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Moringa oleifera leaf extract: An innovative priming tool for rangeland grasses

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Abstract: *Moringa oleifera* leaf extract (MLE) is rich in amino acids, ascorbate, zeatin, minerals, and many other compounds known for their growth-promoting potential. This study was planned to explore the potential of MLE as a seed priming agent to increase the germination rate and plant vigor of 3 range grasses, i.e. *Cenchrus ciliaris*, *Panicum antidotale*, and *Echinochloa crusgalli*. The priming strategies used were hydropriming, $CaCl_2$, PEG-8000 (–1.1 M Pa), MLE (concentrate; 1:10, 1:20, 1:30, and 1:40 dilutions with distilled water), and matripriming using saturated jute mat for 24 h. The primed seeds were placed between 2 moist Whatman No. 1 filter papers in petri plates to evaluate germination and seedling vigor. Though all of the priming strategies excelled as compared to the control, matripriming and priming with 30× diluted MLE (1:30) were the most effective; thus, they were selected for further experimentation. In the second phase, these optimized priming strategies were used along with hydropriming and an unprimed control in pot studies. Both matripriming and MLE (1:30) priming significantly increased the germination. Matripriming was more effective in increasing the number of leaves, number of tillers, and shoot vigor for *Cenchrus ciliaris* and *Panicum antidotale*, but roots were more vigorous when primed with MLE (1:30). In the case of *Echinochloa crusgalli*, the results were reversed: the number of leaves, number of tillers, and shoot vigor were improved when primed by MLE (1:30), whereas matripriming maximally increased the root length and weight. It was concluded that both MLE (1:30) and matripriming with jute mat can be effectively used as priming agents for these rangeland grasses. Both strategies are low-cost, environmentally friendly, and can easily be adapted by farmers and range managers.

Key words: *Cenchrus ciliaris*, *Echinochloa crusgalli*, matripriming, moringa leaf extract, *Panicum antidotale*

Introduction

Moringa oleifera is a highly nutritive multipurpose plant grown for fresh vegetable, livestock fodder, green manure, biogas, medicine, biopesticide, and seed production (Fuglie 1999). Moringa leaf extract (MLE), being rich in amino acids, K, Ca, Fe, ascorbate, and growth regulating hormones like zeatin, is an ideal plant growth enhancer (Makkar and Becker 1996; Basra et al. 2009a, 2009b). Such plant growth promoters influence plant growth in several ways and also promote defense mechanisms against abiotic stresses by harmonizing the plant growth regulator's (PGR) endogenous concentration. Plant growth promoters are usually used as a foliar application or a seed-priming agent.

Seed priming is a pregermination treatment in which seeds are held at low water potential to allow imbibition by preventing radicle extension (Bradford

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1986). Different techniques have been carried out for seed priming, like hydropriming, osmopriming, matripriming, hardening, osmohardening, and hormonal priming (Hardegree and Emmerich 1992a, 1992b; Basra et al. 2004, 2005), to improve the germination rate, field emergence, seedling vigor, stand establishment, and economic yields in many crops (Du and Tuong 2002; Harris et al. 2002; Farooq et al. 2008), and to induce tolerance against biotic and abiotic stresses (Senaratna et al. 2000; Shakirova et al. 2003). Seed priming techniques are successfully being used for cereals, vegetables, and range grasses, and a number of PGRs, such as auxins (IAA, IBA, NAA), gibberellins (GA_3), cytokinins (kinetin, zeatin, benzyl adenine), salicylic acid, and ethylene, are also being used as priming agents (Ashraf et al. 2008). Zeatin is the most naturally occurring cytokinin that not only promotes the growth of plants but also has antiaging potential and protective effects in plants (Marcu 2005).

Being rich in zeatin, ascorbic acid, vitamin E, phenolic compounds, and minerals (Makkar and Becker 1996; Fuglie 1999; Foidl et al. 2001; Nagar et al. 2006), MLE has the potential to promote plant growth; hence, it is used as a natural plant growth enhancer. Zeatin plays an important role in cell division and cell elongation (Taiz and Zeiger 2006). Seed priming with diluted MLE has been reported to effectively improve germination and seedling growth in maize (Iftikhar 2009) and sunflower (Basra et al. 2009a). Ascorbic acid priming and foliar application have been reported to be growth- and yield-improving tools in various crops, especially under saline conditions (Jyotsna and Srivastava 1998). Calcium and potassium play essential roles in crop growth and development through osmoregulation, enzyme activation, photosynthesis, and various other physiological processes (Hasegawa et al. 2000; Epstein and Bloom 2005).

Range grasses have poor vegetation cover and plant density under field conditions due to poor seed germination. Incorporating useful seed priming agents can enhance the germination capacity in range species (Hardegree and Emmerich 1992a, 1992b). Matripriming (by using cellulose cups) and PEG-8000 have proved most effective in range grass priming (Hardegree 1996), but these techniques are expensive and difficult to adopt by the farmers. The present study was designed to introduce organic and inexpensive seed priming tools like moringa leaf extract (MLE) and jute mat as natural and easily adaptable priming agent sources to improve germination and seedling growth in common range grasses *Cenchrus ciliaris*, *Panicum antidotale*, and *Echinochloa crusgalli*.

Materials and methods

Seed material

Seeds of 3 range grasses, *Cenchrus ciliaris*, *Panicum antidotale*, and *Echinochloa crusgalli*, were collected from the Punjab Forest Research Institute (PFRI) in Faisalabad, Pakistan. The grasses were subjected to different seed priming treatments and were tested for germination and seedling vigor.

Germination phase

The initial germination percentage of *C. ciliaris*, *P. antidotale*, and *E. crusgalli* was 33.33%, 35%, and 46.6%, respectively.

Seed treatments

Seeds of each of the 3 species were subjected to different priming tools, i.e. hydropriming (Farooq et al. 2006), osmopriming with 2.2% $CaCl_2$ (Basra et al. 2004), PEG-8000 (–1.1 MPa) (Ruan et al. 2002), MLE (concentrated and diluted with distilled water at the ratios of 1:10, 1:20, 1:30, and 1:40), and matripriming within 2 layers of jute mat saturated with distilled water (Khan 1992; Beckman et al. 1993) for 24 h at 20 ± 2 °C. Young moringa leaves were harvested, thoroughly washed, and stored overnight at freezing temperatures. The next morning, the liquid of the frozen moringa leaves was extracted using a locally fabricated manual machine. The extract was sieved many times through cheesecloth and then diluted with distilled water to prepare the required dilutions for seed priming. Except for during matripriming, continuous aeration was supplied during the soaking period. The ratio of seed weight to solution volume was kept at 1:5 (Farooq et al. 2006). After priming, seeds were given 3 washings with distilled water and redried to near their original weight under shade at 23 ± 3 °C (Basra et al. 2002). The seeds were used immediately after redrying.

Germination test

In petri dishes with Whatman No. 1 filter paper were placed 25 seeds of each of the 3 grasses in a completely randomized design (CRD) in 3 replications; the dishes were then placed in an incubator (Sony: MIR-254) for 21 days at 25 °C with 16 h of daylight and 8 h of darkness. Seed germination was counted daily according to the Association of Official Seed Analysts method (AOSA 1990) until the final seed emerged. The angular transformation of the final germination percentage (FGP) was calculated according to the formula arcsine$\sqrt{\text{FGP}}$. The time until 50% germination (T50) was calculated according to the following formula (Farooq et al. 2005):

$$T50 = t_i + \frac{(\frac{N}{2} - n_i)(t_j - t_i)}{n_j - n_i},$$

where N is the final number of seeds germinated, and n_i and n_j are the cumulative numbers of seeds germinated at adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$. Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981):

$$MGT = \frac{\sum DN}{\sum N},$$

where n is the number of seeds germinated on day D and D is the number of days as counted from the beginning of germination.

Germination index (GI) was calculated by the formula given by the Association of Official Seed Analysts (1983):

$$GI = \frac{\text{No. of germinated seeds}}{\text{Days of firs count}} + \text{------} + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}.$$

Seedling vigor evaluation

The treatments with the best observed results in the germination test, i.e. MLE (1:30) and matripriming, were used along with hydropriming in the next phase. Untreated seeds were used as the control. For each treatment, 25 seeds were planted in earthen pots filled with 3 kg of soil (clay loam), sand, and leaf compost (1:1:1) in a CRD in 3 replications. The experiment was conducted in March-May 2008 in the greenhouse of the Department of Forestry at the University of Agriculture, Faisalabad, Pakistan. After germination and seedling establishment, the seedlings were thinned to 3 plants in each pot.

To evaluate seedling vigor, 60-day-old seedlings were harvested. Data regarding shoot length, root length, number of leaves and tillers per plant, and root and shoot fresh and dry weight were recorded. Fresh root and shoot biomass were weighed immediately after harvesting and then oven-dried at 70 ± 2 °C until they reached a constant weight for the determination of dry weight.

Statistical analysis

Analysis of variance of the data for each attribute was computed using the MSTAT-C computer program (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

Germination phase

All of the priming treatments significantly ($P < 0.05$) affected the germination rate and uniformity of all seeds of the range grasses. Matripriming increased the FGP of *Cenchrus ciliaris*, *Panicum antidotale*, and *Echinochloa crusgalli* by 81.67%, 90.0%, and 90.0%, respectively, as compared to the control and other priming treatments (Table 1). GI was also enhanced significantly by matripriming in *Panicum antidotale* and *Echinochloa crusgalli* (15.82 and 13.22, respectively), and MLE (1:30) was also statistically similar to matripriming. In the case of *Cenchrus ciliaris*, MLE (1:30) was more effective (12.29) as compared to the others (Table 1). All priming treatments reduced MGT and T50 as compared to the control (Table 2). MLE (1:30) priming was recorded as the best treatment for efficiently reducing MGT (15.06, 11.62, and 10.31 days) and T50 (5.76,

Table 1. Effect of seed priming on final germination percentage (FGP), angular transformation of FGP (°), and germination index (GI) of Cenchrus ciliaris, Panicum antidotale, and Echinochloa crusgalli.

Treatments	Parameters								
	FGP						GI		
	C. ciliaris		*P. antidotale*		*E. crusgalli*		*C. ciliaris*	*P. antidotale*	*E. crusgalli*
	%	°	%	°	%	°			
Control	31.67	34.23 g	40	39.21 cd	43.33	42.12 cd	3.62 f	6.61d e	4.92 f
Hydropriming	40	39.21 ef	41.67	40.18 cd	41.67	47.88 b	6.69 e	8.10 bc	6.24 ef
Matripriming	81.67	64.70 a	90	71.95 a	90	59.06 a	10.97 b	15.82 a	13.22 a
$CaCl_2$	50	45.00 d	40	39.21 cd	53.33	46.92 bc	8.26 c	6.56 e	9.54 b
PEG-8000	65	53.76 c	43.33	41.16 c	48.33	43.08 bcd	7.59 cde	7.16 cde	7.21 de
MLE (Conc.)	46.67	43.09 de	41.67	40.20 cd	26.67	43.09 bcd	6.99 de	7.83 c	7.86 cd
MLE (1:10)	31.67	34.23 g	26.67	31.07 e	41.67	40.18 d	6.67 e	7.39 cde	8.11 bcd
MLE (1:20)	25	29.92 h	35	36.24 d	55	47.88 b	7.80 cd	7.81 cd	9.15 bc
MLE (1:30)	73.33	58.93 b	76.67	61.15 b	83.33	54.75 a	12.29 a	16.33 a	14.21 a
MLE (1:40)	36.67	37.26 fg	45	42.12 c	45	41.16 d	8.05 cd	9.13 b	8.92 bc
LSD 5%	12.04	4.07	13.64	4.92	10.54	5.38	1.083	1.219	1.574

Means showing different letters in a column are significantly different at a 5% probability level.

Table 2. Effect of seed priming on mean germination time (MGT) and time taken for 50% germination (T50) of Cenchrus ciliaris, Panicum antidotale, and Echinochloa crusgalli.

Treatments	Parameters					
	MGT			T50		
	C. ciliaris	*P. antidotale*	*E. crusgalli*	*C. ciliaris*	*P. antidotale*	*E. crusgalli*
Control	18.67 a	17.17 a	18.71 a	11.96 a	9.21 a	8.71 a
Hydropriming	17.35 b	16.47 abc	16.12 b	10.19 b	8.45 ab	7.68 b
Matripriming	15.47 ef	13.02 d	12.58 c	6.24 ef	4.89 d	4.47 d
$CaCl_2$	16.50 c	16.58 abc	16.04 b	7.47 cde	7.23 bc	6.66 c
PEG-8000	16.57 c	16.40 bc	16.34 b	8.18 cd	6.67 c	7.21 bc
MLE (Conc.)	16.36 c	17.05 bc	16.28 b	7.83 cd	7.41 bc	7.16 bc
MLE (1:10)	16.41 c	16.71 ab	16.78 b	8.66 c	8.37 ab	7.75 ab
MLE (1:20)	16.18 cd	16.77 ab	16.38 b	6.99 def	7.28 bc	7.16 bc
MLE (1:30)	15.06 f	11.62 e	10.31 d	5.76 f	5.07 d	3.68 d
MLE (1:40)	15.81 de	15.88 c	16.18 b	7.70 cde	7.53 bc	6.27 c
LSD 5%	0.544	0.7145	0.9437	1.475	1.234	0.9946

Means showing different letters in a column are significantly different at a 5% probability level.

5.07, and 3.68 days) in *Cenchrus ciliaris*, *Panicum antidotale*, and *Echinochloa crusgalli*, respectively, followed by matripriming, as compared to the control and other priming treatments. Plumule and radicle length were also increased by the application of seed priming treatments in all 3 rangeland grasses. Plumule length in *Cenchrus ciliaris* and *Echinochloa crusgalli* (6.13 and 9.08 cm, respectively) was maximally increased by matripriming, while in *Panicum antidotale*, MLE (1:30) was more effective (7.58 cm) in comparison with unprimed seeds (Table 3). The same treatments were the most effective in increasing radicle length (Table 3). Maximum radicle length in *Cenchrus ciliaris* and *Panicum antidotale* (3.19 and 1.08 cm, respectively) was recorded when the seeds were subjected to matripriming, while in the case of *Echinochloa crusgalli*, maximum radicle length was recorded in MLE (1:30)-primed seeds. Matripriming and MLE (1:30) were found to be the best priming treatments, so these were selected for seedling vigor evaluation.

Seedling vigor evaluation

In the second phase, all presowing seed treatments significantly ($P < 0.05$) affected the overall plant growth and vigor in all 3 range grasses. Matripriming and MLE priming were the most effective priming strategies. Matripriming increased the shoot vigor (length and fresh and dry weight), number of leaves (105.90 and 84.11), and tillers (12.78 and 9.89) of *Cenchrus ciliaris* and *Panicum antidotale*, respectively (Figures 1-5), but their roots were more vigorous when primed with MLE (Figures 6-8). MLE increased the shoot vigor, number of leaves (96.78), and tillers (9.44) in the case of *Echinochloa crusgalli* (Figures 1-5), while matripriming maximally increased its root length (40.22 cm) and fresh and dry weight (28.5 and 8.37 g, respectively) (Figures 6-8). The root-to-shoot ratios of *Cenchrus ciliaris* and *Panicum antidotale* were significantly increased (0.52 and 0.52, respectively) when their seeds were primed with MLE, while matripriming increased the root-to-shoot ratio (0.29) of *Echinochloa crusgalli* (Figure 9).

Table 3. Effect of seed priming on plumule and radicle length of Cenchrus ciliaris, Panicum antidotale, and Echinochloa crusgalli.

Treatments	Parameters					
	Plumule Length			Radicle Length		
	C. ciliaris	*P. antidotale*	*E. crusgalli*	*C. ciliaris*	*P. antidotale*	*E. crusgalli*
Control	2.36 f	2.15 g	4.54 d	0.92 g	0.34 f	1.12 g
Hydropriming	3.48 e	3.24 f	5.24 cd	1.70 ef	0.43 ef	2.22 f
Matripriming	6.13 a	6.93 b	9.08 a	3.19 a	1.08 a	3.36 ab
$CaCl_2$	4.53 d	4.56 e	5.27 cd	2.18 cd	0.59 cd	2.53 def
PEG-8000	4.81 bcd	5.10 d	5.72 c	2.47 bc	0.49 de	2.26 ef
MLE (Conc.)	4.65 cd	5.16 d	5.67 c	1.65 f	0.47 def	2.41 def
MLE (1:10)	4.81 bcd	5.62 c	5.87 c	2.43 bc	0.54 cde	3.03 bc
MLE (1:20)	5.10 bc	5.72 c	6.71 b	2.04 de	0.75 b	2.81 cd
MLE (1:30)	6.36 a	7.58 a	9.72 a	3.42 a	1.18 a	3.67 a
MLE (1:40)	5.18 b	5.89 c	7.16 b	2.64 b	0.65 bc	2.64 cde
LSD 5%	0.5024	0.4275	0.8079	0.3846	0.1319	0.4030

Means showing different letters in a column are significantly different at a 5% probability level.

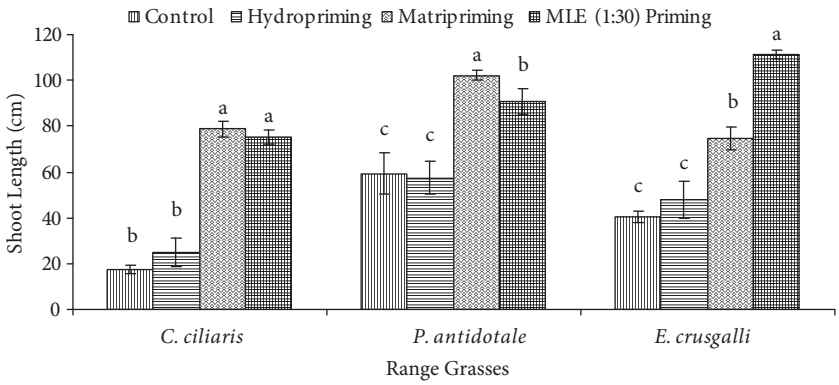

Figure 1. Effect of seed priming on shoot length of *C. ciliaris*, *P. antidotale*, and *E. crusgalli*. Means showing different letters are significantly different at a 5% probability level.

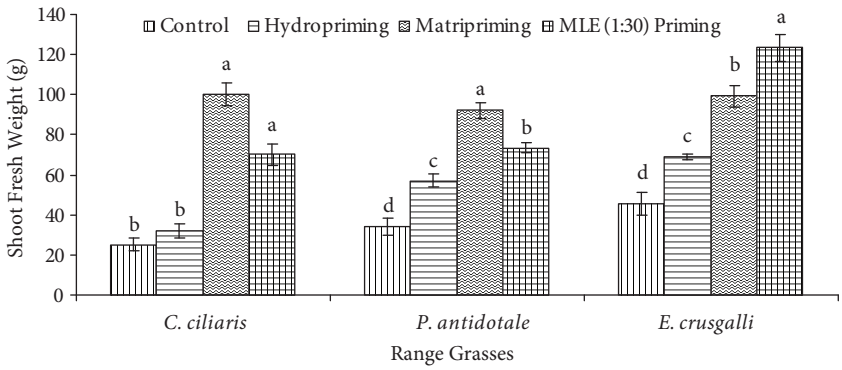

Figure 2. Effect of seed priming on shoot fresh weight of *C. ciliaris*, *P. antidotale*, and *E. crusgalli*. Means showing different letters are significantly different at a 5% probability level.

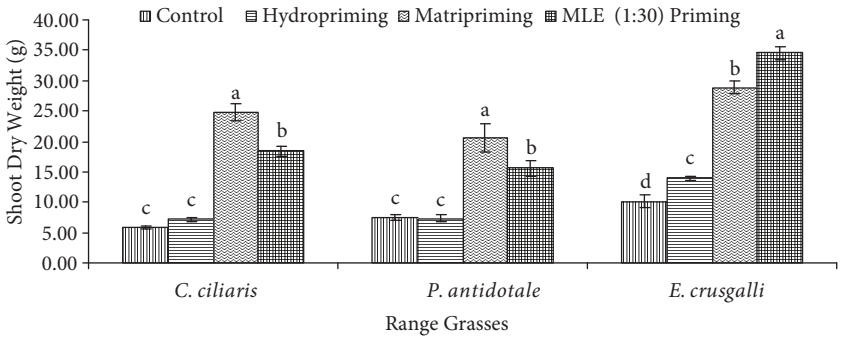

Figure 3. Effect of seed priming on shoot dry weight of *C. ciliaris*, *P. antidotale*, and *E. crusgalli*. Means showing different letters are significantly different at a 5% probability level.

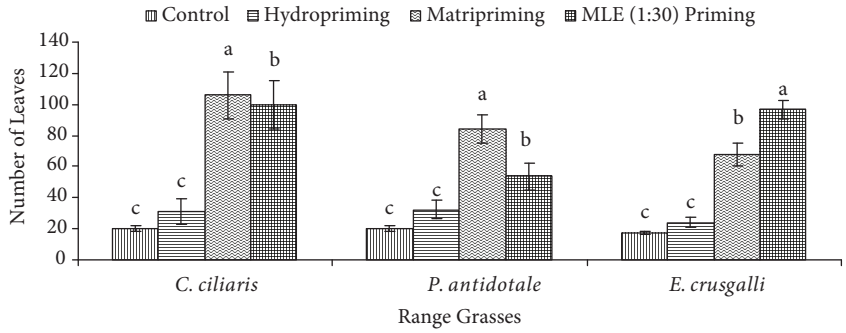

Figure 4. Effect of seed priming on number of leaves of *C. ciliaris*, *P. antidotale*, and *E. crusgalli*. Means showing different letters are significantly different at a 5% probability level.

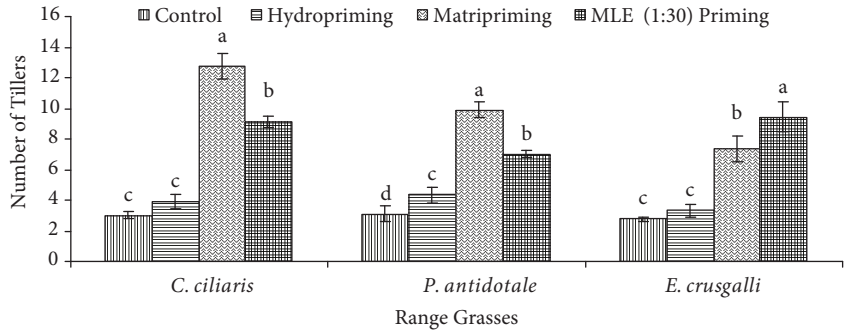

Figure 5. Effect of seed priming on number of tillers of *C. ciliaris*, *P. antidotale*, and *E. crusgalli*. Means showing different letters are significantly different at a 5% probability level.

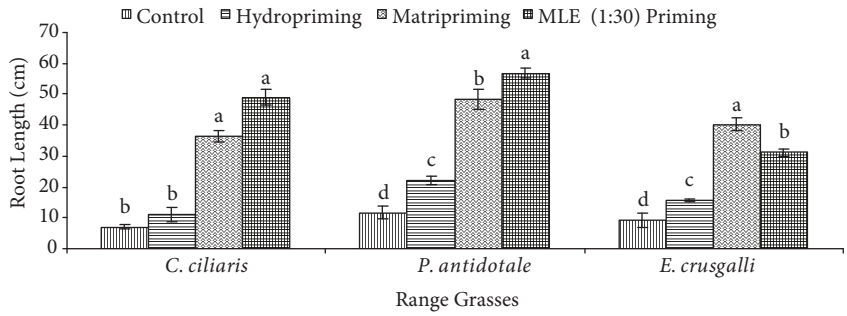

Figure 6. Effect of seed priming on root length of *C. ciliaris*, *P. antidotale*, and *E. crusgalli*. Means showing different letters are significantly different at a 5% probability level.

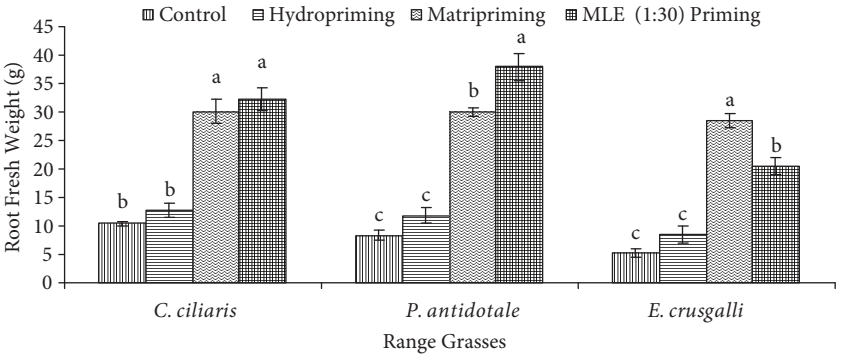

Figure 7. Effect of seed priming on root fresh weight of *C. ciliaris*, *P. antidotale*, and *E. crusgalli*. Means showing different letters are significantly different at a 5% probability level.

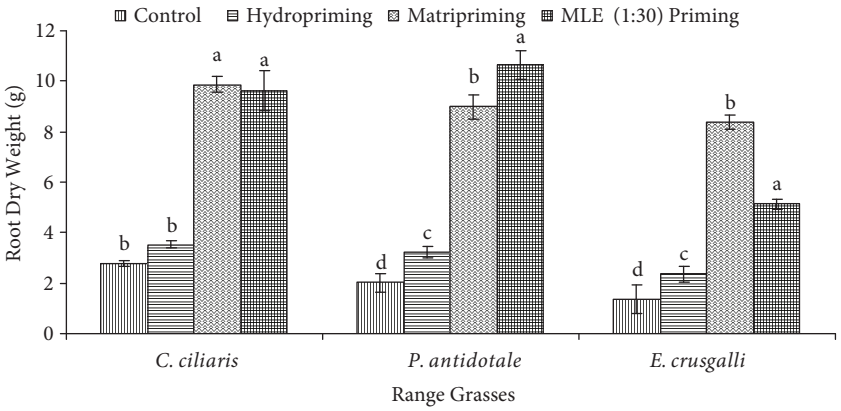

Figure 8. Effect of seed priming on root dry weight of *C. ciliaris*, *P. antidotale*, and *E. crusgalli*. Means showing different letters are significantly different at a 5% probability level.

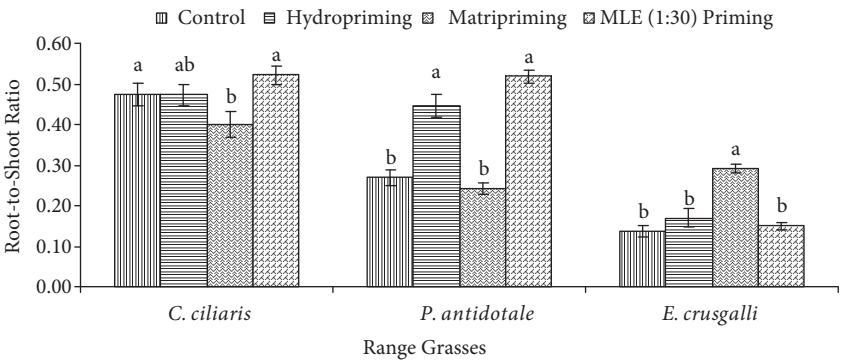

Figure 9. Effect of seed priming on root-to-shoot ratio of *C. ciliaris*, *P. antidotale*, and *E. crusgalli*. Means showing different letters are significantly different at a 5% probability level.

Discussion

All of the seed priming treatments significantly improved seed germination and plant vigor in the present study.

Rangeland grasses have a poor germination rate and can lose vigor due to a variety of environmental stresses (Qamar et al. 2000). Seed priming can improve germination and plant vigor in vegetables (Brocklehurst and Dearman 1983; Brocklehurst et al. 1984; Karssen et al. 1989; Gray et al. 1990; Bradford and Haigh 1994; Welbaum et al. 1998), crops (Harris et al. 1999, 2000; Musa et al. 1999), and range grasses (Hardegree 1992a, 1992b). The present study shows that matripriming and MLE priming for 24 h are both effective tools for enhancing the germination and plant vigor of range grasses *Cenchrus ciliaris*, *Panicum antidotale*, and *Echinochloa crusgalli*. In the first phase, the FGP of unprimed *Cenchrus ciliaris*, *Panicum antidotale*, and *Echinochloa crusgalli* seeds was 31.67%, 40.0%, and 43.33%, respectively, which was improved 2-fold by matripriming and MLE priming for all 3 range grasses.

In the present study, matripriming was more effective than MLE priming in improving the FGP and GI of all 3 range grasses, with a significantly early and synchronized germination. The improved germination rate, germination percentage, and uniformity caused by the seed priming strategies may be due to germination-enhancing metabolites and metabolic activities occurring during the imbibition phase (Shakirova et al. 2003; Basra et al. 2004; Basra et al. 2005).

The highest numbers of leaves were counted in matriprimed seeds of *Cenchrus ciliaris* and *Panicum antidotale*, while in *Echinochloa crusgalli*, MLE priming was more effective in increasing the number of leaves. The reason for the effectiveness of matripriming is not clearly known, but some reports suggest that matripriming regulates the matric potential through adsorptive, interfacial tension and attractive and adhesive forces between the carrier, air, and matric layer-water interfaces (Gray et al. 1990), which control the seeds' water uptake mechanism that allows the seeds to imbibe water slowly and minimize the effects of salts present in the water (Khan 1992). Wu et al. (1999) reported faster germination and a higher germination percentage in loblolly pine seeds treated with matripriming. The same results were recorded for the number of tillers and shoot vigor in all 3 range grasses. However, in the case of below-ground biomass (root length and root fresh and dry weight), the results were reversed. MLE priming was more effective in *Cenchrus ciliaris* and *Panicum antidotale*, while *Echinochloa crusgalli* responded well to matripriming treatment. These findings are in agreement with those of Jett et al. (1996), who reported longer root lengths of seeds subjected to matripriming. Osmopriming and hydropriming affect the germination and plant growth of many crops, as reported by Afzal et al. (2002), but various PGRs effectively improve the germination and plant vigor (Jeong et al. 1994). Exogenously applied PGRs like cytokinins and gibberellins improve plant establishment by making up for environmentally induced deficiencies (Hurly et al. 1991). Healthier or longer roots indicate healthy and vigorous plant growth. MLE priming was more effective in increasing root-to-shoot ratio in the case of *Cenchrus ciliaris* and *Panicum antidotale*, while matripriming was effective for *Echinochloa crusgalli*. The increased root-to-shoot ratio from MLE and matripriming may be attributed to cell wall extension and increased metabolic activities at low water potential, as in matripriming (Afzal et al. 2002).

MLE is a rich source of PGR hormone, zeatin, ascorbic acid, Ca, and K (Fuglie 1999; Foidl et al. 2001), which are involved in several plant growth and development processes. Seed priming with growth regulators improves plant vigor (Jyotsna and Srivastava 1998; Afzal et al. 2002). Cytokinin often plays its role by interacting with other plant hormones like auxins and abscisic acid (Iqbal et al. 2006). Iftikhar (2009) observed increased emergence and vigorous plant development in maize seeds primed with MLE (1:30) due to the presence of Ca, K, ascorbic acid, and cytokinin hormone.

Moringa leaf extract and matripriming are organic and inexpensive, and are more effective than other priming strategies in which expensive salts or synthetic PGRs are used. These findings open new doors for plant researchers to explore natural and organic sources of priming agents.

Conclusion

We conclude that matripriming (using saturated jute mat) and leaf extract of *Moringa oleifera* (diluted with water 30×) are very effective alternatives to popular priming strategies in which synthetic chemicals are used. These sources are not only organic but also are inexpensive, easily adapted, and environmentally friendly. However, there is a need to explore these sources more deeply to further identify their importance in comparison with synthetic priming sources. Proper management and implementation of such new ideas and scientific findings can easily overcome the low productivity and limited plant cover of the range areas, to provide not only soil cover but also feed for the livestock that is essential to any economy.

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