Physiol.*, **52:** 274-277 (1973).
10. Ros Barcelo, A., Morales, M. and Pedreno, M.A., Specific compartmentalization of peroxidase isozymes in relation to lignin biosynthesis in plant cell. In: Lewis NG, Sarkanen S, eds, Lignin and lignin Biosynthesis: *American Chemical Society, Washington.* **697:** 84-95 (1998).
11. Sagisaka, S., Injuries of cold acclimated poplar twigs resulting from enzyme inactivation and substrate depression during frozen storage at ambient temperatures for a long period. *Plant Cell Physiology* **26:** 1135-1145 (1985).
12. Sakamoto, A. and Murata, N., The role of glycine betaine in the protection of plants from stress: clues from transgenic plant . *Plant Cell Environn.*, **25:** 167-171 (2002).

13. Sarugama, H. and Tanida, M., Effect of chilling on activated oxygen Scavenging enzymes in low temperature sensitive and tolerant cultivars of rice (*Oryza sativa* L.*) Plant Science Limerick*. **109(2):** 105-113 (1995).
14. Sennerby, F. L., and Fircks, H.A., Ultra structure of cells in the cambial region during winter hardening and spring hardening in the Salix dasyclados Wim. Grown at two nutrient levels. *Tree structure and Function* **1(3):** 151-163 (1987).
15. Shannon, L. M., Plant isozymes. *Annaual reviewof biochemistry* **38:** 189-210 (1969).
16. Tang, Risheng, S., Huang, B. and Tong, H.Y., Regulation of rice seedling growth by seedling vigorous agent and its mechanism. *Jiangsu J. of Agri. Sci.*, **15(2):** 77-81 (1999).
17. Tsuji, Y., Kawai, S., Katayama, Y., Morohoshi, N., Yamada, K. and Ebinuma, H., Formation and characterization of transformed woody plants inhibited lignin biosynthesis. Conference on *Forest Biotechnology*. (1995).
18. Wang, H., Jian, L.C. and Zhang, J.R., Changes of the level of Ca^{2+} in cell of rice seedlings under low temperature stress. *Acta Botanica Sinica*, **36(8):** 587-591 (1994).
19. Zhi, Y., Xian, L.I., Ling, L.I., Xing, H. and Tianli, Y., Effects of temperature and several chemicals on metabolic changes during dormancy release in NJ72, *Nectaria. A Sgriculture science in China.* **2(5):** 549-555 (2003).