International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(5): 99-103

Received: 10-07-2017
Accepted: 11-08-2017

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Effect of different hydro and osmopriming materials on germination and seedling growth of mung bean

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Abstract
A laboratory experiment was carried out to study the effect of different seed priming chemicals (IBA, GA_3, H_3BO_3 and PEG-6000) at different concentrations for improving induced stress tolerance potential regarding germination and seedling growth of mungbean. The experiment was laid out in Completely Randomised Design (CRD) replicated thrice. Application of IBA (10 ppm), GA_3 (10 ppm) and H_3BO_3 (0.05%) in mungbean resulted as increase in germination percentage to the tune of 5.39%, 3.53% and 4.56%, respectively as compared to control as well as other priming treatments. GA_3 at 5 ppm concentration resulted in highest shoot length, however, regarding root length IBA at 100 ppm resulted best. Application of GA_3 at all concentrations had higher vigour index. Among all the seed priming treatments PEG-6000 had no additional benefit on seed germination of mungbean though it had pronounced effect on root growth.

Keywords: Hydro priming, Mungbean, Osmo priming, Seed germination

Introduction
Green gram (*Vigna radiata* (L.) Wilczek) is a tropical legume which is an important dietary pulse crop of India enriched with lysine and tryptophan. It is an important pulse crop of India with a wide soil and climatic adaptability. Being a short duration crop green gram is commonly cultivated as a rotation crop/intercrop in India. Efforts were made to maximize yield, is largely hampered by adverse effect of a biotic stress such as drought especially during *pre-kharif* season which results into a lower crop yield due to poor germination and failure of the crop to establish in some cases. Various abiotic stresses, especially drought and salinity are major limitations to its production. Salt stress alone was found to cause reduction in germination percentage, shoot and root lengths, fresh weight and seedling vigour in green gram (Misra *et al*. 1996; Promila and Kumar 2000; Misra and Dwivedi 2004) [22, 25, 23]. Better germination and vigour of the seedlings are the main basic foundations for the success of good stand establishment of any crop plant. Seed priming is a technique in which seeds are soaked in solutions of low water potential that initiates pre-germinative metabolic activity but prevents radical protrusion. The beneficial effects of seed priming are attributed to early germination; improving germination rate, breaking of dormancy, improving seedling vigour, strengthening stand establishment and increasing yield. Besides all these beneficial effects, seed priming also reduce leakage of metabolites, repairs deteriorated seed parts, improve RNA and protein synthesis. Seed priming has been developed as an indispensable method to produce tolerant plants against various stresses. It is a pre-germination treatment method which improves seed performance and provides faster and synchronized seed germination. In seed priming, the seed is either soaked in water (hydropriming), solutions of Polyethylene Glycol (PEG) (osmopriming), salt ($CaCl_2$, $CaSO_4$, NaCl etc.) or some definite chemicals prior to germination (Patade *et al.* 2009) [24]. Seed priming with optimal concentrations of plant growth hormones, such as auxin (IAA), gibberellins (GA), abscisic acid, and ethylene, has proven that germination performance as well as growth and yield of many crop species under both normal and stress conditions could be improved effectively (Hurly *et al.* 1991, Anosheh *et al.*, 2014) [16, 2]. Priming is believed to bring about some biochemical changes within the seeds, which ultimately favours germination and further growth stages. According to Gurusinge *et al.* (1999) [13], priming enhances the early events of germination. Even though some metabolic changes take place within the seed during priming, are not enough to induce

radical protrusion (McDonald, 2000) [21]. Due to the effect of some of these biochemical changes likely to take place prior to seed germination, plants raised from primed seeds showed various advantages over the non-primed ones, such as sturdy and quick cellular defense response against abiotic stresses. The seedlings emerging from primed seeds also showed early and uniform germination. Moreover, the overall growth of plants was enhanced due to the effect of various seed priming treatments. The priming effects depend on the nature of priming agent, priming duration, concentration of priming agent and the plant (Jeong *et al.* 2000) [18]. Priming for enhanced resistance to abiotic stress is operating via various pathways involved in different metabolic processes (Jisha *et al.* 2013) [19]. Considering all the facts the current study is planned to assess the effects of different seed priming materials on the germination and seedling growth of green gram crop under different hydro and osmopriming conditions.

Materials and Methods

Germination of seeds

The present experiment was conducted at research laboratory of College of Agriculture (Extended Campus), Uttar Banga Krishi Viswavidyalaya, Majhian, Dakshin Dinajpur, West Bengal, India during March, 2017. Healthy and uniform seeds of mungbean var. PDM 11 were selected and sterilized with 0.1% Mercuric Chloride solution for 1 min then rinsed with sterilized distilled water. Selection of suitable priming compounds was made on the basis of findings of different research workers (Das and Choudhury, 1996; Grandi *et al*. 1999; Harris *et al*. 2001; Basra *et al.* 2002; Farooq *et al*. 2006; Kaur *et al.* 2006) [9, 11, 6, 10, 20]. The treatments used were: (a) Control (untreated water soaked seeds), (b) three (3) concentrations of Indole Butyric Acid (IBA) i.e., 5 ppm, 10 ppm and 15 ppm; (c) three (3) concentrations of Boric Acid (H_3BO_3) i.e., 0.05%, 0.5% and 1%; (d) three (3) concentrations of Gibberellic Acid (GA_3) i.e., 5 ppm, 10 ppm and 15 ppm and (e) three (3) concentrations of Polyethylene glycol (PEG-6000) i.e., 5%, 10% and 25%. The mungbean seeds were primed in aerated solutions for 24 hrs at 25 ± 2°C. The primed seeds were set to germinate in an incubator at 27°C. The effect of the treatments on different parameters of seedling growth such as germination %, shoot length, root length, fresh shoot weight, fresh root weight, dry shoot weight, dry root weight and vigour index were recorded from 7 days old seedlings.

Germination test and seedling growth

Germination potential of mung bean seeds was estimated in accordance with the AOSA method (AOSA, 1990) [3]. In an incubator, three replicates of 25 seeds each were sown in 12 cm diameter petri dishes, between the layers of moist Whatman-42 filter papers at 27 °C. The Petri dishes were arranged in a complete randomized design (CRD) with three replicates. Starting on the first day of imbibition, counts of germinating seeds were made at 12 hour intervals as far maximum germination was attained.

The Seedling Vigour Index (SVI) was calculated according to following formula (Abdul Baki and Anderson, 1973) [1]:

SVI = (Average Root length (cm) + Average Shoot length)*Germination Percentage

The shoot and root length of seedlings was also recorded.

The Relative Seed Germination rate (RSG) and Relative Root Growth (RRG) and Germination Index (GI) were calculated using the equations (Jayarambabu *et al*, 2014) [17]:

Relative Seed Germination Rate = $(S_c / S_s) \times 100$

Relative Root Growth= $(R_s / R_c) \times 100$

Germination Index = (RSG / RRG) ×100

Where S_s is the number of seeds germinated in sample, S_c is the number seeds germinated in control

Statistical Analysis

The experiment was arranged according to completely randomized design with three replicates, each treatment having 25 seeds. Data recorded were analyzed statistically using Fisher's analysis of variance technique and Duncan's Multiple Range Test at 5% probability level to compare the differences among treatment means (Steel *et al.* 1997) [27].

Results and Discussion

The experimental result revealed significant differences for all characters among thirteen (13) treatments in mungbean. Different seed priming treatments affected significantly the germination and seedling growth of mungbean over control (Table 1). There was significant effect of seed priming treatments on germination percentage (Figure 1). Enhanced germination percentage over control was highlighted for almost all the treatments except PEG 6000 (all concentration) and H_3BO_3 at 1% where it was reduced to 8.53% (5% PEG) and 6.40%, respectively, from that of control. Highest germination to the extent of 95.88% was recorded in the treatment with 10 ppm of IBA followed by 0.05% of H_3BO_3 (95.12%) and 10 ppm of GA_3 (94.19%), though these treatments are statistically at par. Germination percent improved over control in all concentration of GA_3 suspension save at 15 ppm GA_3 concentration and it was reduced progressively as concentration increased for PEG-6000 suspension. In contrast, H_3BO_3 showed decreasing trend with increase in concentration. The increment in germination percentage was observed to the tune of 5.39%, 4.56% and 3.53% in IBA (10 ppm), H_3BO_3 (0.05%) and GA_3 (10 ppm), respectively, as compare to control. Seed germination could be considered as index of plant growth, development and yield and as a beginning of physiological process seed germination required adequate water absorption.

Experimental results revealed the fact that different seed priming treatments also has an effect on the germination index (GI) (Figure 4). Data on GI revealed that at 10 and 15 ppm concentration of GA_3 the highest values were obtained whereas, the treatment T_{12} (25% PEG) recorded the least GI.

The data regarding length of plumule and radicle has been presented in the Table 1 and Figure 2. Highest promotional effect on shoot length was depicted by the treatment 5 ppm of GA_3 (18.16 cm) which was statistically at par with other two concentrations of GA_3. The treatments T_{12} (25% PEG), T_1 (5 ppm IBA),T_{11} (15% PEG) and T_4 (0.05% H_3BO_3) showed more shoot length than the control (T_{13}) whereas, 15 ppm concentration of IBA, 0.5% H_3BO_3, 0.5% H_3BO_3, and 5% concentration of PEG recorded lower shoot length than the control (Fig. 2). Most favourable effect on the character root length was highlighted by respective treatments of 10 ppm of IBA followed by 15 ppm IBA, 25% of PEG and 10 ppm of GA_3. Root elongation over control following treatments with 10 ppm and 15 ppm of IBA was noticed but it was less with lowest concentration of IBA (5 ppm of IBA). The data also depicts that the lower level of priming concentration is more beneficial than higher ones. Similar to the data on shoot length the maximum was recorded with T_{12} (25% PEG) which was 23.7% higher than control (T_{13}), whereas, the least fresh weight of shoot was associated with T_6 (1% H_3BO_3) which was 50.4% lower than the control.

Contrast to shoot fresh weight the treatments receiving different concentrations of IBA gave higher fresh root weight with 10 ppm IBA treatment was highest (0.145 g). All the H_3BO_3 treatments performed adversely as compared to control with least fresh root weight with highest concentration of H_3BO_3 (T_6). Except 25% PEG treatment all other treatments receiving 5% and 15% PEG performed negatively as compared to the control (Table 1 and Fig. 3).

The data on shoot and root dry weight revealed the fact that there was no significant difference between the treatments.

Highest Seed Vigour Index was noticed in 5 ppm of GA_3 (2386.88) followed by 10 ppm and 15 ppm GA_3; however, there was no significant difference between the treatments with three different concentrations of GA_3. The treatment T_6 (1% H_3BO_3) performed the worst as far as Seed Vigour Index was concerned (Table 1 and Fig. 4).

Seed vigor is an important quality parameter which needs to be assessed to supplement germination and viability tests to gain insight into the performance of a seed lot in the field or in laboratory. It is the sum total of those properties of the seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence. The seed lot showing the higher seed vigor index is considered to be more vigorous (Abdul-Baki and Anderson, 1973) [1].

The study revealed that seed priming and presoaking techniques using different seed priming materials can significantly improve mungbean plant performance by increasing seed germination and seedling vigour although response varied with different solutions and concentrations. Seed priming with different concentrations of solutions significantly improved germination performance, but no remarkable differences were observed for seedling growth. Generally, seed germination entails three distinct phases: (i) imbibition, (ii) lag phase, and (iii) radicle growth and emergence (Bradford *et al*., 1990) [7]. Earlier and more uniform germination and emergence was observed in primed seeds as indicated by IBA, H_3BO_3 and GA_3. Treated seeds had high germination percentages and quicker germination. One hypothesis is that benefits of priming can be due to metabolic repairment of damage during treatment and that change in germination events i.e., changes in enzyme concentration and formation and reduces lag time between imbibition and radicle emergence (Bradford *et al*. 1990) [7]. Better genetic repairment, i.e. earlier and faster synthesis of DNA, RNA and proteins are also some of the basis for enhanced growth (Bray *et al*. 1989) [8]. The differences observed in the germination percentage of seed subjected to different hormonal treatments at different concentrations shows significant impacts of the various pre-treatments on seed germination. When compared with different priming reagents, GA_3 of all concentrations showed greater influence on germination and participated in regulation of many growth and developmental processes in plants which was confirmatory to the present report (Hedden and Phillips, 2000; Sevik and Guney, 2013) [15, 26] and was particularly important in regulating stem elongation (Zhang *et al.* 2007) [28]. GA treated seed was closely associated with their rapid utilization in the synthesis of various amino acids and amides (Gupta and Mukherjee, 1982) [12], which could be the reason for the increased germination rate. The application of PEG-6000 reduced the seedling vigour as compared to other seed priming treatments. The reduction in the seedling vigour has also reported by early research work (Basra *et al*. 2003) [4]. Priming reagents (especially for PEG priming) had the beneficial effects on shoot and root biomass. This was mainly due to the accelerated metabolism occurring in primed seeds, which increases the imbibition speed as compared to unprimed seeds. Reduction in germination and seedling vigour in osmopriming treatments might be the result of toxicity (stress) of the solutes used, as earlier found in KNO_3 osmopriming in rice (Basra *et al.* 2003, 2005) [4, 5].

Table1: Effect of Different priming materials for seed germination and seedling growth of mungbean

Treatments	% germination	Shoot length	Root length	Fresh shoot wt/ seedling	Fresh root wt/ seedling	Shoot Dry wt/ seedling	Root Dry wt/ seedling	Seedling Vigor index
T_1: *IBA (5 ppm)*	89.42^{ef}	12.20^{c}	6.60^{de}	0.260^{abc}	0.126^{abc}	0.016^{a}	0.011 ^{a}	1681.10^{d}
T_2: *IBA (10 ppm)*	95.88^{a}	10.50^{cde}	10.75^{a}	0.202^{cde}	0.145^{cde}	0.018 ^{a}	0.013 ^{a}	2037.45^{b}
T_3: *IBA (15 ppm)*	92.31b^{cd}	9.10^{e}	9.10^{b}	0.222^{bcd}	0.134^{bcd}	0.017 ^{a}	0.012 ^{a}	1680.04^{d}
T_4: *H_3BO_3 (0.05%)*	95.12^{a}	10.60^{cde}	7.60^{bcd}	0.210^{cde}	0.090^{cde}	0.014 ^{a}	0.018 ^{a}	1731.18^{cd}
T_5: *H_3BO_3 (0.5%)*	91.28^{cde}	8.80^{e}	5.80^{ef}	0.160^{de}	0.080^{de}	0.013 ^{a}	0.016 ^{a}	1332.69^{ef}
T_6: *H_3BO_3 (1.0%)*	83.21^{h}	6.40^{f}	4.12^{g}	0.140^{e}	0.070^{e}	0.012 ^{a}	0.015 ^{a}	875.37^{g}
T_7: *GA_3 (5 ppm)*	93.75^{ab}	18.16^{a}	7.30^{cde}	0.278^{abc}	0.120^{abc}	0.022 ^{a}	0.019 ^{a}	2386.88^{a}
T_8: *GA_3 (10 ppm)*	94.19^{ab}	17.20^{a}	7.90^{bcd}	0.278^{abc}	0.110^{abc}	0.019 ^{a}	0.015 ^{a}	2364.17^{a}
T_9: *GA_3 (15 ppm)*	90.21^{de}	17.75^{a}	7.75^{bcd}	0.295^{ab}	0.113^{ab}	0.023 ^{a}	0.020 ^{a}	2300.36^{a}
T_{10}: *PEG (5%)*	87.23^{fg}	9.33^{de}	4.66^{fg}	0.173^{de}	0.080^{de}	0.017 ^{a}	0.011 ^{a}	1220.35^{f}
T_{11}: *PEG (15%)*	86.45^{g}	11.25^{cd}	6.18^{def}	0.201^{cde}	0.090^{cde}	0.021 ^{a}	0.014 ^{a}	1506.82^{de}
T_{12}: *PEG (25%)*	85.14^{gh}	14.50^{b}	8.50^{bc}	0.340^{a}	0.121^{a}	0.023 ^{a}	0.018 ^{a}	1958.22^{bc}
T_{13}: *Control (Water)*	90.97^{de}	10.50^{cde}	7.70^{bcd}	0.276^{abc}	0.115^{abc}	0.015 ^{a}	0.009 ^{a}	1655.65^{d}
CD (P=0.05)	2.30	1.93	1.56	0.07	0.04	NS	NS	245.27

NS- Non Significant at P = 0.05

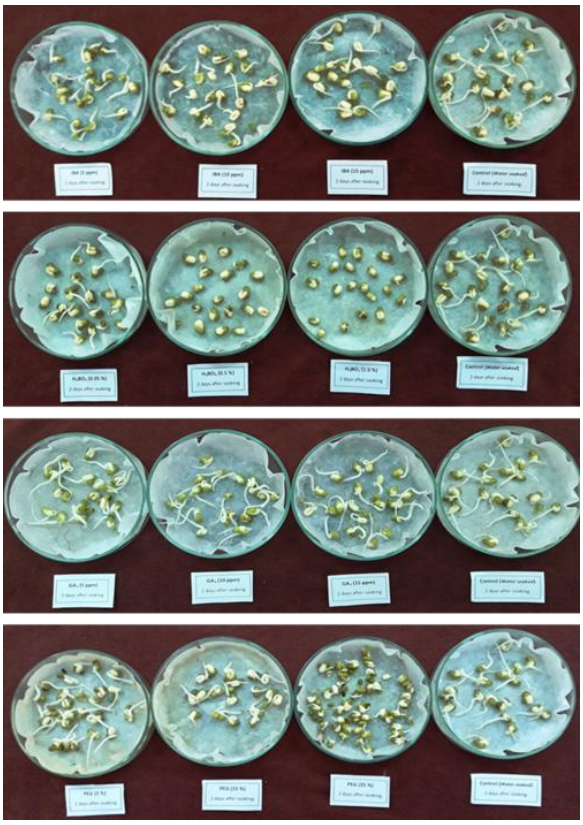

Plate 1: Germinated mungbean seeds (2 days after the seed priming)

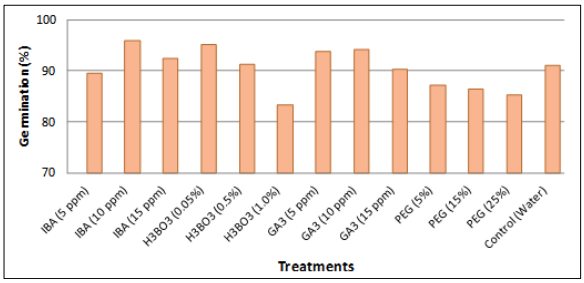

Fig 1: Effect of treatments on Germination percentage of mungbean seeds

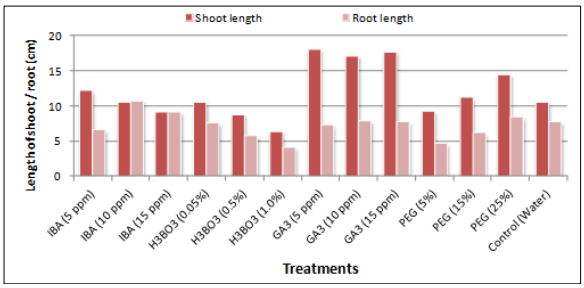

Fig 2: Effect of tretments on shoot and Root length of mungbean

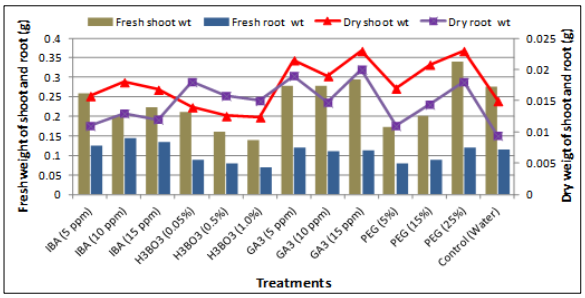

Fig 3: Effect of treatments on fresh dry weight of shoot and roots of mungbean

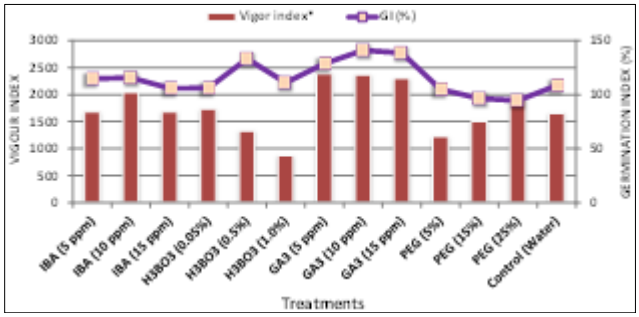

Fig 4: Effect of treatments on vigor index and Germination index of mungbean seeds

Conclusion

Results of the present study suggest that seed priming is very effective tool for inducing seed vigour in mung bean. The application of IBA (10 ppm), GA_3 (10 ppm) and H_3BO_3 (0.05%) as seed priming increased the germination percentage and seedling vigour substantially as compared to other priming treatments. There is further need to investigate these priming treatments under field conditions in relation to crop production. Application of IBA (10 ppm), H_3BO_3 (0.05%) and GA_3 (10 ppm) in mungbean resulted in increase of germination percentage upto 5.40%, 4.56% and 3.54% respectively, over control. Increases of upto 16.19% (5 ppm of IBA), 72.95% (5 ppm of GA_3) and 38.09% (25% of PEG) in shoot length and 39.61% (10 ppm of IBA), 2.59% (10 ppm of GA_3) and 10.38% (25% of PEG) in root length were evidenced. Upswing in shoot fresh weight upto 23.18% (25% of PEG) and 6.88% (15 ppm of GA_3) and in fresh root weight upto 26.08% (10 ppm of IBA), 4.34% (10 ppm of GA_3) and 5.21% (25% of PEG) led to an enhancement up to 35.55% (5 ppm of IBA), 13.91% (1% of H_3BO_3), 82.42% (5 ppm of GA_3) and 46.82% (5% of PEG) in vigor index over control. The results indicated that the final germination percentage and seedling growth varied with different reagents significantly. Our results confirmed that seed pretreatment is an effective technique to improve the germination percentage, seedling growth and seed vigour.

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