

Original Research Article

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Evaluation of Rice Genotypes for Chlorophyll Content and Scavenging Enzyme Activity under the Influence of Mannitol Stress towards Drought Tolerance

S. Vanisri¹, M. Sreedhar^{2*}, L. Jeevan¹, A. Pavani¹, Anurag Chaturvedi²,
M. Aparna², D. Pavan Kumar², T. Sunitha¹, K. Aruna¹, V.G.N. Tripura Venkata¹,
Ch. Surender Raju³ and R. Jagadeeswar³

¹Institute of Biotechnology, Prof. Jayashankar Telangana State Agricultural University,
Rajendranagar, Hyderabad – 500030, Telangana, India

²Quality Control Laboratory, Prof. Jayashankar Telangana State Agricultural University,
Rajendranagar, Hyderabad – 500030, Telangana, India

³Rice Section, Agriculture Research Institute, Prof. Jayashankar Telangana State Agricultural
University, Rajendranagar, Hyderabad – 500030, Telangana, India

**Corresponding author*

ABSTRACT

In an effort to determine the biochemical markers for identifying genotypes for drought tolerance, changes in chlorophyll content and activities of scavenging enzymes were determined in the seedlings of 20 rice genotypes, each with different genetic background. Water deficit conditions were induced by treating with mannitol. The 14th day seedlings of 20 rice genotypes were selected for the stress treatment with mannitol at 20 per cent concentration which was added to the supporting medium. Control with cultivar Vandana was taken by using the same micro nutrient medium Yoshida. The estimation of chlorophyll content and activity of scavenging enzymes in the leaf tissue was carried out after 24 hrs exposure to stress. The results of the present study revealed significant increase in the activity of SOD and POD in rice genotypes exposed to water limiting situation. These findings indicated increased antioxidant activity in correspondence with raised levels of free radicals. The observed increase of SOD and POD of the antioxidant system indicated that increase in oxidative stress caused by drought might have been overwhelmed by this enzymatic system. The leaf SOD activity of Rajendra (34.39), Vandana, IR 64(31.48), Anjali (30.38) and Varalu (30.23) at 20 per cent mannitol was higher than that of check Vandana at no mannitol stress. Genotype Rajendra was superior to check Vandana at 20% mannitol stress. The results have clearly indicated the free radical scavenging ability of these varieties under the influence of drought stress by correspondingly enhancing the production levels of SOD. The POD activity in Vijetha (138.60), MTU 1010 (133.40), Vandana (136.50), BPT 5204 (127.77), IR 64 (112.90), Prasanna (114.30), B 133 (114.80) and Azucena (102.50) was found to be desirable and had reflected an increased ROS scavenging capacity of these genotype. Significant reduction in leaf chlorophyll content was also observed under mannitol stress. The study indicated that chlorophyll b was found to be more sensitive than chlorophyll a for water stress conditions. The data showed that status of scavenging enzymes and chlorophyll could provide a meaningful tool for depicting drought tolerance of a rice genotype.

Keywords

Scavenging enzymes,
Chlorophyll, Rice,
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Introduction

Even though, rice crop provides food and nutritional security for millions in India, the pressure exerted of late by environment had jeopardized its progress in terms of productivity. Irrigation water had been the singular determining factor in modern rice cultivation, in view of its scarce availability in almost all the rice growing regions of the world and India is no exception. Most of the assured rice ecosystems have started transforming in to fragile niches for its culture, putting in doubt the productive potential of our large acreage especially in rainfed and upland rice ecosystems. Dwindling ground water tables, late receipt of inflows in to reservoirs, early cessation of seasonal monsoons both in *kharif* and *rabi* have made the phenomenon of drought a regular occurrence in rice cultivation, which hitherto was not a production constraint of potential threat. Grassy growth nature of rice and its profuse tillering ability particularly in adverse conditions have accorded buffering capacity to the crop to circumvent the fluctuations in water availability. But the severity of water shortages in time and space has rendered the crop unable to perform optimally under such challenging conditions.

Generally, drought stress physically manifests when the available water in the soil is depleted and atmospheric conditions aid in continuous loss of water by transpiration and evaporation. Drought tolerance is observed in almost all plants but its extent varies from species to species and even within species as also observed in case of rice crop. In relative terms, drought stress is considered to be associated with moderate loss of water, which leads to stomatal closure and limitation of gas exchange. Desiccation involves higher magnitude of water loss, leading to gross derailment of metabolism and cell structure and eventually to the cessation of enzyme

catalyzed reactions. Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism and finally the death of plant.

Drought exposure usually leads to oxidative stress resulted from stomatal closure which causes the over-reduction of photosynthetic electron chain and formation of reactive oxygen species (ROS) in chloroplasts and mitochondria. Generally, the loss in the integrity and function of cellular membrane was believed to be directly correlated with the massive accumulation of ROS under drought stress. ROS including superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (HO^\cdot) and singlet oxygen (1O_2) could disrupt normal metabolism of plants through oxidative damages to important cellular components like lipids, proteins, nucleic acids, photosynthetic pigments (Chl *a*, *b*) and enzymes. It is believed that reactive oxygen species significantly increased the peroxidation of membrane lipids, which result in injury to large molecules such as proteins, nucleic acids, carbohydrates and lipids. Further, drought stress caused the imbalance in production and scavenging of oxygen free radicals within cells, which promote the membrane lipid peroxidation which could lead to the destruction of cellular integrity, cell dysfunction and ultimately affect the survival and reproduction of plants. In order to overcome oxidative stress, plants have developed enzymatic and non-enzymatic antioxidant defense mechanisms to scavenge ROS (Smirnoff, 1993). The most important antioxidant enzymes are superoxide dismutase (SOD) and peroxidase (POD). SOD converts O_2 into H_2O_2 and O_2 and POD scavenges H_2O_2 into H_2O .

With this back ground, the present study was executed involving certain rice cultivars possessing known attributes for combating the drought stress at various stages of crop

phenology from seedling to adult plant. The selected cultivars were subjected to water stress conditions simulated by mannitol treatment and the ability to counter the stress was estimated by assaying the scavenging enzyme activity for SOD and POD. Further, data was also generated to assess the variation in photosynthetic pigments Chl *a*, *b* during the period of drought. An attempt was made to understand the variability for these parameters in the selected rice cultivars.

Materials and Methods

Germination and seedling growth

Seed of 20 rice cultures were collected from sources as listed above. Seeds were surface sterilized with 0.5% Sodium hypochlorite solution for 5 min. thoroughly rinsed 7-8 times with distilled water and kept for germination in petri dishes under aseptic conditions in an incubator maintained at a relative humidity of 65% at 25° C for 48 hrs.

After 3rd day, seedlings were transferred into the micro nutrient Yoshida medium in plate/cups and thermo coal sheets were kept as support for the seedlings and seedlings were grown up to 14 days with natural sunlight at 30°C day/25°C night temperature and 80 per cent relative humidity at the Quality Control Laboratory, Professor Jayashankar Telangana State Agricultural University, Hyderabad during *kharif* 2014.

Mannitol treatment

14th day seedlings were selected for the stress treatment by using Mannitol at 20 per cent concentration which was added to the supporting medium. Control with cultivar Vandana was taken by using the same micro nutrient medium Yoshida. The estimation of chlorophyll content and activity of scavenging enzymes in the leaf tissue was carried out

after 24 hrs exposure to stress as described below.

Enzyme extraction and assay

Enzyme extraction for Superoxide Dismutase and Peroxidase was carried out by following the method of Costa. One gram of plant tissue collected from both mannitol treated and control samples were homogenized with extraction buffer containing 50 mM phosphate buffer (pH 7.4) and 1 mM EDTA.

Superoxide Dismutase (SOD) activity

The assay is based on formation of blue colour formazone by nitro-blue tetrazolium and O₂⁻ radical, which absorbs at 560nm, and the enzyme (SOD) decreases this absorbance due to reduction in the formation of O₂⁻ radical by the enzyme (Dhindsa *et al.*, 1981). Superoxide dismutase activity was estimated by recording the decreases in optical density of formazone made by superoxide radical and nitro- blue tetrazolium dye by the enzyme (Dhindsa *et al.*, 1981).

The reaction mixture (3 ml) contained 13.33mM Methionine (0.2ml of 200mM), 75µM Nitroblue tetrazolium chloride (NBT) (0.1 ml of 2.25mM), 0.1mM EDTA (0.1 ml of 3mM), 50mM phosphate buffer (pH 7.8)(1.5ml of 100 mM), 50mM Sodium Carbonate (0.1ml of 1.5M), 0.05 to 0.1 ml enzyme, 0.9 to 0.95 ml of water (to make a final volume of 3.0ml). Reaction was started by adding 2µM riboflavin (0.1ml) and placing the tubes under two 15 W fluorescent lamps for 15 min.

A complete reaction mixture without enzyme served as control. Then the reaction mixture was kept under light for 15 min and again kept under dark to stop the reaction. The absorbance was recorded at 560nm, and one unit of enzyme activity was taken as that

amount of enzyme, which reduced the absorbance reading to 50% in comparison with tubes lacking enzyme.

$$1 \text{ Unit (of enzyme)} = \frac{\text{Control - Test}}{\text{Control} / 2}$$

Peroxidase (POD) activity

Peroxidase activity is assayed as increase in optical density due to the oxidation of guaiacol to tetra-guaiacol (Castillo *et al.*, 1984).

The reaction mixture (3 ml) contained 1 ml of Phosphate buffer (50 mM. pH 6.1), 0.9 ml of distilled water, 0.1 ml of Enzyme extract, 0.5 ml of Guaiacol (16mM) and 0.5 ml of Hydrogen peroxide (2 mM). Absorbance due to the formation of tetra -guaiacol was recorded at 470 nm and enzyme activity was calculated as per extinction coefficient of its oxidation product, tetra-guaiacol. Enzyme activity is expressed as μM tetra-guaiacol formed per min g fresh weight or per mg protein.

$$\text{Enzyme extract units/ litre} = \frac{3.18 \times 0.1 \times 1000}{6.39 \times 1 \times \Delta t \times 0.1}$$

Chlorophyll content

Chlorophyll content of pot cultured plantlets was determined Lichtenthaler (1987). Leaf tissue was added to a pre chilled mortar in an ice bath and were ground with pestle in calcium carbonate (CaCO_3) (Spectrum, CA) at a ratio of 1g of leaf tissue to 2g of CaCO_3 together with 10ml of 80% (v/v) acetone. The sample extracts were filtered using Whatman no. 1 filter paper followed by washing with 80% (v/v) acetone. The extraction volume was made up to 50ml with 80% (v/v) acetone. Sample extracts were subjected to UV-VIS

spectrophotometric determination (CECIL Aquarius CE 7200, Double Beam Spectrophotometer) of chlorophyll at 646nm and 663nm. The chlorophyll *a* (*Ca*) and chlorophyll *b* (*Cb*) content in milligram per litre was determined according to the formulae given below and further expressed in milligram per gram of fresh weight of plant material.

Chlorophyll *a*,

$$Ca = 12.25(\text{OD } 663) - 2.79(\text{OD } 646)$$

Chlorophyll *b*,

$$Cb = 21.50(\text{OD } 646) - 5.10(\text{OD } 663)$$

Total chlorophyll,

$$C a + b = 7.15(\text{OD } 663) + 18.71(\text{OD } 646)$$

Results and Discussion

Mannitol, a member of sugar alcohols, is an osmotic adjustment chemical to control osmotic potential in the culture media or nutrient solutions in order to induce water deficit conditions for the material to be screened against drought stress. Mannitol stress is a well known factor that affects antioxidant status and increases free oxygen radical generation in the treated environment. Mannitol stress was reported to induce overproduction of reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals and hydrogen peroxide, which react rapidly with almost all structural and functional organic molecules. To avoid oxidative damage, plants have evolved various protective mechanisms to counteract the effects of ROS at cellular and ultra cellular level. One of the protective mechanisms is the enzymatic system, which operates with sequential and simultaneous actions of a number of enzymes including

peroxidase and super oxide dismutase (Sreedhar *et al.*, 2013). Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers. Both the chlorophyll a and b are prone to soil drying. Drought stress changes in the content and ratio of chlorophyll 'a' and 'b' were observed. The functional state of photosynthesis has been considered an ideal physiological activity to monitor the health and vitality of plants (Clark *et al.*, 2000). In the present investigation also the genotypes differed significantly for chlorophyll a content (expressed in mg/g FW). All the genotypes were significantly superior when compared with experimental mean except Rasi. Significantly higher values were noticed in Tellahamsa, Satya, Vijetha, Tulasi, Anjali, Erramallelu, IR 64, B 133, Swarna, Varalu, Prasanna, Vandana and N22 at 20 per cent mannitol stress. Check variety Vandana recorded lower chlorophyll a content of 2.62 compared to Control (2.92) at no mannitol stress (Table 2, Fig. 1). Only one genotype N22 recorded higher chlorophyll a values compared to check Vandana though statistically not significant. The genetic and physiological attributes of N22 in countering drought situations are well documented and results of the present study also indicated the same. Among other genotypes that exhibited higher values of chlorophyll a IR 64, Varalu, Erramallelu, Satya were important in view of their proven ability to sustain long periods of field drought. These genotypes possess inherent ability to stay green and sustain the vegetative growth without much physiological disturbance during transition to reproductive phase in view of short duration and moderate tillering habit. Maintenance of higher levels functional photosynthetic pigments indicated the plant's capacity to tolerate stress created by water shortages. The chloroplast pigments, chlorophylls "a" and "b" play an important role in photochemical reactions. Earlier studies showed that drought

stress significantly reduced the leaf chlorophyll "a" and "b" content of drought sensitive as well as drought tolerant genotypes. This is in line with earlier reports in rice (Pattanagul, 2011), and wheat (Moaveni, 2011). The decrease of chlorophyll content under water limited conditions is reported to take place because of instability of protein complexes and its photo-oxidation under the influence of drought (Anjum *et al.*, 2011). Further, Ravi Ranjan Kumar *et al.*, (2011) reported that decrease of total chlorophyll with drought stress implies a lowered capacity for light harvesting. Since the production of reactive oxygen species is mainly driven by excess energy absorption in the photosynthetic apparatus, this might be avoided by degrading the absorbing pigments. Under drought stress, degradation of chlorophyll takes place due to the increased activity of chlorophyllase enzyme. Drought stress is a serious limiting factor to rice production and yield stability particularly in rain-fed rice areas. In rice the effect of drought varies with the variety, degree and duration of stress and its coincidence with different growth stages. Rice is more susceptible to drought because it is unable to regulate its transpirational water loss as effectively as other cereals. As a result drought rice rapidly becomes damaged by the effects of low tissue water potential. An inverse relationship usually occurs between the photosynthetic activity and *in vivo* chlorophyll fluorescence (Sikuku *et al.*, 2010).

Unlike chlorophyll a, it was clear that a progressive stress adversely affected chlorophyll b content. Earlier, Ashraf *et al.*, (1994) also reported that drought stress will reduce concentration of chlorophyll b more than chlorophyll a. In the present investigation also the mean chlorophyll b content was 1.52 compared to 1.92 of chlorophyll a indicating the profound

influence of drought on drastic reduction of this parameter. The results also revealed that chlorophyll *b* was more sensitive to drought compared to its counterpart. Similar to chlorophyll *a* all the genotypes differed significantly for chlorophyll *b* (expressed in mg/g FW) also. Among the genotypes evaluated, IR 64, Tellahamsa, B133, BPT 5204, Buddha, Erramalleu, Anjali, Prasanna, MTU 1010, Satya and Rasi were found to be superior than check entry Vandana (1.02) at 20 per cent mannitol stress. Lowest chlorophyll *b* value of 0.76 was recorded in respect of Tulasi.

However check Vandana at no stress recorded 2.09 chlorophyll *b*. Among these superior entries Rasi (4.33), Satya (3.20), MTU 1010 (3.06), Prasanna (3.05) (Table 2, Fig. 1) were the best registering highest values for chlorophyll *b*. Considering the levels of both photosynthetic pigments under the influence of drought Satya, IR 64 and Erramalleu were found to be superior in the context of sustaining water stress situations.

Drought stress had a significant effect on the content of chlorophyll *a* and *b*, and on the total of chlorophyll. A decrease of total chlorophyll with drought stress implies a lowered capacity for light harvesting. Since the production of reactive oxygen species is mainly driven by excess energy absorption in the photosynthetic apparatus, this might be avoided by degrading the absorbing pigments. Total Chlorophyll (expressed in mg/g FW) content was also affected during the present investigation which showed that long progressive stress along with some other environmental factors may affect photosynthetic ability of the plant system (Ravi Ranjan *et al.*, 2011). Hsu and Kao (2003) also demonstrated that PEG induced water stress caused decrease in total chlorophyll content in rice leaves. Erramalleu (5.23), MTU 1010 (5.49), Anjali (5.53), Satya (5.82), Prasanna (5.92) and Rasi (6.56) were found to be very effective with regard to total chlorophyll content and were found to be significantly superior to check Vandana (5.01).

Table.1 Details of rice genotypes used in the study

S.No.	Source of seed	No. of entries	Details of entries
1	Directorate of Rice Research, Rajendra Nagar.	6	Tulasi, Vandana, Anjali, Prasanna, Rasi, Naveen
2	Bangalore	3	Buddha, BI33, Dular
3	Regional Agricultural Research Station, Maruteru.	3	Vijetha, MTU-1010, Swarna
4	Rice Section, ARI, Rajendranagar, Hyderabad.	5	Satya, Rajendra, Tellahamsa, N22, Azucena, BPT-5204
5	International Rice Research Institute, Philippines.	1	IR-64
6	Regional Agricultural Research Station, Warangal.	2	Varalu, Erramallelu

Table.2 Mean performance of rice genotypes for chlorophyll and enzyme parameters under mannitol stress

S.No	Genotype	Chl a (mg/g FW)	Chl b (mg/g FW)	Total Chl (mg/g FW)	SOD (eu/100µl)	POD (eu/100µl)
1	TULASI	2.66	0.76	3.42	22.68	57.40
2	N22	2.94	1.42	4.36	8.77	33.20
3	ANJALI	2.70	2.83	5.53	30.38	87.10
4	SATYA	2.62	3.20	5.82	14.79	27.40
5	VARALU	2.85	1.63	4.48	30.23	67.60
6	RAJENDRA	2.57	1.86	4.43	34.39	63.80
7	PRASANNA	2.88	3.05	5.92	24.90	114.30
8	AZUCENA	2.59	1.34	3.93	24.60	102.50
9	B133	2.77	2.00	4.78	26.45	114.80
10	BUDDA	2.53	2.45	4.99	17.29	56.10
11	ERRAMALLELU	2.70	2.52	5.23	2.43	49.60
12	RASI	2.23	4.33	6.56	1.29	74.50
13	NAVEEN	2.45	1.46	3.90	9.53	53.10
14	VIJETHA	2.63	1.76	4.39	14.12	138.60
15	MTU-1010	2.43	3.06	5.49	13.68	133.40
16	SWARNA	2.83	1.44	4.27	0.50	51.10
17	TELLAHAMSA	2.62	1.93	4.54	15.05	96.60
18	IR64	2.74	1.91	4.65	31.48	112.90
19	BPT5204	2.50	2.04	4.54	2.67	127.77
20	VANDANA	2.62	1.02	3.63	32.05	136.50
21	VANDANA Control	2.92	2.09	5.01	31.83	114.80
	Experimental Mean	1.92	1.52	3.44	13.42	62.52
	SEm±	0.15	0.13	0.07	0.95	3.63
	CD (0.05)	0.43	0.38	0.20	2.76	10.58

Table.3 Analysis of variance for chlorophyll and enzyme parameters

Source of variation	D.F	Chl a	Chl b	Total Chl	SOD	POD
Treatments	20	0.09*	2.12**	1.87**	389.03**	3809.68**
Error	42	0.04	0.03	0.01	1.79	26.37

*Significant at 5 per cent level, **Significant at 1 per cent level

Fig.1 Chlorophyll content of rice genotypes under mannitol stress

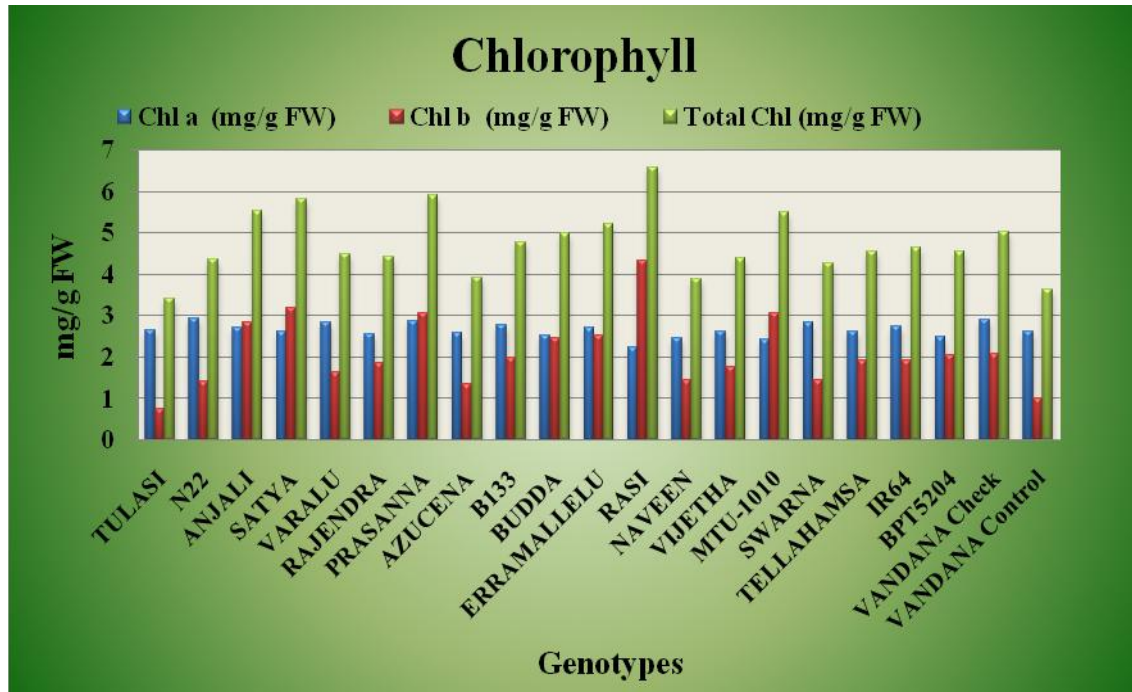
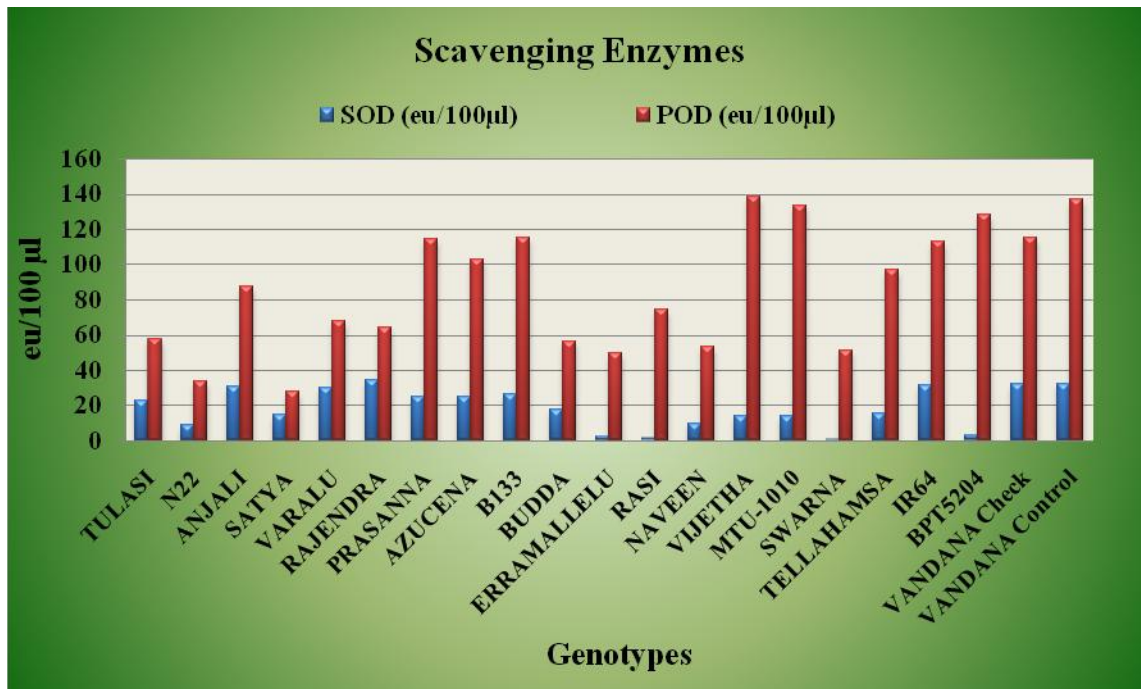


Fig.2 Scavenging enzyme activity in rice genotypes under mannitol stress



Apart from degradation of chlorophyll pigments, the prolongation of drought stress was accompanied with the increase of SOD

activity. The activity of SOD under drought stress levels was significantly higher than that of the control, which indicated the enhanced

activity of SOD and reducing the rate of membrane lipid peroxidation to resist the effects imposed by the external environment. Further, POD activity was also in the same trend as that of SOD under all levels of drought stress. The POD activity under all drought stress levels was significantly higher than that of the control group. During the long process of evolution, plants developed a series of anti-oxidation protective system to avoid injuries caused by the external environment. SOD and POD were vital constituents of this system and played major roles in maintaining normal physiological functions in the plants. Previously, Yong *et al.*, (2006) found that the rules of SOD, CAT and POX activity change are similar, which indicated that these three enzymes cooperated with each other during water deficits. Changes of protective enzymes was probably induced by the expression of certain protective genes in the early stage of drought stress so that the cellular protective enzymes were strengthened to defend the attacks of ROS to membrane lipids and protect the structure and function of cellular membrane system. This might be part of the self protection mechanisms in plants (Sun Cunhua *et al.*, 2010).

SOD converts superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2), POD reduces H_2O_2 to water using various substrates as electron donors. In order to determine the nature of the antioxidant responses of 20 rice varieties to drought stress during seedling stage, we measured the enzymatic activity of SOD, POD in seedlings of 20 cultivars treated with 20% mannitol. As shown in Figure 2 and Table 2, 20% mannitol treatment increased the activity of SOD in leaves of rice cultivars (expressed in eu/100 μ l). More precisely, the leaf SOD activity of Rajendra, Vandana, IR 64, Anjali and Varalu at 20 per cent mannitol was higher than that of check Vandana at no mannitol stress. The results have clearly indicated the free radical scavenging ability of these varieties under the influence of drought stress by correspondingly enhancing the production levels of SOD.

Drought tolerance or sensitivity in plants is well-correlated with inherent antioxidant responses. Tolerant plant species generally have a better capacity to protect themselves from drought induced oxidative stress, via the enhancement of antioxidant enzyme activity (Sekmen *et al.*, 2007). Further, it was reported by earlier researchers that under drought conditions, the activities of SOD, CAT and ascorbate peroxidase (APX, EC 1.11.1.11) increased to a greater extent, resulting in lower levels of lipid peroxidation and electrolyte leakage, in a drought-tolerant clone than in a drought-sensitive *Coffea canephora*. The drought-resistant *Phaseolus acutifolius* also revealed higher activities of SOD, CAT, POD and APX, and lower level of lipid peroxidation than the drought-susceptible *P. vulgaris* (Turkan *et al.*, 2005). Khanna-Chopra and Selote (2007) attributed lower membrane injury to the higher activities of POD and APX in a drought-tolerant wheat cultivar than in a drought sensitive cultivar under severe drought stress. In this study, significant differences were observed in the scavenging enzyme activity of rice genotypes under 20% mannitol stress conditions. The levels of antioxidant enzymes are higher in tolerant plants than in sensitive species under environmental stresses created experimentally. In the present study, a higher SOD activity observed in tolerant rice genotypes and suggested that the drought-tolerant rice cultivars possessed a better O_2^- -scavenging ability. These results were also in agreement with reports of increased SOD activity in drought-tolerant cultivars of sorghum (Jagtap and Bhargava, 1995).

Further, H_2O_2 , a toxic species, is a byproduct of the activity of SOD to prevent cellular damage, and must be eliminated by conversion to H_2O in subsequent reactions involving POD which regulate H_2O_2 levels in plants. Data from the present investigation showed that POD activity (expressed in eu/100 μ l) in leaves was higher under drought stresses. Furthermore, the elevated POD activity in Vijetha, MTU 1010, Vandana, BPT 5204, IR 64, Prasanna, B 133 and Azucena leaves treated with 20% mannitol

had reflected an increased ROS scavenging capacity and decreased damage to lipids of the plasma membrane under stress conditions (Fig. 2 and Table 2). PODs are involved not only in scavenging H₂O₂ but also in plant growth, development, lignification, suberization, and cross-linking of cell wall compounds (Passardi *et al.*, 2005). Accordingly, in the present study, drought tolerant genotypes often recorded higher POD activity than sensitive plants under stress conditions.

In this context, it is pertinent to mention that, exposure of plant cells to Mannitol stress leads to the formation of ROS and therefore, compounds that scavenge ROS may confer drought protect effects. SOD removed superoxide formed during drought exposure and also inhibits formation of more reactive pro oxidants. In the present study, this was evident due to significant increase in the activity of SOD exposed to water limiting situation. These findings indicated increased antioxidant activity. The observed increase of SOD of the antioxidant system indicated that increase in oxidative stress caused by drought might have been overwhelmed by this enzymatic system.

A perusal of varietal release, notification proposals and official cultivation recommendation of cultivars clearly indicated that varieties studied in the present investigation viz., Tulasi, Vandana, Anjali, Prasanna, Naveen, Varalu and Azucena thrived and performed well under water limiting upland rainfed situations. The results of the present investigation also confirmed the suitability of these varieties for rain fed areas through estimation of scavenging enzymes and chlorophyll content. Further, it could be concluded that estimation of changes in leaf chlorophyll content and scavenging enzyme activity could be successfully employed as biochemical markers in assessing the drought tolerance capacity of rice genotypes. Hence, these results can be successfully applied in screening and identification of drought tolerant genotypes in crop improvement programmes.

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