Solid matrix priming with chitosan enhances seed germination and seedling invigoration in mung bean under salinity stress

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Abstract

The objective of present study was to evaluate the response of the mung bean seeds of ‘Sonali B1’ variety primed with chitosan in four different concentrations (0, 0.1%, 0.2% and 0.5%) under salinity stress of five different concentrations (i.e., 0, 4, 6, 8 and 12 dS*mm^{-1}) and halotolerance pattern by applying Celite as matrix at three different moisture levels (5%, 10% and 20%). Improved germination percentage, germination index, mean germination time, coefficient of velocity of germination along with root and shoot length was observed comparing with control. Germination stress tolerance index (GSI), plant height stress tolerance index (PHSI) and root length stress tolerance index (RLSI) were used to evaluate the tolerance of the mung bean seeds against salinity stress induced by chitosan. Results of GSI, PHSI, RLSI showing noteworthy inhibitory effect of salinity stress in control set was significantly less pronounced in chitosan treated seedlings. Chitosan can remarkably alleviate the detrimental effect of salinity up to the level of 6 dS*m^{-1}, beyond which no improvement was noticed. In conclusion present investigation revealed that chitosan is an ideal elicitor for enhancing the speed of germination and seedling invigoration that synchronize with emergence of radicle and salinity stress tolerance.

Keywords: Chitosan, mung bean, salinity, solid matrix priming

Introduction

Salinity of soil is a significant abiotic stress. It hampers crop yield worldwide because it reduces germination percentage and early seedling growth (Jamil et al., 2006). There is a common tendency to investigate new methods that decrease the negative effect of abiotic stress and increase yield along with quality crops. Seed priming is one such method. The work done by Cayuela et al. (1996) on tomatoes, by Pill et al. (1991) on asparagus, by Singh (1995) on sunflower, revealed improved seed germination and plant growth after priming under salinity stress. Chitosan may alleviate the harmful effects of salinity in many plants. It increased germination percentage and improved vigour in cabbage (Chandrkrachang, 2002), pearl millet (Manjunatha et al., 2008), sunflower (Cho et al., 2008) and rape-seed (Sui et al., 2002). Chitosan treatment of rice seeds promoted better seed germination and seedling growth withstanding the stress condition (Ruan and Xu, 2002). Priming with liquid media can cause soaking injury due to rapid liquid uptake into the cotyledons, causing cell death (Orphanos and Heydecker, 1968). Besides, osmotic solutions are needed in large volumes, thereby hindering aeration. As a result, enriched air is frequently needed for continuous aeration (Pill, 1995). A solid or semi-solid medium is an alternative to a liquid medium which is known as solid matrix priming (SMP) or matric conditioning (Copeland and McDonald, 1995). In SMP, seeds are mixed with a solid or semi-solid material with specified amount of water and utilize the chemical and physical characteristics of a solid material to restrict the water uptake of seeds.

In this study, an effort was made to evaluate the response of the mung bean seeds primed with chitosan under salinity stress and halotolerance pattern by applying Celite as matrix at three different moisture levels.

Materials and methods

Mung bean seeds were collected from Pulse and Oilseed Research Station, Berhampur, West Bengal, India. After surface sterilization, seeds were mixed with chitosan treated celite (1:1 proportion) with different solutions of chitosan (0, 0.1%, 0.2% and 0.5% dissolved in 1% acetic acid solution whose pH was adjusted to 6.00 with the help of 1% sodium hydroxide solution) and kept in plastic air tight zip packet at three different moisture levels (5%, 10% and 20%) for priming for 24 hours at room temperature. After incubation, matrix was sieved. Then, seeds were placed in five different saline solutions including control. The electrical conductivity of saline solution was measured with a conductivity meter ($dS*m^{-1}$ = deci Siemen per meter). Finally seeds were kept in Seed Germinator (REMI) for germination adjusted to 25 ± 2°C in a dark. Data were recorded for 10 days. Seedlings were evaluated for the following germination parameters.

(1) Germination Index (GI) = $\Sigma (G_t/T_t)$ where G_t is the number of seeds germinated on t^{th} day and T_t is the number of days up to t^{th} day (Ruan and Xue, 2002).

(2) Mean germination time (MGT) = $\Sigma dn/\Sigma n$, (Ellis and Roberts, 1981); Where (n) is the number of seeds which were germinated on day (d), and (d) is the number of days counted from the beginning of germination.

(3) Coefficient of velocity (CV) = $100 (\Sigma N_i/\Sigma N_{i.} T_i)$ (Scott et al.,1984), where N is the number of seeds germinated on day i and T is the number of days from sowing.

(4) Speed of germination (SG) = (Number of germinated seeds/Days of 1^{st} count) + …….. + (Number of germinated seeds / Days of final count) (Ruan et al., 2002)

Stress tolerance analysis

Following the formulae given by Ashraf et al. (2006)

i. Promptness index (PI) = nd2 (1.00) + nd4 (0.75) + nd6 (0.5) + nd8 (0.25) where n is the number of seeds germinated at day d

ii. Germination stress tolerance index (GSI) = (P.I of stressed seeds / P.I control seeds) × 100

iii. Plant height stress tolerance index (PHSI) = (Plant height of stressed plant / Plant height of control plants) × 100

iv. Root length stress tolerance index (RLSI) = (Root length stressed plant / Root length of control plants) × 100

Phytotoxicity analysis

The following formulae of Asmare (2013) were used for evaluation of root and shoot phytotoxicity:

Root phytotoxicity (%) = (Root length of Control – Root length of treatment) / Root length of control ×100

Shoot phytotoxicity (%) = (Shoot length of Control – Shoot length of treatment) / Shoot length of control × 100

Statistical analysis

The experiment was arranged as a complete randomized block design (CRD) with three replica (n=3). The data were statistically evaluated using Duncan's multiple range test (DMRT) at 5% level to compare the differences among treatment means.

Results

Present investigation revealed that GI, CV, shoot and root length were improved in all chitosan concentrations applied here in comparison with control although 0.2% chitosan concentration showed optimum results. It was also observed that GI (Figure 1), CV (Figure 2), shoot & root length were notably decreased with higher salinity starting from control (0 $dS*m^{-1}$) to 12 $dS*m^{-1}$. In case of CV and GI, under higher moisture, chitosan could not produce significant improvement in higher salinity. But with decreasing moisture level chitosan was found to be very effective in higher salinity like 8 and 12 $dS*m^{-1}$. The maximum value of GI was obtained in 0 $dS*m^{-1}$ salinity level under 0.2% chitosan treatment irrespective of moisture level, though 0.1% chitosan treatment was also found to be very effective at low moisture level. In low moisture level, the significantly lower MGT values were obtained in almost all chitosan treated seeds whereas enhanced results were obtained in higher moisture level (10% and 20%), under 0.2% chitosan treatment (Figure 3). The speed of germination (SG) was decreased as the salinity levels increased (Figure 4).

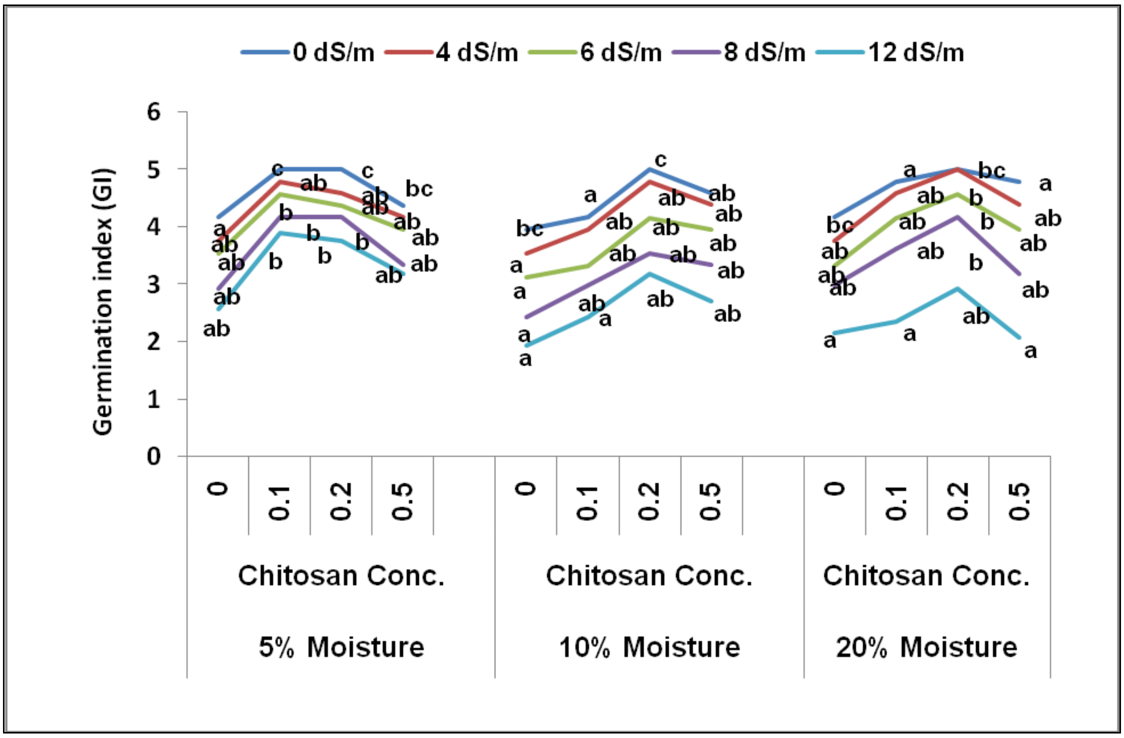

Figure 1. Germination Index (GI) of mung bean seeds

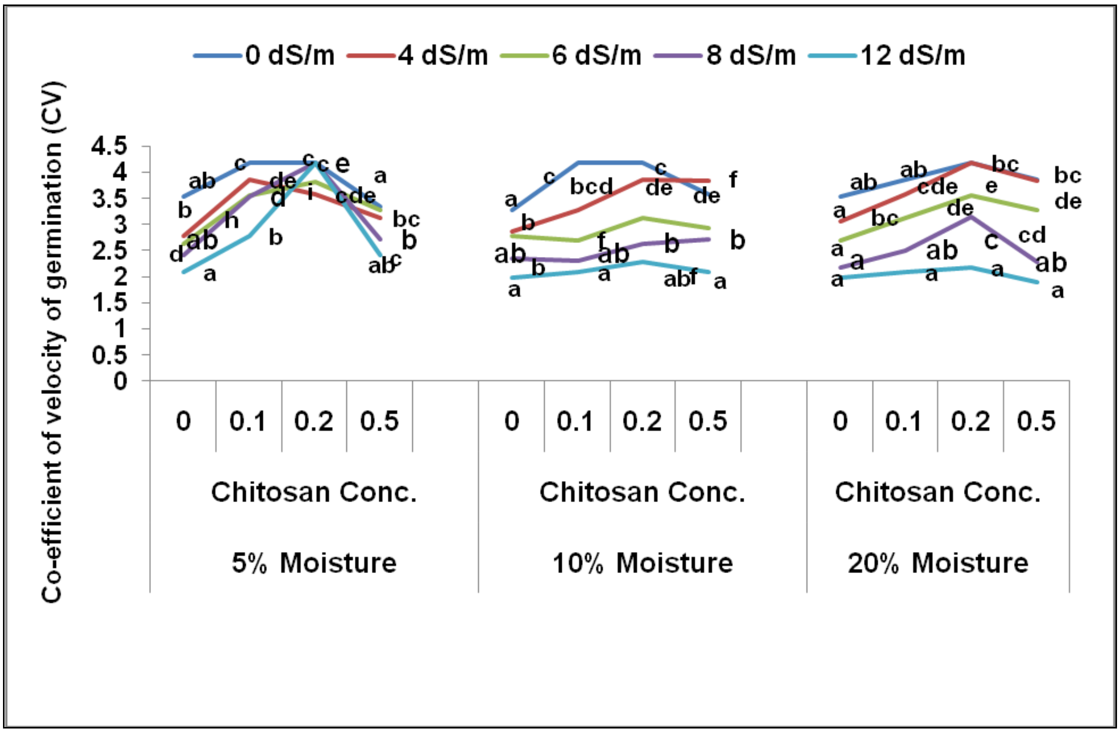

Figure 2. Coefficient of velocity (CV) of mung bean seeds

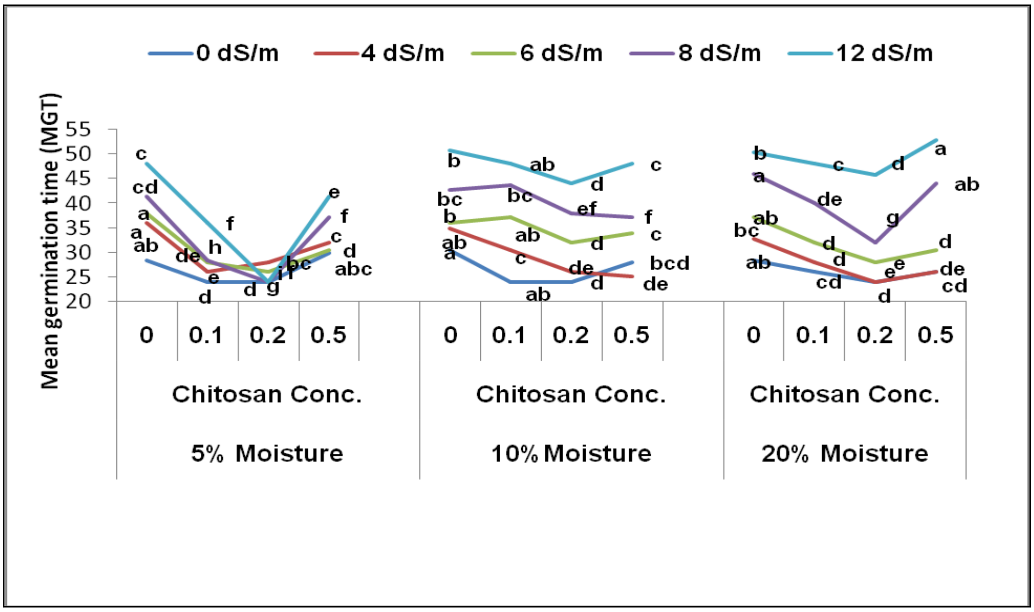

Figure 3. Mean germination time (MGT) of mung bean seeds

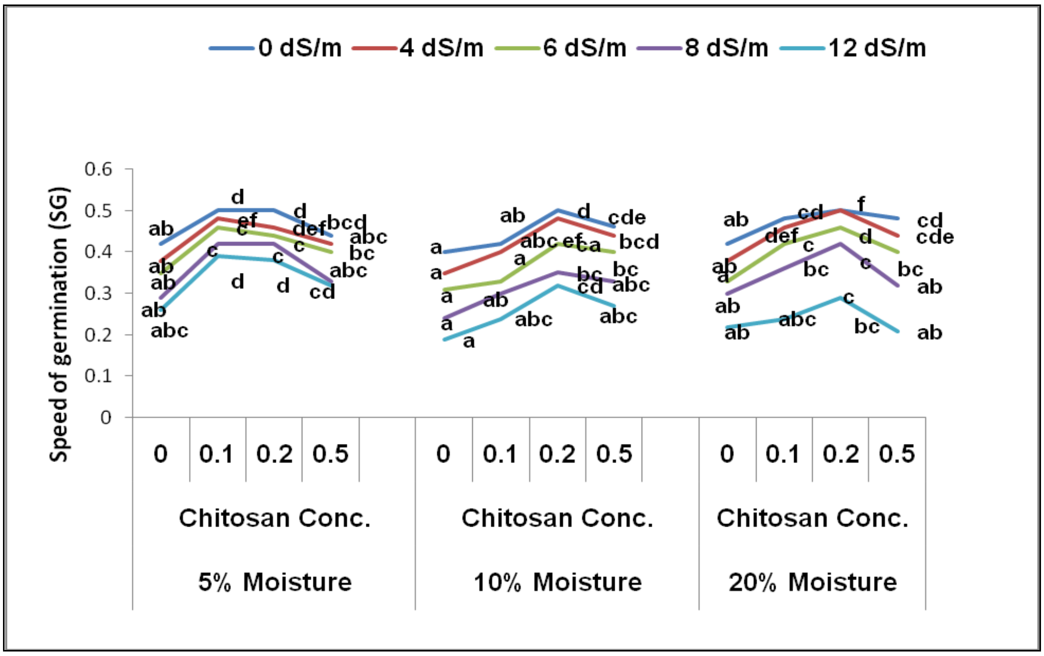

Figure 4. Speed of germination (SG) of mung bean seeds

Irrespective of moisture levels, the highest SG was found at the control and the least was at 12 dS*m^{-1}.

Stress tolerance analysis

Salinity stress inducing water stress significantly reduced the overall PHSI, RLSI and GSI. PHSI was found to be lowered along with increasing salinity concentrations. The minimum PHSI and RLSI values were obtained at all moisture level under salinity stress 12 dS*m^{-1} in unprimed seeds. The maximum PHSI values were obtained at 10% moisture level under salinity stress 4 dS*m^{-1} (Figure 5) whereas in case of RLSI, optimum results were obtained at 20% moisture level under same salinity stress (Figure 6) indicating that the salinity stress can be managed very efficiently up to 6 dS*m^{-1} with chitosan priming. Chitosan priming (0.2%) also considerably improved GSI values against all salinity stress at high matrix moisture (10% and 20%) (Figure 7). In case of PHSI and RLSI data, analysis of variance showed noteworthy differences among different chitosan and salinity concentrations.

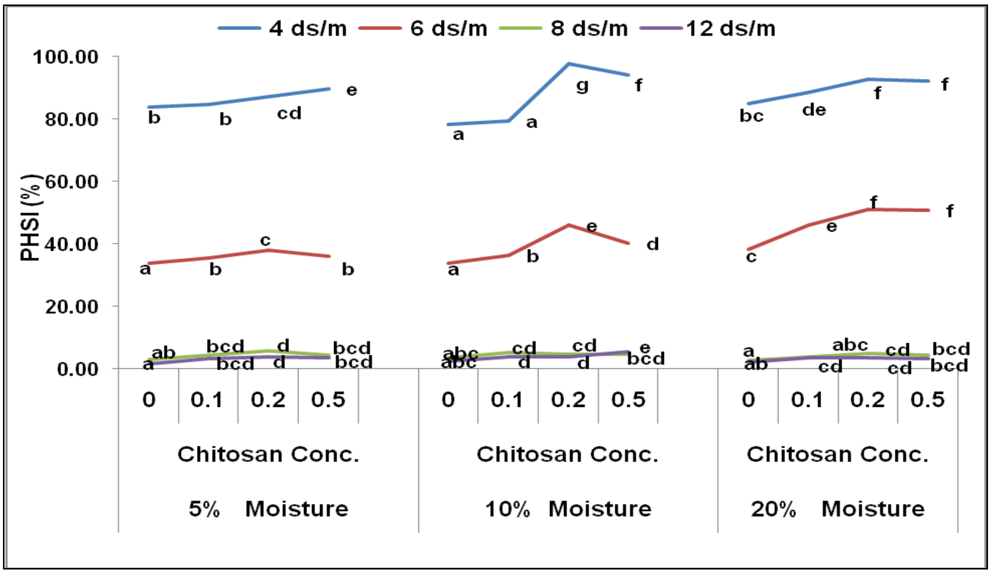

Figure 5. Plant height stress tolerance index (PHSI) of mung bean seeds

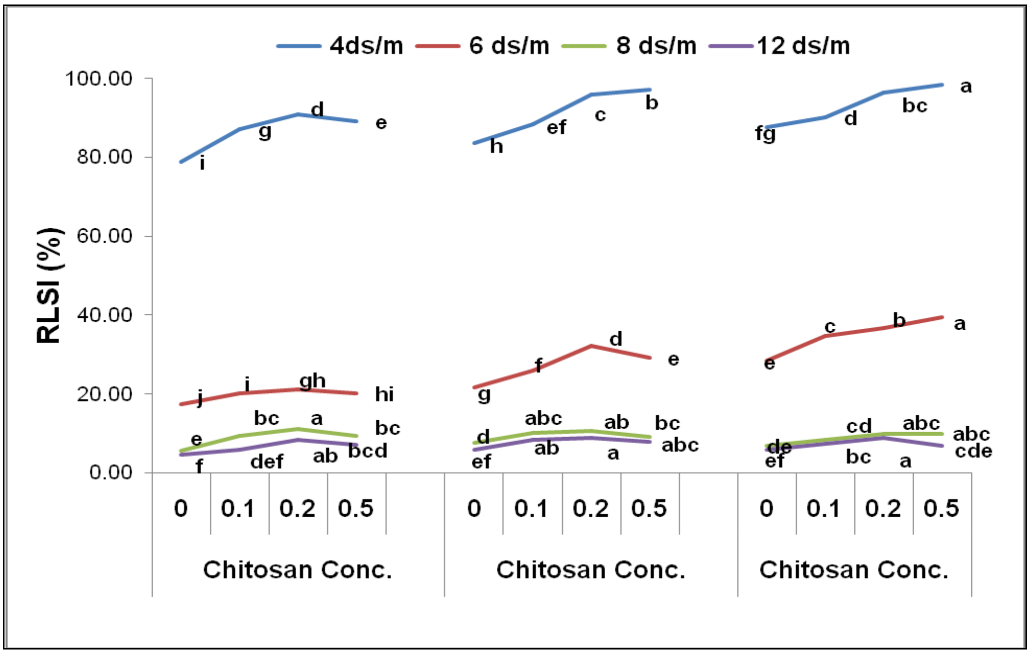

Figure 6. Root length stress tolerance index (RLSI) of mung bean seeds

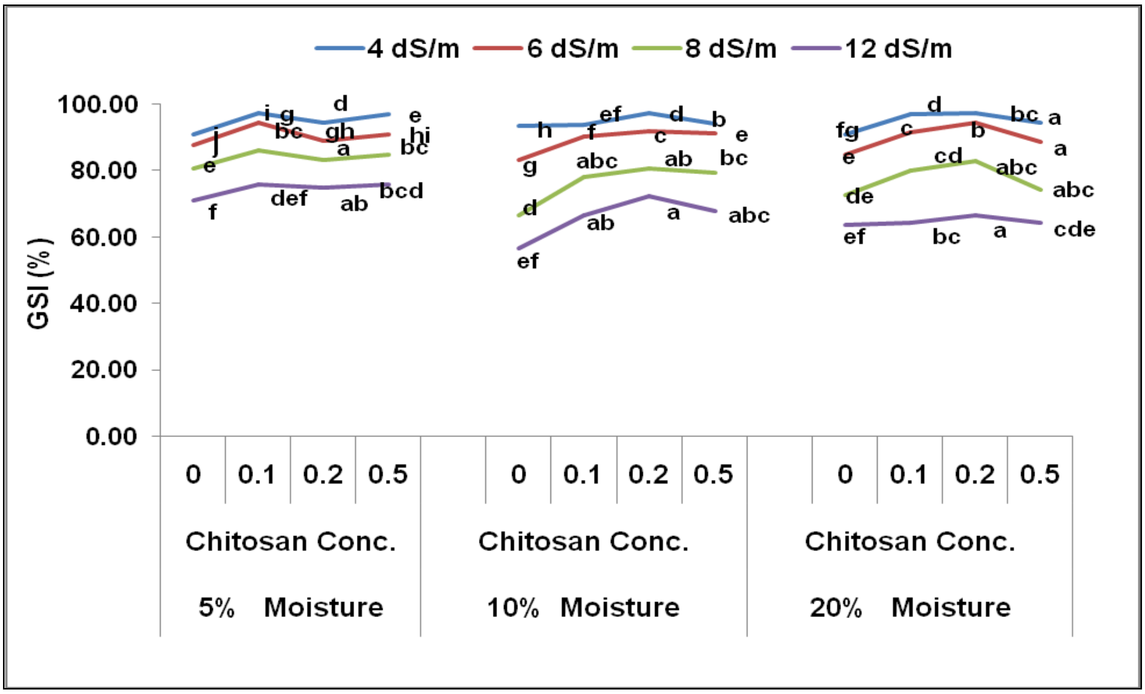

Figure 7. Germination stress tolerance index (GSI) of mung bean seeds

Shoot phytotoxicity

The threshold level of shoot phytotoxicity increased in comparison to the control in each case of moisture level due to chitosan treatment. Figure 8 shows the effect of chitosan on the shoot phytotoxicity (%) in mung bean in three different moisture levels (Table 1).

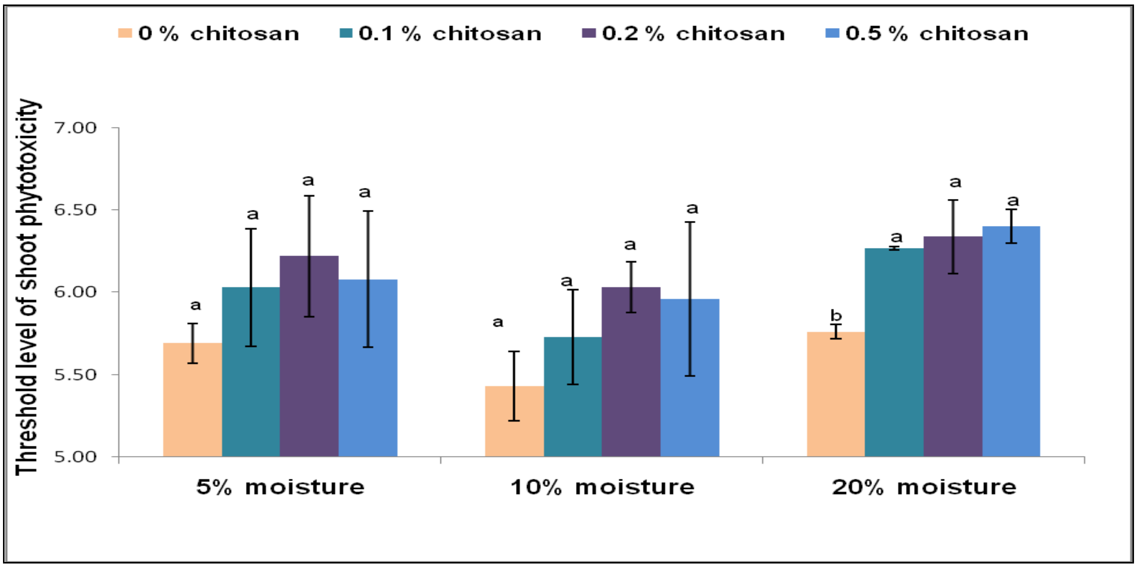

Figure 8. Effect of chitosan on the shoot phytotoxicity (%) in mung bean in three different moisture levels

Table 1. Shoot phytotoxicity threshold level

Treatment	Best fit regression type	Equation	R^2	T_{50}	Standard Error of Estimation (SEE)	DMRT indices
T_1	Linear	y = 19.43x - 60.51	0.998	5.69	0.12	A
T_2	Linear	y = 24.61x - 98.48	0.984	6.03	0.36	A
T_3	Linear	y = 26.82x - 116.7	0.983	6.22	0.37	A
T_4	Linear	y = 25.72x - 106.5	0.978	6.08	0.42	A
T_5	Linear	y = 18.65x - 51.34	0.994	5.43	0.21	A
T_6	Linear	y = 20.17x - 65.61	0.989	5.73	0.29	A
T_7	Linear	y = 23.96x - 94.44	0.997	6.03	0.15	A
7_8	Linear	y = 24.81x - 97.81	0.972	5.96	0.47	A
T_9	Linear	y = 20.08x - 65.73	0.999	5.76	0.044	B
T_{10}	Linear	y = 26.48x - 116.0	1.000	6.27	0.003	A
T_{11}	Linear	y = 28.10x - 128.2	0.993	6.34	0.224	A
T_{12}	Linear	y = 27.56x - 126.3	0.998	6.40	0.103	A

T_1, T_2, T_3, T_4 are 0%, 0.1%, 0.2% and 0.5% chitosan treatment respectively under 5% moisture level; T_5, T_6, T_7, T_8 are 0%, 0.1%, 0.2% and 0.5% chitosan treatment respectively under 10% moisture level; T_9, T_{10}, T_{11}, T_{12} are 0%, 0.1%, 0.2% and 0.5% chitosan treatment respectively under 20% moisture level; T_{50} = Phytotoxicity threshold level.

Maximum threshold level of shoot phytotoxicity was observed at 20% moisture under all treatments with chitosan in comparison with low moisture level. Although in all moisture levels, threshold levels of shoot phytotoxicity have been increased in comparison with that of untreated seeds. In the present study, 0.5% & 0.2% chitosan treatment showed maximum threshold level of shoot phytotoxicity.

Root phytotoxicity

Just like shoot, the threshold level of root phytotoxicity was also increased in comparison to the control at all moisture level due to chitosan treatment. Figure 9 shows the effect of chitosan on the root phytotoxicity (%) in mung bean at three different moisture levels (Table 2).

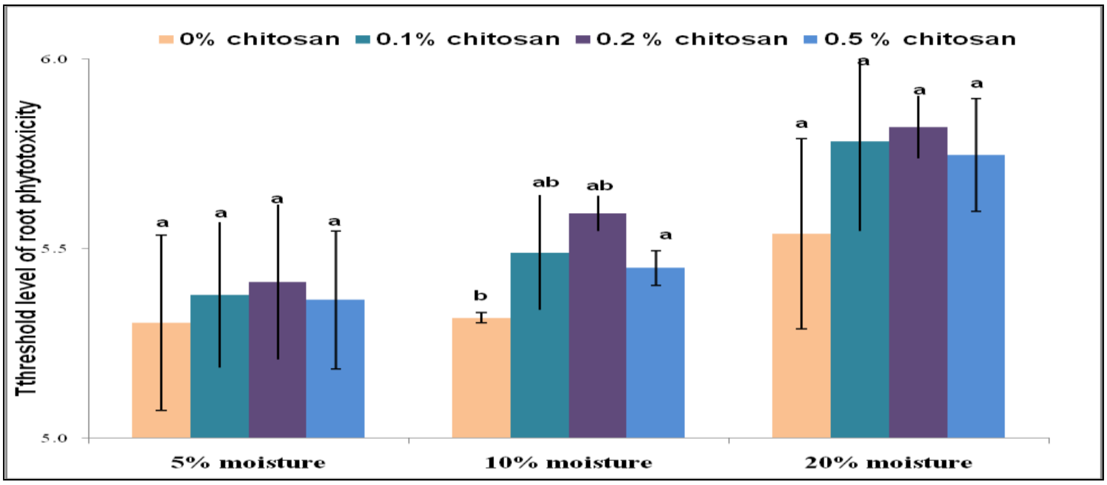

Figure 9. Effect of chitosan on the root phytotoxicity (%) in mung bean in three different moisture levels

Table 2. Root phytotoxicity threshold level

Treatment	Best fit regression	Equation	R^2	T_{50}	SEE	DMRT indices
T_1	Linear	y = 44.06x - 183.7	0.973	5.30	0.231	a
T_2	Linear	y = 44.73x - 190.5	0.981	5.37	0.192	a
T_3	Linear	y = 45x - 193.5	0.979	5.41	0.203	a
T_4	Linear	y = 45.80x - 195.7	0.983	5.36	0.182	a
T_5	Linear	y = 39.10x - 157.9	0.999	5.32	0.014	b
T_6	Linear	y = 37.42x - 155.4	0.988	5.49	0.151	ab
T_7	Linear	y = 37.98x - 162.4	0.998	5.59	0.046	a
7_8	Linear	y = 41.17x - 174.3	0.998	5.45	0.046	ab
T_9	Linear	y = 39.42x - 168.3	0.968	5.54	0.251	a
T_{10}	Linear	y = 40.87x - 186.3	0.972	5.78	0.236	a
T_{11}	Linear	y = 43.89x - 205.4	0.996	5.82	0.083	a
T_{12}	Linear	y = 43.25x - 198.5	0.988	5.75	0.149	a

T_1, T_2, T_3, T_4 are 0%, 0.1%, 0.2% and 0.5% chitosan treatment respectively under 5% moisture level; T_5, T_6, T_7, T_8 are 0%, 0.1%, 0.2% and 0.5% chitosan treatment respectively under 10% moisture level; T_9, T_{10}, T_{11}, T_{12} are 0%, 0.1%, 0.2% and 0.5% chitosan treatment respectively under 20% moisture level; T_{50} = Phytotoxicity threshold level; SEE = Standard Error of Estimates

Maximum threshold level of root phytotoxicity was observed at 20% moisture under all treatments with chitosan in comparison with low moisture level. In the present study, 0.2% chitosan treatment showed highest threshold level of root phytotoxicity.

Discussion

Present study has investigated the impact of matrix assisted chitosan priming on salinity stress at three different moisture levels. This study showed that chitosan had greatly affected mung bean germination revealing that germination parameters at different salinity levels were significantly different in primed seeds from untreated seeds. Kaya et al. (2003) also reported similar results. Hopper et al. (1979) investigated that metabolic activities in primed seeds during germination process initiate much earlier than radicle coming out and thus, had better effectiveness for water intake from growing media. NaCl creates an osmotic potential outside the seeds and prevents water absorption. Sometimes other toxic ions like chlorides are produced (Grieve and Fujiyama, 1987). As a result, germination is delayed or inhibited (Khajeh-Hosseini et al., 2003; Tobe et al., 2004). Almansouri et al. (2001) also found similar findings of delaying and retarding plant growth due to osmotic and saline stresses. Murillo-Amador et al. (2002) opined similarly that under NaCl stress, absorption of water by seeds was reduced due to too many toxic ions. But chitosan priming can nullify such adverse effects of salinity and stimulate better growth than control under salinity stress in wheat (Ma et al., 2011). Present investigation revealed that 0.1% chitosan in low moisture (5%) and 0.2% chitosan treatment at high moisture level (10% and 20%) were found to be most effective in mitigating the adverse effect of salinity and they become tolerant to salinity up to certain level. This result is in agreement with the observations by Mahdavi and Rahimi (2013) in ajowan under salt stress. Maximum CV were obtained in low salt stress and reduced in higher salinity. Okcu et al. (2005) reported similar results in pea under drought & salt stress. Çiçek and Çakirlar (2002) confirmed that seedling growth of maize was reduced under salt stress. This study showed that under various salt stresses chitosan priming considerably enhanced mung bean seedling growth. Likewise, Katembe et al. (1998) reported that under salinity stress seedling growth could be invigorated by seed priming in two species of *Atriplex*. Present data in mung bean were in agreement with the previous findings of Stofella et al. (1992) where radical length was considerably enhanced by seed priming in pepper. The decreasing tendency of SG due to increasing salinity was in the conformity with the reports of others (Mohammad et al., 1989). The reduction of speed of germination at high salt levels might be mainly due to osmotic stress (Heenan et al., 1988). Having diverse potential applications, SMP not only increases the germination performance of seeds, but also provide a delivery system for selective fungicides and bio-control organisms to control various soil-borne pathogens (Harman and Taylor, 1988). Moreover, for improving seed emergence SMP could be used as a pre-sowing seed treatment when sown in cold soils especially for the plants sensitive to cool temperature (Marsh, 1993). Priming could allow greater membrane integrity in the embryo as a result of which the developing seedlings reduce ion leakage through the membranes. Mereddy et al. (2000) established improved germination performance by SMP in okra. Several types of materials can be used for SMP but most optimal conditions for moisture content and priming time for each matrix must be determined. In general, extended priming periods are responsible for declined germination rates and lower vigor. It was also confirmed from the present study that 5% and 10% moisture levels were optimal for SMP of mung bean seeds in comparison with 20% moisture level, although in soybean more than 5% moisture was found to be detrimental (Mercado and Fernandez, 2002).

Earlier it was confirmed that legume plants including mung bean had a very high phytotoxicity (low tolerance to salinity) than that of cereals, technical and fodder plants (Lixandru et al., 2007). Rye was considered as the most tolerant field crop to soil salinity having sensitivity threshold level at 8 $dS*m^{-1}$ whereas bean as the most sensitive having sensitivity threshold level at 1$dS*m^{-1}$ (Sandu et al., 1986). It was also confirmed that the most sensitive plant to salinity is bean while maize was comparatively less sensitive, on the other hand, oats and Sudan grass were moderately tolerant (Rhoades and Loveday, 1990). Present investigation revealed that the problem of phytotoxicity against salt stress can be managed up to certain level by chitosan treatment. Chitosan can remarkably alleviate the detrimental effect of salinity up to the level of 6 $dS*m^{-1}$. However, its effect was not significant in 8 and 12 $dS*m^{-1}$ salinity level.

Conclusion

It can be concluded that chitosan priming improves germination and growth of many crops. In this study reduction in germination attributes and seedling growth of mung bean under salinity stress was observed more prominently in control seeds than primed seeds. 0.1% Chitosan under 5% and 0.2% chitosan under 10% and 20% moisture levels were proved to be ideal elicitor for seedling invigoration to speed up germination and synchronize emergence of radical. Present investigation revealed that chitosan priming could be proper seedling invigoration treatment under saline environments particularly for the early growth phase of mung bean seedlings. However, more specific studies are required to highlight the effects of Chitosan priming regarding growth, development and yield of mung bean in field conditions.

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