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Original Research

Role of Sodium nitroprusside on mitigation of salt stress in Sweet corn

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ABSTRACT:

Effect of salinity and the role of sodium nitroprusside (SNP) on sweet corn was examined as a completely randomized design with three replications in Islamic Azad University of Sabzevar. Factors were sodium nitroprusside at the concentration of 200 ppm (vegetative, reproductive and vegetative + reproductive) and salinity (0,1.5, 3 and 4.5 $dS.m^{-1}$) during various growth stages. When SNP was applied at vegetative + reproductive stage, the heighest of all the parameters were recorded except carotenoids which was high in the groups treated with SNP at reproductive stage. On the whole, salinity stress imparted the growth of the plant negatively whereas SNP application at vegetative and reproductive stage had better growth effects.

Keywords:

Foliar application, Salinity, Sodium nitroprusside, Sweet corn, Chlorophyll.

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INTRODUCTION

Salinity stress, especially in arid and semi-arid condition, is an important limitation to crop production. Salt stress directly or indirectly affected biochemical, morphological and anatomical characteristics of crop species including germination Neha *et al.* (2017), growth Marvi *et al.* (2011), cell division Negrão *et al.* (2017), photosynthesis Verena *et al.* (2017), nutrient metabolic and uptake Alejandro *et al*. (2017), crop development and yield Katerji *et al.* (2003) and so on. Different methods are used to reduce the inevitable effects of salinity in plants such as planting of tolerant cultivar Marvi *et al.* (2011), nutrient management (Ali *et al.*, 2011; Ardakani *et al*., 2016; Manesh *et al.*, 2013), agronomy practice Manesh *et al.* (2013) and nowadays foliar application of osmo-protectants or compatible solutesas well as glycine andbetaine Leonid *et al.* (2017), salicylic acid Hadi *et al.* (2017), proline Sabagh *et al.* (2017), ascorbic acid, 24-epibrassinolide and sodium nitroprusside (Praveen *et al.*, 2017).

Sodium Nitroprusside (SNP) is a nitric oxide releasing compound (NO), whose role in plants has been the subject of many research studies (Praveen *et al.*, 2017; Lei *et al.*, 2007; Tian and Lei, 2007; Wang *et al.*, 2010). 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 (Meng *et al.*, 2015). Although NO is less well known in plant functions, recent advances research have shown it has a major role in regulating many plant growth functions, growth and development, response to environmental condition that affecting morphology, message transmission, seed germination, root growth, the formation of shoots, the maturity of fruits (Chunfang *et al.*, 2009). However, the protective role of NO in plants depends on NO concentration, tissue type, age and plant species, and stress type (Praveen *et al.*, 2017). 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 reduces the adverse effects of salinity (Akio *et al.*, 2002). Protect against oxidative damage and increase tolerance to osmotic stress was reported by application of 0.2 mM in rice seedling Lopez-Carrion *et al.* (2008) and 1 mM in barely seedling (Li *et al.*, 2008). Also, in 8-day old rice seedlings, pretreatment of 1 mM sodium nitroprusside (SNP) over two days increased the salt tolerance to sodium chloride (Akio *et al.*, 2002). Huaifu *et al*. (2007) reported that application of NO under salt stress in cucumber increased antioxidant enzyme activates and chlorophyll and proline content that resulting in enhancing seedling growth.

The mufahon in the locus 'Su' (sugary) on chromosome number 4 of the corng resulted in sweet corn. The endosperm of the seeds showed accumulation of soluble sugars due to the genetic variation. 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 damage the plant (temperatures above 35°C and less than 10°C (Tracy and Hallauer, 1994). In contrast to corn, which is one of the grains used to feed domesticated animals or the production flour, sweet corn is utilized as a vegetable for human. Presently sweet corn is one of the most well known vegetables globally and its utilization is expanding because of its flavor and rich in nutrients. The estimation of this yield for processing (canning and freezing) and as a fresh vegetable is the second and fourth respectively (Afsharmanesh, 2013). In 2003, the zone under sweet corn cultivation globally was 1,019,698 ha with an average of 8602 $kg.ha^{-1}$.

Salinity is the most widely recognized environmental stress all through the world, including Iran (Mohammad *et al*., 2010). There is an absence of

Table 1. Analysis of variance table for the studied parameters

SOV	df	Plant height (cm)	Plant dry weight (g. plant^{-1})	Chlorophyll a (mg. g^{-1} FW)	Chlorophyll b (mg. g^{-1} FW)	Total chlorophyll (mg. g^{-1} FW)	Carotenoid content (mg. g^{-1} FW)	Sodium content (mg. g^{-1})	Potassium content (mg. g^{-1})
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.

research on the impacts of salinity on sweet corn, in any case, it is accepted that sweet corn is a semi-senstive to salinity. (Eugene and Hoffman, 1977). This plant has been resistant to salinity during germination but with increasing salinity level, germination is delayed. 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 a lot of changes in sweet corn, including tissue hydration, ion toxicity, food insecurity, and so on. Research on sweet corn hybrids response to salt stress have shown that although hybrids are identical response to salt stress, but seed germination, root length, stem length, fresh and dry weight of roots and stems are reduced with increasing of salinity. Also, salinity increases the amount of malondialdehyde, proline and H_2O_2 in the seedling (Shtereva *et al*., 2015). We hypothesized that SNP application improves the physiological traits of Sweet corn grown under salt conditions. Thus, the objective of conducting the present study was to explore up to what extent foliar-applied SNP could alter chlorophyll content and Ion content under saline conditions of sweet corn grown under salt conditions.

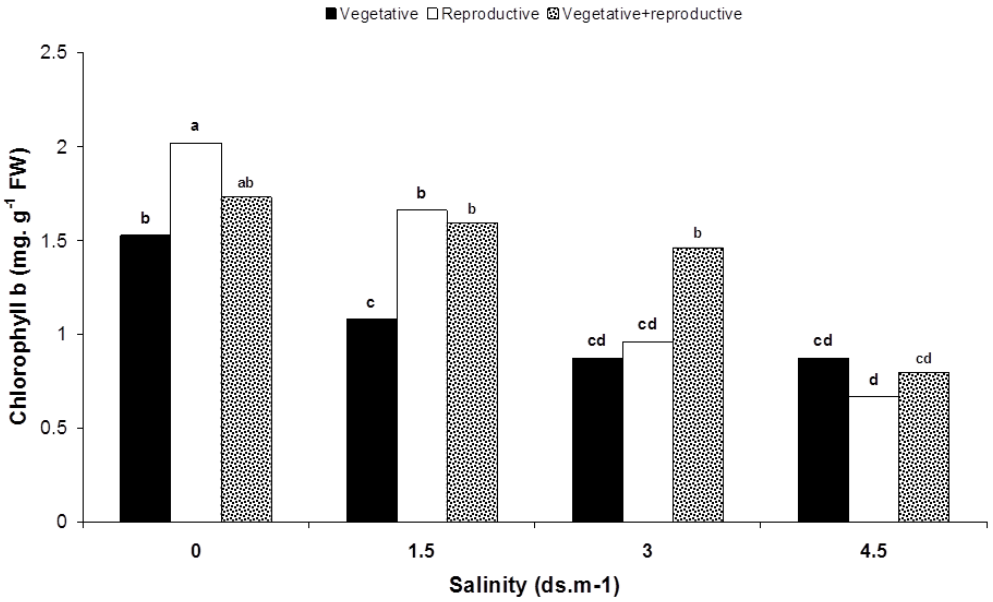

Figure. 1. Interaction between SNP application time and salinity on chlorophyll b content

Table 2. Effect of SNP application time on parameters studied

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

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

MATERIALS AND METHODS

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

Sweet corn (Gold seed kSC403 cultivar) 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. 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 mg of urea, 150 mg $Ca(H_2PO_4)_2.H_2O$, 100 mg of K_2SO_4, 40 mg of $FeSO_4.7H_2O$, 20 mg of $MnSO_4.H_2O$, 20 mg of $ZnSO_4.7H_2O$, 10 mg of $CuSO_4.5H_2O$ and 5 mg 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, three leaves stage and stem elongation).

To measure the height, the length of three randomly selected plants in each pot was measured by the meter. To 20-40 mL of 80% acetone, one gram of

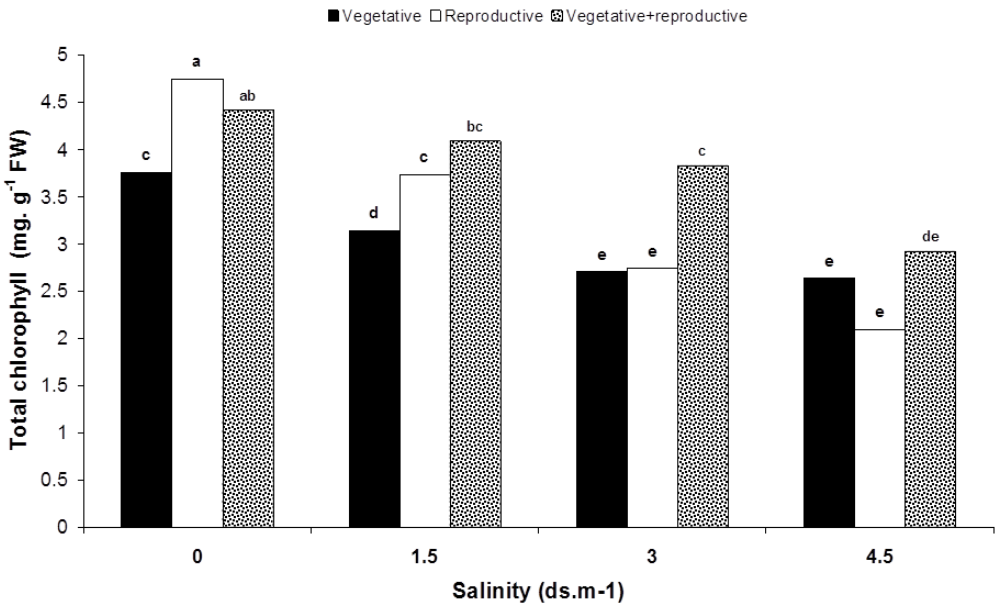

Figure 2. Interaction between SNP application time and salinity on total chlorophyll content

Table 3. Effect of salt stress on parameters studied

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

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

freshly cut fresh leaves were added and centrifuged at 5000-10000 rpm for five minutes. Till the residue becomes colourless, the supernatant was discarded and the process was repeated. Using the solvent as blank, the absorbance of the solution was read at 470 nm, 645 nm and 663 nm (Arnon, 1967):

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 chlorophyll a) - 104 (mg chlorophyll b)/227

Hamada and El-Enany (1994) method was used to measure sodium and potassium elements. For this purpose, 0.5 g dry matter of leaves were washed and then 10 mL of concentrated nitric acid was added and placed in the laboratory temperature for 48 h. In order to remove all vapours, the specimens were placed on a heated oven thermostat for two hours. After leaving acidic vapours and viewing a colorless solution, 100 mL of the distilled water was added to each sample. Using Whatman filter paper No 1, the samples were smooth and sodium and potassium values were measured by photometric photometry (Hamada and El-Enany, 1994). 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.

RESULTS AND DISCUSSION

The final plant height, plant dry weight, chlorophyll a, chlorophyll b, total chlorophyll,

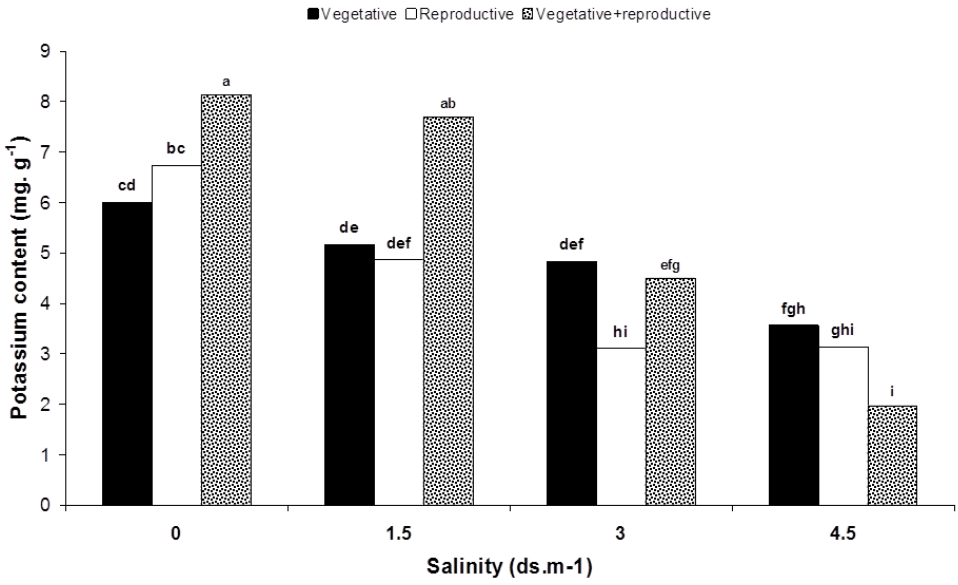

Figure 2. Interaction between SNP application time and salinity on potassium content

carotenoids, leaf chlorophyll index, leaf sodium and potassium content were affected by the time of 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).

Plant height

The highest plant height was observed when SNP used is vegetative + reproductive (115.33 cm) and the lowest is in vegetative time (101.375 cm). It seems that low plant height at SNP application in the vegetative stage was due to the low absorption of SNP due to less leaves per plant. In the vegetative stage, the number or surface area of the leaf has not been produced sufficiently 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 increasing the activity of antioxidant enzymes that protects the plant from damage caused by free oxygen radicals (Huaifu *et al.*, 2007). In wheat, it has also been reported that SNP in saline conditions increases salinity tolerance by increasing the amount of proline in the leaf (Chunfang *et al.*, 2009). It has been reported in cotton that SNP consumption not only increases plant growth but also increases stem and root lengths. It also increases the osmotic pressure of the cell and improved cytoplasmic viscosity that led to the increase stem length. Although high levels of SNP had a negative effect on stem elongation (Liu *et al.*, 2013).

The highest plant height was observed in the control treatment (129 cm) and the lowest was in 4.5 dSm^{-1} (88.17 cm). There was no significant difference between salinity treatments at 1.5 dSm^{-1} levels and control. There are several reasons for reducing plant height with increasing salinity stress. For example, in high salinity level, the plant has difficulty to water absorption, which reduces available water, it also reduces the cell division that depends on the stress of turger pressure. On the other hand, under stress conditions, the photosynthesis of the plant is also affected. Reducing photosynthesis reduces the contribution of photosynthetic materials to growth, which will also reduce plant height. Reduction and disruption of nutrient uptake are also due to decreasing of plant height. Reducing plant growth under salt stress may be due to reduced water absorption and metabolic activity, sodium and chloride toxicity along with food deficiency (Cirillo *et al.*, 2016; Eshghizadeh *et al.*, 2011; Khan *et al.*, 2016; Desire *et al.*, 2009).

Plant dry weight

SNP foliar application at vegetative + reproductive stages had maximum dry weight (0.022 g/ plant) and the lowest in vegetative time (0.014 g/plant). 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 are less than the reproductive stage, therefore, at this stage, lower absorption of SNP is done by the plant. On the other hand, SNP application in the reproductive phase may delay or decrease the leaf loss, which also increases the dry weight of the plant.

Salt stress significantly reduced dry weight. The data of dry weight (Table 2) revealed that the maximum dry weight (0.22 g/plant) produced in the control, whereas the lowest dry weight was recorded at 4.5 $dS.m^{-1}$ (0.01 g/plant). There were significant difference between treatments. 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 the use of a part of photosynthesis or growth material to produce 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 reason of dry weight loss of the plant at high salinity level.

Chlorophyll a

The highest amount of chlorophyll a was observed when SNP was used at vegetative + reproductive (2.15 mg/g) and it has the lowest at the vegetative stage (1.98 mg/g). It seems that the main reason for increasing the amount of chlorophyll a when SNP applied in two times compared to one times is due to SNP application may lead 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 (Inês *et al.*, 2015). Similar results have been reported on the increase of photosynthetic pigmentation due to SNP consumption in chickpea (Parvaiz *et al.*, 2016) and sunflower (Nejadalimoradi *et al.*, 2014).

Salt stress dramatically reduced leaf chlorophyll a content. The main reason for the reduction of photosynthetic pigments in high salinity may be due to the prevention of the adsorption of Mg^{+2} due to the high amounts of sodium, which leads to inhibitory chlorophyll synthesis. It is believed that high levels of sodium inhibit protein synthesis and weaken the binding of chlorophyll and chloroplast in which leading to chlorophyll degradation (Nejadalimoradi *et al.*, 2014). In Lin and Shi (2010) experiments, with increasing salinity, to 10 $dS.m^{-1}$, net photosynthesis rate, stomatal conductance and chlorophyll a content of sunflower had a decreasing trend (Liu and Shi, 2010). Reduction of chlorophyll content in salt stress conditions may be due to the activity of chlorophyllase enzymes. Some regulators, such as abscisic acid and ethylene, stimulate the activity of this enzyme (Neda *et al.*, 2013).

Chlorophyll b

Chlorophyll b content was higher in two times SNP application at vegetative + reproductive stages in other treatments. There were no significant difference between SNP application at reproductive stage and vegetative+ reproductive. Spraying at the reproductive stage increased 56.67% chlorophyll b content in comparison with SNP application at the 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 stage was due to the selected leaf. As shown in Table 3, the maximum chlorophyll b content (12.19 cm) was measured in control. There were significant differences among treatments. Increasing of salinity to 10 $dS.m^{-1}$ in sunflower decreased chlorophyll content, stomata conductance and chlorophyll (Liu and Shi, 2010).

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 the end of the growth stage. This suggested that in high salinity levels, the tolerance of sweet corn to salinity is low so delay in SNP application until reproductive stage couldn't have the mitigation of salinity stress. Salinity has damaged the membrane tissue and increased chlorophyllase enzyme activity, and degrade a large part of chlorophyll (Singh and Tuteja, 2010).

Total chlorophyll content

The highest total chlorophyll content of the leaves was observed when SNP was used in two times at vegetative + reproductive (3.81 mg/g) and its lowest was at vegetative stage SNP application (3.058 mg/g). Since total chlorophyll content is the sum of chlorophyll a and b, both chlorophyll a and chlorophyll b are 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 (Magdy *et al.*, 2012).

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. As shown in Figure 2, at the 0 and 1.5 $dS.m^{-1}$, salinity levels, delay in SNP sprays produced more total chlorophyll content while at higher salinity levelsearly SNP spraying produced higher chlorophyll content. In studying the effects of salinity on the physiological and morphological characteristics of grape varieties, it has been shown that salinity decreased the chlorophyll index in leaves significantly (Ahmad, 2012).

Carotenoid content

The highest level of carotenoids in the leaf was observed when SNP was used at reproductive stage (0.55 mg/g) and its lowest during vegetative (0.445 mg/ g). There was no significant difference among treatment. The research on tomato showed that pre-treatment of SNP had no significant effect on carotenoids in this plant (Alejandro *et al.*, 2017).

The highest amount of leaf carotenoids was obtained in the control treatment (0.61) and the lowest in salinity level 4.5 $dS.m^{-1}$, (0.38), which showed a significant difference between treatments. There showed no significant difference between control and 1.5 $dS.m^{-1}$. Contrary to the above results, it is believed that the carotenoids have an antioxidant role, therefore, in salinity stress conditions, their amounts increase.

Leaf sodium content

At 4.5 $dS.m^{-1}$ leaf Sodium content was maximum and control had the minimum of sodium. There was no significant difference between control and 1.5 $dS.m^{-1}$ salinity level. Increasing salinity levels to 1.5, 3 and 4.5 dSm^{-1} dramatically increased sodium content to 15, 35 and 40%, respectively. It was reported that the concentration of Na^+ and Cl^- ions in sugar beet under salinity stress increased significantly (Cherki *et al.*, 2002).

Leaf potassium content

The highest amount of potassium was observed when SNP was used two times at reproductive + vegetative (5.75 mg/g) and the lowest was measured at reproductive time (4.45 mg/g). The use of this solution at the vegetative stage (4.108 mg/g) showed a higher effect on the amount of potassium in the leaf compared to the reproductive stage (4.458 mg/g). Koohi Faeq *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 treatment 6.96 $dS.m^{-1}$ and lowest in 4.5 $dS.m^{-1}$ (2.89), which showed a significant difference between treatments. 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 (Sérgio *et al.*, 2008). They also reported that the concentration of potassium ion in sugar beet decreased in salinity stress conditions, which is consistent with the results of this study on sweet corn. It has also reported that wheat growth decreases with decreasing potassium ion in salinity conditions (Sharma *et al.*, 2005).

The means comparison showed that in high salinities, 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 (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, which also indicated that SNP can have both inhibitory effects and stimulatory effects on

the potassium levels. It has been reported that in salt stress conditions, Na^+ adsorption increases Ca^{++} and K^+ 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 SNP effect in a hormone signal in salt tolerance and by increasing the ratio of potassium to sodium, it increases the activity of the H^+ ATPase enzyme. In addition, the protective effects of SNP in salt stress conditions may be associated with increased osmotic regulation associated with salt discharging.

CONCLUSION

In conclusion, the foliar spraying of SNP in salt-sensitive of 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 depend on the 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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