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Integrated Seed Priming with Growth Promoting Substances Enhances Germination and Seedling Vigour of Spring Maize at Low Temperature

Shakeel Imran[1]*, Irfan Afzal[1], Shahzad M.A. Basra[1] and Muhammad Saqib[2]
[1]*Department of Crop Physiology, University of Agriculture, Faisalabad, Pakistan*
[2]*Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad, Pakistan*
*For correspondence: shakeel874@yahoo.com

Abstract

Poor germination due to low temperature at early stages badly affects the performance of spring maize. The present study was conducted to look into the effect of seed priming with moringa leaf extract (MLE, 3%), sorghum water extract (SWE, 10 mL L^{-1}), ascorbic acid (ASA, 50 mg L^{-1}), salicylic acid (SA, 50 mg L^{-1}) and hydrogen peroxide (H_2O_2, 2 µM) alone or in combinations on germination and emergence of hybrid maize at 12°C and 25°C. Untreated seeds were used as control. At optimum temperature, most of growth promoting substances significantly improved germination attributes while at low temperature, higher final germination percentage (FGP), germination index (GI) and reduced time to 50% germination (T_{50}), mean germination time (MGT) were attributed to SWE+ASA, MLE+H_2O_2, ASA+H_2O_2, MLE+SWE+H_2O_2, MLE+SA+H_2O_2, ASA+SA+H_2O_2, MLE+SWE+ASA+H_2O_2 and SWE+ASA+SA+H_2O_2 priming treatments as compare to control and hydropriming. Higher shoot length, root length and seedling dry weights were also linked with these treatments at both the temperatures. However, in net house study, better emergence attributes and improved seedling performance was observed in seeds treated with MLE+H_2O_2 followed by SWE+ASA, SWE+ASA+SA+H_2O_2 and ASA+SA+H_2O_2 as compared to other priming treatments. Improved performance of maize seedlings was due to high α-amylase activity and sugar contents. Results revealed that these seed priming combinations were very effective in improving seed germination under cool conditions, while field appraisal of these combinations is imperative.

Keywords: Seed priming; Growth promoting substances; Low temperature and germination

Introduction

To ensure sustainable food production, maize with the highest yield potential among the cereals and being a traditional crop offers the best option to greatly improve the food and feed availability in the country to cope with shrinking land resources and alarmingly increasing population (FAO, 2000). Early sowing of maize crop can contribute to increased grain yield but it required cold tolerance (Rodriguez *et al.*, 2007), because early growth stages are sensitive to low temperature (Greaves, 1996). Inconsistent and poor germination at suboptimal temperature is main barrier in its early planting (Stewart *et al*., 1990). Maize seeds are sown when the soil temperature is 10°C or lower, often injured by cold water imbibition stress (Cohn and Obendorf, 1978). Sometimes, soil temperature goes below 0°C at night time in South Asia, causing delay in germination and reduced seedling emergence in maize (Basra *et al.*, 2011). Unfavourable temperatures occurring during spring altered seedling establishment and photosynthetic activity (Stirling *et al.*, 1991) and decreased productivity and yield stability of maize crop (Leipner *et al.*, 1999). Moreover, low temperature may induce chilling injury through production of reactive oxygen species, which cause oxidative damage to various macromolecules and cellular structures (Noctor and Foyor, 1998) and limit essential plant nutrients especially potassium (Carry and Berry, 1978).

Synchronised germination and improved crop stand at suboptimal temperatures can be achieved through seed priming (Basra *et al.*, 1988; Afzal *et al.*, 2008). Integration of plant growth regulators, vitamins or nutrients during seed priming results in enhanced seed performance and early plant growth and development, particularly under adverse conditions, such as temperature extremes or salinity (Wahid *et al.*, 2008; Bakht *et al.*, 2011; Rasheed *et al.*, 2011). Moringa leaves are rich in zeatin, cytokinin, ascorbate, potassium, calcium, protein, vitamin A and C (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 as well as cool conditions (Basra *et al*., 2011; Afzal *et al.*, 2012a). Sorghum water extract (SWE) has allelopathic effect on crops, which contains high amount of phenolic compounds (Farooq *et al*., 2011). It has been shown that phenolic compounds, among the plants secondary metabolites, are effective in developing tolerance to abiotic stresses (Wahid, 2007).

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The beneficial role of synthetic growth promoters like ascorbic acid (AsA) and salicylic acid (SA) under normal and stressful environments can be not overlooked. Seed priming with AsA enhanced germination capacity, seedling growth, chlorophyll, photosynthesis and antioxidants in wheat under saline conditions (Afzal *et al*., 2006; 2011). Pre-soaking of maize seeds in SA solution reduced emergence time and improved emergence under cold conditions, induced seedling resistance to cold stress, manifested as significantly more plant fresh and dry weights in maize (Janda *et al*., 1999). Wahid *et al*. (2007) reported that pre-treatment of seed with H_2O_2 signals the induction of antioxidants in seeds, which enables the seedlings to encounter the ion-induced oxidative damage. Prasad *et al*. (1994) showed that maize seedlings injected with H_2O_2 become more tolerant against chilling stress. Chilling tolerance was enhanced with exogenous application of H_2O_2 in mung bean seedlings by increasing the glutathione level (Murphy *et al*., 2002).

Though it is evident from the literature that growth promoting substances has pivotal role in development and yield of various crops against abiotic stresses (Afzal *et al*., 2006; Wahid *et al.*, 2007; Afzal *et al.*, 2011), the integration of these substances needs to be explored in spring maize under cool conditions. Nonetheless, resource poor farmers cannot use expensive plant hormones, antioxidants or nutrients for seed priming. A combination of natural (MLE and SWE) and synthetic (AsA, SA or H_2O_2) growth promoters would be very environment friendly, economic and useful under prevailing conditions. Maize seed germination is severely affected by low temperature and exogenous application of growth promoting substances can improve seed germination and seedling vigour. Therefore, the present study intended to improve chilling tolerance in spring maize by priming with different combinations of growth promoting substances at early stages.

Materials and Methods

One seed lot of hybrid maize cv. Pioneer-32F10 with 89% germination was obtained from Pioneer Pakistan Seeds Ltd. The initial moisture content was 12.7%. Seeds were surface sterilized in 1% sodium hypochlorite solution for 3 min and then rinsed with sterilised water and air-dried. Preliminary experiments in the lab indicated better performance of AsA in wheat and maize (Afzal *et al.*, 2006; 2008), MLE in maize (Afzal *et al.*, 2012a) and H_2O_2 in rice (Afzal *et al.*, 2012b) however, integration of these substances with other growth promoters needs to be explored for spring maize. For priming, seeds were soaked in aerated solutions of MLE (3%), SWE (10 mL L^{-1}), AsA (50 mg L^{-1}), SA (50 mg L^{-1}) and H_2O_2 (2 µM). These priming treatments were tested alone or in combinations for germination assays which resulted into thirty two treatments including control and hydropriming (HP).

Preparation of Extracts

Moringa leaves were collected from established moringa trees. Before extraction, mature leaves were washed and kept at freezing temperature for overnight. Extraction was done with a locally assembled machine. The extract was sieved and diluted 30 times with distilled water to prepare a 3% solution of MLE (Afzal *et al.*, 2012a).

Sorghum plant herbage was harvested at maturity, dried for few days under shade and then chopped with a fodder cutter into small pieces (2 cm). This chopped material was taken and soaked in water with 1:10 (material: water) ratio for 24 h and extract passed through a sieve. Filtrates were boiled at 100°C to reduce the volume by 20 times. The concentrated crop water extract was stored at room temperature and 10 mL L^{-1} was used for seed priming (Cheema *et al.*, 2004). The solutions of synthtic growth promoing agents were prepared on the basis of active ingredients and purity percentages.

Priming Protocol

For priming, maize seeds were soaked in respective aerated solutions for 24 h at room temperature. After each treatment, seeds were rinsed thoroughly with distilled water and dried back closer to original moisture under shade condition.

Germination and Emergence Assays

Primed and non-primed seeds were sown on two moistened layers of filter papers in an incubator (Sanyo, England) at 12°C and 25°C for fifteen days. Seeds were placed in each Petri dish with four replications and considered germinated when radicle was visible. Germination was counted on daily basis. Seedlings were harvested and observations were taken regarding seedling growth according to ISTA protocols (ISTA, 2010).

Out of all priming combinations, eight treatments were selected for further seedling emergence evaluation in a net house on the basis of germination attributes and seedling vigour. The daily average temperature ranges between 15-17°C (Fig. 1). Untreated and water soaked seeds were used as control. Twenty five seeds were sown in sand filled plastic trays and harvested after three weeks. Data regarding emergence and seedling vigour were recorded using ISTA protocols (ISTA, 2010). 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 Coolbear *et al.* (1980) while germination index (GI) was calculated as described in ISTA seed testing handbook.

Biochemical Analysis

The α-amylase activity was determined after extraction of maize seed (2 g) in potassium phosphate buffer (pH: 7.0); phenyl methyl sulfonyl fluoride (PMSF) (10 mM) was

added as proteases inhibitor. The sample was centrifuged at 10000 × *g* at 4°C for 10 min and the supernatant was used for determination of the α-amylase activity by the modified DNS method (Varavinit *et al.,* 2002).

For measurement of total soluble sugars, 1 g of ground seed was hydrolyzed with 2.5 N HCl for 3 h in a boiling water bath. After cooling, the liquid was neutralized with solid Na_2CO_3 until effervescence ceased; the volume was adjusted to 100 mL with distilled water, centrifuged at 10,000 × *g* for 10 min at 4°C and the supernatant was collected. Total soluble sugars were computed by the rapid and convenient anthrone reagent method stated by Hedge and Hofreiter (1962) and Thimmaiah (2004). The reducing sugars were measured by DNS method reported by Miller (1959) and Sadasivam and Manickam (1992) from the maize sample (1 g) extracted in 80% ethanol twice (5 mL each time).

Experiments were conducted in randomized completely block design (CRD) with four replicates. Treatment means, standard errors and bar graphs were computed using Microsoft Excel program.

Results

Germination Assay

Low temperature significantly decreased germination time of maize seeds as indicated by approximately threefold lower MGT and T_{50} of maize seeds germinated at low temperature. Though priming treatments maximally improved germination time of maize seeds under low and optimum temperature conditions (Table 1). Higher FGP, GI and lower MGT, T_{50} were observed in seeds treated with SWE+ASA, MLE+H_2O_2, ASA+H_2O_2, MLE+ASA+SA+H_2O_2 and SWE+ASA+SA+H_2O_2, MLE+SWE+ASA+SA and MLE+SA+H_2O_2, while minimum in control. In the same way, maximum shoot length, root length and seedling dry weight were recorded in MLE+H_2O_2, SWE+ASA and SWE+ASA+SA+H_2O_2 chased by ASA+SA+H_2O_2 and ASA+H_2O_2 whereas minimum were observed in control (Table 2). Remaining treatments showed intermediate effects at both temperatures (Table 1 and 2).

Seedling Emergence Evaluation

Eight priming treatments were selected on the basis of better germination and seedling vigour from germination experiment and further evaluated in net house conditions (Table 3). Maximum FEP was observed in seeds primed with ASA+SA+H_2O_2, SWE+ASA+SA+H_2O_2 followed by SWE+ASA and MLE+SA+H_2O_2 while minimum was recorded in control and HP seeds. Lowest E_{50} was noted in seeds treated with MLE+H_2O_2 and SWE+ASA whereas highest was found in control and HP. Minimum MET was recorded in seeds primed with MLE+H_2O_2 and SWE+ASA as maximum was noted in control. Greater values of EI were linked with MLE+H_2O_2 and SWE+ASA+SA+H_2O_2 whilst smallest were associated with control and ASA+H_2O_2. In the same way highest shoot length was measured in seeds primed with ASA+SA+H_2O_2 and SWE+ASA+SA+H_2O_2 followed by MLE+SWE+H_2O_2 and MLE+SWE+ASA+SA while lowest in untreated seeds. Maximum root length was achieved from priming with ASA+SA+H_2O_2 and SWE+ASA+SA+H_2O_2 chased by ASA+H_2O_2 and MLE+SA+H_2O_2 as minimum was obtained from HP seeds trailed by control and SWE+ASA. Highest seedling dry weight was observed in seeds primed with SWE+ASA+SA+H_2O_2, ASA+SA+H_2O_2 and MLE+H_2O_2 followed by SWE+ASA and MLE+SWE+H_2O_2 while minimum was recorded in MLE+SWE+ASA+SA and control.

Biochemical Attributes

All priming agents significantly increased α-amylase activity, total and reducing sugars of maize seeds as compared to control and hydroprimed seeds; however, priming with MLE+H_2O_2 and SWE+ASA maximally improved α-amylase activity, total as well as reducing sugars. In addition, priming with SWE+ASA+SA+H_2O_2, ASA+SA+H_2O_2 was also effective to improve germination metabolites of maize seeds (Fig. 2).

Discussion

Good early vigour is highly important for spring maize which improves its competitive ability with weeds (Revilla *et al.,* 1999). While germination and early vigour of maize seedlings was considerably hampered by the negative effects of low temperature (Table 1). Low temperature during seed germination results in decreased cellular respiration and injured membranes (Xing and Rajashekar, 2001), elevated abscisic acid (ABA) levels and enhanced expression of antioxidants and increased ROS (Nayyar *et al.,* 2005), leading to poor and erratic germination. In this study almost every priming treatment improved FGP and GI with an effective reduction in T_{50} and MGT, as compare to control and HP (Table 1). It is well documented that priming not only promotes seed germination but also improves seedling growth under cool conditions (Afzal *et al.,* 2008; Afzal *et al.,* 2012a, b). Higher germination due to priming has also been linked with stimulation of antioxidant activities in various crops (Bailly, 2004). The application of priming agents in combinations was much effective as compared to sole application under both normal and low temperature conditions (Table 1 and 2). A better performance of priming treatments with MLE and SWE was due to the presence of antioxidants, nutrients, vitamins and phenolics that might be transferred to the growing embryos during lag phase in priming (Basra *et al.,* 2011) leading to more synchronised germination subsequently vigorous seedling growth upon exposure to low temperature (Afzal *et al.,* 2012a).

Table 1: Effect of seed priming treatments on germination potential of spring maize under optimum (25°C) and low temperature (12°C) conditions

Seed Priming	25°C FGP (%)	12°C FGP (%)	25°C T_{50} (days)	12°C T_{50} (days)	25°C MGT(days)	12°C MGT(days)	25°C GI	12°C GI
Control	89.25 ±1.44	80.25 ± 1.72	1.94 ± 0.03	6.71 ± 0.04	3.94 ± 0.03	11.71 ± 0.12	15.97 ± 0.63	4.05 ± 0.23
Hydropriming	91.50 ± 1.20	83.50 ± 1.37	1.76 ± 0.04	6.65 ± 0.03	3.86 ± 0.04	10.54 ± 0.11	17.97 ± 0.89	5.67 ± 0.28
MLE	93.25 ± 1.28	92.50 ± 1.52	1.61 ± 0.04	6.06 ± 0.02	3.61 ± 0.04	10.59 ± 0.07	18.84 ± 0.52	6.13 ± 0.43
SWE	92.75 ± 1.44	91.50 ± 1.37	1.68 ± 0.05	6.16 ± 0.04	3.68 ± 0.05	10.37 ± 0.05	19.11 ± 0.51	6.82 ± 0.37
ASA	93.50 ± 2.23	92.25 ± 1.52	1.73 ± 0.05	6.32 ± 0.23	3.73 ± 0.05	10.31 ± 0.12	19.50 ± 0.71	6.94 ± 0.69
SA	92.25 ± 3.31	90.50 ± 1.45	1.83 ± 0.04	6.34 ± 0.02	3.83 ± 0.04	10.50 ± 0.03	19.53 ± 0.54	6.34 ± 0.27
H_2O_2	93.75 ± 2.33	90.25 ± 1.52	1.73 ± 0.06	6.25 ± 0.03	3.73 ± 0.06	10.77 ± 0.12	19.35 ± 0.48	5.61 ± 0.26
MLE+SWE	93.00 ± 1.82	87.25 ± 1.85	1.66 ± 0.03	6.26 ± 0.03	3.66 ± 0.03	11.09 ± 0.27	19.25 ± 0.67	4.70 ± 0.61
MLE+ASA	92.50 ± 2.33	91.00 ± 1.15	1.78 ± 0.05	6.17 ± 0.03	3.78 ± 0.05	10.65 ± 0.21	19.56 ± 0.50	5.82 ± 0.57
SWE + ASA	96.25 ± 1.59	95.75 ± 1.98	1.52 ± 0.04	5.44 ± 0.02	3.54 ± 0.08	8.85 ± 0.39	22.01 ± 0.79	7.69 ± 0.24
MLE+SA	94.75 ± 2.33	88.25 ± 2.02	1.54 ± 0.03	6.34 ± 0.03	3.54 ± 0.03	11.37 ± 0.09	18.46 ± 0.32	3.57 ± 0.29
SWE+SA	92.25 ± 1.96	92.50 ± 5.53	1.70 ± 0.03	7.85 ± 0.05	3.70 ± 0.03	11.28 ± 0.12	19.26 ± 0.74	4.48 ± 0.45
ASA+SA	93.75 ± 2.96	87.50 ± 5.53	1.63 ± 0.05	7.60 ± 0.03	3.63 ± 0.05	11.73 ± 0.11	18.79 ± 0.53	2.67 ± 0.12
MLE+ H_2O_2	96.75 ± 0.98	96.00 ± 1.05	1.54 ± 0.04	5.57 ± 0.2	3.50 ± 0.06	8.75 ± 0.23	21.48 ± 0.90	7.57 ± 0.63
SWE+ H_2O_2	94.75 ± 2.96	89.75 ± 1.52	1.77 ± 0.05	6.14 ± 0.02	3.77 ± 0.05	10.93 ± 0.12	19.56 ± 0.42	5.24 ± 0.38
ASA+ H_2O_2	99.51 ± 1.79	96.50 ± 1.10	1.57 ± 0.04	5.61 ± 0.50	3.65 ± 0.03	9.59 ± 0.35	19.61 ± 0.60	7.13 ± 0.34
SA+ H_2O_2	95.00 ± 2.05	82.50 ± 5.53	1.64 ± 0.03	6.50 ± 0.02	3.64 ± 0.03	11.19 ± 0.22	16.73 ± 0.56	4.07 ± 0.33
MLE+SWE+ASA	94.25 ± 1.78	81.25 ± 4.93	1.62 ± 0.05	6.73 ± 0.02	3.72 ± 0.05	13.04 ± 0.32	17.98 ± 0.74	3.38 ± 0.56
MLE+SWE+SA	93.75 ± 1.91	83.75 ± 2.76	1.65 ± 0.03	6.67 ± 0.03	3.74 ± 0.03	12.81 ± 0.15	17.98 ± 0.40	3.42 ± 0.43
MLE+ASA+SA	94.75 ± 2.18	83.00 ± 4.72	1.55 ± 0.03	7.10 ± 0.04	3.75 ± 0.03	14.34 ± 0.43	17.98 ± 0.40	1.80 ± 0.51
SWE+ASA+SA	93.25 ± 2.02	85.75 ± 2.51	1.40 ± 0.03	6.51 ± 0.02	3.70 ± 0.03	14.33 ± 0.36	17.98 ± 0.40	2.17 ± 0.57
MLE+SWE+ H_2O_2	98.50 ± 1.79	96.25 ± 1.52	1.55 ± 0.02	5.70 ± 0.31	3.45 ± 0.02	9.45 ± 0.13	19.48 ± 1.14	7.28 ± 0.32
MLE+ASA+ H_2O_2	92.50 ± 1.91	87.50 ± 2.89	1.61 ± 0.03	6.40 ± 0.03	3.61 ± 0.03	12.32 ± 0.18	17.98 ± 0.40	5.58 ± 0.61
SWE+ASA+ H_2O_2	93.50 ± 2.13	90.00 ± 4.72	1.62 ± 0.02	6.29 ± 0.02	3.72 ± 0.02	12.73 ± 0.51	17.98 ± 0.40	4.80 ± 1.41
MLE+SA+ H_2O_2	97.50 ± 1.79	95.50 ± 1.79	1.49 ± 0.01	5.72 ± 0.26	3.39 ± 0.01	9.68 ± 0.38	20.23 ± 0.70	7.35 ± 0.44
SWE+SA+ H_2O_2	94.75 ± 2.88	87.25 ± 3.51	1.63 ± 0.04	6.71 ± 0.07	3.63 ± 0.04	12.62 ± 0.56	17.98 ± 0.40	2.12 ± 0.69
ASA+SA+ H_2O_2	99.50 ± 1.20	95.50 ± 1.74	1.53 ± 0.05	5.71 ± 0.32	3.43 ± 0.05	8.74 ± 0.12	20.98 ± 0.63	7.65 ± 0.48
MLE+SWE+ASA+SA	97.75 ± 2.64	94.75 ± 2.23	1.56 ± 0.03	5.88 ± 0.54	3.47 ± 0.03	9.95 ± 0.27	19.98 ± 0.71	7.43 ± 0.39
MLE+SWE+ASA+ H_2O_2	93.00 ± 2.45	91.00 ± 1.70	1.67 ± 0.03	6.46 ± 0.14	3.67 ± 0.03	12.77 ± 0.18	17.98 ± 0.40	5.16 ± 0.58
MLE+ASA+SA+ H_2O_2	93.25 ± 1.91	90.00 ± 4.72	1.60 ± 0.03	6.67 ± 0.12	3.60 ± 0.03	12.76 ± 0.29	17.98 ± 0.40	4.53 ± 0.34
SWE+ASA+SA+ H_2O_2	98.75 ± 2.02	96.25 ± 2.51	1.49 ± 0.03	5.80 ± 0.37	3.49 ± 0.03	8.91 ± 0.49	20.73 ± 1.20	7.59 ± 0.48
MLE+SWE+ASA+SA+ H_2O_2	94.75 ± 2.20	87.50 ± 5.53	1.66 ± 0.04	6.35 ± 0.06	3.66 ± 0.04	12.13 ± 0.11	17.98 ± 0.40	4.63 ± 0.94

Note: Where apparent, data are mean ± standard error. FGP=Final germination percentage, T_{50}=Time taken to 50% germination, GI=Germination index, MGT= Mean germination time

Higher root and shoot lengths are the contribution of early and rapid germination resulting in higher seedling dry weights. Therefore, better root and shoot lengths can improve the ability of maize seedlings to produce assimilates with under cool conditions. Thus strong seedling vigour and accelerated growth results in faster nutrient uptake which contributes towards better production of maize in spring season when temperature is very low at initial stage (Revilla *et al.*, 1999). Out of 32 priming agents, the response of H_2O_2 with other priming agents was very prominent (Table 1 and 3). The pronounced effect of H_2O_2 was due to signalling effect that activates antioxidants in seeds and prevents the maize seedling from oxidative damage during cool conditions. However this finding is inconsistent with the study of Afzal *et al.*, (2012b) who reported that priming with H_2O_2 failed to improve emergence and seedling growth in rice cultivars.

As a whole all priming combinations performed well but few of them resulted in poor germination and seedling growth that was might be due to the antagonistic effects among different growth promoting agents. An increase in sugars and α-activity was found in most promising treatments as compared to untreated or hydroprimed seeds

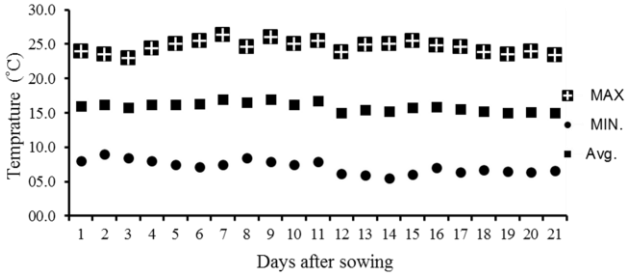

Fig. 1: Data of daily air temperature during emergence test conducted in net house

(Fig. 2) that are correlated with higher seed vigour and the results confirm the findings of Horii *et al.* (2007). Priming with MLE+H_2O_2 and SWE+ASA followed by SWE+ASA+SA+H_2O_2, ASA+SA+H_2O_2 increased α-amylase activity maximally. Total as well as reducing sugars of maize seeds increased which contributed better germination potential and higher seedling dry weight of maize (Afzal *et al.*, 2012a). Various workers have reported chilling tolerance by ASA, SA and H_2O_2 in maize (Farooq *et al.*, 2008; 2009; Ahmad *et al.*, 2013).

Table 2: Effect of seed priming treatments on seedling vigour of spring maize under optimum (25°C) and low temperature conditions (12°C)

Seed Priming	25°C	12°C	25°C	12°C	25°C	12°C
	Shoot L.(cm)	Shoot L.(cm)	Root L.(cm)	Root L.(cm)	SLDW(mg)	SLDW(mg)
Control	8.87 ± 0.62	4.67 ± 0.32	9.35 ± 0.33	7.55 ± 1.54	0.152 ± 0.011	0.147 ± 0.008
Hydropriming	8.00 ± 0.26	5.05 ± 0.57	10.02 ± 2.49	8.03 ± 1.60	0.159 ± 0.008	0.152 ± 0.005
MLE	8.30 ± 0.88	4.95 ± 0.40	11.20 ± 1.15	7.40 ± 1.98	0.171 ± 0.005	0.163 ± 0.012
SWE	8.62 ± 0.79	4.55 ± 0.33	10.65 ± 1.00	9.80 ± 0.75	0.169 ± 0.006	0.155 ± 0.004
ASA	9.55 ± 0.42	5.57 ± 1.01	11.70 ± 0.95	9.73 ± 0.82	0.167 ± 0.008	0.156 ± 0.005
SA	9.32 ± 0.34	4.97 ± 1.17	11.35 ± 0.33	8.68 ± 1.49	0.158 ± 0.008	0.151 ± 0.009
H_2O_2	8.00 ± 0.26	4.65 ± 0.35	11.10 ± 1.51	6.18 ± 0.46	0.170 ± 0.011	0.163 ± 0.009
MLE+SWE	8.30 ± 0.88	4.82 ± 0.41	11.20 ± 1.15	6.68 ± 0.65	0.178 ± 0.011	0.165 ± 0.010
MLE+ASA	8.62 ± 0.70	5.95 ± 0.50	10.79 ± 0.91	9.95 ± 1.10	0.170 ± 0.009	0.164 ± 0.010
SWE + ASA	11.60 ± 0.36	6.85 ± 0.32	13.65 ± 0.91	10.73 ± 0.86	0.195 ± 0.011	0.191 ± 0.002
MLE+SA	9.82 ± 0.42	4.17 ± 0.65	11.42 ± 0.53	7.10 ± 1.44	0.170 ± 0.009	0.163 ± 0.012
SWE+SA	9.90 ± 0.46	4.75 ± 0.48	11.92 ± 1.06	9.25 ± 0.53	0.164 ± 0.011	0.157 ± 0.010
ASA+SA	9.37 ± 0.49	4.07 ± 0.48	10.15 ± 0.79	7.43 ± 1.34	0.160 ± 0.008	0.151 ± 0.010
MLE+ H_2O_2	11.66 ± 0.83	6.72 ± 0.82	13.37 ± 1.11	10.90 ± 0.62	0.197 ± 0.013	0.189 ± 0.003
SWE+ H_2O_2	9.27 ± 0.74	2.85 ± 0.43	11.60 ± 0.43	4.50 ± 0.58	0.170 ± 0.003	0.156 ± 0.004
ASA+ H_2O_2	10.63 ± 0.736	6.25 ± 0.82	12.70 ± 0.33	10.55 ± 0.87	0.188 ± 0.009	0.181 ± 0.006
SA+ H_2O_2	5.45 ± 1.33	4.37 ± 1.01	7.07 ± 1.17	6.33 ± 2.00	0.166 ± 0.014	0.156 ± 0.017
MLE+SWE+ASA	7.90 ± 0.28	3.67 ± 0.70	8.57 ± 0.51	4.65 ± 1.56	0.170 ± 0.005	0.158 ± 0.008
MLE+SWE+SA	7.55 ± 1.63	1.82 ± 0.29	8.95 ± 1.54	1.33 ± 0.10	0.172 ± 0.006	0.168 ± 0.006
MLE+ASA+SA	5.80 ± 1.16	2.27 ± 0.46	8.00 ± 1.63	2.68 ± 0.62	0.169 ± 0.007	0.157 ± 0.046
SWE+ASA+SA	9.07 ± 0.44	2.67 ± 0.43	9.57 ± 0.53	1.75 ± 0.21	0.163 ± 0.006	0.159 ± 0.021
MLE+SWE+ H_2O_2	10.26 ± 0.46	6.57 ± 0.80	12.10 ± 0.48	9.90 ± 1.65	0.184 ± 0.005	0.179 ± 0.040
MLE+ASA+ H_2O_2	9.52 ± 0.6	3.70 ± 1.12	11.57 ± 1.14	5.40 ± 1.51	0.159 ± 0.002	0.151 ± 0.011
SWE+ASA+ H_2O_2	9.17 ± 1.52	2.60 ± 0.28	11.15 ± 1.57	2.65 ± 0.41	0.164 ± 0.007	0.159 ± 0.010
MLE+SA+ H_2O_2	10.82 ± 0.79	6.30 ± 0.52	12.60 ± 0.79	9.73 ± 0.68	0.186 ± 0.051	0.180 ± 0.012
SWE+SA+ H_2O_2	8.72 ± 0.57	4.52 ± 0.31	10.82 ± 1.16	3.80 ± 0.58	0.168 ± 0.007	0.156 ± 0.012
ASA+SA+ H_2O_2	11.30 ± 0.36	7.50 ± 0.70	13.35 ± 1.80	10.83 ± 1.20	0.193 ± 0.005	0.186 ± 0.017
MLE+SWE+ASA+SA	10.30 ± 1.80	6.32 ± 0.61	12.22 ± 0.80	9.78 ± 0.61	0.189 ± 0.005	0.183 ± 0.009
MLE+SWE+ASA+ H_2O_2	9.45 ± 1.70	5.27 ± 1.20	10.95 ± 0.94	5.45 ± 0.93	0.167 ± 0.004	0.196 ± 0.004
MLE+ASA+SA+ H_2O_2	11.80 ± 0.72	6.62 ± 1.71	13.25 ± 0.65	7.20 ± 1.02	0.164 ± 0.008	0.143 ± 0.037
SWE+ASA+SA+ H_2O_2	11.54 ± 1.29	7.55 ± 1.04	13.42 ± 1.92	10.75 ± 0.76	0.191 ± 0.008	0.187 ± 0.011
MLE+SWE+ASA+SA+ H_2O_2	9.80 ± 1.30	6.35 ± 0.60	9.67 ± 1.04	7.18 ± 0.33	0.172 ± 0.008	0.157 ± 0.002

Note: Where apparent, data are mean ± standard error. Shoot L.=Shoot length, Root L.=Root length, SLDW=Seedling dry weight

Table 3: Effect of seed priming treatments on emergence potential and seedling vigour of spring maize under net house conditions (Sand culture)

Seed Priming	FEP (%)	E_{50} (days)	EI	MET(days)	Shoot L(cm)	Root L.(cm)	SLDW(g)
Control	81.75 ± 1.72	6.53 ± 0.10	11.53 ± 0.10	16.72 ± 0.20	17.15± 0.79	22.05± 0.77	1.13 ± 0.11
Hydropriming	84.75 ± 1.28	6.05 ± 0.08	11.25 ± 0.08	19.85 ± 0.59	18.25 ±0.67	21.93± 1.21	1.19 ± 0.11
SWE+ASA	93.00 ± 1.24	5.20 ± 0.09	10.20 ±.098	22.10 ± 0.27	21.28 ± 0.59	29.43± 0.66	1.32 ± 0.06
MLE+H_2O_2	94.25 ± 1.44	5.16 ± 0.09	10.16 ± 0.09	22.50 ± 0.41	22.55 ±0.81	28.65± 0.49	1.35 ± 0.10
ASA+H_2O_2	85.50 ± 1.20	5.87 ± 0.04	10.87 ± 0.04	17.66 ± 0.51	19.07± 0.15	25.20± 1.43	1.25 ± 0.07
MLE+SWE+H_2O_2	89.00 ± 1.05	5.81 ± 0.04	10.81 ± 0.04	19.01 ± 0.43	20.37± 0.35	24.70± 0.44	1.28 ± 0.03
MLE+SA+H_2O_2	92.00 ± 1.05	5.90 ± 0.05	10.90 ± 0.05	20.38 ± 0.64	19.85± 0.52	25.00± 0.87	1.26 ± 0.11
ASA+SA+H_2O_2	95.25 ± 1.78	5.64 ± 0.07	10.34 ± 0.07	21.47 ± 0.16	23.67± 0.31	27.45± 0.30	1.42 ± 0.06
MLE+SWE+ASA+SA	90.25 ± 1.28	5.95 ± 0.03	10.95 ± 0.03	19.32 ± 0.30	21.17± 1.07	26.63± 0.05	1.16 ± 0.16
SWE+ASA+SA+H_2O_2	94.50 ± 0.74	5.35 ± 0.05	10.29 ± 0.05	22.33 ± 0.40	23.65± 0.28	27.28± 0.97	1.44 ± 0.04

Note: Where apparent, data are mean ± standard error. FGP=Final emergence percentage. E_{50}=Time taken to 50% emergence, EI=Emergence index, MET=Mean emergence time, SLDW=Seedling dry weight

In conclusion, seed priming was helpful to improve chilling tolerance in maize through increased vigour associated with carbohydrate metabolism and hydrolytic enzyme activities. Seed priming in combinations of MLE+H_2O_2, SWE+ASA, SWE+ASA+SA+H_2O_2 and ASA+SA+H_2O_2 were more effective in improving low temperature tolerance in hybrid maize as compared to alone application. Seed priming is generally cost-effective, which supports spring maize seedling survival at low temperature stress.

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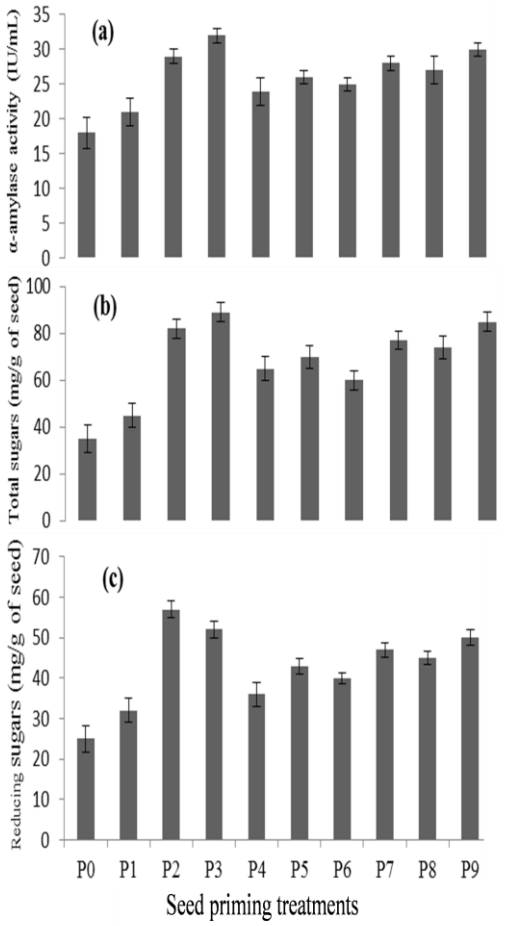

Fig. 2: Effect of different seed priming treatments on biochemical attributes of hybrid maize seeds. Vertical bars are standard error of means. Where, P_0=Control, P_1=Hydropriming, P_2=SWE+ASA, P_3=MLE+H_2O_2, P_4=ASA+H_2O_2, P_5=MLE+SWE+H_2O_2, P_6=MLE+SA+H_2O_2, P_7=ASA+SA+H_2O_2, P_8=MLE+SWE+ASA+SA, P_9 = SWE+ASA+SA+H_2O_2

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