Pakistan J. Agric. Res. Vol. 26 No. 4, 2013

ASSESSMENT OF SOME PRIMING TECHNIQUES IN MUNGBEAN *(VIGNA RADIATA)* : A GREEN HOUSE STUDY

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ABSTRACT:- Priming technique used for achieving better crop stand, in which seeds are partially hydrated to a point where germination process begin but radical emergence does not occur. A pot experiment was conducted in a green house. The seeds were invigorated by traditional soaking (hydropriming), osmo-conditioning using phosphorous (KH_2PO_4; 200 mM and 400 mM), mannitol (20 and 40 gl^{-1}), polyethylene glycol (50 and 100 gl^{-1}), sodium molybdate dihydrate (0.2 and 0.4 gl^{-1}) and hormonal priming by salicylic acid (10 and 20 ppm). Untreated dry seeds were used as control. All the invigoration treatments significantly improved plant vigor in terms of final germination biomass, root, shoot length and nutrient uptake of mungbean seedlings compared to control (non-primed dry seedling). The application of seed priming also improved the protein concentration at the early stage of seedlings. Phosphorous application through priming significantly improved germination up to 95%, seedling vigour index up to 23.05 and protein content up to 2.17 (g^{-1}FW).

Key Words: Vigna radiata; Osmo-priming; Hydropriming; Germination; Emergence; Protein Content; Seedling Vigour; Pakistan.

INTRODUCTION

Crop production is widely limited by poor stand establishment and nutrient deficiencies (Jones and Wahbi, 1992). Particularly in drought prone environment, the emergence trends are irregular and can extend over long periods (Bougne et al., 2000). Poor crop establishment is often cited as a major constraint for mungbean production and high yields have been correlated positively with early seedling vigor (Naseem et al., 1997). Healthy plants with well-developed root system can more effectively mobilize limiting nutrients from the soil and can better withstand adverse conditions.

In seed priming, seeds are partially hydrated to allow metabolic events to occur without actual germination, and then re-dried (near to their original weight) to permit routine handling (Bradford, 1986). Seed priming has been found a realizable technology to enhance rapid and uniform emergence, high vigor, and better yields for vegetable and field crops (Janmohammadi et al., 2009; Rouhi et al., 2011). Seed priming can be accomplished through different

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methods such as hydro priming, osmopriming, solid matrix priming and using plant growth regulators (Harris et al., 1999).

Mungbean (*Vigna radiata* L.) is an important pulse crop contributing 16 % to the total pulse production in Pakistan, grown on 183,000 ha under rainfed conditions (GoP, 2010). Poor crop establishment is a major restraint for mungbean production (Rahmianna et al., 2000) and high yields can be associated with early vigor (Harris et al., 2005). Any attempt to overcome this problem will play a vital role for increasing mungbean production especially in rainfed areas.

Sekiya and Yano (2009) concluded that seed P-enrichment can reduce requirement of fertilizer P for subsequent production of wheat shoots. This is due to an improved seedling growth caused by increased seed P content, and an improved P-uptake capacity caused by increased root production with relatively less investment of dry matter, particularly fine roots. The higher contents of phosphorous in the seeds help to with stand the P deficiency in soil, and can enhance N_2-fixation and nodulation of legume crop (Grandi et al., 1999).

The present study intended to evaluate and compare the effect of different seed priming techniques on germination, emergence, protein concentration and seedlings vigour enhancement for mungbean under green house conditions.

MATERIALS AND METHOD

Seeds of mungbean cultivar Chakwal Mung-97 (CM-97) were sterilized by using 30% sodium hypochlorite for 5 min and then washed thrice with distilled water. For nutrient priming, seeds were soaked in aerated solution of phosphorous (@ 200 and 400 mM) and molybdenum (@ 0.2 and 0.4 gl^{-1}) for five and four hours, respectively. The sources for phosphorous and molybdenum were potassium dihydrogen phosphate (KH_2PO_4) and sodium molybdate ($Na_2MoO_4.2H_2O$), respectively. For osmopriming, seeds were soaked in aerated solutions of mannitol ($C_6H_{14}O_6$ @ 20 and 40 gl^{-1}) and polyethylene glycol (PEG_{6000} @ 50 and 100 gl^{-1}) for six and four hours, respectively. For hormonal priming the seeds were soaked in aerated solution of salicylic acid ($C_7H_6O_3$ @ 10 and 20 ppm) for about four hours.

After seed treatments, for vigour analysis, the seeds were washed thrice with distilled water. Air dried soil was placed in 10 cm tall plastic pots with 6cm diameter. The soil used in the pot experiment was sandy loam having pH of 7.9, ECe of 0.34 dSm^{-1}, available potash and phosphorous were 123 and 4.6 ppm, respectively. Ten seeds were planted in each pot and thinned to six plants per pot after complete emergence. Same amount of water was applied at regular intervals to all pots. Experiment was conducted in the greenhouse at 25 ± 5 °C and 70-75% relative humidity. The plants were harvested 21 days after sowing (DAS), and length of seedlings root and shoot were measured. The seedlings were dried at 75 °C for 48h and the dry matter was finally determined.

For recording of emergence, final germination percentage (FGP) and mean germination time (MGT), control and treated seeds (25 in each) were sown in plastic trays having

moist sand, replicated thrice and were placed in growth chamber in completely randomized design (CRD). Emergence was recorded daily according to the method of AOSA (1990). Mean emergence time (MET) was calculated according to the equation of Ellis and Roberts (1981) as under:

$$MET = \Sigma Dn / \Sigma n$$

where, n = number of seeds, which emerged on day D (D = number of days counted from the beginning of emergence).

The time to reach 50% emergence (E_{50}) was calculated according to the following formula (Coolbear et al., 1984) and modified by Farooq et al. (2005):

$$E_{50} = ti + (N/2 - ni) (tj - ti) / nj - ni$$

where N = final number of emergence (ni, nj = cumulative number of seeds emerged by adjacent counts at times ti and tj when ni < N/2 < nj.

The plants were harvested 20 days after planting and seedling vigour data were recorded according to the seedling evaluation (AOSA, 1990). Seedling length and seedling dry weight were measured after the 20 days. Vigor index was calculated according to the following formula:

Seedlings vigor index = [seedling dry weight (g) × germination percentage]

Phosphorous and nitrogen concentration was measured according to the method described by Anderson and Ingram (1993), using UV-1700 spectrophotometer. Nutrient uptake by the seedling was calculated by multiplying the respective nutrient concentration with the total biomass and expressed as mg plant^{-1}.

Nutrient uptake = Nutrient concentration × Total dry matter

For protein extraction seven days old seedlings were randomly collected from each treatment and control separately and estimated by method of Bradford (1976).

Statistical Analysis

The data collected for various characteristics were subjected to Analysis of Variance in MSTAT-C software (MSTATC, East Lansing, Mich) and the means obtained were compared by Duncan Multiple Range Test (DMRT) at 5 % level of significance (Steel et al., 1997).

RESULTS AND DISCUSSION

Seedling Vigour

The results showed that different seed priming treatments had significant ($p<0.05$) effect on mean emergence time in days (MET), time to 50% seeds to emergence in days (E_{50}) and final germination percentage (FGP). All the priming treatments decreased the MET and E_{50} compared to control. Maximum mean emergence time (5.52 days) was observed in control where untreated dry seeds were sown. Minimum MET (4.51 days), E_{50} (3.41 days) and maximum final germination (95 %) was observed in T_5 (KH_2PO_4 @ 200 mM).

The application of P as 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. Similarly, the increase in seedling vigour due to salicylic acid may be due to enhance oxygen uptake and the efficiency of mobilizing nutrients from the cotyledons to the embryonic axis (Kathiresan et al., 1984) and decreased catalase and peroxidase levels as recorded in pea (*Pisum sativum*) seedlings (Srivastava and Dwivedi, 1998).

It is also reported that early emergence in treated seed may be due to the faster production of germination metabolites (Lee and Kim, 2000; Basra et al., 2005), better genetic repair, i.e. earlier and faster synthesis of DNA, RNA and proteins (Bray et al., 1989) or the breakdown of dormancy and the seed biochemical processes outset, which led to faster germination and emergence (Basra et al., 2006). Curtailed emergence time, lowered E_{50} and MET (Table 1) appeared to be linked to the efficient mobilization and utilization of seed reserves (Lee and Kim, 2000; Basra et al., 2005), thereby leading to earlier commencement of germination events (Lee et al., 1998). Rouhi et al. (2011) concluded that all the improved traits relating to germination and improved final germination were due to seed priming.

Shoot and Root Length

The data depicts that seed priming had significant impact on the root as well as shoot length of seedlings (Table 2). All the priming treatments significantly increased the root as

Table 1. Mean emergence time (MET), time to 50% seed to germinate (E_{50}), final germination percentage (FGP) and seedling vigour index (SVI) of mung bean

Treatment	MET (days)	E_{50} (days)	FGP (%)	SVI
T_1	5.52 ^{a}	4.44 ^{a}	80 ^{de}	10.41 ^{de}
T_2	5.12 ^{cd}	4.00 ^{cd}	86 ^{bc}	13.05 ^{bcd}
T_3	5.37 ^{abc}	4.26 ^{ab}	89 ^{b}	15.45 ^{bc}
T_4	5.20 ^{bcd}	4.09 ^{bcd}	79 ^{e}	14.01 ^{bcd}
T_5	4.51 ^{e}	3.41 ^{f}	95 ^{a}	23.05 ^{a}
T_6	5.02 ^{d}	3.91 ^{de}	82 ^{cde}	16.62 ^{b}
T_7	5.38 ^{ab}	4.29 ^{ab}	84 ^{cd}	14.10 ^{bcd}
T_8	5.32 ^{abc}	4.18 ^{bc}	79 ^{e}	13.84 ^{bcd}
T_9	5.05 ^{d}	3.72 ^{e}	73 ^{f}	15.98 ^{bc}
T_{10}	5.20 ^{bcd}	4.07 ^{bcd}	63 ^{g}	14.65 ^{bc}
T_{11}	5.24 ^{bcd}	4.23 ^{abc}	70 ^{f}	12.07 ^{cde}
T_{12}	5.27 ^{abcd}	4.23 ^{abc}	65 ^{g}	8.83 ^{e}
LSD	0.25	0.23	2.17	2.34

T_1 = Control; T_2 = Hydropriming; T_3 = Mo @ 0.2 gl_{-1}; T_4 = Mo @ 0.4 gl^{-1}; T_5 = KH_2PO_4 @ 200 mM; T_6 = KH_2PO_4 @ 400 mM; T_7 = SA @ 10 ppm; T_8 = SA @ 20 ppm; T_9 = Manitol @ 20 gl^{-1}; T_{10} = Manitol @ 40 gl^{-1}; T_{11} = PEG @ 100 gl^{-1}; T_{12} = PEG @ 50 gl^{-1}
Means followed by same letter do not differ significantly according to DMRT at P < 0.05.

well as shoot length of seedlings as compared to control. The data revealed that T_5 (KH_2PO_4 @ 200 mM) and T_9 (mannitol @ 20 gl^{-1}) gave the best results. The lowest root length (4.56 cm) was observed in control. The highest shoot length was achieved from the seeds which were phosphorous enriched (T_5). There was an increase of 52% in shoot length observed in T_5 compared to control. The data depicts that the lowest shoot length was attained in control followed by T_4 (Table 2).

The increase in root/shoot ratio with priming treatments may be due to that, priming induced nuclear replication in root tips of fresh seedlings. These observations are in conformity with earlier work on bell pepper (*Capsicum annuum*) seeds (Stofella et al., 1992). Zheng et al. (2002) reported earlier and uniform emergence in rice (*Oryza sativa*) seeds osmoprimed with KCl and $CaCl_2$ and mixed salts under flooded conditions. However Nascimento and West (1999) reported early germination of primed seeds but not recorded any improvement in the growth of seedlings in muskmelon (*Cucumis melo*) seeds under laboratory conditions. Confounding results, where priming did not show any beneficial results, also reported by different research workers (Mwale et al., 2003; Giri and Schillinger, 2003).

The increased vigour in 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;

Table 2. Root/shoot length and fresh/dry weight of mung bean seedlings

Treatment	Root length (cm)	Shoot length (cm)	Fresh wt. plant^{-1} (mg)	Dry wt. plant^{-1} (mg)
T_1	4.56 ^{f}	17.73 ^{f}	241 ^{c}	140 ^{c}
T_2	5.78 ^{b}	23.93 ^{bc}	283 ^{c}	163 ^{c}
T_3	5.41 ^{c}	24.07 ^{bc}	328 ^{bc}	187 ^{bc}
T_4	4.68 ^{ef}	20.97 ^{e}	333 ^{bc}	190 ^{bc}
T_5	6.48 ^{a}	27.03 ^{a}	461 ^{a}	260 ^{a}
T_6	4.69 ^{ef}	23.27 ^{bcd}	386 ^{ab}	219 ^{ab}
T_7	4.91 ^{de}	22.13 ^{de}	315 ^{bc}	181 ^{bc}
T_8	4.70 ^{ef}	23.17 ^{bcd}	331 ^{bc}	189 ^{bc}
T_9	6.55 ^{a}	24.57 ^{b}	404 ^{ab}	229 ^{ab}
T_{10}	4.93 ^{de}	24.05 ^{bc}	385 ^{ab}	219 ^{ab}
T_{11}	5.07 ^{b}	23.02 ^{cd}	269 ^{bc}	155 ^{bc}
T_{12}	5.92 ^{d}	23.07 ^{cd}	331 ^{c}	189 ^{c}
LSD	0.27	1.48	97.44	53.25

T_1 = Control; T_2 = Hydropriming; T_3 = Mo @ 0.2 gl^{-1}; T_4 = Mo @ 0.4 gl^{-1}; T_5 = KH_2PO_4 @ 200 mM; T_6 = KH_2PO_4 @ 400 mM; T_7 = SA @ 10 ppm; T_8 = SA @ 20 ppm; T_9 = Manitol @ 20 gl^{-1}; T_{10} = Manitol @ 40 gl^{-1}; T_{11} = PEG @ 100 gl^{-1}; T_{12} = PEG @ 50 gl^{-1}
Means followed by same letter do not differ significantly according to DMRT at P < 0.05.

Thomson and Bolger, 1993).

Biomass of Seedlings

There was significant ($p<0.05$) effect of different seed priming techniques on fresh and dry weight of 21 day's old seedlings (Table 2). The maximum fresh and dry weight was obtained in T_5 (KH_2PO_4 @ 200 mM). All the priming treatments increased the fresh and dry weight of the seedlings except T_2 and T_{12}.

These results are in agreement with those of Pill and Necker (2001) who reported that primed compared to non-primed plants resulted in greater shoot dry weights. Improved biological yield as a result of seed priming might be due to earlier and uniform emergence as concluded by Hussain et al. (2006). The priming-related increase in the biomass of mungbean might be due to a combination of better emergence and better performance per plant (Parera and Cantliffe, 1994); however Shah et al. (2012) found not only significant increase in biomass due to nutrient priming under green house condition but improved fresh and dry weight observed in field study.

Nutrient Uptake and Protein Content

There was significant effect of different seed priming techniques on nutrient uptake and protein concentration in mung bean seedlings. There was significant increase in N and P-uptake observed due to seed priming treatments as compared to control, where non soaked dry seeds were

Table 3. Nutrient uptake and protein contents of mungbean seedlings

Treatment	N-uptake (mg plant^{-1})	P-uptake (mg plant^{-1})	Protein (g^{-1}FW)
T_1	1.43 ^{b}	0.20 ^{c}	1.81 ^{fg}
T_2	2.36 ^{ab}	0.37 ^{abc}	1.86 ^{ef}
T_3	2.70 ^{a}	0.30 ^{bc}	1.92 ^{cd}
T_4	2.38 ^{ab}	0.41 ^{abc}	1.97 ^{b}
T_5	3.11 ^{a}	0.56 ^{a}	2.17 ^{a}
T_6	2.30 ^{ab}	0.36 ^{abc}	1.94 ^{bc}
T_7	2.29 ^{ab}	0.35 ^{abc}	1.87 ^{de}
T_8	2.71 ^{a}	0.43 ^{ab}	1.90 ^{cde}
T_9	2.34 ^{ab}	0.46 ^{ab}	1.88 ^{de}
T_{10}	3.18 ^{a}	0.32 ^{bc}	1.78 ^{g}
T_{11}	1.66 ^{ab}	0.28 ^{bc}	1.91 ^{cde}
T_{12}	2.22 ^{b}	0.31 ^{bc}	1.89 ^{cde}
LSD	0.87	0.22	0.05

T_1 = Control; T_2 = Hydropriming; T_3 = Mo @ 0.2 gl^{-1}; T_4 = Mo @ 0.4 gl^{-1}; T_5 = KH_2PO_4 @ 200 mM; T_6 = KH_2PO_4 @ 400 mM; T_7 = SA @ 10 ppm; T_8 = SA @ 20 ppm; T_9 = Manitol @ 20 gl^{-1}; T_{10} = Manitol @ 40 gl^{-1}; T_{11} = PEG @ 100 gl^{-1}; T_{12} = PEG @ 50 gl^{-1}
Means followed by same letter do not differ significantly according to DMRT at P < 0.05.

planted (Table 3). The data revealed that maximum N-uptake (3.18 mg plant^{-1}) was observed in T_{10} (mannitol @ 20 gl^{-1}) followed by T_5 (KH_2PO_4 @ 200 mM). The data also revealed that seed priming also improved the P-uptake in the mungbean seedlings as compared to control. Maximum P-uptake has been observed in T_5 (KH_2PO_4 @ 200 mM) followed by T_{10}, compared to control. The nutrient priming using KH_2PO_4 @ 200 mM significantly increased the N and P content of mungbean seedlings. The enhanced vigour and early germination of primed plants may have lead to more nutrient uptake as compared to non-primed plants. Nutrient priming has been found more economical and convenient as compared to soil application in low fertile soils (Slaton et al., 2001). Zhang et al. (1998) concluded that soaking seeds in P solutions before sowing increased the P nutrition of rice during early growth. There has also been reported enhanced P content up to 300 mg (kg seeds)$^{-1}$ soaking in 500 mM P solution. While some previous studies also showed that nutrient enrichment by priming in macronutrient solutions had little or even a negative effect on later plant growth (Scott, 1989; Ros et al., 2000). Shah et al. (2012) concluded that the application of low rate of nutrient to seeds increased their concentration and could be effective in reduction of fertilizer dose and cost.

All the seed priming treatments improved the protein contents in the mungbean seedlings (Table 3). Heydecker and Coolbear (1977) reported that hydration of seeds during priming enhanced the embryos' ability to synthesize protein and RNA, resulting in earlier germination even under unfavourable environments. Gallardo et al. (2001) also found that seed priming improved the protein contents in *Arabidopsis thaliana* seed during germination.

The present study strongly support that seed priming enhances the seedling vigour of mungbean due to improved protein contents. Nutrient priming using phosphorous (KH_2PO_4 @ 200 mM) and osmopriming with mannitol (@ 20 gl^{-1}) proved to be the most appropriate priming treatments. The application of PEG_{6000} in osmopriming could not improve the seedling vigour as compared to other seed priming treatments used in this study. Farmer can easily adopt this cost effective technology by soaking seeds in diluted solution of any phosphorous salt/fertilizer.

ACKNOWLEDGEMENTS

This study was made possible through the financial support of Higher Education Commission (HEC) of Pakistan under the head, Indigenous 5000-fellowship programme.

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