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Enhancing germination tolerance of maize to salinity stress using lipo-chitooligosaccharides

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ABSTRACT

Worldwide salinity is the foremost soil environmental factor adversely affecting the seed germination, plant growth and yield. Recent studies show that lipo-chitooligosaccharides (LCO-nod factors) is a unique molecule which is responsible for abiotic stress tolerance. Hence a laboratory experiment was taken up with a sole objective of seed priming with LCO (4 ml/kg) on maize seedling growth which were exposed to different levels of salinity (0, 2, 4, 6, 8 and 10 dS/m) and was laid out in a factorial completely randomized design with four replications. As a result of salinity stress the seedling characters of maize were negatively influenced. However nod factor treated maize seeds had relatively higher germination percentage, germination stress tolerance index and mean daily germination and were found to have decreased phytotoxicity to roots and shoots. Thus seed priming with nod factor (4 ml/kg) improved the tolerance to salinity at seedling stage of maize by inducing physiological changes in seeds.

Keywords: Salinity; lipo-chitooligosaccharides; germination percentage; germination stress; tolerance index; mean daily germination

INTRODUCTION

Salinity is one of the most critical issues in agriculture around the world which affects the 6 per cent of the total land area of the planet which is approximately 800 million hectares (Anon 2012). It has adverse effect on seedling growth by creating an osmotic potential on the rhizosphere of the crop which leads to soil fertility loss.

Maize is one of the most important cereals which is grown under various environmental conditions of the world. In India maize which is the third most significant cereal after wheat and rice is grown on about 71 million hectares with a production of 22.26 MT and productivity of 2476 kg/ha (Anon 2014). It is being consumed as prime food and commonly fed to the livestock. Improvement in establishment of characteristics of maize has received considerable attention around the world particularly in salt affected soils. Enhancing the tolerance for salinity would be of substantial value for this sensitive crop when grown under saline conditions.

Germination is a very sensitive phase in the crop growth that reduces the water absorption. Saline environment delays germination of seeds as well as the final germination percentage (Zeinali et al 2002). Increasing the salt concentration not only reduces the germination but also inhibits the seedling establishment. Seed resistance to increased salinity during germination is essential for plant survival in the field consequently for its further development and high yield performance.

Pre-sowing seed treatments have been shown to enhance stand establishment in non-saline areas (Khan 1992) and have potential in saline areas as well (Basra et al 2005). In the past two decades improving seed germination and seedling emergence under various stress conditions by means of physiological treatments have been investigated intensively. It is thought that the depressive effect of salinity on germination could be related to a decline in endogenous levels of hormones (Debez et al 2001)). However incorporation of plant growth regulators during pre-soaking, priming and other pre-sowing treatments in many vegetable crops have improved seed performance under stress conditions.

Seed priming is one of the biochemical methods that enhances seed performance and provides better germination under adverse conditions. One among the biochemical molecules is lipo-chitooligosaccharides (nod factor/ LCO), bacteria-to-plant signals which are produced as a result of plant-microbe interactions. Nod factor is a unique signal molecule which is known to handle adverse environmental conditions and recently has been considered to have hormone like substances. Supanjani et al (2009) used LCO at concentrations of 10^{-8} M and 10^{-10} M and reported that LCOs increased germination speed (8 hours from 58 hours), increased 69 per cent of total root length and increased about 30 per cent of total surface/ projected area of the roots in cauliflower. Leaf area, hypocotyl length and seedling weights of cauliflower were not affected by LCO treatment.

At present research is in progress and information on seedling tolerance of maize to salinity through nod factors (LCO) is inadequate. Although from the agronomical point of view the most important traits of maize crop submitted to salinity reflect their adaptation at the early crop establishment phase. Hence the present study was planned to furnish the knowledge about the effect of nod factors (LCO) on seedling growth of maize under saline conditions.

MATERIAL and METHODS

To investigate the effects of salinity-mediated seed priming of LCO on maize germination and seedling establishment under saline conditions with a completely randomized factorial experiment with four replications was carried out at Tamil Nadu Agricultural University under laboratory conditions. Seeds of maize hybrid CoHM (6) were primed by soaking in LCO @ 4 ml/kg of seed for 12 h at room temperature.

The experiment consisted of 2 factors such as six salinity levels (0, 2, 4, 6, 8 and 10 dS/m) and 2 levels of priming (LCO primed and unprimed seeds). The experiment was carried out in 48 Petri dishes, 24 for LCO primed seeds and 24 for unprimed (control) seeds. The salinity levels were obtained by dissolving 1.28, 2.56, 3.84, 5.12 and 6.4 g of NaCl each in one litre distilled water. Distilled water (0 dS/m) was used as control. Seeds were sown in 10 cm dia Petri dishes lined with Whatman # 1 filter paper and were supplied with 10 ml of each treatment solution daily.

Following parameters were measured:

Germination percentage: The emergence of plumule was taken as index of germination. Initiation and completion of germination were recorded daily for 14 days and germination percentage was calculated using the following formula:

$$\text{Germination percentage (\%)} = \frac{\text{Number of germinated seeds}}{\text{Number of total seeds}} \times 100$$

Germination stress tolerance index (GSI): The germination stress tolerance index (GSI) and promptness index (PI) were estimated using following formula (Ashraf et al 2008):

$$PI = nd_1\,(1.00) + nd_2\,(0.75) + nd_3\,(0.50) + nd_4\,(0.25)$$

where nd_1, nd_2, nd_3 and nd_4= Number of seeds germinated on the 1st, 2nd, 3rd and 4th day respectively.

A germination stress tolerance index (GSI) was calculated in terms of percentage as follows:

$$GSTI = (\text{PI of stressed seeds} / \text{PI of control seeds}) \times 100$$

Mean daily germination (MDG): This is an index of daily germination speed and it was calculated using the formula:

$$MDG = \frac{FGP}{D}$$

where FGP= Final germination per cent, d: Test period

Phytotoxicity to roots and shoots: Phytotoxicity to roots and shoots was calculated based on the following formula:

$$\text{Phytotoxicity of roots and shoots} = \frac{\text{Shoot/root length in control} - \text{Shoot/root length in stress}}{\text{Shoot/root length in control}}$$

Statistical analysis: The data on germination percentage were transformed using arc sine values prior to statistical analysis. Significant differences between treatments were determined using LSD test at the 5 per cent level.

RESULTS

Both salinity level and priming had significant effects on the aforesaid parameters. Moreover the interaction of salinity level and priming with lipo-chitooligosaccharides (LCO) had significant effect on all seedling parameters tested at 5 per cent significant level. Priming with LCO relatively increased the germination parameters (germination percentage, mean daily germination) and growth parameters (phytotoxicity of roots and shoots) of maize under different levels of salt concentration as compared to non-primed seeds.

Data on maize germination percentage showed the effect of LCO priming and non-priming at different salinity concentrations. In general germination percentage for both primed and non-primed seeds decreased significantly with increasing NaCl salinity level. However this reduction was significantly higher for non-primed seeds compared to primed seeds (Fig 1). Germination percentage (GP) in seed primed with LCO relatively increased as compared to control. Analysis showed the germination percentage in seeds with LCO was 96, 91, 83, 73, 65 and 57 for the salinity levels of 0, 2, 4, 6, 8 and 10 ds/m respectively.

Germination stress tolerance index (GSI) was significantly reduced by the induction of salinity stress. However it varied for the different levels of salinity as shown in Fig 1. The highest germination stress tolerance index (GSI) of 92.4 was noted under 0 dS/m salinity level and the lowest (46.5) was in 10 dS/m. The LCO primed seeds were successful in increasing relatively highest GSI of 95.2 at 0 dS/m while at 10 dS/m the GSI was lower but relative increase was noticed compared to non-primed seeds. Among the salinity levels both for priming and non-priming 10 dS/m NaCl level was at the bottom in performing the tolerance.

In general increasing NaCl concentration reduced the mean daily germination (Fig 2). The maximum MDG of 6.6 was observed in control (0 dS/m) compared to all the levels of salinity for both LCO primed and non-primed seeds. Higher level of salinity inhibited the daily mean germination and registered MDG of 4.09 and 2.23 at 10 dS/m for LCO primed and non-primed seeds.

The osmotic stress had significant effect on phytotoxicity of roots (PhR) and phytotoxicity of shoots (PhSh) both for LCO primed and non-primed seedlings (Fig 3). PhR and PhSh were significantly increased with increasing osmotic stress, the lowest values recorded at 0 dS/m (0% each). The highest value of PhR (59.1 and 80.7) and PhSh (77.6 and 84.7) was observed at 10 dS/m for both primed and non-primed seeds respectively.

DISCUSSION

Generally increasing salinity causes a decrease in maize germination which might be due to the toxic effects of Na^+ and Cl^- in the process of germination (Khajeh-Hosseini et al 2003). It alters the water imbibitions by seeds due to lower osmotic potential of germination media, causes toxicity which changes the activity of enzymes of alpha amylase activity, nucleic acid metabolism, changes protein metabolism, interrupts hormonal balance and reduces the utilization of seed reserve food (Gomes-Filho et al 2002). LCO effect was significant on seed germination. The increase in germination over non-primed seeds accounted for 13, 29, 32, 36, 39 and 45 per cent under 0, 2, 4, 6, 8 and 10 dS/m NaCl salt concentration. LCO primed seeds of maize might have better competency for water absorption from the growing media that enabled metabolic activities in seeds during germination process of a start much earlier than radicle and plumule appearance. Similarly increased solubilization of seed storage proteins like the beta subunit of the globulin and enhanced antioxidative activity in primed seeds facilitating germination.

The salt tolerant cultivars of the crop can be identified on the basis of germination stress tolerance index because during germination, seed vigour and seed storage conditions also affect. However in the present study healthy seeds having similar size and good viability were used. So in this case the reduction in germination stress tolerance index might have been due to the effect of salinity. Reason attributed for the retarded seed germination and root emergence at higher salinity level might be due to osmotic effect which is deleterious and prevents the plants from maintaining their proper nutritional requirements necessary for their healthy growth which leads to lesser tolerance for salinity (Krishnamurthy et al 2007). Results of germination stress tolerance index revealed that at the salinity level 2 dS/m, the LCO primed seeds had 89 of GSTI which was affordable for growing while for salinity level 4 dS/m which is close to 2 dS/m proved

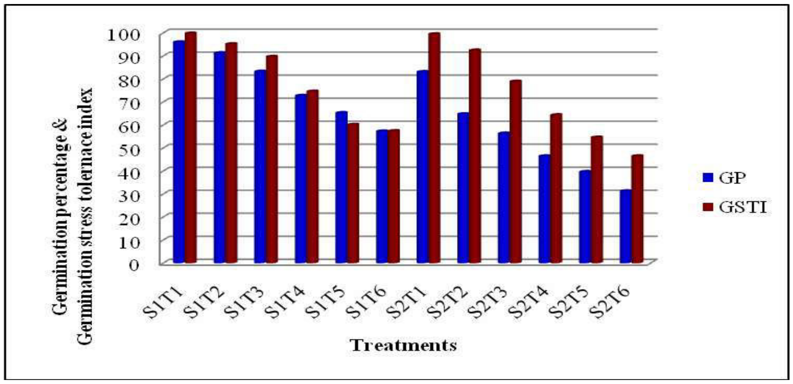

Fig 1. Influence of LCO on germination percentage and germination stress tolerance index of maize under salt stress

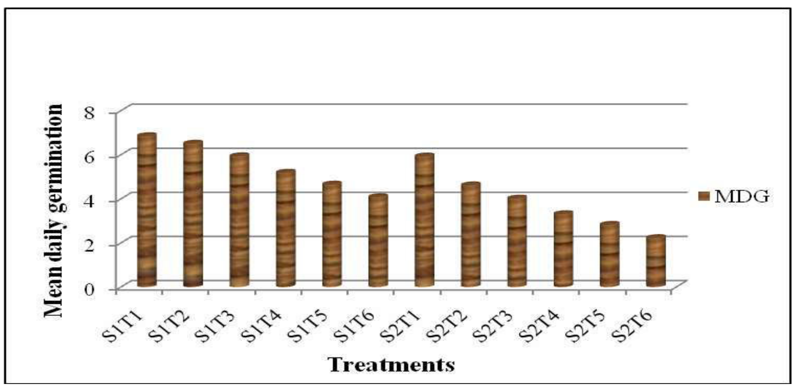

Fig 2. Influence of LCO on mean daily germination of maize under salt stress

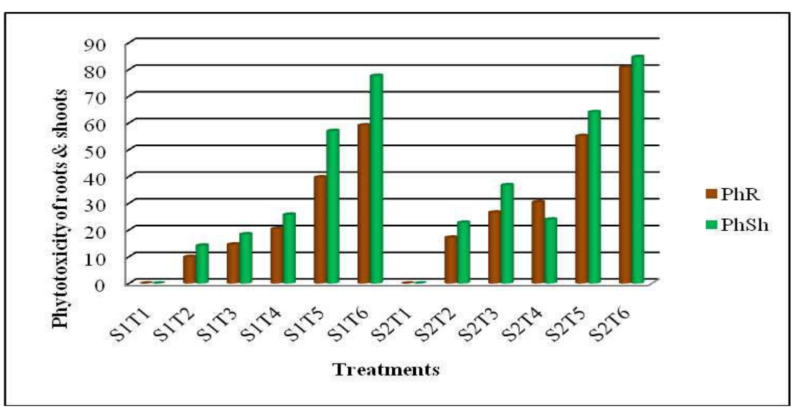

Fig 3. Influence of LCO phytotoxicity on roots and shoots of maize under salt stress

suitable for cultivation. It is well known fact that if crop stand is good and well established at the initial level the yield will be higher than that crop with poor stand. Results of Hamid et al (2008) also confirm the present findings. The reason for relative increase in GSTI of LCO primed seeds might be due to the osmotic potential acceleration which induces the uptake of water necessary for mobilization of nutrient required for germination.

Daily mean germination acts as an indicator for salt tolerance. The reason for higher mean daily germination in control might be due to lack of NaCl concentration. Range of mean daily germination ranged from 6.86 to 4.09 for priming and 5.94 to 2.23 for non-priming for control and 10 dS/m respectively. Decrease in germination by increasing of salinity level was possibly due to reduced osmotic potential, high levels of toxic ions and seed food reserve.

Information on phytotoxicity is important to identify the phytotoxicity tolerance of the crop. Increase in NaCl concentration increased the phytotoxicity which is supported by the findings of Asmare (2013). High salinity inhibits root and shoot elongation due to slowing down of the water uptake by the crop. However LCO primed seeds had relatively low phytotoxicity of root and shoot that indicated that it was better in tolerating higher NaCl concentration. This may be attributed to earlier germination induced by LCO priming. During priming the embryo expands, compacts the endosperm and accelerates the activities of hydrolytic enzymes. The compaction force facilitates root and seedling projection after rehydration (Mohammadi 2009). This might have resulted in vigorous seedlings with more root and shoot length than the seedlings from non-primed seeds. The result of this study is in agreement with the work of Mordi and Zavareh (2013).

CONCLUSION

Seed priming with lipo chitooligosaccharides (LCO/nod factors) improved the tolerance to salinity at seedling stage of maize by inducing physiological changes in maize. The facts mentioned above make it possible to recommend this LCO seed priming to plants grown under conditions of soil salinity. Further research is needed to optimize the effectiveness of LCO seed priming on number of crops.

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