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Potential of Moringa (*Moringa oleifera*) Leaf Extract as Priming Agent for Hybrid Maize Seeds

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ABSTRACT

Although commercial growth enhancers have been successfully used to improve growth and development of crop plants under chilling stress, they are expensive and usually not available. Leaves of *Moringa oleifera* are rich source of zeatin, ascorbate, phenolic compounds, calcium and potassium, so being explored as natural crop growth enhancer. Two pot experiments were conducted to study the potential of moringa leaf extract (MLE) as a seed priming agent for hybrid maize. Seeds of hybrid maize cv Dekalb-5219 were exposed to MLE (100% extract & diluted to 10 & 30 times with distilled water), benzyl aminopurine (BAP 50 mg/L), ascorbate (50 mg/L), $CaCl_2$ (2.2%), hydropriming and overnight water soaking for 18 h. Non-primed (control) seeds were used for comparison. Regarding effective concentration based optimization of extraction method, seed priming with freshly harvested MLE 1:30 (diluted to 30 with distilled water) was the most effective concentration and extracted method as depicted by higher emergence rate and better early seedling growth of spring maize. However, ethanol extracted MLE and stored MLE were failed to improve the emergence. During second experiment all the priming treatments were effective in improving emergence rate and subsequent seedling growth as indicated by lower E_{50}, MET, higher FEP, CUE, EE and enhanced root shoot lengths and seedling fresh and dry weights as compared to that of untreated seeds. Enhancement of maize growth by priming with MLE 1:30 and ascorbate is attributed more likely due to improved chlorophyll and phenolic contents of seedlings as compared to overnight soaking, hydropriming including control.

Key Words: Moringa leaf extract; Seed priming; Germination capacity; Low temperature
Abbreviations: MLE=Moringa leaf extract; E_{50}=Time to 50% emergence; MET=Mean emergence time; FEP=Final emergence percentage; CUE=coefficient of uniformity of emergence; EE=Emergence index; BAP=benzyl aminopurine

INTRODUCTION

Good crop establishment is a major constraint to maize production in spring season. Low soil temperatures in the spring delay and reduce seedling emergence in maize, which require optimal temperature between 25-28ºC for germination. Maize planted when soil temperature is 10ºC or even lower, which often impairs the imbibitions of water (Cohn & Obendorf, 1978). During chilling stress, reactive oxygen species (ROS) are generated which may react with important macromolecules causing oxidative damage and impairing the optimal cellular functions (Farooq *et al.,* 2008). ROS in plants are scavenged by a variety of antioxidant enzymes and/or lipid- and water-soluble molecules (Foyer *et al.,* 1994). Phenolic reserves may have been used for lignin biosynthesis or as antioxidants to counteract and scavenge free-radicals and protect from oxidative stress. High temperature during maturity stage of spring sown maize severely affects the gain filling. Alternatively maize crop is now being sown during late winter so that crop matures before excessive temperature rise. However, seeds and seedlings often experience adverse physical conditions in the seedbed and such temperature extremes may adversely affect germination and post-germination growth. Therefore, the emergence and stand establishment of maize are often slow and extremely erratic due to chilling condition.

Application of plant growth regulators or nutrients during pre-soaking, priming and other pre-sowing treatments in many crops have improved seed performance that results in overall plant growth and productivity particularly under adverse conditions, such as temperature extremes or salinity (Taylor & Harman, 1990; Pill & Finch-Savage, 1998; Afzal *et al.,* 2008; Bakht *et al.*, 2011). Typical responses to priming are faster and closer spread of times to emergence over all seedbed environments and wider temperature range of emergence, leading to better crop stands, and hence improved yield and harvest quality, especially under suboptimal and stress growing conditions in the field (Halmer, 2004). Furthermore, the primed seeds often germinate and emerge more rapidly than non-primed seeds, especially under low temperatures (Bodsworth &

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Bewley, 1981; Murray, 1990; Zheng *et al.,* 1994).

Although proper exogenous application of plant hormones along with nutrients, antioxidants, organic and inorganic chemicals promotes plant growth and development, however, these are not cost effective and out of the reach of farmers. So efforts should be made to explore the cheaper and best alternatives of expensive priming agents. Among different natural sources used to extract plant growth regulators, moringa (*Moringa oleifera* L.) is gaining a lot of attraction (Foidl *et al.,* 2001).

Moringa belongs to family Moringaceae. There are about 13 species of moringa of which *M. oleifera* is most widely grown. Since leaves of moringa are rich in zeatin, it can be used as natural source of cytokinin (Fuglie, 1999). In addition, moringa leaf is also rich in ascorbates, carotenoids, phenols, potassium and calcium, which have plant growth promoting capabilities and often applied as exogenous plant growth enhancers (Foidl *et al.,* 2001). Antioxidants such as ascorbic acid and glutathione, which are found at high concentrations in moringa chloroplasts and other cellular compartments, are crucial for plant defense against oxidative stress (Noctor & Foyer, 1998). In view of all these reports, it is hypothesized that priming with leaf extract from moringa, having a number of plant growth promoters, mineral nutrients and vitamins in a naturally balanced composition, which may promote the plant growth. No information is so far available on the induction of chilling tolerance in hybrid maize by priming with moringa leaf extract. Therefore, the objectives of this study were to optimize the extraction method and dose of moringa leaf extract as priming agent in spring maize.

MATERIALS AND METHODS

Seeds of hybrid maize cv. Dekalb-5219 were obtained from Monsanto Pakistan AgriTech (Pvt.) Ltd. Before the start of experiment, seeds were surface sterilized in 1% sodium hypochlorite solution for 3 min, then rinsed with sterilized water and air-dried.

Fresh moringa leaves were collected from a mature moringa tree and juice was extracted by a locally fabricated juice extraction machine following the method of Foidl *et al.* (2001). Experiment 1 was conducted to optimize extraction method and evaluate fresh and one month stored MLE on spring maize under lab conditions. The young leaves/branches of moringa were grinded with a little amount of water (1 L/10 kg fresh material). Moringa leaves were extracted with water and ethanol and then diluted with distilled water for 30 and 40 times (1:30 & 1:40). Seeds were primed with fresh and one month stored MLE for 18 h. The extraction method was optimized on the basis of better emergence and growth characteristics.

After optimization of concentration and extraction method, another experiment was conducted to compare the efficacy of MLE as priming agent with other priming agents. For this purpose seeds were primed with respective aerated solutions of MLE (diluted to 10 & 30 times), BAP (50 mg L^{-1}), ascorbate (50 mg L^{-1}) for 18 h. Non-primed seeds were considered as controls. However, hydropriming for 18 h and overnight soaking were also used for better comparison of seed treatments. Continuous aeration was provided using small aquarium pump. After each soaking treatment, seeds were dried on filter sheets for 48 h at room temperature.

Emergence and seedling vigor evaluation: Control and treated seeds were sown in 10 kg plastic pots (25 in each) containing moist sand, replicated four times and were placed in net house during spring season. Daily day and night temperatures were monitored and temperature varied from 15ºC to 18ºC during day time and less than 10ºC were monitored at night time. Emergence was recorded daily according to the Seedling Evaluation Handbook of Association of Official Seed Analysts (1983). The experiment was preceded for four weeks. During this, mean emergence time (MET) was calculated according to the equation of Ellis and Roberts (1981):

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

Where n is the number of seeds, which were emerged on day D, and D is the number of days counted from the beginning of emergence.

Emergence energy was calculated by following formula (Basra *et al.,* 2005).

$$EE\ (\%) = \frac{\text{No. of seedlings emerged 4 days after sowing}}{\text{Total no. of seeds sown}} \times 100$$

The coefficient of uniformity of emergence (CUE) was calculated using the following formulae:

$$CUE = \sum n / \sum \left[\left(\bar{t} - t \right)^2 . n \right]$$

Where 't' is the time in days, starting from day 0, the day of sowing, and n is the number of seeds completing emergence on day 't'.

Shoot and root lengths at the time of harvest were measured with the help of scale in each replication and averaged to get mean shoot length per replication whereas fresh and dry weights of seedlings were taken with the help of an electric balance at the time of harvest for each replication. Dry weight was determined after oven drying the samples at 65°C.

Biochemical analysis: For chlorophyll determination, 1 g leaf sample was grinded in 10 mL of 80% acetone. From this 1 mL aliquot was taken and total volume of 5 mL was made by adding 4 mL of acetone. Poured it in cuvettes and read at 663 and 645 OD·s using UV-spectrophotometer (Bruinsma, 1963). Substitute the values in the formula below.

A = 8(OD 663) + 20(OD645) = µg chlorophyll/mL

Total soluble phenols were determined according to method described by Singleton and Rossi (1965). Leaf sample (0.5 g) was homogenized in 10 mL of 80% acetone and centrifuged it at 4000 rpm for 10 min. After centrifugation, 1.58 mL water and 100 µL of Folin-Ciocalteu reagent was added. Wait for between 30 s and 8 min and then mixed with 300 µL of sodium carbonate solution, and kept it at 25°C for 2 h. The absorbance of each sample was taken at 760 nm against the blank and plotted absorbance against concentration. With the calibration curve determined the phenolic level in the sample.

The experiment was laid out in completely randomized design with four replications. The experiments were conducted twice and data were pooled for the analysis of variance and to determine the significant differences between treatments. Duncan multiple range test was applied to compare the treatment means.

RESULTS

Expt. 1: Optimization of extraction method: During study regarding optimization of extraction method, all the priming agents significantly increased final emergence and took less time to emerge as compared to untreated seeds, however, the response of these priming agents was found different in improving seedling vigor of maize (Table I). Except priming with stored MLE 1:30 and hydropriming, all extracted methods improved shoot length. Root length was negatively affected by priming with moringa leaf extracted through various methods except fresh MLE 1:30. Seedling fresh weight was improved only by all priming agents, whereas seedling dry weight was only improved by priming with fresh MLE 1:30 followed by stored MLE 1:30. Overall, maximum improvement was recorded in growth of maize plants raised from seeds primed with freshly extracted moringa leaves diluted to 30 times for 18 h.

Expt. 2: Moringa as priming agent: All the priming treatments were effective in reducing the days to 50% emergence (E_{50}), mean emergence time (MET), while enhancing final emergence percentage (FEP) percentage as compared to that of untreated seeds (Table II). Maximum value for CUE was recorded in priming with MLE 1:30 (0.99), which is statistically at par with hormonal priming with ascorbate (0.89). Number of secondary roots was also improved by most of priming treatments except 100% MLE, MLE diluted to 10 times and BAP. The present study also indicate that all the priming treatments significantly improved root and shoot lengths, however, priming with $CaCl_2$ failed to improve shoot length of maize plants (Fig. 1). All priming treatments resulted in higher seedling fresh weight compared with that of control whereas seedling dry weight was only improved by priming with MLE 1:30, BAP and ascorbate for 18 h (Fig. 2).

All priming treatments increased chlorophyll contents as compared to untreated seeds except priming with BAP as compared to untreated seeds. Maximum chlorophyll contents were recorded in seed primed with MLE 1:30 followed by 50 mg/L ascorbate treatments. Similar trend was recorded regarding total phenolic contents in maize seedlings (Fig. 3).

Fig. 1: Effect of different seed priming treatments on (a) root length and (b) shoot length of hybrid maize

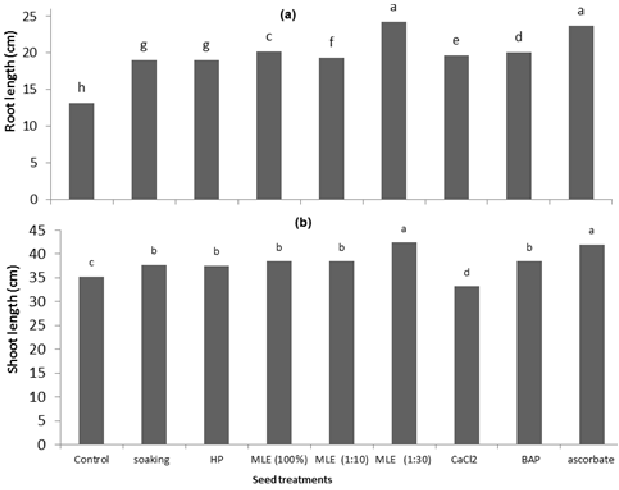
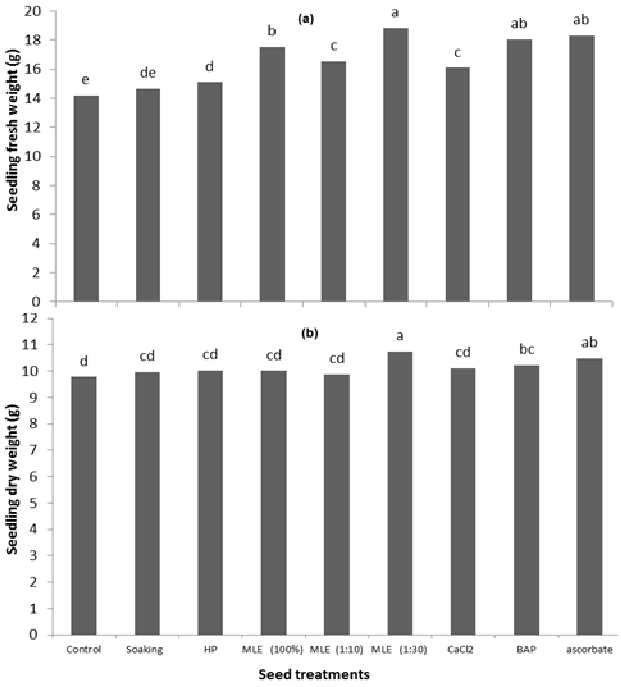


Fig. 2: Effect of different seed priming treatments on (a) seedling fresh weight and (b) dry weight of hybrid maize



DISCUSSION

Emergence rate, root shoot lengths, seedling biomass

Table I: Effect of different extraction methods of moringa and its concentration on emergence and seedling growth of hybrid maize

Treatments	E50 (days)	MET (days)	FEP	Soot length (cm)	Root length (cm)	Seedling fresh wt (g)	Seedling dry wt (g)
Control	5.10 a	5.49 a	72.33 c	23.43 f	16.97 de	12.95 d	4.35 c
HP	3.71 e	4.55 c	96.22 a	24.27 ef	17.65 d	14.26 c	4.89 bc
Fresh MLE (1:30)	3.34 g	3.79 e	98.78 a	37.47 a	21.47 a	18.55 a	6.88 a
Stored MLE (1:30)	3.85 d	4.49 c	79.88 b	23.07 f	17.80 cd	17.57 b	5.49 b
Ethanol extracted MLE(1: 30)	3.85 d	4.52 c	83.37 b	31.91 b	16.32 ef	15.93 b	5.11 bc
Fresh MLE (1:40)	4.14 b	4.86 b	96.78 a	26.62 d	18.93 bc	14.96 bc	5.03 bc
Stored MLE (1:40)	3.97 c	5.38 a	83.33 b	28.57 c	15.49 f	14.38 c	4.94 bc
Ethanol extracted MLE (1: 40)	3.49 f	4.14 d	96.54 a	36.26 a	17.75 cd	14.36 c	4.72 bc

Means within a column followed by the same letters are not significantly different at $P \leq 0.05$
HP = hydropriming, MLE = moringa leaf extract

Table II: Effect of seed priming on emergence potential and seedling establishment of maize plants

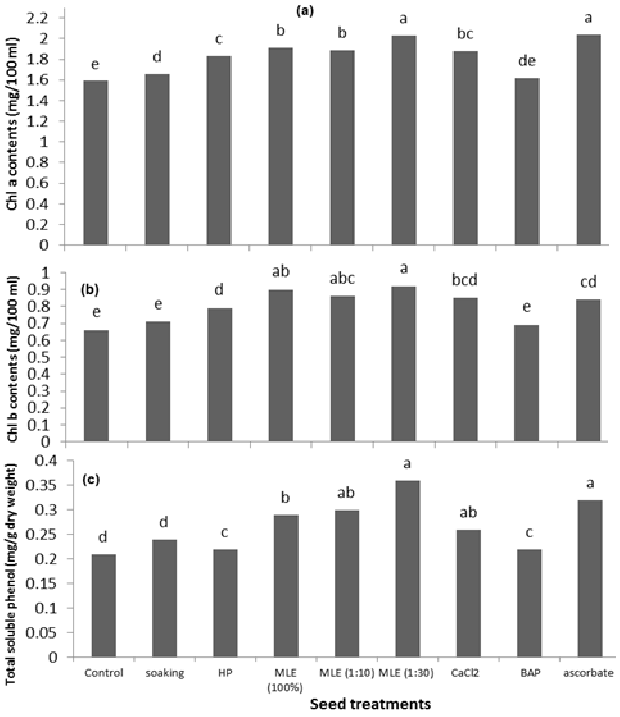
Treatments	E50 (days)	MET (days)	EE	FEP	CUE	No of secondary roots
Control	3.81 a	4.14 a	39.99 e	39.77 d	0.34 e	9.47 c
Overnight soaking	2.80 a	3.81 b	51.25 d	74.96 bc	0.35 e	10.67 b
Hydropriming	2.29 bc	3.21 d	59.77 cd	81.65 b	0.38 e	10.93 b
MLE (100%)	2.85 b	3.51 c	65.33bc	70.16 c	0.56 d	10.40 bc
MLE (diluted to 1:10)	2.52 bc	3.11 d	74.93 b	76.66 bc	0.6 c	9.80 bc
MLE (diluted to 1:30)	1.65 bc	2.19 f	93.00 a	96.67 a	0.99 a	13.20 a
$CaCl_2$ (2.2%)	2.64 bc	2.78 e	67.22 bc	69.99 c	0.54 cd	10.07 b
BAP (50 mg/L)	2.86 bc	3.62 bc	66.66 bc	75.09 bc	0.70 b	10.73 bc
Ascorbate (50 mg/L)	1.51 c	2.23 f	93.36 a	98.89 a	0.80 a	12.33 a

Means within a column followed by the same letters are not significantly different at ≤ 0.05
MLE = moringa leaf extract, BAP = Benzyl amino purine

are all important contributors of seed vigor. Higher emergence rate is the main foundation, which ensures an improvement of overall seedling performance. The results of present study indicated that seed priming with MLE 1:30 was the most effective concentration and extracted method as depicted by higher emergence rate and better early seedling growth of spring maize. However, ethanol extracted MLE and stored MLE were failed to improve the emergence. The better performance of MLE 1:30 might be due to higher nutrients and vitamins, which were lost during freezing moringa leaves (Begum *et al.,* 2009). During priming with MLE, which is rich in potassium, calcium and ascorbate, most of the N and Ca^{2+} appeared to be partitioned to embryo, which enhanced seedling emergence and subsequent growth of maize seedlings (Farooq *et al.,* 2010).

Priming not only promotes germination rate and subsequent growth under cool conditions but also helps in broadening the range of temperature during germination, which ultimately enhances crop yield (Murray, 1990; Zheng *et al.,* 1994; Farooq *et al.,* 2008). Although most of priming agents significantly enhanced emergence vigor of maize but maximum improvement was recorded in plants raised from seeds primed with MLE 1:30 followed by ascorbate priming (Table II). Priming with MLE 1:30 and ascorbate not only improved seedling emergence (lower values of MET, E_{50}) but also enhanced the seedling vigour as indicated by higher root and shoot lengths, and seedling fresh and dry weights (Fig. 1 & 2). Better performance of maize plants raised from seeds primed seeds with MLE 1:30 and ascorbate might be due to the maintenance of tissue water contents, increase in antioxidant activities, and carbohydrate

Fig. 3: Effect of different seed priming treatments on chlorophyll contents and total soluble phenols of hybrid maize



metabolism (Farooq *et al.,* 2008). These results are also in accordance, to some extent, with the studies of Patel and Saxena (1994) who reported an increase in fresh and dry

weights of seedlings raised from seeds treated with kinetin and GA_3 as compared to seeds treated with NAA and ethrel. Use of higher concentrations of MLE as priming agents were not feasible as seed priming with 100% MLE and diluted to 1:10 performed similar to overnight soaking and hydropriming and failed to improve number of secondary roots and seedling dry weight (Table II; Fig. 2).

Exposure of maize plants to chilling stress leads to the generation of ROS, which may react with important macromolecules causing oxidative damage and impairing the optimal cellular functions (Farooq *et al.,* 2008). Plant phenolics have a beneficial role during oxidative burst, improvement of vigour and germination under stress, soluble phenolics were contributed to high antioxidant activity (Randhir *et al.,* 2004). In previous studies involving faba beans and pea, a significant increase in total phenolics was observed, which correlated with enhanced seedling height and weight (Mccue *et al.,* 2000; Randhir & Shetty, 2003). Burguieres *et al.* (2007) also correlated an increase of phenolic contents in pea seedlings with the increase in growth response of seedlings due to exposure of seeds to folic acid or vitamin C. The findings of present study also suggest that an increase in total phenolic contents correlated with enhanced seedling vigor of maize raised from seeds primed with fresh MLE 1:30 and ascorbate (Figs. 1-3). Lower chlorophyll contents and phenolics in plants raised from non-primed seeds and seeds exposed to overnight soaking might be due to absence of important hormones/nutrients or antioxidant compounds, which are abundantly present in moringa leaves.

In conclusion, seed priming with moringa leaf extract diluted to 30 times with water and ascorbate for 18 h increased the ability of maize plants to grow successfully. Further research is needed to extend priming with MLE to other plant systems to improve both seed vigor and phenolics.

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