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RESEARCH ARTICLE

Hydro-Priming Methods for Initiation of Metabolic Process and Synchronization of Germination in Mung Bean (*Vigna Radiata* L.) Seeds

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Abstract

Seed priming is a commercially applied technique for improving seed vigor under variable field conditions. The present study was carried out to optimize the methods (direct: soaking in water 1:1 W/V and indirect: preconditioning under high RH ≥ 85%) and duration (hours)of hydro-priming, attempting to correlate the critical seed water content with priming-induced metabolic-restart in dry mungbean (*Vigna radiata*L.)seeds. Although the rate of water absorption and the amount of water absorbed was more by direct compared to the indirect imbibition method, the priming-induced germination responses were identical after 6hof priming treatment. The seed water content absorbed during this period was crucial for initiating the chain of biochemical events, required for early synchronized germination in primed seeds which was evident by improved membrane permeability, high activity of catalase and superoxide dismutase, and more integrated chloroplast and mitochondria in primed seeds. We demonstrate that the critical water content, required for priming-induced benefits for germination vigor, is a relative unit with respect to the duration of priming and the rate of water absorption. Seed priming is a co-ordinately regulated mechanism for controlling germination capacity of seeds by modifying the permeability characteristics of biological membranes and enzyme activity. This study helps to enhance our understanding on the potential of seed priming for synchronized germination and early seedling establishment in the field and beneficial for the resource poor farming community for better return of their limited financial/farm resources under variable environmental/field conditions.

Key words : Hydro-priming, membrane permeability, mitochondrial/chloroplast-integrity, seed-water relations, synchronized germination

Introduction

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 vigor 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 (Ellis 1992).

One of the seed invigoration methods for rapid, uniform, and increased germination is post-storage priming technology. The priming was originally introduced in the context of polyethylene glycol (PEG) osmoticum treatments at water

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potentials just below full imbibition; these treatments resulted in more rapid germination on subsequent sowing (Heydecker et al. 1973). Therefore, seed priming is a controlled hydration technique that triggers the metabolic-restartduring early phase of germination before radicle protrusion (McDonald 2000). 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 antioxidative activity, and the repair processes are associated with the enhancement of germination processes through priming (Bailly et al.2000; Chen and Arora 2011; Rajjou et al. 2012; Sharma and Maheshwari 2015; Sharma et al. 2018).

In the last two decades, seed priming has been recognized as a common seed treatment to increase the rate and uniformity of emergence in many vegetable and flower species. Different types of priming methods such as hydro-priming, solid matrix-priming, and osmo-priming are in practice and are economically viable for increasing the crop yields at the field level (Bradford 1986; Heydecker and Coolbear 1977). Hydro-priming involves non-controlled water uptake, whereas other methods are associated with controlled water uptake by using osmotic solutions. In the triphasic model of water uptake by dry orthodox seed, phase I (rapid water uptake) and II (lag phase with least water uptake or the activation phase) represent the most delicate phases for the process of germination and are crucial for successful seed priming treatment (Bewley 1997). Although hydro-priming is the easiest and the most economical method, the major disadvantage of hydro-priming is the non-controlled water uptake that may proceed until radicle protrusion if the process is not stopped at a precise moment before phase III begins (rapid water uptake followed by radicle emergence). Hence, the most critical stage of hydro-priming is to determine the right amount of water required to hydrate seeds that initiates germination metabolism and carry outrepair processes while preventing the radicle emergence, i.e. the beginning of phase III. The disadvantages associated with other methods include the accumulation of salts in seeds that could determine toxicity when salts are used as osmoticumfor priming treatment (Bradford 1995). Although PEG is an inert material and is most widely used as a priming agent that prevents seed toxicity, the major drawback resulting from the use of PEG is the reduction ofoxygen availability in the solution and non-uniform aeration during priming operations because of its viscosity. Optimizing the aeration during priming is essential because seeds need air to germinate and lack of oxygen may adversely affect seed viability. Besides the relatively high cost and environmental hazards by PEG, its disposal is a serious practical constraint that restricts the use of PEG as priming agent at commercial and/or the farmer's level.

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. 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). It appears therefore that providing further clues on the methodsare the only way for reliable benefits of priming by the farming community. 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 labor 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 1999, 2000). Hence, it is more appropriate to optimize the water absorption patterns and the duration of priming for crops/genotypes of economic importance for making hydro-priming an economically viable and farmer-friendly technique.

Mung bean (*Vigna radiata* L.) 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 vigor 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, whether mung bean seeds are used for crop production and/or consumed as sprouts and little work on seed priming has been done for mung bean seeds. Apparently, potential benefits of priming can be achieved only when the seed-water relationship is properly understood for mungbean seeds. In this study, we have tried to study the quantitative aspects of water absorption during seed priming on four genotypes of mung bean with the objective (1) to optimize the duration and methods of hydro-priming techniques for metabolic re-start short of radicle emergence (2) to determine the genotypic differences with respect to priming responses and (3) to understand the cellular and biochemical mechanisms of hydro-priming in mung bean seeds.

Materials and Methods

Seed material

High-quality fresh seeds of four genotypes of mung bean viz. RMG 62, RMG 268, RMG 344, and MUM 2 were obtained from the mung bean breeder of Rajasthan Agricultural Research Institute (RARI), Swami Keshwanand Rajasthan Agricultural University, Jaipur (Rajasthan) India. The initial germination of these genotypes ranged between 94-98% and

the initial seed moisture ranged between 8.0 to 8.5% in different genotypes.

Seed priming

The seed priming treatment was conducted in two separate sets of experiments. The first set of experiments was conducted to study the seed-water absorption patterns of mung bean genotypes by using two methods of priming for different time intervals (2, 4, 6, 8, 24 h). The non-primed seed samples (0 days) were used as the untreated control. The first method of priming involved soaking of seeds directly in water in a beaker (1:1 W/V ratio) at room temperature (24 ± 1°C). The de-ionized distilled water was used for giving the hydro-priming treatment and uniform aeration was ensured throughout the priming treatment. Whereas for the second method of priming, seedswere equilibrated at 85% RH and temperature 24 ± 1°C in a pre-conditioned humidity chamber for similar durationas was used for the first method. The experiments were conducted in a completely randomized design with four replications. The fresh weight (FW) of each seed lot was recorded before giving the treatment. The primed seeds were removed after exposing them for the appropriate duration, the excess water was removed using filter paper, and thereafter the hydrated weight (HW) was recorded. The primed seeds were then dried at 80°C in a hot air oven until a constant weight was achieved and the dry weight (DW) was recorded. The percentage water absorption (HW-FW/HW*100) at each stage was calculated on hydrated weight basis after making corrections for the initial seed moisture for the respective genotype. The rate of water absorption was expressed as mg water g^{-1} fresh weight $hour^{-1}$.

The second experiment was conducted for biochemical/molecular estimations by subjecting seed-priming treatment for 6 h following both methods of priming (direct and indirect), because 6 h of priming was theoptimum duration for best germination responses irrespective, of the methods of priming and the genotypes (preliminary studies). A total of 100 g of seeds of each genotype were subjected to hydro-priming treatment for 6h using the direct priming method (1:1 W/V ratio in water) along with full aeration at 24±1°C temperature. The non-primed seed samples were used as the untreated control. The primed seeds were then re-dried at RH ≥ 35% and 24±1°C untilthe original moisture (~8%) was achieved and dried seeds were then stored and used for conducting the physiological/biochemical experiments.

Seed germination and vigor

Dry seeds, primed for 6 h by the direct and indirect priming methods were used for germination, seed vigor analysis, and for the measurement of electrical conductivity. The germination analysis was conducted on four replicates of 100 seeds each placed between moist paper towels in the dark as per the International Seed Testing Association (ISTA 2011). These moist paper towels were then kept in an electronically controlled growth chamber maintained at 90% relative humidity and 24±1°C temperature. Seeds with radicle length of 1 cm were considered as germinated and the number of seeds germinated was scored daily until a plateau was reached on day 7, indicating the completion of germination. Germination % data were arcsine-transformed and then were subjected to statistical analysis using the student t-test at $P<0.05$ level of significance.

After the final germination count, 10 seedlings were randomly selected treatment-wise and the length (cm) and fresh weight ((g) was measured. Ten seedlings were kept in a hot air oven at 80°C until the constant dry weight (g) was obtained. The percentage germination, vigor index I (Percentage germination X seedling length) and vigor index II (Percentage germination X seedling dry weight) were then calculated as per ISTA (2011) rules. The hard seeds were counted as germinated, while recording the germination count as per ISTA (2011) rules.

Electrical conductivity

The electrical conductivity (EC) was measured to determine the membrane permeability characteristics of seeds (Simon 1974). Dry seeds, primed for 6 h by the direct and indirect methods were used for the measurement of electrical conductivity of seeds as per the International Seed Testing Association (ISTA 2011). The experiment was conducted with four replications of 50 seeds for each treatment, including the untreated control (ISTA 2011). The primed and non-primed/control seeds were soaked in 50 ml de-ionized distilled water in a 100 ml flask and were then kept overnight at 20°C. The conductivity of leachates (dSm^{-1}) was measured using an automated conductivity meter, model CC-663, Century Pvt. Ltd. India.

Enzyme assay

Since both the methods of priming produced similar germination responses, dry seeds primed for 6h by the direct method were used for determining the activity of catalase (CAT) and superoxide dismutase (SOD) following the standard procedures.

Catalase (CAT) activity

CAT (EC 1.11.1.6) activity was determined spectrophotometrically at 240 nm with three replications following the method of Aebi (1984). Enzyme extract for catalase was prepared by homogenizing 0.5 g fresh seed tissue of the primed and non-primed/control samples of four genotypes in ice cold 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM ethylenediaminetetraacetic acid (EDTA) and 1% polyvinyl pyrollidone (PVP). The homogenate was filtered and then centrifuged at 4°C for 20 min at 20000x g using a cooling centrifuge (model CM-12, Remi Instruments Private Limited, Ahmedabad, India). The supernatant was used for enzyme assay. The reaction assay mixture contained 0.5 ml of enzyme extract and 2.0 ml of 0.1 M sodium phosphate buffer (pH 6.8). The reaction was initiated by the addition of 0.5 ml of 10 mM hydrogen peroxide and the absorbance was measured at 240 nm for 3 min at 30 s time interval using

Table 1. SSRs used for amplification of chloroplast and mitochondrial genome in mung bean genotypes in this study.

S. No.	Primer	Sequence	No. of base
1	pcVa1	F-CGCCGAAAAAGGAAGGAAAGG	21
		R-TGGATTTCGATGATATCAAATCGGA	25
2	pcVa2	F-TCGTACAATCAGCGTAATCCAGA	23
		R- TGAACTGACGCTTAACCTGGT	21
3	mtVa2	F-AACCTCCTGGAGAGAGCGAT	20
		R-AAACGAAAGCGCTAACGAGC	20
4	mtVa3	F-GTCGGGAGGGAAGGCTAGTA	20
		R-AGTCTTTCTCGCATGCGTGA	20

Nano-Drop (UV-Vis Spectrophotometer, model 2000 C, Thermo Scientific, USA). The total soluble protein in the crude extract was determined by the method of Lowry et al. (1951) and the specific activity of catalase was calculated and expressed as $\mu molmg^{-1}$ $proteinmin^{-1}$.

Superoxide dismutase (SOD) activity

SOD (EC 1.15.1.1) activity was measured spectrophotometrically at 420 nm with three replications following the method of MarklundandMarklund (1974). Enzyme extract for superoxide dismutase (SOD) was prepared by grinding 0.5 g fresh tissue of the primed and non-primed/control seed samples with 10 ml of chilled 0.1M potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA. The homogenate was filtered and then was centrifuged for 15 min at 20000x g using a refrigerated centrifuge (model CM-12, Remi Instruments private limited, Ahmedabad, India). The supernatant was used for enzyme assay. The reaction assay mixture contained 1 ml of enzyme extract in 2 ml sodium phosphate buffer (0.1 M). The enzyme reaction was initiated by adding 1.5 mlpyrogallol (3 mM) and the absorbance was measured at 420 nm using Nano-Drop (UV-Vis Spectrophotometer, model 2000 C, Thermo Scientific USA) for 3 min at 30 s time intervals. The enzyme specific activity was calculated and expressed as $\mu mol\ mg^{-1}protein\ min^{-1}$. The protein content in the enzyme extract was calculated by Lowry et al. (1951).

DNA isolation and mining of Simple Sequence Repeats (SSRs)

Dry seeds, primed for 6h by the direct method were used for the amplification of chloroplast and mitochondrial DNA. The DNA from the primed and non-primed/control seeds was isolated by the following modified DNA isolation protocol (Sharma et al. 2011).The chloroplast genomesequence (NC_013843; Tangphatsornruang et al. 2010) and mitochondrial genome sequence(NC_015121; Alverson et al. 2011) of *V. radiata* were retrieved in fasta and GenBank format from NCBI (National Centre for Biotechnology Information; www.ncbi.nlm.nih.gov). Mining of chloroplast and mitochondrial SSRs was carried out using MISA (http://pgrc.ipk-gatersleben.de/misa/). The identified sequence containing SSRs were used for designing primer pairs using Primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/primer3/input.htm) with default parameters of GC content, melting temperature, primer and PCR product size (Untergasser et al. 2012). 20 SSRs were synthesized with the help of Integrated DNA Technologies, Primer-Quest tool, Singapore (Table 1). DNA was quantified using Nano-Drop (UV-Vis Spectrophotometer, model 2000 C, Thermo Scientific, USA) and purity was checked by taken the absorption ratio at 260 and 280 nm. Only DNA samples with A260/280 ratio ranging from 1.8 to 2.0 in primed and non-primed/control samples were used for the experimentation. Each DNA sample was then diluted using nuclease-free water to make a final concentration of 100 $ng\mu l^{-1}$ for further analysis. The PCR amplification was then performed in DNA thermal cycler (Model CGI-96, Corbett Research, Australia) using the specific primer set following the method of Sharma et al. (2011).

Statistical analysis

The treatments were distributed in completely randomized design with four replications for measuring germination, vigor, water absorption, and electrical conductivity, and three replications for measuring enzyme activity. The germination percentage data were arcsine-transformed and then subjected to statistical analysis using student's t-test at 0.05% level of probability. The DNA amplification of chloroplast and mitochondria was conducted on two independent biological experiments replicated two times to ensure the stability of expression.

Results

Water absorption patterns

Although, the two methods of priming showed similar absorption patterns but followed significantly different absorption rates. Significantly, higher rate of water absorption was observed by the direct compared to indirect method. Nevertheless, at the end of priming duration (24 h) the rate of both the methods were identical, irrespective of the genotypes (Fig. 1). The peak rate of absorption was observed by 2h after priming by the direct method followed by a decline to a minimum level by the end of the priming duration (24 h). On

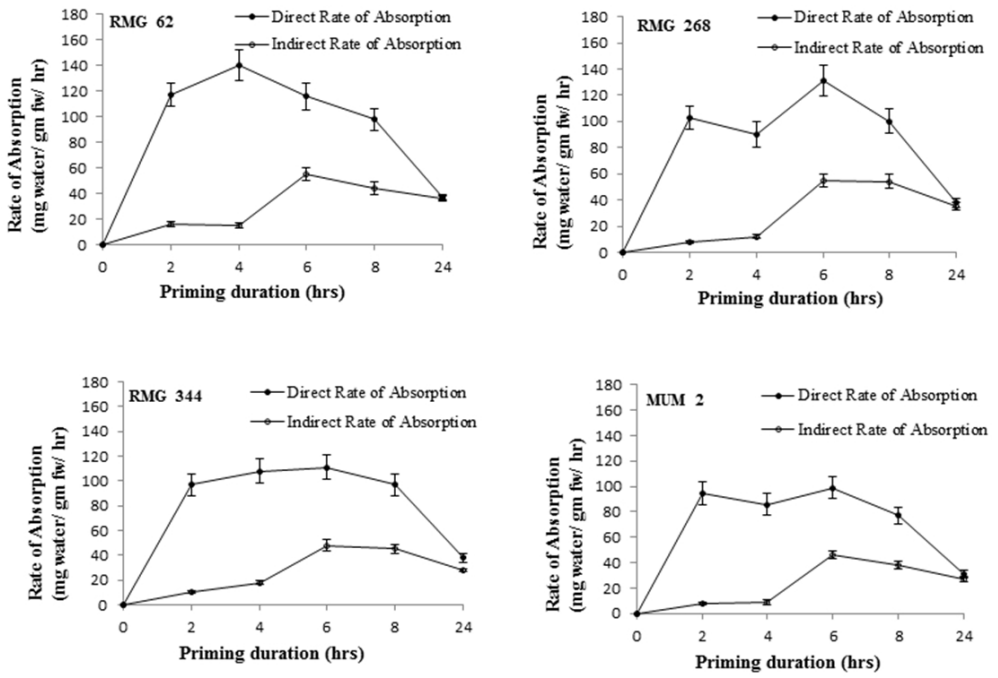


Fig. 1. The rate of water absorptionby direct (●) and indirect (○) hydro-priming methodsat 24±1°C temperature for different time intervals (0-24h) in four genotypes of mung bean seeds. Each value is a mean of four independent replications.

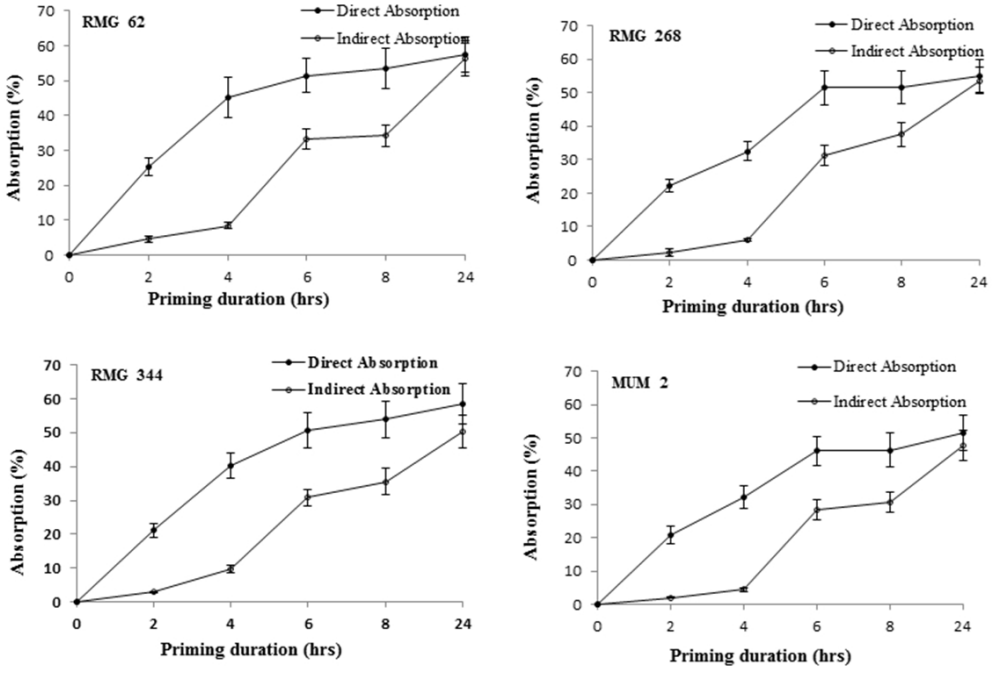


Fig. 2. The percentage of water absorption (hydrated weight basis) by direct (●) and indirect (○) hydro-priming methods at 24±1°C temperature for differenttime intervals (0-24h) in four genotypes of mung bean seeds. Each value is a mean of four independent replications.

the contrary, comparatively slow rate by the indirect method was observed during the initial period up to 2 h, reached to a peak value by 6 h and then declined to the same level by 24 h after priming.

The seed water content (%) was low initially by 2 hand increased linearly by 6 h followed by a slow uptake by 8 h and thereafter, there was a rapid increase by 24 h after priming, indicating a sigmoidal pattern of water absorption, irrespective of the methods of priming and the genotype. Although the two methods of priming showed similar absorption curves, there were significant genotypic differences in the water content absorbed by different methods of priming (Fig. 2).

MUM2 absorbed less water than that of others, irrespective of the methods of priming which might be because of the presence of 30% hard seeds in MUM2. However, the water content absorbed from 2 to 6h after priming, ranged between 26 to 29% in different genotypes, irrespective of the methods of priming, indicating that the lag/activation phase (phase II) of water uptake was independent of the methods of hydro-priming and the availability of water (Fig. 2). Similar sorption patterns were observed earlier by several workers (McDonald 2000; Sun et al. 1997).

Seed germination and vigor

Priming seeds for 6 h significantly improved the rate and uniformity germination of seeds compared to non-primed/control, irrespective of the methods of priming and the genotypes (Fig. 3). Germination was fast and immediate in primed seeds, showing approximately 90% germination by day 2 in all the genotypes, except MUM 2 in which 45% (indirect) to 78% (direct) germination was observed by day 2 after planting. The germination reached to ~100% by day 5 in primed seed samples, irrespective of the methods of priming in all the genotypes except, MUM 2 which showed 80 (indirect) to 90% (direct) germination by day 5 after planting. In non-primed/control seeds of RMG 62, RMG 268 and RMG 344; the initial germination was 20-50% by day 2 and increased gradually to 94-100% by day 5 with non-significance dif-

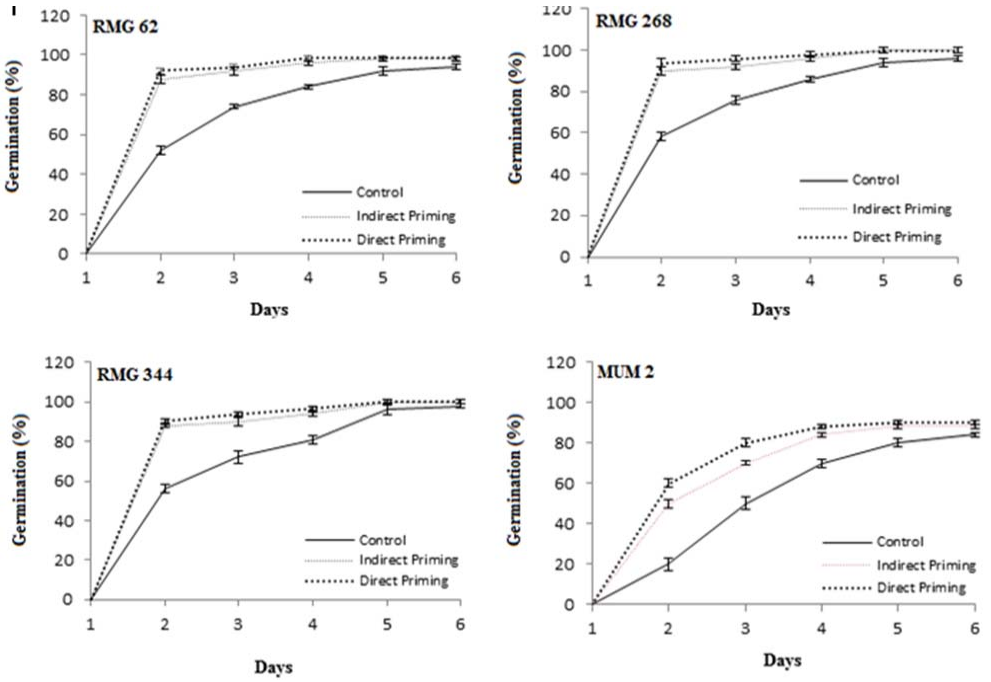


Fig. 3. Thegermination percentage of four genotypes of mung bean seeds subjected to 6 h hydro-priming treatment by indirect (⋯) and direct (⋯) methods at 24±1°C temperature. The non-primed seeds (—) represents the control. Each value is a mean of four independent replications. The vertical bar represents ±SD at 0.05 level of significance.The germination percentage data were arcsine-transformed and then subjected to statistical analysis using student's t-test at < 0.05% level of probability.

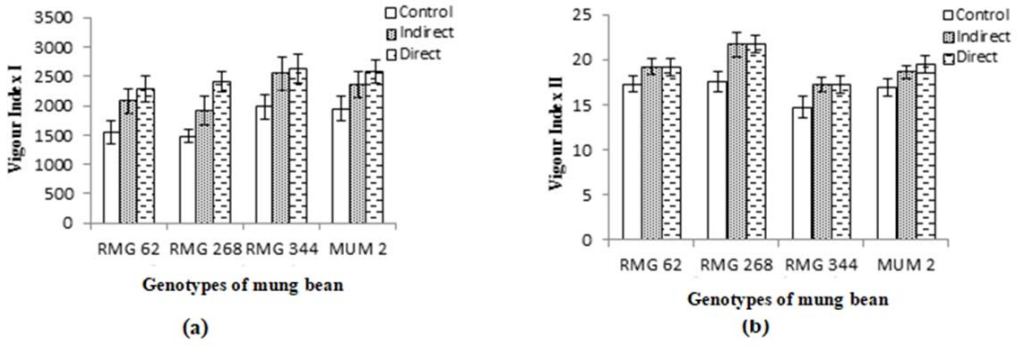


Fig. 4. The vigor index I and II of four genotypes of mung bean seeds subjected to 6 hhydro-priming treatment by indirect ▒ and direct ⋯ methods at 24±1°C temperature. The non-primed seeds □ represents the control. Each value is a mean of four independent replications. The vertical bar represents ±SD at 0.05 level of significance.

ferences among the genotypes. Whereas in MUM 2 control samples, the germination increased from 20% by day 2 to 80% by day 5 which was significantly lower than that of the remaining genotypes, indicating the genotypic variability in the germination potential (Fig. 3). Seed priming for 6h significantly improved vigor Index I (Percentage germination X seedling length) and vigor index II (Percentage germination X seedling dry weight), irrespective of the genotypes and the methods of priming (Fig. 4). These results agree with earlier reports on the beneficial effects of priming for improving germination and seed vigor (McDonald 2000).

Electrical conductivity

Results indicated that 6 h hydro-priming by either method significantly reduced the electrical conductivity of seeds compared to non-primed/control irrespective of the genotypes and the methods of hydro-priming, indicating the improvement in the membrane integrity with respect to the permeability properties of seeds. However, there was no difference in the seed leachates between the two methods of priming, suggesting that the different rates of water absorption had no adverse effect on the permeability characteristics of primed seeds (Fig. 5).

Enzyme activity

The activity of antioxidant enzymes (CAT and SOD) increased significantly in primed seed samples subjected to 6h of hydro-priming treatment compared to non-primed/control, irrespective of the genotypes, indicating that the activity of ROS scavenging enzymes was re-established by priming. However, significant genotypic differences were observed in CAT and SOD activity (Fig. 6).

Amplification of chloroplast and mitochondrial DNA

Primed mung bean seeds had more integrated mitochondria and chloroplast DNA compared to non-primed seeds, indicating early activation of these organelles in response to priming (Fig. 7). Although the evidenceis still fragmentary, there are reports that the repair of pre-existing mitochondria occurs upon imbibition (Grelet et al. 2005) and greater transcript peaks of mitochondria and chloroplast genes have been reported during imbibition of riceseeds (Howell et al. 2009).

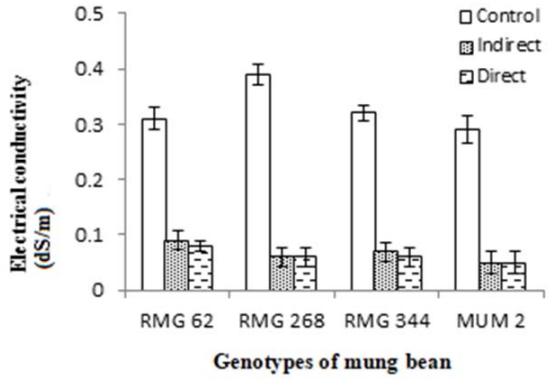
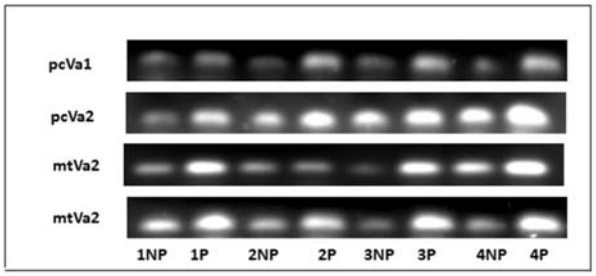


Fig. 5. The electrical conductivity (dSm^{-1})of four genotypes of mungbean seeds subjected to 6 h hydro-priming treatment by indirect ▒ and direct ⁝ method. The non-primed seeds □ represents the control.Each value is a mean of four independent replications. The vertical bar represents ±SDat 0.05 level of significance.



* NP= Non-Primed, P= Primed; 1=RMG 62, 2= RMG 268, 3= RMG 344, 4=MUM 2

Fig. 7. The integrity of chloroplast and mitochondria DNA for four genotypes of mung bean seeds (1=RMG 62, 2=RMG 268, 3=RMG 344, 4=MUM 2) subjected to hydro-priming treatment for 6h by the direct method. The non-primed/control is represented by (NP) and the primed is represented by (P). The PCR amplification of chloroplast and mitochondrial DNA was carried using specific SSRs (Table 1).

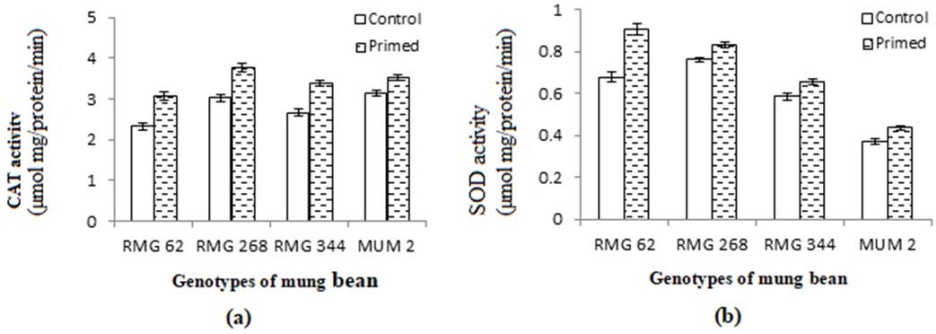


Fig. 6. The activity of antioxidant enzymes (µmol mg^{-1} $proteinmin^{-1}$) (a) catalase (CAT) and (b) superoxide dismutase (SOD) of four genotypes of mung bean seeds subjected to 6 h hydro-priming treatment by ⁝ direct method. The non-primed seeds □ represents the control. The values indicate the mean of three replications with SD ± at 0.05 level of significance.

Discussion

The available literature indicates that osmotic priming using PEG as an osmotic agent is widely used method of priming avoiding imbibitional membrane injury that might occur during rapid uptake of water, if seeds are soaked directly in water (Heydecker et al. 1973). 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 results of this study indicated that the promotive response of priming was independent of the method and the rate of water absorption, whether the hydro-priming was done by direct seed soaking (1:1 W/V ratio) or indirectly by keeping seeds in a conditioned atmosphere (RH ≥ 85%) at 24±1°C temperature. The former resulted higher rates of water absorption compared to the latter method (Fig. 1). Nevertheless, both the methods of priming resulted in synchronized and early germination and improved seed vigor compared to non-primed/control seeds in all the genotypes except MUM 2 which although it showed significantly improvement germination/vigor over the untreated control but had significantly lower germination and vigor compared to other genotypes (Figs. 3 and 4). However, the difference in germination capacity might be because of presence of 30% hard seeds in MUM2 which probably, in part, was also responsible for the less amount of water absorbed throughout the priming period (Fig. 2). The promotive response of post-storage priming treatment agrees with several studies carried out by previous workers (Heydecker et al. 1973; Probert et al. 1991; Yeh and Sung 2008).

Both the methods of priming followed different rates of water absorption depending upon the availability of water and the genotype, but showed a similar reverse sigmoidal curve (Fig. 2) without adversely affecting the priming-induced benefits of germination and vigor (Figs. 3 and 4). A similar pattern of absorption was obtained by others (McDonald 2000). We analyzed the water absorption by primed seeds 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 by the indirect method was too low (2 to 5%) initially by 2 h after priming compared to the direct method (21 to 25%) due to a significantly lower rate of water absorption in the former than by the latter method (Fig. 1). Although the seed water content by 6h after priming was still more by direct (46 to 51%) compared to indirect (28 to 33%) priming, the difference in the amount of water absorbed by the two methods of hydro-priming was reduced from ~20 times by 2h to ~2 times by 6 h after priming, indicating that a comparatively greater amount of water was absorbed by indirect priming, resulting in narrowing the difference in water content from 2 to 6 h after priming. The greater reduction in the amount of water absorbed by the direct compared to the indirect method from 2 to 6h after priming suggests that self-regulatory mechanisms exist to optimize the water requirement for priming responses by adjusting their respective rates of 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 2 h compared to seeds exposed in humid conditions which showed an increased rate of water absorption by 6 h after priming (Fig. 1). The water absorption was almost negligible (genotypic average indirect/direct: 3/1%) between 6-8 h after priming, suggesting that the priming period from 2 to 8 h represents phases I and II of water absorption patterns. The increased duration of priming resulted in a second rise of seed water absorption (Fig. 2) which was also associated with the seed coat rupture/radicle emergence, irrespective of the genotype and the methods (data not shown) by 24 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. At the end of the priming duration (24 h), the rates of water absorption by both the methods were at par, resulting in further narrowing the difference in the seed water content (genotypic average indirect/direct: 48/58%) between the methods (Figs. 1, 2). Since, very little water was absorbed between 6 to 8 h after priming; it was considered that priming duration for 6h was the optimum period for mung bean seeds. It is, therefore, suggested that the water absorbed during 6 h priming period with uniform aeration, irrespective of the method of water absorption, 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 salt leakage during the initial hours of priming compared to that of the indirect method. But the results indicated that except for genotypic differences, there was no significant difference in the final germination and the vigor index (Figs. 3 and 4) between the two methods of priming, indicating that the initial (2 h) higher rate of water absorption by direct priming had no adverse effect on the priming-induced germination responses. Nevertheless, the salt leakage upon direct seed soaking might be associated with a transient increase in the membrane permeability without any severe damage to seed quality. This contention agrees with Hoekstra and Van der Wal (1988) and is supported by the data that there was no significant difference in the electrical conductivity by 6h after priming in response to different rates of water absorption (Fig. 5). However, the salt leakage may be an important consideration for undertaking seed priming treatments by the direct soaking method when the initial seed moisture is considerably low.

It is therefore suggested that the critical water content required for priming-induced benefits for germination and seed vigor enhancement is a relative unit, independent of the priming methods but dependent on the duration of priming, which is governed by a self-regulated internal system through adjusting the rates of water absorption by seeds. Nevertheless, the critical water content may be species-specific because water

absorption is a physical process and is governed by the seed morphology, physiology, and water availability (McDonald 2000). The data support the contention of Ashraf and Foolad (2005) that the positive effects of seed priming on seed invigoration depends on priming duration. Hence, it is necessary to optimize the duration of priming for each crop species so that the water content should not be exceeded. Primed seeds exhibited slower rates of deterioration compared to non-primed mung bean seeds (Sharma et al. 2018). The over-priming may lead to the loss of desiccation tolerance and poor storage stability when such seeds are re-dried and stored. This might be the reason why differential responses were observed by different scientists on the longevity of primed seeds. Nevertheless, priming has been shown to be both beneficial (Probert et al. 1991; Yeh and Sung 2008) and detrimental (Tarquis and Bradford 1992) to subsequent longevity, but the issue is still debatable. The variable effects of pre-storage priming treatment in relation to seed longevity may be because of anaerobic conditions during priming treatments, priming duration, and/or the post-priming seed drying rates as well as the seed moisture during storage.

Many studies have indicated that a relatively short priming treatment is advantageous for extending the longevity and improving the vigor 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 (Vertucci and Leopold 1987). A significantly higher activity of CAT and SOD in primed compared to non-primed seeds (Fig. 6) indicates that there was an early induction of metabolic activities for supporting rapid and synchronized germination in primed seeds (Fig. 3). The increased activity of CAT and SOD 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 non-primed/control supports this contention (Fig. 5) 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 non-primed/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 (Fig. 7). 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 CAT and SOD and the improved integrity of chloroplast/mitochondria by priming treatments (Figs. 6 and 7) were in coordination for early metabolic restart and the synchronized germination in the primed seeds (Fig. 3).

It is concluded that the seed hydro-priming by direct seed soaking is as beneficial as by putting seeds indirectly in humid conditions for priming treatments and the priming effects are independent of the initial rates of water absorption, provided uniform aeration is ensured during the treatment. Nevertheless, duration of priming needs to be optimized for each crop before giving the priming treatment to a seed lot. The amount of water absorbed by 6h of priming is optimum for mung bean seeds for initiation of metabolic processes for enhanced and synchronized germination. It is recommended that hydro-priming by either method (direct/indirect) may be adopted by farmers for convenience without additional financial liability to optimize the benefits of their available resources for increasing the crop productivity under a wide variety of field conditions including biotic/abiotic stresses.

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