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Research Article

Effect of osmotic stress on root architecture and defensive system in wheat genotypes at seedling stage

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Abstract

The present investigation was conducted to assess the changes in root architecture and defensive system in contrasting genotypes under PEG induced osmotic stress and to determine the optimum osmotic stress level, which could differentiate the drought resistance among genotypes. Changes in physiological and biochemical characteristics were also evaluated. Seedlings were subjected to osmotic stress by supplementing root medium with three different concentrations of PEG 6000. Results highlighted statistically significant differences in most of the traits for treatment T4 (-1.00MPa) as critical for differentiating the drought tolerant and drought susceptible genotypes at seedling stage itself. Water status and chlorophyll content decreased in all the concentrations of PEG. Increase in the activities of superoxide dismutase and catalase enzyme was observed in drought tolerant genotypes C306 and PBW175 which also suggested their water stress tolerance behavior in comparison to other genotypes. This study provides evidence that the tolerant genotypes are well equipped with better physiological traits and good defensive system. This seedling assay could be used by wheat breeders in their breeding programmes to select drought tolerant wheat genotypes at an early stage.

Key words: *Triticum aestivum;* osmotic stress; antioxidant; WinRHIZO root analyzer.

1. Introduction

The yield of wheat, grown on large acreage in India, is adversely affected by various biotic and abiotic stresses including drought stress. It is recognized globally that, almost 50% of the wheat cultivated in the developing world (50 mha) is sown under rain-fed systems that receive less than 600mm of rain per annum and most of these are inhabited by the poorest farmers of the developing countries (CIMMYT Business Plan, 2006-10). Considerable attention has been given by plant breeders and biotechnologists to increase the crop productivity and to minimize losses caused by adverse environmental conditions. The responses of plants to water stress depend on plant species, plant age, phases of growth and development, level and duration of drought and physical parameters. These variations in the level of resistance to drought stress are known to exist amongst genotypes of plant species, e.g. in maize (Lorens *et al.*, 1987), wheat (Winter *et al.*, 1988).

Drought triggers a wide variety of plant responses from cellular level to phenotypic and yield level. Water deficit caused by drought effect the morphology of root and shoot, water status, chlorophyll content which are connected with the onset of protective mechanisms in plants (Jackson *et al.*, 1996). The first organ exposed to water stress is the root (Manske and Vlek 2002). The root system of a plant determines its ability to capture available water and nutrients, and therefore is critical for drought tolerance. So the root architecture modification under osmotic stress can be one of the important parameter of study. Root length, shoot length, root and shoot fresh weight all are reported to be decreased correspondingly with osmotic stress in some wheat varieties (Marcinska *et al.*, 2013). Drought stress also causes an increase in the level of reactive oxygen species (ROS) (Devi *et al.*, 2012).

Development of stress tolerant varieties is an objective of many breeding programs but success has been limited due to inadequate screening techniques and lack of clear phenotypic data induced by particular stress. Field screening for abiotic stresses, like drought, is difficult due to uncertain environmental changes, like rainfall, which do not show as much clear correlation between drought tolerance and different physiological changes, as observed in controlled lab conditions. PEG has been used in a number of studies to develop osmotic stress under laboratory condition (Sayar *et al*., 2010). The wheat genotypes i.e. C306, PBW175, NI5439 used in this study has shown to be relatively drought tolerant (DT) while two genotypes i.e., DBW17, PBW343 are taken as drought susceptible (DS) (Devi *et al*., 2012). The aim of this study is (i) to investigate the impact of polyethylene glycol induced osmotic stress on root growth and defensive system in five wheat cultivars differing in drought tolerance, (ii) to determine the optimum stress level, induced by PEG, to expose the differences in the physiological and biochemical parameters among genotypes for water stress and to find an easy technique for screening the genotypes, for drought tolerance in lab conditions.

2. Material and methods

2.1 Plant material: To study the effect of osmotic stress, seedlings of three drought tolerant (C306, PBW175 and NI5439), and two drought susceptible genotypes (PBW343 and DBW17) were examined in the experiment. Hoagland solution was used as a nutrient media. A poly ether compound, polyethylene glycol (PEG 6000) (Hi Media) was used to provide osmotic stress in lab.

2.2 Growth media preparation: Different levels of osmotic stress were induced in lab by using five PEG concentrations, with -0.3 MPa (T1), -0.50MPa (T2), -0.75 MPa (T3), -1.00MPa (T4) and -1.25 MPa (T5) of water potential along with control (without PEG), prepared by dissolving in Hoagland solution (pH-7.1). Hoagland solution was prepared by adding 1M KH_2PO_4, 1M KNO_3, 1M $Ca(NO_3)_2$, 1M $MgSO_4$, 1M $Fe(NO_3)_2$ stock macronutrient solutions and microelement stock solution (0.286% H_3BO_3, 0.022% $MnCl_2.7H_2O$, 0.001% $Na_2MoO_4.H_2O$) per liter of double distilled water (DDW).

2.3 Experimental design: Initially, the seeds were disinfected in 0.1% $HgCl_2$ (w/v) for three minutes and washed thrice in DDW, were placed in petri plates with filter paper moistened with DDW. Experiment was conducted in five replications of each stress level under sterile condition. Seven days old seedlings were put on muslin cloth wrapped above bottles containing different concentration of PEG with half strength Hoagland solution and maintained in a hydroponics culture in culture room for 12days. The roots were allowed to percolate into the solution. Bottles were kept in culture room under controlled temperature (22±2°C) and 60-70 % relative humidity. The time of transfer of bottles into culture room was defined as zero day of treatment and various parameters (morphology, physiological and biochemical) were analyzed at 4th, 8th and 12th day of treatment under different osmotic potentials (C, T1, T2, T3, T4, T5).

2.4 Growth measurements: Shoot length (SL) was measured directly by using a scale and root measurements were done through WinRHIZO software after scanning the roots to analyze root length (RL), root surface area (RSA) and root volume (RV). Root fresh weight (RFW) and shoot fresh weight (SFW) was recorded directly by weighing after removing surface water by blotting. Relative water content (RWC) was estimated by using RWC% = (Fresh Weight –Dry Weight)/(Turgid Weight – Dry Weight) x 100 where in TW was recorded by imbibing shoots individually in distilled water for 24 h (Barrs and Weatherley, 1962) and DW was recorded by placing the at 80 °C till constant weight was obtained. Chlorophyll content of leaves was measured by using chlorophyll meter (atLEAF$^+$, FT GREEN LLC; Wilmington, DE, 19801, USA).

2.5 Extraction and determination of Enzyme Activities: For spectrophotometric determination of enzyme activity and protein content estimation, 100 mg leaf sample was grinded in chilled pestle and mortar with extraction buffer (100 mM potassium phosphate buffer, pH 7.5) containing 0.5mM EDTA solution and centrifuged at 15,000rpm to collect the supernatant and stored at 4^{o}C. Super-oxidase dismutase activity (SOD) was determined by using the method SPCYO01 of Sigma (Mccord and Fridovich, 1969). Catalase enzyme activity (CAT) was calculated by using extinction coefficient of H_2O_2 (39.4 $mMcm^{-1}$) (Rao *et al*., 1996) and Peroxidase activity (POX) was calculated by using extinction coefficient 26.6 $mM^{-1}cm^{-1}$ (Jebara *et al*., 2005). Protein content was measured according to Bradford's method (Bradford, 1976) and Bovine serum albumin (BSA) was used for preparation of standard curve.

2.6 Statistical analysis: All experimental data were analyzed as mean ± standard error of five replicates. One way analysis of variance (ANOVA) was adopted to analyze the data(CropStat7.2). Differences among treatment and genotype means were assessed by comparison for all pairs using Tukey-Kramer HSD test at $p \leq 0.05$(JMP 9, SAS programme).

3. Results and discussion

3.1 Growth measurements: In this study, changes in different growth parameters i.e. root length (RL), root fresh weight (RFW), shoot fresh weight (SFW) have been observed during 0-12th day of the experiment and were compared in different concentration of PEG with control.

In all figures, error bars indicate SE (n = 6) and mean values followed by the same letter are not significantly different (p = 0.05)

3.2 RWC and chlorophyll content: With progressive increase in water potential, comparable decrease in RWC was recorded in all the selected genotypes from 4th to 12th day. Highest percentage reduction in RWC was recorded in drought susceptible genotypes DBW17 (60%) and PBW343 (58%), followed by drought tolerant genotypes C306 (43%) and PBW175 (38%) on 12th day, under treatment T4 (Fig.1a). This genotypic variation in RWC may be attributed to differences in the ability of the variation to absorb more water from the solution or the ability to control water loss through the stomata (Marcinska *et al.*, 2013).Highest percentage reduction (73%) was observed in genotype DBW17 (13) at maximum osmotic stress (T4) on 12thday while PBW175 showed least reduction in chlorophyll content (41%) (Fig.1b). This differential rate of decrease in chlorophyll content across the genotypes showed the presence of osmotic stress and genetic variability for chlorophyll retention which could be due to impaired chlorophyll synthesis or membrane injury caused by production of reactive oxygen species (ROS) under water stress in susceptible genotypes (PBW343 and DBW17) (Huseynova, 2012).

3.3 Shoot and Root fresh weight: In this study, the effect of osmotic treatment and genotype was found highly significant for shoot fresh weight (SFW) but not for the interaction between genotype and the osmotic treatment (Table 1). SFW was progressively reduced in all the five genotypes from 4th to 12th day with increasing PEG concentration. In control conditions, highest SFW was observed for genotype C306 (0.531gm), while the lowest was recorded in genotype PBW343 (0.375gm). Under osmotic stress, genotype DBW17 (79%) showed maximum reduction in SFW and lowest in PBW175

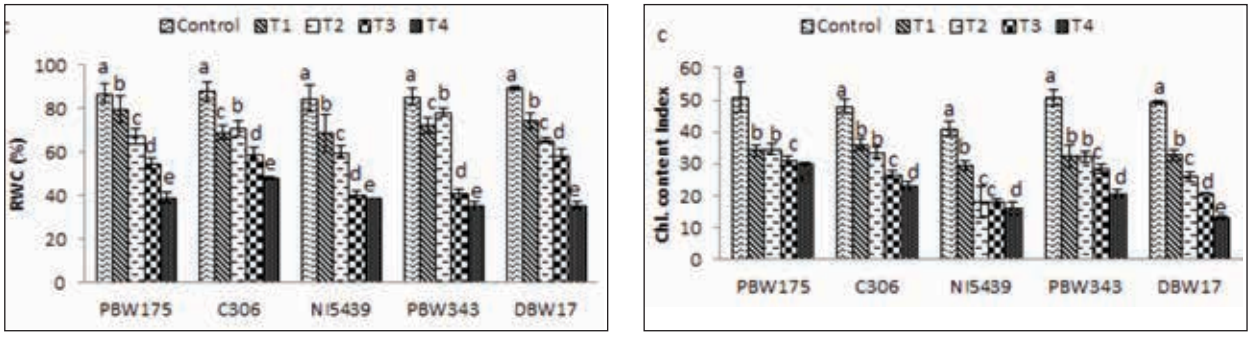

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Fig. 1 (a) Variations in relative water content (RWC) and (b) chlorophyll content index at 12th day at different levels of osmotic stress in selected genotypes.

(58%) on 12th day in T4 treatment (Fig. 2a). Water stress effect on plant growth can also be seen from seedling root fresh weight (RFW) (Rauf *et al.*, 2007). In the present investigation, significant difference has been found for RFW in all the selected genotypes and in four treatments (T1, T2, T3, T4) except T5 as plants did not survive on this concentration. On 12th day, 39% reduction in RFW was observed in PBW175 comparatively less to other genotypes C306 (51%) followed by PBW343 (67%) and DBW 17 (69%) under treatment T4 (Fig. 2b). Usually, drought resistant genotypes accumulate more biomass in leaves than susceptible ones (Kerepesi and Galiba, 2000). Presence of PEG reduces the mobilization of nutrients and decrease the ease with which the plant takes up water for

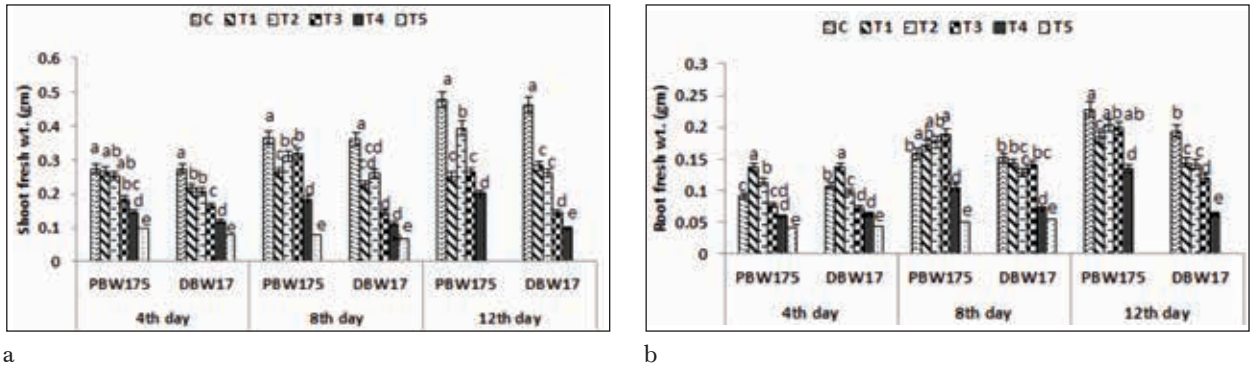

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Fig. 2 (a) Variation in shoot fresh weight (SFW) and (b) root fresh weight (RFW) at different levels of osmotic stress.

the activities of normal growth in plant. Decrease in root and shoot biomass has recently been reported in wheat seedlings treated with PEG (Sayar *et al.*, 2010) showing water stress effect on growth of plant.

3.4 Effect of osmotic stress on root system: Root length (RL) was highly influenced by osmotic stress and significant differences were noticed among four osmotic treatments (T1-T4) (Table 1)**.** **G**enotype PBW175 at treatment T4 showed minimum reduction in RL (39%) followed by C306 (49%), NI5439 (52%), PBW343 (53%) and DBW17 (61%) showed good water stress adaptability of PBW175 (Fig.3a). Individually, C306 has maximum RL in control as well as in all the treatments on 12th day but its percent reduction (52%) observed was comparatively more than PBW 175 (up to 39%)in all the treatments. At T5 treatment shoot as well as root length reduction was higher in all genotypes showing no significant difference in tolerant and susceptible genotypes. Root length was negatively related to osmotic potential. Similar findings have been reported by different researchers (Marcinska *et al.*, 2013; Rauf *et al.,* 2007) indicated negative effect of osmotic stress on wheat seedling growth. Various root characters such as root length, root thickness and root dry weight have been considered as screening tool for drought tolerance (Champoux *et al.*, 1995). The diversity between different plant varieties for root traits, is associated with their differences in stress resistance to drought stress (Manske and Vlek, 2002). Consequently, the genotypic variations for root traits could be consider as one of the selection criteria, to screen genotypes for drought tolerance (Babu, 2010). Under progressive increase in osmotic potential, in all the genotypes, a significant decline in root volume (RV) and root surface area (RSA) was recorded. RV and RSA are considered as useful traits for better growth and good resource uptake (Narayanan *et al.*, 2014). For RV, the effect of osmotic stress and genotype was found highly significant, along with the interaction between them (Table 1). RV reduced significantly under all the treatments (T2, T3, T4) for all the genotypes tested from 4th day to 12th day. In this study, percentage RV reduction was found maximum in DBW17 (89%) and minimum in PBW175

Table 1. Analysis of variance of seedling shoot traits: shoot fresh weight (SFW), shoot length (SL), relative water content (RWC) and chlorophyll content index and root traits: root length (RL), root volume (RV), root surface area (RSA) and root fresh weight (RFW) showing mean square values

Traits	Between genotypes	Between treatments	Interaction
SFW (gm)	2068.63**	815.61**	109.48
SL (cm)	148.24**	34.69**	1.10
RWC (%)	372.18**	695.11**	48.40*
Chl C Index	256.15	150.05**	27.74*
RL (cm)	2068.63**	815.601	109.48
RV(cm^3)	2249.16**	2563.81**	585.07
RSA (cm^2)	156.45**	48.30*	15.64
RFW (gm)	0.21**	0.85**	0.79

**Significant at the p≤0.05, **Significant at the p≤0.01*

(58%) on 12th day in treatment T4 (Fig. 3b). For RSA also, significant difference was observed for genotype and treatment but no difference was found between genotype and treatment interaction. On 12th day, none of the genotype showed significant difference for RSA under T1 and T4 treatment. Similar to RV, lowest percentage reduction in RSA was also observed in PBW175 (48%) followed by C306 (52%), NI5439 (58%), PBW343 (62%) and DBW17 (63%) in treatment T4 on 12th day (Fig.3c). High value of root traits (root length, root surface area and root volume) in drought tolerant cultivars (PBW175 and C306) indicates more ability in their water absorption and maintains turgor which is necessary for better growth under drought. The studies of genotypic variation in the root characteristics are of important parameter in breeding for improved wheat cultivars for water-limited environments. The results obtained from this study co-relate with the earlier publication wherein root length,

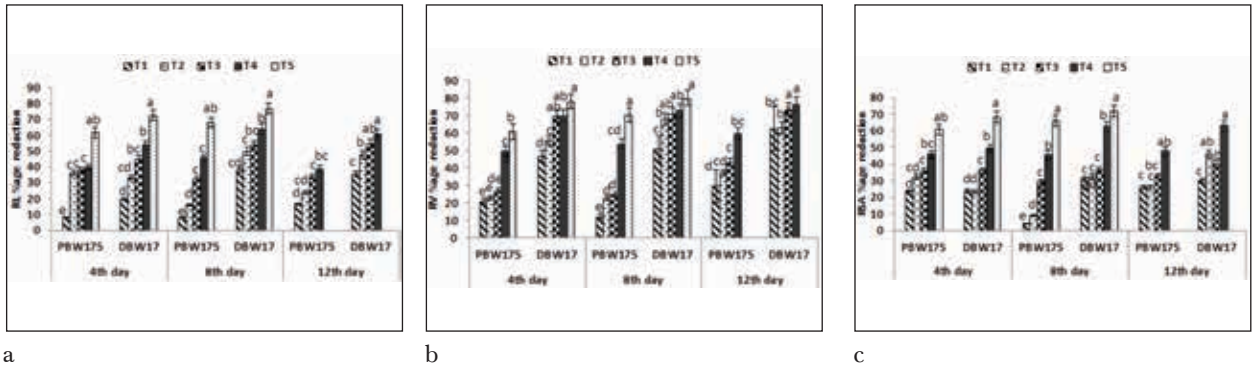

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Fig. 3 Percentage reduction in (a) root length (RL), (b) root volume (RV) and (c) root surface area (RSA) at different levels of osmotic stress (C, T1, T2, T3, T4, T5).

shoot length, root and shoot fresh weight decreases with progressive growth days under PEG in *Arabidopsis thaliana* as compared to controlled condition (Weele *et al.*, 2000).

3.5 Effect of osmotic stress on total protein and antioxidant enzyme activity: The total protein increased progressively in all the genotypes from 4th day till 12thday (Fig. 4). This increase was however more prominent in drought tolerant genotypes (PBW175 and C306) than drought susceptible genotypes (PBW343 and DBW17).The increase in protein may be due to the increased expression of protective proteins and the proteins/enzymes involved in combating the oxidative stress as a defense mechanism in response to drought conditions (Hameed *et al.*, 2011; Sheoran *et al.*, 2015). In case of NI5439, no significant differences were obtained in the total protein content on 8th and 12th day. In comparison to other treatments, the protein content was lowest after treatment T4. This may be because of expected protein denaturation under severe stress (Fazeli *et al.,* 2007). But this decrease in total protein affect the activity of antioxidant enzymes, as expressed more under drought stress. SOD activity increased significantly in genotype C306 from 4th to12th day in all the four treatments (T1-T4). On 8th day, except DBW17 a gradual increment in SOD activity was observed in all the genotypes up to T3 treatment and in T4 treatment the drought tolerant

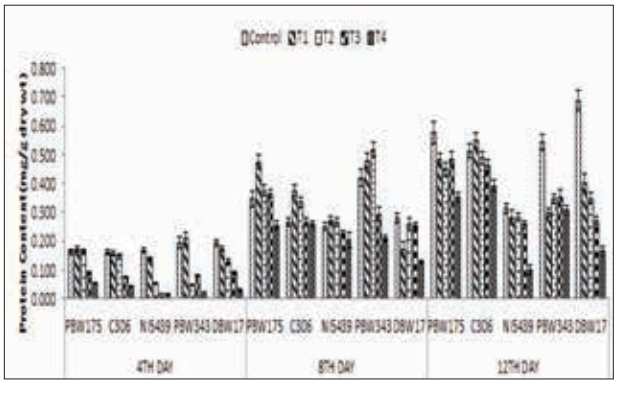


Fig. 4 Variations in protein content (mg g^{-1} fresh weight) at different levels of osmotic stress in selected genotypes.

genotypes and PBW343 showed still higher SOD activity than control. On 12th day except C306, all the genotypes showed a gradual decrease in the activity. Highest increase in SOD activity was observed in C306 (5743.13 units/mg protein) at treatment T4 followed by PBW175 (3776.387 units/mg protein) in T3 treatment (Fig.5a). Therefore, it is noteworthy that the tolerant genotypes may have the inherent capability to sense the stress conditions. In other reports also comparatively higher SOD activity has been reported in tolerant cultivars than the susceptible ones (Sairam *et al.*, 1998), suggesting that higher antioxidant enzymes activity has a role in imparting tolerance to these cultivars against environmental stresses.

POX and CAT are considered as the most important enzymes involved in the regulation of intracellular level of H_2O_2 (Sairam *et al.,* 1998). On 4th day, no significant increase in POX activity was observed in all the five genotypes under all the treatment except PBW175 and C306 which showed increment only under treatment T4. On 8th day, significant increase in POX activity was recorded in drought tolerant and drought susceptible genotypes under four treatments except PBW343. POX activity decreased in all the genotypes on 12th day subjected under the four treatments, except genotype C306 (Fig.5b). Higher POX activity under stress condition has been shown to be associated with higher water retention and subsequent stress tolerance (Hameed *et al.*, 2011). Catalase activity showed slight increase in only two genotypes DBW17 and C306 on 4th day. Except genotype NI5439, CAT activity significantly increased in all the genotypes up to treatment T2 on 8th day. The highest activity was recorded in genotype C306 (0.000902 µmol/min./mg protein) in treatment T4 on 8thday. Except C306, all the genotypes, showed decrease in the enzyme activity on 12th day (Fig.5c). These results showed more conversion of H_2O_2 into H_2O in all the drought tolerant and susceptible genotypes on 8th day up to T2 treatment. With further increase of the osmotic stress i.e. up to T4 treatment only two genotypes C306 and PBW175 showed

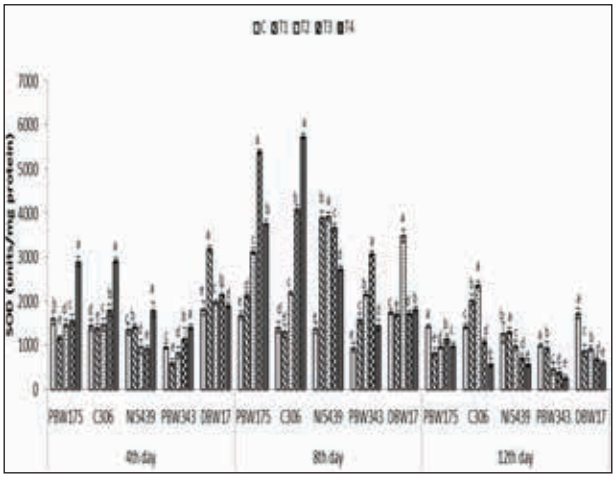
Table 2. Analysis of variance of various biochemical traits: Protein, Superoxide dismutase (SOD), Peroxidase (POX) and Catalase (CAT) showing mean square values

Source of Variation	Protein (mg g^{-1} f. wt.)	**SOD (units g^{-1}f. wt.)**	**POX (units g^{-1} f. wt.)**	**CAT (µMmin^{-1}mg protein^{-1})**
Genotype	0.150*	0.181*	0.444**	1.494**
Treatment	0.160**	701402	0.623	0.266
Interaction	0.412	385819	0.697	0.237

**Significant at the p≤0.05, **Significant at the p≤0.01*

increment in CAT activity. In wheat seedlings, increase in the catalase activity has been observed by other researchers also (Zhang and Kirkham, 1994). Again these results suggested that the tolerant genotypes can withstand the adverse conditions by increasing the activity of the antioxidant enzymes like CAT. Variability in increasing the activities of these antioxidants among these selected wheat genotypes showed their significant differential ability to acquire drought tolerance (Table 2).

The results of our study revealed that water stress was responsible for the induction of oxidative stress and therefore related damage as shown by decrease in RWC, chlorophyll content and enhanced oxidative responses



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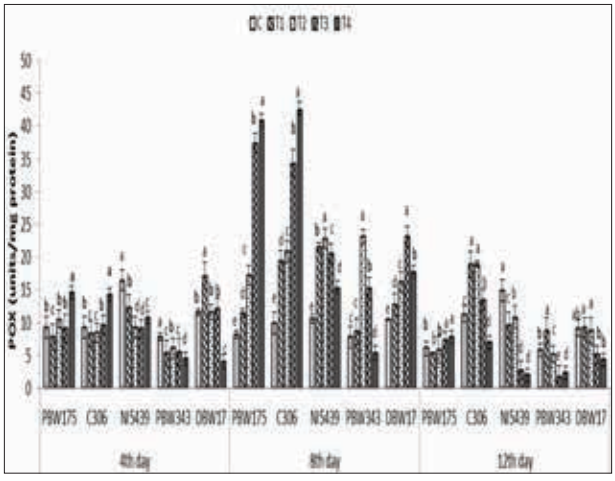

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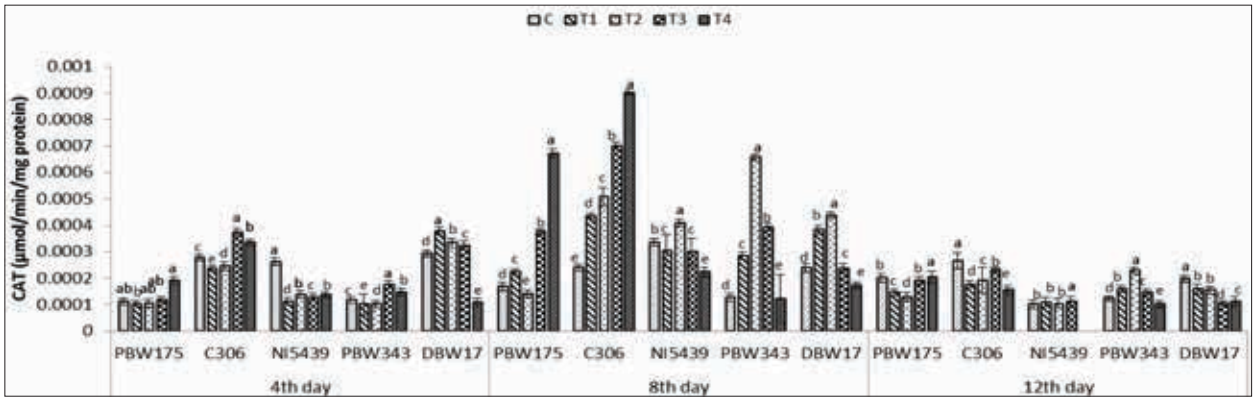

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Fig. 5 Activity of antioxidant enzymes (units g^{-1} fresh weight): (a) superoxide dismutase (SOD), (b) Peroxidase (POX) and (c) Catalase (CAT) ($\mu Mmin^{-1}mg\ protein^{-1}$) at different levels of osmotic stress in selected genotypes.

was evident by the differential response of antioxidative enzymes activities in drought tolerant and drought susceptible genotypes. Out of the five genotypes taken for the study, the three drought tolerant genotypes (C306, PBW175, NI5439) showed better root characters and a much more pronounced antioxidative mechanism even at the longer duration and increased severity of drought and hence, they seem to be protected from the determinantal effects caused by oxidative stress. The genotype PBW343 (high yielding, occupied vast area of NWPZ of India) showed high SOD activity and lower CAT and POX activity than control under T4 treatment showed that H_2O_2 scavenging systems as represented by POX and CAT are more important in imparting tolerance against drought induced oxidative stress than SOD alone. On the basis of statistical analysis, using ANOVA interaction, the variation among genotypes and stress treatment was identified for selected traits studied in this experiment (Table 1, 2). Significant difference between the genotypes (tolerant and susceptible) was clearly observed at the osmotic treatment T4 (Table 3). Therefore, this particular osmotic stress could be used in future research programs to identify the promising genotypes for drought tolerance.

Results showed that treatment T4 i.e. -1.00MPa of PEG was the optimum concentration which could be used to differentiate the drought tolerant and susceptible genotypes of wheat at seedling stage. The genotypes PBW175 and C306 showed maximum tolerance to osmotic stress having highest root growth along with high antioxidant enzyme activity among genotypes at seedling stage.

Table 3. Mean square values and significance levels of the measured traits: Chlorophyll content (Chl), Relative water content (RWC), shoot length (SL), root length (RL), root fresh weight (RFW) and shoot fresh weight (SFW).

Traits	Treatments	Between genotypes	Between days	Interaction
Chl content index	C	24.2*	26.0*	5.7
	T1	21.9	74.1	9.8
	T2	75.6**	158.6**	12.6
	T3	35.3	288.9***	14.6
	T4	68.3*	312.7***	16.6*
RWC%	C	22.5***	23.4**	1.4
	T1	46.3***	75.5***	2.8
	T2	47.6	107.5**	14.4
	T3	146.2	146.2**	146.2
	T4	170.0*	412.2*	61.8*
SL (cm)	C	29.8 ***	8.4**	0.6
	T1	22.1***	6.9***	0.4
	T2	34.3***	2.0***	0.1
	T3	44.9***	8.5**	0.6
	T4	46.4***	0.5	1.4
RL (cm)	C	467.1***	330.2***	22.7
	T1	75.5**	164.2***	8.0
	T2	10.5	12.3**	1.9
	T3	84.1*	199.2**	17.1
	T4	75.5**	164.2***	8.0
RV	C	9.4*	15.2**	1.5
	T1	0.2	3.4**	0.4
	T2	0.6	10.0***	0.6
	T3	0.2	10.1***	0.6
	T4	0.2	3.4***	0.4
RSA	C	45.2***	8.1*	2.5
	T1	0.3	6.3	2.4
	T2	10.5*	12.3**	1.9
	T3	0.9	4.1	4.2
	T4	0.29	6.3	2.4

*Significant at level p=0.05, ** Significant at level p=0.01, *** Significant at level p=0.001

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