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ROLE OF SODIUM NITROPRUSSIDE ON MITIGATION OF SALT STRESS IN SWEET CORN

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ABSTRACT.To evaluate the effect of foliar application of sodium nitroprusside (SNP) on physiological characteristics of sweet corn under salt stress a factorial experiment in completely randomized design with three replications was conducted in the research greenhouse of Islamic Azad University of Sabzevar.Factors were application time of SNP at the concentrations of 200 ppm (vegetative, reproductive and vegetative + reproductive) and salinity (0,1.5, 3 and 4.5 dS m^{-1}).The results showed that increasing salinity levels from 0 to 4.5 dSm^{-1} decreased the height by 31.81%, plant dry weight by 34.34%,the number of ear by 46.34%, chlorophyll a content by 30.54%, chlorophyll b content by 55.81%, carotenoid content by 37.40%, total chlorophyll content and the amount of potassium by 54.86% and increased the amount of sodium by 63.86%. Application of sodium nitroprusside twice in vegetative and reproductive stage resulted in maximum height, plant dry weight,chlorophyll a, chlorophyll b, total chlorophyll and foliar application had higher levels of carotenoids. Overall the results indicated that sweet corn is sensitive to salinity and cannot tolerate salinity more than 3 dS m^{-1}. At low salinity condition SNP foliar application at vegetative + reproductive stage and in high salinity level at the vegetative stage can reduce the effects of salinity.

1. INTRODUCTION

Salinity stress, especially in arid and semi-arid conditions, is an important limitation to crop production. Salt stress directly or indirectly affects biochemical, morphological and anatomical characteristics of crop species including germination [1], growth [2], cell division [3], photosynthesis [4], nutrient metabolic and uptake [5], crop development and yield [6] and so on. Different methods are used to reduce the inevitable effects of salinity in plants such as planting of tolerant cultivar [2], nutrient management [7-9], agronomy practice [9] and nowadays foliar application of osmo-protectants or compatible solutesas well as glycine and betaine [10], salicylic acid [11], proline [12], ascorbic acid, 24-epibrassinolide and sodium nitroprusside [13]. Sodium nitroprusside (SNP) is a nitric oxide releasing compound (NO), whose role in plants has been the subject of many research studies [13-16]. Nitric oxide is itself an active nitrogen species, which is thought to be able to mediate as a messenger molecule in adaptive responses to biological and non-biological stresses in plants, and to collect ROS as an antioxidant agent and eliminate it [17]. Although NO is less well known in plant functions, recent advances in research have shownthat it has a major role in regulating

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many plant growth functions, growth and development, response to environmental conditions that affects morphology, signal transduction, seed germination, root growth, the formation of shoots, the maturity of fruits[18]. However, the protective role of NO in plants depends on NO concentration, tissue type, age and plant species, and stress type[13]. Regarding the effect of NO on reducing the effects of salinity, it has been observed that the use of sodium nitroprusside as a NO component which reduces the adverse effects of salinity[19]. Protective role of NO against oxidative damage and increase tolerance to osmotic stress was reported by application of 0.2 mM in rice seedling [20]and 1 mM in barely seedling[21]. Also, in 8-day old rice seedlings, pretreatment of 1 mM SNP over two days increased the salt tolerance to sodium chloride[19].Fan et al., (2007) reported that application of NO under salt stress in Cucumber increased antioxidant enzyme activities and also chlorophyll and proline content that resulted the enhacement of seedling growth.

Sweet corn (*Zea mays* L. var. *saccharata*) is a corn with a mutation on the locus Su (Sugary) on chromosome number 4. The genetic variation causes the accumulation of soluble sugars and polysaccharides in the endosperm of seeds. Sweet corn is one of the tropical plants known to be the third most widely consumed cereal after wheat and rye, and during its growth period it requires a lot of heat and is sensitive to frost. Also, high and low temperatures can damaged (temperatures above 35 °C and less than 10 °C[22].Unlike corn, which is one of the cereals used to feed livestock or the production of flour, sweet corn is used as a vegetable and fresh food for human. Now sweet corn is one of the most popular vegetables in different parts of the world and its consumption is increasing due to its delectability and rich vitamin content. The value of this crop for processing (canning and freezing) and as a fresh vegetable is the second and fourth respectively [23]. In 2003, the area under sweet corn cultivation in the world was 1,019,698 hectares with an average production of 8602 kg per hectare which 8.772.112 tons of corn were produced. About 27 percent of corn acreage and 46 percent of the world belongs to the United States and the largest producers of corn are America, Nigeria, France, Hungary, Peru, South Africa and Japan respectively [24].

Salinity is the most common environmental stresses throughout the world, including Iran[25]. There is a lack of research on the effects of salinity on sweet corn, however, it is believed that sweet corn is semi-sensitive to salinity[26].This plant is resistant to salinity during germination, but increased levels of salinity delay germination. High soil salinity and low temperature in sweet corn delay the emergence of leaves and the formation of the first internodes and reduce the green cover. Continuing stress in subsequent growth stages reduces plant height, and the number of seeds. Salinity stress also causes tissue hydration, ion toxicity, food insecurity, and so on. Studies on sweet maize hybrids have shown that they are the same as salt stress, although hybrids show the same response to germination of salt stress seeds, but root length, stem length, fresh and dry weights and stems of the roots have decreased with increasing salinity. Also, salinity increases the amount of malondialdehyde, proline and H_2O_2in the seedling [27].

We hypothesized that SNP application improves the physiological traits of sweet corn grown under salt conditions. Thus, the objective the present study is to explore up to what extent foliar-applied SNP could alter chlorophyll content and ion content of sweet corn grown under salt conditions.

2. MATERIAL AND METHOD

This research was carried out as a factorial experiment based on a completely randomized design with three replications in greenhouse of Islamic Azad University in 2015.The experimental factors included salinity in four levels (0, 1.5, 3, 4.5 dS m-1) and nitroprusside application at 200 ppm in three growth stage of sweet corn (vegetative, reproductive and vegetative+reproductive). 4-5 leaves and tassel observation in 50% of plant were considered as vegetative and reproductive stage, respectively. Salt treatments were conducted by the addition of NaCl and $CaCl_2$ in the equivalent proportion of 1:1 in tap water (ECi = 0.3 dS m-1).

Sweet corn (Gold seed kSC_40_3cultivar) was planted in pots with a diameter of 25 cm at a depth of 5 cm. After ensuring of complete and optimal development of plants, 5 plants per pot were maintained, and the rest of the plants are excluded. Soil contain 50% sand and 50% field soil. Soil was kept moist during whole duration of experiment. Pot soil moisture content was maintained in a range of 70 to 100% of field capacity. According to the results of soil analysis, the required fertilizers (300 milligrams of urea, 150 milligrams Ca $(H_2PO_4)_2.H_2O$, 100 milligrams of K_2SO_4, 40 milligrams of $FeSO_4.7H_2O$, 20 milligrams of $MnSO_4.H_2O$, 20 milligrams of $ZnSO_4.7H_2O$, 10 milligrams of $CuSO_4.5H_2O$ and 5 milligrams H_3BO_3 per kg of soil) were added to the soil before planting and mixed well. Urea fertilizer was consumed in three stages (pre-cultivating, 3leaves stage and stem elongation). The plants were maintained in a greenhouse under environmental conditions (27 (±5)°C, 65 (±10)% RH and a 14 h light, 10h dark photoperiod.

At the beginning of the reproductive stage, plants were completely removed from the pots and length of three randomly selected plants in each pot was measured for determination of plant height by the meter.

One gram of finely cut fresh leaves were taken and ground with 20 – 40ml of 80% acetone. It was then centrifuged at 5000 –10000 rpm for 5mins. The supernatant was transferred and the procedure was repeated till the residue becomes colorless. The absorbance of the solution was read at 470nm, 645 nm and 663nm against the solvent (acetone) blank [28].The concentrations of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following equation:

Total Chlorophyll: 20.2 (A645) + 8.02 (A663)

Chlorophyll a: 12.7 (A663) – 2.69 (A645)

Chlorophyll b: 22.9 (A645) – 4.68 (A663)

Carotenoids: 100 (A470) - 3.27 (mg chl. a) – 104 (mg chl. b) / 227

Hamada and El-Enany[29]method was used to measure sodium and potassium elements. For this purpose, 0.5 g dry matter of leaves washed and then 10 ml of concentrated nitric acid was added and kept at room temperature for 48 hours. In order to remove all vapors, the specimens were placed on a heated oven thermostat for 2 hours. After leaving acidic vapors and viewing a colorless solution, 100 ml of distilled water was added to each sample. Using Whatman filter paper, the samples were get smooth and sodium and potassium values were measured by photometric photometry [29].

The data were analyzed using SAS software and the averages were compared by Duncan multiple range test. Tables and graphs were drawn in Excel software.

3. RESULTS AND DISCUSSION

The final plant height, plant dry weight, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, leaf chlorophyll index, leaf sodium and potassium content were affected by SNP application and salinity, whereas the interaction effect of SNP
application and salinity for chlorophyll B, total chlorophyll and leaf chlorophyll index were significant (Table 1).

TABLE 1: Analysis of variance for plant height, plant dry weight, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids content, sodium content and potassium content.

SOV	df	Plant height	Plant dry weight	Chloro-phyll a	Chloro -phyll b	Total chlorophyll	Carotenoids content	Sodium content	Potassium content
Time (A)	2	623**	21.74**	0.75 **	0.31 **	1.75 **	0.04 **	2.90 **	3.80 **
Salinity (B)	3	3154**	10.01**	0.98 **	1.62 **	5.12 **	0.09 **	12.64 **	29.52 **
A×B	6	62.24 ^{ns}	1.12 ^{ns}	0.11 ^{ns}	0.16 **	0.48 **	0.006 ^{ns}	0.19 ^{ns}	3.86 **
Error	24	98.84	1.09	0.09	0.04	0.05	0.008	1.38	0.66
CV		14.43	24.78	11.64	16.51	6.74	17.28	20.63	16.39

ns: not significant; (*) and (**) represent significant difference over control at $P < 0.05$ and $P < 0.01$, respectively.

3.1. Plant height

The highest plant height was observed when SNP used in vegetative+reproductive (115.33 cm) and the lowest in vegetative time (101.375 cm, Table 2). It seems that low plant height at SNP application in vegetative stage was due to low absorption of SNP due to less leaves per plant. In the vegetative stage, the number or surface area of the leaves has not been sufficient so that SNP application has not shown its beneficial effects due to decreased absorption, while at the beginning of the reproductive stage, although the vegetative growth process has to be cut off, SNP application has increased the plant height by increasing the length of the thistle. It was reported that SNP application could increase plant growth in saline conditions by raising the activity of antioxidant enzymes that protects the plant from damage caused by free oxygen radicals [30]. In wheat, SNP in saline conditions increases tolerance by raising the amount of proline in the leaf[18]. In cotton that SNP consumption not only promotes plant growth but also augments stem and root lengths. It also rises osmotic pressure of the cell and improves cytoplasmic viscosity that leads to elongate stem length. On the other hand high levels of SNP had a negative effect on stem elongation[31].

TABLE 2: Effect of SNP application time on plant height, plant dry weight, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, sodium and potassium contents.

SNP application time	Plant height	Plant dry weight	Chlorophyll a	Chlorophyll B	Total chlorophyll	Carotenoids content	Sodium content	Potassium content
	Cm	g. plant^{-1}	mg. g^{-1} FW				mg. g^{-1}	
Vegetative	101 b	1.38 c	1.97 b	1.08 b	3.05 c	0.45 b	5.77a	4.89 b
Reproductive	111 a	1.85 b	1.99 b	1.32 b	3.32 b	0.57 a	6.15a	4.45 ab
Vegetative + Reproductive	115 a	2.2 a	2.41 a	1.39 a	3.81 a	0.51 ab	5.17a	5.87 a

Values followed by the same letter within the same columns do not differ significantly at p = 5% based on Duncan.

The highest plant height was observed in the control (129 cm) and the lowest was in 4.5 dSm-1 (88.17 cm, Table 3). There was no significant difference between salinity treatments at 1.5 dSm-1 levels and control. There are several reasons for the reduction of plant height with increasing salinity stress. For example, in high salt levels cause plants to absorb water difficultly which reduces available water and cell division depending on turgor pressure. On the other hand, under stress conditions, photosynthesis rate of the plant is also affected. Reducing photosynthesis lessen the contribution of photosynthetic metabolites to growth, which will also reduce theplant height. Reduction and disruption of nutrient uptake are also due to decreasing of plant height. Reduction in plant growth under salt stress occur due to decreased water absorption and metabolic activity, sodium and chloride toxicity along with food deficiency[32-35].

3.2.Plant dry weight

SNP foliar application at vegetative + reproductive stages revealed maximum dry weight (0.022 g.plant-1) and at vegetative stage the lowest value was observed (0.014 g.plant-1, Table 2). Spraying at the reproductive stage in comparison with the vegetative stage increased 35.71% of plant dry weight. The higher dry weight with SNP application at reproductive stage in comparison to the vegetative stage may be due to the fact that in the vegetative stage, the level and number of leaves were less than the reproductive stage, therefore, at this stage, lower absorption of SNP was carried out by the plant. On the other hand, SNP application in the reproductive phase might have delayed or decreased the leaf loss, which also increased the dry weight of the plant.

TABLE 3: Effect of salt stress on plant height, plant dry weight, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, sodium and potassium contents.

Salt stress ($dS.m^{1}$)	Plant height	Plant dry weight	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids content	Sodium content	Potassium content
	Cm	g. $plant^{-1}$	mg. g^{-1} FW				mg. g^{-1}	
0	129 a	2.22 a	2.54 a	1.75 a	4.31 a	0.61 a	4.34c	6.95 a
1.5	120 a	1.97 ab	2.21 b	1.44 b	3.65 b	0.58 a	5.23bc	5.91 b
3	99 b	1.66bc	1.99 bc	1.09 c	3.08 c	0.48 b	6.11ab	4.14 c
4.5	88 c	1.44 c	1.77 c	0.77 d	2.54 d	0.38 c	7.11a	2.88 d

Values followed by the same letter within the same columns do not differ significantly at *p* = 5% based on Duncan.

Salt stress significantly reduced dry weight. Results revealed that the maximum dry weight (0.22 $g.plant^{-1}$) was produced in the control, while the lowest dry weight was recorded at 4.5 $dS.m^{-1}$ (0.01 $g.plant^{-1}$). There were significant differences between treatments (Table 3). Increasing salinity to 4.5 $dS.m^{-1}$ reduced 45.95% of plant dry weight. Reducing dry weight with increasing salt stress may be due to usage of a part of photosynthesis or growth metabolites for the production of secondary metabolites in order to cope with salinity, which reduces the photosynthesis assimilate for the other parts. On the other hand, the decrease in height, number of leaves and disruption in absorption and transfer of nutrient in high salinity are the main reasons of dry weight loss of the plant at high salinity conditions.

3.3.Chlorophyll a

The highest amount of chlorophyll a was observed when SNP was used at vegetative + reproductive (2.15 $mg.g^{-1}$) and it has the lowest at vegetative stage (1.98 $mg.g^{-1}$, Table 2). The main reason for increasing the amount of chlorophyll a when SNP applied twice compared to once might be due to the production of more pre-structures of chlorophyll production. It has been reported that the use of SNP by preventing the activity of ROE reduces oxidative damage in photosynthetic pigments, which increases the total chlorophyll content of the leaf [36]. Similar results have been reported on the increase of photosynthetic pigmentation due to SNP consumption in chickpea[37] and sunflower[38].

Results revealed that, salt stress dramatically reduced leaf chlorophyll a content (Table 3). The main reason for the reduction of photosynthetic pigments in high salinity may be due to prevent the absorbtion of Mg^{+2} due to high amounts of sodium, which leads to the inhibition of chlorophyll synthesis. Previous studies suggest that high levels of sodium inhibit protein synthesis and weaken the binding of chlorophyll and chloroplast in which leading to chlorophyll degradation[39]. Lin and Shi (2010) showed that, with increase salinity up to 10 dSm^{-1}, net photosynthesis rate, stomatal conductance and chlorophyll a content of sunflower had a decreasing trend[39]. Reduction of chlorophyll content in salt stress conditions may be due to the activity of chlorophyllase enzymes. Some regulators, such as absisic acid and ethylene, stimulate the activity of this enzymes[40].

3.4.Chlorophyll b

Chlorophyll b content was higher in twice SNP application at vegetative + reproductive stages than in other treatments (Table 2). There was no significant difference between SNP application at reproductive stage and vegetative+ reproductive. Spraying at the reproductive stage increased chlorophyll b content 56.67 % when compared with the application at vegetative stage. The amount of chlorophyll depends on the type of leaf and the time of sampling. Therefore, it seems that no significant difference between the amount of chlorophyll b in the reproductive and vegetative was due to selected leaf sample. As shown in table 3, the maximum chlorophyll b content (12.19 cm) was measured in control. There were significant differences among treatments. Increase of salinity to 10 $ds.m^{-1}$ in sunflower decreased chlorophyll content, stomatal conductance and chlorophyll content [39].

Delay in the SNP application produced a higher chlorophyll b content at low salinity stress whereas, at high salinity, spraying in early growth stage produced more chlorophyll b than spraying at the end of growth stage (Figure 1). This suggests that in high salinity levels, the tolerance of sweet corn to salinity is low so delay in SNP application until reproductive stage could not induce mitigation of salinity. Salinity can damaged by the membrane tissue and might increasedchlorophyllase enzymes activity, causing a large part of chlorophyll to be degraded[41].

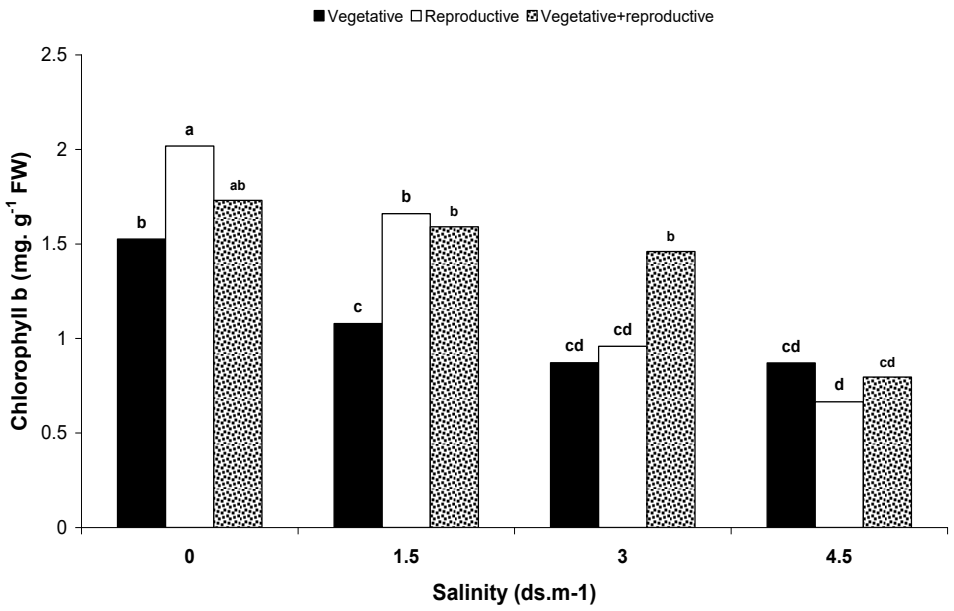

FIGURE 1: Interaction between SNP application time and salinity on chlorophyll b content.

3.4. Total chlorophyll content

The highest total chlorophyll content of the leaves was observed when SNP was used twice at vegetative + reproductive (3.81 mg.g^{-1}) and lowest at vegetative stage (3.058 mg.g^{-1}, Table 2). Since total chlorophyll content is the sum of chlorophyll a and b, both of them were less at vegetative stage than the other stages, so the total amount of chlorophyll is the lowest. The higher total chlorophyll content by SNP spraying in vegetative + reproductive stages due to higher levels of chlorophyll a and b in these two stages. In cotton, it was reported that SNP application reduces the damage caused by salt stress to chlorophyll [42].

With increasing salinity stress, the total chlorophyll content decreased linearly, so that the control treatment had the highest chlorophyll content and 4.5 dS m^{-1} treatment had the lowest total chlorophyll (Table 3). As shown in figure 2, at the 0 and 1.5 dS m^{-1}, salinity levels, delay in SNP sprays induce the production of more total chlorophyll while at higher salinity levels early SNP spraying resulted in higher chlorophyll content. In the studies about the effects of

salinity on the physiological and morphological characteristics of grape varieties, it has been shown that salinity induce the decrease of chlorophyll index in leaves significantly [43].

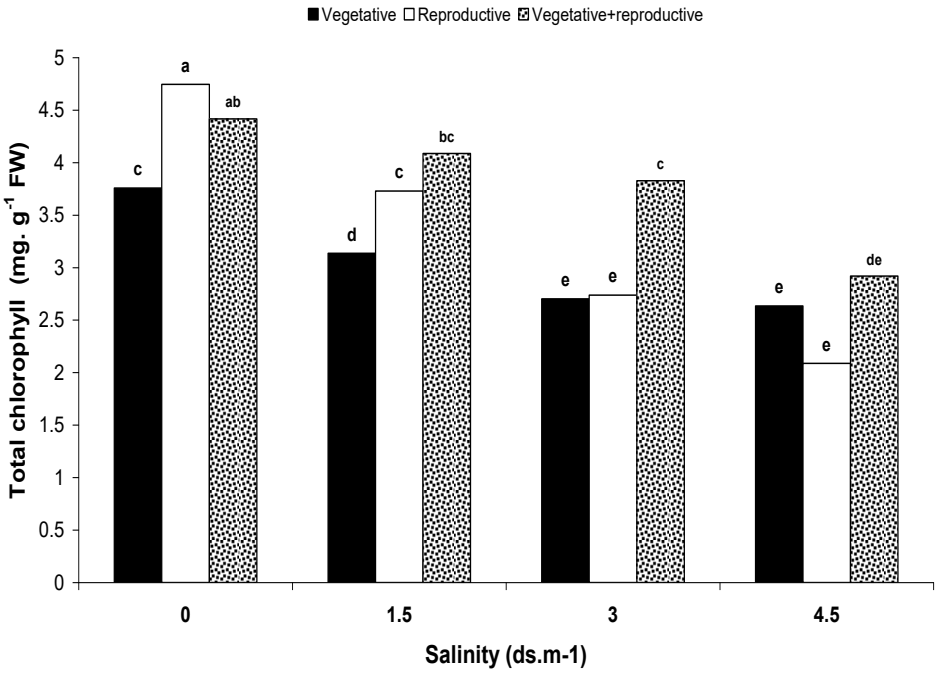

FIGURE 2: Interaction between SNP application time and salinity on total chlorophyll content.

3.5. Carotenoids content

The highest level of carotenoids in the leaf was observed when SNP was used at reproductive stage (0.55 mg.g^{-1}) and its lowest during vegetative stage (0.445 mg. g^{-1}, Table 2).There was no significant difference among treatment. A previous research on tomato showed that pre-treatment of SNP had no significant effect on carotenoids in this plant [5].

The highest amount of leaf carotenoids was obtained in the control (0.61 mg.g^{-1}) and the lowest at the salinity level of 4.5 dS m^{-1}, (0.38mg.g^{-1}), which showed a significant difference between treatments. There was not significantly different between control and 1.5 dS m^{-1}(Table 3). Contrary to the above results, previous sties suggest that the carotenoids have an antioxidant role, therefore, in salinity stress conditions, their amounts increase [15].

3.6. Leaf sodium content

Results display that, at 4.5dS m^{-1} leaf sodium content was maximum and control had the minimum of sodium. No significant difference was observed between control and 1.5 $ds.m^{-1}$ salinity level (Table 3). Increasing salinity levels to 1.5, 3 and 4.5 dS m^{-1} dramatically increased sodium content 15, 35 and 40%, respectively. It was also reported in sugar beet that salt stress has induced a significant increase in the concentrations of Na and Cl ions [44].

3.7. Leaf Potassium content

The highest amount of potassium was observed when SNP was used twice at reproductive + vegetative (5.75 $mg.g^{-1}$) and the lowest was measured at reproductive stage (4.45 $mg.g^{-1}$). The use of the solution at the vegetative stage (4.108 $mg.g^{-1}$) showed a higher effect on the amount of potassium in the leaf compared to reproductive stage (4.458 $mg.g^{-1}$, Table 2). KoohiFaeq et al. (2011) showed a significant decrease in potassium content in leaves and roots with increasing salinity.

The highest amount of potassium in leaf was in control (6.96 $mg.g^{-1)}$ and lowest in 4.5 ds.m-1 (2.89 $mg.g^{-1}$), which the values were significantly different from each other. (Table 3). The reduction of potassium in salinity conditions can be due to sodium competition for binding to plasma membrane carriers and potassium leakage due to instability of the plasma membrane [45]. Previous studies have reported that the concentration of potassium ion in sugar beet decreases in salinity stress conditions, which is consistent with the results of the current study on sweet corn. It has also been reported that wheat growth decreases with decreasing potassium ion in salinity conditions[46].
As shown in figure 3, in high salinity conditions, delay in SNP spray application could not reduce the effects of salinity stress, and the increase in the frequency of spraying due to increased concentrations of SNP had inhibitory effects on potassium uptake, which may be due to reducing root activity to potassium absorption. While in the control treatment, and low salinity levels (1.5 $dS.m^{-1}$), increasing the amount of spraying (spraying at the vegetative+reproductive stage) increased the content of potassium in sweet corn leaf (Figure 3), which also indicates that SNP can have both inhibitory and stimulatory effects on potassium levels. It has been reported that in salt stress conditions, sodium absorbtion increases calcium and potassium decreases. In such a situation, the addition of appropriate amounts of SNP reduces sodium uptake and increases the absorption of potassium, magnesium and calcium, which may be the effect of SNP in hormone signaling that might be implicated in salt tolerance. As the ratio of potassium to sodium increases, the activity of H+ ATPase enzyme also increases. In addition, the protective effects of SNP in salt stress conditions may be associated with increased osmotic regulation associated with salt discharging.

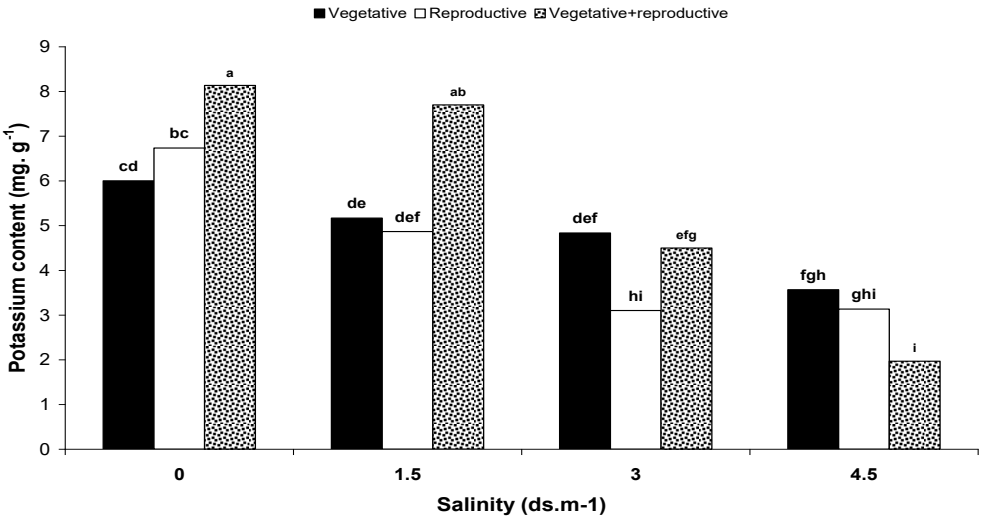

FIGURE 3: Interaction between SNP application time and salinity on potassium content.

4. CONCLUSION

In conclusion, the foliar spraying of SNP in salt-sensitive sweet corn was an effective way to stimulate physiological and morphological traits when plants were exposed to salt stress. The time of exogenous application of SNP to mitigation of salinity in Sweet corn depends on salinity levels. At low salinity condition sodium nitroprusside foliar application in vegetative + reproductive stage and in high salinity level once in the vegetative stage can reduce the effects of salinity.

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