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RESEARCH ARTICLE

# Physiological responses of seven varieties of soybean [*Glycine max* (L.) Merr.] to salt stress

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## Abstract

In agriculture, salinity is one of the most significant abiotic stress that plants confront and harms agricultural productivity, physiological growth and development processes. In the present study, there were 7 different varieties of soybean (Ajmeri, William-82, D.A, PSC-60, Rawal-1, NARC-1 and NARC-2) were tested under NaCl concentration level (0 mM and 150 mM) to determine their physiological performance under control and experimental conditions. The present investigation aimed to select salt tolerant varieties. Under salt stress, different varieties have differ significantly in the biological yield, chlorophyll contents, antioxidant activity and ionic concentrations. The results showed that among the seven varieties evaluated NARC-1 and NARC-2 were producing higher biological yield and antioxidant activity than others under 150 mM NaCl. NARC-1 and NARC-2 under 150 mM NaCl concentration produced significantly higher biomass in comparison with other varieties and similarly enhance the antioxidant activity by decreasing the catalase activity. The relative water content (RWC) of plants was measured 15, 30, 45 and 60 days after the treatment was applied, as well as at harvest time, along with the grain yield and characters related to yield. The 7 different soybean varieties tested showed significant differences in grain yield and yield-associated characters when exposed to salinity. The salinity had a greater impact on Ajmeri and William than on NARC-1 and NARC-2. Under salt stress, the grain yield of the NARC-1 and NARC-2 varieties was 70% and 65% respectively, while the yields of the Ajmeri and william-82 varieties were 41% and 38% respectively. The salinity-induced decrease in grain yield was traced to fewer pods per plant, fewer seeds per pod and a lighter weight per 100 grains. However, the number of pods per plant was most affected compared to the other characters. It was also observed that $Na^+$ ion concentrations were elevated in the shoot under salt stress in all varieties. However, NARC-1 and NARC-2 showed low salt concentration in shoot as compared to other varieties. SDS-PAGE revealed significant variations in the protein profile of seedling soybean varieties. NARC-1 and NARC-2 have shown a unique banding pattern under salt stress with a molecular weight of 60 and 130 kDa. The results indicate that salinity (NaCl) triggered an antioxidant response in tolerant varieties (NARC-1 and NARC-2) of *Glycine max* (L.). This study suggested that both varieties have more capability and appropriate survival under salt stress as compared to other varieties.

## Keywords

Antioxidant activity, biological yield, chlorophyll constituents, *Glycine max*, salinity

## Introduction

Legume *Glycine max* (L.) Merr. is widely regarded as one of the most significant crop on the earth (1). As a source of food, fuel and energy, soybean is one of the world's most important crop (2) and is recognized as the fourth most important yield producing crop in the world (3). Protein, hormones, phospholipids and antioxidants are all found in abundance in this plant (4). Tocopherols, sugars, fatty acids and organic acids, as well as sterols and volatile chemicals, are abundant in soybeans (5). Dicot crops have been classified on the basis of variable salt tolerance level. However, soybean is considered as "salt sensitive crop" because of their ability to thrive under adverse conditions (6).

Biotic and abiotic stresses exert a negative impact on yield potentials of the field-grown crops such stresses cause poor growth and development in crops. Salinity, as one of the challenge for irrigation farming, affects soil fertility and limit production (7). Salinity causes adverse effects on the growth and development of plants by interfering with vital metabolic processes (8). It is a limiting factor for plant biomass accumulation, antioxidant enzyme degradation, malondialdehyde (MDA) and hydrogen peroxide imbalance, proteins and peptides shortage and stress-related protein accumulation (9). The chlorophyll contents of plants also become reduced as salinity stress deteriorates photosynthetic pigments in a plant which directly affects the plant's production and growth (10). Additionally, salt stress damages proteins, down-regulation of photosynthesis-related proteins and reduces photosynthesis that leading to a reduction in plant growth (11). Different physio-biochemical changes occur in plants grown under salinity stress (12). Salinity is involved in the overproduction of reactive oxygen species (ROS) in plants which cause oxidative damage to proteins and plant cells (13). To minimize the effects of all these problems it is necessary to screen such varieties which are salinity tolerant and can grow on land affected by high salts concentrations (14). Salt tolerance in plants depends upon growth stages, type of plant, the nutrients provided to the plant and its environment. Various biochemical and physiological markers have been used for salinity tolerance along with changes in genetic makeup identified in soybean for its growth in saline conditions (15).

This work has been undertaken to relate the effect of salt on seven soybean varieties that are commonly grown in Pakistan. The study aims to screen out the varieties that performed well even under salinity and indicate a preference for breeding soybean varieties to develop salt tolerance.

## Materials and Methods

There were 7 soybean varieties seeds obtained from the Ayub Agriculture Research Institute in Faisalabad in Pakistan: Ajmeri, William-82, D.A., PSC-60, Rawal-1 NARC-1 and NARC-2. Under natural day/night conditions (14h light/10h dark) at 28 °C in a greenhouse at Bahauddin Zakariya University Multan, Pakistan, the experiment was conducted. Before conducting experiments, soybean seeds were treated with 5% sodium hypochlorite for 5 min to sterile the surface. Seeds were sowed in 21 cm plastic pots with a diameter of 15.5 cm and a depth of 20 cm, filled with thoroughly washed 4 kg sand. The muslin fabric was used to cover the drainage holes in the bottom of each pot. Five replicates were used for the experiment's arrangement, which was completely randomized. A complete solution of Hoagland's solution was used to irrigate all pots. In just one week, the seeds sprouted and were thinned to a maximum of eight plants per pot. The number of plants in each pot was reduced to five after the second week of growth. The two weeks old seedlings were subjected to salt treatment 0 mM and 150 mM NaCl up until they matured. Two plants for each sample were taken 15, 30, 45, 60 and 80 days for chemical and molecular study, some samples were maintained at -80 °C, while others were dried for additional phytochemical investigation.

### *Fresh and dry weight*

Fresh biomass of plants was subjected to standard method (16) to separating root and shoot with sterilized blade after 2 weeks of salinity treatment and dry biomass was to be recorded by placing the plants in oven at 75 °C for 72 hrs (17) and measured by electric balance and the mean of 3 plants was recorded for accuracy of data.

### *Relative water content (RWC)*

It was determined that the leaflets of the second fully expanded leaf from the top of the main stem had a relative water content (RWC) that could be measured at 15, 30, 45 and 60 days after imposition of salt stress. After the leaves had been harvested and brought to the laboratory, a measurement of the leaf's fresh weight was performed. Following an 8 hr soaking in distilled water, the leaf samples were blotted for surface drying and then the water saturated leaf weight was determined. After being dried in an oven at a temperature of 80 °C until they reached a weight that was constant, the leaf dry weight was determined. The following is the formula that was used to determine RWC (18):

$$\text{RWC (\%)} = (\text{fresh weigth} - \text{dry weight} / \text{turgid weight} - \text{dry weight}) \times 100$$

### *Total proteins and free amino acids*

The water-soluble protein quantification was done by described method (19) and the protein samples were prepared by blending 1 g leaf in sodium phosphate buffer (50 mM; pH 7.5). Protein contents were quantified as mg/g of seedling using standard Bovine Serum Albumin (BSA). The absorbance at 595 nm wavelength of protein samples was obtained through UV visible spectrophotometer (Hitachi U-2900, Tokyo Japan). The free amino acids were estimated using a described standard procedure (20). Total free amino acids were estimated by pyridine-ninhydrin as standard and absorbance were taken at 570 nm.

### *Chlorophyll estimation*

Leaf photosynthetic pigments were evaluated by using described acetone method (21). Plant leaves were blended and mixed in 80% acetone and optical densities (OD) were

taken at 750, 663, 652, 645 and 470 nm using a spectrophotometer (Hitachi U-2900, Tokyo Japan).

### *Malondialdehyde Content (MDA) Estimation*

Malondialdehyde contents were determined by described thiobarbituric acid method (22). The sample (0.5 g) was grinded in pre-chilled pester and mortar on precooled phosphate buffer (pH 7.8). Centrifuge the sample up to 8000-13000 rotation per minute for 15 min at 4 °C and finally mixed with 0.5% thiobarbituric acid along with 5% trichloroacetate. The final mixtures were heated at 95 °C for 30 min placed water bath and cooled to room temperature. Finally, mixtures were centrifuged at 6000 × g for 15 mins and obtained supernatants were subjected to detection at a wavelength of 450, 532 and 600 nm. The MDA concentrations were calculated by following described formula:

$$MDA\ (\mu mol/ml) = 6.45 \times (D532 - D600) - 0.56 \times D450$$

### *$H_2O_2$ determination*

$H_2O_2$ was observed by using a described protocol (23) 0.5 g of a leaf was blended in 5 ml of 0.1% (w/v) trichloroacetate. The paste was centrifuged at 10000 × g for 30 min. The obtaining supernatant was diluted with an equal volume of sodium phosphate buffer and finally dissolved in 1 ml of potassium iodide buffer. It was shaken well and recorded absorbance at 390 nm with a spectrophotometer.

### *Sodium and potassium ions Analysis*

Ions analysis was performed by oven-dried roots and shoots at 72 ℃ for 24 hrs. Oven dry samples were ground and 0.2 g powder was digested with 0.1% Conc. $HNO_3$ at 115 ℃ for 4 hrs. The digested samples were subjected to a flame photometer to determine the sodium and potassium ions (24).

### *Dynamic study of catalase activity (CAT)*

The catalase enzyme activity was evaluated through $H_2O_2$ decomposition at 240 nm (25). The samples were placed in a precooled container filled with liquid nitrogen and 0.5 g of sample was blended in 8 ml sodium phosphate buffer. The samples were mixed with 0.1 ml of $H_2O_2$ and readings were recorded at 240 nm with a period of 0-30 seconds.

### *SDS-PAGE*

The protein banding profile of seedlings was analyzed using SDS-PAGE as the standard described protocol (26). After electrophoresis, gels were placed in a solution of methanol/acetic acid/water solution (50:10:40) with 0.25% Coomassie brilliant blue R-250 for 30 minutes (27) and destained with the same solvent without Coomassie dye. The protein banding pattern of plant samples and protein marker (Bio-basic BG00363) were compared on SDS-PAGE to determine the molecular weight of protein.

### *Statistical analysis*

Data were statistically examined through One Way Completely Randomized Analysis of Variance (1WCR ANOVA) by using COSTAT software (Cohort Software, Berkeley, California) (28).

## Results and Discussion

Morphological and physiological processes were adversely affected by salt stress. Salinity reduces growth in soybean by interfering with physiological pathways, as well as identifying salt-sensitive, moderate and tolerant soybean types in the current study. The morphological and development characteristics of seven soybean varieties were compared under well water condition (Fig.1). The results revealed that some varieties (Ajmeri, William-82, D.A., PSC-60, Rawl-1) were found to be salt sensitive but NARC-1 and NARC-2 were showed salt-tolerant varieties. These results demonstrated that the wild plants retained a higher water status under salinity conditions than the more sensitive cultivars.

Seven varieties were found to have substantial differences in fresh and dry weight reduction (Table 1). Salt stress showed a significant drop in the fresh and dry plant

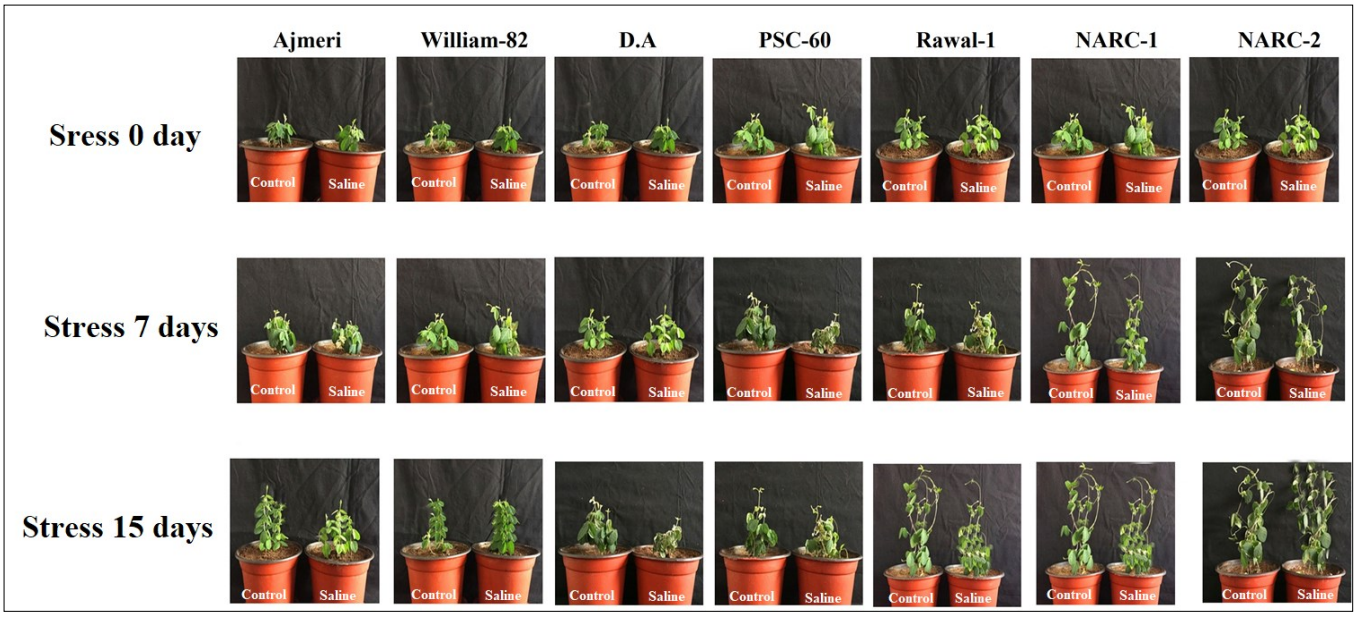


**Fig. 1**. Salt stress-related phenotypes of soybean varieties (Ajmeri, William-82, D.A., PSC-60, Rawl-1 NARC-1, and NARC-2) under salt stress (0 mM and 150 mM) for 7 to 15 days.

**Table 1**. Analysis of variance for biomass (g/plant) of Soybean varieties after salinity treatment.

| Source of Variance | Df | Shoot f. wt. (g/plant) | Shoot d. wt. (g/plant) | Root f. wt. (g/plant) | Root d. wt. (g/plant) |
|---|---|---|---|---|---|
| *Variety* | 6 | 5.029* | 0.679*** | 0.776*** | 1.936*** |
| *Salinity* | 1 | 500.585*** | 8.447*** | 11.160*** | 7.541*** |
| *Variety×Salinity* | 6 | 2.832* | 0.091* | 0.068* | 0.040* |
| *Error* | 42 | 2.122 | 0.042 | 0.152 | 0.031 |

*,*** shows Significant level of 0.05 and 0.001 respectively

biomass of the salt-sensitive varieties Ajmeri and William-82. Two varieties (NARC-1 and NARC-2) revealed higher biomass as compared to the control and other varieties. Thus, 7 varieties were classified in response to salinity in the 3 sets, i.e., salt tolerant (NARC-1 and NARC-2), moderate salt-tolerant varieties (Rawal-1, D.A and PSC-60) and the salt-sensitive Ajmeri and William-82 (Fig. 2). Changes

Total free amino acid contents were reduced in all tested 7 varieties as compared to the control. The variation in the content of the amino acid under salinity of all soybean varieties is illustrated in Fig. 4. Mean of data was calculated by two-way completely randomized ANOVA showed a significant increase in the NARC-1 and NARC-2 ($p<0.001$) as compared to the other varieties (Table. 2). The

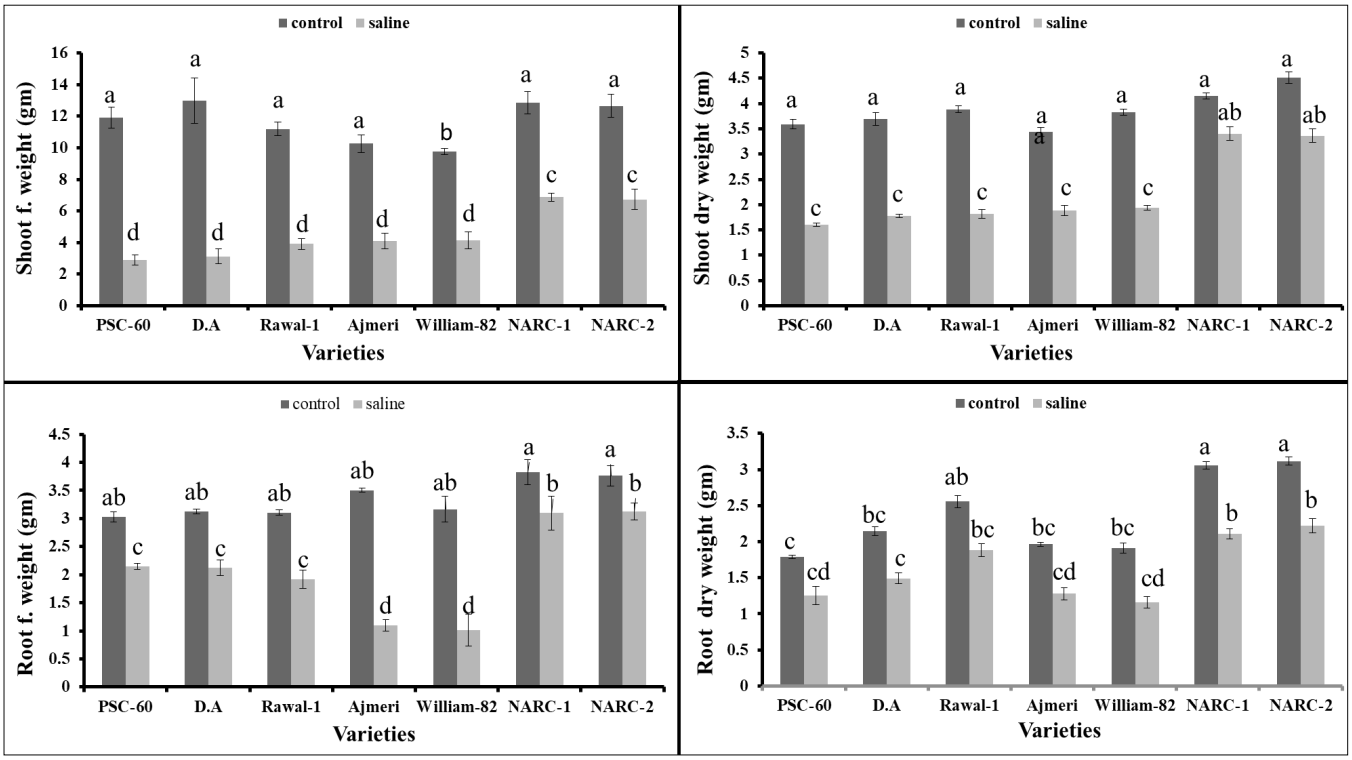


**Fig. 2**. Fresh and dry biomass (g/plant) of seven soybean varieties after under salt stress.

plant dry weight have been reported like that of the plant fresh weight. Significant reduction in both shoot and root growth have been observed under saline conditions (29). Morphologically, the symptoms of salt stress injury were detained plant growth due to hindrance in the cell expansion (30).

Therefore, limited water absorption causes serious cellular growth and developmental consequences which lead to a reduction in stem and root growth (31). On the first day of salt treatment, the RWC differences between the control varieties of soybean and the salt-treated varieties were not statistically significant (Fig. 3). After 30 days of salt treatment and again at harvest (60 days), there was a significant difference in RWC between non-stressed and salt-treated varieties of soybean. According to the findings of our research, out of a total of 7 varieties, NARC-1 and NARC-2 have maintained better water relations when subjected to salt stress.

previous studies supported the above results that free amino acids contents were increased under salt stress (32). Plants that accumulate free amino acid content in the cell during salt stress have been suggested as a chief contribution to the osmotic adjustment (33), inhibit ROS production and stabilized the protein as well as cellular membranes integrity (34).

The total soluble proteins were estimated with Bradford's assay. It is indicated that NARC-1 and NARC-2 showed significantly higher protein concentrations as compared to other varieties (Table 2). The control had a greater protein quantity as compared to all tested varieties under salinity (Fig. 4). It is commonly known that the physiological status of plants was determined through the presence of the total soluble proteins. It was reported that salt stress stimulated signal transduction pathways that modulated the gene expression

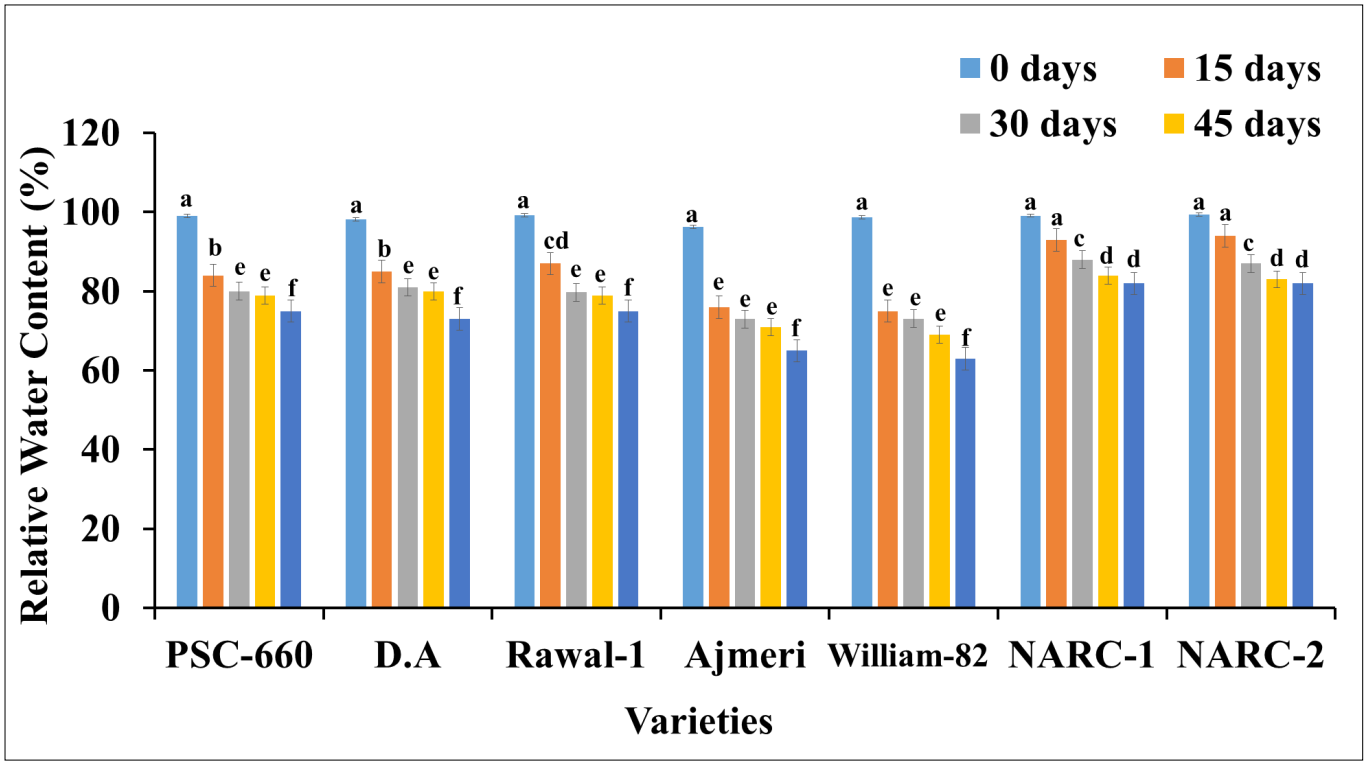


**Fig. 3**. Leaf relative water content of soybean varieties under salt stress.

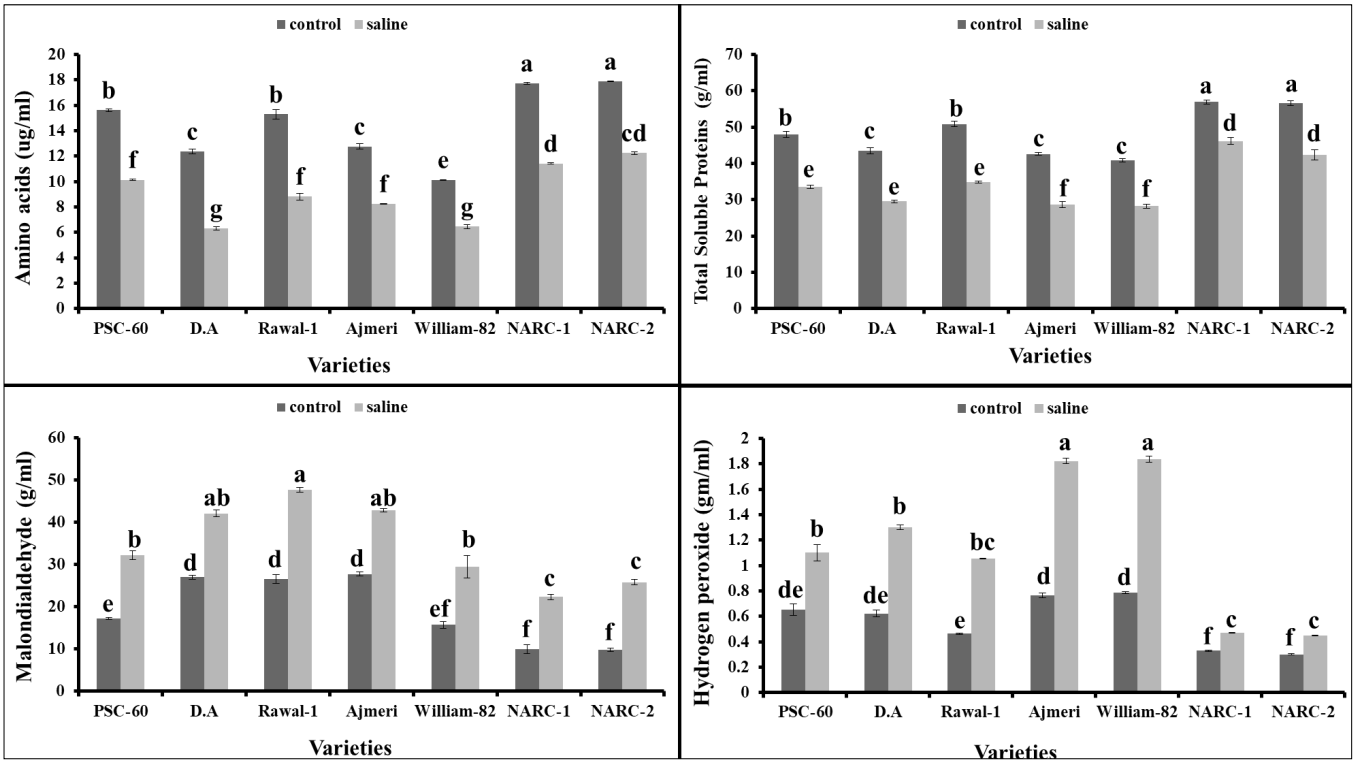


**Fig. 4**. Soluble proteins, free amino acids, MDA and $H_2O_2$ content (mg/g) of soybean varieties.

**Table 2**. Analysis of variance for biochemical attributes of Soybean varieties.

| Source of Variance | Df | proteins (mg/g) | Amino acids (mg/g) | MDA (mg/g) | $H_2O_2$ (mg/g) |
|---|---|---|---|---|---|
| *Variety* | 6 | 366.479 *** | 131209.65*** | 605.654*** | 0.805*** |
| *Salinity* | 1 | 2638.079*** | 1027320.5*** | 3344.785*** | 12.620*** |
| *Variety×Salinity* | 6 | 5.435** | 5033.534*** | 15.136* | 0.161*** |
| *Error* | 42 | 2.811 | 415.703 | 5.261 | 0.003 |

*, **, *** shows significant level at 0.05, 0.01 and 0.001 respectively

and relative proportion of protein (35). Under salt stress reduction in the protein, concentration was observed by many authors (36). Another report stated that protein concentration was decreased in the 3 soybean cultivars

in the saline environment (37). The decline in the protein concentration due to the presence of NaCl causes a toxic effect on protein synthesis (38). The high salt concentration affects many physiological processes in plants like lower water potential and induces osmotic stress that leads to developing secondary oxidative stress which interferes with $CO_2$ assimilation and protein synthesis (8).

MDA and $H_2O_2$ contents were elevated in soybean grown under salt stress (Table 2). $H_2O_2$ and MDA contents have been significantly increased under salt stress in all tested varieties of soybean as compared to their control. William-82 and Ajmeri showed relatively higher levels of $H_2O_2$ and MDA contents than other varieties. The NARC-1 and NARC-2 showed significantly lower $H_2O_2$ and MDA compared to other varieties (Fig. 4). $H_2O_2$ has been accumulated in plants at the arrival of various biotic and abiotic stresses (39). Osmotic stresses accelerate the production of ROS such as $H_2O_2$, which develops harmful effects on subcellular compartments and metabolic processes by causing oxidative cellular destruction (40). ROS production is also involved in membrane destruction and increases MDA content in the cells (41, 42). A higher level of MDA is involved in the peroxidation of lipids in cell membranes and is considered an indicator during oxidative stress (43). MDA is considered a biomarker of biotic and abiotic stresses that are strongly correlated with the increasing level of MDA with electrolyte leakage and loss of membrane integrity.

The grain yield and characteristics associated with yield were adversely affected by NaCl salinity across all seven soybean varieties (Table 3). However, the salinity had a greater impact on the characters of PSC-60, D.A., Rawal-1, Ajmeri and William-82 than it did on the characters of NARC-1 and NARC-2. PSC-60, D.A., Rawal-1, Ajmeri and William-82 produced a significantly higher number of pods per plant than NARC-1 and NARC-2 did when the conditions were controlled. However, when the salinity was increased to 150 mM NaCl, NARC-1 and NARC-2 produced 72% pods per plant, whereas PSC-60, D.A., Rawal-1, Ajmeri and William-82 produced only 32%, 33%, 27%, 25% When compared to other varieties, Ajmeri and William-82 were the ones whose number of seeds per pod was significantly impacted the most. NARC-1 and NARC-2 had a relative number of 72% and 66% of seeds per pod respectively, when compared to the control; all of the other varieties had a relative number of seeds per pod that was less than 50%. The relative weight of 100 seeds for NARC-1 and NARC-2 was 80% and 83% respectively, whereas for the other varieties, it was less than 50% with the exception of PSC-60, which was 53%. The salinity also had a significant impact on the decrease in the grain yield. When compared to the grain yield of NARC-1 and NARC-2 (12.65 and 12.32 g/plant), the grain yield of Ajmeri and William-82 control was noticeably higher (14.98 and 15.09 g/plant respectively). However, the decrease in grain yield caused by NaCl salinity was more noticeable in Ajmeri and William-82 than it was in NARC-1 and NARC-2. This was the case in both of these locations. The cumulative reduction in all of the yield associated characters was what was thought to be responsible for the lower grain yield that salinity caused. However, among the characteristics that were associated with yield, salinity had a significant impact on the number of pods produced by each plant in soybean varieties. When compared to seed setting and seed development, the findings suggested that salinity had the greatest impact on pod setting. When compared to those of Ajmeri and William-82, the characters of NARC-1 and NARC-2 have a lower level of affected yield and yield associated traits. Salinity stress limited the number of pods produced per plant, the number of seeds produced within each pod, the individual grain weight and the grain yield produced by each plant in soybean (44). Soil salinity caused significant damage to plants at any stage of their development, primarily as a result of the osmotic and ionic stress that it produced (45, 46).

**Table 3**. Effect of salinity on grain yield and yield associated characters of seven soybean varieties

| Varieties | No. of Pods/Plant | | No. of seeds/Pod | | 100-Seeds Weight (g) | | Grain Yield (g) / Plant | |
|---|---|---|---|---|---|---|---|---|
| | Control | Saline | Control | Saline | Control | Saline | Control | Saline |
| PSC-60 | 86.54 | 27.34 (32%) | 1.91 | 1.37 (47%) | 6.34 | 3.36 (53%) | 13.34 | 6.65 (50%) |
| D.A | 91.21 | 30.1 (33%) | 2.84 | 1.35 (47%) | 5.89 | 2.77 (47%) | 11.72 | 5.38 (45%) |
| Rawal-1 | 70.3 | 19.21 (27%) | 2.81 | 1.32 (46%) | 6.64 | 3.24 (49%) | 10.98 | 5.58 (51%) |
| Ajmeri | 71.56 | 18.23 (25%) | 2.64 | 1.19 (45%) | 9.67 | 4.63 (48%) | 14.98 | 6.15 (41%) |
| William-82 | 68.6 | 16.12 (23%) | 2.7 | 1.21 (44%) | 9.53 | 4.16 (44%) | 15.09 | 5.69 (38%) |
| NARC-1 | 36.23 | 26.12 (72%) | 2.98 | 2.16 (72%) | 7.87 | 6.26 (80%) | 12.65 | 8.84 (70%) |
| NARC-2 | 32.24 | 23.37 (72%) | 3.21 | 2.12 (66%) | 7.12 | 5.93 (83%) | 12.32 | 8.12 (65%) |

Salt stress in soybean significantly influenced the $K^+/Na^+$ ratio (Table 4). However, under salinity more severe salt stress, as consequence, many metabolic processes are inhibited that depend on the $K^+$ ions. It was concluded that

**Table 4**. Analysis of variance for $Na^+$ and $K^+$ ion (mg/g) of seven Soybean varieties.

| Source of Variance | Df | Root $K^+$ ion | Shoot $K^+$ ion | Root $Na^+$ ion | Shoot $Na^+$ ion |
|---|---|---|---|---|---|
| *Variety* | 6 | 807.792*** | 998.967*** | 1267.909*** | 14631.485*** |
| *Salinity* | 1 | 27542.237*** | 23776.11*** | 119200.85*** | 1083953.5*** |
| *Variety×Salinity* | 6 | 480.879*** | 377.706*** | 975.772*** | 32076.643*** |
| *Error* | 42 | 46.323 | 45.337 | 187.471 | 185.340 |

*** shows Significant level of 0.001.

significant rise in $Na^+$ content and a reduction in the $K^+/Na^+$ ratio. The NARC-1 and NARC-2 showed higher $K^+$ ion concentration in shoots rather than $Na^+$ ions and similar results have been observed as compared to all other varieties (Fig. 5). Plants are affected under salt stress in 3 different possible ways: It minimizes the water potential, disturb ions balance and also deteriorate ions homeostasis (47). Significant uptake of sodium ions causes severe growth retardation or leads to death of salt-sensitive of glycophytes species (48) i.e. barley (49) and soybean (50). For plants, both $Na^+$ and $K^+$ are competitive ions. Potassium ion plays a vital role in the various cellular processes proceeded in plants. If $K^+$ ions become outcompeted under $K^+$ ion observed as a higher concentration in the tolerant varieties than in the susceptible led to a reduction in the toxicity of sodium ions.

Several reports had been revealed that the biochemical action of green pigment (chlorophyll) is extraordinarily vulnerable to drastic effects of ions and cellular dehydration that altered chemical change by the destruction of chlorophyll directly or dissembling chemical action instrumentality (51). Our findings showed that salinity caused a significant loss of green pigment (Table 5) that's supported by the finding of (52).

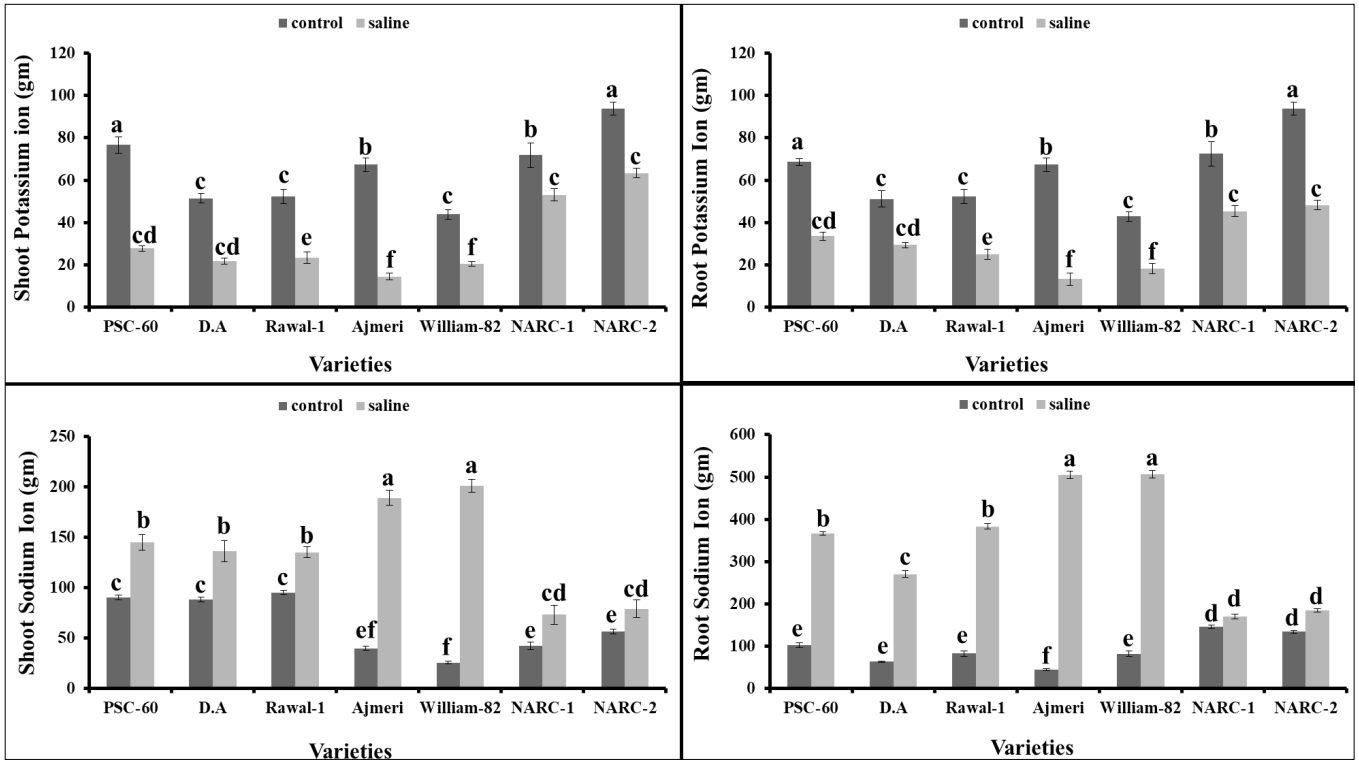


**Fig. 5**. Ion analysis for seven varieties of soybean under salt stress.

**Table 5**. Analysis of variance in chlorophyll contents for seven varieties of Soybean.

| Source of Variance | Df | Chlorophyll a (g/ml)/ plant | Chlorophyll b (g/ml)/ plant | Chlorophyll a/b (g/ml)/ plant | Total chlorophyll (g/ml)/ plant |
|---|---|---|---|---|---|
| *Variety* | 6 | 1.260*** | 0.304*** | 3.382*** | 0.403* |
| *Salinity* | 1 | 39.009*** | 30.386*** | 0.016 ns | 24.698*** |
| *Variety×Salinity* | 6 | 0.929** | 0.313*** | 1.887*** | 0.332* |
| *Error* | 42 | 0.212 | 0.022 | 0.131 | 0.129 |

*, **, *** showing significant level of 0.05, 0.01 and 0.001 respectively.

The reduction in chlorophyll content; however, was less in soybean varieties NARC-1 and NARC-2 (Fig. 6), indi-

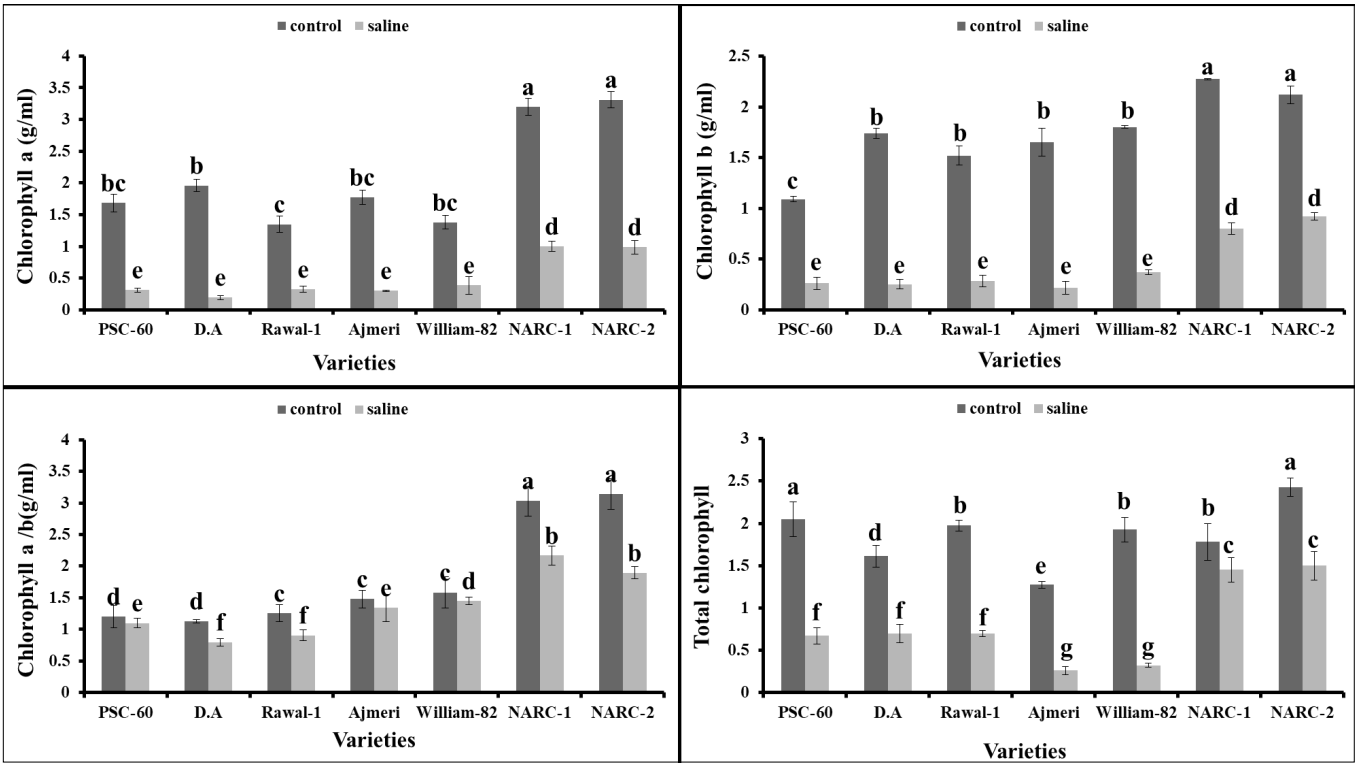


**Fig. 6**. Chlorophyll estimation for seven varieties of soybean under salt stress.

cating that these varieties resisted chlorophyll destruction and thus had the flexibility to stay up chemical action performance under saline conditions. The decline in chlorophyll and carotenoid under salt stress reported in this study agrees with several studies reported about glycophytes (53). The membrane deterioration is the major cause of the decline in chlorophyll contents under salt stress (54). It was reported that the generation of $H_2O_2$ and $OH^-$ were considered the major destruction agents of chlorophyll contents and chloroplast ultra-structure (55). Salt stress also interferes with chlorophyll synthesis (56) and also developed instability in chloroplastic membrane and pigment complex protein. It was reported that salinity reduced the activity of 5-aminolevulinic acid dehydratase (ALA-D) which involves in porphyrin ring formation during chlorophyll synthesis (57).

The variation in antioxidant enzyme activity such as catalase (CAT) in soybean varieties during salt stress is presented in Table 6. The CAT activity was observed to be

**Table 6**. Analysis of variance for catalase activity of Soybean varieties

| Source of Variance | Df | CAT (mM/gFW) |
|---|---|---|
| *Variety* | 6 | 0.198*** |
| *Salinity* | 1 | 3.083*** |
| *Variety×Salinity* | 6 | 0.035*** |
| *Error* | 42 | 4.562 |

*** shows significant at 0.001 level.

significantly decreased in control as compared to salt-treated plants. Our data revealed that increasing level of CAT activities in NARC-1 and NARC-2 and moderately tolerant D.A and Rawal-1, PSC-60 than in the salt-sensitive William-82 and Ajmeri (Fig. 7) under salt stress. This result provides information about the antioxidant defense system and suggested that NARC-1 and NARC-2 detoxify $H_2O_2$ and MDA cellular destruction.

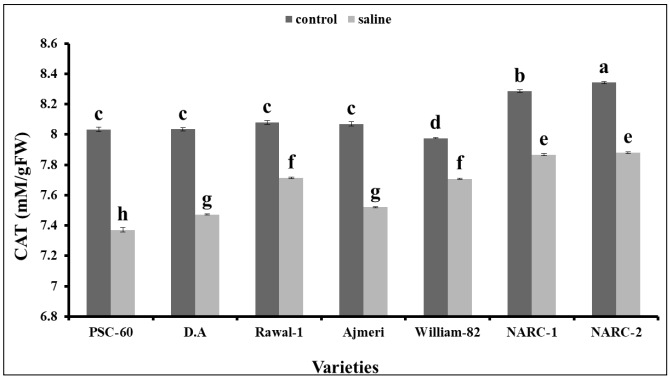


**Fig. 7**. Catalase activity of seven soybean varieties under salt stress.

. The SDS-PAGE showed different protein banding patterns of seven soybean varieties grown under salt stress. In control assays, all types displayed a comparable banding pattern between 10 kDa and 50 kDa (Fig. 8A, B). Salinity, on the other hand, resulted in the disappearance of the protein band (MW 10 kDa) in five kinds of Ajmeri. However, the salt-grown seedling has shown some variance. The 60 kDa and 130 kDa bands found on NARC-1 and NARC-2 were the only ones of their kind (Fig. 8B; Lane 3 and 4 respectively).

Gene expressions were influenced by salinity exposure via the synthesis of novel polypeptides and the loss and over-expression of proteins, respectively (58). The polypeptides with a molecular weight of 40, 34, 32, 29 and 14 kDa were expressed in the salt treat callus of *Mesembryanthemum crystallinum* (59). The proteins have a key role in salinity tolerance with molecular weights of 68, 52, 46, 43, 35, 33, 18 and 11 kDa (60). These proteins have been suggested that these peptides have a vital role in osmoprotectant or protecting cellular structures (61).

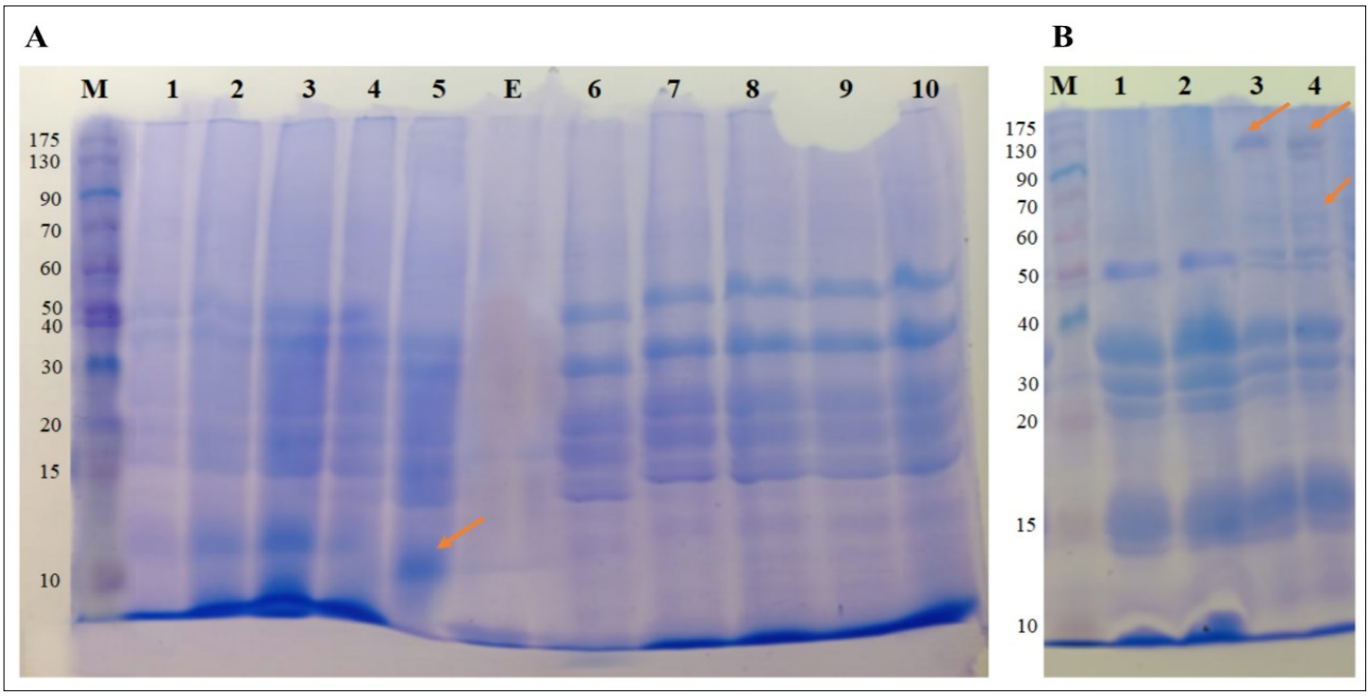


**Fig. 8.** SDS-PAGE of Soybean seedling under control and salt stress. (**A**) is showing the banding profile of Ajmeri, D.A, PSC-60, Rawal-1 and William-82 under control (Lane 1 to 5) and salt stress (Lane 6 to 10). (**B**) is protein banding pattern of NARC-1 and NARC-2, Lane 1 and 2 loaded with control and lane 3, 4 loaded with salt treated plant samples of NARC-1 and NARC-2 respectively. The headed arrows showing the disappearance and newly formed protein under salt stress. Lane M containing protein marker to compare the electrophoretic mobility of protein samples in the gel.

## Conclusion

In conclusion, we confirmed the high salt tolerance associated with the relative grain production of the soybean varieties NARC-1 and NACR-2 in comparison to other soybean varieties evaluated. The high relative grain output of NARC -1 and NARC-2 was correlated with the least impacted number of pods per plant, number of seeds per pod and 100-seed weight by NaCl salinity compared to other cultivars examined in this study. This salt tolerance was achieved by NARC-1 and NARC-2 through increased antioxidant activity and decreased peroxidation activity. On the basis of our findings, we suggest that this inherited characteristic of the NARC-1 and NARC-2 soybean will be valuable in research initiatives aimed at creating more salt-tolerant germplasm as well as in genetic and physiological studies aimed at figuring out the mechanisms underlying increased crop yields on high-salinity soils. Further investigations have to be undertaken to recognize molecular markers or salinity-responsive genes to realize a stronger understanding of mechanisms underlying resistance in soybean varieties.

## Authors contributions

NK carried out the experimental work and write-up improvement. AS made the drafting of original manuscript. The authors have read and agreed to the published version of the manuscript.

## Compliance with ethical standards

**Conflict of interest**: No conflict of interest in this study.

**Ethical issues**: No animals/humans were used for studies that are the basis of this research.

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