Journal of Plant Sciences
2015; 3(5): 272-278
Published online October 18, 2015 (http://www.sciencepublishinggroup.com/j/jps)
doi: 10.11648/j.jps.20150305.15
ISSN: 2331-0723 (Print); ISSN: 2331-0731 (Online)

Plant Water Relations and Proline Accumulations in Soybean Under Salt and Water Stress Environment

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To cite this article:
Md. Shawquat Ali Khan, M. Abdul Karim, Abullah-Al-Mahmud, Shahana Parveen, Md. Mahfuz Bazzaz, Md. Altaf Hossain. Plant Water Relations and Proline Accumulations in Soybean Under Salt and Water Stress Environment. *Journal of Plant Sciences.* Vol. 3, No. 5, 2015, pp. 272-278. doi: 10.11648/j.jps.20150305.15

Abstract: The study was carried out with three soybean genotypes viz. Galarsum, BD 2331 and BARI Soybean-6 in a vinyl house of Banghabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh during January to March, 2012 to analyze leaf water status, leaf temperature, xylem exudation and proline accumulation under salt and water stress environment. Treatments included control, water shortage, 50 mM NaCl irrigation, 50 mM NaCl irrigation with water shortage, 75 mM NaCl irrigation, and 75 mM NaCl irrigation with water shortage environments. The relative water content, xylem exudation, leaf water potential of soybean plants were sharply decreased at 75 mM NaCl salt combined with water stress environment. However, these changes were lower in Galarsum and recorded 74.28 % relative water content, 7 mg hr^{-1} xylem exudation rate and -1.03 MPa leaf water potential. Leaf temperature was more in BD 2331 and BARI Soybean-6 than Galarsum. Galarsum accumulated higher amount of proline in leaves under salt and water stress environment. At 75 mM NaCl salt combined with water stress treatment, the highest proline content was also recorded in Galarsum (2.34 µmoles g^{-1} fresh weight). Plant water status and biochemical changed sharply under combined salt and water stress condition. Among the soybean genotype, Galarsum was more capable than BD 2331 and BARI Soybean-6 to manage salt under water stress environment.

Keywords: Salt Stress, Water Stress, Relative Water Content, Leaf Water Potential, Xylem Exudation, Leaf Temperature, Proline Content

1. Introduction

Soybean (*Glycine max* L.) is a minor crop in Bangladesh though it was reported as one of the most nutritious crops in the world [1]. It has become an important crop for its increasing demand as human food and also livestock feeds. In the coastal region with semi-arid climate, soybean is sown in mid-January in the southern Bangladesh. It is exposed to salinity and drought during pod formation and seed filling stage. The climate in the arid and semi-arid regions reflects the fact that with the progression of crop growth stages the precipitation decreases, and temperature and evapo-transpiration increase, resulting in rising salt concentration in the soil solution [2]. Thus salt and water stress prevails at the same time in dry season in Bangladesh, which very often adds extra harm on plant growth [3].The adverse effects of both salt and water stress are primarily due to the restriction of water uptake by the roots [3], which decreased relative water content [4]. Therefore, plants are unable to maintain metabolic activities or turgidity for normal growth because of the low osmotic potential in soil. At the same time, plants absorb damaging amounts of Na^+ and Cl^- [5, 6, 7]. Na^+ is the primary cause of ion specific damage, resulting due to a range of disorders in enzyme activation and protein synthesis [8]. The osmotic adjustment is considered as one of the important mechanisms of water deficit tolerance of plants [9], which promotes the protection of the plant cell structures including membrane and chloroplasts [10]. Plants adjust to high salt concentrations or water stress by lowering tissue osmotic potentials by the accumulation of inorganic ions and or organic substances to permit the maintenance of cell turgor [11, 12]. Proline is one of the osmoprotectants formed in tissues enable the plant to maintain low water potentials that allows additional water uptake from the stress environment, thus buffering the immediate effect of water deficit within the organism [13]. Physiological changes in plants growing under salt or water-deficit conditions have been developed as effective indices for resistant screening in plant breeding programs [14, 15].Therefore, physiological changes in soybean genotypes are required to compare the water and salt stress tolerance of the crop. Thus, the aim of this experiment was to analyze the leaf water status, temperature, xylem exudation and proline accumulation in soybean to salt and water stress environmental conditions.

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 Banghabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur, Bangladesh during winter, 2012. The location situated at about $24^0 23'$ north latitude, $90^0 08'$ east longitude and an altitude of 8.4m above sea level and adjacent to capital Dhaka city. Three tested genotypes of soybean (*Glycine max* L.) namely Galarsum, BD 2331 and BARI Soybean-6 were compared by six environmental conditions of salinity and water stress. These genotypes were selected based on their performance in previous study [16].

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 and establishment, two uniform healthy seedlings per pot were allowed to grow for three weeks in equal environment. Protective measures were taken to control Jassids and white flies at vegetative stage. The crop was harvested at 55 days after emergence.

2.3. Design and Treatments of the Experiment

At 21 days after emergence, three genotypes of soybean were compared by six environmental conditions of salinity and water stress. The environmental conditions were 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 Completely Randomized Design (CRD) with 3 replications.

2.4. Data Collection

Different physiological parameters like Relative water content (RWC), Leaf water potential (LWP), Leaf temperature, Xylem exudation rate and proline accumulations were determined after 4 weeks of treatments imposition.

Measurement of Relative Water Content: Terminal leaflet (without petiole) of the fully expanded uppermost trifoliate leaves of three plants for each treatment were collected at 8:00 am. Fresh weight (FW) of the collected leaves was measured immediately. Thereafter, the leaves were put on distilled water for 24 hours floating at room temperature in the dark. These leaves were weighed to record the turgid weight (TW) after removing excess water by gently wiping with a paper towel. The leaves were then dried in an oven for 48 hours at 72^0C to determine their dry weight (DW). The values of the fresh, turgid and dry weights of the leaves were used to calculate RWC as follows:

$$\text{Relative water content (RWC)}=\{(FW-DW)/(TW-DW)\}X100$$

Measurement of Xylem exudation: Xylem exudation rate was measured at 5 cm above the stem base of the plant between 6:00 -7:00 am. At first clean and dry cotton was weighed. A slanting cut on the stem was made with a sharp knife. Then the weighed cotton was placed on the cut surface. The exudation of sap was collected from the stem for 1 h at normal temperature. To prevent evaporation the cotton was covered with cellophane bag. The final weight of the cotton with sap was taken. The exudation rate was calculated by deducting cotton weight from the sap plus cotton weight and expressed per hour basis as follows:

$$\text{Exudation rate} = \{(\text{Weight of cotton + sap}) - (\text{Weight of cotton})\} / \text{Time (h)}$$

Measurement of Leaf Water Potential: The uppermost fully expanded trifoliate leaves with petiole were cut with a sharp razor and leaf water potential was measured with Scholander Bomb Technique following Tyree and Hammel [17]. The measurement was made at dawn.

Estimation of Proline: To estimate proline accumulation, samples were collected from top third fully expanded young trifoliate leaves of soybean genotypes. The collected leaf samples were immediately kept in an ice-bag and brought to the laboratory. Proline was determined by ninhydrin method [18].

Measurement of Leaf Temperature: Leaf temperature was measured by a hand held Infrared thermometer (SMART SENSOR, AR802A, China).

2.5. Data Analysis

Data were analyzed by STAR (Statistical Tool for Agricultural Research) program and the treatments means were compared by using Tukeys's Honest Significant Difference (HSD) Test. Differences at $P \leq 0.05$ were considered significant.

3. Results

3.1. Relative Water Content

The water status of leaves of three soybean genotypes was significantly reduced by salinity and water stress treatments (Table 1). The relative water content (RWC) was obtained 81.21% in Galarsum, 81.77% in BD 2331 and 81.68% in BARI Soybean-6 in the control plants. However, the lowest RWC (68.76 %) was obtained in BARI Soybean-6 under 75 mM NaCl salt water stress which was identical with BD 2331(69.95 %) and Galarsum (74.28 %) in the same treatment and also in combined salt (75 mM) and water stress treatment. It was observed that RWC was highly affected in the combined salt and water stress conditions in both the salinity levels (50 and 75 mM NaCl) and BARI Soybean-6 showed more susceptible under 75 mM NaCl salt stress. Under only water stress treatment, the RWC was reduced from 81.21 to 75.81% in Galarsum, 81.77% to 75.64% in BD 2331 and 81.68% to 79.24% in BARI Soybean-6. The results are in agreement with the findings of Omami and Hammes [19] in Amaranth, Mannan et al. [20] in Soybean under salinity stress and Choudhury [21] in French bean under water stress.

Table 1. *Relative water content (%) as affected by salinity and water stress after 4 weeks of the treatment imposition. Different letters indicate a significant difference at P ≤ 0.05. Lettering was made for observing the variation in genotype x environmental response.*

Treatment	Genotypes		
	Galarsum	**BD 2331**	**BARI Soybean - 6**
Control	81.21 ± 1.36ab	81.77 ± 2.02def	81.68 ± 2.86fgh
Water stress (WS)	75.81 ± 1.27abcdef	75.64 ± 1.78abc	79.24 ± 1.42ab
50 mMNaCl	76.72 ± 2.78abcd	76.70 ± 0.89cde	76.41 ± 3.46cdef
50 mMNaCl + WS	75.33 ± 1.15bcdefg	74.64± 0.31bcd	72.52 ± 2.40cde
75 mMNaCl	74.28 ± 1.07def	69.95 ± 2.36fgh	68.76 ± 3.12h
75 mMNaCl + WS	71.68 ± 1.23cdef	69.38 ± 1.14efg	70.49 ± 1.46gh
HSD (0.05)		6.04	
CV (%)		2.62	

3.2. Leaf Water Potential

The leaf water potential (LWP) of three soybean genotypes was affected by salinity and water stress treatments (Figure1). The leaf water potential was decreased under both the stress conditions. In the control plants, LWP was recorded of -0.78, -0.77 and -0.79 MPa in Galarsum, BD 2331 and BARI Soybean-6, respectively. Under only water stress treatment, the BARI Soybean 6 maintained the highest value of LWP (-0.92 MPa) and BD 2331 maintained the lowest (- 0.95 MPa). Galarsum maintained LWP of -0.915 MPa. In compared to the salt stress and, the combined salt and water stress conditions, LWP was highly affected in the combined salt and water stress conditions in both the salinity levels (50 and 75 mM NaCl) and decreased more in higher salinity level. At 75 mM NaCl salt stress, the highest LWP recorded in Galarsum (-0.91 MPa) which was followed by BD 2331 (-0.92 MPa) and the lowest recorded in BARI Soybean-6 (-0.95 MPa). At 75 mM NaCl salt combined with water stress treatment, the highest LWP was recorded in Galarsum (-1.03 MPa) which was followed by LWP of BARI Soybean-6 (-1.06 MPa) and the lowest recorded in BD 2331 (-1.08 MPa).The results are in agreement with the findings of Omami and Hammes [19] in amaranth under salt and water stress, Mannan et al. [20] in soybean, Kabir et al. [22] in Mungbean under salinity stress, Choudhury [21] in French bean under water stress. Kusvuran [23] also reported that leaf water potential decreased in all the melon genotypes under salt and drought stress.

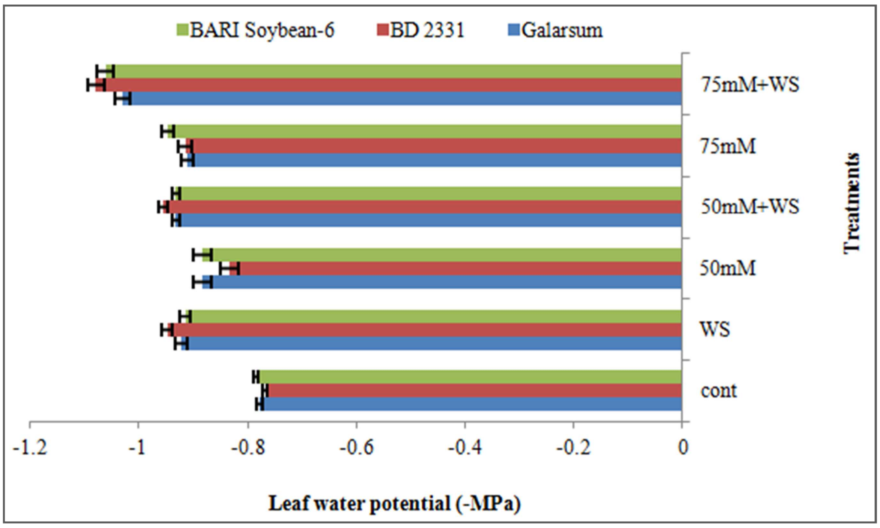

Figure 1. *Leaf water potential (MPa) as affected by salinity and water stress after 4 weeks of the treatment imposition. Bar represents mean ± S.E. of the genotypes at the same level of treatment (P ≤ 0.05). Here, Cont = Control, WS = Water stress and mM = millimolar NaCl concentration.*

3.3. Leaf Temperature

Remotely sensed infrared leaf temperature is one of the technologies for screening genotypes under stress [24, 25, 26]. All the soybean genotypes were not significantly differed by salinity and water stress treatments, but showed higher leaf temperature as compared to control (Table 2). Water deficit and salinity cause stomatal closure, a reduced transpiration rate, and elevated canopy foliage temperature [27]. Transpiration is an endothermic reaction in plants requiring energy in the form of heat. About 580 cal of heat is required to evaporate one gram of water at 25º C. This latent heat of vaporization is important for cooling the plant leaves by transpiration. As a consequence of the reduction in transpiration rates of leaves, leaf temperature increases.

However, the highest leaf temperature recorded in BARI Soybean-6 (32.04º C) which was followed by BD 2331 (32.01º C) and Galarsum (31.84º C) at 75 mM NaCl salt combined with water stress conditions. Similar increases in leaf temperature under drought and salinity have been reported by other workers in Sugar beet [28], Maize [29] and Potato [30].

Table 2. *Leaf temperature (° C) as affected by salinity and water stress after 4 weeks of the treatment imposition. Different letters indicate a significant difference at P ≤ 0.05. Lettering was made for observing the variation in genotype x environmental response.*

Treatment	Genotypes		
	Galarsum	BD 2331	BARI Soybean- 6
Control	29.76 ± 0.76	30.21 ± 1.22	29.69 ± 1.46
Water stress (WS)	31.48 ± 0.80	31.58 ± 1.21	31.02 ± 1.92
50 mMNaCl	30.68 ± 1.06	30.73± 0.98	30.86 ± 1.20
50 mMNaCl + WS	31.05 ± 1.07	31.81 ± 0.76	31.67 ± 1.21
75 mMNaCl	31.48 ± 1.15	31.93 ± 1.43	32.02 ± 1.62
75 mMNaCl + WS	31.85 ± 0.88	32.01 ± 0.67	32.05 ± 1.11
HSD (0.05)		NS	
CV (%)		3.79	

3.4. Xylem Exudation

Xylem exudation rate is the flow rate of plant sap against gravitational force through the xylem vessels from the root stump which is exposed to the cut end of a stem. Exudation rate of soybean plant sap decreased under salinity and water stress treatments (Figure 2).

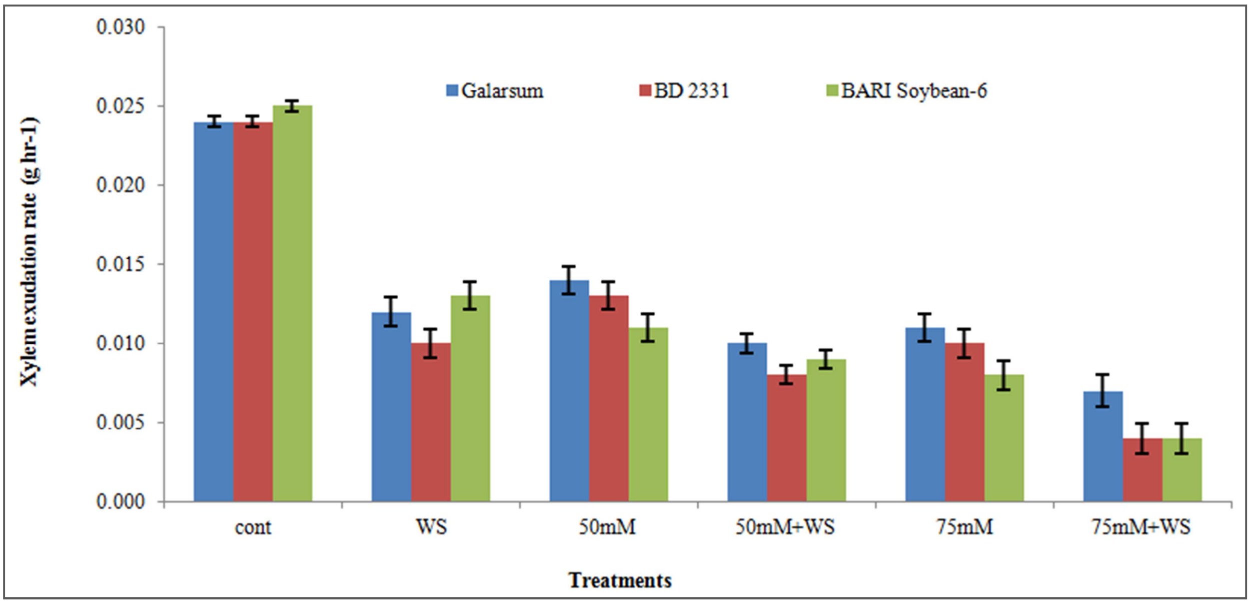

Figure 2. *Xylem exudation rate as affected by salinity and water stress after 4 weeks of the treatment imposition. Bar represents mean ± S.E. of the genotypes at the same level of treatment (P ≤ 0.05). Here, Cont = Control, WS = Water stress and mM = millimolar NaCl concentration.*

Decreased in exudation rate indicated the lower uptake of water by the plant under stress. The exudation rate was recorded as 24, 24 and 25 mg hr^{-1} in the control plants of Galarsum, BD 2331 and BARI Soybean-6, respectively. Under only water stress treatment, the lowest exudation rate was recorded in BD 2331 (10 mg hr^{-1}) and the highest in BARI Soybean-6 (13 mg hr^{-1}). Exudation rate was highly affected in the combined salt and water stress conditions in both the salinity levels (50 and 75 mM NaCl) than only salt stress. In 75 mM NaCl salt combined with water stress condition, the lowest exudation was recorded both in BD 2331 and BARI Soybean-6 (4 mg hr^{-1}), and the highest in Galarsum (7 mg hr^{-1}). RWC and exudation rate are directly associated with the flow of transpiration stream [22, 31].

3.5. Proline Accumulation

The soybean genotypes were varied in proline accumulation by salinity and water stress treatments (Figure 3). The proline accumulation was found higher in all genotypes under salinity and water stress as compared to control. Under only water stress treatment, the highest amount of proline was accumulated by BARI Soybean-6 (1.77 µmoles g^{-1} fresh weight). At 75 mM NaCl salt stress condition, the highest amount of proline was recorded in Galarsum (1.62 µmoles g^{-1} fresh weight) and the lowest in BARI Soybean-6 (1.34 µmoles g^{-1} fresh weight). Soybean plant accumulated higher amount of proline under combined salt and water stress than only salt stress treatment. In 75 mM NaCl salt combined with water stress condition, the highest proline content was also recorded in Galarsum (2.34 µmoles g^{-1} fresh weight) which was followed by proline content of BD 2331 and the lowest from BARI Soybean-6 (2.1 µmoles g^{-1} fresh weight).

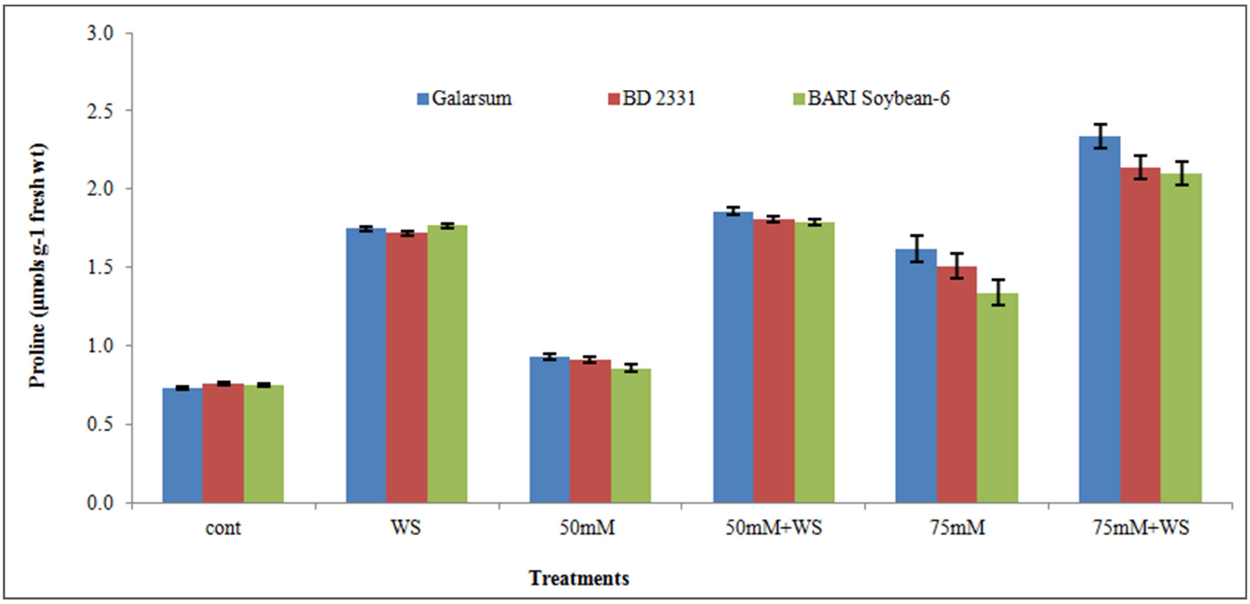

Figure 3. *Proline content in leaf as affected by salinity and water stress after 4 weeks of the treatment imposition. Bar represents mean ± S.E. of the genotypes at the same level of treatment (P ≤ 0.05). Here, Cont. = Control, WS = Water stress and mM = millimolar NaCl concentration.*

4. Discussion

Both salinity and water shortage affect plant processes by creating water stress in plant cells, though their mechanisms in exerting deleterious effects differ in many ways. Salinity has damaging effect on plant growth mostly due to the toxicity of specific ions or as a result of osmotic stress [32]. Relative water content, leaf water potential and leaf temperature are important characteristics that influence plant water relations. Salt and water stressed plant had lower relative water content and leaf water potential than non-stressed ones [19]. Exposure of plants to salt stress and drought stress substantially decreased the leaf water potential, relative water content and transpiration rate with a concomitant increase in leaf temperature [33, 20]. The variation in RWC under different water stress was attributed to the genetic ability of the resistant trait to undergo certain modifications in their metabolic pathway, thus declining their osmotic and water potentials with a concomitant preliminary decrease in their RWC. Orcutt and Nilsen [4] stated that salinity decreased water potential of the salt solution and the plant cannot uptake water freely, and consequently RWC decreased. RWC is closely related with cell volume; it may more closely reflect the balance between water supply to the leaf and transpiration rate [34]. This influences the ability of plant to recover from the stress and consequently affects yield and yield stability [35].

Resistant genotypes showed higher water potential under both of the stress conditions. Anyia and Herzog [36], Xu and Zhou [37], and Echevarrıa-Zomeno et al. [38] have suggested that leaf water potential may differentiate between resistant and sensitive cultivars of different crops.

Galarsum maintained higher LWP in the salt and water stress treatment than BD 2331 and BARI Soybean-6. It might be due to osmotic adjustment in plant cell. There is substantial evidence that plants adjust to high salt concentrations or water stress by lowering tissue osmotic potentials by the accumulation of inorganic ions and or organic substances to permit the maintenance of cell turgor [11,12].

When the stem of a plant is cut off just above the ground level, large quantities of sap may be seen to exude from the root stump, a phenomenon which is called exudation. Under any kind of stress, the exudation rate of a plant becomes slower than that under normal conditions. Higher exudation means a plant absorbs more water from the soil than that a plant with lower exudation rate. The decreased rate of plant sap exudation was also observed by Mannan et al. [20] in soybean, Choudhury [21] in French bean and Kabir et al. [22] in Mungbean.

White and Izquierdo [39] reported that under severe stress conditions plant cells accumulate metabolites and make the osmotic potential of the cell more negative to maintain turgor pressure. The osmotic potential may be regulated through shifts in concentration of some osmoprotectants like proline, sugar etc. This mechanism is considered to be an important adaptation of plants to stress condition [40, 41]. Proline is a non-protein amino acid formed in most tissues subjected to water stress, and together with soluble sugars, is readily metabolized following recovery from drought [42]. The role of proline in adaptation and survival of plants under drought stress were reported by Watanabe et al. [43] and Saruhan et al.

[44]. Enhanced proline accumulation with increased salinity levels was also observed by Khawale et al. [45] in different grape cultivars. Increased in leaf proline content under salt and water stress might be caused by the induction or activation of proline synthesis from glutamate or decrease in its utilization in protein synthesis. High levels of proline enable the plant to maintain low water potentials that allows additional water uptake from the stress environment, thus buffering the immediate effect of water deficit within the organism [13]. Higher amount of proline accumulation in the leaf tissues of the soybean genotype Galarsum under the salt and water stress might be due to osmoregulation in cells.

5. Conclusion

The relative water content, xylem exudation, leaf water potential of soybean plants were decreased more under salt and water stress environments than only salt or water stress. However, these changes were lower in Galarsum than BD 2331 and BARI Soybean-6. Leaf temperature was more in BD 2331 and BARI Soybean 6 than Galarsum. Galarsum accumulated higher amount of proline in leaves under salt and water stress. The results revealed that the genotype Galarsum was more capable than BD 2331 and BARI Soybean-6 to manage salt under water stress environment.

Acknowledgements

We are very grateful to the Director General of Bangladesh Agricultural Research Institute (BARI) for the financial support through a fellowship and also the Department of Agronomy, Bangabandhu Sheikh Mujibur Rahman Agricultural University for providing the research facilities.

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