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ALLEVIATION OF ADVERSE EFFECTS OF SALT STRESS IN *TRITICUM AESTIVUM* BY FOLIAR APPLICATION OF COMPATIBLE SOLUTES

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Abstract:
Abiotic stresses, such as drought, salinity and oxidative stress are serious threats to agriculture. Salinity causes growth inhibition in most of the plants. Plants have defense mechanisms that allow them to acclimatize in saline environment. One of them is the accumulation of certain osmolytes like Proline, glycine betaine, Mannitol, Sorbitol, synthesized by plants as a response to abiotic stress. Wheat (*Triticumn* spp.) is one of the world's major cereal crops which is tolerant at germination stage but highly sensitive to salinity at later stages. In the present study protective effect of foliar spray of different osmolytes and KNO_3 on salt stressed wheat plants was studied. The foliar treatment of 50 mM and 100mM osmolytes like Mannitol, Sorbitol and KNO_3 was given to plants with the supplementation of 100 mM NaCl. Effect of osmolyte treatment was studied on different growth parameters like root and shoots length, root and shoot fresh weights and dry weights, chlorophyll contents, proline content and K^+ to Na^+ ratio. Results were very promising; 100mM osmolyte and KNO_3 application was more effective than 50mM concentrations. Tested parameters were positively affected by the foliar application of the Osmolytes under saline and control conditions and showed better tolerance towards salinity.
Keywords: Salinity, Foliar Spray, Compatible Solutes, Salt Stress

Introduction

Salt-affected soil is one of the most serious abiotic stress factors that reduce plant growth and development, therefore leading to a decline in crop productivity [1]. Abiotic stresses, such as salinity, drought and high temperatures, are major problems to agriculture. Salinity is very big problem of in coastal areas. Konkan Region of Maharashtra is having a coastal length of 720 km. The sea water enters through the creeks during high tides and submerges large area of cultivable land.

Salinity stress affects many physiological and metabolic processes such as growth, photosynthesis, protein, and lipid metabolism in plants. The deleterious effects of salinity on plant growth are associated with: Low osmotic potential of soil (water stress), nutritional imbalance and specific ion effect. Soil salinity initially represses plant growth in the form of osmotic stress and ion toxicity [3]. Osmotic stress in the initial stage of salinity stress causes various physiological changes, such as interruption of membranes, nutrient imbalance, impairs the ability to detoxify reactive oxygen species (ROS), antioxidant enzymes, decreased photosynthetic pigments and decrease in stomatal aperture [2,5]. One of the most detrimental effects of salinity stress is due to accumulation of Na^+ and Cl^- ions in tissues of plants that causes severe ion imbalance [2].

Plants developed different adaptive mechanisms that allow them to acclimatize under salt stress. In response to salt stress plants synthesize and accumulate low molecular weight organic compounds in the cytosol and organelles. These compounds are collectively called compatible osmolytes because they accumulate and act without perturbing intracellular functions of enzymes. Some of the compatible osmolytes combat against salinity stress are sugars (glucose); polyols (sorbitol, mannitol, galactitol); amino acids (proline); quaternary amino acid derivatives like (glycine betaine) [4,7]. These Osmolytes are responsible for osmoregulation, maintaining the active conformation of macromolecules during stress conditions and participate in detoxification of Reactive Oxygen Species [1,6]. The foliar application of proline, glycine betaine and KNO_3 was reported to have protective role and can improve the salt tolerance in plants [8, 10, 11,12].

Wheat (*Triticum*spp.) is one of the world's major cereal crops. Wheat as a crop is more tolerant at germination stage but highly sensitive to salinity at later stage [9]. The objective of present study was to improve salt tolerance and therefore the productivity of wheat using foliar application of different osmolytes like Mannitol, Sorbitol and KNo_3. The study also includes comparative effect of these osmolytes on the growth of the wheat plant under salt stress.

Material and Methods:

2.1 Plant material and design of the Experiment: Seeds of *Triticum aestivum* were surface sterilized with 70% ethanol for 1 minute followed by the treatment of 2% Sodium hypochlorite for 10 minutes. Before sowing the seeds sand was thoroughly washed to remove any traces of salt. The experiment was run using three replicates for

each treatment. The pots were maintained under greenhouse conditions. The pots were irrigated with 20 ml of Hoagland solution for 8 days of germination and then salt treatment was started. The salt (NaCl) concentration was gradually increased form 0mM, 50mM and finally up to 100 mM at the intervals of two days. After one week of the final NaCl treatment, foliar spray of Mannitol, sorbitol and KNO3 was applied at 50mM and 100mM concentrations two times at the interval of 5 days. The treatments were as follows: Control (distill water), 100mM NaCl, 100mM NaCl+50mM Sorbitol as foliar Spray,100mM NaCl+100mM Sorbitol as foliar spray, 100mM NaCl+50mM Mannitol as foliar Spray,100mM NaCl+100mM Mannitol as foliar spray,100mM NaCl + 50mM KNO3 as foliar Spray and 100mM NaCl+100mM KNO3 as foliar spray. After a week from the final treatment the plants were carefully uprooted from the soil, washed and different parameters were analyzed.

2.2 Measurements of the growth parameters: The growth of roots and shoots was observed by measuring their length by using scale. Fresh and dry weights of shoot and root were taken (FW).For the measurement of the dry weight of the root and shoot the samples were dried in an oven at 70 °C for 24 h and the dry weight (DW) obtained.

2.3 Chlorophyll Content: The chlorophyll *a and b* content was determined with the method as described by Arnon, D.T., 1949[13]. Arnon's equation was used to convert absorbance measurements to mg Chl g-1 leaf tissue.

2.4 Proline Content: The proline (PRO) content was estimated by the method of Bates et al., 1973[14]. Proline content was expressed as µmoles per gram tissue = µg of proline per ml × ml toluene ×5g sample /115.5.

2.5 Sodium to Potassium ratio: Na^+ and K^+ were determined with a flame photometer with series of standards of Na^+ and K^+.

Result and Discussion:

Salt stress exerted overall a negative impact on all the growth parameters. The data presented in table1. showed that salt stress significantly reduced the shoot and root length; fresh and dry weights of root and shoots in wheat but application of osmolytes and KNO_3 i.e. 50mM and 100mM showed a positive effect on all the growth parameters under salt stress.100mM level of exogenous osmolytes was more effective than lower levels. KNO_3 was found to be more affective as compared to sorbitol and mannitol against salt stress as indicated in Fig 1a & b. The report of Jabeen and Ahmad also confirms the positive effect of KNO3 on growth under salt stress [15]

100Mm concentrations of KNO_3 and sorbitol resulted in proline reduction in salt-stressed tissues of plant as per the figure 2a. & table 2. Our results are in corroboration with the findings of Elhindi et.al. 2016 [12] but sorbitol spray at 100Mm concentration enhanced the proline content. Intracellular proline which is accumulated during salinity stress not only provides tolerance towards stress but also serves as an organic nitrogen reserve during stress recovery. Proline accumulation in response to salt stress has previously been reported in rice by Nakamura et al. [16]. Exogenous Glybet treatment was found to be an alternative way to enhance proline accumulation for the tolerance mechanism [17].

Photosynthetic pigments (chlorophyll *a* and chlorophyll *b*) were enhanced due to the application of all the osmolytes and KNO3. Chlorophyll content was reduced under salinity as compared to control. There are many reports which support the increase in chlorophyll content after exogenous treatment of osmolytes and KNO_3 [10, 12].

Table: 1. Effect of foliar spray of osmolytes and KNO3 on growth parameters of *Triticum aestivum* grown under salt stress.

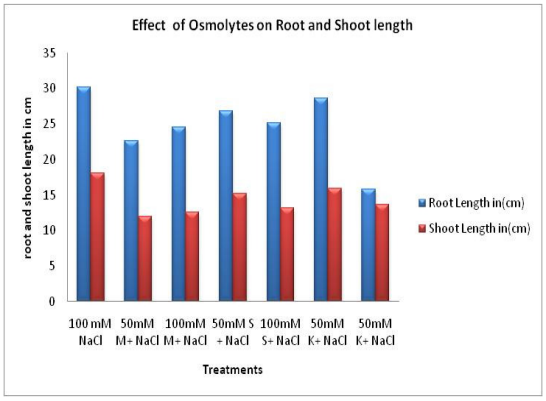
Treatments	Root Length in(cm)	Shoot Length in(cm)	Shoot FW(gm)	Shoot DW(gm	Root FW(gm)	Root DW(gm
Control	30.2	18.1	3.15	0.42	3.60	0.82
100 mM NaCl	22.6	12	1.3	0.35	3.48	1.12
50mM Mannitol+ NaCl	24.6	12.6	2.45	0.55	4.30	0.89
100mM Mannitol+ NaCl	26.8	15.2	1.35	0.37	4.10	0.78
50mM Sorbitol +NaCl	25.2	13.2	2.00	1.90	3.14	0.91
100mM Sorbitol+ NaCl	28.6	15.9	0.56	0.46	2.85	0.57
50mM KNO_3+NaCl	15.8	13.6	3.35	0.66	3.45	1.10
100mM KNO_3+NaCl	29	16.2	2.42	0.60	1.98	0.96

Table: 2. Effect of foliar spray of osmolytes and KNO3 on proline and chlorophyll contents of *Triticum aestivum* grown under salt stress.

Treatments	Proline in (µgs)	Chlorophyll a mg/gm of leaf	Chlorophyll b mg/gm of leaf	C = x+c
Control	30	1.210	1.309	4.570
100 mM NaCl	38	0.948	0.940	4.331
50mM Mannitol+ NaCl	18	0.971	0.875	3.803
100mM Mannitol+ NaCl	28	1.600	1.571	5.989
50mM Sorbitol +NaCl	38	1.093	1.056	3.884
100mM Sorbitol+ NaCl	40	1.580	1.370	6.147
50mM KNO_3+NaCl	26	1.373	1.446	5.005
100mM KNO_3+NaCl	28	1.535	1.527	5.872

Table: 3. Effect of foliar spray of osmolytes and KNO3 on K^+ / Na^+ Ratio of Triticum *aestivum* grown under salt stress

Treatments	Conc.of Na^+ in ppm	Conc.of K^+ in ppm	K^+/ Na^+ Ratio
Control	840	180	0.214
100 mMNaCl	1540	160	0.103
50mM M+ NaCl	950	170	0.178
100mM M+ NaCl	560	150	0.267
50mM S +NaCl	1320	150	0.113
100mM S+ NaCl	1370	200	0.145
50mM K+NaCl	1480	220	0.148
100mM K+NaCl	1450	260	0.279



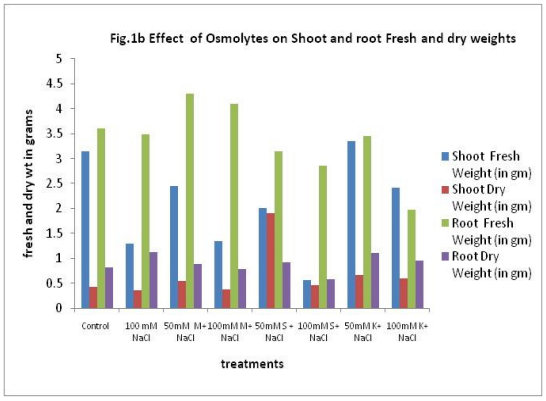
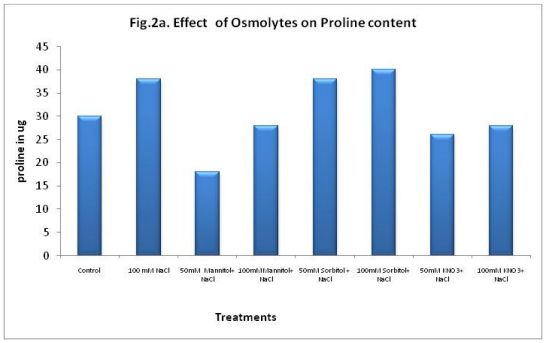


Figure: 1. Effect of foliar spray of osmolytes and KNO_3 on (a) root and shoot length (b) Shoot and Root fresh and dry weights of *Triticum aestivum* grown under salt stress.



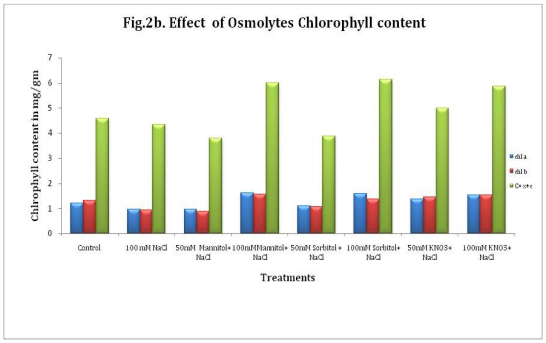


Figure: 2. Effect of foliar spray of osmolytes and KNO3 on (a) Proline content (b) Chlorophyll Content of *Triticum aestivum* under salt stress.

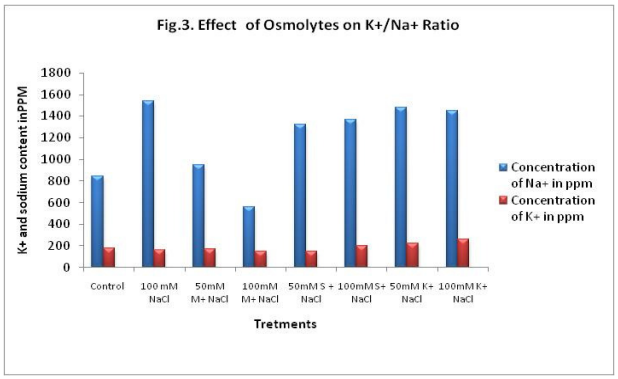


Figure: 3. Effect of foliar spray of osmolytes and KNO3 on K^+/Na^+ Ratio of *Triticum aestivum* grown under salt stress.

Analysis of data presented in Fig.3. Shows that salt stress increases the sodium concentration and reduced the potassium concentration. Both levels of exogenous osmolytes i.e. 50mM and 100mM were almost equally effective. Application of compounds like Salicylic acid, KNO_3 are reported to increase shoot K+ concentrations in plants and decreased sodium uptake [12,21]. This is in accordance with findings in experiments on maize [18), mung bean [20] and barley [19].

Conclusion:

The present study was conducted to determine whether the foliar spray of different co-osmolytes and KNO_3 exert a positive impact on different growth parameters of wheat under salt stress. On the basis of findings of the present studies it can be concluded that salt stress negatively affects the growth and physiology of wheat but the exogenous application of co-osmolytes and KNO_3 significantly ameliorates the harmful effects of salt stress.

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References

1. Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000): Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Mol Biol, vol.51: Pp. 463-499.
2. R. Munns and M. Tester (2008): Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, vol. 59: Pp. 651–681.
3. Bhaskar Gupta and Bingru Huang (2014): Mechanism of Salinity Tolerance in Plants: Physiological, Biochemical, and Molecular Characterization. International Journal of Genomics, Volume 2014:Pp. 1-18
4. Ashraf M, Harris PJC (2004): Potential biochemical indicators of salinity tolerance in plants. Plant Sci. vol.166: Pp3-16.
5. A. Rahnama, R. A. James, K. Poustini, and R. Munns, "Stomatal conductance as a screen for osmotic stress tolerance in durum wheat growing in saline soil," Functional Plant Biology, vol. 37, no. 3, pp. 255–263, 2010.
6. Paul H. Yancey (2005): Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. Journal of Experimental Biology, 208: Pp. 2819-2830
7. Sairam, R.K., Tyagi, A., Chinnusamy, V. (2006): Salinity tolerance: cellular mechanisms and gene regulation. In: Huang, B. (Ed.), Plant-Environment Interactions, third ed. CRC Press, Boca Raton, FL.
8. H.G. Abd El-Gawad (2015): Effect of Organic Osmolytes on Pea Seed Yield under Drought Stress Conditions Imposed at Different Growth Stages. Journal of Horticultural Science & Ornamental Plants 7 (2):Pp. 56-65,
9. Francios, L.E., Mass, E.V., Donovon, T.J. and Young, V.L. (1986): Effect of salinity on grain yield, quality, vegetative growth and germination of semi dwarf and durum wheat. Agron. J., vol 78(6): Pp. 1053-1058
10. Aqsa Talat, Khalid Nawaz, Khalid Hussian, Khizar Hayat Bhatti, Ejaz Hussain Siddiqi, Aneela Khalid, Sehrish Anwer and M. Usman Sharif (2013): Foliar application of proline for salt tolerance of two wheat *(Triticum aestivum L.)* Cultivars. World Applied Sciences Journal vol. 22 (4): Pp.547-554
11. B. Kahlaoui, M. Hachicha , E. Misle, F. Fidalgo, J. Teixeira (2016) Physiological and biochemical responses to the exogenous application of proline of tomato plants irrigated with saline water. Journal of the

Saudi Society of Agricultural Sciences [in press].

12. Khalid Mohammed Elhindi, Salah El-Hendawy, Eslam Abdel-Salam, Urs Schmidhalter Shafiq ur Rehman, Al-Adl Hassan (2016): Foliar application of potassium nitrate affects the growth and photosynthesis in coriander (*Coriander sativum L.)* plants under salinity. J. Progress in Nutrition Vol. 18 (1): Pp. 63-73
13. Arnon, D.T. (1949): Copper enzyme in isolated chloroplasts, polyphenal oxidase in Beta vulgaris. Plant Physiol. 24: Pp.1-15.
14. Bates, L.S., Waldren, R.P., Teare, I.D. (1973): Rapid determination of free proline for water – stress studies. Plant Soil 39:Pp. 205–207.
15. Jabeen N. and Ahmad R. (2011): Foliar Application of Potassium Nitrate Affects the Growth and Nitrate Reductase Activity in Sunflower and Safflower Leaves under Salinity.*Not Bot Horti Agrobo,* 39(2):Pp.172-178
16. Nakamura I, Murayama S, Tobita S, Bong BB, Yanagihara S, Ishimine Y, Kawamitsu Y (2002) Effect of NaCl on the photosynthesis, water relations and free proline accumulation in the wild *Oryza* species. Plant Prod Sci 5: 305-310.
17. Suriyan CHA-UM, Chalermpol KIRDMANEE (2010): Effect of glycine betaine on proline, water use, and photosynthetic efficiencies, and growth of rice seedlings under salt stress. Turk J Agric ,34:Pp. 517-527
18. Agami R.A. (2013): Alleviating the adverse effects of NaCl stress in maize seedlings by pre-treating seeds with salicylic acid and 24-epibrassinolide. South African J. Bot. 88: Pp.171-177.
19. Fayez K.A., Bazaid S.A. (2014): Improving drought and salinity tolerance in barley by application of salicylic acid and potassium nitrate. Journal of the Saudi Society of Agricultural Sciences 13(1):Pp. 45-55
20. Khan M.I.R., Asgher M., Khan N.A. (2014): Alleviation of salt-induced photosynthesis and growth inhibition by salicylic acid involves glycinebetaine and ethylene in mungbean *(Vigna radiata* L.).Plant Physiol. Biochem. 80: Pp.67-74.
21. Mohammed Wasfy Mohammed ELWAN, Rewaa Salah Ahmed El-Shatoury (2014): Alleviation of NaCl stress in summer squash 'eskandrani' by foliar application of salicylic acid. Journal of Horticultural Research vol. 22(2): Pp.131-137