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Evaluation of different seed priming techniques in mung bean (*Vigna radiata*)

A. Umair[1], S. Ali[1], K. Bashir[1] and S. Hussain[2]
[1]PMAS Arid Agriculture University, Rawalpindi
[2]Pesticide Control Lab, AARI, Faisalabad

Abstract

Seed priming is an effective and shortgun approach to improve the emergence and stand establishment of field crops. Impact of priming on the germination and seedling vigour of mung bean (CM-97) were tested under laboratory conditions. The seeds were soaked in water (hydropriming), solutions of KH_2PO_4, Manitol, Polyethylene glycol (PEG_{6000}), $Na_2MO_4.2H_2O$ (osmocoditioning) and salicylic acid (SA) (hormonal priming).having two levels for each chemical (treatment). After giving all the priming treatments by soaking seeds for five hour, seeds were dried and sown in petri dishes in growth chamber at 27 ± 2 ^oC for seven days. All the treatments significantly improved the germination percentage, root and shoot length, seedling dry weight and seedling vigour index (SVI) while time to fifty percent seeds to germinate (T_{50}) and mean germination time (MGT) decreased, significantly. Osmopriming using P at 0.60% applied in the form of KH_2PO_4 significantly improved seed vigour and final germination percentage. The use of polyethylene glycol was expensive and gave similar results as for control (dry seeded). It is concluded that seed priming (osmo-priming and hormonal-priming) can be used as effective tool for invigouration of mung bean seeds, for vigour enhancement.

Key words: Osmo-priming, hydropriming, *vigna radiate* L., seedling vigour, seedling vigour index

Introduction

Mungbean is an important source of protein in South and South East Asia where the diet is mostly cereal based. It is cultivated both as Rabi as well as Kharif season crop throughout Pakistan. Mung bean production in Punjab Province is dependent mainly on surface irrigation but it is also grown under rainfed conditions. In the Southern region of Pakistan, rainfall is scanty and mung bean is grown only with surface irrigation. Poor crop establishment is a major constraint for mung bean production (Naseem *et. al*., 1997; Rahmianna *et al*., 2000) and high yields can be associated with early vigour (Kumar *et al.*, 1989).

Seed priming is a practice by which seeds are partially hydrated to a point where germination processes begin but radical emergence does not occur. Seed priming can be found effective for legumes *i.e*., yields of mung bean and chickpea were increased substantially by priming (hydropriming) seeds for 8 h before sowing mostly under irrigated conditions (Harris *et al.,* 1999; Musa *et al.,* 2001; Rashid *et al.*, 2004).

Improved seed invigouration treatments are being used to reduce the germination time, to get synchronized germination, improve germination rate, and improve seedling stand in many horticultural (Bradford *et al*. 1990; Rudrapal and Nakamura 1998) and field crops like wheat, maize (Dell' Aquilla and Tritto, 1991; Basra *et al.,* 2002) and more recently rice (Farooq *et al*. 2004). These invigouration treatments include hydropriming, osmoconditioning (Basra *et al*. 2005), osmohardening (Farooq *et al*. 2006) and hardening (Farooq *et al*. 2004). These treatments can also be employed for earlier and better nursery stand establishment (Lee *et al*., 1998).

Most of the seed priming treatments have been employed on mung bean cultivars of NM-92 and NM-98. There are some other cultivars of mung bean which are being cultivated on Barani land. One of the cultivar is CM-97 which is small seeded mungbean variety. There is not any study to evaluate the response of CM-97 to different seed priming treatments.

This study was initiated to explore the effects of various priming strategies including osmopriming and hormonal priming on mung bean (CM-97) seed germinability and vigour under laboratory conditions.

Materials and methods

Seed materials

Seeds of mung bean cultivar CM-97 were obtained from Barani Agricultural Research Institute (BARI), Chakwal. The seeds were sterilized by using 30% sodium

*Email: adnanumair@gmail.com

hypochlorite for five minutes and then washed three times with sterilized 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) and previous observations (unpublished data). The treatments used were: T1 = Control (untreated dry seeds), T2 = Hydropriming, T3 = Molybdenum at 0.02% (applied as $Na_2MoO_4.2H_2O$), T4 = Molybdenum at 0.04%, T5 = Phosphorous (P) at 0.60% (KH_2PO_4 salt was used as P source), T6 = Phosphorous at 1.24%, T7 = Salicylic acid at 10 mg L^{-1}, T8 = Salicylic acid at 20 mg L^{-1}, T9 = Manitol at 2%, T10 = Manitol at 4%, T11 = Polyethylene glycol (PEG_{6000}) at 5%, T12 = Polyethylene glycol (PEG_{6000}) at 10%.

Seeds were primed in aerated solutions (1:5 w/v) for 5 h at 25 ± 2 ^oC. The primed seeds were set to germinate in an incubator at 27 ^oC. After soaking, seeds were given three surface washings with distilled water (Khan, 1992) and re-dried under shade, near to original weight with forced air. The seeds were then sealed in polythene bags and stored in refrigerator till further use (Basra *et al.,* 2002).

Germination test

The experiment was conducted at research laboratory of PMAS Arid Agriculture University, Rawalpindi in 2007. Germination potential of mung bean seeds was estimated in accordance with the AOSA method (AOSA, 1990). In an incubator, three replicates of 25 seeds each, were sown in 12 cm diameter petri dishes, between the layers of moist Whatman-45 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 imbibitions, counts of germinating seeds were made at 12 hour intervals as far maximum germination was attained. The time to reach 50% germination (T_{50}) of final germination was calculated according to the following formula (Coolbear *et al.,* 1984) and modified by Farooq *et al.* (2005):

$$T_{50} = ti + (N/2 - ni) (tj - ti) / n_j - n_i$$

Where N is the final number of germination and ni, nj cumulative number of seeds germinated by adjacent counts at times ti and tj when ni < N/2 < nj. Mean emergence time (MGT) was calculated according to the equation of Ellis and Roberts (1981) as under:

$$MGT = \sum \mathbf{Dn} / \sum \mathbf{n}$$

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.

The seedling vigour index was calculated according to following formula (Islam *et al*., 2009)

SVI = (seedling length (cm) × germination percent)/100

The seedlings shoot and root length was also recorded. Germination index (GI) was calculated as described in the Association of Official Seed Analysis (1990) as the following formulae:

$$GI = \frac{\text{No. of germinated seeds at first count}}{\text{Days of first count}} + \ldots + \frac{\text{No. of germinated seeds at final count}}{\text{Days of final count}}$$

Statistical Analysis

The experiment was arranged according to completely randomized design with three replicates, each replicate 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).

Results

Different seed priming treatments affected significantly the germination vigour of mungbean in terms of time required for fifty percent of seeds to germinate (T_{50}) (Figure 1) and mean germination time (MGT) (Figure 2). Significant reduction in T_{50} and MGT observed in all the seed priming treatments as compare to control. Maximum reduction for T_{50} and MGT was observed in phosphorous application (P at 0.6%) as compared to all other treatments as well as control. The data also describes that the lower level of priming solution concentrations were more suitable than the higher ones.

There was significant effect of seed priming treatments on germination percentage (Figure 3). Maximum (95.33%) germination was observed in T5 (P at 0.6%). There was an increase of 19 percent has been observed in T5 as compare to control. The germination percentage decreased in T4, T7, T9 up to T12. There was also significant effect of different seed priming treatments on the germination index (GI) (Figure 4). The maximum GI was observed in T5 which was 53% higher than control followed by T4 and T2, respectively. All the priming treatments improved the GI compare to control.

The data regarding length of plumule and radicle has been presented in the Figure 5 and 6. Maximum shoot length was achieved in T3 (Mo at 0.02%) which was 18% higher as compared to control. The data also depicts that the lower level of priming concentration is more beneficial than higher ones. The minimum root and shoot length was in control where non-primed seeds were used. There was similar trend observed for root length of mung bean as affected by different seed priming treatments. The highest root length was observed in T5 followed by T3 and T6. All the priming treatments improved shoot as well as root length of mung bean seedlings.

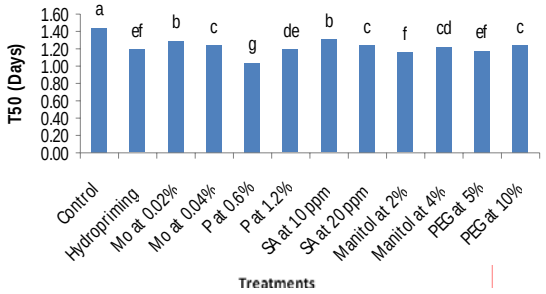

Figure 1: Impact of different seed priming treatments on time to fifty percent seeds to germinate of mung bean

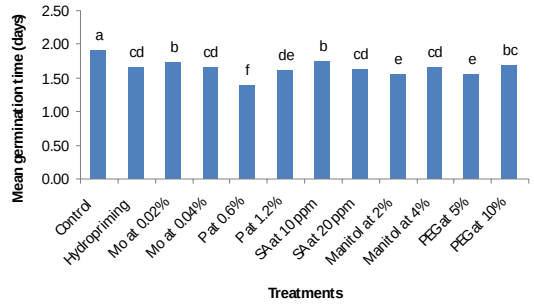

Figure 2: Impact of different seed priming treatments on mung bean mean germination time (MGT) under controlled conditions

Mo = Molybdenum: P = Phosphorous: SA = Salicylic acid and PEG = Polyethylene glycol, $p < 0.05$; Means with the same letter(s) are not significantly different according to DMRT

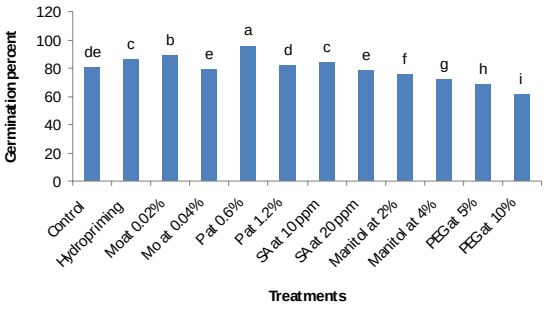

Figure 3: Impact of different seed priming treatments on germination in mung bean

The data also depicts that there was significant effect of different seed priming treatments on seedling vigour index (SVI) (Figure 7). The maximum SVI was observed in T5 (60 % increase over control) followed by T4 and T2 respectively. All the priming treatments improved the SVI as compare to control except T10 and T12. The SVI decreased in T10 and T12 by 10 % compared to control. Other priming treatments showed similar response for vigour enhancement.

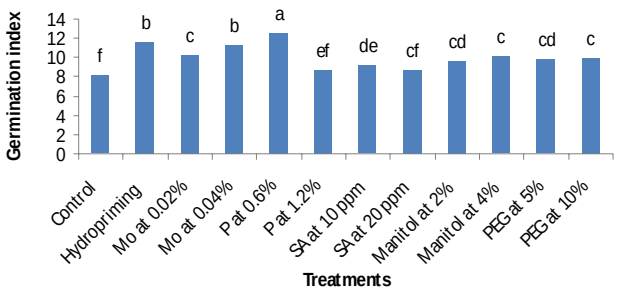

Figure 4: Impact of different seed priming treatments on mung bean mean germination index

Mo = Molybdenum: P = Phosphorous: SA = Salicylic acid and PEG = Polyethylene glycol, $p < 0.05$; Means with the same letter (s) are not significantly different according to DMRT

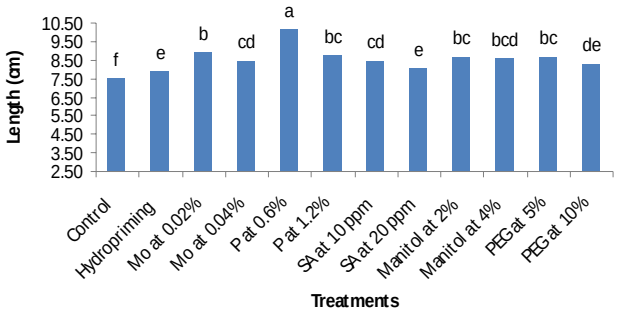

Figure 5: Impact of different seed priming treatments on shoot length of mung bean

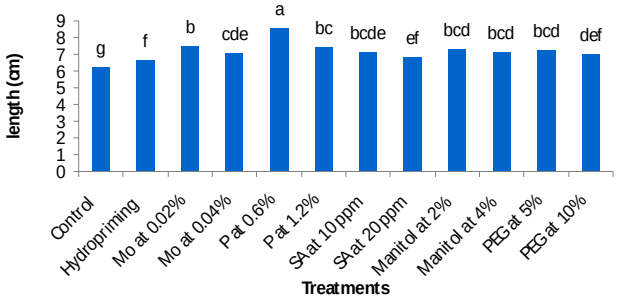

Figure 6: Impact of different seed priming treatments on root length of mung bean

Mo = Molybdenum: P = Phosphorous: SA = Salicylic acid and PEG = Polyethylene glycol, $p < 0.05$; Means with the same letter (s) are not significantly different according to DMRT

The analysis of variance showed that there was significant impact of different seed priming treatments on dry weight of mung bean seedlings under control conditions as shown (Figure 8 and 9). The data describes that all the priming treatments increased the dry matter yield of shoot as well as root as compare to control. The highest dry matter of shoot and root was observed in T5 (P at

0.6%) followed by T8 and T10. The lower levels of treatments gave better results as compared to higher ones.

Discussion

Earlier and more uniform germination and emergence was observed in primed seeds as indicated by lower MET and T_{50} and higher GI, root and shoot length. Lesser MET and T50 indicate the earlier and rapid germination. These results are in line with the findings of different research workers (Ozbingol *et al*., 1999; Khan *et al*., 2009) who reported priming tomato seeds with PEG_{8000} (-1.0 M Pa) significantly reduced T_{50}. Reduction in T_{50} may be attributed to early reserve breakdown and early reserve mobilization. It might also be due to possible early activation or *de novo* synthesis of cell wall degrading enzymes (Hisashi and Maciaa, 2005). However Nascimento and West (1999) reported early germination of primed seeds but not recorded any improvement in the growth of seedlings in muskmelon seeds under laboratory conditions.

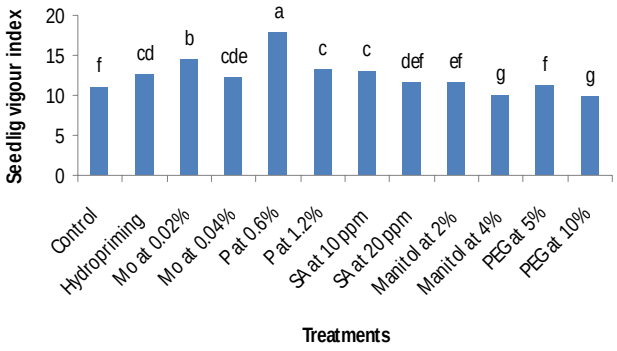

Figure 7: Impact of different seed priming treatments on seedlings vigour index of mung bean

Mo = Molybdenum: P = Phosphorous: SA = Salicylic acid and PEG = Polyethylene glycol, p<0.05; Means with the same letter (s) are not significantly different according to DMRT

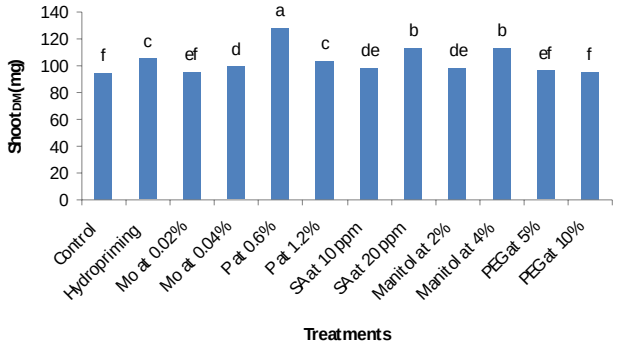

Figure 8: Impact of different seed priming treatments on shoot dry matter of mung bean seedlings

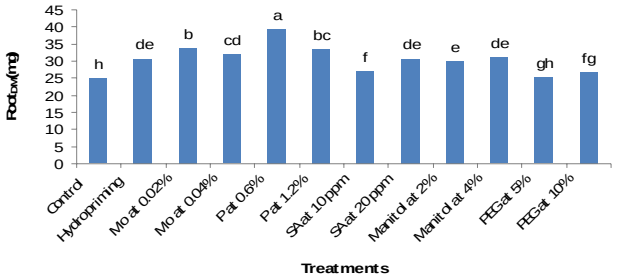

Figure 9: Impact of different seed priming treatments on root dry matter of mungbean seedlings

Mo = Molybdenum: P = Phosphorous: SA = Salicylic acid and PEG = Polyethylene glycol, p < 0.05; Means with the same letter (s) are not significantly different according to DMRT

Treated seeds had high germination percentages and quicker germination time. One hypothesis is that benefits of priming can be due to metabolic repair 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). Better genetic repair, i.e. earlier and faster synthesis of DNA, RNA and proteins are also some of the basis for enhanced growth (Bray *et al*., 1989). Gray and Steckel (1983) also concluded that priming increased embryo length, which resulted in early initiation of germination in carrot seeds.

The application of P in the form of seed treatment resulted in increased seedling vigour. These results are in line with the findings of Grandi *et al.* (1999) who also found that P enrichment by soaking seeds in 200 mM KH_2PO_4 solution improved the seedlings establishment. The increased vigour of P-enriched seed might be due to increased P content both inside the seeds and on the seed surfaces which leads to better establishment of seedlings (Bolland and Baker 1988; Zhang *et al*. 1990; Thomson and Bolger 1993; Ros *et al*. 1997). Similarly, the increase in seedling vigour due to salicylic acid may be due to enhanced oxygen uptake and the efficiency of mobilizing nutrients from the cotyledons to the embryonic axis (Karthiresan *et al*., 1984) and decreased catalase and peroxidase levels as recorded in pea seedlings (Srivastava and Dwivedi, 1998).

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). 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).

Our results showed that molybdenum enrichment of seeds by applying sodium molybdate improved the vigour of the mung bean seeds as molybdenum (Mo) has erratically affected the seed proteins, thus improving the quality of grains (Chatterjee and Nautiyal, 2001). The fully developed seeds from Mo deficient wheat were less vigourous as they showed lowere percentage of germination, seed vigour index and higher EC of seed leachate and production of abnormal seedlings (Chatterjee and Nautiyal, 2001). Faster emergence rate after priming may be explained by an increased rate of cell division in the root tips as previously found for wheat (*Triticum aestivum)* (Bose and Mishra, 1992; Basra *et al.,* 2002) and fine rice (*Oryza sativa*) (Basra *et al.,* 2003).

Results of the present study suggest that seed priming is very effective tool for seed invigouration in mung bean (*Vigna radiate* L.). The application of phosphorous (P at 0.60%) through seed priming increased the germination rate and seedling vigour substantially as compared to other priming treatments. The use of polyethylene glycol (PEG) in osmopriming, mung bean could not show better results as compared to other priming treatments including control. There is further need to investigate these priming treatments under field conditions in relation to crop production. Seed priming could be proved low cost technique for resource poor farmers for getting good germination and crop stand in arid climate. Further studies are required for alternative treatments, optimizations of temperatures, substrates etc., and/or combining of different seed priming techniques.

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