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RESEARCH ARTICLE

HYDROPRIMING TREATMENTS FOR IMPROVED GERMINATION AND EARLY SEEDLING GROWTH OF MUNGBEAN SEEDS (*Vigna radiata* [L.]. R. Wilczek)

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ABSTRACT

Hydropriming treatments in freshly harvested mungbean seeds (cv. Sabitri) showed a significant improvement on early seedling growth as measured by root and shoot length of the seedlings than the untreated control. The short duration soaking followed by lightly air-drying treatment (0.5 h to 4 h) along with pre-conditioning treatments viz. moist sand conditioning for 12 h and moist sand conditioning followed by soaking for 1 h have shown better results in improving vigour of the seedlings (root and shoot length). Long duration showed adverse effect on germinability. The studies on membrane functions as determined by electrical conductance and leaching of sugar revealed that reduced leakage of electrolytes and sugar were noted in the short duration priming treatments (0.5 h to 4 h soaking) and pre-conditioning treatments viz. moist sand conditioning as well as moist sand conditioning followed by soaking treatments (0.5h to 4 h) than the untreated control. Long duration soaking showed adverse effect on germination percentage and a marginal improvement on enzyme activity. On the basis of the results of the present investigation, short duration hydropriming treatment alongwith pre-conditioning treatments viz. moist sand conditioning and moist sand conditioning followed by soaking and then lightly air-drying may be employed in improving early germination and seedling growth of mungbean seeds.

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INTRODUCTION

Mungbean (*Vigna radiata* [L.].R. Wilczek) is an important food legume in South East Asia. It is also an excellent source of high-quality dietary protein. Pulses are essential to global food security by delivering high-nutrition protein to people and critical nutrients to soil. Globally, India is the largest producer (70% of world production) as well as consumer of mungbean. About 1.5-2.0 mt of mungbean is being produced annually (10-12% of total pulse production) from about 3-4 m ha of area with an average productivity of 500 kg ha^{-1} in the country. In eastern India including the state of West Bengal, mungbean is mainly cultivated as an important rainfed pre-*kharif* crop. Since mungbean seeds tend to absorb water vapour in humid atmosphere, decline in germination and viability of seeds become a major problem upon ageing of seeds, especially under hot and humid climatic situations as prevalent in our country. In many agricultural areas, the major cause of poor stand establishment and low crop yield is due to prevalence of unfavourable environmental conditions for seed germination and seedling emergence. Poor crop establishment is a major constraint for mungbean production (Rahmianna *et al*., 2000), particularly in drought-prone environment, exhibiting irregular emergence trends that can extend over a long period of time. Conversely, rapid germination of seedlings can help in faster emergence, producing deep roots before the upper layers of the soil are dried and crusted, which may result in better crop establishment and higher yield (Ashraf and Foolad, 2005). Healthy plants with well-developed root system can effectively mobilize limiting nutrients from the soil and can also better withstand adverse conditions. Synchronized germination and early seedling establishment are critical stages in crop production for higher grain/seed yield and increased tolerance against various biotic/abiotic stresses. However, under normal growth conditions it is difficult to achieve synchronous germination for two reasons. Firstly, the threshold stimulus required to complete the germination varies among individual seed and secondly, the viability of desiccation-tolerant orthodox seeds gradually decreases during the dry storage period largely due to ageing processes and/or deterioration events. Besides, the seed quality with respect to viability and vigour is also compromised during various stages of its production processes due to fluctuating environmental conditions, more particularly humidity and temperature. Taken together, the time from sowing to seedling establishment is a crucial period in crop growth with a direct impact on stress tolerance, final yield, and quality.

One of the seed invigoration methods for rapid, uniform, and increased germination is post-storage priming technology. Seed priming is a controlled hydration technique that triggers the metabolic-restart during early phase of germination before radicle protrusion (McDonald 2000). Seed priming is a simple and effective low-cost technology towards improving seed germination, seedling emergence, stand establishment, crop growth, nodulation and productivity of mungbean. Not only that, it can also be appropriate for better managing climate risk, crop husbandry and guaranteeing the full expression of crop yield potential (Padgham, 2009).Although, the mechanisms involved in seed priming have not yet been fully delineated, the recent molecular and technological advances demonstrate that the advancement of germination metabolism, enhanced anti-oxidative activity, and the repair processes are associated with the enhancement of germination processes through priming (Bailly *et al*.2000; Sharma and Maheshwari 2015). Mung bean (*Vigna radiata* [L.].R. Wilczek) is an important short duration pulse crop cultivated in South and Southeast Asia under conserved/rain-fed conditions. Seeds are rich in essential amino acids such as leucine, isoleucine, and valine and are preferably consumed as sprouts for better nutritional value and for a rich source of minerals. Low seed vigour is a common problem in legume establishment. Mung bean seeds deteriorate faster during dry storage which results in poor seed germination and establishment leading to reduced crop yields. An assurance of early and synchronized germination is important, potential benefits of priming can be achieved only when the seed-water relationship is properly understood for mungbean seeds. In this study, attempts have been made to study the quantitative aspects of water absorption during seed priming of mung bean with the objective (1) to optimize the duration of hydro-priming techniques for metabolic re-start of radicle emergence and (2) to understand the biochemical mechanisms of hydro-priming in mung bean seeds.

MATERIALS AND METHODS

Freshly harvested (1-month old) seeds of mungbean (cv. Sabitri) were taken for the experiment. Fifty grammes of seeds were soaked in water alone for various durations, viz., 0.5, 1, 2, 4, 6 and 8 hours. Moist sand conditioning of seeds was done by pre-moistening the seeds with air-dried moist sand (6% moisture content) in the container and then seeds were thoroughly mixed with the moist sand (seed : sand :: 1:3) and kept covered for 12 hours under ambient conditions (85±1% R.H. and temp 30±1°C). After the stipulated period, seeds were sieved to let the sand pass and then lightly air-dried. In case of moist sand conditioning soaking, after moist sand conditioning for 12 hours, seeds were soaked in double volume of water for 1 hour and then lightly air-dried under the fan. Seeds were placed for germination immediately after treatment following the method of Punjabi and Basu (1982) with minor modifications. Before placing the seeds on the glass plate, the seeds were thoroughly slurry-dressed or pre-treated with Mancozeb to control fungal growth during germination. The germination percentage, root and shoot length of the seedling were recorded after 5 days of germination. Root and shoot length of normal seedling was measured to the nearest millimetre. Over 400 seeds for each treatment were employed for germination test (ISTA, 1996). To study membrane permeability of treated and untreated seeds, the electrical conductance and leaching of sugar was measured following the method of Anderson *et al*. (1964) and Mc Cready *et al*. (1950) respectively. Twenty seeds from each treatment were soaked in 25 ml of distilled water for 30 minutes at 29 ± 1°C and then seed steeped water was decanted off and electrical conductance of seed leachate was recorded on a Conductivity Bridge (cell constant = 0.756). The amount of sugar leached out was determined by adding 4 ml of ice-cold freshly prepared Anthrone reagent (0.2% Anthrone in 98% sulphuric acid) to a mixture of 1 ml of pre-cooled seed leachate and 1 ml of distilled water (after standardization) in a hard glass test tube and kept in cold for 30 minutes for the development of bluish green colour. The intensity of the colour was measured on a Systronics Spectrophotometer at 580nm.The dehydrogenase enzyme activity of treated and untreated seeds was estimated following the method of Kittock and Law (1968). Four uniformly sprouted embryos were dipped in 2 ml of 0.2% tetrazolium chloride solution and incubated for 3h in the dark at 29 ± 1°C. After incubation, the solution was decanted off and the embryos were thoroughly washed with distilled water and surface dried. Then four ml of 2-methoxy ethanol were added and kept overnight for the extraction of red colour formazan. The absorbance of the solution was recorded on a Systronics Spectrophotometer at 470nm.

RESULTS AND DISCUSSION

The data on germination percentage did not show any significant difference between untreated control (0 h) and hydropriming treatment immediately after treatments (Table 1). But there was a marginal improvement on germination percentage which was noted when seeds were soaked for 0.5h and 1h. Similarly, moist sand conditioning followed by soaking for 1h have shown more or less similar results in improving germination percentage immediately after treatment (Table 1). However, all the priming treatments, 0.5h to 4h alongwith moist sand conditioning for 12 h and moist sand conditioning followed by soaking for 1 h has shown better results in improving early seedling growth as measured by root and shoot length of the seedlings (Table 1). Long duration soaking (6 h and 8 h) showed adverse effect on germinability. Similarly, the vigour index has shown same type of response.

Physiological and biochemical studies revealed that reduced leakage of electrolytes and sugar were noted in the short duration hydropriming treatment and the moist sand conditioning treatments than the untreated control (Table 2). The long duration viz. 6 h and 8 h soaking showed adverse effect on germination percentage as well as leaching of electrolytes and sugar. The dehydrogenase enzyme activity was significantly higher in the moist sand conditioning, moist sand conditioning followed by soaking and then light air-drying and short duration soaking (0.5h to 4h) treatment than the untreated control (Table 2). Among the treatments, moist sand conditioning and moist sand conditioning followed by soaking has shown better results in maintaining higher dehydrogenase enzyme activity. The priming, irrespective of methods, principally involves an initial uptake of water (imbibition) which is a key event to induce seed metabolic activities before radicle emergence and thus reduces the lag period required to switch on the germination process (McDonald 2000). The promotive response of post-storage priming was independent of the rate of water absorption. Despite potential benefits of priming for seed quality enhancement, the technique has not achieved widespread circulation, as there are critical points undermining its practical use by the farming community.

Table 1. Effect of hydropriming treatments for improved germination and early seedling growth of mung bean seeds (cv. Sabitri)

Hydropriming treatment	Germination		Mean root length (mm)	Mean shoot length (mm)	Vigour Index
	%	Arc-Sin value			
Soaking duration (h)					
0	93	75.11	34	47	7533
0.5	100	90.00	56	69	12500
1	100	90.00	47	66	11300
2	94	75.82	50	68	11092
4	96	78.46	51	70	11616
6	82	64.90	54	59	9266
8	80	63.43	37	58	7600
Pre-conditioning treatment					
Moist Sand Conditioning (12 h)	100	90	58	67	12500
Moist Sand Conditioning (12 h) followed by soaking (1 h)	97	80.03	56	72	12416
L.S.D at 0.05P	-	NS	9.3	9.7	-
L.S.D at 0.01P	-	NS	13.5	14.1	-

Table 2. Effect of hydropriming treatments on membrane permeability and enzyme activity of mung bean seeds (cv. Sabitri)

Hydropriming treatment	Germination		Electrical conductivity (μscm^{-1})	Leaching of sugar (O.D. value recorded at 580 nm)	Dehydrogenase enzyme activity (O.D. at 470 nm)
	%	Arc-sin value			
Soaking duration (h)					
0	91	72.64	57.65	0.086	0.305
0.5	100	90.00	15.36	0.035	0.375
1	96	78.46	36.70	0.046	0.366
2	93	74.66	38.33	0.048	0.348
4	87	68.87	46.49	0.065	0.324
6	80	63.43	48.96	0.075	0.315
8	67	54.94	79.74	0.114	0.285
Pre-conditioning treatment					
Moist Sand Conditioning (12 h)	98	81.87	33.81	0.040	0.624
Moist Sand Conditioning (12 h) followed by soaking (1 h)	92	73.57	34.38	0.044	0.568
L.S.D at 0.05P	-	NS	9.3	9.7	-
L.S.D at 0.01P	-	NS	13.5	14.1	-

Other details are same as Table 1.

The available scientific literature suggests that recent research has more closely addressed the subject of biochemical changes than the issues of priming methods (Paparella *et al*. 2015). Whatever be the methods, it is the water uptake by seeds during phases I and II of the imbibition process that triggers the metabolic re-start and onset of the germination process (McDonald 2000). Hydro-priming is the most practical technique without much labour cost and disposal concern associated with other priming agents. Hence, the amount of water that initiates the metabolic events to a point short of radicle emergence, the method of water absorption, and duration of its absorption are important considerations for seed quality enhancement and synchronization of the germination process through hydro-priming. The water uptake during priming is influenced by the availability of water in the vicinity of seed, duration of treatment, and the physical/ chemical characteristics of seeds (McDonald 2000). Hence, it is more appropriate to optimize the water absorption patterns and the duration of priming for crops of economic importance for making hydro-priming an economically viable and farmer-friendly technique. The water absorption by primed seeds was analysed for different hours and the results are discussed in relation to the triphasic water absorption pattern by seeds (Bewley 1997). The water absorbed by seeds was low initially by 0.5 hour after priming due to a significantly lower rate of water absorption. The self-regulation of water absorption rates might be governed by diffusional/imbibitional forces acting on the seed-water interface. This contention is supported by our results that peak rates of water absorption by the direct seed soaking was achieved by 4 h which showed an increased rate of water absorption by 2 h or 4 h after priming.

The increased duration of priming resulted in a second rise of seed water absorption which was also associated with the seed coat rupture/radicle emergence, by 6 or 8 h after priming, indicating the initiation of phase III of water absorption pattern (Bewley 1997). Nevertheless, it is not possible to define these phases in relation to their respective duration. Since, very little water was absorbed between 6 to 8 h after priming; it was considered that priming duration for 1-4 h was the optimum period for mung bean seeds. It is, therefore, suggested that the water absorbed during 1-4 h priming period with uniform aeration may be crucial for mung bean seeds for initiating metabolic events (phased I, II) and for enhancing germination process while preventing radicle emergence (phase III). Possibly, the higher rate of water absorption by the direct soaking method might have resulted in a greater solute leakage during the initial hours of priming. But the results indicated that the initial (2-4 h) higher rate of water absorption by direct priming had no adverse effect on the priming-induced germination responses.

Moist Sand Conditioning for 12 hours and Moist Sand Conditioning for 12 hours followed by soaking for 1 hour also gave commendable results. The results of Laghari *et al.*, (2016) showed that the soaking of mung bean seed with distilled water for 4 hours gave a significant increase in germination percentage, radicle and shoot length, and seedling vigour index. Interestingly, higher duration of exposure of hydropriming also favoured to assimilate the metabolites, thereby benefitting for better crop stand. The effect on germination might be explained due to the efficient mobilization as well as utilization of seed reserves.

Faster was the production of germination metabolites, the better was the genetic repair, i.e. earlier and faster synthesis of DNA, RNA and proteins or the breakdown of dormancy and the seed biochemical processes outset, which led to better germination and emergence. Biochemical evidences in favour of germination advancement by hydration-dehydration pre-treatments have been greatly emphasized by Sen and Osborne, 1974. According to Sen and Osborne (1974), the rate of RNA and protein synthesis in wetted-dried rye embryos were similar to those in embryos continuously germinated for the same period of total hydration. Heydecker (1974) was also of the opinion that hydration caused an advancement of the germination process which was compatible with the subsequent drying back. Studies in the present laboratory (Mandal and Basu, 1982 and 1987) suggested that the beneficial effects of pre-sowing soaking might also be obtained with shorter soaking duration. Many studies have indicated that a relatively short priming treatment is advantageous for extending the longevity and improving the vigour of stored seeds (Dearman *et al*. 1986). However, others have reported that the storage life of seeds is shortened following priming (Sun *et al*. 1997). Nonetheless, it is necessary to mention that different rates of water absorption and the duration of absorption may alter the redistribution of absorbed seed water among different binding sites (weak water-binding sites, strong water-binding sites, and multimolecular-binding sites) which is unclear and needs to be studied in relation to seed longevity. A significantly higher activity of dehydrogenase enzyme in primed compared to non-primed seeds indicates that there was an early induction of metabolic activities for supporting rapid and synchronized germination in primed seeds. The increased activity of dehydrogenase enzyme may also protect the cell against membrane damage which occurs naturally due to lipid peroxidation during storage (Bailly *et al*. 2000). A lower EC value in primed compared to nonprimed/ control supports this contention because EC has been found to be associated with the membrane permeability properties of cells (Simon 1974). The early onset of metabolic activity in response to imbibition of seeds has been reported to be associated with increased energy requirement (Ehrenshaft and Brambi 1990). High integrated chloroplast and mitochondria in response to priming compared to nonprimed/ control seeds in all the genotypes indicate that the activity of these organelles increased by priming treatments to meet out the additional energy requirement by the germinating seeds. The repair of pre-existing chloroplast and mitochondria and the associated enhanced production of ROS upon imbibition have also been reported by earlier workers (Howell *et al*. 2009). The enhanced production of ROS is tightly regulated with ROS scavenging system (Bailly 2004). It is therefore suggested that the enhanced activity of dehydrogenase enzyme and the improved integrity of chloroplast/ mitochondria by priming treatments were in coordination for early metabolic restart and the synchronized germination in the primed seeds.

Pre-sowing seed priming with vermiculite and warm water (50ºC) soaking for 60 minutes improved the emergence responses of bitter gourd seeds under 25ºC and 20ºC (Lin and Sung, 2001). Pre-sowing wetting-drying treatments for enhancing germination and better seedling establishment have been proved very effective in seeds of a number of species (Hegarty, 1970). Mandal and Basu (1987) reported that pre-sowing hydration treatments improved field emergence and yield of wheat. Heydecker and Coolbear (1977) made an extensive survey of the work on pre-sowing wetting-drying treatments and concluded that the seeds could be invigorated successfully for improving seedling vigour and better crop stand. Greater uncertainties under changing climatic situation often combined with the use of poor quality seeds and crop cultivation in marginal lands lead to slow the germination and emergence, causing patchy stand as well as multiple and delayed replanting of mungbean. Seed priming is a simple, effective and climate-resilient technology for addressing this problem. It has been found that primed seeds emerge more quickly, produce more vigorous seedlings with better developed root systems, reach flowering and maturity earlier, and result in higher yield than non-primed seeds, which is important for avoiding terminal drought (Padgham, 2009). It is recommended that Moist Sand Conditioning for 12 h and Moist Sand Conditioning for 12 h followed by soaking for 1 hour along with hydro-priming for 0.5-4 hours may be adopted in mungbean for improving early germination and seedling growth.

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