The Bioscan 12(2): 691-696, 2017
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

STATUS OF H_2O_2 METABOLISING ENZYMES IN PIGEONPEA (*CAJANUS CAJAN* L.) GENOTYPES UNDER SALT STRESS

NORAH JOHAL[1], JAGMEET KAUR[2]*, SARVJEET SINGH[2], INDERJIT SINGH[2] AND SATVIR KAUR[3]
[1]Department of Botany, Punjab Agricultural University, Ludhiana - 141 004, INDIA
[2]Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana - 141 004, INDIA
[3]Department of Biochemistry, Punjab Agricultural University, Ludhiana - 141 004, INDIA
e-mail: jagskaur@pau.edu

KEYWORDS
Pigeonpea
Salt stress
H_2O_2 metabolising enzymes
Malonidialdehyde
Proline

Received on :
04.01.2016

Accepted on :
13.04.2017

***Corresponding author**

ABSTRACT
The present study was aimed at evaluating the eight pigeonpea genotypes on the basis of physiological and biochemical attributes subjected to varied salinity levels (20, 30 and 40mM NaCl). A significant decline in vigour index in eight days old seedlings was observed consistently among all the genotypes under all concentrations of NaCl. On the basis of physiological parameters (Vigour index) genotypes were cateogarized into tolerant (ICPL 20330, ICPL 88039 and AL 1873), sensitive (AL 1817, AL 1849, AL 1836) and moderately sensitive (AL 201 and PAU 881) ones. The antioxidative enzymatic activity was performed at 30mM and 40mM NaCl. The average fold increase in Peroxidase (POD) (2.8 fold) and Superoxide dismutase (SOD) (1.8 fold) activity was higher among tolerant genotypes as compared to sensitive and moderately sensitive ones. ICPL 20330, showed maximum POD activity (4.4 fold and 3.6 fold in root and shoot respectively) at 40mM NaCl reflecting the tolerance capacity as compared to other genotypes. AL 201 exhibited maximum H_2O_2 (5.6 fold) content in shoots and malonidialdehyde (MDA) (2.8 fold) content in roots at 40mM NaCl concentration which correlates it with the lower enzymatic antioxidative defense system. This study concludes that antioxidative defense system forms an array of network to thrive under salt stress.

INTRODUCTION

Soil salinity, a global increasing stress, has negatively affected irrigated land crop productivity. With the passage of time, more and more cultivable area is being dominated with salinity stress which refrain the normal plant species to survive under normal climate conditions resulting in lower yields (Maruthi *et al.,* 2010). Pulses are mostly very sensitive to stresses especially salinity. Among pulses Pigeonpea (*Cajanus cajan* L.), a diploid orphan perennial legume ($2n = 2x = 22$), of family Fabaceae, is the sixth most important food crop globally (Varshney *et al.,* 2012) and is the main target hit by salinity. Being important, very preliminary information on its screening for salt tolerance is available. Apart from pigeonpea, other pulses crops such as lentil, alfa alfa and mung bean have been screened initially and they showed considerable yield losses on exposure towards salinity (Waheed *et al.,* 2006). Generally, plants on being exposed to high concentration of saline ions *i.e.* Na^+, K^+, Cl^- produce reactive oxygen species (ROS). These ROS mainly include O_2^{2-}, H_2O_2, OH and other singlet oxygen species which hamper the plant growth lead to lipid peroxidation, protein oxidation and degradation. Plants in response to such stresses develop an antioxidant defense system which helps them to thrive under these conditions. This antioxidative system comprises of enzymes such as SOD, POX and CAT (Miller, 2010). In addition to such enzymes, certain compatible solutes such as proline (Jaiswal *et al.,* 2014) is also present in order to stabilize the osmotic pressure of the cell which is disturbed by the influx of Na^+ and Cl^- ions.

Increased resistance to salinity and other environmental stresses is often correlated with an efficient antioxidative system. Strong antioxidant defence system may act as a shield towards abiotic stresses particularly salinity and would reduce the crop losses to minimal. *In vitro* physiological screening of the pigeonpea genotypes at different salinity levels and to ascertain the role of antioxidative enzymes in assigning tolerance towards salinity was undertaken in this present study to underpin the quality genotypes with better future prospects in the forthcoming schema.

MATERIALS AND METHODS

Plant materials, treatments and physiological evaluation

For the study, eight pigeonpea genotypes [ICPL 20330, ICPL 88039, AL 1873, AL 1817, AL 1836, AL 1849, AL 201 and PAU 881] were procured from, Punjab Agricultural University, Ludhiana and ICRISAT, Hyderabad, India. Mature seeds of genotypes were surface sterilized with 0.1% $HgCl_2$ solution and rinsed three times with distilled water. Three salt concentrations *viz.* 20mM, 30mM and 40mM NaCl were undertaken and standard germination tests were performed in the plant growth chamber at constant conditions, temperature $25 \pm$ ºC, relative humidity 60-70% in dark for eight days. Fifteen seedlings from each treatment were randomly collected and used for conducting physiological responses *viz.*, per cent germination and seedling length elucidated in terms of vigour index. Random sampling of seedlings was

also conducted for analysing biochemical assays in roots and shoots.

Enzyme extraction and assays

POD and SOD were extracted from 0.1g of fresh tissue with 2ml 0.1M potassium phosphate buffer (pH 7.5) containing 1 mM ethylene diamine tetraacetic acid (EDTA), 1 % polyvinyl pyrrolidone (PVP) and 10 mM β mercaptoethanol whereas for CAT extraction, 0.1 g of fresh tissue was homogenised with 0.05 M sodium phosphate buffer (pH 7.5) containing 1 % PVP (Kaur *et al*., 2009). All enzyme extractions were conducted at 4ºC and enzyme assays were performed at 25ºC.

POD activity was determined by following the method of Shannon *et al*. (1966) with minor modifications. The extract (0.1 ml) was added to 3 ml of 0.05 M guaiacol prepared in 0.1 M phosphate buffer (pH 6.5) alongwith 0.1 ml of 0.8 M H_2O_2. The reaction was initiated by adding H_2O_2 and rate of change in absorbance was recorded at 470 nm for 3 min at an interval of 30s. The reaction mixture without H_2O_2 was taken as a blank. The activity was expressed as change in absorbance min^{-1} g^{-1} of FW.

SOD activity was assayed according to the method of Marklund and Marklund (1974), with slight modifications. For assay, 1.5 ml of 100 mM Tris HCl buffer (pH 8.2), 0.5 mL of 6 mM EDTA, 1 ml of 6 mM pyrogallol solution and 0.1 mL of enzyme extract was added in spectrophotometric cuvette. Absorbance was recorded at 420 nm in a spectrophotometer after an interval of 30 s up to 3 min. A unit of enzyme activity has been defined as the amount of enzyme causing 50 % inhibition of auto-oxidation of pyrogallol observed in blank. The activity was expressed as units min^{-1} g^{-1} FW.

CAT activity was measured by following the method of Chance and Maehly, (1955). In the spectrophotometric cuvette, 1.9 ml of 50 mM sodium phosphate buffer (pH 7.5) and 0.1 ml of enzyme extract was added. The reaction was initiated by adding 1mL of 39 mM H_2O_2 and utilization of H_2O_2 was recorded at intervals of 30 s for 3 min by measuring the decrease in absorbance at 240 nm. CAT activity was expressed as μmol of H_2O_2 decomposed min^{-1} g^{-1} FW. The extinction coefficient for H_2O_2 has the value of 0.0394 mM^{-1} cm^{-1}.

H_2O_2 content

H_2O_2 content was estimated by following the method of Noreen and Ashraf, (2009). Fresh tissue (0.1 g) was homogenised with 0.1 % (w/v) trichloroacetic acid (2 mL) in a pre-chilled pestle and mortar and the homogenate was centrifuged for 15 min at 12,000 x g. To 0.5 mL of supernatant, 0.5 mL of 10 mM phosphate buffer (pH 7.0) and 1 ml of 1 M potassium iodide were added. The amount of H_2O_2 was recorded at 390nm.

MDA content

The content of MDA was estimated by following the method of Ohkawa *et al*. (1979). Fresh tissue (0.2 g) was homogenised with 5% trichloroacetic acid (TCA) (2ml) and centrifuged at 10,000 x g for 15-20 minutes at 4ºC. The supernatant obtained (1 mL) was reacted with 1ml of TBA reagent. The mixture was heated for 30 minutes over a water bath at 95ºC and terminated in cold water bath. The samples were again centrifuged at 10,000 rpm for 10-15 minutes. The absorbance of supernatant was read at 532 nm and 600nm on UV 2600 spectrophotometer using reagent blank. The content was calculated using extinction coefficient of $155mM^{-1}cm^{-1}$.

Proline

Fresh tissue (0.2 g) was homogenised with 4ml sulphosalycylic (3%) acid. The homogenate was filtered with Whatman no. 1 filter paper and the filtrate obtained was used for estimating proline content (Bates *et al.,* 1973). The filtrate (2 ml) was added to 2 ml acidic ninhydrin and 2 ml of glacial acetic acid. The contents were heated at 100ºC for 1 hr in water bath. The reaction was terminated in ice bath for 10-15 minutes. The reaction mixture was extracted with toluene (4ml) using vortex mixture for 15-20 minutes and the chromophore containing upper layer was used for recording the absorbance at 520 nm. The content of proline was expressed in μmol g^{-1} FW.

Statistical analysis

The results are expressed as mean ± SD of three replicates (n = 3). Data were subjected to Tukey's post-hoc test (SPSS 16.0) for determining differences between treatments and among genotypes. Mean fold increase/decrease were calculated for various parameters studied and differences among genotypes and between treatments were determined.

RESULTS

Vigour Index

Vigour index showed a gradual and significant decrease in all the genotypes in response to saline levels (Fig. 1). Genotype, AL 1817 depicted highest average fold decrease of 1.6 fold at 40mM NaCl. The minimum decline in vigour index was observed in AL 1873 at all salt concentrations.

POD activity

A significant increase in POD activity was observed in roots and shoots of all genotypes at 30 and 40mM NaCl concentration from their respective controls (Table 1). The mean fold increase in POD activity was 1.6 and 2.8 fold and 1.6 and 2.7 fold in roots and shoots, respectively at 30mM and 40mM NaCl. Among genotypes, POD activity was higher

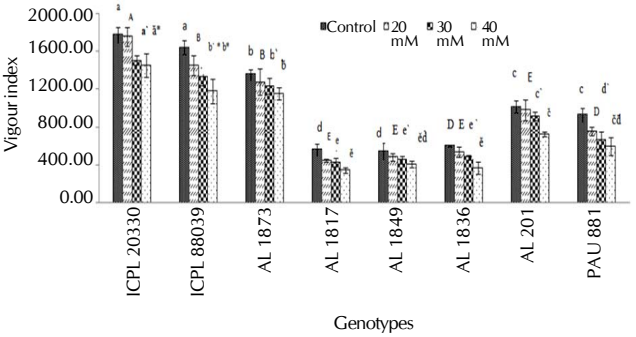

Figure 1: Vigour index in pigeonpea genotypes under salt stress conditions. Error bars denote mean ± SD of three replicates. Bars with different small letter(s), capital letter(s), small letters bearing grave accent and small letter(s) bearing combining breve denote significant differences among controls, 20 mM, 30mM and 40 mM NaCl, respectively at P ≥ 0.05; analysed by tukey's post-hoc test. Asterisk shows significant differences in treated plants from their respective controls

Table 1: Peroxidase activity and SOD activity in root and shoot of Pigeonpea genotypes

Peroxidase activity (“ A min^{-1}g^{-1} FW)							SOD activity (units min^{-1}g^{-1} FW)					
	Root			Shoot			Root			Shoot		
Genotypes	Control	30mM	40mM	Control	30mM	40mM	Control	30mM	40mM	Control	30mM	40mM
ICPL 20330	17.2 ± 0.6^{a}	$26.1 \pm 0.3^{b'*}$	$52.7 \pm 0.4^{a\hat{}*}$	21.3 ± 0.6^{a}	$38.9 \pm 0.7^{a'*}$	$73.6 \pm 0.9^{a\hat{}*}$	88.0 ± 0.7^{b}	$136.9 \pm 0.4^{b'*}$	$160.5 \pm 1.04^{b\hat{}*}$	119.7 ± 0.9^{a}	$160.3 \pm 0.3^{a'*}$	$181.0 \pm 0.4^{a\hat{}*}$
ICPL 88039	10.7 ± 0.2^{b}	$31.7 \pm 0.5^{a'*}$	$47.7 \pm 0.3^{b\hat{}*}$	13.1 ± 0.5^{b}	$27.4 \pm 0.4^{b'*}$	$47.4 \pm 0.7^{b\hat{}*}$	74.6 ± 0.4^{e}	$123.0 \pm 0.5^{c'*}$	$142.2 \pm 0.7^{c\hat{}*}$	92.1 ± 0.4^{d}	$137.0 \pm 0.8^{c'*}$	$165.2 \pm 0.3^{c\hat{}*}$
AL 1873	8.3 ± 0.1^{d}	$12.2 \pm 0.5^{e'*}$	$25.1 \pm 0.4^{d\hat{}*}$	9.2 ± 0.3^{d}	$15.0 \pm 0.1^{d'*}$	$28.5 \pm 0.3^{e\hat{}*}$	98.1 ± 0.4^{a}	$149.1 \pm 0.8^{a'*}$	$166.1 \pm 0.4^{a\hat{}*}$	115.1 ± 0.5^{b}	$151.9 \pm 0.4^{b'*}$	$175.1 \pm 0.4^{b\hat{}*}$
AL 1817	11.0 ± 0.2^{b}	$13.2 \pm 0.2^{d'e'*}$	$20.8 \pm 0.3^{f\hat{}*}$	12.3 ± 0.3^{bc}	$22.6 \pm 0.3^{c'*}$	$35.6 \pm 0.1^{d\hat{}*}$	84.1 ± 0.4^{c}	$111.2 \pm 0.3^{d'*}$	$121.3 \pm 0.6^{f\hat{}*}$	99.4 ± 1.1^{c}	$125.3 \pm 0.9^{d'*}$	$131.8 \pm 0.7^{f\hat{}*}$
AL 1849	11.2 ± 0.07^{b}	$14.7 \pm 0.5^{c'*}$	$17.8 \pm 0.2^{g\hat{}*}$	12.0 ± 0.7^{bc}	$12.4 \pm 0.1^{e'}$	$20.3 \pm 0.4^{f\hat{}*}$	75.1 ± 0.7^{e}	$102.0 \pm 0.4^{f'*}$	$126.1 \pm 0.5^{e\hat{}*}$	89.6 ± 0.3^{e}	$111.0 \pm 0.3^{g'*}$	$126.1 \pm 0.5^{g\hat{}*}$
AL 1836	9.6 ± 0.4^{c}	$13.0 \pm 0.1^{d'e'*}$	$22.7 \pm 0.3^{e\hat{}*}$	11.7 ± 0.4^{c}	$14.9 \pm 0.08^{d'*}$	$17.2 \pm 0.2^{g\hat{}*}$	63.8 ± 0.6^{g}	$96.0 \pm 0.5^{g'*}$	$112.3 \pm 0.7^{g\hat{}*}$	91.4 ± 1.1^{d}	$118.4 \pm 0.7^{f'*}$	$135.2 \pm 0.5^{e\hat{}*}$
AL 201	9.3 ± 0.2^{c}	$14.1 \pm 0.5^{c'd'*}$	$31.2 \pm 0.3^{c\hat{}*}$	11.3 ± 0.2^{c}	$23.4 \pm 0.4^{c'*}$	$39.3 \pm 0.2^{c\hat{}*}$	82.0 ± 0.5^{d}	$108.3 \pm 0.5^{e'*}$	$136.4 \pm 0.3^{d\hat{}*}$	68.0 ± 0.7^{f}	$123.6 \pm 0.5^{e'*}$	$148.5 \pm 0.8^{d\hat{}*}$
PAU 881	7.4 ± 0.3^{e}	$10.6 \pm 0.4^{f'*}$	$17.2 \pm 0.4^{g\hat{}*}$	9.6 ± 0.4^{d}	$10.4 \pm 0.5^{f'}$	$14.2 \pm 0.2^{h\hat{}*}$	65.9 ± 0.5^{f}	$109.9 \pm 0.5^{d'*}$	$109.9 \pm 0.8^{h\hat{}*}$	36.7 ± 0.9^{g}	$71.2 \pm 0.4^{h'*}$	$90.9 \pm 0.3^{h\hat{}*}$
Mean	10.6	17	29.4	12.6	20.6	34.5	78.9	117.1	134.4	89	124.9	144.2

Values are mean ± SD of three replicates. Values with different small letter(s), small letters bearing grave accent and small letter(s) bearing circumflex accent denote significant differences among controls, 30mM and 40 mM NaCl, respectively at P≥ 0.05; analysed by tukey's post-hoc test. Asterisk shows significant differences in treated plants from their respective controls.

Table 2: Catalase activity and H_2O_2 content in root and shoot of Pigeonpea genotypes

Catalase activity (μmol of H_2O_2 decomposed min^{-1}g^{-1}FW)							H_2O_2 content (μmol g^{-1}FW)					
	Root			Shoot			Root			Shoot		
Genotype	Control	30mM	40mM	Control	30mM	40mM	Control	30mM	40mM	Control	30mM	40mM
ICPL 20330	1027.4 ± 2.0^{e}	$926.3 \pm 3.0^{e'*}$	$541.6 \pm 0.8^{a\hat{}*}$	1302.5 ± 2.1^{d}	$756.8 \pm 1.5^{b'*}$	$433.3 \pm 3.3^{d\hat{}*}$	94.7 ± 0.3^{f}	$220.1 \pm 2.9^{f'*}$	$433.1 \pm 1.8^{f\hat{}*}$	120.8 ± 0.5^{e}	$252.7 \pm 1.8^{d'*}$	$309.5 \pm 4.0^{e\hat{}*}$
ICPL 88039	793.3 ± 0.7^{h}	$430.9 \pm 1.9^{e'*}$	$243.2 \pm 1.4^{d\hat{}*}$	1073.1 ± 5.8^{e}	$649.3 \pm 1.6^{c'*}$	$359.7 \pm 9.4^{e\hat{}*}$	75.4 ± 1.1^{h}	$108.2 \pm 2.4^{g'*}$	$232.7 \pm 1.1^{g\hat{}*}$	86.4 ± 1.1^{f}	$187.3 \pm 2.2^{f'*}$	$218.6 \pm 3.4^{f\hat{}*}$
AL 1873	832.3 ± 0.6^{g}	$532.5 \pm 1.9^{c'*}$	$423.5 \pm 1.2^{b\hat{}*}$	987.1 ± 1.6^{f}	$424.1 \pm 2.6^{f'*}$	$331.1 \pm 1.7^{g\hat{}*}$	88.1 ± 0.5^{g}	$258.4 \pm 1.1^{e'*}$	$526.4 \pm 12.6^{e\hat{}*}$	87.2 ± 1.9^{f}	$199.5 \pm 0.8^{e'*}$	$318.5 \pm 5.8^{e\hat{}*}$
AL 1817	1612.5 ± 1.3^{c}	$767.6 \pm 2.1^{b'*}$	$269.6 \pm 1.7^{d\hat{}*}$	1832.6 ± 4.1^{b}	$919.9 \pm 3.8^{a'*}$	$611.1 \pm 1.2^{b\hat{}*}$	196.3 ± 1.2^{b}	$564.9 \pm 9.2^{b'*}$	$717.2 \pm 3.7^{c\hat{}*}$	143.7 ± 1.6^{c}	$283.7 \pm 2.8^{c'*}$	$373.0 \pm 6.7^{d\hat{}*}$
AL 1849	1814.3 ± 2.2^{b}	$469.1 \pm 0.9^{d'*}$	$148.2 \pm 1.7^{f\hat{}*}$	1933.4 ± 9.2^{a}	$744.9 \pm 18.6^{b'*}$	$648.9 \pm 5.2^{a\hat{}*}$	223.1 ± 2.5^{a}	$602.9 \pm 2.6^{a'*}$	$864.7 \pm 1.5^{a\hat{}*}$	165.8 ± 2.3^{a}	$301.3 \pm 1.2^{b'*}$	$426.1 \pm 23.9^{c\hat{}*}$
AL 1836	1877.6 ± 12.5^{a}	$232.1 \pm 1.4^{g'*}$	$162.8 \pm 1.5^{h\hat{}*}$	1731.1 ± 2.8^{e}	$580.3 \pm 2.3^{d'*}$	$510.5 \pm 2.9^{c\hat{}*}$	183.1 ± 2.1^{c}	$508.3 \pm 6.6^{c'*}$	$765.9 \pm 12.5^{b\hat{}*}$	155.4 ± 1.8^{b}	$311.0 \pm 2.8^{b'*}$	$466.2 \pm 11.8^{b\hat{}*}$
AL 201	963.1 ± 2.02^{f}	$535.8 \pm 2.1^{c'*}$	$321.4 \pm 1.9^{c\hat{}*}$	774.4 ± 1.3^{g}	$341.4 \pm 1.1^{g'*}$	$258.5 \pm 3.1^{h\hat{}*}$	112.9 ± 2.3^{d}	$323.0 \pm 2.8^{d'*}$	$541.3 \pm 17.6^{e\hat{}*}$	57.6 ± 2.0^{h}	$165.2 \pm 9.5^{g'*}$	$323.0 \pm 9.0^{e\hat{}*}$
PAU 881	1070.1 ± 14.1^{d}	$362.8 \pm 3.4^{f'*}$	$109.4 \pm 0.7^{g\hat{}*}$	1059.3 ± 16.9^{e}	$502.4 \pm 10.5^{e'*}$	$346.7 \pm 2.1^{f\hat{}*}$	104.6 ± 0.9^{e}	$249.9 \pm 4.1^{e'*}$	$608.2 \pm 4.5^{d\hat{}*}$	127.8 ± 3.1^{d}	$325.0 \pm 3.6^{a'*}$	$622.8 \pm 2.2^{a\hat{}*}$
Mean	1248.8	532.1	277.4	1336.7	614.9	437.2	134.8	354.5	586.2	118.1	253.2	382.2

Values are mean ± SD of three replicates. Values with different small letter(s), small letters bearing grave accent and small letter(s) bearing circumflex accent denote significant differences among controls, 30mM and 40 mM NaCl, respectively at P≥ 0.05; analysed by tukey's post-hoc test. Asterisk shows significant differences in treated plants from their respective controls

in roots and shoots of tolerant (1.9, 3.5-fold, respectively and 1.9, 3.4-fold, respectively) genotypes than sensitive (1.3, 1.9-fold, respectively and 1.4, 2.0-fold, respectively) and moderately sensitive (1.5, 2.9-fold, respectively and 1.6, 2.6-fold, respectively) genotypes at 30 and 40mM NaCl. Genotype ICPL 88039 showed maximum increase in POD activity *viz*., 3.0 and 4.4 fold, respectively in roots and 2.1 and 3.6-fold, respectively in shoots at 30mM and 40mM, respectively.

SOD activity

Genotypes under study exhibited significant variation in SOD activity and within different salt concentrations (Table 1). A consistent increase in SOD activity was observed in roots (1.5, 1.7- fold, respectively) and shoots (1.4, 1.6 fold, respectively) in all genotypes on exposure to salt stress. The activity was higher in roots of tolerant (1.6 and 1.8 fold, respectively) genotypes than sensitive (1.4 and 1.4 fold, respectively) and moderately sensitive (1.5 and 1.7 fold, respectively) genotypes whereas shoot exhibited maximum SOD activity in moderately sensitive (1.9, 2.3-fold, respectively) genotypes at 30 and 40mM NaCl, respectively. The accumulation of the root SOD ranged from 1.3-1.9 fold at 30 and 40mM NaCl in all genotypes. PAU 881 showed highest accumulation of SOD at 30mM (1.9 fold) and 40mM NaCl (2.5 fold) in shoots.

CAT activity

Under the effect of NaCl, a significant decline in CAT activity was observed in the roots and shoots of all treated genotypes (Table 2). The levels of CAT decreased exponentially in roots (2.3, 4.5-fold, respectively) and shoots (2.1, 3.0-fold, respectively) with increasing salt concentrations. Sensitive genotypes showed maximum decline in CAT activity (3.6, 9.1 fold, respectively and 2.4, 3.1 -fold, respectively) of root and shoot in collation to tolerant (1.4, 2.2 fold, respectively and 1.8, 3.0-fold, respectively) and moderately sensitive (2.3, 4.7-fold, respectively and 2.2, 3.0-fold, respectively) genotypes at 30 and 40mM NaCl respectively. AL 1836, a sensitive genotype, registered maximum average fold, respectively decrease in roots and shoots at 30mM (8.1 and 3.0 fold, respectively) and 40mM (11.5 and 3.4 fold, respectively) salt concentration whereas minimal CAT activity in roots (1.1 and 1.9 fold, respectively) and shoots (1.7 and 3.0 fold, respectively) was observed in genotype, ICPL 20330.

H_2O_2 content

Salt stress significantly and consistently induced H_2O_2 levels at both the salt concentrations in the roots and shoots of the tested genotypes (Table 2). Elevation in the H_2O_2 content of roots was remarkably higher (2.6 and 4.3-fold, respectively) than of shoots (2.1 and 3.2 fold, respectively) at 30mM and 40mM NaCl, respectively. The mean fold increment in root and shoot H_2O_2 content was highest in moderately sensitive (2.6 and 5.3 fold, respectively and 2.6 and 5.1-fold, respectively) genotypes at respective concentrations (30mM and 40mM NaCl). Genotype AL 1873, exhibited maximum H_2O_2 content at 30mM (2.9 fold) and 40mM (6.0 fold) in roots. In shoots, AL 201, possessed highest accumulation of H_2O_2 (2.9 and 5.6-fold, respectively) on account of increasing salt concentration *viz*., 30mM and 40mM.

MDA content

A significant increase in MDA content was observed in roots of all tested genotypes after salt treatment (Fig. 2). The accumulation of MDA in roots was observed to be 1.3 and 1.7 fold, respectively at both NaCl concentrations in comparison to their respective controls. A profound effect of salt stress on root MDA content was higher in moderately sensitive genotypes (1.7 and 2.2 fold, respectively) than tolerant (1.1 and 1.2 fold, respectively) and sensitive (1.2 and 1.6 fold, respectively) genotypes. AL 201 exhibited highest MDA content in roots at 30mM (2.4 fold, respectively) and 40mM (2.8 fold, respectively) NaCl concentration. However, salt stress did not elicit the significant variation in shoot MDA content among genotypes.

Proline content

Sodium chloride (NaCl) significantly induced proline levels in roots and shoots of 8 days old seedlings in comparison to their respective control (Fig. 3). A marked increase in proline content of roots (1.5 and 2.6 fold, respectively) and shoots (1.9 and 2.5 fold, respectively) was observed at 30mM and 40mM respectively. Among genotypes, the average fold proline accumulation in shoots was maximum in tolerant genotypes (2.0 and 2.8 fold, respectively) whereas root exhibited highest

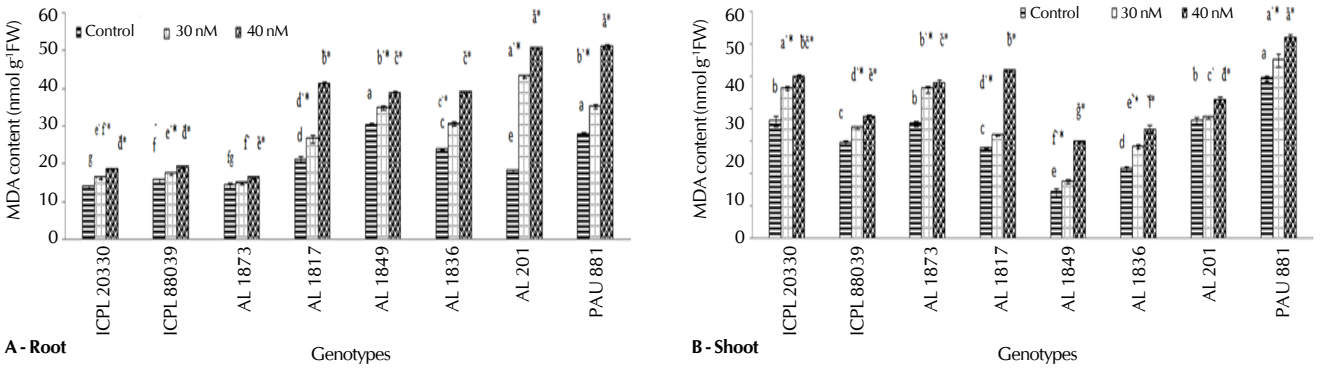

Figure 2: MDA content in A - Root, B - Shoot of pigeonpea genotypes under salt stress conditions. Error bars denote mean ± SD of three replicates. Bars with different small letter(s) small letters bearing grave accent and small letter(s) bearing combining breve denote significant differences among controls, 30mM and 40 mM NaCl, respectively at P ≥ 0.05; analysed by tukey's post-hoc test. Asterisk shows significant differences in treated plants from their respective controls

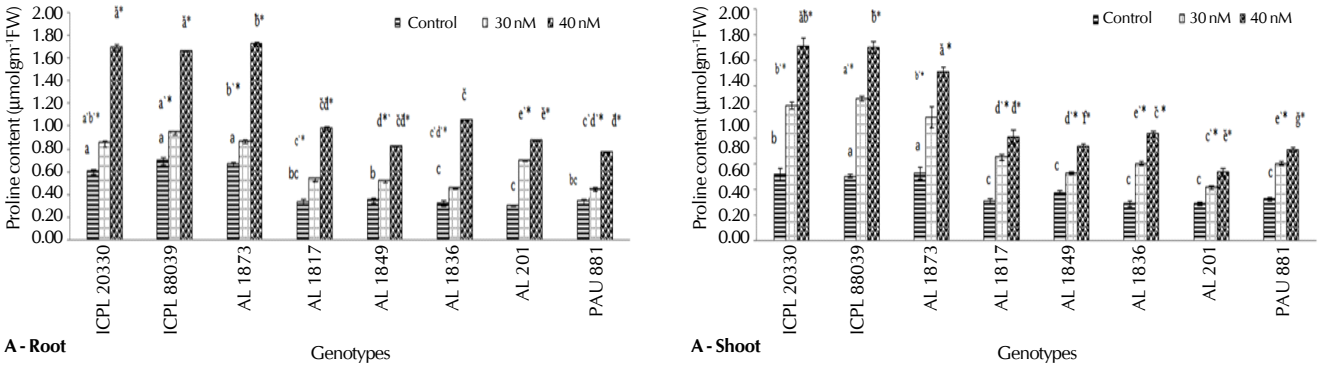

Figure 3: Proline content in A- Root, B-Shoot of pigeonpea genotypes under salt stress conditions. Error bars denote mean ± SD of three replicates. Bars with different small letter (s) small letters bearing grave accent and small letter(s) bearing combining breve denote significant differences among controls, 30mM and 40 mM NaCl, respectively at P ≥ 0.05; analysed by tukey's post-hoc test. Asterisk shows significant differences in treated plants from their respective controls.

proline content in moderately sensitive genotypes (1.8 and 2.6fold, respectively) at 30mM and 40mM NaCl respectively. ICPL 88039, a tolerant genotype, registered highest proline (2.2 and 3.0 fold, respectively) accumulation in shoots as compared to their respective controls.

DISCUSSION

Soil salinity is regarded as one of the most evident factor that affects plant growth and development. In the present investigation of pigeonpea genotypes, salinity stress caused gradual reduction in seed germination and total seedling length which was determined in reference to vigour index (Fig.1). Salinity stress altered the normal physiological process causing reduction in water uptake and decreasing the osmotic potential of plant tissue thereby disturbing the normal physiological process (Mahajan and Tuteja, 2005) that resulted in lack of assimilates for plant growth, reduced tissue length and thus vigour index.

Another important plant response to withstand deleterious effects of salinity stress is the activation of defense system which results from the upregulation of set of antioxidative enzymatic compounds such as SOD, POD, CAT and non-antioxidative defensive compounds such as proline, glycine betaine (Gill *et al.,* 2010). POD and CAT disproportionate toxic H_2O_2 produced in response to stress into water and oxygen. The increased POD activity in roots and shoots alongwith lower content of H_2O_2 in the tolerant genotypes as compared to sensitive and moderately sensitive genotypes indicates the ability of the tolerant genotypes to protect themselves from deleterious effects of H_2O_2. The decreased POD activity in sensitive genotypes (PAU 881) might be responsible for the accumulation of H_2O_2 (Table 1) which causes cytoxicity in plant tissue through hydroxyl radicals produced in Haber-Weiss reaction (Meloni *et al.,* 2003). The above fact is verified from the similar studies in soybean (Aghaleh and Niknam, 2009) and alfalfa xinmu no. 1 (Wang *et al.,* 2009) where increased peroxidase activity at higher salt concentrations proved the contribution of POD in providing salt tolerance to plants by catalyzing H_2O_2.

High SOD activity in tolerant genotypes as compared to sensitive and moderately sensitive genotypes in response to salt stress indicated its contribution in providing resistance to tolerant genotypes (Table 1). SOD scavenges the hydroxy radicals and converts them into toxic H_2O_2 which has dual functions. At lower concentration, H_2O_2 acts as a signaling molecule whereas at higher concentration it causes cytotoxicity as in case of sensitive and moderately sensitive genotypes. These observations revealed that increase in H_2O_2 content are partly due to upregulation of SOD. The upregulation of resistance mechanism in salt tolerant genotypes might be due to overproduction of superoxide radicals which when converted to H_2O_2 further act as a signaling molecule for the activation of various enzymatic and non enzymatic antioxidants. The cytotoxicity of H_2O_2 in tolerant genotypes is further prevented by the enhanced action of POD and CAT as revealed earlier. Rasool *et al*. (2013) reported high SOD activity in salt tolerant SKUA-06 and SKUA-07 chickpea genotypes.

There was a significant decrease in CAT activity in roots and shoots of germinated seedlings with increasing salt stress, irrespective of the tolerance capacity and sensitivity of genotypes (Table 2). The high accumulation of H_2O_2 content in sensitive genotypes is further strengthened by the fact that more decline in CAT activity occurred in sensitive genotypes as compared to tolerant genotypes. The minimal decline in CAT activity of tolerant genotype (ICPL 20330) correlates with the fact that there might be lower production of salt induced ROS which is also evident from the lower values of H_2O_2 content. The observation is in consistence with Hu *et al*. (2012) in which decline in CAT activity has been observed in ryegrass when exposed to salt stress.

The increase in roots and shoots H_2O_2 content has been observed in all the genotypes. However, relatively lower increase in H_2O_2 content of tolerant genotypes as compared to sensitive genotypes (Table 2) suggested the potential of tolerant genotypes in activating strong antioxidative defence mechanism in response to salt stress (Maffei *et al.,* 2007). Wang *et al*. (2013) reported the similar trend in salt tolerant cultivar of rice i.e. Zhenghan 2 which has lower H_2O_2 content than Yujing 6 when exposed to NaCl.

The higher MDA and lower proline content observed in the roots and shoots of sensitive genotypes at 30 and 40mM NaCl than tolerant and moderately sensitive genotypes (Fig. 2) indicates membrane damage in plant cells. The membrane damage occurs on account of peroxidation of polyunsaturated fatty acids in membrane by hydroxyl radicals produced in response to induced salt stress (Hernandez and Almansa, 2002). On being subjected to such damage there is an accumulation of certain osmolytes such as proline. Accumulation of significant proline levels in tolerant genotypes confers the fact of proline being an important osmolyte that contribute in providing resistance to tolerant genotypes (Fig. 3) by protecting the membrane damge. The significant decrease in proline levels and high MDA content of sensitive genotypes might be due to increased hydroxyl radicals that leads to the decreased solubility of cell wall rich components mainly hydroxyproline resulting in cell wall toughening and thus limiting the exchange of nutrients and gases. Liang *et al*. (2003), reported membrane damage in terms of increased MDA content in Barley cultivar and in tobacco plant (Ruiz *et al.,* 2005) in response to salt stress.

In conclusion, physiologically screened tolerant genotypes were found to possess higher antioxidative status. Higher status of POD, SOD, CAT, proline and lower H_2O_2, MDA contents in tolerant genotypes under salt stress appeared to contribute in providing resistance to these genotypes by lowering the salinity induced oxidative stress. This study might provide better insights in understanding the integrated contribution of various biochemical components in upregulating defense responses under salt stress. Significant changes in growth parameters observed, served as indicators of salt stress. Thus, the results reinforce that salinity affected both physiological and biochemical traits.

REFERENCES

Aghaleh, M. and Niknam, V. 2009. Effect of salinity on some physiological and biochemical parameters in explants of two cultivars of soybean (*Glycine max* L). *J. Phytol.* **1:** 86-94.

Bates, L. S., Waldren, R. P. and Teare, I. D. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil.* **39:** 205-207.

Chance, B. and Maehly, A. C. 1955. Assay of catalases and peroxidases. *Method Enzymol.* **2:** 764-775.

Gill, R. S., Gupta, A. K., Taggar, G. K. and Taggar, M. S. 2010. Role of oxidative enzymes in plant defenses against insect herbivory. *Acta Phytopathol Hun.* **45:** 277-290.

Hernandez, J. A. and Almansa, M. S. 2002. Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. *Physiol. Plantarum.* **115:** 251-257.

Hu, L., Li, H., Pang, H. and Fu, J. 2012. Response of antioxidant gene protein and enzymes to salinity stress in two genotypes of perennial ryegrass (*Lolium perenne*) differing in salt tolerance. *J. Plant Physiol.* **169:** 146-156.

Jaiswal, A., Pandurangam, V. and Sharma, S. K. 2014. Effect of Salicylic Acid in Soybean (*Glycine Max* L. Meril) under salinity stress. *The Bioscan.* **9:** 671-676.

Kaur, H., Gupta, A. K., Kaur, N. and Sandhu, J. S. 2009. Differential response of the antioxidant system in wild and cultivated genotypes of chickpea. *Plant Growth Regul.* **57:** 109-114.

Liang, Y., Chen, Q., Liu, Q., Zhang, W. and Ding, R. 2003. Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L). *J. Plant Physiol.* **160:** 1157-1164.

Maffei, M. E., Mithofer, A. and Boland, W. 2007. Insects feeding on plants: rapid signals and responses preceding the induction of phytochemical release. *Phytochemistry.* **68:** 2946-2959.

Mahajan, S. and Tuteja, N. 2005. Cold salinity and drought stresses: An overview. *Arch. Biochem. Biophys.* **444:** 139-158.

Marklund, S. and Marklund, G. 1974. Involvement of superoxide anion radical in the autoxidation of pyragallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.* **47:** 169-174.

Maruthi, Y. A., Rao, S. R., Chaitanya, D. A., Hossain, K., Kumar, R. S., Sitaratnam, M. and Rao, R. T. 2010. Evaluation of Water Quality in the vicinity of some Saltpans, Visakhapatnam District, Andhra Pradesh. *The. Bioscan.* **3:** 665-672.

Meloni, D. A., Oliva, M. A., Martinez, C. A. and Cambraia, J. 2003. Photosynthesis and activity of superoxide dismutase peroxidase and glutathione reductase in cotton under salt stress. *Environ. Exp. Bot.* **49:** 69-76.

Miller, G. 2010. Reactive oxygen species homeostasis and signaling during drought and salinity stresses. *Plant Cell Environ.* **33:** 453-467.

Noreen, Z. and Ashraf, M. 2009. Change in antioxidant enzymes and some key metabolites in some genetically diverse cultivars of radish (*Raphanus sativus* L). *Environ. Exp. Bot.* **67:** 395-402.

Ohkawa, H., Ohishi, N. and Yagi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95:** 351-358.

Rasool, S., Ahmad, A., Siddiqui, T. O. and Ahmad, P. 2013. Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. *Acta Physiol. Plant.* **35:** 1039-1050.

Ruiz, J. M., Blasco, B., Rivero, R. M. and Romero, L. 2005. Nicotine-free and salt tolerant tobacco plants obtained by grafting to salinity-resistant rootstocks of tomato. *Physiol. Plant.* **124:** 465-475.

Shannon, L. M., Kay, E. and Lew, J. Y. 1966. Peroxidase isozymes from horseradish roots I Isolation and physical properties. *J. Biol. Chem.* **241:** 2166-2172.

Varshney, *et al.* 2012. Draft genome sequence of pigeonpea (*Cajanus cajan*) an orphan legume crop of resource- poor farmers. *Nature Biotechnol.* **30:** 83-89.

Waheed, A., Hafiz, I. A., Qadir, G., Murtaza, G., Mahmood, T. and Ashraf, M. 2006. Effect of Salinity on Germination, Growth, Yield, Ionic Balance and Solute Composition of Pigeon Pea (*Cajanus cajan* (L.) Millsp). *Pak. J. Bot.* **38:** 1103-1117.

Wang, W. B., Kim, Y. E., Lee, H. S., Kim, K. Y., Deng, X. P. and Kwak, S. S. 2009. Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stress. *Plant Physiol. Biochem.* **47:** 570-577.

Wang, X., Hor, C., Liu, J., He, W., Nan, W., Gong, H. and Bi, Y. 2013. Hydrogen peroxide is involved in the regulation of rice (*Oryza sativa* L.) tolerance to salt stress. *Acta Physiol. Plant.* **35:** 891-900.