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ORIGINAL ARTICLE

Alleviation of the Effects of NaCl Salinity in Spinach (*Spinacia oleracea* L. var. All Green) Using Plant Growth Regulators

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Exposure to environmental stress due to salinity has been reported to result in adverse effects on the growth of plants. Studies have shown that the use of plant growth regulators (PGRs) has an ameliorative effect on plants grown under saline conditions. In the present investigation, effect of gibberellic acid (GA_3), 6-furfuryladenine (Kinetin) and benzyl adenine (BA) on *Spinacia oleracea* L. var. All Green, cultivated under saline conditions has been studied. After a pre-soaking treatment of six hours in 20 mg L^{-1} solutions of GA_3, Kinetin and BA, the seeds were allowed to germinate and grow for forty-five days under saline conditions. On the analysis of mature leaves, it was observed that both chlorophyll a and b, and total chlorophyll showed an increase in PGR-treated plants compared to the untreated set. With the pretreatment, the reducing and non-reducing sugar content, as well as protein content of the leaves showed an increase in accumulation compared to the untreated plants. The accumulation of the stress metabolite proline, which increases under saline conditions, showed a significant decrease in the plants pretreated with PGRs.

Key words: Benzyle adenine, Gibberellic acid, Kinein, Plant growth regulators, Salinity.

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Key words: Benzyle adenine, Gibberellic acid, Kinein, Plant growth regulators, Salinity.

Salinity is one of the most devastating forms of land degradation which severely affects crop production worldwide especially in arid and semiarid regions (Shomeili *et al*., 2011). Attempts have been made to counteract the adverse effects of salinity on plants by pretreating the seeds with various plant growth regulators (Kasim and Dowidar, 2006). A report by Afzal *et al*. (2005) says that seed treatment with Kinetin can increase the ability of wheat to grow successfully under saline conditions. Mohammad (2007) has observed that gibberellic acid can be used to alleviate the adverse effects of salinity on mungbean seedlings. Similarly, it was reported by Bagdi and Afria (2008) that the adverse effects of salinity on growth and yield of wheat cultivar can be ameliorated by the use of benzyl adenine. Chauhan *et al*. (2009) suggested that plant growth regulators help in overcoming the harmful effects of salinity on growth by changing the endogenous growth regulators which affect plant water balance. Hence, in the present study an attempt has been made to study the ameliorative effect of plant growth regulators like gibberellic acid (GA_3), 6-furfuryladenine (Kinetin) and benzyl adenine (BA) on *Spinacia oleracea* L. var. All Green, cultivated under saline conditions.

MATERIALS AND METHODS

Seeds of *Spinacia oleracea* L. were obtained from Indian Agricultural Research Institute, Pusa, New Delhi. After a pre-soaking treatment of six hours in 20 mg L^{-1} solutions of gibberellic acid (GA_3), 6-furfuryladenine (Kinetin) and benzyl adenine (BA), seeds were sown in earthen pots (20 cm diameter, 29 cm height). The pots were irrigated using tap water till the phase of germination. A pot served as control where the seeds were irrigated with tap water. Remaining pots were irrigated with NaCl solutions. In order to acclimatize the plants to NaCl, the concentrations were raised after every three-four days in a stepwise manner starting from 5 mM till a final concentration of 60 mM NaCl. The plants were grown for forty-five days. Mature leaves of these plants were used to estimate organic constituents like chlorophyll a, chlorophyll b, total chlorophyll, reducing sugars, non-reducing sugars, proteins and proline using standard protocols of Arnon (1949), Miller (1959), Lowry *et al*. (1951) and Bates *et al*. (1973) respectively.

RESULTS AND DISCUSSION

From table 1, it is clear that chlorophyll a, chlorophyll b as well as total chlorophyll decreased with salinity as compared to control. A decrease in chlorophyll content has been observed in *Spinacia oleracea* by Kaya *et al*. (2001) under NaCl salinity. Similarly decreases in chlorophyll content have been reported in sweet sorghum (Almodares *et al.*, 2008) and *Vicia faba* (El Sayed, 2011) under saline conditions. The reduction in chlorophyll content can be attributed to the inhibitory effect of accumulated ions such as sodium and chloride, on the biosynthesis of the pigments as suggested by Mohammed (2007). He suggested that the degradation of these pigments as well as the structure of the chloroplast can be affected by NaCl. According to Arulbalachandran *et al*. (2009), the reduction of photosynthetic pigment under salinity occurs due to degradation of chlorophyll by enzyme chlorophyllase and reactive oxygen species generated during photorespiration.

When the seeds were pretreated with plant growth regulators, ameliorative effects were observed as the chlorophyll content were observed to improve with such treatment. The present study showed that GA_3, Kinetin and BA can successfully mitigate the adverse effect of NaCl salinity on pigment content (Table 1). Zeid (2011) also reported an alleviation of adverse effect of salinity on chlorophyll content in barley with GA_3 treatment. Ali *et al*. (2012) recorded restoration of altered pigments by application of GA_3 under saline condition in *Hibiscus sabdariffa*. Enhanced production of total chlorophyll due to grain priming with Kinetin in *Sorghum bicolor* has been observed by Alsokari (2009). Alleviation of salinity stress in wheat by the use of BA for presoaking has proved to be effective as total chlorophyll content has been reported to increase (Bagdi and Afria, 2008).

Reducing as well as non-reducing sugars were found to increase with increasing salinity in the present study (Table 2). While photosynthesis is the main source of carbohydrate accumulation, Munns (1993) reported that the concentrations of sugars (and reserve polysaccharides) always rise after plants are exposed to salinity. Similarly, Siringam *et al*. (2011) reported an increase in sugars in the roots of *Oryza sativa* under salt stress. They related such accumulation of soluble sugars with increased sodium content. Sugars in such cases may function as an osmoticum and help in maintaining water use efficiency in the root cells.

The present study showed further rise in sugars in pretreated seeds. Pretreating seed with Kinetin has improved the sugar content at a maximum level (Table 2). Similarly, Niazi *et al*. (2005) observed that Kinetin pretreatment has enhanced the sugar content in fodder beet grown under NaCl salinity. An increase in sugar content in wheat seedlings with GA_3 pretreatment has been observed by Jat and Sharma (2006) when grown under saline conditions.

In the present study, the protein content was observed to decrease with increase in salt concentration (Table 2). There are several reports indicating a decrease in protein content under saline conditions as in *Dioscorea rotundata* (Jaleel *et al*., 2008), rice (Amirjani, 2010) and tomato (Doganlar *et al*., 2010). Decreased protein content under the influence of NaCl salinity has also been reported in *Triticum aestivum* roots by Tammam *et al*. (2008).

The pretreatment with plant growth regulators showed an improvement in protein content, Kinetin pretreatment was found to be most effective. Similarly Kinetin was recorded beneficial when used for pretreating the seeds of *Hordeum vulgare* thereby increasing the protein content under saline irrigation by Sarwat and El-Sherif (2007). Shomeili *et al*. (2011) recorded a positive effect of GA_3 treatment on protein content in sugarcane under NaCl salinity. Bagdi and Afria (2008) have also observed that presoaking the seeds of wheat with BA helped by improving protein content over control.

In the present study, an increase in the proline contents was observed with increasing salinity (Table 2) Accumulation of osmolytes such as proline are beneficial under stress conditions as they make the water potential low inside the cell and prevent the intracellular loss as suggested by Mahajan and Tuteja (2005). There are several reports where an increase in proline content have been reported under saline conditions as in alfalfa (Mezni *et* al., 2010), *Jatropha curcas* (Patel *et al*., 2010), *Brassica* genotypes (Siddiqui *et al*., 2010) and *Capsicum annuum* (Chookhampaeng, 2011).

Presoaking treatment with all the three PGRs has resulted in lowering the proline content under saline conditions, most effective plant growth regulator being GA_3 (Table 2). Similarly, Kasim and Dowidar (2006) observed lower proline content in GA_3 primed radish seedlings under NaCl treatment. They suggested that, this decrease in the amino acid in GA_3 primed seedlings may be due to their incorporation in some new proteins or their utilization in the synthesis of certain existing proteins.

CONCLUSION

On the basis of above findings it is clear that all the three plant growth regulators used in the present study are found to be effective in mitigating the adverse effect of NaCl on *Spinacia oleracea* L. var. All Green.

Table 1 Effect of seed priming with PGRs on chlorophyll content from the mature leaves of 45 day old *Spinacia oleracea* L. var. All Green grown under saline (60 mM NaCl) environment

NaCl Concentration /PGR Treatment	Chlorophyll a (mg/ 100g)*	Chlorophyll b (mg/ 100g) *	Total Chlorophyll (mg/ 100g) *
0 mM (Control)	45.799 ± 0.258	31.803 ± 2.293	77.760 ± 2.156
60 mM	20.325 ± 0.390	15.122 ± 0.986	35.509 ± 1.363
60 mM + GA_3	40.856 ± 0.846 #	30.587 ± 1.127 #	71.927 ± 0.286 #
60 mM + Kinetin	37.161 ± 0.436 #	27.567 ± 0.527 #	64.709 ± 0.953 #
60 mM + BA	40.391 ± 0.731 #	27.655 ± 1.313 #	68.026 ± 0.905 #

Results are the mean of three determinants.
*One-way ANOVA was carried out and F ratio was significant at 5% level of significance.
Significant at $p < 0.05$ (t-test was carried out to test whether there is significant difference between control and individual salt concentration).

Table 2 Effect of seed priming with PGRs on reducing sugars, non-reducing sugars, protein and proline content from the mature leaves of 45 day old Spinacia oleracea L. var. All Green grown under saline (60 mM NaCl) environment

NaCl Concentration/PGR Treatment	**Reducing Sugars (mg/ 100g) •**	**Non-Reducing Sugars (g/ 100g) •**	**Protein (g/100g FW) •**	**Proline (mg/100g FW) •**
0 mM (Control)	541.6 ± 37.932	1.441 ± 0.054	1.813 ± 0.105	2.48 ± 0.145
60 mM	640.0 ± 26.770	1.953 ± 0.134	1.206 ± 0.067	3.00 ± 0.158
60 mM + GA_3	738.3 ± 28.674	1.931 ± 0.048	1.686 ± 0.049	1.28 ± 0.048
60 mM + Kinetin	788.3 ± 28.963	2.105 ± 0.044	2.000 ± 0.058	1.66 ± 0.066
60 mM + BA	746.6 ± 30.641	1.776 ± 0.047	1.753 ± 0.052	1.48 ± 0.041

Results are the mean of three determinants.
• One-way ANOVA was carried out and no significant difference was observed in the group, so data is not analyzed further for pair-wise comparisons among the treatments.

REFERENCES

Afzal, I., Basra, S.M.A. and Iqbal, A. (2005): The effects of seed soaking with plant growth regulators on seedling vigor of wheat under salinity stress. *Journal of Stress Physiology and Biochemistry*, **1(1):** 06-14.

Ali, H.M., Siddiqui, M.H., Basalah, M.O., Al-Whaibi, M.H., Sakran, A.M. and Al-Amri, A. (2012): Effects of gibberellic acid on growth and photosynthetic pigments of *Hibiscus sabdariffa* L. under salt stress. *African Journal of Biotechnology*, **11(4)**: 800-804.

Almodares, A., Hadi, M.R. and Dosti, B. (2008): The effects of salt stress on growth parameters and carbohydrates contents in sweet sorghum. *Research Journal of Environmental Sciences*, **2(4)**: 298-304.

Alsokari, S.S. (2009): Modulatory role of kinetin on photosynthetic characteristics, yield and yield attributes of cadmium-treated *Sorghum bicolor* plants. *Journal of Applied Sciences Research*, **5(12)**: 2383-2396.

Amirjani, M.R. (2010): Effect of NaCl on some physiological parameters of rice. *EJBS*, **3(1)**: 06-16.

Arnon, D.I. (1949): Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.*, **24**: 1-15.

Arulbalachandran, D., Sankar Ganesh, K. and Subramani, A. (2009): Changes in metabolites and antioxidant enzyme activity of three *Vigna* species induced by NaCl stress. *American-Eurasian Journal of Agronomy*, **2(2)**: 109-116.

Bagdi, D.L. and Afria, B.S. (2008): Alleviation of salinity effects using plant growth regulators in wheat. *Indian Journal of Plant Physiology*, **13(3)**: 272-277.

Bates, L.S., Waldren, R.P. and Teary, I.D. (1973): Rapid determination of free proline for water stress studies. *Plant Soil,* **39**: 205-207.

Chauhan, J.S., Tomar, Y.K., Singh, N.I., Ali, S. and Debarati (2009): Effect of growth hormones on seed germination and seedling growth of black gram and horse gram. *Journal of American Science*, **5(5)**: 79-84.

Chookhampaeng, S. (2011): The effect of salt stress on growth, chlorophyll content, proline content and antioxidative enzymes of pepper (*Capsicum annuum* L.) seedling. *European Journal of Scientific Research*, **49(1)**: 103-109.

Doganlar, Z.B., Demir, K., Basak, H. and Gul, I. (2010): Effects of salt stress on pigment and total soluble protein contents of three different tomato cultivars. *African Journal of Agricultural Research*, **5(15)**: 2056-2065.

El Sayed, H.S.A. (2011): Influence of salinity (NaCl and Na_2SO_4) treatments on growth development of broad bean (*Vicia faba* L.) plant. *American-Eurasian Journal of Agricultural and Environmental Sciences*, **10(4)**: 600-610.

Jaleel, C.A., Sankar, B., Sridharan, R. and Paneerselvam, R. (2008): Soil salinity alters growth, chlorophyll content and secondary metabolite accumulation in *Catharanthus roseus*. *Turk Journal of Biology*, **32**: 79-83.

Jat, N.K. and Sharma, V. (2006): The interactive effect of salinity and PGR on certain bio-chemical parameters in wheat seedlings. *American Journal of Plant Physiology*, **1(2)**: 132-141.

Kasim, W.A. and Dowidar, S. (2006): Amino acids and soluble protein profile of radish seedlings under salt stress as affected by GA_3 priming. *Indian Journal of Plant Physiology*, **11(1)**: 75-82.

Kaya, C., Higgs, D. and Kirnak, H. (2001): The effects of high salinity (NaCl) and supplementary phosphorus and potassium on physiology and nutrition development of spinach. *Bulgarian Journal of Plant Physiology*, **27(3-4)**: 47-59.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951): Protein measurement with Folin Reagent. *Journal of Biological Chemistry*, **93**: 265-275.

Mahajan, S. and Tuteja, N. (2005): Cold, salinity and drought stresses: An overview. *Archives of Biochemistry and Biophysics*, **444**: 139-158.

Mohammad, A.H.M.A. (2007): Physiological aspects of mungbean plant (*Vigna radiata* L.) in response to salt stress and gibberellic acid treatment. *Research Journal of Agriculture and Biological Sciences*, **3(4)**: 200-213.

Mezni, M., Albouchi, A., Bizid, E. and Hamza, M. **(**2010): Minerals uptake, organic osmotica contents and water balance in *Alfalfa* under salt stress. *Journal of Phytology*, **2(11)**: 01-12.

Miller, G.L. (1959): Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, **31(3)**: 426-428.

Munns, R. (1993): Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. *Plant Cell and Environment*, **16**: 15-24.

Niazi, B.H., Rozema, J. and Athar, M. (2005): Effect of pre-germination and post-germination treatment with growth hormones (kinetin and abscisic acid) on ion concentration and biochemical contents of fodderbeet and seebeet under saline conditions. *General and Applied Physiology*, **31(1-2)**: 89-104.

Patel, A.D., Panchal, N.S., Pandey, I.B. and Pandey, A.N. (2010): Growth, water status and nutrient accumulation of seedlings of *Jatropha curcas* L. (Euphorbiaceae) in response to salinity. *Anales de Biologia*, **32**: 59-71.

Sarwat, M.I. and El-Sherif, M. (2007): Increasing salt tolerance in some barley genotypes (*Hordeum vulgare*) by using kinetin and benzyladenine. *World Journal of Agricultural Sciences*, **3(5)**: 617-629.

Shomeili, M., Nabipour, M., Meskarbashee, M. and Memari, H.R. (2011): Effects of gibberellic acid on sugarcane plants exposed to salinity under a hydroponic system. *African Journal of Plant Science*, **5(10)**: 609-616.

Siddiqui, M.H., Mohammad, F., Khan, M.N., Al-Wahibi, M.H. and Bahkli, A.H.A. (2010); Nitrogen in relation to photosynthetic capacity and accumulation of osmoprotectant and nutrients in *Brassica* genotypes grown under salt stress. *Agricultural Sciences in China*, **9(5)**: 671-680.

Siringam, K., Juntawong, N., Cha-Um, S. and Kirdmanee, C. (2011): Salt stress induced ion accumulation, ion homeostasis, membrane injury and sugar contents in salt-sensitive rice (*Oryza sativa* L. spp. *indica*) roots under iso-osmotic conditions. *African Journal of Biotechnology*, **10(8)**: 1340-1346.

Tammam, A.A., Abou Alhamd, M.F. and Hemeda, M.M. (2008): Study of salt tolerance in wheat (*Triticum aestivum* L.) cultivar Banysoif 1. *Australian Journal of Crop Science*, **1(3)**: 115-125.

Zeid, I.M. (2011): Alleviation of seawater stress during germination and early growth of barley. *International Journal of Agriculture: Research and Review*, **1(2)**: 59-67.