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INFLUENCE OF LIPO CHITO OLIGOSACCHARIDES ON GERMINATION OF MAIZE UNDER DIFFERENT LEVELS OF SALINITY

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ABSTRACT

Laboratory experiment was conducted to study the effectiveness of nod factors (LipoChito Oligosaccharides) on seed germination in maize under different levels of salinity (0, 2,4,6,8 and 10 dSm^{-1}).Seeds were treated with nod factors and sown in petriplates. Significant reduction in the germination percentage (31 %), shoot length (3.65 cm), root length (1.2 cm), seedling vigour index (154) and salt tolerance index (4.9) was observed under thenon primed seeds of maize at increasing salinity levels. The higher reduction was recorded under 10 dSm^{-1}. LCO primed seeds relatively increased seed germination (96 %), rootlength (13.97 cm), shoot length (23.2 cm), seedling vigour index (3570) and exhibited salt tolerance as compared to non primed seeds irrespective of thesalinity levels.The regression analysis under salt stress condition showed that aforesaid traitsall together contributed69.4 per cent for salt tolerance.The results indicated that salt stress can reduce the germinable characters suggesting that LCO (nod factors) canincrease the initial seedling growth by invigorating the salt tolerance.

INTRODUCTION

Salinity the most significant and widespread problem around the world, limiting the crop seed germination and establishment (Almansouri *et al.*, 2001). Worldwide salinity has reached a level of 19.5% in irrigated lands (45mha) and 2.1% in drylands (32mha) affecting the agriculturalactivities.

Under saline environment the seed germination is reduced thus resulting an uneven crop stand and establishment and thus reduced crop yields.It is reported that soil salinity causes greater reduction in shoot growth than in root growth (Ramoliya and Pandey, 2003).

Root and shoot length were found to be decreased significantly by saline stress as compared to control. Length of root and shoot were reported to decrease perhaps due to accumulation of ions near the root surface in maize (Khatoon *et al.*, 2010). According to Gholipoor *et al.* (2000), salt stress decreased seedling growth in chickpea.

To alleviate the problem of seed germination under salt stress condition priming techniques are found to produce improvement in seed germination and alsosynchronized establishment. It is an easy and safe agro technique of recent origin, to address the problem of salinity in agriculture(Neto and Tabosa, 2000). The priming is done with materials of inorganic, biological and organic origin which has a unique compound to alleviate stress. One among such compound is nod factor.

Legume-rhizobia symbiosis produces an organic compound called LipoChito-Oligosaccarides (nod factor),an unique molecule that when present at that time of planting, enhances a root and shoot development immediately and independently of variety, soil environment conditions and thereby it improves the plant health, which ultimately enables the plant to manage environmental pressures.

In non legumes, nod factors have been reported to act as a substitute for auxin and cytokinin causing enhanced cell division. Priming with phytohormoness howed beneficial effect on growth and yield of crop species grown under saline conditions by increasing the nutrient reserves through accelerated physiological activities.Miransari and Smith (2009) reported that LCOs seemed to be more effective on barley germination as they significantly increased seed germination (upto 44%). Supanjani *et al.* (2009) used LCO at concentrations of 10^{-8} M and 10^{-10} M. He reported that LCOs increased germination speed 8 hours from 58 hours, increased 69% of total root length, and increased about 30% of total surface/ projected area of the roots in cauliflower. Leaf area, hypocotyls length and seedling weights were not affected by LCO treatment.

Maize one of most important cereal of rice and wheat is reported as salt sensitive species among the cereals. Maize is a dual crop grown for both grain and animal feed, widely cultivated in all the region of the world. In India, it is cultivated in an area of 71 million hectares with a production of 22.26 million tones with an average productivity of 2476 kg ha^{-1} (Anonymous, 2014).

At present, research is in progress to ameliorate the effect of salinity on seed germination of some crops by employing biologically produced compounds like Nod factors. But an understanding of the physiology of seed germination under saline condition is important. Hence, a laboratory study is attemptedto investigate the effects of priming maize in LCOon germination and establishment purely under saline conditions.

MATERIALS AND METHODS

The experiment was conducted in Tamil Nadu Agricultural University,under laboratory conditions to study the effect of LCO priming on germination and seedling establishment of maize under salt stress. Seeds of maize hybrid CoHM (6) were primed by soaking in LCO @4mL/kg of seed for 12h at room temperature.

The experiment was laid out in factorial CRD with 2 factors such assix salinity levels

(0, 2,4,6,8 and 10 dSm^{-1}) and 2 levels of priming (LCO primed and unprimed seeds) and replicated four times. The experiment was carried out in 48 petridishes that is 24 for LCO primed seeds and 24 for nonprimed (Control) seeds. The salinity levels were obtained by dissolving 1.28, 2.56, 3.84, 5.12, 6.4 g of NaCl in a litre distilled water, respectively. Distilledwater (0dSm^{-1}) was used as a control.Seeds were sown on 10cm diapetridishes,lined with whatman No1. Filter paper and were supplied with 10ml of each treatment solution daily.

Everyday germination count was recorded after 48 hrs of sowing, and terminated when no further germination occurred.The experiment was conducted up to 14 days and parameters like germination percentage, root length, shoot length, were observed also seedling vigour index and salt tolerance were calculated. The data of germination percentage was transformed using by arcsine values prior to statistical analysis. For comparing the differences among treatment means,LSD test was applied at 5% probability level. Correlationand regression analysis was performed using the SPSS and Excel.

RESULTS

Results of the study showed that both salinity and LCO influenced seed germinable characters both independently and interactively. The responses of seed germination percentage, root length, shoot length, vigour index and salt tolerance index to LCO priming and NaCl concentration was significant. Outcome of the experiment on the affect of LCO on maize seed germination is presented in figures.

Salt concentrations of NaCl adversely affected the germinable attributes of maize. In general all the germinable characters decreased with increasing salt concentration. Maximum reduction in germination percentage (83 %), root length (16.8 cm), shoot length (8.08 cm), seedling vigour index (2069) and salt tolerance index (100) was found in control which is irrigated with distilled water.

Priming with LCO has significant influence on the germination percentage, shoot length, root length and vigour index of maize under varying level of salinity. The germination percentage was assessed higher in the control (96 %) which is on par with 2dSm^{-1} (91 %). The lower germination percentage was observed in 10 dSm^{-1} (57 %). Similarly, shoot length was found maximum in control (14 cm) followed by 2 dSm^{-1}(11.9 cm). The minimal length was found in 10 dSm^{-1} (3.1 cm). However, lengthiest root was observed in control (16.8 cm) while the lowest was observed in 10 dSm^{-1} (3.65 cm). Priming of LCO (3570) recorded higher vigour index in control of 0 dSm^{-1} while the lowest was observed in 10 dSm^{-1} (749). Salt tolerance index also followed the same trend.

Correlation analysis was performed to find out the association of independent variables namely seedling vigour index (X_1), shoot length (X_2), root length (X_3), germination percentage (X_4)with the dependent variable salt tolerance index. Correlation analysis showed that germination percentage, shoot length, root length and seedling vigour index haveshown positive and significant association withthe salt tolerance indexat one per cent probability level (Table 1).

DISCUSSION

Germination percentage

Table 1: Correlation coefficients between the traits of maize seed germination in different levels of salt stress

Traits	Seedling vigour index	Root length	Shoot length	Salt tolerance index	Germination percentage
Seedling vigour index	1				
Root length	0.980**	1			
Shoot length	0.969**	0.979**	1		
Salt tolerance index	0.804**	0.793**	0.811**	1	
Germination percentage	0.965**	0.925**	0.964**	0.820**	1

*and**: Significant at 0.05 and 0.01 probability level, respectively; In order to find out the relative influence of each independent variable towards salt tolerance, regression analysis was performed (Table 2).

Table 2: Regression of salt tolerance index of maize under different levels of salt stress

S.No.	Variables	Partial Regression Coefficient (b)	SE	't' value
1.	Seedling vigour index	-.059	.102	-.572
2.	Root length	19.670	32.079	.613
3.	Shoot length	-9.150	18.425	-.497
4.	Germination percentage	3.271	4.202	.778

$R^2 = 0.694$

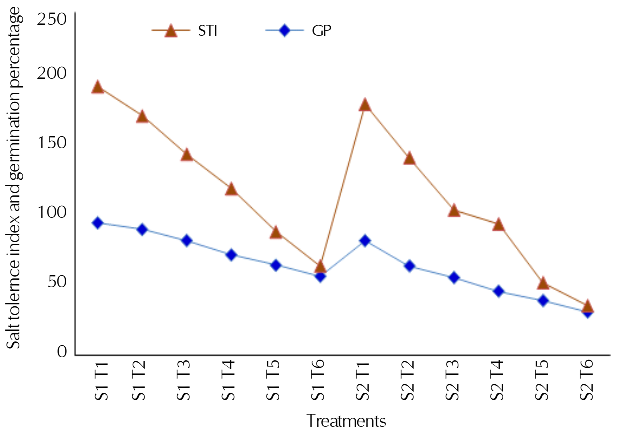

Figure 1: Effect of LCO on germination percentage and salt tolerance index of maize under salt stress

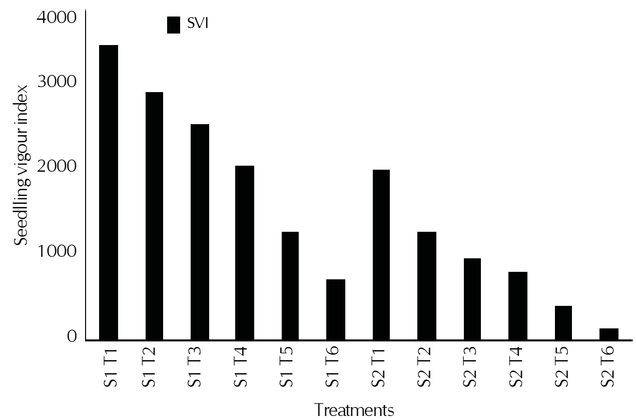

Figure 2: Effect of LCO on seeding vigour index of maize under salt stress

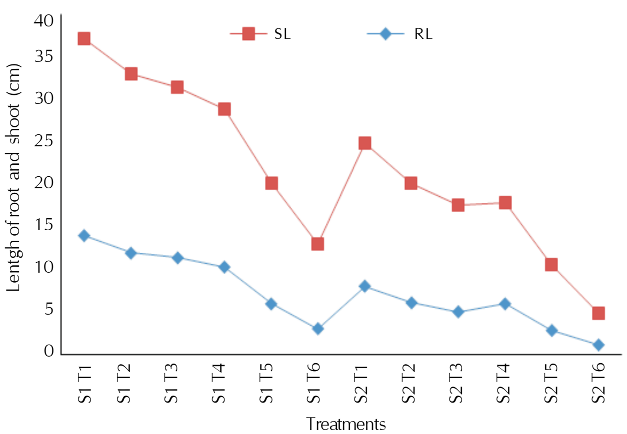

Figure 3: Effect of LCO on root and shoot length of maize under salt stress

Germination percentage is an important index for salt tolerance. From the results of this study, it is clear that the germination and seedling vigour of both primed and nonprimed seed reduced with increase in NaCl concentration (Fig.1). While observing the effect of different salinity stresses imposed using sodium chloride at 2, 4, 6, 8 and 10 dSm^{-1} levels on germination percentage, a significant reduction in germination was noticed at the salinity level of 10 dSm^{-1}. The reason for reduction in germination under salinity is due to the reduction of water potential in the medium which hinders water absorption that plays a critical role in the initiation of activity of the enzyme á-amylase. Salinity caused reduction in germination percentage upto62 per cent at higher NaCl level (10 dSm^{-1}) in maize. But, the detrimental effect was lower at lower concentration and higher at higher concentration. This observation is in accordance with the findings of Kaymakanova (2009).

However, the performance of the bioprimed seed was better in all the NaCl concentrations as compared to the nonprimed seed. LCO effect was significant on seed germination. The increase in germination over nonprimed seeds accounted for 13, 29, 32, 36, 39 and 45 per cent under 0, 2, 4, 6, 8 and 10 dSm^{-1}NaCl salt concentrations. Seed germination was significantly quick in LCO treated seeds than those in control petridishes. The increase in emergence percentage in seeds primed with nod factor under saline conditions may be due to increase in cell division activity by the LCO which leads to enhanced oxygen uptake, increased á- amylase activity and the efficiency of mobilizing nutrients from the cotyledons to the embryonic axis. This finding was in conformation with the outcome of Daychok *et al.* (2000) study who reported the induction of cell division by the nod factors (LCO) in non-legumes.

Salt Tolerance Index

The results revealed that with the increase in salinity level, there was a significantly decreasing trend in all the treatments, indicating that higher the stress lesser the tolerance (Fig.1).

In general STI will be more in lower level of salinity with priming. Though LCO treatment realized in GP the tolerance to salinity is increased in a gradual manner. At 10

dSm^{-1} the STI is around 8.10 in LCO treated which is 38 per cent higher than non primed seed under saline environment.

Seedling Vigour Index

Seedling vigour is an important quality parameter to assess the performance of seed for its germination and viability (Fig. 2). The seedling vigour index was significantly altered by all the six levels of salinity. Salinity at 10dSm^{-1} concentration had a significant reduction in the mean vigour index over control. The maximum vigour index of 2069 was recorded by the 0 dSm^{-1} (Control) followed by 2 dSm^{-1} (1307) in maize. These results are consistent with the finding of Gholipoor *et al.* (2000).

Based on results, salinity level, seed priming and their interaction have significant effect on vigour index. Increasing salinity causes a significant decrease in maize vigour index for both unprimed and LCO primed seeds. However, the relative decrease for LCO primed seeds was significantly different when compared to seedlings from unprimed seeds. Vigour index ranged from 749 (10 dSm^{-1}) to 3570 (control). Vigour index was increased in LCO primed seeds compared to unprimed seeds of maize. This might be due to improvement in seedling length and total germination percentage may be a result of earlier induced germination by primed seeds over unprimed seeds, which resulted in vigorous seedlings with more root and shoot length than the seedlings from unprimed seeds. Similar results were found by Ruan *et al.* (2002) working on rice seeds, priming showed higher vigour index then non primed ones.

Shoot length

There was a significant decrease in the shoot length at salinity levels 8 and 10dSm^{-1}NaCl concentration when compared with the salinity level of 2dSm^{-1}. At the severe stress level of 10dSm^{-1}, the response of maize was differential (Fig. 3). Control showed significantly increased shoot length of 10.3 cm when compared to other salinity levels. The salinity level of 10dSm^{-1} had a significantly reduced shoot length of 3.65 cm when compared to control. This result was in accordance with the finding of Panneerselvam*et al.* (1998). This was in conformation with the result of Khatoon *et al.* (2010) who found that a high sodium chloride concentration caused reduction in shoot length of maize.NaCl treatment caused decrease in dry weight of embryonic axis of groundnut (Basha*et al.*, 2011).

There was a significant increase in the total length of the shoot with priming of LCO. The shoot lengthof LCO primed seeds were 23.2 cm in 0dSm^{-1} and 21.1cm in 2 dSm^{-1} than non primed seeds (control). The percent increase over non primed seeds for various salt concentrations is varied from 27 to 63 per cent. This might be attributed to the inherent mechanism of LCO in the regulation of entire germination process. This was in accordance with the consequence of Supanjani *et al.* (2009).

Root length

The adverse effects of salt stresses on germination per cent also reflected on root growth of maize seedlings (Fig.3). Besides poor germination higher level of salinity stress also showed a significant reduction in root length. The salinity level of 0 dSm^{-1}, (control) recorded significantly higher root length of 8 cm over other salinity level. A significant reduction was observed in the highest level of salinity (10 dSm^{-1}). 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.

The present study also revealed that the priming of LCO maintained better root growth under salinity condition. LCO primed seeds are attribute to have 61 per cent than non primed seeds under the salinity level of 10 dSm^{-1}. This might be due to enhanced germination and seedling growth, along with the mitogenic nature of LCOs, suggests accelerated meristem activity which induces the root growth. This was in agreement with the finding of Soulamanov*et al.* (2002).

Correlation and Regression analysis

Correlation analysis showed that the traits were more in normal condition than saline condition; ultimately salinity places its adverse effects on the aforesaid traits. The reason might be that reduction in enzyme activity of sprouting seeds, which leads to the reduction of radical and coleoptile length by inhibiting the water intake of seeds due to lower osmotic potentials. Inhibition of germination due to salinity has been reported earlier in greengram cultivars (Abdul Jaleel *et al.* 2007).

Regression analysis revealed that the R^2 value was 0.694, which revealed that all the traits together contributed 69.4 per cent variation in the salt tolerance. Regression analysis of salinity effects on traits showed that, there was a negative linear relation between NaCl concentrations with traits. The 'F' value was not significant at one per cent level of probability and the equation is given below.

Regression equation

$$Y = -77.59 - 0.59(X_1) + 19.67(X_2) - 9.15(X_3) + 3.27(X_4)$$

It could be seen from the above equation that the variables *viz.*, seedling vigour index (X_1), shoot length (X_2), root length (X_3), germination percentage (X_4), had not shown any significant contribution at one per cent level of probability.

In this study the effect of LCO priming on maize under different salinity levels was investigated. Based on the results of the study, it is learnt that priming the maize seeds with LCO enhances the germination under saline stress conditions. However the mode of action of salt tolerance by LCO at initial stage (seed establishment) such studies needs further investigation.

REFERENCES

Abdul Jaleel, C., Gopi, R. Sankar, B. Manivannan, P. Kishore kumar, A., Sridharan, R. and Panneerselvam, R. 2007. Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in Catharanthusroseus seedlings under salt stress. *South African J. Botany* **73:**190-195.

Almansouri, M. Kinet, J. M. and Lutts, S. 2001. Effect of salt and osmotic stresses on germination in durum wheat (*Triticumdurum*). *Plant and Soil.* **231:** 243-254.

Anonymous 2014. Ministry of Agriculture, 2014-15. *www.agricoop.nic.in.*

Basha, S. K. M., Fareeda, G., Srilatha Devi, R. K. Uma maheswari, M. and Savithramma, N. 2011. Effect of Nacl Stress Ameliorated with $CaCl_2$ on proteaseand Polypeptide Analysis of Groundnut (*Arachishypogaea* L.). *The Bioscan.* **6(2):** 283-287.

Daychok, J. V., Tobin, A. E. Price, N. P. J. and Von Arnold, S. 2000. Rhizobial nod factorstimulate somatic embryo development in *Piceaabies*. Plant Cell Rep. **3:** 290-297.

Gholipoor, M., Ghasemi-golezani, K., Khoole, F. R. and Moghaddam, M. 2000. Effects of salinity on initial seedling growth of chickpea (*Cicerarietinum* L.). *Acta Agronomica Hungarica.* **48(4):** 337-343.

Kaymakanova, M. 2009. Effect of salinity on germination and seed physiology in bean (*Phaseolus vulgaris* L.), XI Anniversary Scientific Conference. *Biotechnology and Biotechnology, Grasas y Aceites.* **23:** 326-329.

Khatoon, T., Hussain, K., Majeed, A., Nawaz, K. and Nisar, M. F. 2010. Morphological Variations inMaize (*Zea mays* L.) Under Different Levels ofNacl at Germinating Stage. *WarsawAgricultural Science J.* **8(10):** 1294-1297.

Miransari, M. and Smith, D. L. 2009. Alleviating salt stress on soybean (Glycine max (L.) Merr.) - Bradyrhizobiumjaponicum symbiosis, using signal molecule genistein. *European J. Soil Biology.* **45(2):** 146-152.

Neto, A. D. and Tabosa, J. N. 2000. Salt stress in maize seedlings: I. Growth analisys. Revista Brasileirade Engenhariaagrícola e Ambiental. **4:** 159-164.

Panneerselvam, R., Muthukumarasamy, M. and Rajan, S. N. 1998. Amelioration of Nacl Stressby triadimefon in soybean seedlings, *Bio.*

Plant. **41:** 133-137.

Ramoliya, P. J. and Pandey, A. N. 2003. Effect of salinization of soil on emergence, growth and survival of seedlings of Cordiarothii. *Forest Ecology and Management.* **176:** 185-194.

Ruan, S., Xue, Q. and Tylkowska, K. 2002. Effects of seed priming on germination and health of rice (*Oryzasativa*L.) seeds. *Seed Sci. Technol.* **30:** 451-458.

Souleimanov, A, Prithiviraj, B. and Smith, D. L. 2002. The major Nod factorof Bradyrhizobiumjaponicum promotes early growth ofsoybean and corn. *J. Exp. Bot.* **53:** 1929-34.

Supanjani, F., Mabood, A., Habib, D., Donnelly and Smith, D. L. 2006. Nod factor enhancescalcium uptake by soybean. *Plant Physiol. Biochem.* **44(11-12):** 866-872.

Supanjani1, Lee, K. D., Duzan, H. and Smith, D. L. 2009. Effect of Lipo-chitooligosaccharide on Germination and Seedling Growth of Cauliflower. *J. Akta Agrosia.* **12(1):** 75-82.