ORIGINAL RESEARCH PAPER

Biology

INTERACTIVE EFFECTS OF GIBBERELLIC ACID AND SALT STRESS ON GROWTH AND SOME METABOLIC CHANGES OF *ZEA MAYS* L. SEEDLIGNS.

KEY WORDS: Salinity, Gibberellic acid, Photosynthetic pigments.

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ABSTRACT

A pot experiment was designed in a green house to study the effect of gibberellic acid on *Zea mays* L. seedlings grown in 25, 50, 100 mM NaCl. Salinity induced marked decreases in growth parameters (shoot and root lengths, shoot and root fresh and dry weights and leaf area) , photosynthetic pigments (chlorophylls a , b and carotenoids) insoluble sugars and some mineral nutrients as K, Ca and Mg contents. The effects of NaCl on the previous parameters were increased with NaCl concentrations. Application of GA_3counteracted the NaCl deleterious effects on *Zea mays* seedlings by lowering the levels of Na and increasing the K , Ca and Mg levels. It also caused a significant increases in photosynthetic pigments, insoluble sugars and proline .

Introduction

Salinity is one of the major factors that affect plant growth (shoot and root lengths, shoot and root fresh and dry weights and leaf area) and metabolism, leading to severe damage and a loss of productivity mainly in arid and semi arid regions (Vaidyanathan et al., 2003 ; Taibi et al.,2016). High salinity afflicts about 95 million hectars of land worldwide (Szaboles ,1994). Salt stress decreases chlorophylls, carotenoids, photosynthetic activity and carbohydrate contents (El-Shihaby et al.,2002 ; Jasmine and John, 2012).

These reductions are due to two main negative effects : water deficit and ion toxicity associated with excess Na^+ and Cl^+ uptake. They affect the plants by causing changes in membrane chemistry, cell and plant water status, protein synthesis and enzyme activities (Turkan Demiral ,2009).

The solid phase of the soil system, the concentration and composition of the solutes in the soil solution and its pH control the activity of the nutrient ion which is affected by salinity (Jia-minl et al.,2008). The relations between salinity and mineral nutrition of crops are extremely complex affecting the nutrient availability, competitive uptake and transport within the plant. The plant becomes susceptible to osmotic and specific ion injury as well as to nutritional disorders that may result in reduced growth, yield and quality (Rashad and Hussien, 2014)

The plant hormone plays an imported role in regulating the plant growth under stressful environment. Gibberellic acid (GA_3) is a common hormone which is most favourable for promoting and improving the plant growth, ion uptake and transport, and the nutrient utilization under salt stress . They are responsible for seed germination, stem elongation, leaf expantion and flowering, and prevent chlorophyll breakdown (Schwechheimer, 2008 ; Bose et al., 2013).

In view of these reports, the present investigation was undertaken to study the influence of GA_3 on growth, photosynthetic pigments, soluble and insoluble sugars, soluble protein and proline as well as some mineral nutrients contents of *Zea mays* seedlings subjected to salinity. *Zea mays* is a major vegetable crop with greater nutritional value and accounts for higher consumption and economic importance all over the world. Thus the major objective of this study was to determine the extent to which GA_3 could ameliorate the effect of salt stress on *Zea mays* seedlings.

Material and Methods

The green house experiment was performed at the Department of Botany and Microbiology, King Saud University, Riyadh KSA. The seeds of Zea mays (single cross 10) were obtained from the Agriculture Research Center, Giza, Egypt. The seeds were surface sterilized by 1% sodium hypochlorite for 10 min., then rinsed thoroughly with distilled water.

Five seeds were sown in plastic pots filled with vermiculite and sand (1:1). The pots were arranged in a simple randomized design with 5 replicates per treatment. After germination , the pots were divided into 4 groups :- i- control, ii- pots treated with different concentration of NaCl solutions 25, 50,100 mM, iii- pots treated with GA_3 (10^{-2} M) iv- pots treated with 10^{-2}M GA_3 with each of the NaCl concentration. The pots were irrigated with 200 ml water every 3 days. After 15 days from treatments, growth parameters in terms of shoot and root lengths, fresh and dry weights of shoot and root , and leaf area / plant were measured using leaf area meter (CL- 202 Area meter CID, INC).

Photosynthetic pigments (chlorophyll a, chlorophyll b and caroteniods) were determined spectrophotometrically following the method of Metzner et al., 1965.

Soluble carbohydrates was extracted following the method adopted by Homme et al., 1992. The dry residue left after extraction of soluble carbohydrates was used for determination of polysaccharides as described by Naguib , 1963.

Soluble proteins were determined according to the method described by Bradford, 1976 with BIO-RAD protein assay dye reagent using bovine serum albumin (BSA) as a standard.

Proline concentration was estimated colorimetrically according to the method of Bates et al., 1973 .

The mineral contents (Na , K , Ca , Mg , Fe ions) was determined according to the procedure described by A.O.A.C. (1995) using Atomic Absorption Spectrophotometer model AA-670 IF.

Statistical analysis was carried out according to Snedecor and Cochran, 1980 using (F. Test). Significant differences were obtained by calculating LSD at p<0.01 and p-0.05.

Results and Discussion

Application of the test levels of NaCl to *Zea mays* plants adversely influenced their growth pattern (shoot and root lengths, shoot and root fresh and dry weights and leaf area) as compared with control plants (table 1). All plants treated with 25mM NaCl increased non- significantly while those treated with 100 mM deceased high significantly below the control. These results are in agreement with Makkrash, 2012 ; Lai et al., 2014 and Taibi et al., 2016.

Water uptake is inhibited due to reduced osmotic potential in the soil solution caused by NaCl (Garcia-Sanchez et al. , 2002). Due to restriction of water uptake, water contents within the plant decreases and ultimately this leads to reduce growth rate. Growth inhibition might be also due to the reduction in cytoplasmic volume and the loss of cell turgor as a result of osmotic outflow of intercellular water (Summart et al., 2010). Beside the previous reasons, growth reduction is also due to the toxic effects of ions as well as unbalanced nutrient uptake by the plant (Keshavarzi et al. , 2011 ; Farhangi and Torabian , 2017).

GA_3 treated plants exhibited an increase in tolerance to salt treatment. This increase was reflected significantly in the measured growth criteria in plants treated with 25 and 50 mM NaCl more than those treated with 100 mM. Hamayun et al., (2010) observed the role of GA_3 in salinity alleviation on soyabean , also Jasmine and John ,(2012) observed the same results on Okra.

Table 1: Growth parameters of *Zea mays* seedlings subjected to different concentrations of NaCL in presence or absence of GA_3.

Each value is a mean of 3 replicates

NaCl (mM)	GA_3 (M)	Lengths (cm)		Fresh weight (g)		Dry Weight (g)		Leaf area /plant (cm^2)
		Shoot	Root	Shoot	Root	Shoot	Root	
0	0.0	7.08	17.10	1.05	0.64	0.117	0.061	41.22
25		7.23	17.30	1.11	0.67	0.119	0.068	42.13
50		6.50	14.20**	0.91*	0.55*	0.088*	0.051	40.01
100		4.38*	10.04**	0.66**	0.32**	0.053**	0.025**	27.75**
0	10^{-2}	13.37**	19.34**	1.28**	0.85*	0.147*	0.091**	56.22**
25		13.01**	19.70**	1.20*	0.88*	0.132*	0.095**	48.53**
50		12.62**	18.80**	1.17*	0.78	0.126	0.084**	46.38**
100		6.90	15.73	0.87**	0.61	0.083*	0.043	37.77

***significant   **highly significant**

Table 2 shows that the pigments (chlorophyll a, b and carotenoides) content treated plants decreased below the controls. The decreases in total pigments were 7.2 % and 38.1 % in leaves treated with 50 and 100 mM NaCl respectively . Similar results were obvious by El-Tayeb , 2005 on barley , Shah , 2007 on mustard and Turkyilmaz , 2012 on wheat. This decrease is due to the reduction in the leaf area, thus decrease water uptake as well as leaf efficiency (Bach et al., 2017), or it may be due to the inhibition of chlorophyll biosynthesis (Khan, 2003) and / or chlorophyllase degrading enzyme (Reddy and Vora 1986). Also salt stress is known to enhance oxygenase activity of Rubisco and reduce its carboxylase activity (Sivakumar et al , 1998).

GA_3 treated plants exhibited higher values than those of control or the salinity treated leaves. The total pigments increased above those of the control by 49.0 %, 13.7 % and 2.5 % in leaves treated with 25, 50 and 100 mM NaCl respectively. These results are in agreement with Ali et al.,2012; Siddiqui, et al., 2012 and Turkyilmaz, 2012.

Table 2: The effect of different concentrations of NaCl in presence or absence of GA_3 on photosynthetic pigments of *Zea mays* leaves.

Values listed are expressed as mg/g FW.

NaCl (mM)	GA_3 (M)	Chlorophyll (a)	Chlorophyll (b)	Carotenoids	Total pigments
0	0	4.16	2.01	1.7	7.34
25		4.27	2.65*	1.18	8.1
50		3.89	1.98	0.94	6.81
100		3.10*	0.82**	0.62**	4.54**
0	10^{-2}	6.96**	2.75	1.66	11.37
25		6.84**	2.69*	1.41	10.94**
50		5.31**	2.4	1.77**	9.48**
100		4.14	2.31	1.07	7.52

***significant   **highly significan**

The data shows that the salinized *Zea mays* seedlings accumulated soluble carbohydrates up to 100 mM NaCl. On the other hand, salinity is capable of inducing decreases in the insoluble carbohydrates in those treated with 50 and 100 mM NaCl. The maximum soluble carbohydrate production occurred at 100 mM NaCl, where the increase was 39.3 % while the decrease in the insoluble ones was 33 3 % (table 3). GA_3 treatments caused decreases in the content of soluble carbohydrate below that of untreated samples while it increases the insoluble ones.

Table 3: The effect of different concentrations of NaCl in presence or absence of GA_3 on soluble and insoluble carbohydrates, soluble protein and proline contents of *Zea mays* seeding.

NaCl (mM)	GA_3 (M)	Soluble Carbohydrates mg/g	Insoluble Carbohydrates mg/g	Soluble Protein mg/g	Proline µg/g
0	0.0	10.73	42.0	4.42	429
25		10.88	44.4	4.56	577*
50		12.34**	38.1	5.59*	635**
100		14.95**	28.0*	8.52**	723**
0	10^{-2}	9.37**	52.5	4.44	480
25		10.06	57.3*	4.38	620**
50		10.91	40.5	4.77*	714**
100		11.24**	35.6	6.17**	807**

***significant   **highly significant**

The accumulation of organic solutes enhances plant survival under different environmental conditions, among such solutes are the insoluble carbohydrates and proline which are known to be important osmoprotectants (Siddiqui et al., 2008 ; Emam , 2011). The observed increase in the insoluble carbohydrate of salinized Zea mays seedlings treated with GA_3 might be used as a tool by which the plant can cope with salinity stress in the formation of new cells and tissues (Gaber , 1981).

The reduction in the soluble contents in GA_3 treated salinized seedlings (table 3) might be attributed to the regulation of carbohydrate metabolism which were shifted toward insoluble carbohydrate accumulation.

Salt stress stimulated the accumulation of total soluble protein in salinized seedlings, also all NaCl levels showed increase in proline contents (table 3). NaCl at the level of 100 mM activated proline accumulation up to 68.5 % in the absence of GA_3 seedlings , while in the presence of GA_3, the proline accumulation was 88.1 % above the control. Proline plays an adaptive roles in mediating osmotic adjustment and protecting sub- cellular structure in stressed plants (Ashraf and Foolad , 2007 ; Sobahan et al., 2016).

The resuls revealed that salinity is capable of inducing a general increase in the Na ions, the increases were 16.2 %, 115.6 %, 346.4 % in plants treated with 25, 50 and 100 mM NaCl respectively above the control. However K, Ca and Mg ions were significantly decreased as salinity levels increased. No significant effect was obvious concerning Fe ions (table 4). GA_3 decreased the accumulation of Na ions in 50 and 100 mM NaCl treated plants by 35.2 % and 54.7 % respectively below those of their corresponding untreated GA_3 ones , while it induced increases in K, CA and Mg ions above their corresponding ones . Similar results were observed by Soylemez et al. , 2017.

Table 4: The effect of different concentration of NaCl in presence or absence of GA3 on mineral contents of *Zea mays* seeding.

Values listed are expressed as mg/g D.W

NaCl (mM)	GA_3 (M)	Na	K	Ca	Mg	Fe
0	0.0	17.9	31.5	12.41	2.59	4.4
25		20.8	27.36*	9.85*	1.48**	5.42
50		38.6**	26.0**	8.3**	1.79**	4.09
100		79.9**	15.41**	7.78**	1.70**	3.93
0	10^{-2}	16.1	35.64	14.55*	2.61	4.5
25		18.6	34.34	13.91	2.75	5.48
50		25.0*	33.1	12.91	2.74	5.14
100		36.2**	20.95**	10.93	1.99**	4.56

*significant   **highly significant

One of the most severe effects of salt stress is the absorption of Na ions by plant root. High levels of external Na interfere with K acquisition by the roots. The antagonism relation between Na and K ions indicated that the high levels of Na ions compete with the sites of K ions absorption and thus limited the absorption of K (Emam and Helal,, 2012). The high level of Na also disrupt the integrity of root membranes and alter their selectivity that must be sufficient to meet the levels of K required for metabolic processes , regulation of ion transport and osmotic adjustment (Rashad and Hussien, 2014.

Since water uptake is inhibited due to reduced osmotic potential in the soil solution caused by NaCl, water contents within the plant decrease and ultimately this leads to reduce growth rate. So plant needs to enhance the water content in plants grown in salt stress regimes by supplying both organic (sugars and proline) and inorganic (Ca^{2+} and Mg^{2+}) ions in order to lower osmotic potential within the plant (Shahid et al. ,2015 ; Soylemez et al., 2017). GA_3 strongly enhanced the absorption of Ca^{2+} and K^+ that could meet Na^+ higher concentration and controlled the Na/ K and Na / Ca ratios (Rashed and Hussein,2014).

Conclusion

Salinity stress led to a considerable reduction in plant growth, photosynthetic pigments and mineral contents (K, Ca and Mg ions), while increases in Na ions, soluble carbohydrates and proline contents were observed. Application of GA_3 partly overcome the adverse effects of salinity by improving the plant growth, ion uptake and the nutrient utilization under salt stress .

References

1. A.O.A.C.,1995 . Official Methods of Analysis . Association of Official Analytical Chemists . Washington , D., C.
2. Ashraf,M.,Karim,F.,and Rasul,E. 2000. Interactive effects of gibberellic acid (GA3) and salt stress on growth, ion accumulation and photosynthetic capacity of two spring wheat (Triticum aestivum L.) cultivars differing in salt tolerance .Plant Growth Regulation1-11.
3. Ashraf,M. and Foolad,M.A., 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental Experimental of Botany.59,206-216.
4. Bacha, H , Tekaya, M. ,Drine,S ., Guasmi, F., Touil,L., Enneb, H., Triki, T., Cheour, F. and Ferchichi, A., 2017 . Impact of salt stress on morpho-physiological and biochemical parameters of Solanum lycopersicum cv. Microtom leaves .J. Botany 108, 364-369.
5. Bates, L. S.,1973 . Rapid determination of free proline for water-stress studies . Plant and Soil . 39, 205 .
6. Bose, S. K., Yadav, R. K., Mishra, S, Sangwan, R. S., Singh, A. K. , Mishra, B., Srivastava, A. K., Sangwan, N. S., 2013. Effect of gibberellic acid and calliterpenone on plant growth attributes, trichomes, essential oil biosynthesis and pathway gene expression in differential manner in Mentha arvensis L.
7. Bradford, M. M. , 1976 . A rapid and sensitive method for the quantification of microgram quantities of protein utilization the principle of protein dye binding . Anal Biochem.72, 248-254.
8. El-Shihaby, O. A., Alla M. M. N., Younis, M. E. and El-Bastawisy, Z. M. , 2002 . Effect of kinetin on photosynthetic activity and carbohydrate content in waterlogged or sea-water treated Vigna sinensis and Zea mays plants. Plant Biosyst., 136, 277-290.
9. El-Tayeb, M.As ,2005 . Response of barley grains to the interactive effect of salinity and salicylic acid. Plant Growth Regulation 45, 215-224.
10. Emam, M. M. , 2012 . Efficiency of yeast in enhancement of the oxidative defense system in salt-stressed flax seedlings. Acta Biological Hungarica 63(2), 389-401.
11. Emam , M. M. and Helal, N. M. ,2008 . Vitamins minimize the salt-induced oxidative stress hazards. Australian Journal of Basic and Applied Science . 2(4) 1110-1119.
12. Farhangi-Abriz, S. and Torabian, S., 2017. Antioxident enzyme and osmotic adjustment changes in bean seedlings as affected by biochar under salt stress. Ecotoxicology and Environmental Safety. 173, 64-70.
13. Gaber, A. M. , 1981 .Studies on nitrogen metabolism in certain plant tissues . M.Sc. Thesis, Mansoura, Univ. Mansoura, Egypt.
14. Garcia-Sanchez, F., Jifon, J.L., Carvajal, M. and n, J.P. ,2002 .Gas exchange, chlorophyll and nutrient contents in relation to Na+ and Cl- accumulation in "Sunburst" mandarin grafted on different rootstocks. Plant Sci. , 162, 705-712.
15. Hamayun, M. , Khan, S.A, Khan, A.L,Shin,J.H., Ahmad,B., Shin, D.H.and Lee,I.J. , 2010 . Exogenous gibberellic acid reprograms soyabean to higher growth and salt stress tolerance. J. Agric. Food Chem. 58(12) , 722-7232.
16. Jia-minl, H., Xiao-li,W., Fu-qinl, Z. and Feng,F. , 2008 . Effects of NaCl and Ca+2 on membrane potential of experimental cells of maize roots. Abri. Sci. Chin., 7(3), 291-286.
17. Jasmine, M.S. and John, M.A. ,2012 . Effects of gibberellic acid on seedling growth, chlorophyll content and carbohydrate metabolism in okra (Abelmoschus esculentus L. Moench) genotypes under saline stress. Res.J.Chem.Sci.,2(7) ,72-74.
18. Keshavarzi, M.H.B., Rafsanjani, M.O., Moussavinik,S.M. and Lak,A.P. ,2011 . Effect of salt (NaCl) stress on germination and early seedling growth of spinach (Spinacia oleracea L.) J. Ann. Biol. Res. 2 (4) 490-497.
19. Khan, N.A. 2003 . NaCl inhibited chlorophyll synthesis and associated changes in ethylene evolution and antioxidative enzyme activities in wheat. Biol. Plant.47,437-440.
20. Lai, D., Mao, Y., Zhou, H. , Li, F. , Wu, M. , Zhang, J. ,He, Z. , Cui, W. and Xie, Y. , 2014. Endogenous hydrogen sulfide enhances salt tolerance by coupling the reestablishment of redox homeostasis and preventing salt-induced K+ loss in seedlings of Medicago sativa . Plant Science. 225,117-129.
21. Makkrash, N.M., 2012. Role of ascorbic acid and a-tocopherol germination and growth of sunflower plants (Helianthus annuus L.) grown under salt stress. M.SC. Thesis, Bot. Dept., KSU. Riyadh. K.S.A.
22. Metzner, H., Rau, H. and Senger, H, 1965. Unterschunger zur synchronisier – barkeit einzelner pigmenmangel. Mutantenvon. Chlorella. Planta. 65, 186.
23. Naguib, M. I., 1963. Colourimetric estimation of plant polysaccharides . Zeitsch. Zucker. !6, 15-26.
24. Rashad, T. R. and Hussien, A.,2014. A comparison study on the effect of some growth regulators on the nutrients content of maize plant under salinity conditions. Annals of Agricultural Science ,59 , 89.
25. Reddy, M.P. and Vora, A. B., 1986 . Changes in pigment composition,hill reaction activity and saccharides metabolism in Bajra (Pennisetum typhoides S & H) leaves under NaCl salinity. Photosynthetica 20, 50-55.
26. Schwechheimer, C., 2008 . Understanding gibberellic acid signaling –are we there yet? Current Opinion in Plant Biology 11(1), 9-15.
27. Shah, S.H., 2007. Effects of salt stress on mustard as affected by gibberellic acid application. Gen. Appl. Plant Physiology 33(1-2) 97-106.
28. Shahid, M.A., Pervez, M.A., Balal R.M.,, Ayyub,C.M., Ghazanfer,U., Abbas,T. Rashid,A., Garcia-Sanchez,F. , Mattson, N. S. and Akram,A. , 2011 . Effect of salt stress on growth, gas exchange attributes and chlorophyll contents of pea (Pisum sativum L.) . Afr. J. Agric. Res., 6, 5808-5816.
29. Siddiqui, M.H., Khan, M.N., Mohammed, F., Khan, M.M.A., 2008 . Role of nitrogen and gibberellin (GA3) in the regulation of enzyme activities and in osmoprotectant accumulation in Brassica juncea L. under salt stress. J.Agron. Crop Sci. 194, 214-224.
30. Siddiqui, M.H., Ali, H.M., Basalah, M.O., 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. Afr. J. Biotechnol. 11(4) 800-804.
31. Sivakumar, P.P., Sharmila, P. and Pardha, S.P., 2000 . Proline alleviates salt stress –induced enhancement in ribulose -1,5-biophosphate oxygenase activity . Biochem. Biophys. Res. Commun.,279, 512-515.
32. Snedecor, G.W. and Cochran, C.G. ,1980 . Statistical Methods 7th ed.Iowa, Iowa State University Press.
33. Sobahan, M.A., Akter, N., Murata, Y. and Munemasa, S., 2016 . Exogenous proline and glycinebetaine mitigate the detrimental effect of salt stress on rice plants. J. Silpakorn U. Science & Tech. 10 (3).
34. Soylemez, S., Kaya, C. and Dikilitas, S.K. ,2917 . Promotive effects of epibrassinolide on plant growth , fruit yield , antioxidant and mineral nutrition of saline stressed tomato plants. Pak. J. Bot., 49(5), 1655-1661.
35. Summart, J., Thanonkeo, P., Panichajakul, S., Prathepha, P. and McManus, M.T., 2016 . Effect of salt stress on growth, inorganic ion and proline accumulation in Thai aromatic rice, KhaoDawk Mail 105, callus culture . Afri. J. Biotechnol. 9(2).
36. Szaboles, I., 1994. Soils and salinization . Handbook of Plant and Crop Stress , Ed.M. Pessaraki, Marcel and Dekker, New York , 3-11.
37. Taibi, K., Taibi, F., Abderrahim, L.A., Ennajah, A., Belkhodja, M. and Mulet, J.M. 2016 . Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in Phaseolus vulgaris L. South African Journal of Botany, 105, 306-312.
38. Turkan, I. and Demiral, T.,2009 . Recent development in understanding salinity tolerance. Environ. Exp. Bot.67, 2-9.
39. Turkyilmaz, B. 2012 . Effects of salicylic and gibberellic acids on wheat (Triticum aestivum L.) under salinity stress. Bangladesh J. Bot. 41(1), 29-34.
40. Vaidyanathan, H., Sivakumer, P., Chakrabarty, R. and Thomas, G., 2003 . Scavenging of reactive oxygen species in NaCl-stressed rice (Oryza sativa L.) : differential response in high-yielding and sensitive varieties. Plant Science 165, 1411-1418.