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# Full Length Article

# Seed Priming Improves the Performance of Late Sown Spring Maize (Zea mays) Through Better Crop Stand and Physiological Attributes

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### **Abstract**

High soil temperature and low water potential is one of major impediment of reduced and delayed germination of spring maize under delayed planting. Seed priming helps to ameliorate the seedling establishment under both normal and less than optimal conditions but very few studies available highlight maize crop establishment improvement by seed priming on physiological basis under field conditions. The present field study evaluated the effects of various seed priming techniques on stand establishment, growth, yield and various physiological attributes of spring maize under normal and late sown conditions. The hybrid maize seed soaked with hydro-priming (water), osmopriming with  $CaCl_2$  ( $\Psi s = -1.25$  MPa), moringa leaf extract (MLE; 1:30) and salicylic acid (SA; 50 mg L<sup>-1</sup>) were grown under two sowing dates (optimum and late conditions). Hydro-priming and dry seeds without priming were taken as controls. Among priming treatments, osmopriming with  $CaCl_2$  reduced the time to 50% emergence ( $E_{50}$ ), mean emergence time (MET), enhanced number of grains per cob, biological yield and improved relative water contents with reduced cell membrane permeability including grain protein contents. Osmopriming with moringa leaf extract (MLE) improved emergence time (EI), Chl a and b contents, grain yield and harvest index (HI) including hormonal priming with SA for maximum HI. Nevertheless, hydro-primed seeds exhibited the minimum days to maturity. The present study suggests that seed osmopriming with MLE and  $CaCl_2$  are effective not only to improve seedling establishment but also yield performance of spring maize through physiological improvement under both optimal and late sown conditions. © 2015 Friends Science Publishers

**Keywords:** Moringa leaf extract; Hydropriming; Stand establishment; Ca<sup>2+</sup>; High temperature

## Introduction

Maize crop often fails to establish quickly and uniformly due to high temperature and crust creation by rapid soil drying (Parera and Cantliffe, 1994) especially during spring season. Many factors constraint to achieve good and uniform crop stand including improper seedbed preparation (Joshi, 1987), poor seed quality, lack of soil moisture contents (Gurmu and Naylor, 1991) and high or low temperature (Weaich *et al.*, 1992) at planting that results in decreased yield of maize because of low plant populations. Maize also faces high temperature during grain formation stage, which substantially reduces the final yield. Prevalence of high temperature at reproductive stage affects pollination and seed setting resulting in reduced grain filling and lowers the final yield (Afzal *et al.*, 2008).

Several approaches are being used to ameliorate the devastating effects of high temperature stress on maize.

These include early spring plantation of maize, growing of short duration varieties and low or high temperature tolerant hybrids but seed invigoration plays important role in improved crop stand and yield performance under normal as well adverse conditions (Rehman et al., 2011). Seed priming is used to lessen emergence time, harmonize germination, enhanced germination rate and crop establishment in a lot of horticultural (Bradford, 1986) and field crops like wheat, rice and maize (Afzal et al., 2002: Farooq et al., 2008; Rehman et al., 2011). Harris et al. (1999) demonstrated that on-farm seed priming clearly enhanced early vigor and seedling establishment of maize, upland rice and chickpea, resulted in accelerated development, earlier flowering and maturity and high yields of these crops. Seed priming with inorganic salts not only promotes seed germination, but may also stimulate subsequent growth and metabolic processes and enhances the final crop yield (Eleiwa, 1989; Sallam, 1999).

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Different osmotica are used in seed priming and each of these has got different characteristics and levels of efficacy. They include polyethylene glycol (PEG), plant growth promoters (salicylic acid, polyamine, cytokinin, GA<sub>3</sub> etc), antioxidant (ascorbate), organic compatible solutes (glycine betaine), inorganic salts (KNO<sub>3</sub>, CaCl<sub>2</sub>, NaCl, KCl etc.) (Taylor et al., 1998) and most recently Moringa oleifera leaf extracts (Rehman et al., 2014). Among the plant growth regulators, salicylic acid (SA) was suggested to be involved in heat stress responses shown by plant (Kawano et al., 1998). SA can also induced long term thermo tolerance, by the involvement of both Ca<sup>2+</sup> homeostasis and antioxidant system (Wang and Li, 2006). Priming with salicylic acid, KCl, and CaCl<sub>2</sub> improved emergence, seedling vigor and temperature tolerance in hybrid maize (Dezfuli and Sharif-zadeh, 2008). Among the different natural sources, moringa leaves are rich in zeatin, a cytokinin, ascorbate, potassium, calcium, protein and vitamin A and C and these leaf extracts can be used as a priming agent or growth enhancers (Yasmeen et al., 2014).

It is hypothesized that different seed invigoration techniques using different osmotica sources may improve stand establishment, various physiological, yield and quality parameters of spring maize under late sown conditions. The objectives of the present study were to evaluate the impact of different seed priming techniques on seedling germination, growth, physiological attributes, yield and related traits and quality of final produce.

# **Materials and Methods**

#### Soil and Site

The present experiment was carried out to evaluate the performance of primed maize seeds under normal and late sown conditions at Agronomic Research Area, University of Agriculture, Faisalabad (31.25° N and 73.09° E) Pakistan, during spring season 2010. The experimental soil belonged to Lyallpur soil series Aridisol-fine-silty, mixed, hyperthermic Ustalfic, Haplargid in USDA classification and Haplic Yermosols in FAO classification scheme. Analytical work was performed in Seed Physiology laboratory, Department of Crop Physiology, University of Agriculture, Faisalabad. Weather data of the whole period of study are presented in (Fig. 1).

#### **Experimental Materials and Details**

Maize (*Zea mays* L.) hybrid FH-810 was obtained from Maize and Millet Research Institute Yusafwala, Sahiwal, Pakistan. The experiment was planed in randomized complete block design (RCBD) having split plot arrangement with three replications keeping plot size of 3 m  $\times$  5 m. Two different sowing dates were kept in main plots and priming techniques in subplots. Primed seeds were sown on February 24, 2010 (normal sowing) and compared

with maize crop planted after 30 days on March 24, 2010 (late sowing). Following seed priming treatments were included: Control (non-primed seed), hydropriming, osmopriming with CaCl<sub>2</sub> ( $\Psi$ s= -1.25 MPa), moringa leaf extract (MLE; 1:30) and salicylic acid (SA; 50 mg L<sup>-1</sup>).

#### **Determination of Seed Moisture Contents**

Initial seed moisture contents were determined according to the recommendation of Ellis *et al.* (1985). A weighed quantity of grinded seeds (10 g each) was taken in petri dishes and placed in an oven at 70°C until a constant weight. The samples were cooled down at room temperature and weighed. The percentage of moisture on wet weight basis was calculated from the loss in weight by following formula:

Moisture % age = 
$$\frac{M_2 - M_3}{M_2 - M_1} \times 100$$

Where:

 $M_1$  = Weight of petri dish (with cover).

 $M_2$  = Weight of petri dish and contents before drying.

 $M_3$  = Weight of petri dish and contents after drying.

The initial seed moisture contents were 12.4%.

## **Seed Priming Protocol**

For all the treatments recommended seed rate was used. The ratio of seed and working solution was kept 1:5 (g mL<sup>-1</sup>) (Farooq *et al.*, 2006). Except control for all priming treatments, seeds were soaked in respective solution for 18 h. For hydro-priming, seeds were soaked in aerated distilled water (Basra *et al.*, 2002). For osmopriming with CaCl<sub>2</sub> seeds were soaked in aerated solution of 2.2% CaCl<sub>2</sub> having  $\Psi$ s= -1.25 MPa (Farooq *et al.*, 2010). Osmopriming of seeds with salicylic acid (SA) and moringa leaf extract (MLE) was done by soaking seed in aerated solution of salicylic acid at 50 mg L<sup>-1</sup> and MLE diluted to 1:30 with water.

## **Post Treatment Operations**

After soaking, surface-washing of seeds was done thrice with tap water and dried under shade with near to original moisture content.

## **Crop Husbandry**

For preparation of seedbed, a pre-soaked irrigation of 10 cm was applied. When soil arrived at field capacity, the field was cultivated twice with the help of cultivator each followed by planking. The primed maize seeds were sown with the help of single row hand drill on properly prepared seed bed. In 75 cm spaced rows with plant to plant distance of 20 cm using seed rate of 25 kg ha<sup>-1</sup>.

Nitrogen (N), phosphorus (P) and potash (K) in the

form of urea, diammonium phosphate (DAP) and potassium sulfate were applied at 250, 120 and 100 kg ha<sup>-1</sup>, respectively. Whole P, K and split of N was incorporated in soil at the time of sowing, while rest of N was used at grain filling stage. In addition to precipitation received throughout the growing season of the crop, ten irrigations were applied keeping in view critical growth stages until crop attained physiological maturity. First irrigation was applied 25 days after sowing (DAS), while subsequent irrigations were applied at seven days interval due to high water requirement of spring planted maize. Confidor (500 mL ha<sup>-1</sup>) was foliar applied to prevent the crop from shoot fly attack. For weed control, hoeing was done. An area of 2 m<sup>2</sup> was harvested and cobs were detached and husk was removed. These cobs were kept for sun-drying for a week and then threshed with the help of maize threshing machine to determine the grain yield.

## Procedure for Recording the Data

## **Seedling Establishment**

Count of emerged seeds was carried out, following the seedling evaluation handbook from Association of Official Seed Analysts (1990) by visiting the field daily. Time period taken to 50% seedlings emergence (E<sub>50</sub>) [Days] was computed using formulae developed by Coolbear *et al.* (1984).

$$E_{50} = t_i + \left[ \frac{N/2 - n_i}{n_j - n_i} \right] (t_j - t_i)$$

Where *N* indicates final emergence count and  $n_i$ , additive number of emerged seeds at adjacent days  $t_j$  and  $t_i$  when  $n_i < (N+1)/2 < n_j$ .

Mean emergence time (MET) was recorded by applying Ellis and Roberts (1981) equation:

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

Where n represents number of emerged seeds on day D, and number of days D is calculated when emergence was started.

Emergence index (EI) was measured according to the handbook of the Association of Official Seed Analysts (1983) by the implementing formulae:

$$EI = \underbrace{\text{Number of germinated seeds}}_{\text{Days of first count}} \underbrace{\text{Number of germinated seeds}}_{\text{Days of ultimate count}}$$

Final emergence percentage (%) was taken at the end of experiment. It represented the ratio of number of emerged seedlings to total seeds planted multiplied by 100.

## **Growth and Development**

For calculation of days to maturity ten plants randomly

selected from each plot were tagged and date of maturity was noted. The grains and husks began to lose moisture and eventually the leaves were dried off, at this stage maximum dry mass attained by kernel which results in development of a line of black cells at the base of kernel and milk line has completely disappeared indicated physical maturity. The mean number of days taken to maturity was worked out from the average of ten plants.

Membrane permeability was estimated following the procedure of Blum and Ebercon (1981). Six segments of leaf approximately of the same size were immersed in distilled water for 12 h succeeded by the estimation of electrical conductivity (EC1) of the electrolytes rich solution with the help of EC meter. Samples were then shifted to autoclave with water for 60 min maintained at 50°C and subsequently cooled at normal temperature. The conductivity of diffused tissues (EC<sub>2</sub>) was once more measured. The ratio between EC1 and EC<sub>2</sub> gave membrane permeability.

For determination of relative water contents (RWC), 0.5 g of fresh leaves  $(W_f)$  was immersed in distilled water until leaves have gained constant weight. The turgid leaves were again weighed  $(W_S)$  and after that kept for drying at 80°C for 24 h to determine their dry weigh  $(W_d)$ . RWC was determined by the using formula of Barr and Weatherley (1962):

RWC (%) = 
$$(W_f - W_d) / (W_S - W_d) \times 100$$

#### **Yield and Related Traits**

For the calculation of number of grains per cob, a random sample of three cobs was selected after shelling and their grains were calculated. At the end their average was taken. Data regarding 1000-grain weight from each treatment was noted in grams (g) by means of an automatic electric balance in the laboratory. Biological yield per plot was recorded after sun-drying of maize crop for one week by hand held weighing balance and expressed in t ha<sup>-1</sup>. After threshing, the clean maize grain was air-dried and weighed to record the grain yield. The grain weight was conformed to 14% moisture contents and denoted in t ha<sup>-1</sup>. Harvest index was computed as the ratio of grain yield to total (above ground) biological yield by using the following formula:

$$HI = \frac{Grain \ yeild}{Biological \ yeild} \times 100$$

### **Biochemical Analysis**

**Chlorophyll determination:** For chlorophyll determination, leaf sample of 0.5 g was grinded in 5 mL of 80% acetone. Poured it in cuvettes and read at 663 and 645 OD·s using UV-spectrophotometer (UV-4000) values were substituted in the following formulas below:

 $A = [(0.0127(OD\ 663) - 0.00269(OD645))*100]/0.5 = mg/g\ of\ fresh\ weight \\ B = [(0.0229(OD\ 645) - 0.00468(OD663))*100]/0.5 = mg/g\ of\ fresh\ weight \\ A = [(0.0229(OD\ 645) - 0.00468(OD663))*100]/0.5 = mg/g\ of\ fresh\ weight \\ A = [(0.0229(OD\ 645) - 0.00468(OD663))*100]/0.5 = mg/g\ of\ fresh\ weight \\ A = [(0.0229(OD\ 645) - 0.00468(OD663)) + 0.00468(OD663)] + 0.00468(OD663) + 0.00468(OD663) + 0.00468(OD663) + 0.00468(OD663)) + 0.00468(OD663) + 0.00468(OD663) + 0.00468(OD663) + 0.00468(OD663) + 0.00468(OD663)) + 0.00468(OD663) + 0.00468(OD663) + 0.00468(OD663) + 0.00468(OD663)) + 0.00468(OD663) + 0.00466(OD663) + 0.00466(OD663) + 0.00466(OD663) + 0.00466(OD663) + 0.00466(OD663) + 0.00460$ 

**Crude protein (%):** For this purpose, 1.0 g of oven dried plant material, 25 mL of concentrated H<sub>2</sub>SO<sub>4</sub> and 5 g digestion mixture was added and digested the material on the gas heater in Kjeldahl digestion flask, cooled it and made up the volume 250 mL. For distillation 10 mL from this solution was taken. Nitrogen evolved as ammonia was collected in receiver having boric acid (4% solution) and mixed indicator bromocresol green and methyl red and titrated against standard (0.1 N) H<sub>2</sub>SO<sub>4</sub>. The reading obtained after titration against H<sub>2</sub>SO<sub>4</sub> was then multiplied by 6.25 to get crude protein percentage (AOAC, 1984).

#### Weather Conditions and Statistical Analysis

Monthly average of maximum, minimum temperature and relative humidity (RH) during crop growing period are given in Fig. 1. Soil temperature recorded daily during seedling emergence of both sowing times are also given in Fig. 2. The collected data was analyzed by using Fisher analysis of variance and significant treatment means were compared by least significance difference (LSD) test at 0.05 probability level (Steel *et al.*, 1997).

#### **Results**

Seed priming treatments considerably reduced the time taken to  $E_{50}$ , MET and improved emergence index, however, seed priming treatments as well as interaction between priming treatments and sowing dates showed non-significant for the final emergence count (Table 1).

Time taken to 50% emergence of maize was significantly affected by different seed priming treatments sown under normal and late sown conditions. Table of treatment means revealed that seed subjected to osmopriming with CaCl<sub>2</sub> took minimum time to complete 50% emergence, which is followed by osmopriming with MLE and hydro-priming under optimum sown conditions. However, under late sown conditions of the seeds subjected to hormonal priming with SA at 50 mg L<sup>-1</sup> took maximum time to complete 50% emergence and control (Table 1). Similarly, seed subjected to priming with SA gave maximum mean emergence time over control and osmopriming MLE under optimum sowing conditions. Maize seed osmoprimed with CaCl2, under late sown conditions resulted in minimum mean emergence time and significantly lower over control and other seed invigoration treatments both under optimum and late sowing conditions. Likewise, emergence index (EI) was improved significantly by seeds subjected to osmopriming with MLE followed by hormonal priming with SA and osmopriming with CaCl<sub>2</sub> as compared to untreated control. Interactive effect showed that maximum EI was found for hormonal priming with SA in respect of optimal sowing while it was least for nonprimed control under late sown conditions. None of the seed priming treatments significantly improved final emergence percentage (FEP) over control under optimal sowing. Reduced FEP was found for seeds primed with SA in late sown maize. However, improved number of plants emerged at optimal sowing date as compared to late sown condition, which indicated that sowing dates considerably affected FEP (Table 1).

Seed priming treatments affected agronomic and yield related traits except 1000-grain weight without any improvement under both optimal and late sowing (Table 2). Significant interactive effect for days to maturity was found for hydropriming with reduced days to maturity under optimal sowing while maximum number of days to maturity was observed in seed exposed to osmopriming with CaCl<sub>2</sub> statistically at par with osmopriming with SA and behaved like control in respect to late sowing. Likewise, seed priming treatments significantly improved the number of grains per cob over control and other treatments (Table 2). Maximum number of grains per cob was found for hydropriming at optimal and for osmopriming with CaCl<sub>2</sub> under late sowing while minimum number of grains per cob was observed in control and seed subjected to hydropriming under late sowing. However, none of the seed priming treatments significantly improved 1000 grain weight above the control in both sowing dates except the seed subjected to osmopriming with CaCl<sub>2</sub> in optimal conditions.

All the seed priming treatments improved the grain yield, biological yield and harvesting index (Table 2) over control under both sowing dates. However, maximum biological yield was recorded from osmopriming with CaCl<sub>2</sub> in optimal and osmopriming with MLE followed by hormonal priming in late sown maize (Table 2). Similarly osmopriming by MLE and SA were the best to improve grain yield under optimal and late sown conditions, respectively. Likewise, highest harvest index was noted for hormonal priming with SA under optimal and late sown conditions as well while minimum harvesting index was observed in plants raised by osmopriming with MLE under late sowing.

Seed priming treatments significantly enhanced the protein contents however; maximum value was recorded in osmopriming with CaCl<sub>2</sub> followed by hormonal priming with SA. Optimally sown seeds exhibited high protein percentage as compared to late sowing. Interactive effect of priming techniques and sowing dates showed that the highest protein content was recorded for seeds subjected to osmopriming with CaCl<sub>2</sub> in late sowing which was similar to hydropriming and osmopriming with MLE under optimal sowing conditions while it was minimum in seeds primed with MLE and control in respect of late sowing.

All the seed priming treatments improved the cell membrane permeability (CMP), relative water contents (RWC), chlorophyll a and b contents (Fig. 2, 3) over control in both sowing dates. However, osmopriming with CaCl<sub>2</sub> and hormonal priming with SA were the best to improve cell membrane permeability in both sowing dates (Fig. 2).

**Table 1:** Effect of different seed priming techniques on stand establishment of spring planted maize under normal and late sown conditions

Treatments	Time taken to 50% emergence (days)	Mean emergence time (days)	Emergence index	Final emergence percentage	
Optimum sowing					
Control	7.42 a ±0.14	$9.09 \text{ b} \pm 0.10$	$2.19 e \pm 0.08$	86.67 a ±2.89	
Hydropriming	6.92 b ±0.29	$9.03 \text{ b} \pm 0.06$	$3.54 b \pm 0.20$	83.33 a ±2.52	
Osmopriming (CaCl <sub>2</sub> )	$6.00 \text{ de } \pm 0.00$	8.13 d ±0.13	$3.67 \text{ b} \pm 0.03$	83.33 a ±0.58	
Osmopriming (MLE)	6.71 b ±0.12	8.97 b ±0.06	$4.02 \text{ a} \pm 0.05$	86.33 a ±0.78	
Osmopriming (SA)	7.19 a ±0.06	9.71 a ±0.03	$3.98 \ a \pm 0.07$	84.00 a ±1.00	
Late sowing					
Control	6.33 c ±0.14	7.69 e ±0.12	$1.75 \text{ f} \pm 0.23$	$70.00 \text{ bc } \pm 1.00$	
Hydropriming	5.88 e ±0.11	$8.20 d \pm 0.10$	$2.19 e \pm 0.10$	$73.3 \text{ b} \pm 2.25$	
Osmopriming (CaCl <sub>2</sub> )	$5.25 \text{ f} \pm 0.25$	$6.61 \text{ f} \pm 0.17$	$2.67 d \pm 0.24$	$70.0 \text{ bc} \pm 01.15$	
Osmopriming (MLE)	5.89 e ±0.06	7.79 e ±0.16	$3.06 c \pm 0.09$	$70.0 \text{ bc} \pm 1.00$	
Osmopriming (SA)	$6.27 \text{ cd} \pm 0.03$	8.50 c ±0.10	$2.89 \text{ cd} \pm 0.21$	66.67 c± 1.65	
LSD interaction $\leq 0.05$	0.27	0.18	0.26	4.40	

MLE= Moringa leaf extract; SA= Salicylic acid; CaCl<sub>2</sub>= Calcium chloride

Means in a column not sharing a common letter differ significantly by Fisher's protected Least Significant Difference (LSD) at 5 % probability Level; ±=Standard Deviation

**Table 2:** Effect of different seed priming techniques on yield related traits, yield and crud protein percentage of spring planted maize under normal and late sown conditions

Treatments	Days to maturity	Number of grains	1000-grain weight	.0 ,	Grain yield	Harvest index	Crud protein
		per cob	(g)	(t ha <sup>-1</sup> )	(t ha <sup>-1</sup> )	(%)	(%)
Control	101.33 cd ±0.58	371.21 d ±0.38	$249.00 \text{ bc } \pm 8.19$	$13.33 \text{ ef } \pm 1.03$	$3.87 de \pm 0.34$	29.03 b ±1.03	4.40 c ±0.05
Hydropriming	$100.33 \text{ e} \pm 0.58$	450.90 a ±3.69	252.33 ab±13.28	$14.53 \text{ cd} \pm 1.05$	4.23 c ±0.34	29.12 b ±0.21	$5.47 \text{ a} \pm 0.09$
Osmopriming (CaCl <sub>2</sub> )	$100.33 \text{ e} \pm 0.58$	425.00 bc±10.68	$255.33 \text{ ab} \pm 5.86$	17.71 a ±1.16	$4.97 \text{ a} \pm 0.51$	26.55 c ±0.91	$5.43 \text{ a} \pm 0.05$
Osmopriming (MLE)	102.00 c ±0.58	423.71 c ±3.42	263.00 a ±2.65	$16.75 b \pm 1.24$	$5.06 \text{ a} \pm 0.45$	$29.70 \text{ b} \pm 0.97$	$5.47 \text{ a} \pm 0.09$
Osmopriming (SA)	$100.67 \text{ de } \pm 0.00$	432.32 b ±2.33	$255.00 \text{ ab } \pm 4.58$	15.16 c ±1.06	$4.70 \text{ b} \pm 0.46$	$33.37 \text{ a} \pm 1.43$	$4.72 \text{ b} \pm 0.06$
Late sowing							
Control	$104.67$ ab $\pm 0.58$	$307.30 \text{ g} \pm 3.18$	$235.00 \text{ cd} \pm 2.00$	$13.10 \text{ f} \pm 1.19$	$3.48 \text{ f} \pm 0.36$	$26.64 c \pm 0.35$	4.31 c ±0.05
Hydropriming	101.67 c ±1.53	$309.00 \text{ g} \pm 3.84$	236.33 cd ±4.16	$14.21 d \pm 1.78$	$3.66 \text{ ef} \pm 0.61$	$24.76 d \pm 2.51$	$4.40 c \pm 0.05$
Osmopriming (CaCl <sub>2</sub> )	$105.00 \text{ a} \pm 2.00$	355.42 e ±9.52	231.67 d ±2.89	$14.10 de \pm 1.16$	3.65 ef±0.45	$28.64 \text{ b} \pm 2.48$	$5.47 \text{ a} \pm 0.09$
Osmopriming (MLE)	$104.00 \text{ b} \pm 1.73$	337.91 f ±0.51	$235.00 \text{ cd} \pm 2.00$	$14.86 c \pm 1.68$	$3.94 d \pm 0.58$	24.59 d ±2.42	$4.39 c \pm 0.08$
Osmopriming (SA)	$104.67$ ab $\pm 0.58$	331.43 f ±0.96	243.00 cd±13.75	14.52 cd±0.51	4.04 cd±0.29	27.18 bc±0.35	$5.46 \text{ a} \pm 0.05$
LSD interaction ≤ 0.05	0.69	8.56	NS	0.72	0.26	1.55	0.12

MLE= Moringa leaf extract; SA= Salicylic acid; CaCl<sub>2</sub>= Calcium chloride

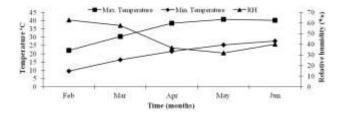
Means in a column not sharing a common letter differ significantly by Fisher's protected Least Significant Difference (LSD) at 5 % probability Level, ±=Standard Deviation

Seed subjected to osmopriming with CaCl<sub>2</sub> showed the greatest CMP at 45 days after sowing followed by hormonal priming with SA at 60 days after sowing under optimal sown. However, in case of late sown maize priming with SA revealed that highest CMP at 90 DAS was followed by seed exposed to osmopriming with CaCl<sub>2</sub> and MLE at 75 and 30 DAS, respectively. Likewise highest RWC was noted in seeds subjected to osmopriming with CaCl<sub>2</sub> at 60 DAS under both conditions followed by seeds exposed to hydropriming at 45 DAS under optimal and 60 DAS in late sown maize (Fig. 2). Furthermore, osmopriming with MLE significantly improved the chlorophyll 'a' and 'b' contents (Fig. 3). Maximum chlorophyll a contents was observed at 60 days after sowing in seeds subjected to osmopriming with MLE followed by osmopriming with CaCl2 under optimal sown. Similarly, osmopriming with MLE also showed greater chlorophyll contents at 75 days after sowing under late sown which are followed by CaCl2 that was statistically at par with seed subjected to hormonal priming with SA. Likewise, osmopriming with MLE revealed highest chlorophyll b contents followed by hormonal

priming with SA at 60 days after sowing under optimal and 75 days after sowing under late sown maize.

## Discussion

Time to 50% emergence, emergence time and emergence important indicators index of uniformity. synchronization of emergence and seedling vigor (Ellis and Roberts, 1981; Bewley and Black, 1994; Lara et al., 2014) and seeds are considered vigorous if complete their emergence within less period of time. The experimental data showed that presence of high temperature at sowing might curtail the emergence and reduced final emergence count (Fig. 1b) but seed priming treatments results in better stand establishment and also gave a vigorous start for maize seedlings under late sown conditions (Table 1). A significant reduction in emergence time and enhancement in emergence index may be due to the fact that seed priming stimulates an array of biochemical changes such as hydrolysis, activation of enzymes and dormancy breaking in the seed (Aziza et al., 2004; Faroog et al., 2010),



**Fig. 1a:** Temporal variations in air temperature and relative humidity from Feb-June, 2010

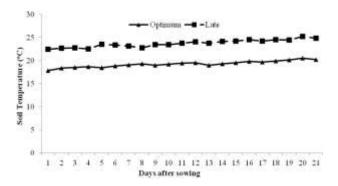
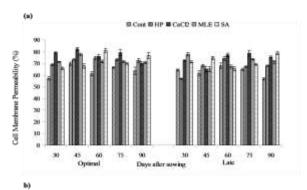


Fig. 1b: Daily variations in soil temperature during emergence

which are requisite to initiate the germination process. Seed treatment with CaCl<sub>2</sub> in rice enhanced the speed of germination, emergence, seedling vigor and vigor index significantly over untreated seed (Rehman *et al.*, 2011) and also improves temperature resistance at seedling establishment in maize (Rehman *et al.*, 2012) as evident from present study findings. However, improved maize performance by seed priming with CaCl<sub>2</sub> under high temperature stress might be due to pivotal role of Ca to improve crop resistance against abiotic stresses (Farooq *et al.*, 2009; Perveen *et al.*, 2015).

Seed priming not only improved the emergence and stand establishment but also growth and yield attributes in maize at both the sowing dates. Hydropriming proved effective to reduce the days to maturity under both optimal and late sowing conditions (Table 2). Basu et al. (2005) reported poor performance of hydropriming on final crop stand of spring summer crop but reduced time to maturity from 113 to 100 days and had little effect on yield components without any seed yield increase (Murungu et al., 2004, Basu et al., 2005). Seed priming treatments showed the significant effect on number of grains per cob and 1000 grain weight and enhanced biological and grain yield including harvesting index (Table 2). However, maximum 1000-grain weight and grain yield was recorded in optimal sowing but it decreased under late sown conditions (Table 2). Kurdikeri et al. (1995) support present observations that maize seeds priming with 0.5% CaCl<sub>2</sub> enhanced grain yield as compared with dry seed.



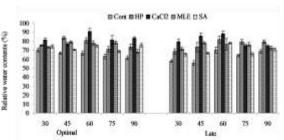
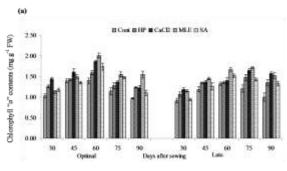


Fig. 2: Effect of seed priming treatments on (a) cell membrane permeability (CMP) (b) relative water contents (RWC) of spring planted maize under optimal and late sown conditions.

**Fig. 2:** Effect of seed priming treatments on (a) cell membrane permeability (CMP) (b) relative water contents (RWC) of spring planted maize under optimal and late sown conditions

Seed priming with inorganic salts like CaCl<sub>2</sub> not only may promote seed germination, but also may stimulate subsequent growth and metabolic processes and enhance final crop yield (Eleiwa, 1989; Sallam, 1999; Rehman *et al.*, 2011). The decrease in grain yield was closely associated with lower 1000 grain weight with late sown cereals crops (Darwinkel *et al.*, 1977). Improved yield performance by seed osmopriming with MLE had been reported in oilseed crop linola under field conditions (Rehman *et al.*, 2014).

Physiological attributes like RWC, CMP and Chl *a* and *b* contents were also improved by seed osmopriming treatments (Fig. 2, 3). Improvement in these attributes enabled the plant to cope with high temperature (Fig. 1a) which results in better plant growth and yield (Table 2). Among priming treatments, osmopriming with CaCl<sub>2</sub> was again ranked top for RWC and CMP followed by hormonal priming with SA (Fig. 2, 3). Reduction in cell membrane permeability for primed seed may be due to better plasma membrane structure by slow hydration. Recently, Kiros and Hunje (2010) reported that seed primed with CaCl<sub>2</sub> showed the highest relative water content and also significantly affected electrical conductivity in soybean. Enhanced chlorophyll contents at maturity observed for seed osmopriming with MLE might be due to presence of zeatin



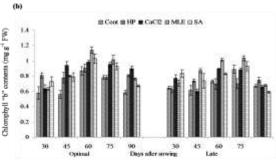


Fig. 3: Effect of seed priming treatments on leaf (a) Chl "a" and (b) "b" contents of spring planted maize under optimal and late sown conditions

**Fig. 3:** Effect of seed priming treatments on leaf (a) Chl "a" and (b) "b" contents of spring planted maize under optimal and late sown conditions

like cytokinin in leaf extracts (Yasmeen *et al.*, 2014). Increased leaf chlorophyll content under heat stress had been reported in creeping bentgrass that might be due to degeneration of chlorophyll and production of new cells (Liu and Huang, 2002). Likewise, improved crude protein percentage for osmopriming with CaCl<sub>2</sub> or SA might be the result of increased net assimilation rate and enhanced LAD providing better and uniform distribution of photoassimilates to growing kernels in present study (Table 2) and needs further investigation of such improvements.

#### Conclusion

Among the various priming agents used in this study, osmopriming with CaCl<sub>2</sub> and MLE seems promising in alleviating high temperature effects on seedling establishment, growth and development ultimately grain yield. Physiologically, the beneficial effect of these priming treatments can be attributed to enhance the RWC, reduced CMP and chlorophyll contents (*a*, *b*) and crud proteins. These treatments can be employed to improve the performance of spring maize crop if planting is delayed.

# References

Afzal, I., S.M.A. Basra, M. Shahid and M. Saleem, 2008. Physiological enhancements of spring maize (*Zea mays L.*) under cool conditions. *Seed Sci. Technol.*, 36: 497–503

- Afzal, I., S.M.A. Basra, N. Ahmad, M.A. Cheema, E.A. Warraich and A. Khaliq, 2002. Effect of priming and growth regulator treatment on emergence and seedling growth of hybrid maize (*Zea mays L.*). *Int. J. Agric. Biol.*, 4: 303–306
- Association of Official Seed Analysis (AOSA), 1990. Rules for testing seeds. *J. Seed Technol.*, 12: 1–112
- AOAC, 1984. Official Methods of Analysis, 14th edition. AOAC, Arlington Virginia, USA
- Association of Official Seed Analysis (AOSA), 1983. *Seed Vigor Testing Handbook*. Contribution No. 32 to the handbook on Seed Testing. Association of Official Seed Analysis., Springfield, Illinois, USA
- Aziza, A., A. Haben and M. Becker, 2004. Seed priming enhances germination and seedling growth of barley under condition of P and Zn deficiency. J. Plant Nutr. Soil Sci., 167: 630–636
- Barr, H.D. and P.E. Weatherley, 1962. A re-examination of the relative turgidity technique for estimating water deficit in leaves. Aust. J. Biol. Sci., 15: 413–428
- Basra, S.M.A., M.N. Zia, T. Mehmood, I. Afzal and A. Khaliq, 2002. Comparison of different invigoration techniques in wheat (*Triticum aestivum* L.) seeds. *Pak. J. Arid Agric.*, 5: 11–16
- Basu, S., S.P. Sharma and M. Daldani, 2005. Effect of hydropriming on field emergence, crop performance and seed yield of maize parental lines during winter and spring summer season. Seed Res., 33: 24– 27
- Bewley, J.D. and M. Black, 1994. *Seeds: Physiology of Development and Germination*, 2<sup>nd</sup> edition, p: 445. Plenum Press, New York, USA
- Blum, A. and A. Ebercon, 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci., 21: 43–47
- Bradford, K.J., 1986. Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. *Hortic. Sci.*, 21: 1105–1112
- Coolbear, P., A. Francis and D. Grierson, 1984. The effect of low temperature pre-sowing treatment under the germination performance and membrane integrity of artificially aged tomato seeds. J. Exp. Bot., 35: 1609–1617
- Darwinkel, A., B.A. Hag and J. Kuizenga, 1977. Effect of sowing date and seed rate on crop development and grain production of winter wheat. *Netherlands J. Agric. Sci.*, 24: 83–94
- Dezfuli, P.M. and F. Sharif-zadeh, 2008. Influence of priming techniques on seed germination behavior of maize inbred lines. *Agric. Biol. Sci.*, 3: 22–25
- Eleiwa, M.E., 1989. Effect of prolonged seed soaking on the organic and mineral components of immature pods of soybeans. *Egypt. J. Bot.*, 32: 149–160
- Ellis, R.A., and E.H. Roberts, 1981. The quantification of ageing and survival in orthodox seeds. *Seed Sci. Technol.*, 9: 373–409
- Ellis, R.A., T.D. Hang and E.H. Robert, 1985. *Handbook of Seed Technology for Genebanks*, Vol. 1. Principles and methodology. International Board for Plant Genetic Resources, Rome, Italy
- Farooq, M., A. Wahid, N. Ahmad and S.A. Asad, 2010. Comparative efficacy of surface drying and re-drying seed priming in rice: changes in emergence, seedling growth and associated metabolic events. *Paddy Water Environ.*, 8: 15–22
- Farooq, M., S.M.A. Basra, H. Rehman and B.A. Saleem, 2008. Seed priming enhances the performance of late sown wheat by improving chilling tolerance. J. Agron. Crop Sci., 194: 152–160
- Farooq, M., S.M.A. Basra, M. Khalid, R. Tabassum and T. Mehmood, 2006. Nutrient homeostasis, reserves metabolism and seedling vigor as affected by seed priming in coarse rice. *Can. J. Bot.*, 84: 1196– 1202
- Farooq, M., S.M.A. Basra, A. Wahid, N. Ahmad and B.A. Saleem, 2009. Improving drought tolerance in rice (Oryza sativa L.) by exogenous application of salicylic acid. *J. Agron. Crop Sci.*, 195: 237–246
- Gurmu, M. and R.E.L. Naylor, 1991. Effects of low water availability on germination of two sorghum cultivars. Seed Sci. Technol., 10: 103– 112
- Harris, D., A. Joshi, P.A. Khan, P. Gothkar and P.S. Sodhi, 1999. On-farm seed priming in semi-arid agriculture: Development and evaluation in maize, rice and chickpea in India using participatory methods. *Exp. Agric.*, 35: 15–29

- Joshi, N.L., 1987. Seedling emergence and yield of pearl millet on naturally crusted arid soils in relation to sowing and cultural methods. Soil Till. Res., 10: 103–112
- Kawano, T., N. Sahashi, K. Takahashi, N. Uozumi and S. Muto, 1998. Salycylic acid induces extracellular superoxide generation followed by an increase in cytosolic calcium ion in tobacco suspension culture: the earliest events in salicylic acid signal transduction. *Plant Cell Physiol.*, 39: 721–730
- Kiros, M.A. and R. Hunje, 2010. Seed priming for enhancing stand establishment, seed yield and quality of soybean. *Karnataka J. Agric.* Sci., 23: 701–707
- Kurdikeri, M.B., B. Aswathaiah, M. Mahadevappa, K.P.R. Prasanna and S.R. Prasad, 1995. Effect of seed invigoration on field performance of maize. *Mysore J. Agric. Sci.*, 29: 208–212
- Lara, T.S., J.M.S. Lira, A.C. Rodrigues, M. Rakocevic and A.A. Alvarenga, 2014. Potassium nitrate priming affects the activity of nitrate reductase and antioxidant enzymes in tomato germination. *J. Agric. Sci.*, 6: 72–80
- Liu, X. and B. Huang, 2002. Cytokinin effects on creeping bentgrass response to heat stress leaf senescence and antioxidant metabolism. Crop Sci., 42: 466–472
- Murungu, F.S., C. Chiduza, P. Nyamudeza, L.J. Clark and W.R. Whalley, 2004. Effect of on-farm seed priming on emergence, growth and yield of cotton and maize in semi-arid areas of Zimbabwe. Exp. Agric., 40: 23–36
- Parera, C.A. and D.J. Cantliffe, 1994. Pre-sowing seed priming. *Hortic. Rev.*, 16: 109–141
- Perveen, A., A. Wahid, S. Mahmood, I. Hussain and R. Rasheed, 2015.
  Possible mechanism of root-applied thiourea in improving growth, gas exchange and photosynthetic pigments in cadmium stressed maize (*Zea mays*). *Braz. J. Bot.*, 38: 71–79

- Rehman, H., I. Afzal, M. Farooq, T. Aziz and S.M.A. Basra, 2012. Improving temperature stress resistance in spring maize by seed priming. In: Proc. 3<sup>rd</sup> Int. Conf. 'Frontiers in Agriculture', Dankook International Cooperation on Agriculture, Dankook University, Cheonansi, Republic of Korea, pp. 28–32. October 3–5, 2012
- Rehman, H., M.Q. Nawaz, S.M.A. Basra, I. Afzal, A. Yasmeen and F.U. Hassan, 2014. Seed priming influence on early crop growth, phenological development and yield performance of linola (*Linum usitatissimum L.*). *J. Integr. Agric.*, 13: 990–996
- Rehman, H., S.M.A. Basra and M. Farooq, 2011. Field appraisal of seed priming to improve the growth, yield and quality of direct seeded rice. *Turk. J. Agric. For.*, 3: 994–1004
- Sallam, H.A., 1999. Effect of some seed soaking treatments on growth and chemical components on faba bean plants under saline conditions. *Ann. Agric. Sci.*, 44: 159–171
- Steel, R.G.D., J.H. Torrie and D.A. Dickey, 1997. Principles and Procedures of Statistics: A Biometrical Approach, 3<sup>rd</sup> edition. McGraw Hill Book Inc. Co., New York, USA
- Taylor, A.G., P.S. Allen, M.A. Bennett, K.J. Bradford, J.S. Burris and M.K. Misra, 1998. Seed enhancements. Seed Sci. Rev., 8: 254–256
- Wang, L.J. and S.H. Li, 2006. Salicylic acid-induced heat or cold tolerance in relation to Ca<sup>2+</sup> homeostasis and antioxidant systems in young grape plants. *Plant Sci.*, 170: 685–694
- Weaich, K., K.L. Bristow and A. Cass, 1992. Pre-emergent shoot growth of maize under different drying conditions. Soil. Sci. Soc. Amer. J., 56: 1272–1278
- Yasmeen, A., W. Nouman, S.M.A. Basra, A. Wahid, H. Rehman, N. Hussain and I. Afzal, 2014. Morphological and physiological response of tomato (*Solanum lycopersicum* L.) to natural and synthetic cytokinin sources: a comparative study. *Acta Physiol. Plant.*, 36: 3147–3155

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