

**EFFECTS OF SEED PRIMING ON SALT TOLERANCE OF MAIZE
(*ZEA MAYS* L.) SEEDLINGS BY LIPO-CHITTOOLIGOSACCHARIDE**

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Abstract

Effects of lipo-chitooligosaccharide seed priming on agronomic traits of maize under different salt concentrations (0, 2, 4, 6 and 8 dS/m) were studied. Results showed that the effects of salinity and seed priming on agronomic characteristics were significant at the probability level of 5% ($P < 0.05$). The increase in salinity up to 10 dS/m negatively influenced all traits. However, seed priming with LCO @ 4ml/kg compensated the adverse effects of salinity on agronomic traits. The regression analysis under salt stress condition showed that aforesaid traits all together contributed 98.4 per cent for salt tolerance. Hence, the novel finding showed that seed priming with LCO signal molecule can enhance the maize tolerance to salinity by improving the physiological changes in seeds which has very high value of implications.

Worldwide totally 800 million hectares of land is affected by salinity which is of 6 per cent of the planets total land area. Salinization is more spreading in irrigated lands because of inappropriate management of irrigation and drainage and in arid and semi arid regions due to high evaporation.

Seed germination is a major factor limiting the establishment of plants under saline conditions (Ghavami and Ramin 2007) by creating an osmotic potential outside the seed inhibiting the absorption of water, or by the toxic effect of Na^+ and Cl^- (Khajeh-Hosseini *et al.* 2003). Bilgin *et al.* (2008) showed that increasing levels of salinity cause significant decrease in corn root growth. Garg *et al.* (2006) reported that increasing salinity due to saline water irrigation reduced the shoot dry matter progressively.

Seed priming can be considered as a simple method for improving salinity tolerance of seeds. Applications of lipo-chitooligosaccharides (nod factors) have been emerged as a new trend of this decade which is a unique molecule and recently considered to have a hormone like substances. In non-host plants, LCOs have been shown to elicit several physiological responses.

Maize (*Zea mays* L.) one of the most important cereal crops growing in the world under range of environmental condition is used as food and its oil for human consumption, feed for livestock and poultry and raw material for agro-based industries. Since, ancient time's maize is known as "Queen of cereal". Maize belonging to poaceae is elsewhere reported as salt-sensitive species and the most salt-sensitive of the cereals. Negative effect of increased salt concentration on seed germination and early growth of maize was reported by Carpici *et al.* (2009). The experiment was designed to further exhibit the significance of nod factors and its effect on maize under salinity conditions.

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To evaluate the effects of LCO or nod factor seed priming on maize growth under saline conditions, a factorial experiment based on a completely randomized design with four replications was carried under natural light conditions at Tamil Nadu agricultural University, Coimbatore, Tamil Nadu, India. Experiment consists of 2 factors which are salinity at 5 levels (0, 2, 4, 6 and 8 dS/m) and priming with 2 levels (LCO primed and nonprimed seeds). Seeds of maize hybrid (CoHM 6) were used. The seeds primed with LCO (4 ml/kg of seeds) and non primed seeds of each five were sown in the pots at a depth of 3 cm and thinned to 3 after emergence. Desired levels of salinity in pots were maintained by watering salt solutions with the respective ECs (dissolved 1.28, 2.56, 3.84 and 5.12 g of NaCl in one litre distilled water to obtain 2, 4, 6 and 8 dS/m, respectively). All the plants were harvested 45 days after sowing.

Shoot length (cm) was measured from the base of the plant to the tip of the top-most completely opened leaf on selected plants from each treatment using a standard ruler/scale in centimeters. Similarly, the root length (cm) was measured from the base of the plant to the tip of the root. On the 14th day, fresh weights of radicles and hypocotyls were measured. Subsequently the radicles and hypocotyls were dried at 80°C for 24 hrs and weighed. Water was poured into a clean measuring cylinder (nearly three fourth of its volume) and the level of water noted. To avoid parallax error, reading was taken at the lowest level of meniscus or curved surface of the liquid. A string was attached to the root and lowered into the water and the new level of water was noted. The difference in the above two readings was calculated and expressed as root volume in cc plant⁻¹. Analysis of variance was applied to determine the effects of salinity levels and priming treatments on the plant growth traits.

Results of analysis of variance indicated that both salinity and priming independently affected plant height at the probability level of 5%, and their interaction was also significant for this trait (Table 1). Salinity had significantly decreased the shoot length of maize in both nonprimed and primed seeds. The increase in salinity up to 8 dS/m caused more reduction in plant height compared with control. These reductions might be due to both osmotic and specific ionic effects on seedlings growth (Akram *et al.* 2010). The data regarding the plant height showed that LCO primed seeds produced the higher plant height (124.18 cm) while non primed seeds produced the shortest plants (109.36 cm). These differences might be due to the enhanced germination and seedling growth, along with the mitogenic nature of LCOs, suggesting accelerated meristem activity which induced growth (increase water absorption and turgor pressure). This result is in accordance with the finding of Souleimanov *et al.* (2002).

Table 1. Effect of lipo- chito oligosaccharides (LCO) on plant height (cm) and plant dry weight (g) of maize under different levels of salinity.

Salinity (dS/m)	Plant height (cm)			Plant dry weight (g)		
	P	NP	Mean	P	NP	Mean
0	141.20	131.40	136.30	83.10	81.60	82.35
2	136.30	116.80	126.55	80.20	77.30	78.75
4	129.40	107.30	118.35	67.40	54.30	60.85
6	112.50	101.70	107.10	52.50	45.70	49.10
8	101.50	89.60	95.55	44.10	31.20	37.65
Mean	124.18	109.36		65.46	58.02	
	P	S	P x S	P	S	P x S
SEd	0.61	0.96	1.36	0.33	0.52	0.73
CD (p = 0.05)	1.25	1.97	2.79	0.67	1.06	1.50

*P - priming; NP - non priming.

Plant dry weight was inversely related to increasing salinity levels (Table 1). At all levels of NaCl, the plant dry weight recorded was higher for LCO primed seeds while lesser in case of non primed seeds. Salinity reduced the shoot biomass production and it is well established fact now that the highest levels of NaCl in the given medium caused stunted growth of plants (Takemura *et al.* 2000). Salinity reduced dry mass of both roots and shoots of maize and damaged the leaves which might be due to accumulation of Na⁺ in leaves. This finding is in agreement with the findings of Khan *et al.* (1995). The tolerance increase in LCO primed seeds might be due to the enhanced growth of root and shoot which leads to increase in plant dry weight.

Both salinity and priming independently affected root length at the probability level of 5%, while their interaction was also significant for the same probability level (Table 2). Salinity had a rather negative effect on root length and this reduction was significant in all the EC levels. With respect to different EC levels, 0 d/Sm (control) manifested considerably higher root length (25.45) which was followed by 2 d/Sm. The decline in root growth under salt stress condition was attributed to the inhibition of hydrolysis of endosperm reserves and reduced translocation of food reserves from endosperm to embryo. Enhancement of root length was highly observed in LCO primed seeds than non primed seeds both in saline and non-saline conditions. This might be attributed to the underlying mechanism of LCO in the regulation of entire germination process.

Table 2. Effect of lipo- chito oligosaccharides (LCO) on root length (cm), root dry weight (g) and root volume (cc/plant) of maize under different levels of salinity.

Salinity (dS/m)	Root length (cm)			Root dry weight (g)			Root volume (cc/plant)		
	P	NP	Mean	P	NP	Mean	P	NP	Mean
0	27.10	23.80	25.45	34.60	33.30	33.95	59.30	56.30	57.80
2	26.20	21.30	23.75	30.80	28.50	29.65	53.20	51.60	52.40
4	25.10	19.70	22.40	27.50	21.30	24.40	48.10	34.20	41.15
6	22.30	15.60	18.95	22.30	17.50	19.90	41.20	23.70	32.45
8	19.20	12.40	15.80	15.60	9.80	12.70	33.60	11.20	22.40
Mean	23.98	18.56		26.16	22.08		47.08	35.40	
	P	S	P x S	P	S	P x S	P	S	P x S
SEd	0.16	0.26	0.37	0.20	0.32	0.45	0.25	0.40	0.56
CD (p=0.05)	0.34	0.53	0.75	0.41	0.64	0.91	0.51	0.81	1.15

*P - priming; NP - non priming.

The crops showed a negative relationship for root growth with increase in salinity. Considering the results of the variance of analysis, priming and salinity levels significantly ($p < 0.05$) affected root volume maize plant. Data presented in Table 2 shows that, root volume was reduced in response to increasing salinity so that the highest root volume was obtained under control and also it was statistically significant at all the salinity levels. The reason for reduction is due to lower availability of O₂ under saline conditions which deprive the plants from energy source and accumulation of high level of sodium inhibits root growth. The rate of reduction was less pronounced in the LCO primed plants than the nonprimed ones. The increment in LCO primed seeds could be a result of hydration which initiates metabolic activities of the seeds for better seedling establishment.

Growth of maize in pots in the under natural light conditions showed significant ($p < 0.05$) effect of salinity level and seed priming on root dry weight (Table 2) and the interaction was also significant ($p < 0.05$). In the same way, root dry weight significantly indicated a decrease with progressive increase in salinity level. Root dry weight was with the highest value at control, while it was noted minimum in 6 and 8 d/Sm. The reason behind the reduction is absorption of sodium

leads to reduction of cell division in root apex. On the basis of root dry weight, it was clear that salinity decreased growth of roots at all concentrations of NaCl.

But the inhibitory effect was more in nonprimed seeds in relation to LCO primed seeds which produced higher root biomass. A positive reaction of LCO on plant growth regulations might be associated with cell division enhanced by LCO.

Correlation coefficient was calculated to find out the degree of association between two characters and helps in understanding the nature and magnitude of association among the characters. The results of correlation analysis between the various agronomic traits towards plant presented in Table 3 showed significant correlation with agronomic traits. Correlation analysis showed that the traits were more in normal condition than saline condition; ultimately salinity places its adverse effects on the aforesaid traits.

To determine the best regression equation and also to define the most influential variables on the dependent variable (plant height), the regression analysis was used (Table 3). Regression analysis showed that the R^2 value was 0.984 which revealed that all these four traits could totally contributed 98.4 % of the per cent variation in salt tolerance for plant height.

Table 3. Correlation coefficients between the agronomic traits of maize under different levels of salinity.

Sl.No.	Variables	Partial regression coefficient (b)	SE	't' value
1.	Plant dry weight	0.414	0.363	1.139
2.	Root length	3.158	0.764	4.135
3.	Root volume	-0.989	0.365	-2.709
4.	Root dry weight	1.260	0.872	1.444

Regression equation: $Y = -34.42 + 0.41(X_1) + 3.16(X_2) - 0.99(X_3) + 1.26(X_4)$

$R^2 = 0.984$

From the study, it was found that seed priming with LCO regulated germination process by overcoming the negative effects of salt stress. Further study is needed in extrapolating the usefulness of nod factors to the crop production. Advanced research is also required to explore LCO induced physiological and biochemical changes in seed under stress condition.

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