

## Enhancement of emergence potential and stand establishment of *Moringa oleifera* Lam. by seed priming

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**Abstract:** *Moringa oleifera* is a miracle tree that can be used in multiple ways, including as foodstuff, livestock fodder, a plant growth enhancer, or a water purifier. While the stem cutting method is easy and successful for tree propagation, the recent introduction of moringa as a field crop for biomass production requires propagation through seeds. The germination of stored moringa seeds is a problem of great concern that may be overcome by employing seed priming techniques. A pot study was conducted to explore the potential of seed priming in moringa. Seeds were subjected to hydropriming, matriming, and priming with moringa leaf extract (MLE) for 8, 16, and 24 h. Most of the priming strategies enhanced the emergence rate, synchronized the emergence, and improved seedling vigor. However, hydropriming (8 h) was more effective in improving emergence, shoot vigor, and chlorophyll b contents, while MLE priming (8 h) produced vigorous roots and increased the chlorophyll a and mineral contents of moringa leaves. Both of these priming sources are natural, cheap, environmentally friendly, and easily adaptable for farmers to grow *Moringa oleifera* from seed.

**Key words:** Chlorophyll, emergence, hydropriming, matriming, moringa, seedling vigor

### Introduction

*Moringa oleifera* Lam., called moringa and native to India and Pakistan, is being populated widely around the world, especially throughout the tropical belt (Makkar and Becker 1997; Olson and Carlquist 2001). Moringa leaves contain protein, fiber, calcium, phosphorous, potassium, sulfur, iron, ascorbic acid, carotene, choline, thiamine, riboflavin, nicotinic acid, and a complete amino acid profile in a sufficient amount (Bau et al. 1994; Sarkar and Peace 1994). Its fresh leaves, containing 19.3% to 26.4% crude

protein (Aregheore 2002), are essential for livestock and can be used to solve worldwide malnutrition or undernourishment problems (Thurber and Fahey 2009). Moringa has the potential to combat vitamin A and other micronutrient deficiencies (Babu 2000; Nambiar 2006). The high proportions of minerals and vitamins in moringa leaves suggest its importance for both human beings and animals.

Moringa is propagated sexually through seeds and vegetatively through stem cuttings (Palada and Chang 2003). Recently, moringa is being exploited as

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a fodder crop in many parts of the world (Sanchez et al. 2006). No doubt, vegetative propagation of moringa is easy, but its biomass production as livestock fodder requires propagation through seeds. Its final germination percentage ranges between 60% and 90% for fresh seeds (Jahn et al. 1986) and it germinates at between 7 and 30 days after sowing (Sharma and Raina 1982). Moringa cultivation through seed is problematic because the seeds usually start losing viability within 2 months of storage. Sharma and Raina (1982) reported 60%, 48%, and 7.5% germination of moringa seeds sown after 1, 2, and 3 months of seed collection. Old moringa seeds have germination problems due to their high oil content and insect attacks. There is a dire need to explore ways to overcome germination problems in moringa seeds.

Various priming strategies have been developed to invigorate seeds, like hydropriming, osmopriming, matripriming, hardening, osmohardening, and hormonal priming (Basra et al. 2005 and 2006; Afzal et al. 2008). The priming treatments reduce emergence time and increase final emergence percentage and emergence index (Farooq et al. 2005, 2006b, 2008). These also synchronize seed emergence, which results in uniform stand and improved yield (Du and Tuong 2002; Harris et al. 2002). Tedonkeng et al. (2004) reported that the germination of older moringa seeds can also be increased by soaking in water but no comprehensive research study had been previously published on exploring the effect of different seed priming techniques on emergence, seedling vigour, chlorophyll contents and mineral quality of moringa.

Moringa leaf extract (MLE) is rich in cytokinin and its application as a seed priming tool was recently exploited on range grasses by Nouman et al. (2012). Its foliar application has also been reported as a high-yielding plant growth promotion agent in melon, peanut, corn, sorghum, onion, and sugarcane (Foidl et al. 2001). However, the physiological and biochemical aspects of using MLE as a priming agent need to be explored. The present study was conducted hypothesizing that the emergence problem of moringa seeds can be overcome by implementing seed priming, and with the objective of exploring the best priming tool and the optimum priming duration.

## Materials and methods

### Seed material

Moringa seeds were collected from a local tree at the University of Agriculture in Faisalabad, Pakistan, in May 2010. Initial germination of the moringa seeds was tested by placing the seeds between 2 layers of moist Whatman filter paper No. 1 in petri plates. The pretreatment germination percentage of the moringa seeds was 54.33%.

### Plant material

Fresh, mature moringa leaves were collected from mature moringa trees (approximately 6 years old) at the University of Agriculture in Faisalabad, Pakistan. After collection, 200 g of leaves were washed and stored overnight at freezing temperatures, which yielded 125 mL of MLE. The extracted MLE was sieved through cheesecloth and diluted 30 times with distilled water to prepare a 1:30 solution of MLE (Foidl et al. 2001; Nouman et al. 2012). Shelled moringa seeds were used for priming, and the ratio of the seed weight to soaking solution volume was kept at 1:5.

### Seed treatments

The priming strategies employed were hydropriming (seeds were soaked in distilled water), matripriming (seeds were kept between 2 layers of jute mat saturated with distilled water), and priming with MLE (1:30), each for 8 h, 16 h, and 24 h. Except for in the matripriming, fresh air was continuously supplied throughout the soaking period. After the treatment application, seeds were washed with distilled water and redried to their original weight under shade at  $27 \pm 3$  °C (Basra et al. 2002). Unprimed (control) and dehulled seeds were also used as treatments.

### Seedling emergence and vigor evaluation

The present experiment was conducted in a controlled greenhouse at  $32 \pm 3$  °C, with 16-h daylight and 8-h night conditions. Unprimed (control) and primed seeds were sown in acid-washed, sand-filled plastic pots (20 seeds per pot) in a completely randomized design with 3 replications. The seeds were watered on alternate days, but after emergence, Hoagland solution was applied once to the seedlings to provide nutrients. Emerged seeds were counted daily until a constant count was reached, according to the

rules of the Association of Official Seed Analysts (AOSA 1990). The angular transformation of the final emergence percentage (FEP) was calculated according to the formula ( $\arcsin\sqrt{\text{FEP}}$ ). The amount of time taken to reach 50% emergence ( $E_{50}$ ) was calculated according to the following equation (Farooq et al. 2005),

$$E50 = t_i + \frac{(\frac{N}{2} - n_i)(t_j - t_i)}{n_j - n_i}$$

where  $N$  is the final number of seeds emerged and  $n_i$  and  $n_j$  are the cumulative numbers of seeds that emerged by adjacent counts at times  $t_i$  and  $t_j$ , respectively, when  $n_i < N/2 < n_j$ .

Mean emergence time (MET) was calculated according to the equation of Ellis and Roberts (1981) as:

$$MET = \frac{\sum Dn}{\sum n}$$

where  $n$  is the number of seeds that emerged on day  $D$  and  $D$  is the number of days counted from the beginning of germination.

Emergence index (EI) was calculated by the formula given by the Association of Official Seed Analysts (AOSA 1983), as described below.

$$EI = \frac{\text{No. of emerged seeds}}{\text{Days of first count}} + \frac{\text{No. of emerged seeds}}{\text{Days of final count}}$$

In each replication, 3 seedlings were randomly selected for seedling vigor evaluation. The mean values of the 3 plants per replication were computed for statistical analyses. Seedling growth in terms of growth characteristics such as shoot and root length, number of leaves per plant, and root and shoot fresh and dry weights were recorded 20 days after sowing. Fresh root and shoot biomass were weighed immediately after harvesting. The roots were uprooted gently and sand particles were removed to obtain precise root weight. The shoots and roots were dried in oven at 70 °C until they reached a constant dry weight.

#### Crude protein analysis

Dried and ground moringa leaves (5 g) were digested in sulfuric acid with a mixture of  $K_2SO_4$ ,  $CuSO_4$ ,

and  $FeSO_4$  (10:5:1) with a micro-Kjeldahl apparatus according to the method described by Chapman and Pratt (1961) for nitrogen digestion, distillation, and quantification. Crude protein was calculated by multiplying the nitrogen content by 6.25.

#### Wet digestion

Moringa leaves were oven dried at 60 °C to a constant weight and ground to pass through a 2-mm sieve. The samples (0.5 g) were digested using the wet digestion method with concentrated nitric acid (10 mL) and concentrated  $HClO_4$  (5 mL). After digestion, they were filtered through Whatman filter paper No. 1 and a volume of 100 mL was obtained by adding distilled water (Rashid 1986).

#### Mineral (Ca and K) analysis

A flame photometer with a potassium filter (Jenway PEP-7, Jenway, UK) was used to determine K levels in diluted extracts of plant material (Chapman and Pratt 1961). Calcium was determined by using an atomic absorption spectrophotometer (Hitachi Z-8200, Hitachi, Japan).

#### Chlorophyll a and b and $\beta$ -carotene determination

A chlorophyll ( $a$  and  $b$ ) and  $\beta$ -carotene detection method, described by Nagata and Yamashita (1992), was used to quantify photosynthetic pigments concentration. Moringa leaf samples of 1 g were ground in 10 mL of 80% acetone and filtered through Whatman filter paper No. 1. The filtered extract was poured into a cuvette and absorbance was noted at 663, 645, 505, and 453 nm using a UV spectrophotometer (UV-4000, O.R.I., Germany). The following formulae were used to calculate chlorophyll  $a$ , chlorophyll  $b$ , and  $\beta$ -carotene.

$$\text{Chlorophyll } a = 0.999A_{663} - 0.0989A_{645}$$

$$\text{Chlorophyll } b = -0.328A_{663} + 1.77A_{645}$$

$$\beta\text{-Carotene} = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453}$$

#### Statistical analysis

The experiment was conducted twice and analyses of variance of the data from each attribute were computed using MSTAT-C (MSTAT Development Team 1989). A least significant difference (LSD) test at a 5% level of probability was used to test the differences among mean values (Steel et al. 1997).

**Results**

The emergence and seedling vigor were significantly ( $P < 0.01$ ) affected by the various priming treatments (Table 1). Hydropriming (8 and 16 h) and MLE priming (8 h) resulted in early seed emergence as compared to the control. The minimum time computed for MET and  $E_{50}$  was recorded when the seeds were subjected to hydropriming for 8 and 16 h (Table 1). An increase of 72% and 56% in FEP was observed when the moringa seeds were subjected to hydropriming for 8 and 16 h, respectively. MLE priming (8 h) increased FEP by 34.2%, which was statistically on par with matrimpriming (8 h). Maximum EI was also found in the seeds subjected to hydropriming (8 h), and this was statistically on par with hydropriming (16 h). Furthermore, hydropriming (24 h) was statistically similar to matrimpriming (8 and 24 h).

Hydropriming (8 h) was the most effective treatment for improving the shoot vigor of moringa seedlings, while MLE priming (8 h) effectively increased root vigor as compared to the control and the other treatments. An increasing effect on moringa seedling vigor was also recorded with the other

priming treatments at longer durations as compared to the control, but priming for 8 h maximally improved seedling vigor. Maximum shoot length was recorded in seeds subjected to hydropriming for 8 h followed by MLE priming for 8 h, which was statistically similar to hydropriming (16 h) and MLE priming (16 h). Maximum root length was recorded after priming with MLE (8 h) as compared to the control, which was statistically on par with hydropriming (8 and 16 h) and MLE priming (16 and 24 h). Maximum fresh and dry shoot weights were also recorded in seedlings raised from seeds hydroprimed for 8 h followed by matrimpriming (16 h) in comparison to nonprimed seeds. Maximum fresh and dry root weights were recorded in seeds primed with MLE (8 h) followed by MLE priming (16 h) and hydropriming (8 h) (Table 2).

Dehulling of moringa seeds, hydropriming (16 and 24 h), matrimpriming (8 and 16 h), and MLE priming (8 and 24 h) all significantly ( $P < 0.01$ ) increased crude protein in moringa leaves as compared to the control (Figure 1), while calcium and potassium contents were significantly ( $P < 0.01$ ) improved by

Table 1. Effect of seed priming techniques on final emergence percentage (FEP), angular transformation of FEP in degrees ( $^{\circ}$ ), emergence index (EI), mean emergence time (MET), and time taken for 50% emergence ( $E_{50}$ ) of *M. oleifera*.

Treatment	FEP		EI	MET (days)	$E_{50}$ (days)
	%	$^{\circ}$			
Control	58.33	49.80 d	8.167 c	9.34 a	6.69 a
Dehulling	66.67	54.89 cd	10.98 bc	8.95 ab	6.06 ab
Hydropriming (8 h)	98.33	85.69 a	27.57 a	7.88 f	3.22 d
Hydropriming (16 h)	93.33	77.71 ab	23.72 a	8.07 ef	3.42 cd
Hydropriming (24 h)	75.00	60.07 cd	16.69 b	8.36 cdef	4.31 cd
Matrimpriming (8 h)	81.67	65.00 bc	16.26 b	8.63 bcde	4.88 bc
Matrimpriming (16 h)	70.00	54.84 cd	11.55 bc	8.92 abc	4.98 bc
Matrimpriming (24 h)	71.67	58.07 cd	16.21 b	8.31 def	3.85 cd
MLE priming (8 h)	78.33	66.84 bc	14.98 b	8.80 abcd	4.94 bc
MLE priming (16 h)	58.33	49.90 d	13.21 bc	8.37 cdef	4.58 bcd
MLE priming (24 h)	68.33	55.77 cd	13.05 bc	8.67 bcd	4.92 bc
LSD 5%		14.188	5.721	0.5667	1.622

Means not showing the same letters in a column differ significantly at a 5% probability level.

Table 2. Effect of seed priming techniques on seedling growth of *M. oleifera*.

Treatment	Shoot length (cm plant <sup>-1</sup> )	Root length (cm plant <sup>-1</sup> )	Shoot fresh weight (g plant <sup>-1</sup> )	Shoot dry weight (g plant <sup>-1</sup> )	Root fresh weight (g plant <sup>-1</sup> )	Root dry weight (g plant <sup>-1</sup> )
Control	18.97 d	1.49 e	2.79 d	0.84 f	2.94 e	0.89 f
Dehulling	22.01 cd	3.16 cd	3.84 cd	1.15 e	4.08 de	1.22 e
Hydropriming (8 h)	30.78 a	4.68 b	6.21 a	1.86 a	6.07 bc	1.82 bc
Hydropriming (16 h)	26.27 b	4.73 b	4.75 bc	1.48 bc	5.05 cd	1.52 d
Hydropriming (24 h)	24.16 bc	3.72 bc	4.26 bc	1.28 de	5.32 cd	1.61 cd
Matrimpriming (8 h)	20.19 d	3.9 bc	4.52 bc	1.36 cd	4.8 cd	1.44 de
Matrimpriming (16 h)	23.69 bc	2.5de	5.12 ab	1.54 b	5.27 cd	1.58 cd
Matrimpriming (24 h)	24.21 bc	3.10 cd	4.35 bc	1.31 d	5.13 cd	1.59 cd
MLE priming (8 h)	26.58 b	6.11 a	4.54 bc	1.36 cd	7.62 a	2.29 a
MLE priming (16 h)	26.07 b	4.48 b	4.95 bc	1.49 bc	6.82 ab	2.06 ab
MLE priming (24 h)	21.91 cd	4.65 b	4.65 bc	1.4 bcd	5.91 bc	1.76 c
LSD 5%	3.257	1.087	1.248	0.1588	1.360	0.241

Means not showing the same letters in a column differ significantly at a 5% probability level.

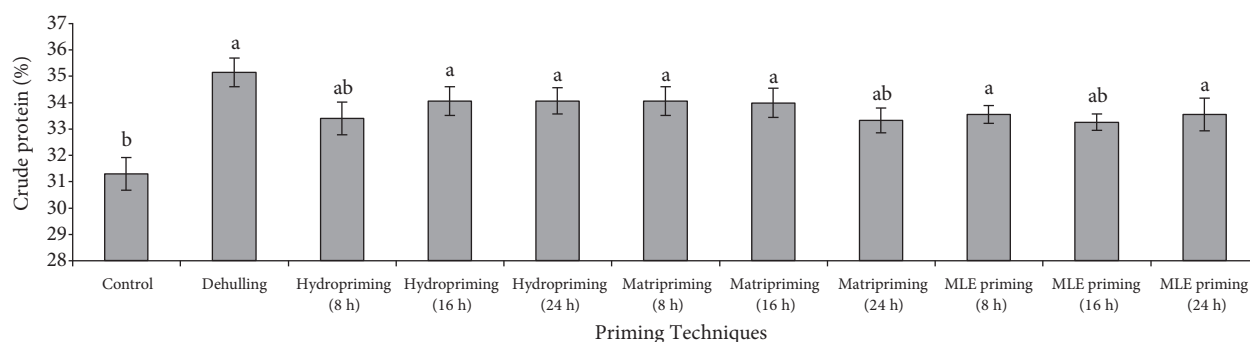


Figure 1. Effect of seed priming on crude protein contents (%) of *M. oleifera*. Vertical bars represent standard errors. Means not showing the same letters differ significantly at a 5% probability level.

MLE priming (8 h) followed by hydropriming (8 and 16 h) (Figures 2 and 3). Hydropriming (16 h), matrimpriming (8 h), and MLE priming (16 and 24 h) were statistically on par with each other in terms of improving calcium contents.

Chlorophyll *a*, chlorophyll *b*, and  $\beta$ -carotene contents were also significantly ( $P < 0.05$ ) affected by seed priming. Maximum chlorophyll *a* contents were recorded in moringa leaves when the seeds were

primed with MLE priming (8 h), followed by MLE and hydropriming (16 h), while maximum chlorophyll *b* contents were recorded in moringa leaves when seeds were subjected to hydropriming (8 h), followed by MLE priming (8 h) (Figure 4).  $\beta$ -Carotene contents were increased by MLE and hydropriming (8 h). Hydropriming and matrimpriming (24 h) did not change  $\beta$ -carotene contents, which were statistically similar to those of unprimed seeds (Figure 5).

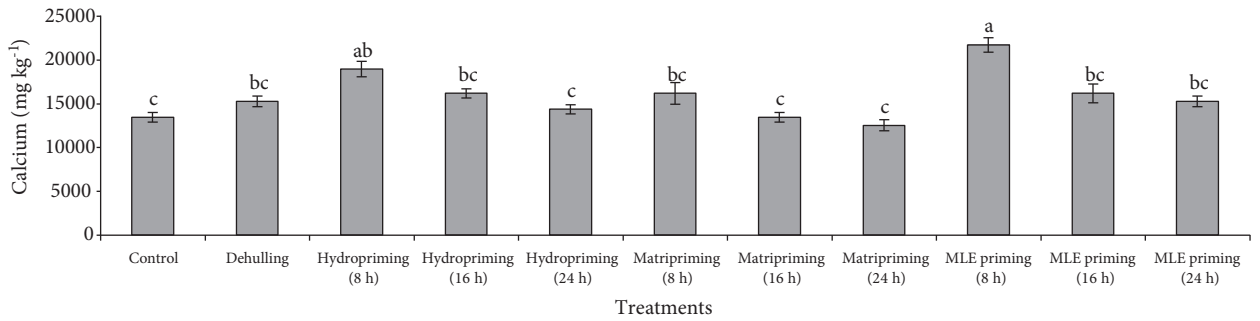


Figure 2. Effect of seed priming on calcium contents (mg kg<sup>-1</sup>) of *M. oleifera*. Vertical bars represent standard errors. Means not showing the same letters differ significantly at a 5% probability level.

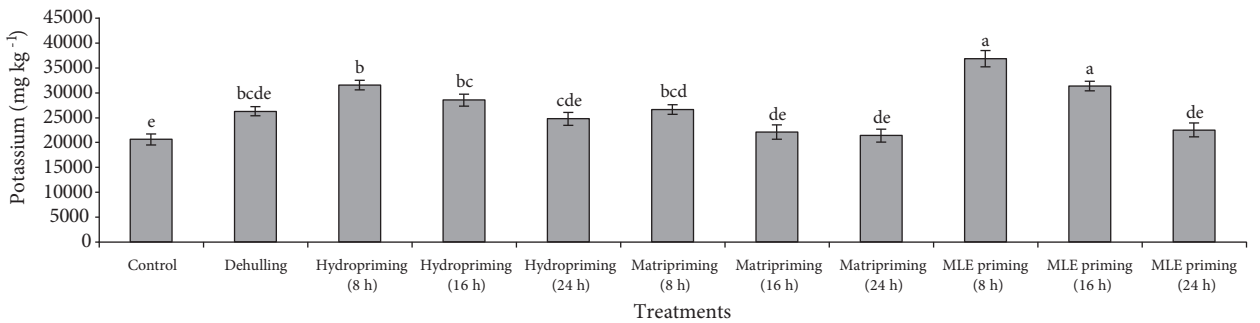


Figure 3. Effect of seed priming on potassium contents (mg kg<sup>-1</sup>) of *M. oleifera*. Vertical bars represent standard errors. Means not showing the same letters differ significantly at a 5% probability level.

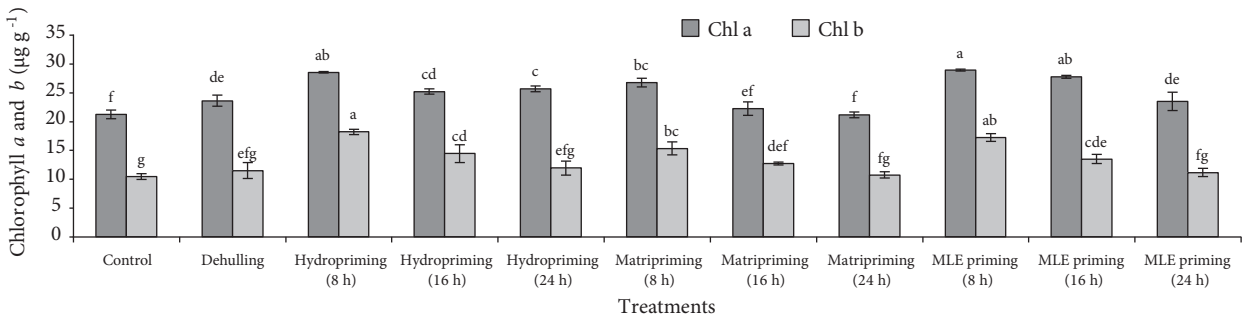


Figure 4. Effect of seed priming on chlorophyll *a* and *b* contents (µg g<sup>-1</sup>) of *M. oleifera*. Vertical bars represent standard errors. Means not showing the same letters differ significantly at a 5% probability level.

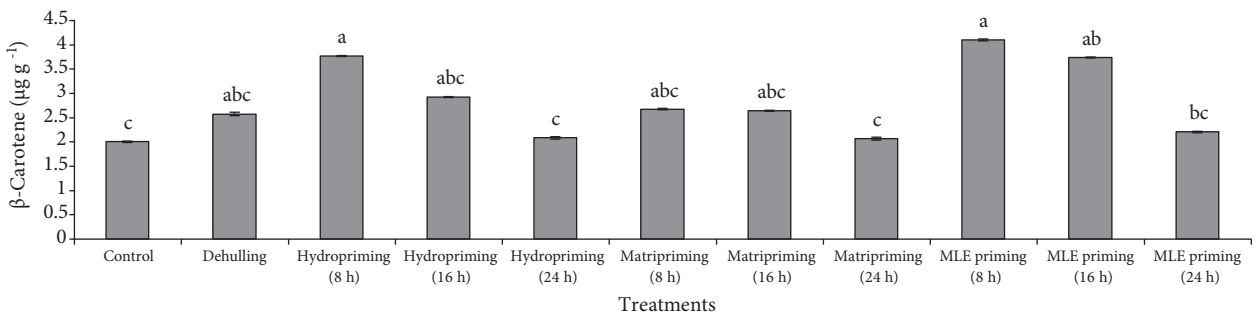


Figure 5. Effect of seed priming on β-carotene contents (µg g<sup>-1</sup>) of *M. oleifera*. Vertical bars represent standard errors. Means not showing the same letters differ significantly at a 5% probability level.



## Discussion

Seed priming improves germination and stand establishment and induces tolerance against adverse conditions like abiotic stress, especially during emergence and early seedling growth. It also increases the yield of most field crops (Harris and Jones 1997), vegetables (Welbaum et al. 1998), fruits (Basra et al. 2007), and tree species (Brancaion et al. 2008). Moringa seeds are not an exception, and it was reported that 12 h of hydropriming increased the germination of moringa seeds collected from Nicaragua and Kenya (Tendonkeng et al. 2004). Hydropriming, matricpriming, and hormonal priming are effective approaches for accelerating and synchronizing seed emergence and improving plant vigor (Hurly et al. 1991; Wu et al. 1999; Farooq et al. 2006b). The present study shows that hydropriming is a more effective priming strategy as compared to other treatments to improve early emergence, shoot vigor, and chlorophyll *b* contents, while MLE priming increased root biomass and chlorophyll *a*,  $\beta$ -carotene, and mineral contents. Hydropriming for 8 h not only enhanced the emergence speed, as exhibited by lower MET and  $E_{50}$  values, but also increased the FEP and EI values in the present study. The early and synchronized emergence might be attributed to increased metabolic activity in the treated seeds (Basra et al. 2005). Hydropriming further increased the shoot length and fresh and dry weight of moringa seedlings, while MLE priming increased the root length and fresh and dry weights. Morton (1991) reported that moringa seedlings gained a height of 20 to 30 cm after 6 weeks of sowing, while in the current experiment, moringa seedlings achieved a height of 30 cm after 20 days of sowing by hydropriming (8 h). Bose and Mishra (1992) also reported that hydropriming resulted in increased multiplication of shoot tip cells. MLE priming (8 h) was more effective in increasing root attributes, followed by hydropriming (8 h). MLE is rich in calcium, potassium, ascorbate, zeatin, auxins, and many phenolic compounds that are responsible for enhancement of plant growth and development (Fuglie 1999; Nagar et al. 2006). This study argues that MLE can also be used as an effective priming tool to increase the root development of moringa seeds. It might affect the increase in cell division within the apical meristem (Farooq et al. 2008). A high level

of root development is an indicator of good plant health. Vigorous root development is a characteristic of the climax vegetation phases in which plants can compete more effectively in the absorption of water and soil nutrients, resulting in good plant health (Allaby 1998).

Maximum chlorophyll *a* and *b* contents were recorded in moringa seedlings when seeds were primed with MLE priming (8 h) and hydropriming (8 h), respectively. Seeds that were hydroprimed and MLE-primed for the same duration also showed better plant growth, which predicts a correlation between plant growth and chlorophyll contents. Ayumi et al. (2004) studied the relation between chlorophyll metabolism and plant growth. It has been found that higher chlorophyll contents indicate maximum production of chemical energy and plant metabolism, which improves plant growth. MLE priming was more effective in improving the mineral contents of moringa leaves. Almost 2-fold calcium and potassium contents were recorded when the seeds were primed with MLE. Harris et al. (2001) reported high mineral contents in different crops due to seed priming. It can be concluded here that MLE is more effective in improving the root biomass and mineral contents of moringa leaves. Farooq et al. (2007) also found a positive correlation between root biomass and mineral contents in rice.

In the present study, dehulling of moringa seeds did not significantly affect the germination rate and seedling growth. It was also found that matricpriming improved the emergence and seedling vigor compared to unprimed and dehulled seeds, but it was not very effective as compared to hydropriming and MLE priming. Priming for longer durations did not affect emergence and seedling vigor. Ong and Monteith (1984) found good germination results when sorghum seeds were soaked for 8 h, while Lee and Kim (1999 and 2000) and Farooq et al. (2006a) reported poor and late germination when the seeds were subjected to longer priming durations. Water prehydration priming (hydropriming) is also an effective tool to induce tolerance in moringa seeds against salt stress (Santos et al. 2011). These findings support the current findings that longer priming durations may affect germination and plant vigor. The present study provides good information about

overcoming moringa seed germination problems and also gives new approaches, like MLE priming, to replace current seed priming techniques.

This study concludes that *M. oleifera* is not an exception to the possibility of increasing emergence rate and vigor by using a seed priming technique. Hydropriming (8 h) effectively improved the emergence, shoot biomass, and chlorophyll *b*

contents while root biomass, chlorophyll *a* contents, and mineral contents were effectively improved by MLE priming (8 h), and both priming strategies were effective in improving  $\beta$ -carotene contents. These 2 approaches (hydropriming and MLE priming) are organic, inexpensive, and environment friendly techniques that require little input but give good results.

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