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Effects of salt and water stress on leaf production, sodium and potassium ion accumulation in soybean

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Abstract: Leaf traits of three soybean genotypes viz., Galarsum, BD 2331 and BARI Soybean 6 were evaluated for their salt and water stress tolerance under the salinity levels of 0, 50 and 75 mM NaCl and water stress with 70% depletion of available soil water at the Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur, Bangladesh. The treatment were imposed in plants on 21 days after emergence and continued up to 56 days of age. The results of this study indicated that leaf traits like leaf number, leaf area and its dry weight of the soybean genotypes were sharply decreased when the plants were exposed to water stress, salt stress and, combined salt and water stress conditions. Least reduction in leaf traits was observed in Galarsum in all stress conditions. All leaf traits decreased more in 75 mM NaCl salinity combined with water stress treatment. The leaf dry weight was decreased to 39.72, 38.58 and 39.43% of the control in Galarsum, BD 2331 and BARI Soybean 6, respectively. The genotype Galarsum also accumulated lower amount of Na^+ and higher amount of K^+ in leaf tissues under salt stress and, combined salt and water stress environments as compared to others.

Keywords: Salt Stress, Water Stress, Ion Uptake

1. Introduction

Soybean is classified as a moderately salt-tolerant crop and the yield will be reduced when soil salinity exceeds 5 dS/m [22]. The adverse effect of salinity on plant is dependent on salt concentration in the substrate, duration of exposure to salinity and stages of plant growth ([5], [23], [12]). It is very common in the arid and semi-arid regions that when the crop growth season progresses, the precipitation decreases, and temperature and evapotranspiration increase, resulting in rising salt concentration in the soil solution [1]. Thus, salt and water stress prevails at the same time in the dry seasons, which very often add extra harm on plant growth [16].

Plants under high saline conditions cannot always absorb sufficient water for metabolic activities or maintain turgidity because of the low osmotic potential in the growth media. At the same time, plants absorb damaging amounts of Na^+ and Cl^- ([5], [13], [15]). Na^+ is the primary cause of ion specific damage, resulting due to a range of disorders in enzyme activation and protein synthesis [33]. Under salt and water stress condition, cell expansion of leaf is reduced due to low turgor which is controlled by the processes related to cellular water uptake and cell wall extension [9] that resulted in decreased leaf area and weight. In the coastal area of Bangladesh, salinity and drought exist together during soybean growing period. High levels of salts in the soil can often cause serious limitations to crop production. As salt concentration in the shoot occurs via transpiration stream, that affect leaves greatly. The response of soybean to salinity stress depends both on genotypes and environmental conditions [11]. Therefore, this study was undertaken to observe the effect of salt and water stress on leaf traits, sodium and potassium ions accumulation in soybean genotypes.

2. Materials and Methods

2.1. Site and Materials of the Experiment

The experiment was conducted in a vinyl house of the Department of Agronomy of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur, Bangladesh during winter, 2012. Three genotypes of soybean viz. Galarsum, BD 2331 and BARI Soybean 6 were used to observe leaf production, sodium and potassium ions accumulation under salt and water stress environments. These genotypes were selected based on their performance in previous study [18].

2.2. Date of Planting and Crop Management

Seeds were washed several times in the tap water for surface cleaning then sown 5 seeds in the soil medium on January 20, 2012 in each plastic pots, 30 cm in height and 24 cm inner diameter. Each pot contained 12 kg air dried sandy loam soil. The soils of each pot were fertilized uniformly with 0.30 g of urea, 0.90 g of triple super phosphate, 0.60 g of muriate of potash and 0.60 g of gypsum. The pots were watered with the amount of 200 ml daily for easy germination. After the emergence, two uniform healthy seedlings per pot were allowed to grow for three weeks.

2.3. Design and Treatments of the Experiment

At 21 days after emergence, all genotypes were divided into 6 groups; Control, Water shortage (irrigation with 70% depletion of available soil water when wilting sign developed), 50 mM NaCl irrigation, 50 mM NaCl irrigation + Water shortage, 75 mM NaCl irrigation, and 75 mM NaCl irrigation + Water shortage. In salt water irrigation and water shortage treatments, initially all pots were irrigated with salt water for a week then followed water shortage and thereafter salt water irrigations. The control plants were irrigated with tap water only with maintained field capacity. The experiment was arranged in two factors Completely Randomized Design (CRD) with 4 replications.

2.4. Data Collection

All leaves were collected at 56 days after emergence and areas were measured by Leaf area meter (Model no. Li-3100C, USA). Samples were then oven dried at 70 ºC for 4 days to measure the dry weight. Dried leaves were ground and then 100 mg ground samples were dry-ashed at 500ºC for 8 hours and then digested with concentrated hydrochloric acid to estimate Na^+ and K^+ concentrations with an Atomic Absorption Spectrophotometer (170-30, Shimadzu Co. Ltd., Tokyo, Japan).

2.5. Data Analysis

Data were analyzed by MSTAT-C program and the treatments means were compared by using Least Significant Difference (LSD).

3. Results and Discussion

3.1. Leaf Number

Leaf number of different genotypes of soybean was significantly affected by salinity and water stress treatments and it ranged from 6.0 to 22.0 per plant (Table 1). Under only water stress condition, Galarsum produced the highest number of leaves (7.7) and BARI Soybean 6 produced the least (6.7).The number of leaves drastically reduced in combined salt and water stress condition than only in salt stress in both the salinity levels (50 and 75 mM NaCl). The leaves decreased more at 75 mM NaCl combined with water stress. Significantly the lowest number of leaves was observed in BARI Soybean 6 (6.0 per plant) and the highest was in Galarsum (8.5 per plant). But the genotypes were not differed at the same level of treatment. The numbers of leaves were decreased to 38.64, 39.92 and 31.25% of the control in Galarsum, BD 2331 and BARI Soybean 6, respectively. Long term exposure to salinity leads to premature leaf senescence [21] and thus reduces the photosynthetic area ([8], [32]). Leaf injury and death is probably due to the high salt load in the leaf that exceeds the capacity of salt compartmentation in the vacuoles causing salt to build up in the cytoplasm to toxic levels [27], [26]. Ludlow and Muchow [19] also reported that reduced leaf growth and accelerated leaf senescence are the common responses to water stress. The reduction in leaf number under salinity and water stress resulted from premature leaf senescence and defoliation.

Table 1. *Leaf number per plant of soybean genotypes as affected by salinity and water stress.*

Treatment	Genotypes		
	Galarsum	BD 2331	BARI Soybean 6
Control	22.0 ± 0.87 (100)	16.7 ± 1.04 (100)	19.2 ± 1.26 (100)
Water stress (WS)	7.7 ± 1.61 (34.85)	7.3 ± 0.58 (43.91)	6.7 ± 0.58 (34.72)
50 mM NaCl	13.5 ± 2.50 (61.36)	15.0 ± 1.32 (89.82)	13.2 ± 1.89 (68.58)
50 mM NaCl + WS	8.7 ± 0.58 (39.39)	7.2 ± 1.76 (42.91)	7.5 ± 2.29 (39.06)
75 mM NaCl	11.2 ± 2.25 (50.76)	11.5 ± 1.50 (68.86)	9.5 ± 1.32 (49.48)
75 mM NaCl + WS	8.5 ± 2.29 (38.64)	6.7 ± 1.53 (39.92)	6.0 ± 0.50 (31.25)
LSD (0.05)		2.58	
CV (%)		14.20	

(Percentages to control in parenthesis)

3.2. Leaf Area

Leaf area of each soybean genotype was significantly affected by the salinity and water stress treatments (Table 2). Under only water stress condition, the highest leaf area recorded in BARI Soybean 6 (484.34 cm^2) and the lowest recorded in Galarsum (391.93 cm^2).Leaf area drastically reduced in 75 mM NaCl salinity combined with water stress condition. But there was no significant difference among the genotypes at this treatment. The highest leaf area was recorded in Galarsum (219.31 cm^2) and the lowest was recorded in BD 2331 (212.55 cm^2). The leaf area decreased to 20.12, 19.76 and 19.16% of the control in Galarsum, BD 2331 and BARI Soybean 6, respectively. Excessive salt uptake can result in the death of leaves and reduce the total photosynthetic leaf area. As a result, there is a reduction in the supply of photosynthate to the plant, affecting the overall carbon balance necessary to sustain growth [27].The leaf area reduction is a common phenomenon of glycophytes grown under salt stress conditions [20]. The reduction in leaf area due to salinity was reported earlier by Khan *et al.* [17] in rice and Chookhampaeng [6] in pepper plant. Drought stress reduces cell and leaf expansion, stem elongation, and leaf area index ([34], [4], [10]). The reduction in leaf area was attributed due to the increasing in leaf senescence and reduced size of leaves developed due to low turgor under saline and water stress conditions.

Table 2. *Leaf area per plant of soybean genotypes as affected by salinity and water stress*

Treatment	Genotypes		
	Galarsum	BD 2331	BARI Soybean 6
Control	1089.98± 118.1 (100)	1075.83 ± 130.6 (100)	1129.61 ± 109.5 (100)
Water stress (WS)	391.93 ± 67.0 (35.96)	425.34 ± 57.8 (39.54)	484.34 ± 66.9 (42.88)
50 mM NaCl	684.39 ± 67.3 (62.79)	541.37 ± 60.6 (50.32)	619.58 ± 64.4 (54.85)
50 mM NaCl + WS	333.79 ± 42.8 (30.62)	314.90 ± 34.02 (29.27)	326.90 ± 13.4 (28.94)
75 mM NaCl	462.13 ± 52.7 (42.40)	301.92 ± 39.6 (28.06)	430.09 ± 50.8 (38.07)
75 mM NaCl + WS	219.31 ± 19.3 (20.12)	212.55 ± 32.34 (19.76)	216.42 ± 43.8 (19.16)
LSD (0.05)		165.1	
CV (%)		19.38	

(Percentages to control in parenthesis)

3.3. Leaf Dry Weight

Table 3. *Leaf dry weight per plant of soybean genotypes as affected by salinity and water stress.*

Treatment	Genotypes		
	Galarsum	BD 2331	BARI Soybean 6
Control	3.90 ± 0.05 (100)	3.58 ± 0.06 (100)	3.61 ± 0.14 (100)
Water stress (WS)	1.45 ± 0.06 (37.18)	1.16 ± 0.05 (32.37)	1.09 ± 0.20 (30.22)
50 mM NaCl	2.54 ± 0.04 (65.16)	2.76 ± 0.31 (77.19)	2.31 ± 0.17 (63.99)
50 mM NaCl + WS	1.57 ± 0.04 (40.26)	1.64 ± 0.09 (45.87)	1.51 ± 0.24 (41.95)
75 mM NaCl	2.45 ± 0.06 (62.93)	2.28 ± 0.12 (63.69)	2.06 ± 0.36 (57.09)
75 mM NaCl + WS	1.55 ± 0.05 (39.72)	1.38 ± 0.04 (38.58)	1.42 ± 0.06 (39.43)
LSD (0.05)		0.25	
CV (%)		7.17	

(Percentages to control in parenthesis)

Leaf dry weight of different genotypes of soybean was significantly affected by salinity and water stress treatments (Table 3). Galarsum produced significantly the highest weight of leaves (1.45 g) under only water stress condition and the lowest leaves weight recorded in BARI Soybean 6 (1.09g).The weight of leaves drastically reduced in combined salt and water stress conditions in both the salinity levels (50 and 75 mM NaCl). The leaves decreased more in 75 mM NaCl salinity combined with water stress. Significantly the lowest leaves weight was observed in BD 2331 (1.38 g), but the genotypes were not differed at this treatment. The leaf dry weight was decreased to 39.72, 38.58 and 39.43% of the control in Galarsum, BD 2331 and BARI Soybean 6, respectively. The reduction in leaf dry weight due to salinity was reported by Karim *et al.*[16] in triticale, Khan *et al.*[17] in rice, Aziz *et al.*[3] in mungbean and Mannan *et al.*[24] in soybean. Leaves dry weight reduced in water stressed cotton plants were reported by Pace *et al.*[29].

Wang *et al.*[35] also reported that leaf biomass decreased significantly in tamarisk (*Tamarix chinensis* Lour) seedlings due to water severity under salt and water stress condition. The reduction in leaf weight was mainly due to decreased in leaf area. Under salt and water stress condition, cell expansion of leaf is reduced due to low turgor that resulted in decreased leaf dry weight.

3.4. Sodium Uptake in Leaves

The accumulation of Na^+ in the leaves of soybean genotypes is affected by salinity and water stress treatments (Figure 1). Under only water stress treatment, significantly the highest accumulation was obtained from BARI Soybean 6 (0.028%) and the lowest (0.022%) both in Galarsum and BD 2331. The accumulation of Na^+ was higher in the salt stress conditions in both the salinity levels (50 and 75 mM NaCl). The results also revealed that the accumulation of Na^+ increased in higher salinity level. However, significantly the highest Na^+ accumulation was obtained from BARI Soybean 6 (0.044%) and the lowest was obtained in Galarsum (0.039%) at 50mM NaCl salt stress treatment. At 75mM NaCl salt stress, significantly the highest accumulation was obtained from BARI soybean 6 (0.146%) which was identical with the accumulation of BD 2331 (0.138%) and the lowest from Galarsum (0.061%).The highest accumulation of Na^+ was also obtained from BARI Soybean 6 (0.094%) under the 75 mM NaCl salt combined with water stress treatment and the lowest from Galarsum (0.036%) in the same treatment. The higher accumulation of Na^+ in leaves under salinity and water stress might be due to higher transpiration rate. Differences in Na^+ accumulation of soybean genotypes revealed that the Galarsum which accumulated lower was more tolerant than that of BD 2331 and BARI Soybean 6. Plant responses to salt and water stress have much in common, salinity leads to many metabolic changes that identical to those caused by water stress, and there are still salt-specific effects. Accumulation of Na^+ in leaves results in necrosis and premature leaf senescence [27].

The results of the study are in agreement with the earlier reports that the tolerant genotypes accumulate less amounts of Na than susceptible ones ([15], [17], [2], [25]). The excess amount of Na^+ creates a toxic effect on plant metabolic processes and therefore, the susceptible cultivars having high amounts of Na^+ suffer more from the effect than the tolerant cultivars ([5], [15]). Kao *et al.*[14] also reported that differences among soybean species in leaf accumulation of Na^+ might be responsible for the differential sensitivity to NaCl treatments.

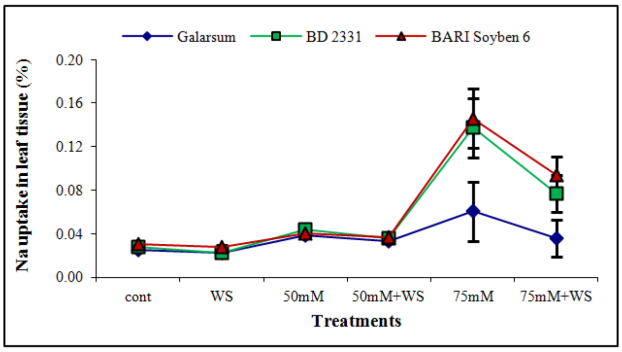

Figure 1. *Sodium (Na) uptake in leaf tissue of soybean under salinity and water stress treatments. Bar represents mean ± S.E. of the genotypes at the same level of treatment.*

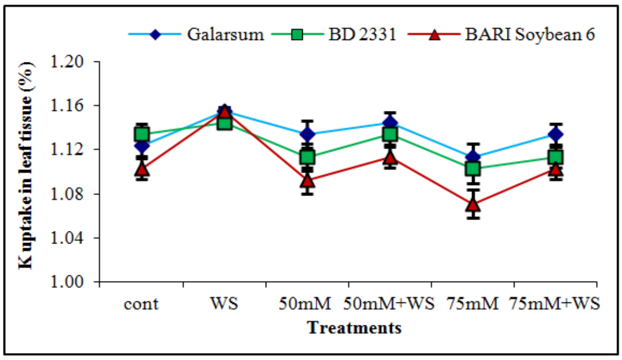

Figure 2.*Potassium (K) uptake in leaf tissue of soybean under salinity and water stress treatments. Bar represents mean ± S.E. of the genotypes at the same level of treatment.*

3.5. Potassium Uptake in Leaves

The accumulation of K^+ in the leaves of soybean genotypes is affected by salinity and water stress treatments (Figure 2). Under only water stress, significantly the highest accumulation was obtained from BARI Soybean 6 (1.155%) and the lowest in BD 2331(1.145%). The accumulation of K^+ was decreased under the combined salt and water stress treatments and decreased higher in higher salt concentration. However, the accumulation was some extent increased in combined salt and water stress than only salt stress condition. At 75mM NaCl salinity, significantly the highest K^+ accumulation was obtained from Galarsum (1.113%) and the lowest from BARI Soybean 6 (1.071%). At 75 mM NaCl salinity combined with water stress, the highest accumulation of K^+ was also obtained from Galarsum (1.134%) and the lowest from BARI Soybean 6 (1.103%). Under water and salt stress conditions, K^+ plays an important role in osmoregulation and the tolerant genotype accumulates higher amounts than others ([5], [31]). Maintenance of high cytoplasmic levels of K^+ is essential for survival of plants in saline habitats [7]. Here, the soybean genotype Galarsum accumulated higher amount of K^+ in leaves than others under salt and water stress conditions.

3.6. Ratio of Potassium and Sodium

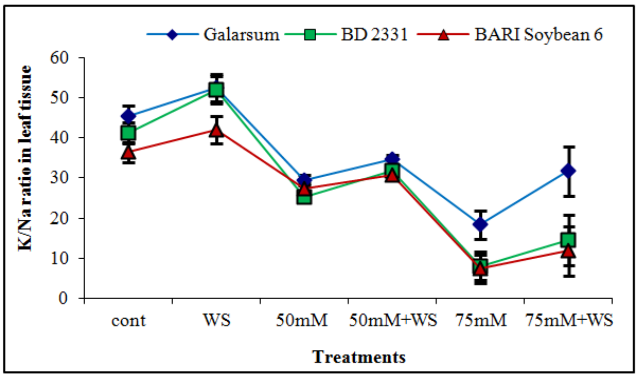

Figure 3. *K/Na ratio in leaf tissue of soybean under salinity and water stress treatments. Bar represents mean ± S.E. of the genotypes at the same level of treatment.*

The ratio of K^+ and Na^+ was decreased under salt and water stress treatments and decreased sharply in higher concentration of salt (Figure 3). Under only water stress, significantly the highest ratio of potassium and sodium was obtained from Galarsum (52.50) and the lowest in BARI Soybean 6 (42.00). At 75 mM NaCl salinity, significantly the highest ratio was also obtained from Galarsum (18.40) and the lowest from BARI Soybean 6 (7.34). The ratio was some extent increased in combined salt and water stress conditions than only salt stress. However, the highest ratio was also obtained from Galarsum (31.72) at 75mM NaCl salt combined with water stress treatment, and the lowest from BARI Soybean 6 (11.79). The results are in agreement with the findings that tolerant genotypes maintain a higher K^+/Na^+ ratio than susceptible ones ([28], [25]).The selective uptake of K in contrast to Na is considered as one of the important physiological mechanisms contributing to salt tolerance in many plant species [30].

4. Conclusion

The results of this study indicated that all the leaf traits of the soybean genotypes drastically decreased with water stress, salt stress and, combined salt and water stress conditions. Variation in salt and water stress tolerance of soybean genotypes was obvious. Reduction in leaf traits (leaf number, leaf area and leaf dry matter) was least in tolerant genotype Galarsum under both salt and, combined salt and water stress conditions. The genotype Galarsum also accumulated lesser amount of Na+ and higher amount of K^+ in leaf tissues under the salt stress and, the combined salt and water stress environments.

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