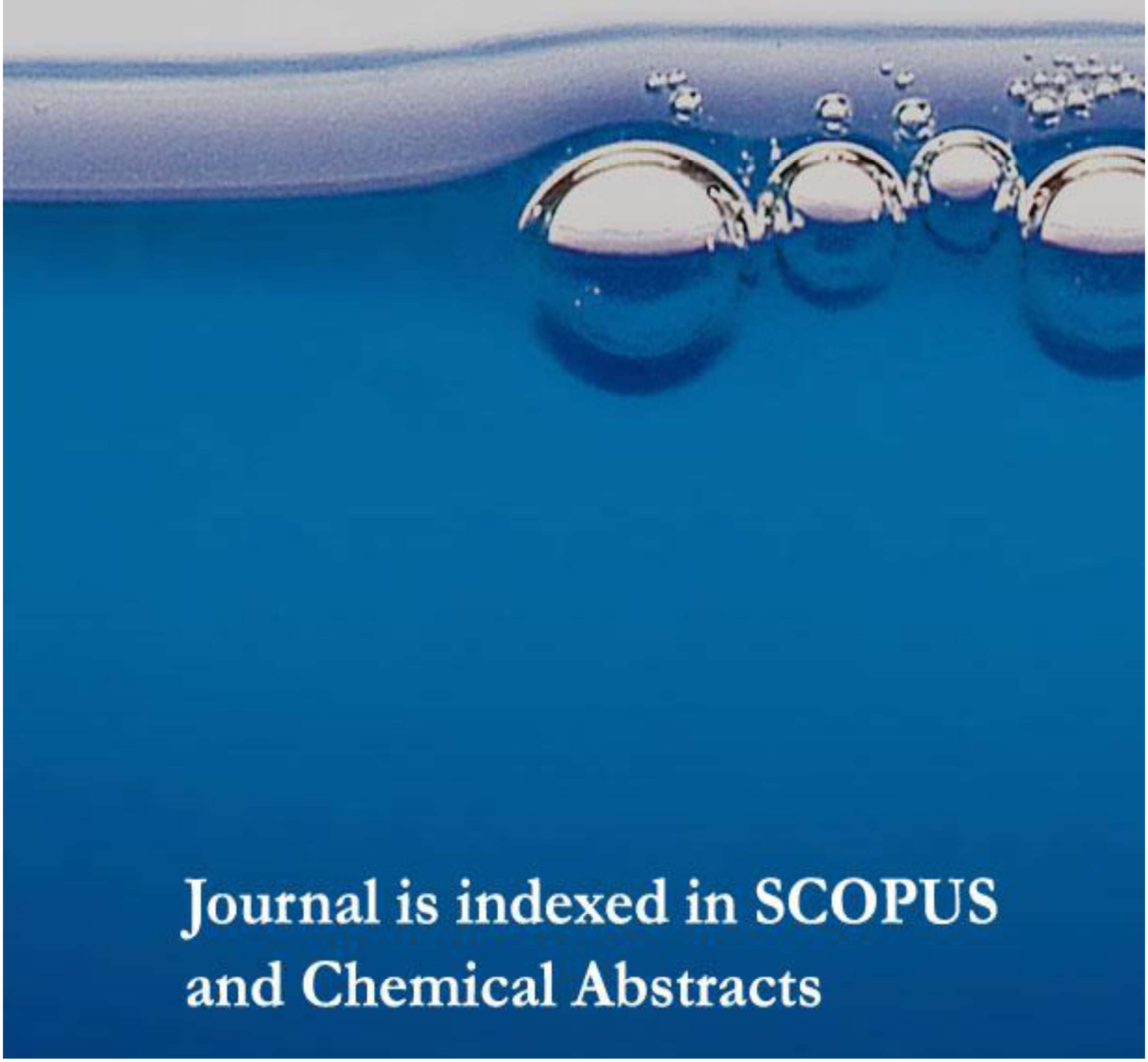
Research Journal of Chemistry and Environment

Vol. 19(10) October 2015



Influence of halopriming and hydropriming on seed germination and growth characteristics of *Zea mays* L. cv. GSF-2 under salt stress

Vaishnav Jayesh and Jain Meeta*
School of Biochemistry, Devi Ahilya University, Takshashila Campus, Khandwa Road, Indore (M.P.), 452001, INDIA
*mjjainmeeta@gmail.com

Abstract
In order to investigate the effect of halopriming and hydropriming on seed germination and growth characteristics in Zea mays L. cv. Ganga Safed-2 genotype under salt stress, a study was conducted as factorial experiment based on the completely randomized design with eight replications. Halopriming by 50, 100, 150, 200 and 300mM NaCl solution and hydropriming by 1/4th Hoagland solution was carried out at five levels of salinity 50, 100, 150, 200 and 300mM NaCl.

The results showed that priming treatment under non saline condition is highly effective. Germination, seed vigor index, seedling length, root length/shoot length ratio and fresh weight were significantly improved in response to priming at all salinity levels. Overall hydropriming treatment was comparatively superior than the halopriming. Germination index was also enhanced by priming seeds with 1/4th Hoagland solution suggesting that hydropriming is a simple low cost and environmentally friendly technique for improving seed and seedling vigor of maize.

Keywords: Germination, growth, halopriming, hydrpriming, maize, salt stress.

Introduction

Salinity, a chemical drought is the most detrimental factor among all abiotic stresses.[26] Salinization of land is a progressively increasing phenomenon. Limited rainfall, high evapo-transpiration, high temperature, poor water quality and poor soil management practices exacerbate salinity effect. In world, saline land occupies over 800 Mha[12,31] and nearly 8.6 million hectares in India and represents a serious threat to agricultural production.[18]

Soil salinity is a major factor limiting plant growth and development at all growth stages.[3,22] The inhibitory effect of salinity has been attributed to osmotic inhibition of water availability as well as the toxic effect of salt ions.[15] Seed germination is the first critical and the most sensitive stage in the life cycle of plants. The seeds exposed to unfavorable environmental conditions like salts and drought stresses may have to compromise the seedlings establishment.[4] Lower levels of salinity delay germination whereas higher levels reduce the final percentage of seed germination.[13] Significant reduction in germination percentage, germination rate, root length, shoots length and weights of four vegetable species (*Beta vulgaris*, *Brassica oleracea capitata* L., *Amaranthus paniculatus* and *Brassica compestris*) due to salinity has been demonstrated by Jamel et al.[20]

Maize is an important cereal crop in the world which provides staple food to many populations. Its cultivation is limited by salinity which causes grain loss of about 11-25% of the total production.[21] Various approaches such as breeding for resistance, chemical treatment of seed etc. have been used over many decades to control salinity effect. However, the limitations of these approaches have prompted the need of other alternative methods. Seed priming, seed soaking and seed coating have been employed to improve germination under salt stress.[7] Common priming techniques include osmopriming i.e. soaking seeds in osmotic solutions such as polyethylene glycol, halo priming i.e. soaking seeds in salt solutions and hydropriming i.e. soaking seeds in water.

Priming not only enhanced emergence rate and even emerge of seedlings[16] but also improved resistance or tolerance to cold,[23] drought[30] and salt[28] Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops.[8,10,17,27] Priming with $CaC1_2.2H_20$ among the different salts of chloride has been proved to be the most effective in inducing salt tolerance in maize at the early growth stages.[5]

The present study was carried out to evaluate the efficiency of halo and hydro priming methods to mitigate salt stress effects; improve germination and seedling establishment of *Zea mays* at various levels of salinity.

Material and Methods

This investigation was performed as factorial experiment under Completely Randomized Design (CRD) with eight replications at School of Biochemistry, Devi Ahilya University, Indore. Seeds of maize (*Zea mays* L. cv.) genotype Ganga Safed-2 (GSF-2) were surface sterilized with 0.1% $HgCl_2$ and then washed in distilled water twice. Seeds were hydroprimed by soaking in ¼th Hoagland Solution (HS) and haloprimed by soaking in different concentrations of NaCl (50, 100, 150, 200 and 300 mM)

**** Author for Correspondence***

prepared in ¼th HS for 4 hr. For germination studies, 10 sterilized unprimed and primed maize seeds were germinated in Petri plates containing 3 layers of filter paper sheets moistened with different concentrations of NaCl (50-300 mM) at 25±3°C in growth chamber.

Final germination percentage was measured on 6th day according to ISTA (International Seed Testing Association, 1993 and 1999) standard method and germination index (GI) was calculated as described in the Association of Official Seed Analysts.[6] Seed Vigor Index (SVI) was calculated by modified formula of Abdul-Baki and Anderson.[1] Mean shoot and root lengths were measured per replication. Fresh weight was measured using electrical balance after blotting the seedling on filter paper. Dry weight of seedlings was taken after drying each replication at 70 °C in the oven to get the constant weight.

Statistics: The data presented in the text are the average of at least eight replicate experiments with ± standard error. Student's 't' test was applied to test the significance of difference obtained for various salt treatments. Duncans Multipal Range Test (DMRT) at 0.05 and 0.01 confidence level was done to compare means between different priming conditions.[29]

Results and Discussion

The results of the experiments demonstrated that supply of 0 – 300 mM NaCl decreased the germination index of both unprimed and primed maize seeds in a concentration dependent manner (Table 1). Further, germination index was increased with halo as well as hydro priming at each salinity level and in non-saline condition also (Table 1). The hydropriming was found to be more effective than halopriming in increasing the germination index in both absence as well as presence of NaCl. Seed vigor index has been shown to be greatly affected by salinity. Decrease was significant and prominent at 150, 200 and 300mM NaCl concentration. The halopriming and hydropriming increased the seed vigor index to almost same extent in the absence and presence of 50 -150 mM NaCl, however, the increase was considerably high at 200 and 300 mM NaCl by the hydropriming (Table 1).

Treatment of maize seeds with different concentrations of NaCl reduced the seedling length with increasing concentration (Table 1). At higher concentration of NaCl (100-300mM) there was substantial decrease in seedling length. Length of both unprimed and primed seedlings was reduced to almost same extent with increasing salinity level. Further, the halopriming caused a marked increase in seedling length at 0, 50, 100 and 300 mM NaCl level. Hydropriming showed a similar trend of increase in seedling length but to a higher extent than halopriming.

Supply of different salt concentration decreased the root length in maize seedlings (Figure 1). The decrease was almost to same extent for both unprimed and primed seeds. Priming methods improved the root development in seedlings at each level of salinity, with the effect being highly significant at 300 mM NaCl. Treatment of maize seeds with NaCl decreased the shoot length in a concentration dependent manner (Figure 1). Reduction in shoot length was more substantial than the root length at each salt concentration. Rather, shoot emergence was completely suppressed at highest salt concentrartion i.e. at 300 mM NaCl. Halopriming and hydropriming of maize seeds enhanced the emergence of shoot greatly at each level of salinity, with the effect being higher for hydropriming.

There was gradual increase in RL/SL ratio in unprimed as well as primed seeds with increasing supply of NaCl (Figure 2). Unprimed maize seeds showed increase in RL/SL ratio to higher extent than the primed seeds (Figure 2) due to less shoot proportion in seedling at each level of salinity (Figure 1). Substantial increase was noted at 200 mM NaCl for unprimed seed. Due to complete suppression of shoot development at 300 mM saline condition RL/ SL ratio could not be measured. Halopriming was found to mitigate salinity stress at 100, 150 and 200 mM NaCl level by 37%, 62% and 58% respectively. Similarly hydropriming also elicited the salinity stress by 43%, 73% and 85% i.e. to a higher extent than halopriming. Further, only hydropriming could be able to emerge shoot at 300 mM NaCl concentration (Figure 1 and 2).

On the 6th day of germination, fresh weight of unprimed, haloprimed and hydroprimed maize seedlings was reduced to almost same extent on treatment with different salt concentrations. Further, halopriming and hydropriming caused increase in the fresh weight to same extent at each level of salinity except at 150 mM NaCl where it was excessively increased by hydropriming (Table 1). Supply of NaCl decreased the dry weight of unprimed and primed maize seeds gradually with increasing salt concentration to similar extent. Halo and hydropriming had no significant effect on dry weight for alleviating the salinity stress.

Germination and seedling establishment are critical stage in plant life cycle. High speed and uniform germination of seed and its ability to germinate under water deficiency affect crop establishment.[11] However, if the stress effect can be alleviated at the germination stage, chances for attaining a good crop establishment would be high.[5] Germination index is an important parameter to know the effect of stress on germination event. It shows speed of germination per day during course of germination.

In the present study, salinity affected the GI of both unprimed and primed maize seeds; however, decrease was less substantial for primed seeds (Table 1). Priming treatment caused the significant mitigation of salinity at 50, 100 and 200 mM NaCl concentration with the effect being slightly higher by hydropriming. Seed vigor index is a measure of development of seedling and its potential to establish into plant under stress condition. It has been found to be significantly and prominently decreased from 150 – 300 mM NaCl in unprimed seeds (Table 1). Halopriming and hydropriming both were able to mitigate the salt stress effect where hydropriming significantly increased the SVI at higher level of NaCl (Table 1). Several reports showed that under undesirable environmental conditions such as salinity and water deficiency, osmopriming leads to cellular, sub-cellular and molecular changes in seed and subsequently promotes seed vigor during germination and emergence in different plant species.[14,24]

Seedling length and RL/SL ratio are physical growth parameter that indicates the development of seedling. In our study, both halo as well as hydro priming significantly increased the seedling length at each salt concentration, with the effect being prominent for the latter. Further, RL/SL ratio increases with increasing salt concentration and shoot length has been found to be suppressed at 300 mM NaCl concentration (Figure 2).

Acceleration in radical emergence by priming via higher enzymatic activities, protein synthesis and ATP production resulting in producing larger root has been suggested by Parera and Canlliffe[25]. Cicek and Cakirlar[9] reported that salinity negatively affect coleoptye and redical development which resulted in delay in emergence time. Shoot length decreased more than root length by salinity at each level. Marked difference was observed at 100 mM NaCl concentration where reduction in root length and shoot length was almost 50% and 75% respectively (Figure 1). The increase in RL/SL ratio may be due to higher cell wall extensiblity and higher metabolic process of root at saline condition.[2] Enhancement in RL/SL ratio has been demonstrated by priming with KNO_3 under saline condition in cotton seeds. [27]

On the basis of results of our investigation, we can say that germination of *Zea mays* L. cv. Ganga Safed-2 genotype is sensitive to salinity. Seed priming resulted in better germination and seedling characteristics. Our findings revealed that hydropriming by 1/4th HS is better than halopriming (with NaCl) in mitigating salinity stress. These effects can be implemented to improve seedling establishment and field performance of this important crop maize.

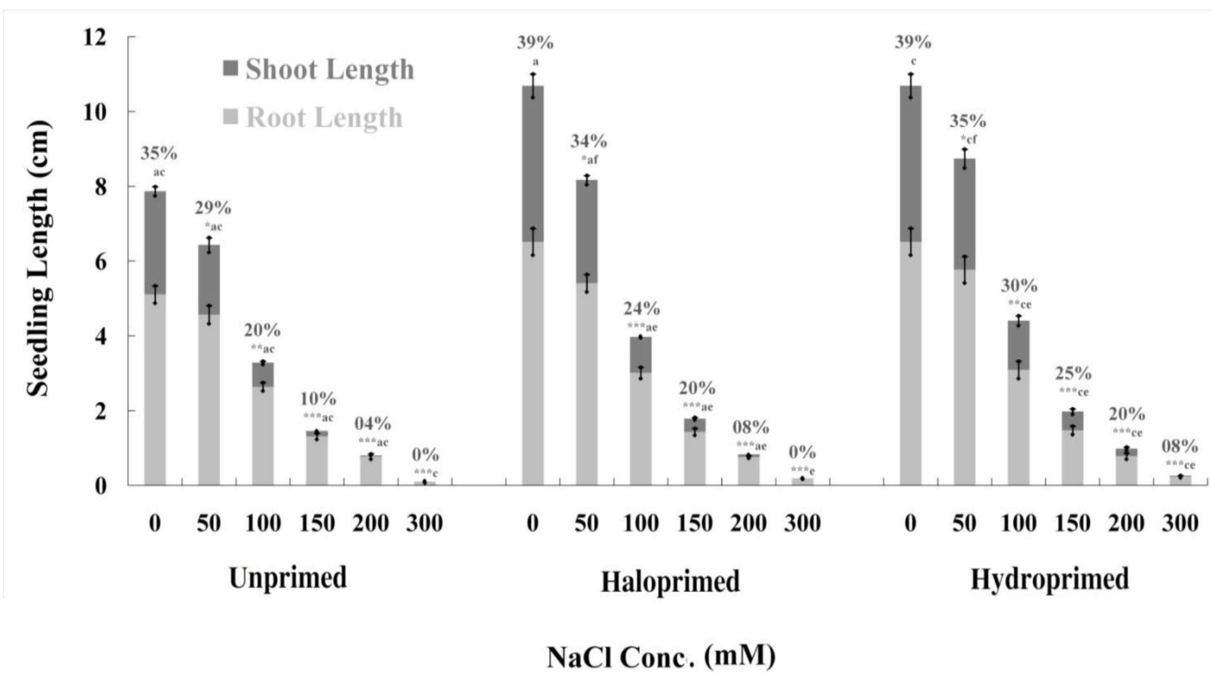


Figure 1: Effect of different priming treatment on seedling length of maize seedlings under salt stress.

Level of significance: 'p' values <0.05*, <0.01** and <0.001*** compared with control at each salinity level.
'a' and 'b' indicate significance of difference between unprimed and haloprimed conditions at 0.01 and 0.05 confidence level respectively.
'c' and 'd' indicate significance of difference between unprimed and hydroprimed treatment at 0.01 and 0.05 confidence level respectively.
'e' and 'f' indicate significance of difference between haloprimed and hydroprimed treatment at 0.01 and 0.05 confidence level respectively.
Percentage (%) values represent shoot length proportion in seedling length.

Table 1
Effect of different priming treatment on germination index, seed vigor index, fresh weight and dry weight of maize seedlings under salt stress.

Parameter	Seed priming	Salinity levels (NaCl conc., mM)					
		0	50	100	150	200	300
Germination Index	Unprimed	3.93±0.31(100)^{ac} *(100)*	3.62±0.28(92)^{ac} *(100)*	2.95±0.19*(75)^{ac} *(100)*	1.94±0.1**(49)^{bc} *(100)*	0.91±0.03***(23)^{c} *(100)*	0.55±0.01***(14)^{c} *(100)*
	Haloprimed	6.51±0.23(100)^{a} *(166)*	5.21±0.2*(80)^{a} *(144)*	4.14±0.8**(64)^{a} *(140)*	2.20±0.3**(34)^{bf} *(113)*	1.13±0.4***(17)^{f} *(124)*	0.58±0.1***(9)^{e} *(105)*
	Hydroprimed	6.51±0.23(100)^{c} *(166)*	5.33±0.20*(82)^{c} *(147)*	4.25±0.35**(65)^{c} *(144)*	2.36±0.16**(36)^{cf} *(122)*	1.31±0.16***(20)^{cf} *(144)*	0.69±0.06***(11)^{ce} *(125)*
Seed vigor index	Unprimed	792±54(100)^{ac} *(100)*	695±35(88)^{bd} *(100)*	388±22**(49)^{bd} *(100)*	128±03***(16)^{bd} *(100)*	39±07***(05)^{c} *(100)*	4.8±1.5***(01)^{c} *(100)*
	Haloprimed	1059±97(100)^{a} *(134)*	729±75*(69)^{bf} *(105)*	419±43**(40)^{b} *(108)*	146±17***(14)^{b} *(114)*	45±08***(04)^{f} *(116)*	5.8±1.6***(01)^{f} *(121)*
	Hydroprimed	1059±97(100)^{c} *(134)*	759±89*(72)^{df} *(109)*	426±57**(40)^{d} *(110)*	154±21***(14)^{d} *(120)*	56±08***(05)^{cf} *(143)*	7±1.3***(01)^{cf} *(146)*
Fresh weight (g)	Unprimed	1.83±0.3(100)^{ac} *(100)*	1.51±0.2(83)^{bc} *(100)*	0.94±0.3**(51)^{d} *(100)*	0.66±0.2**(36)^{bc} *(100)*	0.49±0.0***(27)^{d} *(100)*	0.48±0.2***(26) *(100)*
	Haloprimed	2.2±0.05(100)^{a} *(120)*	1.8±0.03(82)^{b} *(119)*	0.95±0.01**(43)^{f} *(101)*	0.71±0.01**(32)^{bf} *(108)*	0.53±0.00***(24) *(108)*	0.48±0.00***(22) *(100)*
	Hydroprimed	2.2±0.05(100)^{c} *(120)*	1.9±0.07(86)^{c} *(126)*	1.08±0.01**(49)^{df} *(115)*	0.93±0.03**(42)^{cf} *(141)*	0.56±0.02***(26)^{d} *(114)*	0.5±0.03***(23) *(104)*
Dry weight (g)	Unprimed	0.43±0.02(100) *(100)*	0.37±0.02(86)^{d} *(100)*	0.35±0.01*(81) *(100)*	0.33±0.01**(77) *(100)*	0.32±0.01**(74) *(100)*	0.31±0.01**(72) *(100)*
	Haloprimed	0.44±0.01(100) *(102)*	0.40±0.01(95) *(108)*	0.36±0.01*(81) *(103)*	0.33±0.00**(74) *(100)*	0.32±0.00**(74) *(100)*	0.31±0.00**(74) *(100)*
	Hydroprimed	0.44±0.01(100) *(102)*	0.42±0.01(95)^{d} *(114)*	0.38±0.0*(86) *(109)*	0.35±0.0**(70) *(106)*	0.32±0.01**(73) *(100)*	0.32±0.0**(73) *(103)*

Level of significance: 'p' values <0.05*, <0.01** and <0.001*** compared with control at each salinity level.
'a' and 'b' indicate significance of difference between unprimed and haloprimed conditions at 0.01 and 0.05 confidence level respectively.
'c' and 'd' indicate significance of difference between unprimed and hydroprimed treatment at 0.01 and 0.05 confidence level respectively.
'e' and 'f' indicate significance of difference between haloprimed and hydroprimed treatment at 0.01 and 0.05 confidence level respectively.
Values relative to control are given in parentheses.

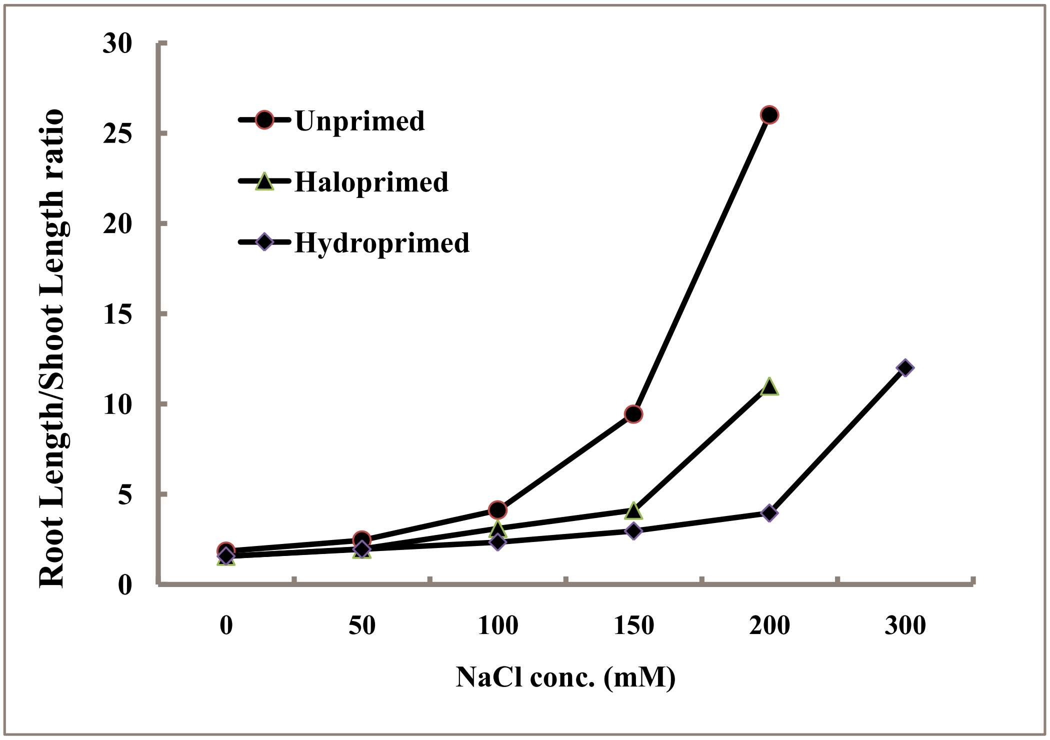


Figure 2: Effect of different priming treatment on RL/SL ratio in maize seedling under salt stress.

References

1. Abdual-baki A.A. and Anderson J.D., Relationship between decarboxylation of glutamic acid and vigour in soybean seed, *Crop Science*, **13**, 222–226 **(1973)**

2. Afzal I., Basra S.M.A., Ahmad R. and Iqbal A., Effect of different seed vigor enhancement techniques on hybrid maize (*Zea mays* L.), *Pakistan Journal of Agricultural Science*, **39(2)**, 109-112 **(2002)**

3. Ahmed S., Effect of soil salinity on the yield and yield components of Mungbean, *Pakistan Journal of Botany*, **4(1)**, 263- 268 **(2009)**

4. Albuquerque F.M.C.D. and Carvalho N.M.D., Effect of type of environmental stress on the emergence of sunflower (*Helianthus annuus* L.), soyabean (*Glycine max* L.) and maize (*Zea mays* L.) seeds with different levels of vigor, *Seed Science Technology,* **31**, 465-467 **(2003)**

5. Ashraf M. and Rauf H., Increasing salt tolerance in maize (*Zea mays* L.) through seed priming with chloride salt: growth and ion transport at early growth stages, *Acta Physiol Plant,* **23**, 407-414 **(2001)**

6. Association of Official Seed Analysis (AOSA), Seed Vigor Testing Handbook, Contribution No. 32 to the handbook on Seed Testing, spring field, IL **(1983)**

7. Basra S.M., Zia A.N., Mahmood T., Afzal A. and Khaliq A., Comparison of different in vigor techniques in heat (*Triricum aestivum* L.) seeds, *Pakistan Journal of Arid Agriculture*, **5**, 11-16 **(2003)**

8. Bradford K.J., Manipulation of seed water relation via osmotic priming to improve germination under stress conditions, *Horticultural Science*, **21**, 1105-1112 **(1986)**

9. Cices N. and Cakirlar H., The effect of salinity on some physiological parameters in two maize cultivars, *Bulgarian Journal of Plant Physiology*, **28**, 66-74 **(2002)**

10. Eskandari H. and Kazemi K., Effect of seed priming on germination properties and seedling establishment of cowpea (*Vigna sinensis*), *Notulae Scientia Biologicae*, **3(4)**, 113-116 **(2011)**

11. Fischer R.A. and Turner N.C., Plant productivity in the arid and semiarid zones, *Annual Review Plant Physiology,* **29**, 277-317 **(1978)**

12. Food and Agricultural Organization (FAO), Land and plant nutrition management service, Available online at: www.fao.org **(2008)**

13. Ghoulam C. and Fares K., Effect of salinity on seed germination and seedling growth of sugar beet (*Beta vulgaris* L.), *Seed Science Technology*, **29**, 357-364 **(2001)**

14. Godfery W.N., Onyango J.C. and Beck E., Sorghum and salinity: Gas exchange and chlorophyll fluorescence of sorghum under salt stress, *Crop Science,* **44**, 506-811 **(2004)**

15. Hakim M.A., Juraimi A.S., Begum M., Hanafi M.M., Ismail M.R. and Selamat A., Effect of salt (NaCl) stress on germination and early seedling growth of four vegetable species, *Journal of Central European Agriculture*, **7(2)**, 273-282 **(2009)**

16. Harris D.A., Joshi P.A., Khan P., Gothkar and Sodhi P.S., On-farm seed priming in semi-arid agriculture, development and evaluation in maize, rice and chickpea in India using participatory methods, *Australian Journal of Experimental Agriculture*, **35**, 15-29 **(1999)**

17. Heydecker W. and Coolbear P., Seed treatments for improved performance survey and attempted prognosis, *Seed Science Technology*, **5**, 353–425 **(1977)**

18. ICAR-CSSRI, Annual Report of Central Soil Salinity Research Institute of Indian Council of Agricultural Research, Karnal, Haryana, India **(2014)**

19. International Seed Testing Association, The accelerated ageing test, ISTA Seed Vigor Testing Handbook **(1995)**

20. Jamel M., Lee D.B., Jung K., Ashraf M., Lee S.H. and Rha E.S., Effect of salt stress on germination and early seedling growth of four vegetables species, *Journal of Central European Agriculture,* **7**, 273-281 **(2006)**

21. Katerji N., van Hoorn J.W., Hamdy A., Karam F. and Mastrorilli M., Effect of salinity on water stress, growth, and yield of maize and sunflower, *Agricultural Water Management*, **30**, 237-249 **(1996)**

22. Khoshsokan F., Babalar M., Chaghazardi H.R. and Fatahimoghasam M.R., Effect of salinity and drought stress on germination indices of two thymus species, *Cercetãri Agronomice in Moldova*, **1(149)**, 27-35 **(2012)**

23. Lü X.H. and Fu J.R., Effects of PEG pretreatment in vigor and hardness on seed vigor of peanut, *Acta Sci Nat Univ Sunyaatseni*, **29(1)**, 63–70 **(1990)**

24. McDonald M.B., Seed priming: Seed Technology and its Biological Basis, ed. by Black M. and Bewley J.D., Sheffield Academic Press Ltd., Sheffield, UK, 287-325 **(2000)**

25. Parera C.A. and Cantliffe D.J., Enhanced emergence and seedling vigor in shrunken-2 sweet corn by seed disinfection and solid matrix priming, *Journal of the American Society of Horticultural Science*, **117**, 400-403 **(1992)**

26. Raveh E. and Levy Y., Analysis of xylem water as an indicator of current chloride uptake status in citrus trees, *Scientia Horticultuae,* **103**, 317–327 **(2005)**

27. Rezaee S., Moghaddam M.R.R. and Bazrgar A.B., Cotton seed germination as affected by salinity and priming, *Indian Journal of Fundamental and Applied Life Sciences,* **5(1)**, 312-318 **(2015)**

28. Ruan S.L., Xue Q.Z. and Wang Q.H., Physiological effects of seed priming on salt-tolerance of seedlings in hybrid rice, Oryza sativa L., *Sci. Agric Sin*, **36**, 463–468 **(2003)**

29. Steel R.G.D. and Torrie J.H., Principles and procedures of statistics, a biometrical approach, 3rd edition, McGraw Hill Book Co. Inc., NY, USA **(1997)**

30. Wang Y.R., Zhang J.Q., Liu H.X. and Hu X.W., Physiological and ecological responses of alfalfa and milk vetch seed to PEG priming, *Acta Ecologica Sinica*, **24**, 402–408 **(2004)**

31. Yang G., Zhou R., Tang T., Chen X., Ouyang J., He L., Li W., Chen S., Guo M., Li X., Zhong C. and Shi S., Gene expression profiles in response to salt stress in *Hibiscus tiliaceus*, *Plant Molecular Biology Reporter,* **29**, 609–617 **(2011)**.

(Received 16th June 2015, accepted 19th August 2015)