

EFFECTS OF SALT STRESS ON GROWTH AND ACCUMULATION OF Na⁺, K⁺ AND Ca²⁺ IONS IN DIFFERENT ACCESSIONS OF *SESBANIA*

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Abstract

Effects of salt stress on seedling growth and ion accumulation in 6 accessions of two *Sesbania* species viz. *S. bispinosa* (Jacq.) W. Wight (4 accessions: #1, #5, #7 and #59) and *S. cannabina* (Retz.) Poir. (2 accessions: #82 and #85), was evaluated in a hydroponic experiment under 0 (control) and 10 dS/m salinity stress. Salt stress significantly affected negatively seedling growth and different ion contents in 6 accessions of *Sesbania* seedling except for Na⁺. The relative values, per cent reduction over control, of all morphological and physiological descriptors, were significantly higher indicating superior salt tolerance in accession #59 compared to other accessions studied. The Na⁺ accumulation was higher in root compared to shoot; however, a reverse scenario was observed for K⁺ and Ca²⁺. No distinct difference in salt tolerance was observed between *S. bispinosa* and *S. cannabina*. Accession #59 could, therefore, be suggested for cultivation at saline-prone areas of Bangladesh. However, it needs further confirmation at the farmer's saline field condition.

Introduction

Soil salinity is one of the major global issues owing to its adverse impact on agricultural productivity and sustainability. Globally about 2,000 ha of arable land are lost to production every day due to salinization; salinization can cause yield decreases of 10-25% for many crops and may prevent cropping altogether when it is severe and lead to desertification (Zaman *et al.* 2018). In Bangladesh out of 2.86 m ha of coastal and off-shore lands, about 1.06 m ha of arable lands are affected by varying degrees of salinity (SRDI 2010); and the salinity affected area is increasing with time due to global climate change and related natural calamities.

Salinity impairs plant growth and development *via* cytotoxicity due to excessive uptake of ions such as sodium (Na⁺) and chloride (Cl⁻), water stress, and nutritional imbalance. Organic matter amendments and Biosaline Agriculture (i.e., the growing of salt-tolerant crops) are two of the most important methods for soil reclamation. The addition of crop residues and other organic materials improves soil structure; its decomposition produces a high level of CO₂ and increases organic acids (e.g., humic, fulvic, etc.), which lower the soil pH. These processes increase the solubility of CaCO₃ and mobilize calcium, thereby replacing exchangeable sodium from the soil exchange complex and reducing soil sodicity also (Pilania and Panchal 2016, Zaman *et al.* 2018). As *Sesbania* spp. (dhaincha) is tolerant to different stress conditions e.g., salinity, water-logging, high and low temperatures, etc. and can be grown in unproductive poor soils for their improvement (Bunma and Balslev 2019). Moreover, the genus *Sesbania* showed a luxuriant growth in soil with a high electrical conductivity up to 10 mS/cm, and some of the *Sesbania* spp. have been recommended for reclamation of saline and sodic soils and as green manure to increase crops yield in saline soils (Chavan and Karadge 1986, Ren *et al.* 2019). Thus the present study was aimed to investigate the performance of *Sesbania* accessions of Bangladesh against the salinity stress at the early growth stage grown under hydroponic conditions.

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Materials and Methods

A two factorial hydroponic experiment was set in CRD (completely randomized design) having three replications. Six *Sesbania* accessions, 4 from *S. bispinosa* (Jacq.) W. Wight (Accession #1, #5, #7 and #59) and 2 from *S. cannabina* (Retz.) Poir. (Accession #82 and #85). The *Sesbania* accessions were collected from different localities of Bangladesh (collection information available upon request).

Plants were grown in hydroponic culture in 32 l polystyrene (RFL) tanks at 0 salinity (as control) and 10 dS/m salinity as treatments. The composition of the full strength nutrient solution followed Pitann *et al.* (2009). Each tank was filled with 32 l water after adding an appropriate amount of different salts for supplying all the nutrient elements. Styrofoam was used as supporting material.

Seeds were sterilized with 1% sodium hypochlorite for 30 min and washed three to four times with distilled water. Then seeds were soaked overnight with water for imbibition. Properly imbibed seeds were placed on a net of the bucket containing water to allow them for germination. For proper germination, the buckets were covered with black materials. After 10 days of germination establishment, the seedlings were transferred to the tank. In each tank, 30 holes were used for six accessions and two to three seedlings were used in each hole. Only water was used in the tank for the first two days to allow the seedlings to repair any root damage during transfer. Then, the water of each tank was replaced with a nutrients solution. Gradually the concentration of the nutrient solution was increased to $1/2$, $3/4$ and full strength, respectively, in the following three consecutive days. In this condition, the seedlings were grown for two weeks without saline condition for adequate growth. Considerable attention was given to maintaining the nutrient solution at a desirable condition. The pH of the solution was monitored daily and maintained around 5.5 by a pH/mv meter (PHS-25, Lida, Shanghai, China). Solution volume was reduced due to evaporation and transpiration. That is why the nutrient solution changed after every seven days. Approximately $200\text{--}250 \mu\text{mol m}^{-2}\text{s}^{-1}$ PPFD (Photosynthetic Photon Flux Density) was provided by three high-pressure sodium (HPS, 400 Watt) lamps maintaining 16 hrs photoperiod for proper growth. Air circulation in the nutrient solution was provided by an air pump to each tank. After 24 days of establishment, the dhaincha seedlings were exposed to salt stress for the next 10 days. The salinity level was measured through EC using an EC meter (SensION EC-5, Hach, Loveland, Colorado, US).

The following data were recorded for the screening at the seedling stage in both control and saline conditions after 10 days of salinization. Root and shoot length were measured in cm by a wooden scale. The root dry weight (RDW), shoot dry weight (SDW), total dry matter (TDM) of seedling were determined by drying the sample in an oven at $80 \pm 2^\circ\text{C}$ until attained to a constant weight (g). The Root–shoot ratio (R:S) of seedlings was calculated from the following formula:

$$\text{Root – Shoot ratio (R: S)} = \frac{\text{Root dry weight (g)}}{\text{Shoot dry weight (g)}}$$

The leaves were separated from the plant and the area was measured by an automatic electronic leaf area meter (LI3100, LI–COR Biosciences, NE, USA). An index of the relative leaf chlorophyll content (leaf greenness) was measured by a chlorophyll meter (SPAD-502, Konica Minolta, Japan). Readings were taken along the middle section of the four leaves of one plant and the mean value was used for analysis. The measurements were made on four plants from each treatment.

The Na⁺ and K⁺ analysis was conducted on acid digested material through the micro-Kjeldahl digestion system (Thomas *et al.* 1967). Dhaincha seedlings after harvesting (10 days after imposing salinity) were rinsed repeatedly with tap water and finally with distilled water and then dried in an oven at 80 ± 2°C to obtain constant weight. Oven-dried samples were ground in a Wiley Hammer Mill, passed through 40 mesh screens, mixed well and stored in plastic vials. Dhaincha plant samples were analyzed to determine the amount of Na⁺ and K⁺ content therein. The digested samples were analyzed for Na⁺ and K⁺ by atomic absorption spectrophotometer (Model PerkinElmer 2380). The K⁺/Na⁺ ratio was calculated from concentrations of Na⁺ and K⁺ in the plant tissues.

The Ca²⁺ content in root and shoot extract in dhaincha plants was determined by the complexometric method of titration using Na₂-EDTA complexing agents (*van Schouwenburg* 1961).

Relative values of all morphological, physiological and biochemical data were calculated from the following formula:

$$\text{Relative value} = \frac{\text{Stress value}}{\text{Control value}} \times 100$$

Collected data were analyzed statistically by the statistical software Statistix 10. The mean differences were evaluated by Duncan's New Multiple Range Test (DMRT) (Gomez and Gomez 1984).

Results and Discussion

Both the root and the shoot length were found to decrease significantly among the accessions with the increase of salinity (Figs 1 and 2). The longest root and shoot were found in accession #59 and the shortest in accession #1 (Fig. 1A and B). The relative value (per cent over control) of root and shoot length was significantly higher in accession #59 in comparison to other accessions (Table 1). Under the saline condition, root growth was more affected than shoot growth (Rahman *et al.* 2001). The reduction in root growth might be due to a reduction in the rate of cell elongation or a reduction in the number of elongating cells (Farooq *et al.* 2015). Nutrient imbalances in the plant may result from the effect of salinity on (i) nutrient availability, (ii) the uptake and/or distribution of a nutrient within the plant, and/or (iii) increasing the internal plant requirement for a nutrient element resulting from physiological inactivation (Grattan and Grieve 1999). These might be some of the causes of growth reduction in the plant which is ultimately expressed as a reduction in dry weight.

The highest root–shoot ratio (0.967) was recorded in accession #1 (1.45) but the lowest (1.09) was found in accession #59 under stress conditions (Fig. 1C). The relative value of root–shoot ratio was highest in accession #1 and the lowest in accession #59 (Table 1).

The root fresh weight (RFW) was higher in accession #59 (31.72 g/10 plants) compared to other accessions under salt stress (Fig. 2A). Under stress, the highest values of shoot and total FW (52.35 and 84.06 g/10 plants, respectively) were recorded in accession #59 (Fig. 2B). The lowest root, shoot and total FW were recorded in accession #1. The relative values of all fresh, dry and total weight were significantly higher in accession #59 in comparison to other accessions showing greater salt tolerance. Accession #1 proved to be salt-sensitive because the shoot and root fresh weight was decreased up to 76.20 and 82%, respectively under salinity, relative to control. Accession #59 effectively grew at salt stress conditions and showed 14.24 and 11.37% reduction in root and shoot fresh weight (Table 1).

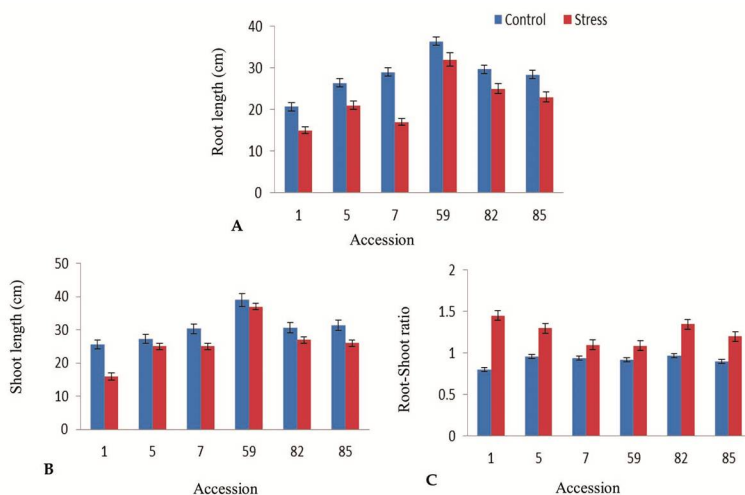


Fig. 1. Morphological descriptors of six *Sesbania* accessions A. Root length, B. Shoot length, and C. Root-shoot ratio under two salinity levels (0 and 10 dS/m). Vertical bar represents SEM (n = 3).

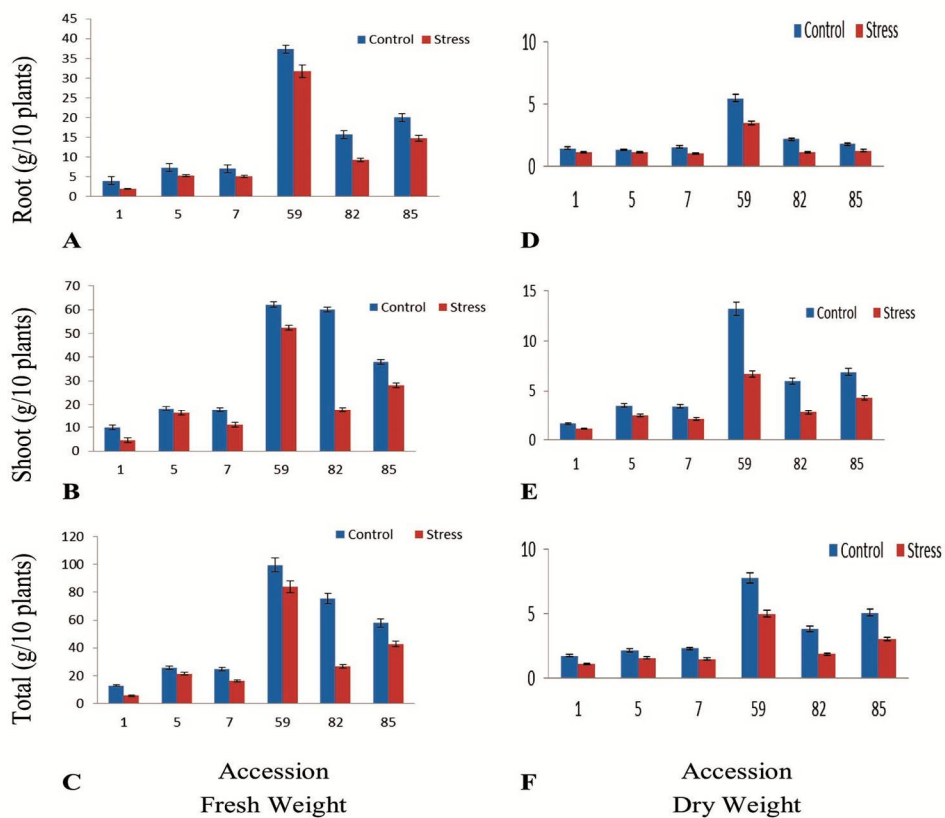


Fig. 2. Weight components of six *Sesbania* accessions. A, D Root; B, E Shoot; and C, F Total under two salinity levels (0 and 10 dS/m). Vertical bar represents SEM (n = 3).

Table 1. Effect of salinity on relative values (% of control) of some morphological descriptors of six *Sesbania* accessions.

Accessions	Root length	Shoot length	Root-shoot Ratio	Leaf area	Root fresh weight	Shoot fresh weight	Total fresh weight	Root dry weight	Shoot dry weight	Total dry weight
Acc#1	57.77 f	67.72 d	98.78 a	35.89 f	18.00 f	23.80 e	21.15 f	33.70 e	25.65 f	12.23 f
Acc#5	65.88 e	74.52 c	87.22 b	43.35 e	51.26 c	26.74 d	54.03 c	26.33 f	36.66 e	24.42 d
Acc#7	74.96 c	63.96 e	86.51 b	47.40 d	38.12 e	52.93 c	47.22 e	58.43 c	46.69 c	16.12 e
Acc#59	94.41 a	95.15 a	78.14 d	87.37 a	85.76 a	88.63 a	88.51 a	89.91 a	85.05 a	82.42 a
Acc#82	68.25 d	56.44 f	81.82 c	53.28 c	55.99 b	51.95 c	51.33 d	54.36 d	42.22 d	54.02 c
Acc#85	82.12 b	83.86 b	87.07 b	64.05 b	46.77 d	61.19 b	70.02 b	64.85 b	59.31 b	64.67 b
CV (%)	1.32	1.34	0.98	0.65	2.41	2.47	2.31	2.79	3.62	2.03

In a column, figures bearing uncommon letters differ significantly at 5% level of significance.

Table 2. Effect of salinity on relative values (% of control) of some physiological and biochemical descriptors of six *Sesbania* accessions.

Accessions	SPAD value	Root				Shoot					
		Na ⁺	K ⁺	Ca ²⁺	K ⁺ /Na ⁺	Ca ²⁺ /Na ⁺	Na ⁺	K ⁺	Ca ²⁺	K ⁺ /Na ⁺	Ca ²⁺ /Na ⁺
Acc#1	51.17 e	194.62 a	45.37 e	29.57 e	45.59 c	16.41 d	187.81 a	33.57 f	34.23 f	43.67 d	17.00 e
Acc#5	57.67 d	124.04 e	74.66 b	46.62 d	54.23 b	17.46 cd	128.43 d	44.45 e	55.09 d	52.33 c	26.05 c
Acc#7	62.72 b	141.91 c	64.76 c	67.76 b	57.11 b	20.78 bc	134.77 c	73.42 b	51.46 e	35.00 e	23.07 d
Acc#59	84.75 a	112.74 f	89.36 a	87.55 a	77.88 a	39.05 a	130.34 d	89.83 a	89.59 a	77.20 a	43.35 a
Acc#82	50.09 f	136.10 d	54.07 d	56.99 c	42.81 c	15.37 d	163.63 b	49.27 d	60.76 c	50.94 c	21.95 d
Acc#85	60.11 c	156.29 b	63.92 c	38.99 d	56.44 b	21.84 b	136.75 c	62.24 c	74.61 b	58.00 b	30.56 b
CV (%)	0.32	1.88	2.80	8.25	3.43	10.00	2.83	3.97	1.04	3.76	3.37

In a column, figures bearing uncommon letters differ significantly at 5% level of significance, SPAD = Soil Plant Analysis Development chlorophyll meter.

Significant variation was found in root DW, shoot DW and total DW among the accessions and between salt stresses (Fig. 2D-F). The highest root, shoot and total DW were recorded in accession #59 under stress conditions (Fig. 2D-F). The lowest root and shoot DW were found in accession #1 under stress condition (Fig. 2D and E). The highest reduction in root (73.67%) and shoot dry weight (74.35%) was noted in accession #1 (Table 1). The relative value of root and shoot DW were maximum in accession #59 (Table 1). In the sunflower plant, the dry weight in leaves, flower discs, and seeds were all significantly reduced from low to high salinity, while exhibiting a nearly 25% loss in stems and no influence was observed in roots (Li and Zhang 2019).

A significant reduction in leaf area and chlorophyll content with the increase of salinity was observed in this experiment (Fig. 3A). The largest leaf area in both control and stress conditions (200.21 and 174.77 cm²/plant, respectively) was recorded in accession #59 and the smallest (59.00 and 25.45 cm²/plant, respectively) in accession #1 (Fig. 3A). The highest reduction in leaf area (64.11%) was noted in accession #1 (Table 1). The highest relative value of leaf area was recorded in accession #59 and the lowest was observed in accession #1 (Table 1). At the seed germination and seedling growth study, accession #59 also produced the maximum biomass and stress tolerance index compared to other *Sesbania* accessions (Sarwar *et al.* 2020).

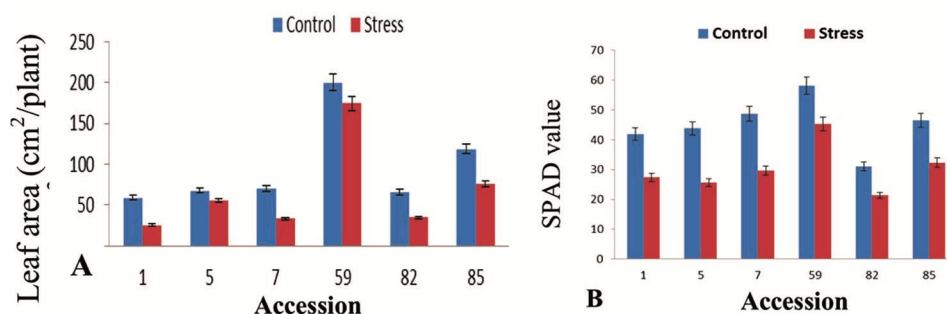


Fig. 3. Leaf area and SPAD value of six *Sesbania* accessions under two salinity levels (0 and 10 dS/m). Vertical bar represents SEM (n = 3).

The SPAD index showed significant variation in all the accessions under salinity stress. The highest value was obtained in accession #59 under control and stress conditions (58.07 and 45.26, respectively) and the lowest value (31.03 and 21.33, respectively) in accession # 82 (Fig. 3B). Relative chlorophyll content was found to vary significantly among the accessions (Table 2). The highest relative value of the SPAD index was recorded in accession #59 and the lowest value was recorded in accession #82 (Table 2). The relative chlorophyll content was reduced due to the presence of Na⁺ ions in plants (Niu *et al.* 2012). Along with other physiological changes, salt stress decreases in chlorophyll a and b, total chlorophyll, and carotenoids concentrations; and salt-tolerant varieties rapidly sequester salt in vacuoles, which slowly leads to alteration in their vital cellular functions (Keisham *et al.* 2018). On the other hand, salt-sensitive varieties are unable to sequester salt in vacuoles and the salt accumulates rapidly in the cytoplasm followed by a reduction of photosynthesis and assimilation.

The Na⁺ content in roots of *Sesbania* is significantly higher than that one in shoots (Fig. 3). The highest Na⁺ concentration in the root and shoot was recorded in accession #1 in both growth conditions (Fig. 4A); whereas the lowest Na⁺ concentration in root and shoot were found in accession #59 (Fig. 4A). The results of relative value revealed that there was a substantial increase in sodium content in all *Sesbania* accessions with the increase of salinity levels. At the highest

level of salinity, maximum Na^+ uptake in root and shoot was recorded in accession #1 while the minimum was recorded in accession #59 (Table 2). The presence of high concentrations of Na^+ and Cl^- can disturb water structure through Kosmo- and chaotropic effects, inhibit enzymes and create a nutritional imbalance (Isayenkov and Maathuis 2019). Halophytic (salt-loving) species can accumulate a high amount of Na^+ , which confirm their capacity to regulate the sodium inflowing in the xylem stream (Flowers and Colmer 2008).

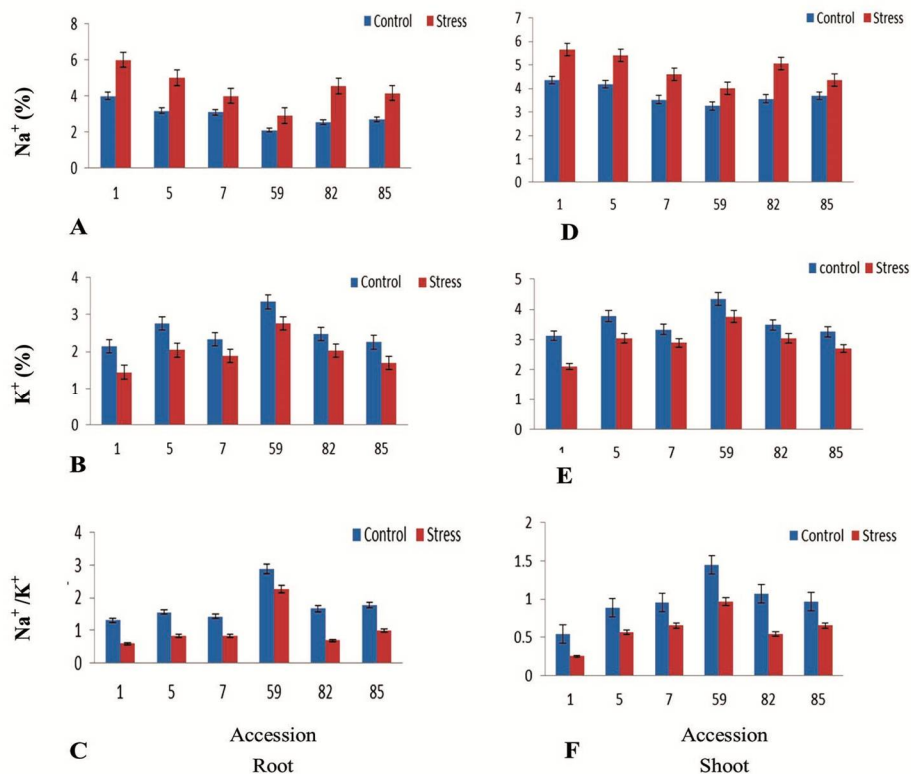


Fig. 4. Ionic concentration in root and shoot of six *Sesbania* accessions. A, D. Na^+ ; B, E. K^+ ; and C, F. Na^+/K^+ under two salinity levels (0 and 10 dS/m). Vertical bar represents SEM (n = 3).

K^+ is one of the essential nutrients in plants, which has played an important role in the growth and metabolism of plants. The steady of K^+ has close ties with the salt tolerance of plants (Pan *et al.* 2011). K^+ content (%) in root and shoot significantly decreased with the increase of salinity among the accessions. The highest K^+ concentration in root and shoot was recorded in accession #59 and the lowest in accession #1 (Fig. 4B). The relative value in case of potassium in root and shoot showed that the highest value was found in accession #59 and the lowest value in accession #1 which indicates that accession #59 successfully maintained a high level of K^+ in root and shoot at salt stress (Table 2). Under the higher salt environment, the plants absorb more K^+ to reduce the damage of Na^+ on plants and be beneficial to the absorption of moisture and nutrients (Tester and Davenport 2003). With the gradual increase of salt, the K^+ content in leaves will be increased and it will be reduced only in the environment of higher salt concentration. Plant parts that contain

more K^+ are also the ones that contain higher Na^+ , therefore, the K^+ and Na^+ content in leaves are higher than those in roots and stems.

The K^+/Na^+ ratio in root and shoot showed significant variations among the accessions with salt stress. The highest K^+/Na^+ ratio was recorded in accession #59 and the lowest was recorded in accession #1 in both root and shoot (Fig. 4C). The increasing uptake of Na^+ with an increase in the salinity levels resulted in a decrease of K^+/Na^+ ratios. The highest relative potassium contents in root at salt stress had resulted in maintaining higher K^+/Na^+ ratios in accession #59, showed better performance under saline conditions and the lowest K^+/Na^+ ratios was recorded in accession #1 performed sensitive to salt stress (Table 2). A similar result of K^+/Na^+ ratios was also found in the case of the shoot (Fig. 4C). The higher charge density of Na^+ compared to K^+ means it behaves as a weak “kosmotrope” that organizes and immobilizes water structure around itself (Isayenkov and Maathuis 2019). Kosmotropy affects hydrogen bonding between water molecules and polar groups of proteins and nucleic acids, potentially interfering with their biochemical activity. The transport systems involved in the uptake and distribution of K^+ and Na^+ in combinations are key determinants of plant salinity tolerance due to their ability to determine tissue and cytosolic K^+/Na^+ ratios, parameters that are generally believed to impact greatly on salt tolerance (Shabala and Cuin 2008).

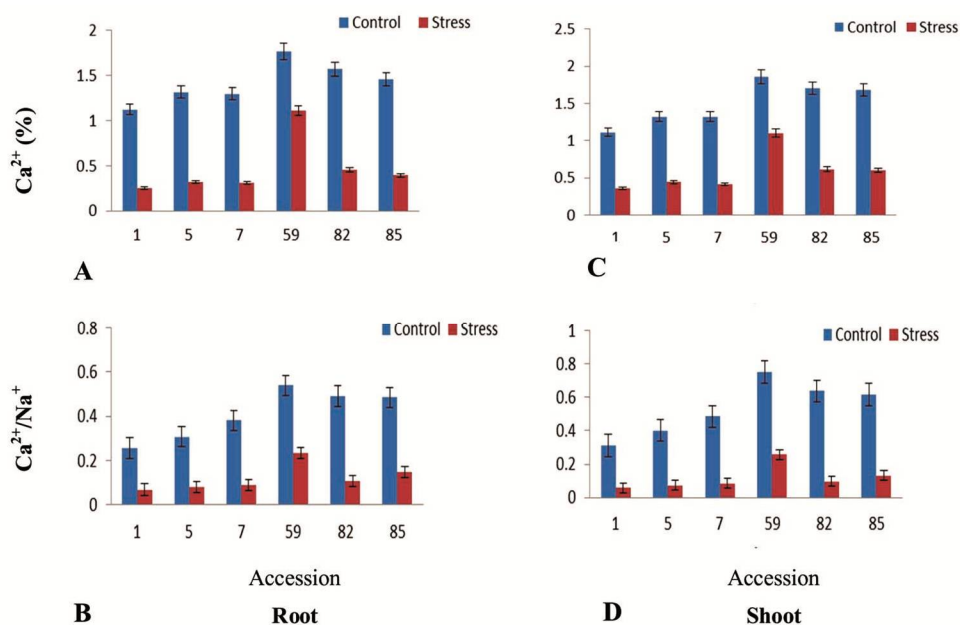


Fig. 5. Ionic concentration in root and shoot of six *Sesbania* accessions. A, C. Ca^{2+} ; and B, D. Ca^{2+}/Na^+ under two salinity levels (0 and 10 dS/m). Vertical bar represents SEM ($n = 3$).

During salinity stress, excessive Na^+ influx results in toxic levels of Na^+ building up in the cytosol, bringing about a range of detrimental cellular effects (Munns 2002, Tester and Davenport 2003). A higher amount of Na^+ was found in accessions #1 and it increased with the increasing salinity levels. On contrary, higher K^+/Na^+ was found in the tolerant plant due to the high amount of Na^+ on the root surface. High external Na^+ negatively affected K^+ acquisition due to similar physiological properties of Na^+ and K^+ (Maathuis and Amtmann 1999). Excess Na^+ also displaced Ca^{2+} from the cell wall and cell membrane as well (Koyama *et al.* 2001).

Ca²⁺ ion concentration in root and shoot significantly decreased among the accessions with salt stress. The highest Ca²⁺ concentration in root (1.77 and 1.11%) and shoot (1.86 and 1.12%) was recorded in accession #59 at control and 10 dS/m salt stress condition, respectively and the lowest Ca²⁺ concentration in root (1.12 and 0.26%) and shoot (1.10 and 0.36%) was found in accession #1 (Fig. 5A). The results of relative value revealed that there was a substantial decrease in calcium content in all *Sesbania* accessions with the increase of salinity levels. At the highest level of salinity, minimum Ca²⁺ uptake in root and shoot was recorded in accession #1 while the maximum was recorded in accession #59 (Table 2). Salinity dominated by Na⁺ salts not only reduces Ca²⁺ availability but reduces Ca²⁺ transport and mobility to growing regions of the plant, which affects the quality of both vegetative and reproductive organs (Grattan and Grieve 1999). The cations K⁺, Ca²⁺ and Mg²⁺ exert a positive function in the energy metabolism of halophytic and/or saline tolerant species because they allow to carry out photosynthesis and maintain the cellular turgor, the osmotic adjustment and the cellular expansion in saline stress (Shabala and Munns 2017). Higher Ca²⁺ was found in accession #59 and the lowest Ca²⁺ was found in accession #1. The Ca²⁺ decreased significantly by higher salinity, which might increase/induce the disease susceptibility of the plant (Yoshioka and Moeder 2020).

Ca²⁺/Na⁺ ratio in root and shoot showed significant variations among the accessions and salt stress. The highest value of Ca²⁺/Na⁺ ratio was recorded in the control condition and the lowest was recorded at 10 dS/m in root and shoot (Fig. 5B). The highest (0.26 and 0.24, respectively) value was recorded in accession #59 and the lowest (0.05 and 0.07, respectively) was recorded in accession #1 in root and shoot under stress condition (Fig. 5B). The increasing uptake of Na⁺ with an increase in the salinity levels resulted in a decrease in Ca²⁺/Na⁺ ratio. The highest relative calcium contents in root at salt stress had resulted in maintaining higher Ca²⁺/Na⁺ ratios in accession #59, showed better performance under saline conditions and the lowest Ca²⁺/Na⁺ ratio was recorded in accession #1 performed sensitive to salt stress (Table 2). A similar result of Ca²⁺/Na⁺ ratio was also found in the case of the shoot (Table 2). High Na⁺ concentration in the root zone inhibits Ca²⁺ uptake and transport resulting in lower Ca²⁺/Na⁺ ratios in salt-stressed plants (Hadi *et al.* 2008). They also suggested the Na⁺/Ca²⁺ ratio could be used for screening salt-tolerant genotypes.

The ever-increasing salinization of arable land will require multipronged solutions, of which crops with increased tolerance is one. No distinct difference in salt tolerance was observed between *S. bispinosa* and *S. cannabina*. Among the *Sesbania* accessions, #59 was found to be more tolerant to salinity (up to 10 dS/m) based on the growth, physiology and biochemical attributes and could be suggested for cultivation in saline-prone areas of Bangladesh. Further study is suggested for the confirmation of present results in the farmer's (saline) field condition.

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