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Research Note

Priming with moringa leaf extract reduces imbibitional chilling injury in spring maize

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Summary

This study investigated the potential of moringa leaf extract (MLE) in seed priming for vigour enhancement of spring maize sown at low temperature (12 ± 1°C). The efficacy of MLE priming (3% w/v) was compared with other priming agents: kinetin (0.5% w/v), KCl (2% w/v) and hydropriming, each for 18 hours in hybrid maize cv. Pioneer-6525. Non-primed seeds were used as the control. Priming with MLE was the most effective in improving germination rate and subsequent seedling growth. This increased seedling performance was the result of reduced time to 50% germination (T_{50}) and mean germination time (MGT), and higher final germination percentage (FGP), germination index (GI) and germination energy (GE). Similarly improved seedling growth following seed priming with MLE or kinetin was due to enhanced root and shoot lengths, the ratio of root:shoot length and maximum seedling fresh and dry weights compared with control seeds. Enhanced seedling maize performance by priming with MLE was attributed to improved chlorophyll content, amylase activity and total sugar contents under cool conditions.

Experimental and discussion

Chilling-stress tolerance is a prerequisite for maize production under cool climatic conditions. However, imbibitional injury has been reported in spring-planted maize seeds due to decrease in daily soil temperature to below 10°C (Bedi and Basra, 1993; Afzal *et al.,* 2008). Sometimes, soil temperature goes below 0°C at night time in south Asia, causing delay in germination and reduced seedling emergence in maize which has an optimal temperature requirement for germination of 25-28°C (Basra *et al.,* 2011). Early sowing of spring maize can contribute to increased grain yield but sensitivity to low temperature at germination and seedling stages results in lower percentage and/or speed of emergence and poor stand establishment (Stewart *et al.,* 1990; Greaves, 1996). Conversely, delayed planting exposes the crop to high temperature during flowering, severely affecting grain filling and yield. The performance of spring maize can be improved by either avoiding this extreme temperature stress by shifting planting date or by use of better adapted or earlier maturing varieties. However, the progress in development of temperature stress tolerant plants is very slow. Seed priming is a treatment that can result in the harmonisation of germination and improved stand establishment at suboptimal temperatures (Basra *et al.,* 1988; Afzal *et al.,* 2008). Incorporation of plant growth regulators or nutrients during priming is found to improve seed performance and early plant growth and development, particularly under adverse conditions, such as temperature extremes or salinity (Pill and Finch-Savage, 1998; Taylor and Harman, 1990; Afzal *et al.,* 2008; Bakht *et al.,* 2011). However, use of plant hormones, antioxidants or nutrients in seed priming is expensive for resource poor farmers. Use of moringa leaf extract (MLE) which is rich in cytokinin and potassium can be a natural and cheaper alternative priming agent to enhance plant growth (Foidl *et al.,* 2001).

Use of MLE as a seed priming agent has been found to improve germination and seedling vigour of maize under optimal conditions (Basra *et al*., 2011), however the effects under low temperature stress conditions have not been considered previously. Moreover, the physiological and biochemical basis of improved performance is not known. Therefore, in this study, the efficacy of MLE compared with other priming agents (KCl and kinetin) was investigated for spring maize stand establishment at low temperature. Further, the physiological and biochemical basis of improved seedling growth by MLE or other seed priming agents was also studied.

One seed lot of hybrid maize cv. Pioneer-6525 with 86% germination was obtained from Pioneer Pakistan Seeds Ltd. The initial moisture content was 12.6%. Seeds were surface sterilised in 1% sodium hypochlorite solution for 3 minutes and then rinsed with sterilised water and air-dried. For priming, seeds were soaked in aerated solution of media with 3, 0.5 and 2% (w/v) fresh moringa leaf extract, kinetin and KCl, respectively, each for 18 hours (Basra *et al.,* 2011). Hydropriming was carried out using distilled water for 18 hours and was considered as a control in addition to untreated dry seeds. Continuous aeration was provided by an aquarium pump. After soaking, seeds were re-dried to near their initial moisture content on filter paper sheets for 48 hours at room temperature.

Germination of the maize seeds was evaluated in accordance with the Association of Official Seed Analysts (AOSA, 1983). Eight replicates of 25 seeds each were germinated in 12 cm diameter Petri dishes on filter paper at 12 ± 1°C in a cooled incubator (Sanyo, England). A seed was scored as germinated when the primary root emerged from the caryopsis. Germination energy (GE) was calculated as percentage germination after four days of sowing. Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981). The time to get 50% germination (T_{50}) was calculated according to the following formula of Coolbear *et al.* (1980):

$$T_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{n_j - n_i}$$

Where N is the final number of germination and n_i, n_j are cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$.

Germination index (GI) was calculated as described in the Association of Official Seed Analysts AOSA (1983) using the following formula:

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

For chlorophyll determination, 1 g fresh leaf sample was extracted in 10 ml of 80% acetone. After extraction, a 1 ml aliquot was taken and 4 ml acetone added. The resulting liquid was transferred to a cuvette and read at 663 and 645 ODs using a UV-spectrophotometer (Bruinsma, 1963). The activity of α-amylase was measured after extraction of 2 g maize seed in potassium phosphate buffer (pH: 7.0) by the modified DNS method (Varavinit *et al.,* 2002). For total soluble sugars, 1 g of ground seed was used and determined by the rapid and convenient anthrone reagent method reported by Thimmaiah (2004). The detailed procedure of α-amylase and total sugars was described previously (Afzal *et al.,* 2011).

Seed priming with MLE improved FGP and reduced T_{50} and MGT compared with untreated and hydroprimed seeds (table 1). Nonetheless, maximum germination index and germination energy were found for kinetin- followed by MLE-priming. Highest shoot and root lengths, root-shoot ratio, seedling fresh and dry weight were produced by priming with MLE. The greatest increase in seedling fresh weight, however was observed following priming with kinetin (table 1). Moreover, among biochemical attributes, highest chlorophyll contents were recorded in seedlings raised from MLE-primed seeds followed by kinetin- and KCl-primed seeds. Similar trends were observed for amylase activity and total soluble sugars in MLE-primed maize seeds followed by seeds primed with kinetin or KCl (figure 1).

Seed priming not only synchronises stand establishment under cool conditions but also helps in broadening the range of temperature required for germination which ultimately enhances crop yield (Murray, 1990; Zheng *et al.,* 1994; Farooq *et al.,* 2008). However, the interaction between priming treatment and initial seed quality can affect the response to priming (Powell *et al.*, 2000). In the current study, all priming agents significantly enhanced germination parameters and seedling performance of maize. Seed priming with MLE alleviated chilling affects in maize by higher germination rate as indicated by lower MGT, T_{50} and higher FGP, GI and GE. MLE is rich in nutrients and vitamins which might transfer to the growing embryo during the priming lag phase (Farooq *et al.,* 2010), ultimately giving enhanced seed germination and subsequent growth of maize seedlings upon exposure to low temperature. This also conforms to our findings that enhanced amylase activity might increase starch metabolism as indicated by high soluble sugars in MLE-primed seeds (figure 1c,d). Nonetheless, Powell *et al.* (2000) described that the improved quality of primed seeds could also be associated with higher germination rate and activation of metabolic repair during priming. While reduced germination from control seeds was likely to be attributed to cold water imbibitional damage (Afzal *et al.,* 2008).

Similarly, improved seedling growth by MLE priming was the result of earlier start of germination and vigorous growth (table 1), confirming earlier findings that seed priming enhances maize performance under cool conditions (Murray, 1990). Nonetheless,

Table 1. Effect of seed priming treatments on germination potential and seedling growth of hybrid maize under cool conditions.

Treatments	FGP (%)	T_{50} (days)	MGT (days)	GI	GE (%)
Untreated	84	3.8 ± 0.12	7.5 ± 0.30	17.0 ± 1.30	44.0
HP	88	2.8 ± 0.11	6.7 ± 0.13	27.3 ± 1.26	58.7
Kinetin (0.5%)	94	1.7 ± 0.13	6.0 ± 0.08	47.3 ± 1.26	94.7
KCl (2%)	89	2.4 ± 0.27	6.5 ± 0.16	34.3 ± 3.15	77.3
MLE (3%)	96	1.5 ± 0.16	6.2 ± 0.04	41.2 ± 1.52	89.3
	Root length (cm)	Shoot length (cm)	Root Shoot ratio	Seedling Fresh wt (mg)	Seedling Dry wt (mg)
Untreated	9.9 ± 0.30	5.3 ± 0.19	1.9 ± 0.01	3.1 ± 0.21	0.5 ± 0.06
HP	16.8 ± 0.94	7.2 ± 0.43	2.3 ± 0.01	4.3 ± 0.34	0.8 ± 0.10
Kinetin (0.5%)	16.3 ± 0.43	7.3 ± 0.05	2.3 ± 0.04	4.9 ± 0.33	0.8 ± 0.07
KCl (2%)	15.1 ± 0.28	6.6 ± 0.03	2.3 ± 0.05	4.2 ± 0.40	0.8 ± 0.12
MLE (3%)	17.0 ± 0.13	7.1 ± 0.09	2.4 ± 0.01	5.3 ± 0.13	1.1 ± 0.08

Note: Where apparent, data are mean ± standard error. HP = Hydropriming; MLE = Moringa leaf extract; MGT = Mean germination time; GI = Germination index; GE = Germination energy, T_{50} = Time taken to 50% germination, FGP = Final germination percentage.

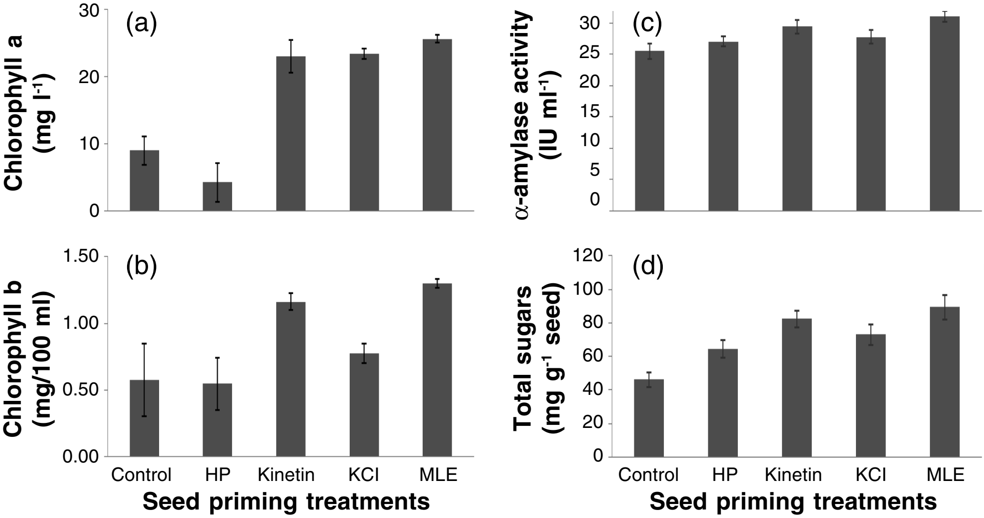

Figure 1. Effect of different priming treatments on biochemical attributes of hybrid maize during germination assay. Vertical bars are standard error of means. HP = Hydropriming, MLE = Moringa leaf extract.

increased seedling performance by seed priming with kinetin in this study is supported by Afzal *et al.* (2011) in which, increased seedling dry weight was observed when wheat seeds were primed with kinetin.

Chlorophyll contents, amylase activity, ascorbate and phenolic contents are strongly associated with higher maize seed vigour (Horii *et al.,* 2007). Rapid and uniform germination from primed seeds could lead to the production of vigorous seedlings with high chlorophyll contents in their leaves (Ghassemi-Golezani *et al.,* 2008). As moringa is rich in zeatin, ascorbate and minerals including potassium which plays a key role in delaying senescence under less than optimum conditions (Basra *et al.,* 2011). This corresponds to improved chlorophyll contents by priming with MLE, kinetin or KCl in maize seedlings under chilling stress found in present study (figure 1a,b).

In conclusion, seed priming was effective in inducing chilling tolerance in maize through modulation of vigour associated attributes such as hydrolytic enzyme activities, chlorophyll and carbohydrate metabolism. Among priming agents, priming with MLE seems more practical being less expensive, non-toxic and the most effective in increasing the ability of maize plants to withstand low temperature. Hence, seed priming with MLE can be recommended after extensive field appraisal across a wide range of environments and genotypes to improve the performance of early spring planted maize.

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