

## Comparative studies on antioxidant enzyme action and ion accumulation in soybean cultivars under salinity stress

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### Abstract

Plant biomass, antioxidant enzymes activity, ions accumulation and proline level in four soybean cultivars were investigated at different NaCl concentrations (20, 40, 60, 80 and 100 mM) applied to plants 15 days after sowing. There was a significant decrease in plant biomass and soluble protein content with each NaCl treatment. Accumulation of Na<sup>+</sup> and Cl<sup>-</sup> was maximum in roots, followed by the stem and leaves in all the treated cultivars; Pusa 9712 being the top accumulator. On the contrary, K<sup>+</sup> and Ca<sup>2+</sup> ion concentrations were inhibited in all the treated cultivars. Activities of antioxidant enzymes (superoxide dismutase, ascorbate peroxidase and glutathione reductase) and proline content increased significantly in all the cultivars with each NaCl treatment. The maximum increase was found in Pusa 9712. However, catalase activity decreased in all the cultivars except in Pusa 9712. On the whole, Pusa 9712 was most efficient in managing protection against salinity stress.

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### Introduction

Salinity, a soil characteristic of arid and semi-arid environments, is now a worldwide problem especially in the areas where irrigation is practiced. Salinity level of the soil in the arid and semi-arid regions is in the range of 20-200 mM, whereas pH is above 7.5. Salinity affects about one billion ha of the world's land area; approximately 60% of the cultivated land, which includes one-third area of the irrigated soil (Chowdhury *et al.*, 1993). Productivity of the salt-affected land is limited as the growth of most crop plants is reduced under saline conditions. However, the wide range of variation that exists among different crop plants may be utilized gainfully for identifying and developing the salt-tolerant candidates.

Of the various crops studied, legumes are more sensitive to salinity in general (Ashraf and Harris, 2004). Different organs, tissues

and cells at different stages of plant development exhibit a varying degree of tolerance to saline conditions (Ashraf, 1994). The uptake and availability of water/moisture to the growing tissue becomes a limiting factor under saline conditions. Water uptake by plants thus attains utmost importance under saline conditions. High concentrations of salts disrupt homeostasis in water potential and ion distribution in plants. Crucial changes in these parameters lead to molecular damage, growth arrest, and even death of the plant. Specific effects of salt stress on plant metabolism, especially on leaf senescence, have been related to accumulation of toxic ions (Na<sup>+</sup> and Cl<sup>-</sup>) or depletion of K<sup>+</sup> and Ca<sup>2+</sup> (Al-Karaki, 2000; Perez-Alfocea *et al.*, 1996). Ion imbalance and hyperosmotic stress, the primary effects of salt stress, often cause secondary stresses such as the oxidative stress.

Rapid accumulation of free proline is a typical response to salt stress. When exposed to high salt content in the soil, many plants accumulate high amounts of proline that provide protection to cell membranes against salt injury (Mansour, 1998). Proline accumulation is also implicated in the osmotic adjustment to salinity (Sultana *et al.*, 1999).

In order to scavenge the active oxygen species (AOS), plants possess specific defense strategies involving both enzymatic and non-enzymatic antioxidant mechanisms (Diwan *et al.*, 2010a,b). Studies have shown that salt tolerance of plants may be improved if the radicals formed in tissues during the activated oxygen damage are scavenged by an elevated antioxidant defense system (Alscher *et al.*, 2002; Shigeoka *et al.*, 2002).

Soybean (*Glycine max* L.), known for its high protein (40%) and oil (20%) contents in dry seeds, is a popular pulse crop and is used in a variety of food. Recent studies have shown that NaCl treatments affect growth and water relations in soybean cultivars (Shereen and Ansari, 2001) and reduce significantly the grain-filling duration, grain weight, grain yield per plant, and the oil and protein contents (Ghassemi-Golezani *et al.*, 2009). Of the several cultivars of soybean, four (Pusa 20, Pusa 1024, PK 416 and Pusa 9712) are under wide cultivation in India.

The present study aims at investigating the effects of salt stress on plant growth, antioxidant enzymes activity, and accumulation of proline and inorganic ions in soybean cultivars with a view to understand the process of their adaptation to salt stress.

### Materials and Methods

**Plant material and salt stress application:** Seeds of soybean (*Glycine max* L.) cultivars Pusa 20, Pusa 1024, PK 416 and Pusa 9712 were obtained from the Indian Agricultural Research Institute, New Delhi. Plant culture was done in the hydroponics systems. Seeds were surface sterilized with 0.1% sodium hypochlorite solution and washed with distilled water for 20 min. The seeds were kept in the dark at 25 °C for 48 hr to germinate in the trays with soil. After four days, the seedlings obtained were transferred to nutrient solution as per the method of Hoagland and Arnon (1950). The seedlings were grown in hydroponics system for 15 days in controlled environmental conditions composed of a photosynthetic photon flux density of 430  $\mu\text{M m}^{-2} \text{s}^{-1}$ , 13 hr of light, 11 hr of darkness and a relative humidity of 60%. The solutions were aerated continuously to provide ample  $\text{O}_2$  and to maintain the solution concentration at the root surfaces. Salt treatments were imposed to 15-day-old seedlings by adding 10, 20, 40, 60, 80 and 100 mM NaCl into  $\frac{1}{2}$  strength nutrient solution. Nutrient solution without NaCl (0.0 mM NaCl) was used for the control group. Observations were recorded at 48, 96 and 144 hr after treatments (HAT) in five replicates for biochemical parameters.

**Measurement of biomass accumulation:** Fifteen plants for each NaCl treatment were collected 48, 96 and 144 HAT and dried in oven at 65°C for 72 hr. Dry weight of the control and the treated

plants was then determined with the help of an electronic balance (Sartorius, Germany) and expressed in mg plant<sup>-1</sup>.

**Determination of sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>) and calcium (Ca<sup>2+</sup>) contents:** The Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> ion contents were estimated by using a flame photometer (Systronic 125, India), following the method described by Tondon (1995). The content of each element in plant tissues was determined, using the standard curve, and expressed in  $\mu\text{mol g}^{-1} \text{d.wt}$ .

**Chloride (Cl<sup>-</sup>), proline and soluble protein content:** The Cl<sup>-</sup> content was estimated by the method of Vogel (1968) and expressed in  $\mu\text{mol g}^{-1} \text{d.wt}$ . Extraction of Cl<sup>-</sup> from plant material was done for 10, 20, 30 and 40 min. Each attempt gave the same ion concentration, thus suggesting that release of Cl<sup>-</sup> ions was complete within 10 min.

The method of Bates *et al.* (1973) was used to determine the proline content of leaf tissues. Concentration of proline was determined against the standard curve of L-proline and expressed in  $\text{mg g}^{-1} \text{f.wt}$ .

Protein in leaf samples was quantified by the method of Bradford (1976), using bovine serum albumin (BSA) as the standard. The content of soluble protein was expressed in  $\text{mg g}^{-1} \text{f.wt}$ .

**In vitro enzyme activity:** The method of Dhindsa *et al.* (1981) was followed with slight modification for the estimation of SOD (EC 1.15.1.1) activity, which was expressed in EU  $\text{mg}^{-1} \text{protein}$ .

The APX (EC 1.11.1.11) activity was measured by following the method of Nakano and Asada (1981). It was calculated by using the extinction coefficient 2.8  $\text{mM}^{-1} \text{cm}^{-1}$  and expressed in enzyme units ( $\text{mg protein}^{-1}$ ). One unit of enzyme is the amount necessary to decompose 1  $\mu\text{mol H}_2\text{O}_2 \text{min}^{-1}$  at 25°C.

The activity of GR (EC 1.6.4.2) was determined by the method followed by Rao (1992). The activity was calculated by using the extinction coefficient of 6.2  $\text{mM}^{-1} \text{cm}^{-1}$  and expressed in enzyme units ( $\text{mg protein}^{-1}$ ). One unit of enzyme is the amount necessary to decompose 1  $\mu\text{mol of NADPH min}^{-1}$  at 25°C.

The CAT (EC 1.11.1.6) was determined by following the method of Aebi (1984). Calculations were made by using the coefficient of absorbance 0.036  $\text{mM}^{-1} \text{cm}^{-1}$ . One enzyme unit (EU) determines the amount of enzyme necessary to decompose 1  $\mu\text{mol of H}_2\text{O}_2 \text{mg}^{-1} \text{protein min}^{-1}$  at 25°C and expressed in enzyme unit ( $\text{mg protein}^{-1}$ ).

**Statistical analysis:** The data collected obtained were analyzed using the SPSS statistical package software, version 10.0 (Chicago, USA). Two-way analysis of variance (ANOVA) was used to determine differences between varieties and treatments. Mean separation was done by DMRT test at  $p \leq 0.05$ . Linear regression was fitted with GENSTAT in order to find out the degree of correlation between the treatments and the different variables in the soybean cultivars studied.

**Table - 1:** Sodium (Na<sup>+</sup>) content (µmol g<sup>-1</sup> d. wt.) of root, stem and leaf of soybean cultivars exposed to NaCl, as observed at 48, 96 and 144 hours after treatments (HAT)

Genotypes	Concentration of NaCl applied (mM)																	
	48 HAT					96 HAT					144 HAT							
	00	20	40	60	80	100	00	20	40	60	80	100	00	20	40	60	80	100
	<b>Root Na<sup>+</sup> content</b>																	
Pusa 20	20 <sup>a</sup>	250 <sup>c</sup>	425 <sup>b</sup>	600 <sup>c</sup>	750 <sup>c</sup>	895 <sup>c</sup>	20 <sup>a</sup>	260 <sup>d</sup>	433 <sup>c</sup>	665 <sup>d</sup>	785 <sup>d</sup>	900 <sup>c</sup>	20 <sup>a</sup>	265 <sup>c</sup>	445 <sup>b</sup>	670 <sup>d</sup>	790 <sup>c</sup>	950 <sup>c</sup>
Pusa 1024	18 <sup>a</sup>	230 <sup>d</sup>	400 <sup>c</sup>	520 <sup>b</sup>	550 <sup>b</sup>	720 <sup>b</sup>	20 <sup>a</sup>	150 <sup>b</sup>	430 <sup>c</sup>	540 <sup>c</sup>	600 <sup>c</sup>	790 <sup>b</sup>	18 <sup>a</sup>	240 <sup>c</sup>	450 <sup>b</sup>	565 <sup>c</sup>	610 <sup>d</sup>	725 <sup>d</sup>
PK 416	20 <sup>a</sup>	140 <sup>b</sup>	380 <sup>c</sup>	500 <sup>b</sup>	520 <sup>b</sup>	700 <sup>b</sup>	18 <sup>a</sup>	235 <sup>c</sup>	445 <sup>b</sup>	555 <sup>b</sup>	565 <sup>d</sup>	700 <sup>c</sup>	20 <sup>a</sup>	155 <sup>d</sup>	400 <sup>c</sup>	490 <sup>b</sup>	550 <sup>b</sup>	600 <sup>b</sup>
Pusa 9712	20 <sup>a</sup>	280 <sup>d</sup>	480 <sup>d</sup>	640 <sup>c</sup>	790 <sup>d</sup>	925 <sup>d</sup>	20 <sup>a</sup>	300 <sup>b</sup>	500 <sup>d</sup>	650 <sup>c</sup>	795 <sup>d</sup>	950 <sup>d</sup>	20 <sup>a</sup>	305 <sup>b</sup>	510 <sup>d</sup>	700 <sup>c</sup>	800 <sup>d</sup>	995 <sup>c</sup>
	<b>Stem Na<sup>+</sup> content</b>																	
Pusa 20	15 <sup>a</sup>	120 <sup>c</sup>	220 <sup>b</sup>	325 <sup>c</sup>	400 <sup>b</sup>	550 <sup>c</sup>	15 <sup>a</sup>	125 <sup>c</sup>	230 <sup>d</sup>	345 <sup>b</sup>	475 <sup>d</sup>	570 <sup>c</sup>	15 <sup>a</sup>	130 <sup>c</sup>	235 <sup>b</sup>	350 <sup>c</sup>	500 <sup>d</sup>	575 <sup>b</sup>
Pusa 1024	15 <sup>a</sup>	100 <sup>c</sup>	210 <sup>b</sup>	310 <sup>d</sup>	385 <sup>c</sup>	510 <sup>d</sup>	15 <sup>a</sup>	115 <sup>c</sup>	215 <sup>b</sup>	320 <sup>d</sup>	385 <sup>c</sup>	395 <sup>d</sup>	15 <sup>a</sup>	120 <sup>c</sup>	225 <sup>b</sup>	330 <sup>c</sup>	400 <sup>b</sup>	325 <sup>c</sup>
PK 416	15 <sup>a</sup>	185 <sup>d</sup>	180 <sup>d</sup>	290 <sup>c</sup>	375 <sup>d</sup>	470 <sup>c</sup>	15 <sup>a</sup>	120 <sup>c</sup>	220 <sup>b</sup>	320 <sup>d</sup>	385 <sup>c</sup>	480 <sup>b</sup>	15 <sup>a</sup>	100 <sup>d</sup>	160 <sup>b</sup>	200 <sup>d</sup>	325 <sup>c</sup>	405 <sup>b</sup>
Pusa 9712	15 <sup>a</sup>	150 <sup>b</sup>	250 <sup>c</sup>	345 <sup>d</sup>	450 <sup>c</sup>	590 <sup>b</sup>	15 <sup>a</sup>	160 <sup>b</sup>	265 <sup>c</sup>	360 <sup>d</sup>	465 <sup>d</sup>	600 <sup>c</sup>	15 <sup>a</sup>	166 <sup>b</sup>	280 <sup>c</sup>	377 <sup>b</sup>	480 <sup>b</sup>	610 <sup>c</sup>
	<b>Leaf Na<sup>+</sup> content</b>																	
Pusa 20	10 <sup>a</sup>	40 <sup>c</sup>	80 <sup>d</sup>	110 <sup>c</sup>	145 <sup>d</sup>	175 <sup>c</sup>	10 <sup>a</sup>	40 <sup>d</sup>	65 <sup>c</sup>	80 <sup>d</sup>	95 <sup>c</sup>	105 <sup>c</sup>	10 <sup>a</sup>	44 <sup>b</sup>	70 <sup>c</sup>	84 <sup>b</sup>	99 <sup>c</sup>	110 <sup>c</sup>
Pusa 1024	10 <sup>a</sup>	30 <sup>d</sup>	70 <sup>c</sup>	90 <sup>b</sup>	130 <sup>c</sup>	150 <sup>b</sup>	10 <sup>a</sup>	35 <sup>b</sup>	55 <sup>d</sup>	70 <sup>b</sup>	88 <sup>d</sup>	100 <sup>c</sup>	10 <sup>a</sup>	40 <sup>c</sup>	60 <sup>b</sup>	74 <sup>c</sup>	95 <sup>c</sup>	100 <sup>c</sup>
PK 416	10 <sup>a</sup>	28 <sup>d</sup>	50 <sup>b</sup>	80 <sup>c</sup>	100 <sup>b</sup>	125 <sup>c</sup>	10 <sup>a</sup>	35 <sup>b</sup>	50 <sup>d</sup>	60 <sup>c</sup>	77 <sup>d</sup>	89 <sup>d</sup>	10 <sup>a</sup>	35 <sup>d</sup>	48 <sup>c</sup>	55 <sup>d</sup>	66 <sup>c</sup>	78 <sup>d</sup>
Pusa 9712	10 <sup>a</sup>	50 <sup>b</sup>	90 <sup>c</sup>	130 <sup>d</sup>	160 <sup>c</sup>	200 <sup>b</sup>	10 <sup>a</sup>	40 <sup>d</sup>	80 <sup>c</sup>	95 <sup>b</sup>	105 <sup>c</sup>	125 <sup>b</sup>	10 <sup>a</sup>	43 <sup>c</sup>	84 <sup>d</sup>	100 <sup>c</sup>	110 <sup>b</sup>	130 <sup>b</sup>

The values are mean of five individual readings. Data followed by the same letters are not significantly different at p<0.05 levels as determined by the Duncan's multiple range test (DMRT)

## Results and Discussion

Variations from the control were non-significant with 10 mM NaCl treatment; therefore, results of this treatment have not been considered. The results of 20, 40, 60, 80 and 100 mM treatments ( $T_1$ - $T_5$ ) are discussed below in relation to the control ( $T_0$ ).

**Biomass accumulation:** NaCl exhibited a considerable inhibitory effect on plant biomass in a dose- and time-dependent manner. Reduction in biomass accumulation was 37% in Pusa 20, 42% in Pusa 1024, 44% in PK 416 and 30% in Pusa 9712 cultivars as observed with  $T_5$  (100 mM NaCl) treatments at 144 HAT compared with the controls. The reduction was maximum in PK 416 and minimum in Pusa 9712 (Fig. 1A).

**Sodium ( $Na^+$ ), chloride ( $Cl^-$ ), potassium ( $K^+$ ) and calcium ( $Ca^{2+}$ ) contents:** The  $Na^+$  content significantly increased in the treated plants with each concentration of NaCl as observed at 48, 96 and 144 HAT, showing the maximum accumulation in roots, followed by the stem and leaves. Accumulation was dose- as well as time-dependent. The maximum  $Na^+$  accumulation was observed in the cultivars Pusa 9712, followed by Pusa 20, Pusa 1024 and PK 416 with each NaCl treatment, as compared with their controls (Table 1).

Compared with the control,  $Cl^-$  content was enhanced in different plant parts of soybean cultivars under salinity stress at each observation (*i.e.* 48, 96 and 144 HAT). The maximum  $Cl^-$  content was found in roots, followed by the stem and leaves in all genotypes. However, Pusa 9712 was the best accumulator (Table 2).

Salinity stress had an adverse effect on  $K^+$  content of all soybean cultivars with  $T_5$  treatment causing the maximum decline. The decline was greatest in roots, followed by the stem and the leaves. Compared with the control, the maximum decline in  $K^+$  content (69% in roots; 67% in stem and 59% in leaves) was observed in PK 416 and the minimum (62, 58 and 54%, respectively) in Pusa 9712, under the influence of  $T_5$  as observed at 144 HAT (Table 3).

NaCl treatment applied at 15 DAS caused a significant decline in  $Ca^{2+}$  content of all soybean cultivars, compared with their controls, as observed at the three time intervals. The maximum reduction in  $Ca^{2+}$  content in root (64%), stem (64%) and leaves (57%) was found in PK 416 and the minimum (52, 46 and 41% respectively) in Pusa 9712, with 100 mM NaCl treatment, as observed at 144 HAT (Table 4).

**Proline and protein content:** Proline accumulation in the leaves of all the cultivars increased significantly under NaCl stress. Compared with the control, the maximum enhancement (4.7 times higher) was observed in Pusa 9712 whereas minimum (3.1 times higher) in PK 416, as recorded at 144 HAT (Table 3). A strong positive correlation was observed between the proline content and the NaCl concentrations applied (Fig. 1B).

NaCl treatment applied at 15 DAS caused a significant reduction in the soluble protein content of all the soybean cultivars, as observed at 48, 96 and 144 HAT. The maximum decline (38%) was observed in genotype PK 416 followed by Pusa 1024 (35%) and Pusa 20 (34%), whereas the minimum (30%) occurred in Pusa 9712 with  $T_5$  at 144 HAT, in comparison to their controls (Table 2). The negative correlation between protein content and NaCl treatments was strong in all the cultivars (Fig. 1C).

**In vitro enzyme activities:** The NaCl-treated plants showed a dose-dependent increase in SOD activity. The extent of increase varied from 39% (in PK 416) to 51% (in Pusa 9712) with  $T_5$  at 144 HAT, over their respective controls (Fig. 2A). The SOD activity showed a strong positive correlation, with  $R^2 = 0.9899$  in cv PK 416 and  $R^2 = 0.9279$  in cv Pusa 9712, with each NaCl treatment.

The APX activity in NaCl-treated plants continued to increase in all the cultivars with the increase in NaCl concentration ( $T_0$ - $T_5$ ) at all stages of plant growth. Of all the cultivars, the maximum increase in activity was recorded in Pusa 9712 ( $R^2 = 0.9312$ ), whereas minimum in PK 416 ( $R^2 = 0.9782$ ) under  $T_5$  treatment at 48, 96 and 144 HAT, respectively, over their controls (Fig. 2B).

Compared with the control, activity of GR also increased under NaCl treatments in all the cultivars as observed at 48, 96 and 144 HAT. The extent of increase varied from 36% (in PK 416) to 53% (in Pusa 9712) with  $T_5$  treatment at 144 HAT (Fig 2C). A strong positive correlation was observed in all the cultivars, with  $R^2$  values ranging from 0.9201 to 0.9673.

The CAT activity, on the other hand, decreased in all the cultivars, except in Pusa 9712, with each NaCl concentration. In Pusa 9712, the activity increased by 15-20% with lower NaCl treatments ( $T_1$ - $T_2$ ), as observed at 144 HAT, compared with the control (Fig. 2D). The activity was dose-dependent, showing a strong negative correlation with NaCl treatments in all the soybean cultivars except in Pusa 9712 ( $R^2 = 0.4919$ ).

The significant decrease in plant biomass, as observed in all soybean cultivars grown under different levels of NaCl stress, may be because salinity can affect external water potential and ion toxicity / imbalance (Hasegawa *et al.*, 2000). A high NaCl content of the soil reduces the uptake of mineral nutrients, particularly of  $K^+$ ,  $Mg^{2+}$  and  $Ca^{2+}$ . In consequence of these changes, biomass production, and hence the growth rate of the plant, is retarded (Pessaraki, 1994). Plants respond to environmental stress by means of osmotic adjustment, normally by increasing the concentration of  $Na^+$  and  $Cl^-$  in their tissues, although accumulations of inorganic ions may cause toxic effect and cell damage. Excess of  $Na^+$  and  $Cl^-$  in the protoplasm leads to ionic imbalance and induces ion-specific effects in enzymes, proteins and membranes (Arshi *et al.*, 2002, 2004). In the NaCl-treated plants, oxidative stress could stem from a decreased stomatal conductance in response to the osmotic imbalance and reduced leaf-water potential (Qureshi *et al.*, 2005),

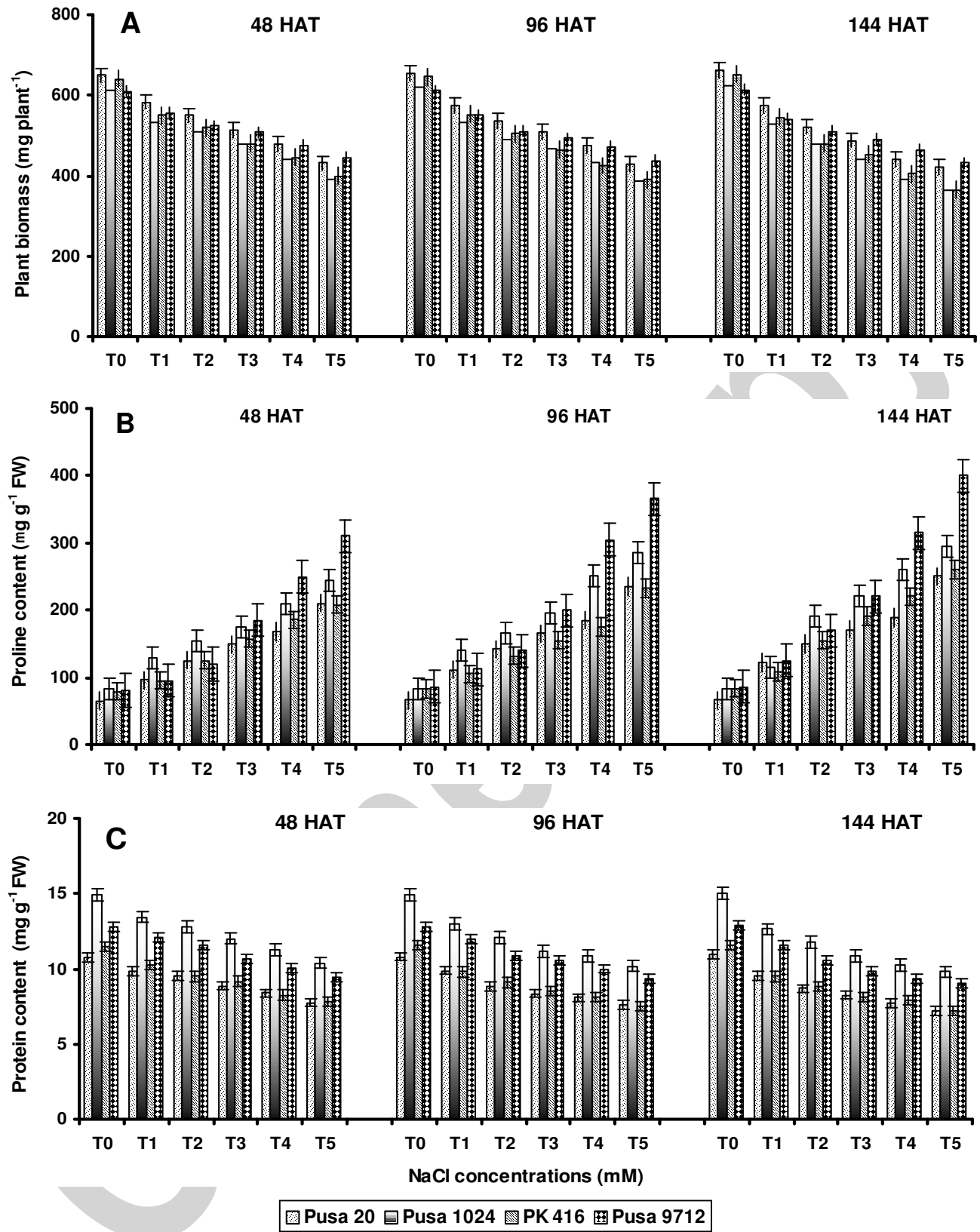


Fig. 1: (A) Plant biomass, (B) Proline content and (C) Protein content in the leaves of soybean cultivars exposed to salinity stress, as observed at 48, 96 and 144 HAT. The values represent the means of five replicates. SE is shown as error bars

**Table - 2:** Chloride (Cl<sup>-</sup>) content ( $\mu\text{mol g}^{-1}$  d.wt.) of the root, stem and leaf of soybean cultivars exposed to NaCl, as observed at 48, 96 and 144 hours after treatments (HAT)

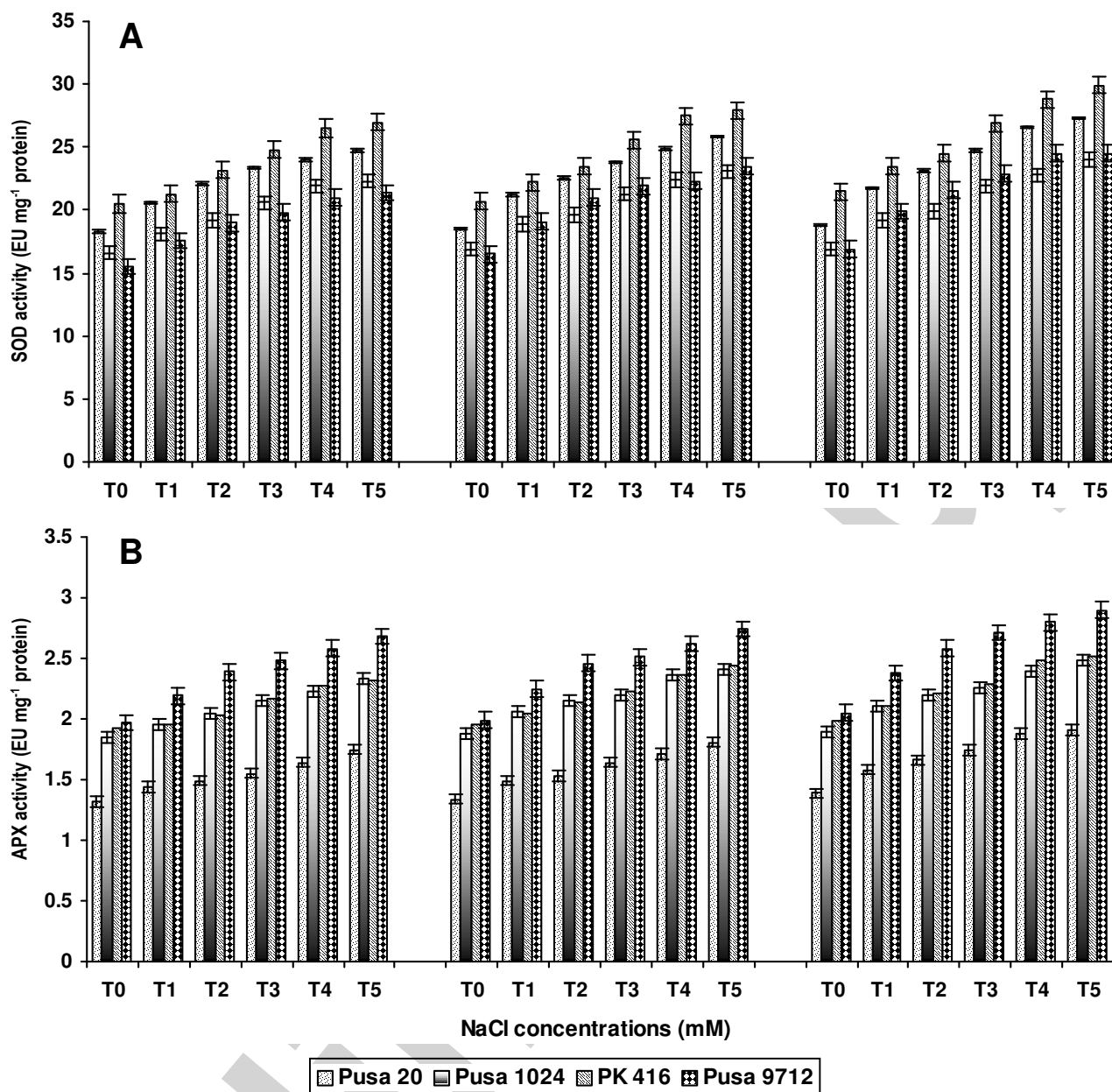
Genotypes	Concentration of NaCl applied (mM)																	
	48 HAT					96 HAT					144 HAT							
	00	20	40	60	80	100	00	20	40	60	80	100	00	20	40	60	80	100
	<b>Root Cl<sup>-</sup> content</b>																	
Pusa 20	5 <sup>a</sup>	60 <sup>d</sup>	80 <sup>c</sup>	110 <sup>b</sup>	140 <sup>c</sup>	170 <sup>d</sup>	7 <sup>a</sup>	80 <sup>b</sup>	109 <sup>c</sup>	141 <sup>d</sup>	175 <sup>c</sup>	200 <sup>d</sup>	15 <sup>a</sup>	90 <sup>b</sup>	110 <sup>c</sup>	150 <sup>d</sup>	185 <sup>c</sup>	205 <sup>b</sup>
Pusa 1024	10 <sup>a</sup>	50 <sup>c</sup>	65 <sup>b</sup>	85 <sup>c</sup>	110 <sup>d</sup>	150 <sup>c</sup>	15 <sup>a</sup>	85 <sup>b</sup>	100 <sup>c</sup>	110 <sup>b</sup>	125 <sup>d</sup>	165 <sup>c</sup>	15 <sup>a</sup>	90 <sup>c</sup>	105 <sup>d</sup>	120 <sup>c</sup>	135 <sup>d</sup>	175 <sup>c</sup>
PK 416	10 <sup>a</sup>	40 <sup>b</sup>	60 <sup>d</sup>	80 <sup>d</sup>	100 <sup>d</sup>	120 <sup>c</sup>	10 <sup>a</sup>	75 <sup>c</sup>	90 <sup>d</sup>	95 <sup>c</sup>	110 <sup>b</sup>	135 <sup>d</sup>	10 <sup>a</sup>	80 <sup>d</sup>	90 <sup>b</sup>	100 <sup>c</sup>	120 <sup>b</sup>	140 <sup>d</sup>
Pusa 9712	15 <sup>a</sup>	80 <sup>d</sup>	100 <sup>c</sup>	130 <sup>b</sup>	165 <sup>c</sup>	200 <sup>b</sup>	17 <sup>a</sup>	105 <sup>b</sup>	125 <sup>c</sup>	155 <sup>d</sup>	180 <sup>c</sup>	205 <sup>b</sup>	20 <sup>a</sup>	110 <sup>c</sup>	130 <sup>d</sup>	165 <sup>b</sup>	190 <sup>c</sup>	210 <sup>b</sup>
	<b>Stem Cl<sup>-</sup> content</b>																	
Pusa 20	5 <sup>a</sup>	55 <sup>b</sup>	70 <sup>d</sup>	100 <sup>c</sup>	120 <sup>d</sup>	160 <sup>b</sup>	10 <sup>a</sup>	90 <sup>d</sup>	100 <sup>d</sup>	130 <sup>c</sup>	150 <sup>d</sup>	160 <sup>d</sup>	10 <sup>a</sup>	80 <sup>c</sup>	95 <sup>d</sup>	110 <sup>c</sup>	150 <sup>b</sup>	180 <sup>c</sup>
Pusa 1024	10 <sup>a</sup>	40 <sup>c</sup>	60 <sup>b</sup>	75 <sup>c</sup>	95 <sup>c</sup>	135 <sup>d</sup>	15 <sup>a</sup>	60 <sup>c</sup>	75 <sup>b</sup>	90 <sup>d</sup>	110 <sup>c</sup>	155 <sup>b</sup>	15 <sup>a</sup>	75 <sup>d</sup>	90 <sup>c</sup>	110 <sup>b</sup>	135 <sup>d</sup>	166 <sup>c</sup>
PK 416	10 <sup>a</sup>	30 <sup>b</sup>	55 <sup>c</sup>	75 <sup>b</sup>	90 <sup>c</sup>	110 <sup>b</sup>	20 <sup>a</sup>	40 <sup>b</sup>	60 <sup>c</sup>	80 <sup>c</sup>	95 <sup>d</sup>	125 <sup>c</sup>	20 <sup>a</sup>	60 <sup>b</sup>	80 <sup>d</sup>	90 <sup>c</sup>	105 <sup>b</sup>	130 <sup>c</sup>
Pusa 9712	15 <sup>a</sup>	75 <sup>d</sup>	95 <sup>b</sup>	110 <sup>c</sup>	150 <sup>d</sup>	185 <sup>c</sup>	15 <sup>a</sup>	85 <sup>d</sup>	105 <sup>c</sup>	125 <sup>d</sup>	165 <sup>c</sup>	180 <sup>d</sup>	15 <sup>a</sup>	90 <sup>c</sup>	110 <sup>b</sup>	135 <sup>d</sup>	175 <sup>c</sup>	190 <sup>b</sup>
	<b>Leaf Cl<sup>-</sup> content</b>																	
Pusa 20	10 <sup>a</sup>	40 <sup>c</sup>	60 <sup>d</sup>	90 <sup>b</sup>	110 <sup>c</sup>	130 <sup>b</sup>	15 <sup>a</sup>	70 <sup>d</sup>	80 <sup>c</sup>	95 <sup>d</sup>	110 <sup>b</sup>	140 <sup>c</sup>	15 <sup>a</sup>	75 <sup>d</sup>	85 <sup>b</sup>	100 <sup>c</sup>	120 <sup>c</sup>	165 <sup>b</sup>
Pusa 1024	10 <sup>a</sup>	35 <sup>c</sup>	50 <sup>b</sup>	70 <sup>d</sup>	80 <sup>d</sup>	120 <sup>b</sup>	10 <sup>a</sup>	50 <sup>c</sup>	65 <sup>d</sup>	85 <sup>c</sup>	100 <sup>b</sup>	135 <sup>c</sup>	10 <sup>a</sup>	60 <sup>b</sup>	75 <sup>c</sup>	100 <sup>d</sup>	120 <sup>c</sup>	150 <sup>b</sup>
PK 416	10 <sup>a</sup>	30 <sup>c</sup>	45 <sup>b</sup>	70 <sup>c</sup>	85 <sup>b</sup>	100 <sup>d</sup>	10 <sup>a</sup>	35 <sup>b</sup>	55 <sup>d</sup>	75 <sup>b</sup>	90 <sup>c</sup>	100 <sup>d</sup>	10 <sup>a</sup>	50 <sup>c</sup>	65 <sup>b</sup>	80 <sup>c</sup>	100 <sup>b</sup>	120 <sup>c</sup>
Pusa 9712	15 <sup>a</sup>	70 <sup>d</sup>	90 <sup>c</sup>	100 <sup>d</sup>	135 <sup>c</sup>	150 <sup>c</sup>	17 <sup>a</sup>	70 <sup>d</sup>	85 <sup>c</sup>	100 <sup>d</sup>	125 <sup>d</sup>	160 <sup>b</sup>	20 <sup>a</sup>	80 <sup>c</sup>	100 <sup>d</sup>	120 <sup>c</sup>	150 <sup>c</sup>	180 <sup>b</sup>

The values are the mean of five individual readings. Data followed by the same letters are not significantly different at  $p \leq 0.05$  levels as determined by the Duncan's multiple range test (DMRT)

Table - 3: Potassium (K<sup>+</sup>) content (μmol g<sup>-1</sup> d.wt.) of the root, stem and leaf of soybean cultivars exposed to NaCl, as observed at 48, 96 and 144 hours after treatments (HAT)

Genotypes	Concentration of NaCl applied (mM)																	
	48 HAT					96 HAT					144 HAT							
	00	20	40	60	80	100	00	20	40	60	80	100	00	20	40	60	80	100
	<b>R o o t K<sup>+</sup> c o n t e n t</b>																	
Pusa 20	85 <sup>a</sup>	74 <sup>c</sup>	60 <sup>d</sup>	55 <sup>b</sup>	48 <sup>d</sup>	40 <sup>c</sup>	85 <sup>a</sup>	70 <sup>d</sup>	66 <sup>c</sup>	52 <sup>b</sup>	44 <sup>d</sup>	32 <sup>c</sup>	85 <sup>a</sup>	70 <sup>c</sup>	62 <sup>b</sup>	55 <sup>c</sup>	50 <sup>d</sup>	40 <sup>c</sup>
Pusa 1024	90 <sup>a</sup>	80 <sup>b</sup>	75 <sup>c</sup>	65 <sup>d</sup>	45 <sup>c</sup>	40 <sup>c</sup>	90 <sup>a</sup>	73 <sup>b</sup>	66 <sup>c</sup>	50 <sup>d</sup>	36 <sup>c</sup>	30 <sup>c</sup>	90 <sup>a</sup>	75 <sup>b</sup>	70 <sup>d</sup>	60 <sup>b</sup>	40 <sup>c</sup>	33 <sup>d</sup>
PK 416	90 <sup>a</sup>	80 <sup>d</sup>	72 <sup>b</sup>	55 <sup>c</sup>	45 <sup>c</sup>	34 <sup>d</sup>	90 <sup>a</sup>	70 <sup>c</sup>	62 <sup>b</sup>	50 <sup>d</sup>	41 <sup>b</sup>	32 <sup>c</sup>	90 <sup>a</sup>	75 <sup>d</sup>	65 <sup>c</sup>	55 <sup>d</sup>	45 <sup>b</sup>	35 <sup>d</sup>
Pusa 9712	85 <sup>a</sup>	80 <sup>c</sup>	71 <sup>b</sup>	60 <sup>d</sup>	50 <sup>b</sup>	41 <sup>b</sup>	85 <sup>a</sup>	75 <sup>b</sup>	66 <sup>d</sup>	55 <sup>c</sup>	45 <sup>b</sup>	39 <sup>d</sup>	85 <sup>a</sup>	75 <sup>b</sup>	65 <sup>c</sup>	54 <sup>d</sup>	44 <sup>b</sup>	41 <sup>b</sup>
	<b>S t e m K<sup>+</sup> c o n t e n t</b>																	
Pusa 20	110 <sup>a</sup>	105 <sup>c</sup>	90 <sup>d</sup>	81 <sup>c</sup>	74 <sup>d</sup>	55 <sup>b</sup>	110 <sup>a</sup>	95 <sup>b</sup>	85 <sup>d</sup>	75 <sup>b</sup>	66 <sup>c</sup>	50 <sup>d</sup>	110 <sup>a</sup>	93 <sup>c</sup>	83 <sup>b</sup>	70 <sup>c</sup>	61 <sup>d</sup>	44 <sup>b</sup>
Pusa 1024	105 <sup>a</sup>	100 <sup>b</sup>	90 <sup>d</sup>	81 <sup>c</sup>	70 <sup>d</sup>	50 <sup>c</sup>	105 <sup>a</sup>	90 <sup>c</sup>	85 <sup>d</sup>	75 <sup>b</sup>	66 <sup>c</sup>	45 <sup>b</sup>	105 <sup>a</sup>	88 <sup>d</sup>	80 <sup>b</sup>	70 <sup>c</sup>	60 <sup>d</sup>	40 <sup>c</sup>
PK 416	110 <sup>a</sup>	100 <sup>b</sup>	83 <sup>c</sup>	75 <sup>d</sup>	60 <sup>c</sup>	47 <sup>c</sup>	110 <sup>a</sup>	95 <sup>d</sup>	80 <sup>c</sup>	70 <sup>d</sup>	55 <sup>b</sup>	42 <sup>b</sup>	110 <sup>a</sup>	92 <sup>b</sup>	78 <sup>b</sup>	60 <sup>d</sup>	50 <sup>b</sup>	39 <sup>c</sup>
Pusa 9712	110 <sup>a</sup>	105 <sup>d</sup>	95 <sup>b</sup>	85 <sup>d</sup>	68 <sup>b</sup>	60 <sup>d</sup>	110 <sup>a</sup>	100 <sup>c</sup>	90 <sup>b</sup>	80 <sup>c</sup>	60 <sup>d</sup>	52 <sup>c</sup>	110 <sup>a</sup>	95 <sup>b</sup>	88 <sup>d</sup>	75 <sup>b</sup>	55 <sup>c</sup>	46 <sup>d</sup>
	<b>L e a f K<sup>+</sup> c o n t e n t</b>																	
Pusa 20	120 <sup>a</sup>	110 <sup>b</sup>	90 <sup>d</sup>	80 <sup>c</sup>	70 <sup>d</sup>	65 <sup>c</sup>	120 <sup>a</sup>	105 <sup>c</sup>	90 <sup>b</sup>	85 <sup>c</sup>	70 <sup>c</sup>	60 <sup>b</sup>	120 <sup>a</sup>	95 <sup>d</sup>	80 <sup>c</sup>	70 <sup>b</sup>	65 <sup>c</sup>	54 <sup>d</sup>
Pusa 1024	120 <sup>a</sup>	115 <sup>c</sup>	102 <sup>b</sup>	90 <sup>b</sup>	75 <sup>c</sup>	60 <sup>d</sup>	110 <sup>a</sup>	100 <sup>d</sup>	90 <sup>b</sup>	85 <sup>c</sup>	75 <sup>b</sup>	69 <sup>c</sup>	110 <sup>a</sup>	105 <sup>c</sup>	90 <sup>b</sup>	80 <sup>d</sup>	66 <sup>c</sup>	47 <sup>b</sup>
PK 416	120 <sup>a</sup>	110 <sup>b</sup>	100 <sup>b</sup>	85 <sup>d</sup>	70 <sup>b</sup>	55 <sup>c</sup>	105 <sup>a</sup>	100 <sup>b</sup>	95 <sup>c</sup>	75 <sup>b</sup>	65 <sup>c</sup>	45 <sup>d</sup>	105 <sup>a</sup>	95 <sup>b</sup>	90 <sup>d</sup>	70 <sup>c</sup>	60 <sup>d</sup>	43 <sup>b</sup>
Pusa 9712	120 <sup>a</sup>	100 <sup>b</sup>	95 <sup>c</sup>	87 <sup>d</sup>	79 <sup>c</sup>	72 <sup>b</sup>	120 <sup>a</sup>	95 <sup>d</sup>	90 <sup>b</sup>	75 <sup>b</sup>	70 <sup>d</sup>	60 <sup>c</sup>	120 <sup>a</sup>	90 <sup>c</sup>	80 <sup>b</sup>	70 <sup>c</sup>	60 <sup>d</sup>	55 <sup>c</sup>

The values are the mean of five individual readings. Data followed by the same letters are not significantly different at p<0.05 levels as determined by the Duncan's multiple range test (DMRT)



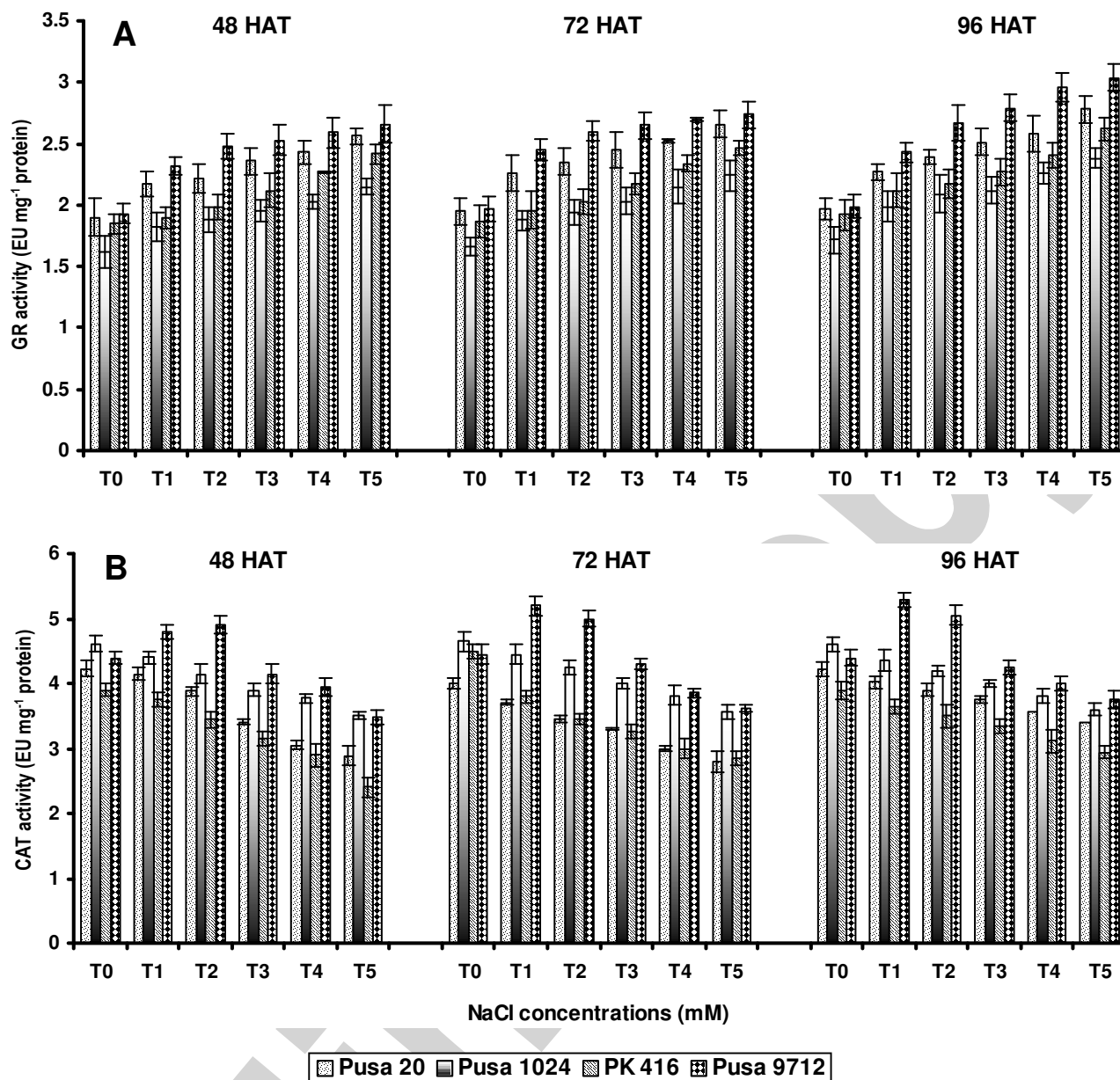
**Fig. 2:** Activities of (A) SOD and (B) APX in the leaves of soybean cultivars exposed to salinity stress, as observed at 48, 96 and 144 HAT. The values represent the means of five replicates. SE is shown as error bars

ultimately leading to a decrease in photosynthesis and biomass accumulation (Blumwald *et al.*, 2000).

Accumulation of inorganic ions, predominantly of Na<sup>+</sup> and Cl<sup>-</sup>, has an important role in the process of osmotic adjustment (Arshi *et al.*, 2005; Gzik, 1996). The Na<sup>+</sup> content was maximum in roots, followed by stem and then leaves in all soybean cultivars studied. Pusa 9712 stored the maximum Na<sup>+</sup> with increase in the level of NaCl treatment. The preferential accumulation in roots may be interpreted as a mechanism of tolerance that works (a) by maintaining a substantial potential for osmotic water uptake into roots,

and (b) by restricting the spread of Na<sup>+</sup> to shoots (Renault *et al.*, 2001). High NaCl concentration strongly inhibits the uptake and accumulation of K<sup>+</sup> and Ca<sup>2+</sup> by roots. Since K<sup>+</sup> is involved in turgor control, inhibition of K<sup>+</sup> uptake should stunt the growth (Renault *et al.*, 2001). High Na<sup>+</sup> levels in the external medium greatly reduce the physicochemical activity of the dissolved calcium and may displace Ca<sup>2+</sup> from the plasma membrane of root cells (Cramer *et al.*, 1986), affecting the Na<sup>+</sup>/K<sup>+</sup> uptake selectivity in favour of sodium. A low Ca<sup>2+</sup> concentration under salinity stress may severely affect the functions of membranes as a barrier to ion loss from cells (Boursier and Lauchli, 1990).





**Fig. 3:** Activities of (A) GR and (B) CAT in the leaves of soybean cultivars exposed to salinity stress, as observed at 48, 96 and 144 HAT. The values represent the means of five replicates. SE is shown as error bars

Proline accumulates rapidly and more frequently than any other amino acids under stressful conditions. It helps in osmotic adjustment and protection of plasma membrane integrity (Mansour, 1998) and acts as a sink of energy or a reducing power (Verbruggen *et al.*, 1996), as a source of carbon and nitrogen (Peng *et al.*, 1981), and/or as a hydroxyl radical scavenger (Hong *et al.*, 2000). Salinity stress may increase activities of proline biosynthetic enzymes and/or inhibit proline dehydrogenase (ProDH) activity (Sumithra *et al.*, 2006). In the soybean cultivars, proline accumulation was greater under high salinity stress. The changes in proline content are suggestive of altered membrane permeability (Ali *et al.*, 1999; Qureshi *et al.*, 2007) and stress tolerance (Mansour, 2000).

The present study demonstrated a dose-dependent, salinity-induced decline in the soluble-protein content. In fact, the low water potential in saline conditions decreases photosynthesis and biomass accumulation, but enhances accumulation of abscisic acid (ABA), proline content and formation of radical scavenging compounds (Bartels and Sunkar, 2005; Yordanov *et al.*, 2003). ABA reduces protein synthesis and accelerates protein degradation as do the osmotic and water stresses (Arshi *et al.*, 2006; Vartanian *et al.*, 1987). Increase in ROS can lead to oxidation of amino-acids-residue side chains, formation of protein-protein cross-linkages and oxidation of the protein backbone, resulting in protein degradation (Bartels and Sunkar, 2005; Berlett and Stadtman, 1997).

**Table - 4:** Calcium (Ca<sup>2+</sup>) content ( $\mu\text{mol g}^{-1}$  d.wt.) in the root, stem and leaf of soybean cultivars exposed to NaCl, as observed at 48, 96 and 144 hours after treatments (HAT)

Genotypes	Concentration of NaCl applied (mM)																	
	48 HAT					96 HAT					144 HAT							
	00	20	40	60	80	100	00	20	40	60	80	100	00	20	40	60	80	100
	<b>Root Ca<sup>2+</sup> content</b>																	
Pusa 20	130 <sup>a</sup>	115 <sup>d</sup>	95 <sup>e</sup>	90 <sup>e</sup>	80 <sup>b</sup>	65 <sup>d</sup>	140 <sup>a</sup>	90 <sup>b</sup>	75 <sup>c</sup>	70 <sup>d</sup>	65 <sup>d</sup>	60 <sup>d</sup>	10 <sup>a</sup>	90 <sup>c</sup>	85 <sup>d</sup>	75 <sup>c</sup>	65 <sup>d</sup>	50 <sup>b</sup>
Pusa 1024	140 <sup>a</sup>	130 <sup>c</sup>	115 <sup>d</sup>	100 <sup>d</sup>	80 <sup>b</sup>	66 <sup>d</sup>	130 <sup>a</sup>	115 <sup>d</sup>	100 <sup>b</sup>	90 <sup>c</sup>	80 <sup>b</sup>	60 <sup>d</sup>	110 <sup>a</sup>	90 <sup>c</sup>	80 <sup>d</sup>	70 <sup>b</sup>	55 <sup>e</sup>	50 <sup>b</sup>
PK 416	140 <sup>a</sup>	125 <sup>c</sup>	115 <sup>d</sup>	90 <sup>b</sup>	75 <sup>c</sup>	55 <sup>e</sup>	140 <sup>a</sup>	115 <sup>d</sup>	100 <sup>b</sup>	80 <sup>d</sup>	66 <sup>d</sup>	50 <sup>b</sup>	110 <sup>a</sup>	85 <sup>d</sup>	70 <sup>c</sup>	66 <sup>d</sup>	54 <sup>c</sup>	40 <sup>d</sup>
Pusa 9712	140 <sup>a</sup>	120 <sup>c</sup>	100 <sup>c</sup>	90 <sup>b</sup>	85 <sup>d</sup>	75 <sup>c</sup>	140 <sup>a</sup>	120 <sup>c</sup>	115 <sup>d</sup>	90 <sup>c</sup>	80 <sup>b</sup>	70 <sup>c</sup>	105 <sup>a</sup>	90 <sup>b</sup>	80 <sup>b</sup>	74 <sup>d</sup>	65 <sup>d</sup>	50 <sup>b</sup>
	<b>Stem Ca<sup>2+</sup> content</b>																	
Pusa 20	110 <sup>b</sup>	100 <sup>b</sup>	90 <sup>c</sup>	80 <sup>d</sup>	70 <sup>c</sup>	65 <sup>d</sup>	110 <sup>b</sup>	95 <sup>c</sup>	90 <sup>b</sup>	80 <sup>d</sup>	70 <sup>c</sup>	60 <sup>c</sup>	140 <sup>a</sup>	120 <sup>d</sup>	100 <sup>c</sup>	90 <sup>d</sup>	80 <sup>c</sup>	70 <sup>d</sup>
Pusa 1024	110 <sup>b</sup>	105 <sup>b</sup>	95 <sup>c</sup>	80 <sup>d</sup>	70 <sup>c</sup>	60 <sup>d</sup>	110 <sup>b</sup>	95 <sup>c</sup>	85 <sup>d</sup>	75 <sup>c</sup>	62 <sup>d</sup>	55 <sup>b</sup>	130 <sup>a</sup>	110 <sup>b</sup>	90 <sup>d</sup>	85 <sup>c</sup>	72 <sup>b</sup>	60 <sup>c</sup>
PK 416	110 <sup>b</sup>	95 <sup>d</sup>	80 <sup>b</sup>	75 <sup>c</sup>	65 <sup>b</sup>	50 <sup>c</sup>	110 <sup>b</sup>	90 <sup>b</sup>	75 <sup>c</sup>	70 <sup>c</sup>	60 <sup>d</sup>	4 <sup>b</sup>	140 <sup>a</sup>	110 <sup>b</sup>	90 <sup>d</sup>	75 <sup>b</sup>	60 <sup>c</sup>	50 <sup>b</sup>
Pusa 9712	105 <sup>b</sup>	100 <sup>c</sup>	90 <sup>d</sup>	85 <sup>b</sup>	80 <sup>d</sup>	70 <sup>b</sup>	105 <sup>b</sup>	95 <sup>d</sup>	85 <sup>b</sup>	80 <sup>b</sup>	75 <sup>c</sup>	64 <sup>d</sup>	140 <sup>a</sup>	120 <sup>c</sup>	115 <sup>b</sup>	100 <sup>d</sup>	90 <sup>c</sup>	75 <sup>d</sup>
	<b>Leaf Ca<sup>2+</sup> content</b>																	
Pusa 20	150 <sup>a</sup>	140 <sup>c</sup>	130 <sup>d</sup>	120 <sup>b</sup>	100 <sup>d</sup>	90 <sup>b</sup>	150 <sup>a</sup>	130 <sup>d</sup>	120 <sup>c</sup>	105 <sup>b</sup>	95 <sup>c</sup>	86 <sup>d</sup>	150 <sup>a</sup>	140 <sup>b</sup>	130 <sup>c</sup>	120 <sup>d</sup>	100 <sup>c</sup>	82 <sup>b</sup>
Pusa 1024	150 <sup>a</sup>	135 <sup>d</sup>	130 <sup>d</sup>	115 <sup>b</sup>	95 <sup>c</sup>	85 <sup>b</sup>	140 <sup>a</sup>	130 <sup>d</sup>	120 <sup>c</sup>	115 <sup>b</sup>	90 <sup>c</sup>	75 <sup>b</sup>	140 <sup>a</sup>	130 <sup>d</sup>	120 <sup>b</sup>	105 <sup>c</sup>	80 <sup>d</sup>	72 <sup>c</sup>
PK 416	150 <sup>a</sup>	130 <sup>b</sup>	110 <sup>c</sup>	100 <sup>d</sup>	80 <sup>b</sup>	70 <sup>d</sup>	150 <sup>a</sup>	130 <sup>d</sup>	115 <sup>d</sup>	100 <sup>c</sup>	85 <sup>b</sup>	68 <sup>d</sup>	150 <sup>a</sup>	125 <sup>c</sup>	105 <sup>d</sup>	90 <sup>b</sup>	75 <sup>c</sup>	65 <sup>b</sup>
Pusa 9712	140 <sup>a</sup>	130 <sup>b</sup>	125 <sup>b</sup>	115 <sup>c</sup>	100 <sup>d</sup>	90 <sup>c</sup>	150 <sup>a</sup>	140 <sup>c</sup>	130 <sup>b</sup>	122 <sup>b</sup>	105 <sup>d</sup>	90 <sup>c</sup>	150 <sup>a</sup>	135 <sup>b</sup>	125 <sup>c</sup>	115 <sup>d</sup>	95 <sup>b</sup>	88 <sup>d</sup>

The values are the mean of five individual readings. Data followed by the same letters are not significantly different at  $p \leq 0.05$  levels as determined by the Duncan's multiple range test (DMRT)

Enzymes of the ascorbate-glutathione-cycle often act as an indicator of plant tolerance against stress. The activities of SOD, APX and GR in the treated soybean cultivars increased with increase in the salinity level. The increase was greater in Pusa 9712 than in other cultivars. SOD and APX play a critical role in the nodule activity of leguminous roots; over-expression of antioxidant enzymes provides additional protection to the process of N<sub>2</sub> fixation in legumes (Maria *et al.*, 2002).

The increased APX activity would increase demand for ascorbate regeneration mediated by an increased GR activity. It is believed that a simultaneous increase in several components of the antioxidant defense system is necessary to gain in stress tolerance (Foyer *et al.*, 1987). Our results are in agreement with earlier findings in relation to mercury, arsenic, chromium and cadmium-induced oxidative stress in Indian mustard (Anjum *et al.*, 2008; Ansari *et al.*, 2009; Diwan *et al.*, 2007, 2010a,b; Khan *et al.*, 2009).

The increase in GR in the salt-treated plants should result in a high pool of GSH, which could be used in ascorbate (ASC) regeneration (Lechno *et al.*, 1997). Analysis of antioxidant defence system in various cell fractions under NaCl treatments revealed that long-term salt treatment causes significant increase in the activity of enzymes of the ascorbate-glutathione cycle (the Halliwell-Asada pathway) in the soluble fraction in salt-tolerant plants (Hernandez and Almansa, 2002). However, no change was observed in the specific activities of cytosolic APX, MDHAR or GR in sensitive plants (Hernandez *et al.*, 2000).

A significant dose-dependent decrease in CAT activity was observed under NaCl stress in all soybean cultivars except in Pusa 9712 with a low NaCl stress. The decline of CAT activity under stressful condition is possibly due to a reduced rate of protein turnover (Hertwig *et al.*, 1992). It could be due also to enhancement of H<sub>2</sub>O<sub>2</sub> levels (Qureshi *et al.*, 2007). The difference in the magnitude of CAT activity among different soybean cultivars could be because of a greater production of ROS in the susceptible cultivars than in the resistant ones growing under salt stress. In resistant cultivars, CAT activity might be induced in response to salt stress to combat ROS.

In summary, our results suggest that salinity stress adversely affected soybean in a dose-dependent manner. It enhanced proline content; which could be involved in stress-resistance mechanisms by acting as an osmoprotectant. Salt stress also increased the activities of antioxidant enzymes to protect plants from oxidative damage. These changes occurred maximally in Pusa 9712, suggesting that this cultivar possesses a regulated antioxidant defense system to facilitate adaptation to salinity. It may therefore show a better growth and yield on saline soils and could be a preferential choice for soybean cultivation in salinity-affected regions.

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