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MORPHO-PHYSIOLOGICAL RESPONSES OF SOYBEAN VARIETIES TO SALINITY STRESS

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Abstract

Soybean (*Glycine max* L. *Merr*) has a tremendous value in agriculture as a good source of high-quality plant protein and vegetable oils in one hand and nitrogen fixing ability on the other, now a day largely growing in coastal salt marshes areas of Bangladesh. The aim of this study was to evaluate morphological and physiological responses of soybean varieties (Shohag, BARI Soybean-6, BARI Soybean-5 and Binasoybean-4) to pot grown plants in different concentrations of salinity level i.e., control (0.3), 4, 8 and 12 dS m^{-1} during 2020-21. Irrespective of the variety, with the increase of salinity levels physiological parameters as well as seed yield were greatly affected. Salinity stress decreased total chlorophyll (Chl *a+b*) and total dry matter (TDM) was reduced due to salinity stress, which ultimately reduced seed yield irrespective of the variety. Sodium (Na^+), calcium (Ca^{2+}), potassium (K^+) ion content and the potassium sodium ratios (K^+: Na^+) in leaf tissue were significantly affected by salinity levels. Under salinity stress, BARI Soybean-6 showed a higher K^+: Na^+ ratio in leaf, which indicates higher tolerance to salinity compared to others. However, H_2O_2 and MDA contents was comparatively lower in the respective variety. This variety also showed higher TDM production, filled pods plant^{-1} and seed yield plant^{-1} in all salinity levels compared to other varieties. Results revealed that, soybean var. BARI Soybean-6showedmore tolerance against salinity stress compared to other varieties.

Introduction

Soybean (*Glycine max* L. *Merr*) is a major source of high-quality protein and oil for human consumption (Katerji *et al*., 2000). Soybean has become an important crop in Bangladesh for its increasing demand as an ingredient in poultry and fish meal.

Salinity stress is the most damaging stress, and rising soil salinity in coastal areas has heightened concern about the possibility of crop damage in fields near the sea. In Bangladesh, 2.85 million hectares area of coastal and off-shore are affected by varying degrees of soil salinity with pH ranges of 6.0-8.4 which is composed of the interface of various ecological and economic systems, including mangroves, tidal flat (Ahmad, 2019; Haque, 2006). Salinity problem has been increasing in Bangladesh, and over the last 35 years, salinity has increased by around 26 percent in the coastal region of Bangladesh (Mahmuduzzaman *et al*., 2014). Due to severe and moderate salinity affect crop growth is hampered in this area because soil salinity affects every aspect of plant growth and development. The inhibition of plant growth due to salinity is attributed to salt-induced ion toxicity, nutrient deficiencies, salt-induced osmotic stress, hormonal imbalance, and salt-induced oxidative stress. Salinity also caused a drastic reduction in grain yield of many crops including soybean (Khan *et al*., 2016), mungbean (Aziz *et al*., 2005), and peas (Duzdemir *et al*., 2009). Since, the growth, development, and yield of a crop are the product of genetic potential interacting with the environment, soybean seed production may be limited by soil salinity (Ghassemi-Golezani *et al*., 2009). Growing salt-tolerant crop or variety is one of the cost-effective strategies for coping with soil salinity. The comparison of the performance of different cultivars under salinity stress is useful to select the best one for cultivation to minimize the yield loss. This study was undertaken to investigate the morpho-physiological basis of salinity tolerance of soybean varieties and their comparative salinity tolerance would help to identify the most salt-tolerant variety and to increase soybean productivity in the saline soils of Bangladesh.

Materials and Methods

A pot experiment was conducted at the vinyl house of Plant Physiology Division, BARI, Gazipur during rabi season of 2020-21. Four variety namely: Shohag, BARI Soybean-6, BARI Soybean-5 and Binasoybean-4, were tested under four salinity levels (control, 4, 8 and 12 dS m^{-1}). Salinity was imposed at 30 days after sowing (DAS) by adding NaCl solution. Salt solution was prepared by dissolving calculated amount of lab-grade NaCl with pond water. Salt solution was applied with an increment of 4 dS m^{-1} in every alternate day until desired salinity levels were attained. In control treatment, pond water was used which salinity levels was 0.3 dS m^{-1}. Salinity levels were maintained by monitoring and adding salt solution when require up to maturity. The experiment was laid out in randomized complete block design with 3 replications. Plastic pots (top dia: 25 cm, bottom dia: 18 cm and height 25 cm; 12 kg soil) was filled up with soil and cow dung (4:1). Seeds were sown in each pot on 29 November 2020. Fertilizers were applied @30-30-80-20-3-1 kg^{-1} ha NPKSZnB (FGR, 2018) in the form of Urea, Triple super phosphate (TSP), Muriate of potash (MOP) Gypsum, Zinc sulphate and Boric acid respectively. Half of N and all other fertilizers were applied as basal and remaining N was applied at 30 days after sowing (DAS). Irrigation was done as and when required for maintaining adequate soil moisture. After emergence plants were thinned to three plants in each pot. Plants from three pots were sampled for leaf area and dry matter measurement at reproductive stages. Plant parts were dried in an oven for 72 hours at 70 $^{\circ}$C and dry weight was recorded. At harvest yield and yield components data were collected from three pots and analyzed statistically and mean separation was done by LSD test at 5% level of significance using data processing software.

Chlorophyll estimation

Leaves of each variety were properly cut into small pieces and weighed 0.5 g and were taken for chlorophyll estimation at 55 DAS. Chlorophyll a, chlorophyll b and total chlorophyll were estimated following Arnon's method (Arnon, 1949). The absorbance of the solution was read at 645 and 663 nm for Chlorophyll a, Chlorophyll b and total chlorophyll.

Calculation:
Chlorophyll a (mg g^{-1}) = {12.7 (D663) - 2.69 (D645)} × V/(1000 × w)
Chlorophyll b (mg g^{-1}) = {22.9 (D645) - 4.68 (D663)} × V/(1000 × w)
Total chlorophyll (mg g^{-1}) = Chl. a+ Chl. b
Where, D = optical density; V = final volume of 80% acetone (ml); w = fresh weight of sample taken (g)

Sodium, potassium and calcium ion uptake measurement

At 45 DAS, fully expanded third leaf from top was collected from each treatment for determination of sodium and potassium ion content in leaf tissue. Cell sap of leaf was extracted from leaf using mortar and pistil. LAQUA twin Sodium Ion Meter (Na-11, Horiba, Japan) LAQUA twin Calcium Ion Meter (Ca-11, Horiba, Japan) and LAQUA twin Potassium Ion Meter (K-11, Horiba, Japan) were used for Na^+, Ca^{2+} and K^+ determination, respectively.

Measurement of Lipid Peroxidation (MDA)

The level of lipid peroxidation was measured by estimating MDA, a decomposition product of the peroxidized polyunsaturated fatty acid component of the membrane lipid, using thio barbituric acid

(TBA) as the reactive material following the method of Heath and Packer (1968). Briefly, the leaf tissue (0.5 g) was homogenized in 3 ml 5% (w/v) trichloro acetic acid (TCA), and the homogenate was centrifuged at 11,500×g for 10 min. The supernatant (1 ml) was mixed with 4 ml of TBA reagent (0.5% of TBA in 20% TCA). The reaction mixture was heated at 95ºC for 30 min in a water bath and then quickly cooled in an ice bath and centrifuged at 11,500×g for 15 min. The absorbance of the colored supernatant was measured at 532 nm and was corrected for non-specific absorbance at 600 nm. The concentration of MDA was calculated by using the extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nmol of MDA g^{-1} FW.

Measurement of H_2O_2

Hydrogen peroxide was assayed according to the method described by Yu *et al*. (2003). The extraction of H_2O_2 was done by homogenizing 0.5 g of leaf tissue with 3 ml of 50 mM K-phosphate buffer (pH 6.5) at 4ºC. The homogenate was centrifuged at 11,500×g for 15 min. The supernatant (3 ml) was mixed with 1 ml of 0.1% $TiCl_4$ in 20% H_2SO_4 (v/v), and the mixture was then centrifuged at 11,500×g for 15 min at room temperature. The optical absorption of the supernatant was measured spectrophotometrically at 410 nm to determine the H_2O_2 content (Є= 0.28 μM^{-1} cm^{-1}) and expressed as nmolg^{-1} FW.

The data on different parameters were analyzed statistically by using Statistix 10 program. The mean separation was done by the LSD at 5% level of probability.

Results and Discussion

Effect of salinity levels on pigment contents

Photosynthetic pigment contents sharply decreased with increase in salinity levels in all the varieties (Figure 1A). Pigment contents were higher in BARI Soybean-6 in all the salinity levels and lower in Shohag. Total chlorophyll content of BARI Soybean-6decreased 4,6and 11%, under 4,8 and 12dS m^{-1}salinity stresses, respectively which was comparatively lower than other three varieties. This result was also in agreement with Mannan and Karim (2011). Netondo *et al*. (2004) also observed that photosynthetic activity decreases when plants are grown under saline conditions leading to reduced growth and productivity. The reduction in photosynthesis under salinity can be attributed to a decrease in chlorophyll content (Jamil *et al*., 2007) and activity of photo-system II (Ganivea *et al*., 1998).

Total dry matter (TDM)

TDM in all the salinity treatments sharply decreased at all the studies soybean varieties (Figure 1B). The TDM of Shohag (sensitive to salt) decreased about 27, 46 and 68% in 4, 8 and 12 dS m^{-1} salinity stresses, respectively against control (No salinity). TDM reduction of Binasoybean-4 was about 19, 33, and 57% in 4, 8 and 12 dS m^{-1} salinity stresses respectively whereas in BARI Soybean-5, the reduction was 16, 33 and 47% in 4,8 and 12 dS m^{-1} salinity stresses, respectively, then the control (No salinity) which leads it to the second position of the studied varieties. However, the maximum TDM production was found in BARI Soybean-6 variety under all salinity levels. Dolatabadian *et al*. (2011) and Kondetti *et al*. (2012) noticed plant height and total biomass significantly decreased due to salinity.

Plant height

The effects of salt stress on the morphological characteristics of four treated soybean varieties were evaluated. Plant height of Shohag, BARI Soybean-6, BARI Soybean-5 and Binasoybean-4 were significantly reduced by increasing salinity levels (Figure 1C). In the present study, plant height decreased in all the varieties with increasing intensity of salinity. However, in all the salinity levels, relatively higher plant height was observed in BARI Soybean-6, which was significantly higher than the other three varieties. On the other hand, relatively lower plant heights were recorded in Shohag, which was followed by Binasoybean-4 in all the salinity levels.

Number of filled pod Plant^{-1}

Number of filled pod plant^{-1} of the varieties varied significantly (Figure 1D). Under control condition, the highest number of filled pod plant^{-1} was observed in BARI Soybean-6, which was identical with Shohag and BARI Soybean-5 but differed from Binasoybean-4. Moreover, under 4, 8 and 12 dS m^{-1} salinity level higher number of filled pod plant^{-1} was recorded in BARI Soybean-6 and Shohag gave the lower number of filled pod plant^{-1} under all salinity level.

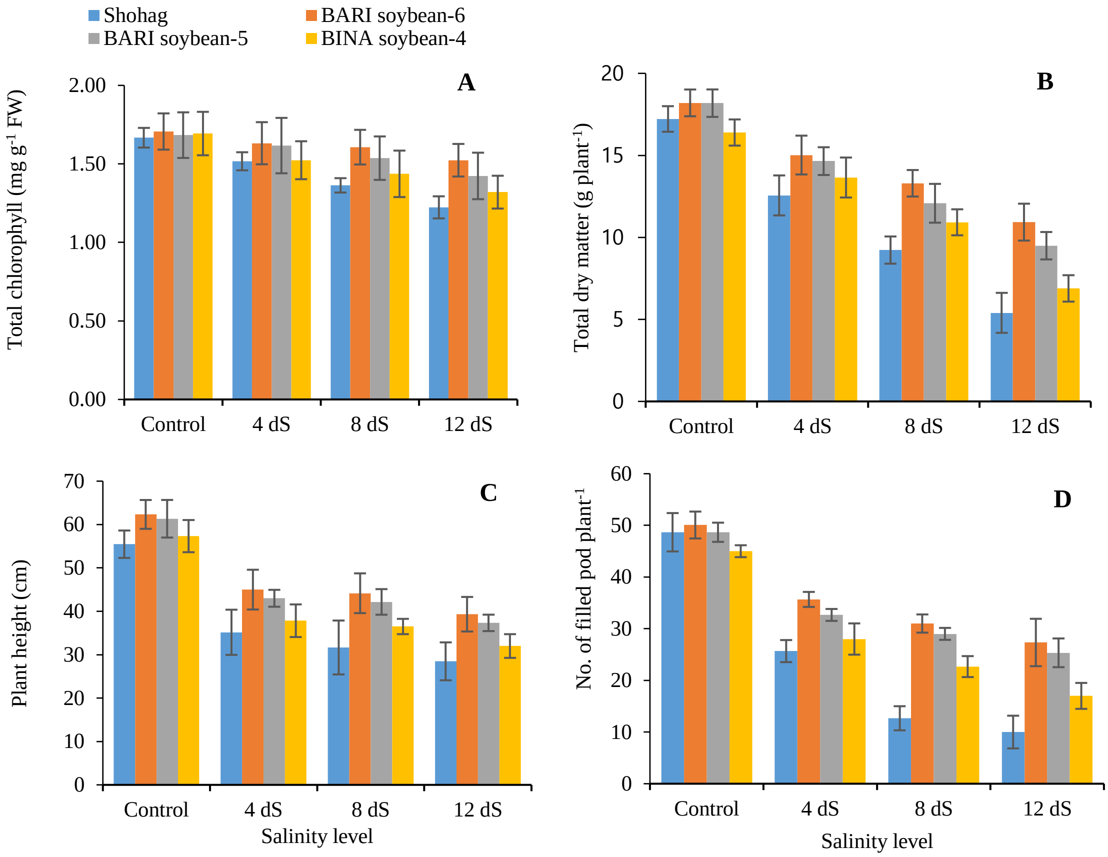

Fig.1. Effect of salinity levels on Total Chlorophyll (A) Total dry matter (B) Plant height (C) and No. of filled pod plant $^{-1}$(D) of soybean varieties. Bars indicate SE values.

Effect of salinity levels on sodium (Na^+), potassium (K^+) content, calcium (Ca^+) content and K^+: Na^+ ratio

The amount of Na^+, K^+, K^+: Na^+ ratio and Ca^+ accumulated in the leaves of Soybean varieties (Shohag, BARI Soybean-6, BARI Soybean-5 and Binasoybean-4) are shown in Figure 2A-D. In the present study, sodium (Na^+) content increased in all the varieties with salinity intensity. Increasing rate was significantly higher in Shohag leaves than rest of the varieties. In Shohag leaves, Na^+ content increased 31, 97 and 142%, in 4, 8 and 12 dS m^{-1} salinity stress, respectively, which was significantly higher than other varieties. Comparatively, increasing rate of Na^+ content was less in BARI Soybean-6, which was followed by BARI Soybean-5 and Binasoybean-4.

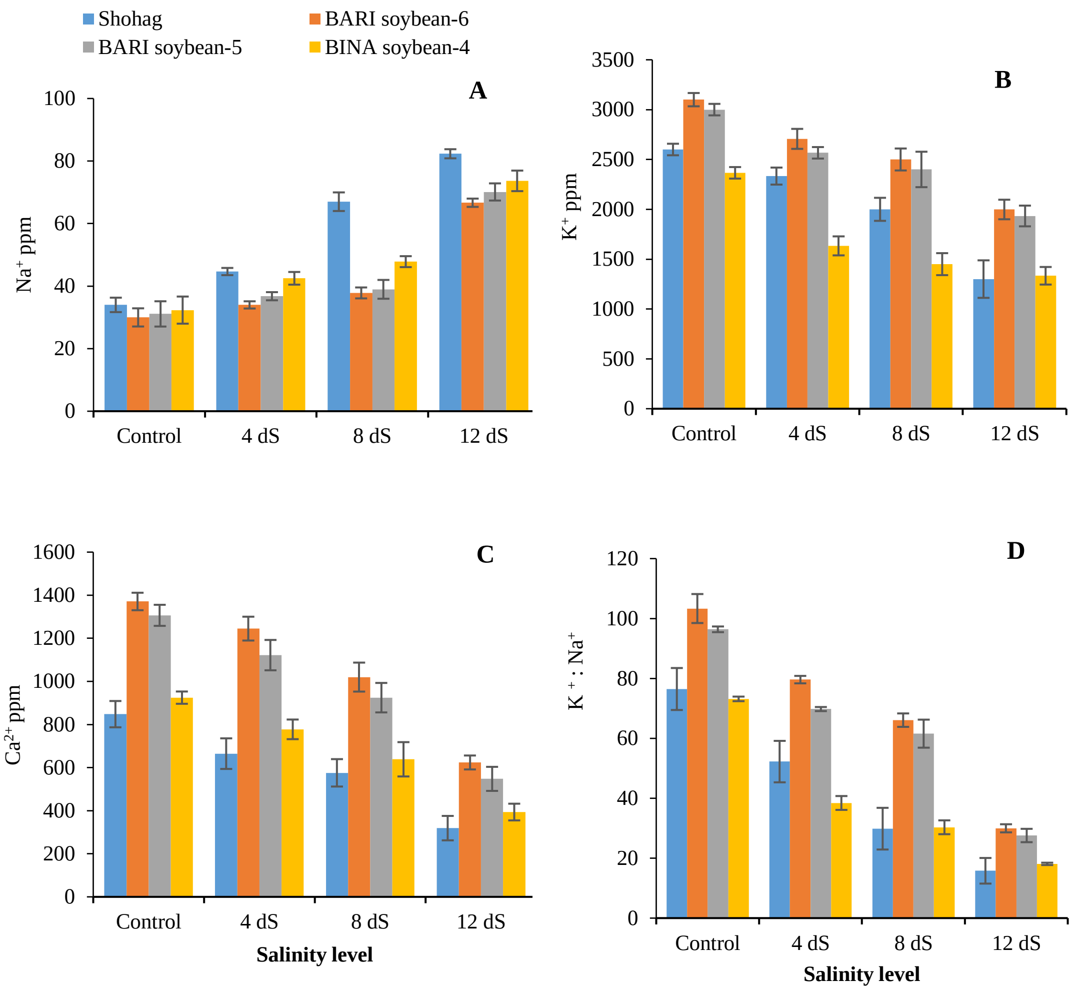

Fig. 2. Effect of salinity levels on Na^+(A), K^+(B), Ca^{2+} (C) and K^+: Na^+ (D) ratio in the leaves of soybean varieties. Bars indicate SE values.

Potassium (K^+) content showed the reverse effect on Na^+ content which means that decreasing trend with increasing salinity level (Figure 2B). The K^+ content is the main cation in plants and is an important component of the cell osmotic potential (Reggiani *et al.*, 1995). The results were supported by reports of Essa (2002) and Sobhanian *et al*. (2010). On the other hand, K^+: Na^+ ratio decreased significantly with salinity levels in all varieties (Figure 3D). BARI Soybean-6 showed higher potassium/sodium ratio than Shohag, BARI Soybean-5and Binasoybean-4in all the salinity treatments. Ca^{2+} contents in leaf decreased with increased salinity levels (Figure 3C). The variety BARI Soybean-6 maintained a higher Ca^{2+} under all salinity levels than the other three varieties. Essa (2002) reported that the main response of the plant to salt stress is a change in Ca^{2+} homeostasis and attributed that the salt tolerance of plants is their ability to avoid Na^+ toxicity and to maintain Ca^{2+} and K^+ concentrations. According to Mannan *et al*. (2013), the relatively high salt tolerance of AGS 313 was associated with the limited accumulation of sodium and high accumulation of different mineral ions in different plant parts, as well as the maintenance of better water relations under salinity than in the case of Shohag. In the present study, Na^+ ion was increased, K^+ and Ca^{2+} ion decreased in all varieties with increasing salinity stress, which is a common phenomenon in all plants. The accumulation of Na^+ ion might be involved in the osmotic adjustment. In this experiment, K^+: Na^+ ratio was higher in BARI Soybean-6.

Effect of salinity levels on H_2O_2 and MDA content

H_2O_2 contents significantly increased in soybean varieties with salinity stress compared with the control (Figure 3A). However, the increment of H_2O_2 contents was lower in BARI Soybean-6 than rest of the varieties. At 12 dSm^{-1} salinity level, the content of H_2O_2 increased by 3.55 folds, in the leaves of BARI Soybean-6, while H_2O_2 content increased by 4.36 folds, in Shohag over their control.

Salinity stress effect on lipid peroxidation was determined as the presence of malondialdehyde (MDA) in soybean leaf tissues. The amount of MDA significantly increased in the soybean seedlings compared to control when exposed to salinity. The increase rate of MDA in Shohag was higher than in the rest soybean varieties (Figure 3B). The contents of MDA were 143,159 and 166% higher at 12 dS salinity stress in BARI Soybean-6, BARI Soybean-5, and Binasoybean-4compared to their control whereas, in case of Shohag MDA content was189% higher than their respective control in the same situation.

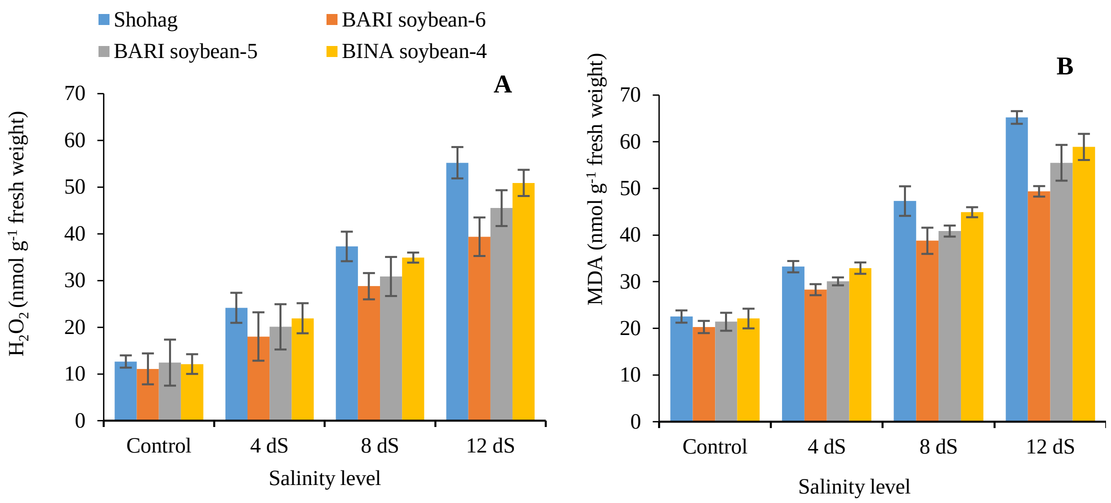

Fig. 3. Effect of salinity levels on H_2O_2 (A) and MDA (B) content of soybean varieties. Bars indicate SE values.

Seed yield plant^{-1}

Seed yield, which is the ultimate goal is very sensitive to salinity conditions. Reduction percentage of seed yield plant^{-1} was higher at higher salinity levels compare to control and gradual increased in reduction in seed yield was obtained with the increment of salinity (Fig 4). The seed yield of soybean varieties was varied significantly among the treatment combinations. The seed yield reduction percentage was higher in Shohag which was 20, 43 and 62% in 4,8 and 12 dSm^{-1} salinity stresses, respectively compared with control. On the other hand, BARI Soybean-6 showed relatively lower seed yield reduction i.e., 6, 28 and 38% in 4,8 and 12 dS m^{-1} salinity stresses, respectively compared with control. BARI Soybean-5and Binasoybean-4 gave moderate seed yield in all salinity level compared with control.

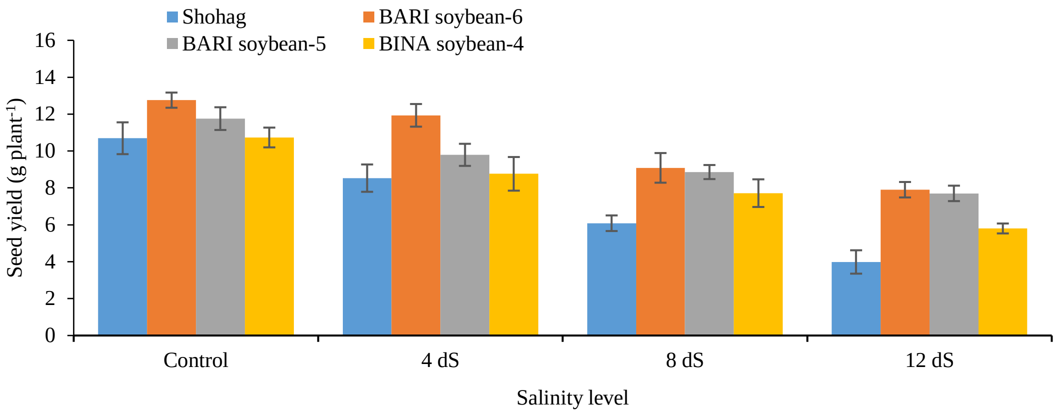

Fig. 4. Effect of salinity levels on Seed yield plant^{-1} of soybean varieties. Bars indicate SE values.

Conclusion

The result showed that plant biomass, higher K^+ concentration, K^+: Na^+ ratio, H_2O_2 and malondialdehyde (MDA) contents as well as yield contributing characters and seed yield as indices of salinity stress tolerance, it has exebit that soybean var. BARI Soybean-6 is relatively tolerant to salinity stress, than the other three varieties.

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