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Full Length Research Paper

Evaluation of seed priming in mung bean (*Vigna radiata*) for yield, nodulation and biological nitrogen fixation under rainfed conditions

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A study was carried out for improving yield and biological nitrogen fixation capacity of mung bean through priming techniques. The seeds were invigorated by traditional soaking (hydropriming), osmo-conditioning (soaking of seeds in aerated, low-water-potential solutions) with potassium di-hydrogen phosphate (KH_2PO_4), mannitol ($C_6H_{14}O_6$), polyethylene glycol (PEG_{6000}), sodium molybdate dihydrate ($Na_2MoO_4.2H_2O$) and salicylic acid ($C_7H_6O_3$) while untreated seeds were kept as control. The experiment was carried out at two locations under different climatic conditions during the year 2007 to 2008. All the priming treatments significantly improved the dry matter yield (4001 to 5262 kg ha^{-1}) and seed yield (713 to 948 kg ha^{-1}) compared to the control. The highest biological nitrogen fixation (46.39 kg ha^{-1}) was observed in phosphorous primed plants compared to the control. In conclusion, overall, priming of mung bean seed with phosphorous (P at 0.6%) was found very effective for improved germination and vigour of mung bean seeds under field conditions. It is easy and cost effective technology for resource poor farmers of the region.

Key words: Rainfed, *Vigna radiata*, seed priming, BNF and N_{dfa}.

INTRODUCTION

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 vigor (Kumar et al., 2002). Unfavorable environmental conditions are major cause of poor stand establishment and low crop yield. However rapid germination of seedlings could emerge and produce deep roots before the upper layers of the soil are dried and crusted, which may result better crop establishment and higher crop yield (Ashraf et al., 2005). One way for achieving good crop stand, enhanced Biological Nitrogen Fixation (BNF) capacity of legumes and getting more benefit from low fertile soils is seed priming which is a technique in which germination processes begin but radicle emergence does not occur (Taylor et al., 1998; Harris et al., 2004). Seed priming found effective for legumes that is, yields of legume crops were increased considerably by priming seeds before sowing (Musa et al., 2001; Rashid et al., 2004; Harris et al., 2004).

In addition to better establishment, primed crops grew more vigorously, flowered earlier and yielded higher (Farooq et al., 2008). It has also been reported that seed priming improves emergence, stand establishment, tillering, allometry, grain and straw yields, and harvest index (Farooq et al., 2008). Application of *Rhizobium* with seed priming significantly increased nodulation and nitrogenase activity, but had little effect on yield (Harris et al., 2004).

Plants take up inorganic phosphate (Pi) from soil solution which is usually less than 1µM. This Low inorganic phosphate is due to fixation of P (10.5-15.5 Tg annum^{-1}) with aluminum, calcium and iron (Sekiya et al., 2009). This huge accumulation occurs mainly in agricultural soils mostly due to irrational application of P fertilization (Bennett et al., 2001; Carpenter, 2005). One

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Table 1. Physical and chemical properties of soils of experimental sites.

Soil characteristic	Unit	Rawalpindi	Chakwal
Texture	-	Sandy loam	Sandy Loam
pH	-	7.6	7.7
E. Ce	dSm^{-1}	0.34	0.28
TOC*	g 100 g^{-1}	0.59	0.43
Total N	mg g^{-1}	0.33	0.23
NO_3-N	µg g^{-1}	9.2	6.7
Available P	µg g^{-1}	4.6	2.3
Extractable K	µg g^{-1}	123	114
Bulk density	Mg m^{-3}	1.44	1.38
Soil moisture	g 100 g^{-1}	12.4	11.6

TOC: Total organic carbon.

way to increase the P availability and reduce its accumulation is seed priming which may reduce the need for fertilizer P in subsequent crop growth (Sekiya et al., 2009). The higher contents of phosphorous in the seeds helps to with stand the P deficiency in soil, and can enhance N_2-fixation and nodulation of legume crop (Grandi et al., 1999).

Keeping in view the importance of priming for improved yield, nodulation and nitrogen fixation in mung bean, we studied the impact of seed priming on biomass, grain yield, nodulation and biological nitrogen fixation (BNF) of mung bean, under rainfed conditions of Potohar.

MATERIALS AND METHODS

Experiments were conducted on mung bean *(Vigna radiata)* during summer (Kharif) seasons (2007 and 2008) at two different locations of Potohar area (Research farms of PMASAAU, Rawalpindi and BARI, Chakwal). Initially, laboratory and green house studies were conducted (un-published), to find out the effect of priming on the germination and seedling growth of the mung bean by different priming treatments. Randomized complete block design (RCBD) was employed in field. Seeds of mung bean cultivar CM-97 were obtained from (BARI), Chakwal.

The seeds were sterilized by using 30% sodium hypochlorite for five minutes and then washed three times with distilled water. Selection of suitable priming compounds was made on the basis of findings of different research workers (Grandi et al., 1999; Harris et al., 2001; Basra et al., 2002; Kaur et al., 2006; Farooq et al., 2006). The treatments used were: T1 = Control (dry seeding), T2 = Hydropriming (soaking in distilled water), T3 = molybdenum ($Na_2MoO_4.2H_2O$) at 0.02% (applied in the form of sodium molybdate dihydrate), T4 = molybdenum at 0.04%, T5 = phosphorous at 0.06% (applied in the form of KH_2PO_4), T6 = phosphorous at 1.2%, T7 = salicylic acid ($C_7H_6O_3$) at 10 ppm, T8 = salicylic acid at 20 ppm, T9 = mannitol ($C_6H_{14}O_6$) at 2%, T10 = mannitol at 4%. The seeds were soaked in aerated solutions of all the treatments for five hours. A non-soaked, non-dried treatment was included as a control. After soaking, seeds were given three surface washings with sterilized water (Khan*,* 1992) and re-dried, near to original weight with forced air under shade. The seeds were then sealed in polythene bags and stored in refrigerator till further use (Basra et al., 2003). Inoculation was also done at the time of sowing.

Plant population, biomass and yield parameters were determined from an area of 1 m^2 from each treatment plot. The ^{15}N Natural Abundance Method (^{15}NNAM) was adopted for quantification of biological nitrogen fixation (Gathumbi et al., 2002). After drying at 70°C, the plant samples were ground to fine powder in a Wiley's Mill. One gram of sub sample for legume and reference non legume (maize) was sent to Stable Isotope Unit, University of Waikato, Hamilton (New Zealand) for analysis of $\delta\,^{15}N$ on Mass Spectrometer (Craswell et al., 1991).

RESULTS

Dry matter and seed yield (kg ha^{-1})

Data regarding dry matter and seed yield as affected by different seed priming treatments combined over years and locations is presented in the Table 2. The data shows that difference between two locations was significant ($p<0.05$). Mean dry matter and seed yield recorded at Rawalpindi was higher by 22 and 6.1% than at Chakwal. The data also depicts that the dry matter and seed yield was reduced up to 16 and 10% during second year (2008) as compared to first year (2007). The highest dry matter and seed yield was observed in Rawalpindi during the first year and the lowest was observed in Chakwal during the second summer season of 2008.

All the priming treatments significantly increased the dry matter and seed yield compared to the control. The data, combined over year and location showed that the maximum dry matter (5262 kg ha^{-1}) and seed yield (948 kg ha^{-1}) was recorded in T5 (P at 0.60%) which was 25 and 30% higher compared to the control. The lowest dry matter (4001 kg ha^{-1}) and seed yield (713 kg ha^{-1}) was achieved in the control plot. The data also showed that there were similar results in T3 (molybdenum at 0.02%), T6 (P at 1.2%), T7 (SA at 20 ppm) and T9 (Mannitol at 2%) while T4, T8 and T10 are also statistically at par for

Table 2. Impact of different seed priming treatments on seed and dry matter yield of mung bean (kg. ha^{-1}).

Treatment	Location			Location		
	Rawalpindi	Chakwal	Mean	Rawalpindi	Chakwal	Mean
	Seed yield (kg ha^{-1})			Dry matter yield (kg ha^{-1})		
Control	784	642	713 g	4737	3265	4001 f
Hydropriming	830	703	766 e	4975	3614	4295 d
Molybdenum (0.02%)	849	795	822 c	5125	4305	4715 b
Molybdenum (0.04%)	822	772	797 d	5016	4162	4589 c
Phosphorous (0.6%)	972	925	948 a	5873	4650	5262 a
Phosphorous (1.2%)	819	832	825 c	5224	4430	4827 b
Salicylic acid (10ppm)	830	825	827 c	5143	4346	4745 b
Salicylic acid (20ppm)	823	808	815 cd	5007	4196	4602 c
Mannitol (2%)	862	846	854 b	5114	4338	4726 b
Mannitol (4%)	812	784	798 d	4904	4087	4496 c
Treatments LSD		20.06			107.6	
Average over years and locations						
Y1 2007	872 a	830 a	851 a	5380 a	4453 b	4916 a
Y2 2008	807 b	756 c	781 b	4770 a	3696 c	4233 b

Nitrogen uptake

Data regarding nitrogen uptake as affected by different seed priming treatments combined over years and locations is presented in Table 3. The data establish that the higher N uptake was observed in Rawalpindi than Chakwal; however, the difference was statistically at par. The highest nitrogen uptake was observed in Rawalpindi during the first year (2007) and the lowest nitrogen uptake was observed in Chakwal during the second summer season of 2008.

All the seed priming treatments significantly improved the N uptake in straw as well as in seed nitrogen uptake (90.6 kg ha^{-1}) was obtained in T5 (P at 0.60%) followed by T6 (P at 1.2%). The overall increase of 12% was observed due to different priming treatments. All the priming treatments also increased the seed nitrogen uptake (Table 3). The data, combined over years and locations showed that the highest seed nitrogen uptake (37.5 kg ha^{-1}) was obtained in T5 (P at 0.60%) followed by T9 (P at 1.2%). The overall increase of 31% in seed N was observed due to different priming treatments.

Nitrogen uptake in grain was higher (Table 3) during the first year (2007) as compare to the second year (2008). Over all, there was a decrease of 10% observed during summer season of 2008. There was significantly higher nitrogen uptake in grain at Rawalpindi recorded which was 20% higher than Chakwal.

All the seed priming treatments increased the nitrogen uptake in mung bean grains as compared to the control (Table 3). The highest N uptake in mung bean during both years and locations was observed in phosphorous primed plants (P at 0.6%) which were 55% higher than the control. The lowest nitrogen uptake (24 kg ha^{-1}) was of mung bean (Table 3). The data combined over years and locations showed that the highest dry matter yield and T3, T6, T7 and T8 were similar in

Table 3. Impact of different seed priming treatments on N-uptake in shoot and grain of mung bean (kg.ha^{-1}).

Treatment	Location			Location		
	Rawalpindi	Chakwal	Mean	Rawalpindi	Chakwal	Mean
	Nitrogen-uptake in shoot (kg ha^{-1})			Nitrogen-uptake in grain (kg ha^{-1})		
Control	70.4	52.8	62.1 d	27.3	22.3	24.2 d
Hydropriming	83.8	54.9	66.9 bc	31.5	26.6	29.6 cd
Molybdenum (0.02%)	84.5	64.0	72.8 bc	36.3	27.7	32.9 abc
Molybdenum (0.04%)	85.4	67.5	72.5 b	34.5	27.6	31.5 abc
Phosphorous (0.6%)	90.6	74.1	83.8 a	40.3	34.2	37.5 a
Phosphorous (1.2%)	86.9	67.9	78.6 b	36.64	29.2	32.7 abc
Salicylic acid (10ppm)	81.3	65.9	73.6 b	34.9	29.3	32.4 abc
Salicylic acid (20ppm)	79.5	63.8	70.7 bc	37.8	31.2	34.8 abc
Mannitol (2%)	81.2	69.0	73.6 bc	39.9	31.3	35.5 ab
Mannitol (4%)	79.7	61.9	67.3 cd	30.8	30.7	30.8 bc
Treatments LSD		6.69			5.39	
Average over years and locations						
Y1 2007	87.8 a	67.3 a	77.1 a	36.0 a	30.8 b	33.5 a
Y2 2008	73.3 ab	55.6 b	64.5 b	34.9 a	27.3 c	30.9 b

seed yield (Table 2). achieved in dry seeded control plot. The data also describes that similar behavior was expressed by the rest of priming treatments.

Percent nitrogen derived from air (% N_{dfa})

Impact of different seed priming treatments combined over years and location (Table 4) showed significant ($p<0.05$) effect on percentage of nitrogen derived from air (% N_{dfa}). Percent nitrogen derived from air (% N_{dfa}) was higher at Rawalpindi (35.72%) compared to Chakwal (31.20%). The data also depicts that it was highest during the first year (2007) as compared to second (2008). The highest % N_{dfa} was found at Rawalpindi during the first year and the lowest was at Chakwal during the second year

The entire priming treatments showed significantly higher %N_{dfa} over the control. Effect of osmopriming using 0.6% phosphorous as priming agent showed highest % N_{dfa} (38.97 %) during both seasons and locations which was 22% higher than the control, while other priming treatments were statistically at par. The lower levels of each priming solutions showed better results in term of %N_{dfa}.

As earlier described, higher % N_{dfa} at Rawalpindi during the first year may be due to favorable climatic conditions especially rainfall as the amount of fixed N depends upon a number of factors including climatic conditions (Havlin et al., 2007). Favorable climatic conditions during the first year might have resulted in higher % N_{dfa} as amount of rainfall during the first year was 1,321 mm (average of two locations from July to September) compared to 982 mm during the second year.

Moreover, increased sucrose synthase (SS) activity due to priming may be one of the reasons for increased % N_{dfa} under rainfed conditions. Higher SS activity in the nodules of primed plants is responsible for normal nodule development (Anthony et al., 1999; Kaur et al., 2005). Higher %Ndfa due to molybdenum (Mo) might be due to the fact that it is needed primarily on the seed coat in order to enhance nodulation with nitrogen-fixing bacteria, which requires Mo for the proper function of the nitrogen-fixing enzyme, nitrogenase (Marschner, 1995; Achakzai and Kayani 2003; Achakzai et al., 2003). Johansen et al. (2007) also reported molybdenum as significant component of nitrogenase enzyme which is involved in the process of biological

Table 4. Impact of different seed priming treatments on N_{dfa} (%) and N_2-fixed (kg ha^{-1}).

Treatment	Location			Location		
	Rawalpindi	Chakwal	Mean	Rawalpindi	Chakwal	Mean
	N_{dfa} (%)			N_2-fixed (kg ha^{-1})		
Control	30.85	33.09	31.97 c	37.36	26.19	31.78 g
Hydropriming	31.94	34.97	33.45 ab	38.06	28.47	33.27 f
Molybdenum (0.02%)	34.42	40.06	37.24 ab	42.06	38.61	40.34 c
Molybdenum (0.04%)	35.15	38.42	36.79 ab	41.89	35.70	38.80 e
Phosphorous (0.6%)	38.67	39.27	38.97 a	52.76	40.02	46.39 a
Phosphorous (1.2%)	36.42	38.30	37.36 ab	47.53	40.09	43.81 b
Salicylic acid (10ppm)	36.18	37.94	37.06 ab	44.46	37.07	40.77 c
Salicylic acid (20ppm)	35.39	38.18	36.79 ab	42.22	35.87	39.04 de
Mannitol (2%)	35.27	37.15	36.21 ab	43.67	36.56	40.12 cd
Mannitol (4%)	36.18	38.36	37.27 ab	42.52	35.39	38.95 de
Treatments LSD		5.75			1.41	
Average over years and locations						
Y1 2007	36.84 b	37.42 a	37.13 a	49.01 a	38.67 b	43.84 a
Y2 2008	33.26 c	37.73 a	35.50 b	37.49 b	32.12 c	34.80 b

nitrogen fixation in the nodulated legumes.

Nitrogen fixation

Impact of different seed priming treatments indicated significant ($p<0.05$) effect on biological nitrogen fixation (BNF) (Table 4). The data depicts that there was higher nitrogen fixation value (51.22 kg ha^{-1}) during the first year (2007) as compared (43.04 kg ha^{-1}) to the second (2008) and it was higher at Rawalpindi site as compared to Chakwal.

The data depicts that different seed priming treatments had significant ($p<0.05$) effect on capacity of mung bean for biological nitrogen fixation compared to the control (35.47 kg ha^{-1}). The highest (57.48 kg ha^{-1}) was recorded in T5 (P at 0.6 %) followed by T3 (Mo at 0.02 %)

The data also describes that the lower concentrations of each priming technique resulted in higher BNF as compared to higher ones. The regression analysis revealed strong association of N_2-fixed with dry biomass (R^2 = 0.77) and nitrogen uptake (R^2 = 0.71) as illustrated in Figure 1.

The results for regression analysis are in line with the findings of Hayat et al. (2008), who also noted strong correlation between N uptake and N_2-fixed. There was highest BNF recorded in P primed plants; this might be due to the fact that it strengthens the rooting system of plants. Its availability is important for the development of nodules on roots (Shah et al., 2004). Phosphorus deficiency inhibits acetylene reduction activity due to reduction in nodule weight as compared to the plants receiving P treatments (Olivera et al., 2004).

Significant difference between the two sites may be due to environmental factors including drought, temperature and soil nutrient status which dramatically affect the process at molecular/functional level and thus, play a part in determining the actual amount of nitrogen fixed by a given legume in the field (Serraj, 2004). The Priming treatments significantly increased

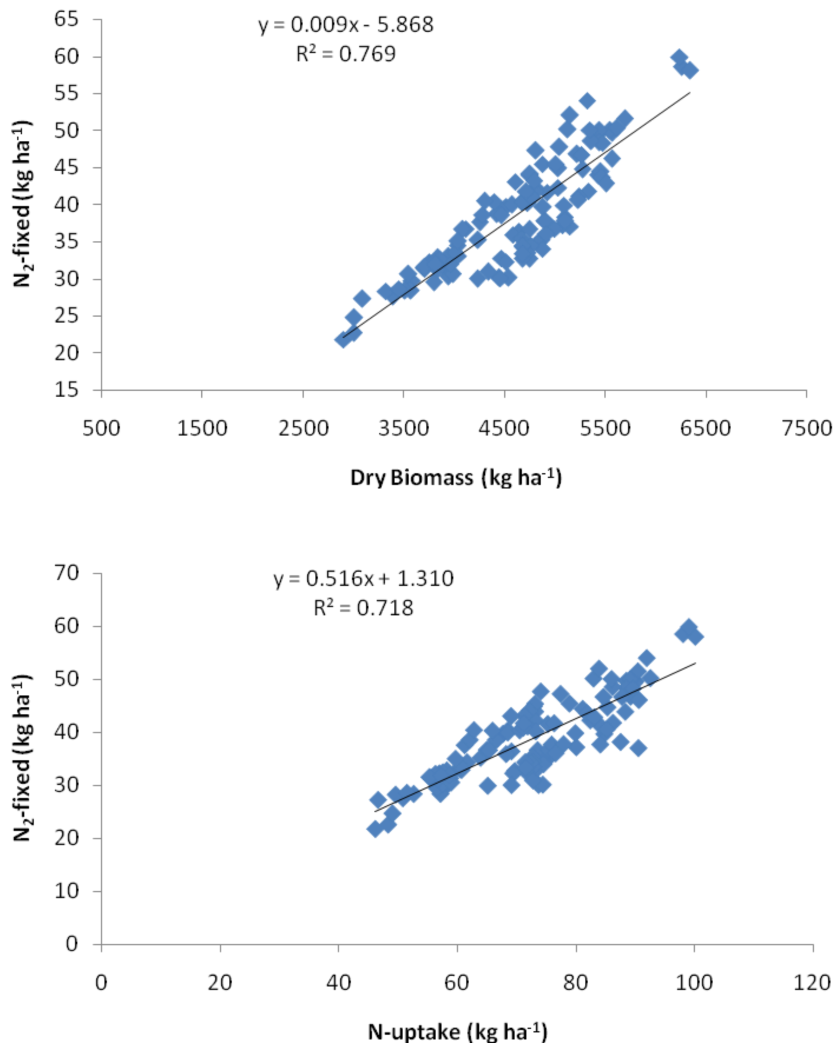

Figure 1. Relationship between Dry biomass and Shoot N yield with N_2-fixation of mung bean.

the nitrogen fixation under rainfed conditions. All the application of Mo also improved the BNF substantially; this might be due to the fact that Mo enriched seeds could be a feasible option to extraneous seed treatment, allowing favoured inoculant strains of *Bradyrhizobium* to uphold high rates of biological nitrogen fixation (Campo et al., 2009).

DISCUSSION

Favorable climatic conditions during the first year might have resulted in higher dry matter and seed yield compared to the second year. The variations of dry matter and seed yield for different season were also reported by different scientists (Ali et al., 1999; Hayat, 2008). Mung bean is moisture sensitive crop and its production decreased under moisture stress conditions. The most critical time for water is during flowering and early pod development of crop (Richards and Thurling, 1978). The root system of mung bean is mainly located in the upper 20-25 cm depth (Kjellstrom, 1991) which, under dry conditions is transformed into short tuberized roots. Thus, it is unable to extract proper moisture and nutrition for growing plants. Water stress at any stage of growth may cause changes in plant morphology, physiology and consequently affects crop growth (Hashem et al., 1998).

The results are in line with the findings of Harris et al. (2004) who reported higher plant dry weight and seed yield following seed priming. The enhanced phenology in mung bean due to primed seed is associated with faster emergence and reduced germination imbibitions periods (Harris et al., 1999). The increase in the dry matter and grain yield of mung bean was due to better emergence and better performance per plant (Parera and Cantliffe, 1994). Rashid et al. (2006) reported that priming enhanced germination, better establishment and increased yields in many diverse environments for a number of crops (Khan et al., 2008).

There was an increase of dry matter yield in P primed plants as plants require it, for root expansion, stooling, flowering, fruiting and seed setting, from early stages of growth (Grant et al., 2005). It plays primary role in many of the physiological processes such as the utilization of sugar and starch, photosynthesis, energy storage and transfer (Havlin et al., 2007). These results are in line with the findings of Sekiya et al. (2009) who found that ~0.35 M potassium phosphate was the most efficacious concentration for P-enrichment of the wheat seed. These results confirm the finding of earlier researchers (Rashid et al., 2004) that a quintuple increase in yield in mung bean due to priming was observed.

The enhancement in Nitrogen-uptake during 2007 might be due to more rainfall as compared to 2008 as mung bean is moisture sensitive crop and its production decreased under moisture stress conditions. The most critical time for water is during flowering and early pod development of crop (Richards and Thurling, 1978). The root system of mung bean mainly located in the upper 20-25 cm depth (Kjellstrom, 1991) which, under dry conditions, transformed into short tuberized roots, unable to extract proper moisture and nutrition for growing plants. Water stress at any stage of growth may causes changes in plant morphology, physiology and consequently affects crop growth (Hashem et al., 1998).

Conclusion

The osmopriming, nutrient priming and hormonal priming increased seed yield of mung bean (*Vigna radiate*) considerably. There was also significant increase in nodulation, nitrogen fixation and nutrient uptake due to seed priming. Phosphorus application at the rate of 0.6% in the form of KH_2PO_4 significantly enhanced yield, nodulation and nitrogen fixation. Seed priming can be applied in a number of crops including cereals and legumes without any harm under rainfed conditions. The physiological basis of enhanced growth and yield should be investigated in mung bean. Higher levels of priming media are not suitable for seedling growth and establishment in mung bean. Further studies are required for alternative treatments, optimizations of temperatures, substrates etc., and/or combining of different seed priming techniques.

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