INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–0134/2018/20–9–2140–2148
DOI: 10.17957/IJAB/15.0747
http://www.fspublishers.org

Full Length Article

Seed Priming Induced High Temperature Tolerance in Wheat by Regulating Germination Metabolism and Physio-biochemical Properties

Wajid Mahboob*, M.A. Khan, M.U. Shirazi, Summiya Faisal, Asma and Saba Mumtaz
Plant Physiology Division, Nuclear Institute of Agriculture, Tando Jam, Pakistan
*For correspondence: wajid_uaf@yahoo.com

Abstract

High temperature is one of the major limiting factors in wheat (*Triticum aestivum* L.) production in arid and semiarid regions of world. The crop experiences moderate to severe high temperature during most of its reproductive growth stages. In this pot study, the efficacy of seed priming treatments to improve the performance of wheat genotype (NIA-Amber) under optimal and late sowing was evaluated. Wheat seeds were subjected to hormonal priming with salicylic acid (SA), osmopriming with calcium chloride ($CaCl_2$), sorgaab (Sor) and moringa leaf extract (MLE) for 12 h. Results revealed that priming treatments significantly improved seed emergence and seedling establishment under both sowing dates. However, seedlings raised from SA priming, showed least time taken to 50% emergence (E_{50}) while osmopriming with MLE exhibited lesser mean emergence time and maximum emergence rate, coefficient of uniformity index and final emergence percentage over control. Furthermore, flag leaf area, spike length, 100 grain weight, grain yield per plant and chlorophyll content were improved in plants raised from seeds primed with SA, MLE and Sor. Contrarily, utmost relative water contents, glycine betaine and total phenolics were observed in osmopriming with $CaCl_2$ while maximum cell membrane thermo-stability exhibited by osmopriming with MLE, thus SA treatment illustrated maximum proline and total soluble sugars under high temperature stress. In crux, seed priming improves wheat performance under optimal as well as late sown conditions through improved germination metabolism and augmented accrual of osmoprotectants, resulting in accelerated growth and development even under high temperature.

Keywords: Chlorophyll; Compatible solutes; Moringa leaf extract; Osmopriming; Phenolics; Relative water content; Seedling establishment

Introduction

Wheat ranks amongst top three staple food crops of the world. Pakistan is one of the major wheat producers in the world with annual production of 25.27 million tons (Anonymous, 2017). Several factors affect the growth and yield of the crop but seed is by far the most important factor (Banard and Calitz, 2011). Temperature is a key abiotic factor affecting seed germination and performance of wheat crop. Usually a temperature range of 20-30°C is ideal for germination and seedling growth any fluctuation in temperature results in delayed germination (Buriro *et al.*, 2011). High soil temperature affects the seed germination in a number of ways. It induces seed dormancy in sensitive varieties while in resistant varieties it reduces coleoptile length which results in poor seed emergence in hot soils even if there is good seed germination (Edwards, 2008). The negative effects of climate change are much intense in developing countries with extended and severe hot dry weather (Sivakumar *et al.*, 2005) resulting in 20-30% wheat yield reduction (Rosegrant and Agcaoili, 2010).

High temperature stress is a great modulator of growth and productivity, which is a major problem for the production of agricultural crops all over the world (Bita and Gerats, 2013). Late sown wheat is usually subjected to extreme low temperature at germination stage resulting in poor germination as well as very poor tillering capacity (Farooq *et al.*, 2008) later at the grain filing stage high temperature reduces the kernel weight and grain yield (Wardlaw and Wrigley, 1994; Hussain *et al.*, 2013). Significant reduction in number of spikelet per spike takes place if temperature exceeds 11.9°C (Johnson and Kanemasu, 1983). The rise in pre-anthesis temperature up to 31°C or more will induce pollen sterility which results in less number of grains (Wheeler *et al.*, 1996), while high temperature at post-anthesis stage significantly affects the grain yield (Hasan and Ahmed, 2005). It is reported that yield of the main cereal crops badly affected by small rise in temperature (Bita and Gerats, 2013), even 1°C increase in temperature above the optimum level caused 4% reduction in wheat yield (Wardlaw and Wrigley, 1994).

Resistant wheat varieties have their own defense mechanisms to withstand high temperature like

To cite this paper: Mahboob, W., M.A. Khan, M.U. Shirazi, S. Faisal and Asma, 2018. Seed priming induced high temperature tolerance in wheat by regulating germination metabolism and physio-biochemical properties. *Int. J. Agric. Biol*., 20: 2140–2148

accumulation of compatible solutes, activation of antioxidant systems (Farooq *et al.,* 2017). However, several physiological approaches may be employed to reduce high temperature stress in wheat. Among these approaches, seed priming is a low cost and safe solution to improve crop stand establishment (Farooq *et al*., 2006). Several studies successfully employed seed priming with salts to enhance seedling vigor, growth and yield of wheat plant grown under late sown conditions (Basra *et al*., 2003; Farooq *et al*., 2008; Hussain *et al*., 2013; Mahboob *et al*., 2015). The best line of defense of plants/plantlets against stresses is perhaps plant hormones (Wani *et al.,* 2016) and osmoprotectants (Salma *et al*., 2015).

Among plant hormones, salicylic acid (SA) plays a vital role in accelerated cell division in apical meristem of wheat seedling which ultimately improves crop performance through better plant growth and yield (Shakirova *et al*., 2003). SA promotes plant growth and increases the thermos-tolerance ability by the regulating antioxidant system as well as Ca^{2+} homeostasis (Wang and Li, 2006). Calcium played vital role in plant-signaling events which makes it a major physiological transducer of diverse stresses (Rincon and Hanson, 1986). From natural sources, sorgaab (sorghum water extract) has several chemical substances i.e., alkaloids, flavonoids and phenolic acids (Einhellig and Leather, 1988) which are involved in the free radical scavenging induced by heat stress (Ishige *et al*., 2001). Likewise, moringa leaf extract having ample amount of cytokinins (zeatin), ascorbate, protein, calcium, potassium, vitamin A and C, can exogenously be supplied as a seed priming substance or plant growth promoters (Yasmeen *et al*., 2013). There is no doubt in the potential of these naturally occurring chemicals to improve seedling vigor and crop growth and development. However, nature and concentration of chemical, priming technique with duration of priming along with the nature of crop species affects the response of these chemicals.

This study was carried out with the objective of evaluating the influence of pre-sowing natural and synthetic chemicals seed treatments to explore the possibility of improving wheat performance under late sowing conditions.

Materials and Methods

Planting Material

Seeds of wheat genotype NIA-Amber were used in this experiments which were collected from Plant Breeding and Genetics Division, Nuclear Institute of Agriculture (NIA), Tandojam, Pakistan.

Seed Priming Techniques and Post Treatment Handling

Seed priming treatments and optimized doses were selected from previous experiments (data not shown). Seeds and priming solutions ratio was kept as 1:5 (w/v). Seed priming technique was practiced by soaking wheat seeds in respective solutions of salicylic acid (50 mg L^{-1}; salicylicate priming), 2.2% $CaCl_2$ (having Ψs= -1.25 MPa; osmopriming), Sorgaab and MLE (diluted to 30 times) for 12 h. Untreated wheat seeds were considered as non-primed control. After soaking, seeds were given three surface washings with distilled water and then re-dried under shade near to their original weight, sealed in polythene bags and stored in a refrigerator until use.

Experimental Details

Treatments were laid out in a factorial arrangement under completely randomized design with three replications. Control and primed wheat seeds (fifteen per pot) were sown in soil-filled plastic pots (10 kg) at two different sowing dates November 15, 2013 (optimum sowing) and December 15, 2013 (late sown) in pot house at Nuclear Institute of Agriculture Tandojam, Pakistan. The experimental soil belongs to shahadra soil series having clay loam texture with pH 7.1, EC 0.99 dSm^{-1}, organic matter 0.67%, total organic carbon 0.25%, total nitrogen 0.034%, AB-DTPA extractable phosphorous 3.85 mg kg^{-1} and exchangeable potassium 197.3 mg kg^{-1}. After the completion of emergence, thinning was practiced to maintain ten plants in each pot.

Observations/Measurements

Stand establishment: The daily count of emerged wheat seedlings was carried out by implying the method proposed by the Association of Official Seed Analysis (1990) till a constant number was obtained. Time period required for seedling emergence to 50% (E_{50}) was measured by using formula of Coolbear *et al.* (1984), whereas Ellis and Roberts (1981) standards were implemented to compute mean emergence time (MET).

The protocol proposed by Association of Official Seed Analysts (1990) was used to observe the Emergence index (EI) by following formulae:

$$EI = \frac{Count\ of\ seeds\ germinated}{Number\ of\ days\ to\ first\ count} + --- + \frac{Count\ of\ seeds\ germinated}{Number\ of\ days\ to\ final\ count}$$

By using formula of Bewley and Black (1985), coefficient of uniformity of emergence (CUE) was estimated. After completion of emergence, final germination percentage was taken as ratio of count of germinated seeds to total planted seeds multiplied by 100.

Agronomic and Yield Related Attributes

The flag leaf area was estimated at flowering stage of wheat by using leaf area meter (LI-3100, USA). Whereas, spike length, 100 grain weight and per plant grain yield were quantified at maturity. Three plants sample was selected from each treatment and spike length was assessed with the help of measuring rod. After that, manual threshing of wheat plants was carried out to measure the grains weight by using an electrical weighing balance (AND-3000, Japan) and average was taken for final grain yield per plant. Furthermore, wheat seeds (100) were counted with the help of seed counter (Numigral, UK) and weighed by using same electrical weighing balance. Wheather data for the whole period of the study was noted and presented on weekly basis (Fig. 1).

Quantification of Chlorophyll Content

Chlorophyll *a*, *b* and total contents were measured by using the method proposed by Arnon (1949). Plant material (0.5 g) was completely homogenized in 10 mL of 80% acetone to extract the chlorophyll pigments. The optical density of the supernatant was measured at 663 and 645 nm by spectrophotometer (Hitachi-150-20, Japan). The following formulae were used to calculate the chlorophyll contents:

$$Chlorophyll\ a = 0.0127D_{663} - 0.00269D_{645}$$
$$Chlorophyll\ b = 0.0229D_{645} - 0.00468D_{663}$$
$$Total\ chlorophyll = 0.0202D_{645} + 0.00802D_{663}$$

Quantification of Compatible Solutes

The proline content was measured following the method of acid ninhydrin proposed by Bates *et al.* (1973). Wheat fresh leaf tissues (0.5 g) were ground in 10 mL of 3% (w/v) sulfosalicylic acid solution and filtered. The filtrate (2 mL) was homogenized with 2 mL each of acid ninhydrin and glacial acetic acid and placed in water bath to incubate at 100ºC for 1 h, and then put it in ice to terminate reaction. After adding toluene (4 mL) and practicing vortex, free proline containing chromophore was aspirated and optical density was measured at 520 nm using spectrophotometer (Hitachi-150-20, Japan). A standard curve was used to quantify proline content and following equation exercised for calculation:

$$\mu moles\ proline/g\ fresh\ weight = \frac{\text{ug proline/ml} \times \text{ml toluene}}{[115.5\ \text{ug/umol}] \div [\text{g sample/5}]}$$

The method of Grieve and Gratan (1983) was used for determination of glycine betaine. Fresh leaf material (1.0 g) was homogenized with 10 mL of distilled water and after filtration 1 mL of extract was acidified by HCl (2N) and then 0.5 mL of this solution was mixed with 0.2 mL of potassium tri-iodide solution. The final mixture was placed in ice bath for 90 min with random shaking followed by addition of 2 mL distilled water (chilled) and 10 mL of cooled 1, 2 dichloroethane. A continuous stream of air was passed for 30-45s to mix the double layered solution. The top aqueous deposit was redundant and absorbance of organic layer was subjected to read at 365 nm in double beam Spectrophotometer (Hitachi-150-20, Japan).

To estimate total phenolics, leaf sample (0.5 g) was homogenized in 10 mL of 80% acetone. The gallic-acid-equivalent soluble phenolics were appraised by the method of Waterhouse (2001).

Cell Membrane Stability and Relative Water Contents

Six segments of leaf approximately of the same size were obtained from stock plants and were evaluated for cell membrane thermostability by following the procedures described by Blum and Ebercon (1981). In case of relative water contents, fresh leaves sample of 0.5 g (W_f) was dipped in distilled water in anticipation of leaves have achieved constant weight. After gaining turgidity leaves were weighted for a second time (W_S) and after that kept for drying for 24 h at 80°C to estimate dry weight (W_d). Relative water contents were calculated by following formula:

$$RWC(\%) = \frac{(Wf - Wd)}{(Ws - Wd)} \times 100$$

Statistical Analysis

Standard statistical procedures were employed to find out the significant difference among the treatment means (Steel *et al.,* 1997). To present graphical demonstration of data and to estimate standard errors meant for treatments comparison Microsoft Excel (Microsoft Corporation, Los Angeles, CA, USA) was used.

Results

Seedling stand Establishment

The restricted seed germination, seedlings stand establishment and their limited growth was observed under late sown conditions. Results also revealed a significant positive effect of priming over seedling emergence and stand establishment under all experimental conditions (Table 1). Among all treatments, osmopriming with MLE was more effective to establish seedling stand under control as well late sowing. However, hormonal priming with salicylic acid (SA) showed least time taken to 50% emergence under both conditions and also expressed minimum mean emergence time (MET) and maximum emergence index (EI) under optimal sowing. Whereas, under late sown wheat, seedlings raised from osmopriming with MLE exhibited minimum MET and maximum EI, emergence rate (ER), coefficient of uniformity index (CUI) and final emergence percentage (FEP) over control. These results were followed by hormonal priming with SA and osmopriming with sorgaab.

Growth and Yield Parameters

Growth and yield traits of the wheat crop were also significantly affected upon exposure to seed priming treatments and heat stress (Table 2). High temperature reduced flag leaf area and spike length by 23% and 15%

Table 1: The effects of seed priming on stand establishment of wheat seedlings under optimal and high temperature conditions

Priming treatments	E_{50} (Days)		MET (Days)		ER (Plants/day)		CUI		EI		FEP (%)	
	Optimal	Late sown	Optimal	Late sown	Optimal	Late sown	Optimal	Late sown	Optimal	Late sown	Optimal	Late sown
Control	4.0 a	3.45 b	5.35 a	5.19 ab	1.58 fg	1.44 g	0.168 f	0.13f	1.75 f	1.77 f	82.2 ef	70.0 g
Sorgaab-priming	3.28 bc	3.32 bc	5.02 b	5.06 b	1.75ef	1.69 ef	0.25 de	0.23e	2.11 de	2.04de	86.2 cd	73.3 g
Salicylicatepriming	2.32 f	2.65 e	4.25 d	4.69 c	2.36 ab	2.00 cd	0.51a	0.36c	3.51 a	2.72 c	96.6 ab	80.0 f
Osmpriming ($CaCl_2$)	3.36 bc	3.32 bc	5.09 b	5.15 ab	1.84 de	1.61efg	0.28d	0.21 e	2.16 d	1.90ef	94.4 b	84.5 de
MLE-priming	3.16 c	2.91 d	4.51 c	4.50 c	2.50 a	2.22 bc	0.52 a	0.42 b	3.37 a	3.11 b	98.9 a	88.9 c
LSD at (P ≤0.05)	*0.21*		*0.22*		*0.244*		*0.041*		*0.26*		*3.88*	

Means sharing the same letter for a parameter, do not differ significantly at p ≤ 0.05; E_{50}= time to 50% emergence; MET= mean emergence time; ER= emergence rate; CUE= coefficient of uniformity of emergence; EI= emergence index; FEP= Final emergence percentage

Table 2: The effects of seed priming on growth and yield attributes of wheat under optimal and high temperature conditions

Priming treatments	Flag leaf area (cm^2)		Spike length(cm)		100-grain weight (g)		Grain yield/Plant (g)	
	Optimal	Late sown	Optimal	Late sown	Optimal	Late sown	Optimal	Late sown
Control	28.4 c	21.8 f	8.36 cd	7.41 e	3.07 c	2.75 d	1.86 ef	1.55 g
Sorgaab-priming	31.3 b	23.7 ef	9.61 ab	8.21cd	3.11 bc	2.93 cd	2.12 bc	1.82 f
Salicylicatepriming	33.2 ab	26.4 cd	8.92 bc	8.70 c	3.49 a	3.16 bc	2.01 cd	1.92 def
Osmpriming ($CaCl_2$)	34.0 a	25.3 de	9.72 a	7.69 de	3.35 ab	3.09 bc	2.29 a	1.89 ef
MLE-priming	32.2 ab	24.7de	10.0 a	7.67 de	3.60 a	3.02 cd	2.22 ab	1.94 de
LSD at (P ≤0.05)	*2.53*		*0.79*		*0.28*			*0.120*

Means sharing the same letter for a parameter do not differ significantly p ≤ 0.05 by least significant difference test

respectively as compared to control. Thus, all seed priming treatments have significantly mitigated the adverse effects of high temperature.

However, osmopriming with $CaCl_2$ and MLE were most effective in improving flag leaf area and spike length respectively, in control conditions. Under high temperature regime, hormonal priming with SA illustrated maximum flag leaf area, spike length and 100 grain weight relative to other priming treatments. Hence, non-primed control failed to cope with high temperature and revealed minimum values for flag leaf area, spike length, 100-grain weight and grain yield per plant.

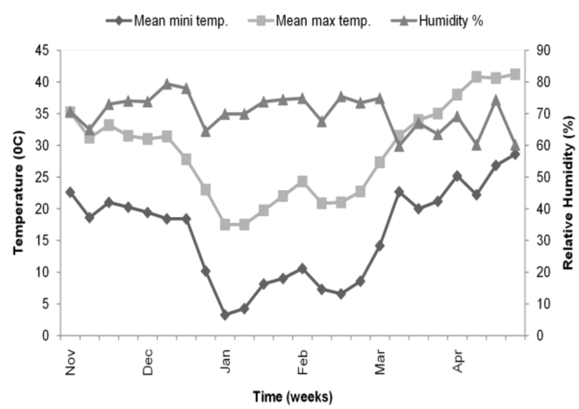

Fig. 1: The average temporal variations in air temperature and relative humidity on weekly basis during crop season

Physiological and Biochemical Attributes

The degradation in chlorophyll a, b and total contents were found in stressed plants in response to late sown conditions. All the seed priming treatments significantly improved chlorophyll contents over untreated control (Fig. 2a, b and c). However, wheat seeds subjected to osmopriming with $CaCl_2$ and Sor produced higher chlorophyll *a* content under both conditions. On other hand, maximum chlorophyll *b* and total chlorophyll contents were observed in osmopriming with MLE which is statistically at par with Sor and SA priming under high temperature regime. Hence, among seed treatments, hormonal priming with SA showed maximum stability in chlorophyll contents under stress conditions. Non-primed control illustrated minimum chlorophyll *a*, *b* and total contents on exposure to optimal as well as late sown conditions.

Results of Fig. 3a indicated that high temperature stress substantially reduced leaf relative water (RWC) content in wheat but seed priming significantly improved RWC by 8.8 and 8.6% with respect of untreated control under optimal and late sowing conditions. Among all treatments, seed priming with $CaCl_2$ showed more consistent results for improved RWC under optimal as well as late sown conditions. Results also revealed significant variation in cell membrane thermo-stability (CMT) in response to high temperature under suboptimal conditions (Fig. 3b). However, seed priming treatments significantly enhanced membrane stability relative to untreated control. Wheat seeds exposed to MLE were most effective in improving CMT followed by $CaCl_2$ under optimal conditions whereas both were also revealed maximum CMT and were found statistically at par in high temperature regime.

Contents of primary and secondary metabolites (Proline, glycine betaine, sugars and total phenols) in

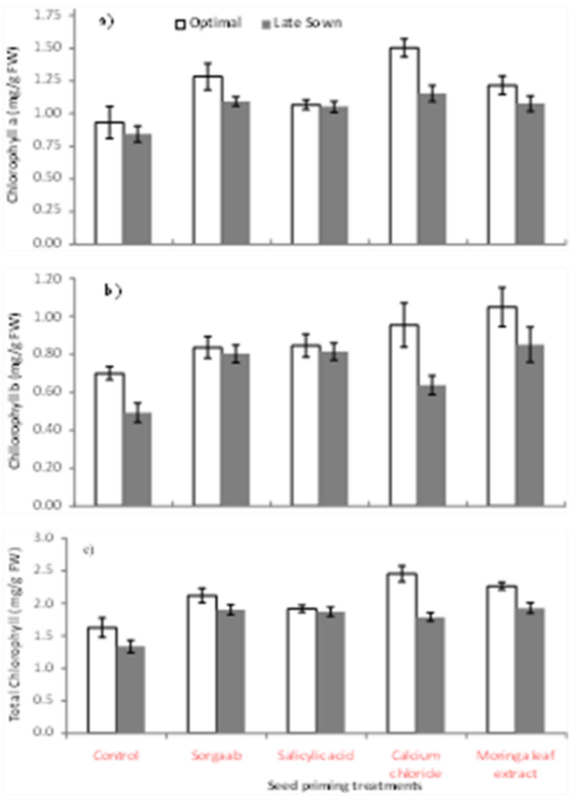

Fig. 2: The effect of seed priming on (a) leaf chlorophyll *a*, (b) chlorophyll *b* and (c) total chlorophyll (c) ± S.E. in wheat under optimal and late sown conditions

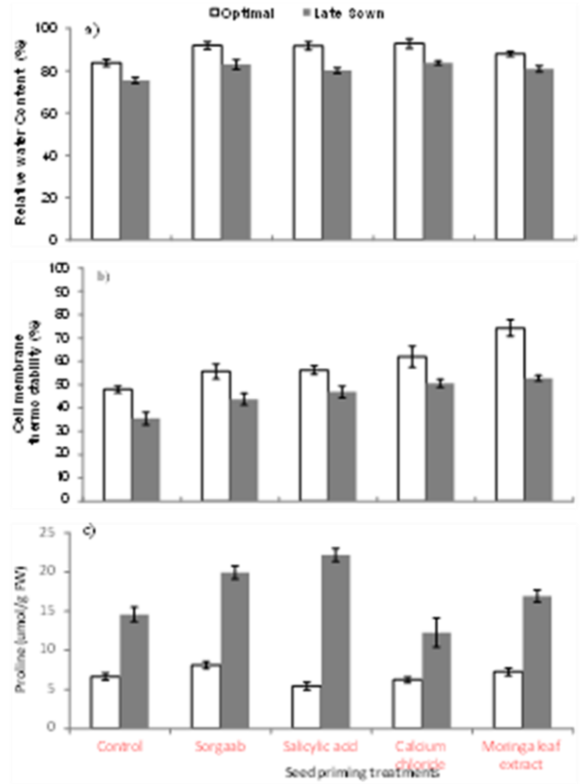

Fig. 3: The effect of seed priming on (a) leaf relative water content, (b) cell membrane thermostability and (c) proline ± S.E. in wheat under optimal and late sown conditions

wheat plants enhanced as result of seed priming treatments under optimal and late sown condition as well (Fig. 3c and 4a, b, c).

Under optimal conditions, maximum proline content was found in osmopriming with sorgaab which is followed by priming with MLE (Fig. 3c). Seed priming with SA failed to perform well and produced minimum proline content as compared to untreated control while it exhibited higher content of endogenous proline which is followed by Sor priming under late sown environment. However, osmopriming with $CaCl_2$ was unable to improve proline content relative to non-primed control. Moreover, significant ($P \leq 0.05$) variation in glycine betaine (GB) ranged from 6.58 to 13.77 (umol/g FW) was found in plants raised from different priming treatments under late sowing regime. Hormonal priming with SA negatively affected GB production and resulted in minimum GB content over non-primed control under all experimental conditions (Fig. 4a). On exposure to suboptimal temperature, osmopriming with $CaCl_2$ and Sor have efficiently improved GB content over untreated control which is followed by MLE. Likewise, wheat plants also showed a significant rise in Total soluble sugars (TSS) level in response to high temperature stress imposed through late sown conditions (Fig. 4b). On average, leaf soluble sugars level increased by approximately 60% from 6.93 mg/g in optimal sowing to 11 mg/g fresh weight under stress conditions.

It is cleared from results that osmopriming with SA failed to produce improved TSS as compared to control and other priming treatments under optimal conditions but resulted in highest production of TSS on exposure to high temperature. However, osmopriming with $CaCl_2$ had accumulated maximum soluble sugars under control conditions and also maintained better sugars content in high temperature regime where all the priming treatments performed better than untreated control (Fig. 4b). The present results illustrated that on an average by 20.5% more phenolic compounds produced in wheat plants under late sowing as compared to optimal conditions (Fig. 4c). Under late sowing regime, wheat plants raised from seeds subjected to osmopriming with $CaCl_2$ produced maximum leaf TPC that is followed by osmopriming with MLE. Thus, the seed priming with sorgaab was statistically at par with untreated control and behaved alike whereas hormonal priming with SA failed to improve over control and showed minimum content of total phenols.

Correlation Matrix

Under optimal conditions, grain yield showed a strong

Table 3: Correlation coefficient of various attributes influenced by seed priming techniques in wheat under optimal and late sowing conditions

	TCC	Pro	GB	TSS	TPC	RWC	CMT	GY
RWC (Optimal) (Late)	0.605*	-0.109	0.172	0.480	0.474	-	-	-
	-0.901*	0.826*	0.311	0.099	0.099			
CMT (Optimal) (Late)	0.504	0.005	0.739*	0.548*	0.242	0.345	-	-
	-0.719*	0.253	0.178	0.006	0.244	0.826*		
GY (Optimal) (Late)	0.731*	0.069	0.692*	0.702*	0.286	0.509	0.810*	-
	0.179	0.117	0.339	0.638*	0.358	0.022	0.338	

*= Significant at p ≤ 0.05; TCC, Total chlorophyll contents; GY, Grain yield; RWC, Relative water contents; CMT, Cell membrane thermostability; GB, Glycine betaine; TSS, Total soluble sugars; TPC, Total phenolics content

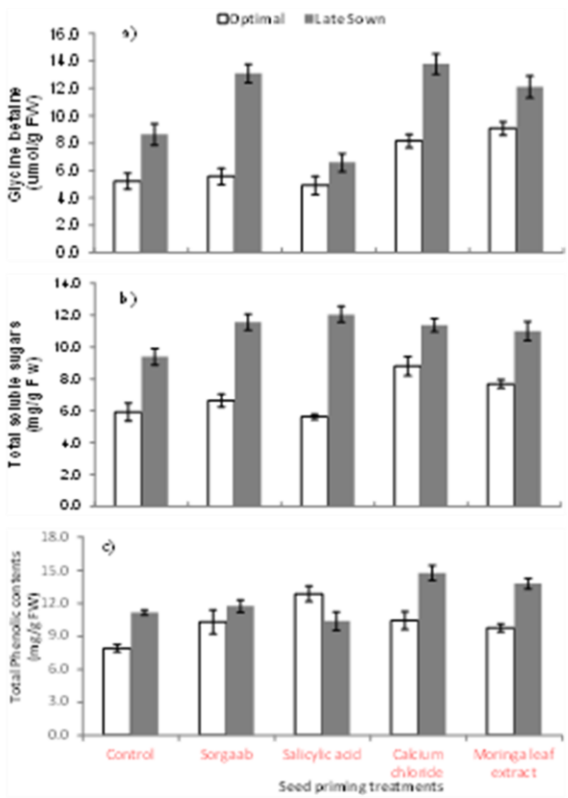

Fig. 4: Effect of seed priming on (a) leaf glycine betaine, (b) total soluble sugars and (c) total phenolics content ± S.E. in wheat under optimal and late sown conditions

positive correlation with TCC, GB, TSS and CMT, hence exhibited a positive but week correlation with Pro, TPC, RWC and CMT in late sown wheat (Table 3). There was also a positive correlation of CMT with TCC, Proline, GB, TSS and TPC under control regime while TCC were negatively related with CMT. Proline and TCC were negatively correlated with RWC under control and late sowing respectively, while also illustrated positively correlation with TCC, GB, TSS and TPC (Table 3).

Discussion

Time to 50% emergence, mean emergence time, coefficient of uniformity index and emergence index are important indicators of seedling vigor and uniform emergence (Lara *et al*., 2014; Mahboob *et al*., 2015). In our experiment, seedling emergence was affected under late sown conditions while priming techniques has significantly mitigated the deleterious effects of high extremes (Table 1). Among seed priming agents, MLE was most effective in improving germination and energetic seedling establishment followed by seed priming with SA. The boost in emergence might be due to the fact that these chemicals have significant role in metabolic repair (Burgass and Powell, 1984) as well as in augmenting the buildup of metabolites, which accelerates the food supply during germination (Kaur *et al.*, 2005; Farooq *et al*., 2006), resulting in better performance even under stress conditions (Farooq *et al.*, 2017). These chemicals also affect the activity of hydrolytic enzymes as well as speed up the metabolism of the embryo thus results in accelerated growth (Bam *et al*., 2006), as was also observed in our study.

In late sown wheat, flag leaf area and spike length significantly reduced on exposure to high temperature which may also cause a decline in 100 grain weight and grain yield per plant (Table 2). However, obvious detrimental results of high temperature might be attributed to impeded pollination and seed set (Farooq *et al.*, 2011), reduced number of ear heads and decreased grains per spike (Nawaz *et al*., 2013). The drastic effects of escalated temperature were mitigated through seed priming treatments by improving wheat performance which was visible through better flag leaf area, spike length, 100 grain weight and grain yield per plant (Table 2). The superior growth and yield of wheat plants exposed to SA priming might be due to its role in the enhancement of cell division in the apical meristem of wheat seedling which results in vigorous plant growth (Shakirova *et al.*, 2003) as was also observed in our study. Damage to photosynthetic system under high temperature stress can be significantly reduced by the application of SA (Hui-Jie *et al*., 2011) since, it has significant role in the activation of antioxidant enzymes in the plants (Ananieva *et al*., 2004). Moreover, the improved performance of wheat subjected to seed priming with MLE might be attributed to zeatin and cytokinin (Benzyl adenine) that enhanced the grain yield in wheat by improving its source sink capability (Gupta *et al.*, 2003), both have significant role in decreasing leaf senescence and chlorophyll degradation which ultimately contributes in better spike length, 100-grain weight and final grain yield under late sown conditions (Table 2). Similar findings were also reported by Farooq *et al*. (2017) in wheat and Mahboob *et al*. (2015) in maize, strengthening the results of present study.

It is obvious from the data that chlorophyll a, b and total chlorophyll contents of wheat leaves degraded by escalated temperature under late sown conditions (Fig. 2a, b and c). This chlorophyll loss is closely associated with damage to thylakoid membrane, due to enhanced activity of chlorophyllase under high temperature, which ultimately reduces the plant photosynthetic activity (Sharkey and Zhang, 2010). However, seed priming effectively attenuated the adverse effects of high temperature by improving chlorophyll contents (Fig. 2) might be attributed to better biosynthesis and stability of chlorophyll. The ameliorated contents of chlorophyll in plants subjected to $CaCl_2$ seed priming might be due to the ability of Ca^{+2} to decrease the activity of chlorophyllase (Issam *et al*., 2012). Overall, seeds primed with MLE produced higher photosynthetic pigments that might be because of the presence of several phenolic compounds in MLE having ability to neutralize free radicals (Adedapo *et al*., 2008). Moreover, MLE is also rich in zeatin, a natural cytokinin, that delays senescence of leaves with improved chlorophyll contents during stressful environment and produced higher grain yield (Yasmeen *et al*., 2013; Mahboob *et al*., 2015), as has been indicated by positive correlation of chlorophyll contents with grain yield (Table 3).

Normal plant cells have higher water contents as compared to stressed-plant; hence, leaf relative water content (RWC) provides a direct measure of cellular water contents. Present results showed a significant reduction in leaf RWC and CMT on exposure to high temperature in late sown wheat (Fig. 3). Improved RWC and CMT results from osmopriming with $CaCl_2$ and MLE, might be due to the fact that both contain calcium ions that played a significant role in the alleviation of stress-induced cell wall extensibility (Zidan *et al*., 1990) and/or water conductance of the plasma membrane (Azaizeh *et al*., 1992) consequently ameliorates water uptake and demonstrated better growth and yield that confirmed the findings of Mahboob *et al.* (2015) in maize. Higher cell membrane thermos-stability helps the plant to maintain its water potential even at high temperature, which confirmed a positive correlation between leaf RWC and CMT in late sown wheat (Table 3).

Among the primary metabolites, accumulation of proline, glycine betaine and soluble sugars is an important tolerance mechanism exhibited by plants under stress conditions like heat (Wahid *et al*., 2007). The data revealed significant improvement in the production of primary metabolites due to seed priming in normal as well as late sown conditions (Fig. 3c, 4a and b). However, in late sowing maximum accumulation of proline was observed in hormonal priming with SA (Fig. 3c) that might be due to increase in γ-glutamyl kinase and decrease in proline oxidase activities as were reported by Khan *et al*. (2013). Similar to our findings, Ahmad and Hasan (2011) also reported increased proline production under high temperature stress. Glycine betaine also has a crucial role in heat stress tolerance by protecting plant cells through osmotic adjustment, maintenance of RWC and stabilizes proteins to secure the photosynthetic apparatus (Wang *et al*., 2014). It is obvious from data that under high temperature, all the priming treatments improved GB accumulation as compared to untreated control, however, SA treatment showed significant reduction in the synthesis of GB (Fig. 4a). High accumulation of GB contents improved the resistance to high-temperature stress (Sakamoto and Murata, 2002) since GB production protects Rubisco activase near thylakoids which prevents inactivation of Rubisco even at higher temperature (Allakhverdiev *et al*., 2008). It is obvious from data that accumulation of GB is closely associated with the stability of cell membranes, therefore a positive correlation was observed between these parameters which is attributed to better plant growth (Table 3).

Results showed a significant increase in the concentration of total soluble sugars, in plants raised from primed seeds, when exposed to high temperature (Fig. 4b), which substantiate the reports of Wahid *et al.* (2007). These sugars play a critical role in mitigating the effects of high temperature, either by osmotic adjustment or by protecting cellular structures by maintaining water balance (Farooq *et al*., 2008), that might be contributed to better grain yield and expressed a strong positive correlation with grain yield under all experimental conditions (Table 3). High temperature stress also induced a change in biosynthesis of total phenolic contents (TPC) (Rivero *et al*., 2001) and generally associated with tolerance to heat stress (Wahid *et al*., 2007). Being equipped with the ability to acts as hydrogen donators, phenolics may help in stabilization of membranes by detoxifying reactive oxygen species (Rice-Evans, 2001). The maximum improvement in TPC was observed in wheat plants raised from seeds primed with $CaCl_2$ and MLE (Fig. 4c) as were also reported by Yasmeen *et al.* (2013). However, enhanced phenolic contents as a result of seed priming, could be the result of increased activity of Phenylalanine ammonia-lyase (PAL) which is considered as the main acclamatory response of cells to heat stress (Wahid *et al*., 2007) and positively associated with RWC, CMT and grain yield (Table 3).

Lower GB and TPC in plants grown from non-primed and SA soaked seeds might be due to absence of important nutrients or antioxidants, which are abundantly present in other priming solutions.

Conclusion

Keeping in view all results, we concluded that among the seed priming treatments osmopriming with moringa leaf extract and hormonal priming with salicylic acid can be successfully used to improve wheat quality attributes like seedling establishment, spike length, CMT, RWC, antioxidant phenolics and compatible solutes even at high temperature under delayed planting.

Acknowledgements

The authors are grateful to Dr. Mahboob Ali Sial, Plant Breeding and Genetics Division, Nuclear Institute of Agriculture, Tandojam, Pakistan, for providing the seeds of wheat genotype "NIA-Amber" used in this study.

References

Adedapo, A.A., F.O. Jimoh, A.J. Afolayan and P.J. Masika, 2008. Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of *Calpurnia aurea*. *BMC Compl. Altern. Med*., 8: 53

Ahmad, J.U. and M.A. Hassan, 2011. Evaluation of seedling proline content of wheat genotypes in relation to heat tolerance. *Bang. J. Bot.,* 40: 17–22

Allakhverdiev, S.I., V.D. Kreslavski, V.V. Klimov, D.A. Los, R. Carpentier and P. Mohanty, 2008. Heat stress: an overview of molecular responses in photosynthesis. *Photosynthesis Res*., 98: 541–550

Ananieva, E.A., K.N. Christov and L.P. Popova, 2004. Exogenous treatment with salicylic acid leads to increased antioxidant capacity in leaves of barley plants exposed to paraquat. *J. Plant Physiol.,* 161: 319–328

Anonymous, 2017. *Agriculture Statistics of Pakistan*. Ministry of Food and Agriculture (Economics Wing), Islamabad, Pakistan

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta vulgaris*. *Plant Physiol*., 24: 1–15

Association of Official Seed Analysis (AOSA), 1990. Rules for testing seeds. *J. Seed Technol*., 12: 1–112

Azaizeh, H., B. Gunse and E. Steudle, 1992. Effects of NaCl and $CaCl_2$ on water transport across root cells of maize (*Zea mays* L.) seedlings. *Plant Physiol.,* 99: 886-894

Bam, R.K., F.K. Kumaga, K. Ofori and E.A. Asiedu, 2006. Germination, vigour and dehydrogenase activity of naturally aged rice (*Oryza sativa* L.) seeds soaked in potassium and phosphorous salts. *Asian J. Plant Sci*., 5: 948–955

Banard, A. and F.J. Calitz. 2011. The effect of poor quality of seed and various levels of grading factors on the germination, emergence and yield of wheat. *S. Afr. J. Plant Soil*, 28: 23–33

Basra, S.M.A., M. Farooq and A. Khaliq, 2003. Comparative study of pre-sowing seed enhancement treatments in fine rice (*Oryza sativa* L.). *Pak. J. Life Soc. Sci.,* 1: 5–9

Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, 39: 205–207

Bewley, J.D. and M. Black, 1985. *Seeds. Physiology of Development and Germination.* Plenum Press, New York, USA

Bita, C.E. and T. Gerats, 2013. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress tolerant crops. *Front. Plant Sci.* 273 (4) doi: 10.3389/fpls.2013.00273

Blum, A. and A. Ebercon, 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop Sci*., 21: 43–47

Burgass, R.W. and A.A. Powell, 1984. Evidence for repair processes in the invigoration of seeds by hydration. *Ann. Appl. Biol*., 53: 753–757

Buriro, M., F.C. Oad, M.I. Keerio, S. Tunio, A.W. Gandahi, S.W.U. Hassan and S.M. Oad, 2011. Wheat seed germination under the influence of temperature regimes. *Sarhad J. Agric.,* 27: 539–543

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

Edwards, J., 2008. Factors affecting wheat germination and stand establishment in hot soils. Oklahoma Cooperative Extension Service

Sivakumar, M.V.K., H.P. Das and O. Brunini. 2005. Impacts of present and future climate variability and change on agriculture and forestry in the arid and semi-arid tropics. *Climatic Change*, 70: 31–72

Einhellig, F.A. and G.R. Leather, 1988. Potentials for exploiting allelopathy to enhance crop production. *J. Chem. Ecol.,* 14: 1829–1844

Ellis, R.A. and E.H. Roberts, 1981. The quantification of ageing and survival in orthodox seeds. *Seed Sci. Technol.,* 9: 373–409

Farooq, M., H. Bramley, J.A. Palta and K.H.M. Siddique, 2011, Heat stress in wheat during reproductive and grain filling phases. *Crit. Rev. Plant Sci.,* 30: 491–507

Farooq, M., M. Rizwan, A. Nawaz, A. Rehman and R. Ahmad, 2017. Application of natural plant extracts improves the tolerance against combined terminal heat and drought stresses in bread wheat. *J. Agro. Crop Sci.,* 1–11

Farooq, M., S.M.A. Basra, H. Rehman and B.A. Saleem, 2008. Seed priming enhances the performance of late sown wheat (*Triticum aestivum* L.) by improving the chilling tolerance. *J. Agron. Crop Sci*., 194: 55–60

Farooq, M., S.M.A. Basra, M. Khalid, R. Tabassum and T. Mahmood, 2006. Nutrient homeostasis, reserves metabolism and seedling vigor as affected by seed priming in coarse rice. *Can. J. Bot.,* 84: 1196–1202

Grieve, C.M. and S.R. Gratan, 1983. Rapid assay for determination of water soluble. Quaternary ammonium compounds. *Plant Soil*, 70: 303–307

Gupta, N.K., S. Gupta, D.S. Shukla and P.S. Deshmukh, 2003. Differential responses of BA injection on yield and specific grain growth in contrasting genotypes of wheat (*Triticum aestivum* L.). *Plant Growth Regul.,* 40: 201–205

Hasan, M.A. and J.U. Ahmed, 2005. Kernel growth physiology of wheat under late planting heat stress. *J. Natl. Sci. Found. Sri Lanka,* 33: 193–204

Hui-Jie, Z., Z. Xue-Juan, M. Pei-Fang, W. Yue-Xia, H. Wei-Wei, L. Li-Hong and Z. Yi-Dan, 2011. Effects of salicylic acid on protein kinase activity and chloroplast D1 protein degradation in wheat leaves subjected to heat and high light stress. *Acta Ecol. Sin.,* 31: 259–263

Hussain, I., R. Ahmad, M. Farooq and A. Wahid, 2013. Seed Priming improves the performance of poor quality wheat seed. *Int. J. Agric. Biol*., 15: 1343–1348

Ishige, K., D. Schubert and Y. Sagara, 2001. Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. *Free Rad. Boil. Med.,* 30: 433–446

Issam, N., M. Kawther, M. Haythem and J. Moez, 2012. Effects of $CaCl_2$ pretreatment on antioxidant enzyme and leaf lipid content of faba bean (*Viciafaba* L.) seedlings under cadmium stress. *Plant Growth Regul.,* 68: 37–47

Johnson, R.C. and E.T. Kanemasu, 1983. Yield and development of winter wheat at elevated temperatures. *Agron. J.,* 75: 561–565

Kaur, S., A.K. Gupta and N. Kaur, 2005. Seed priming increases crop yield possibly by modulating enzymes of sucrose metabolism in chickpea. *J. Agron. Crop Sci.,* 191: 81–87

Khan, M.I.R., N. Iqbal, A. Masood, T.S. Per and N.A. Khan, 2013. Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation. *Plant Signal. Behav.,* 8: 26374–26384

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

Wani, S.H., V. Kumar, V. Shriram and S.K. Sah, 2016. Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. *Crop J.,* 4: 162–176

Salma, I., C. Abdelly, A. Bouchereau, T. Flowers and A. Savouré. 2015. Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. *Ann. Bot*., 115: 433–447

Mahboob, W., H.U. Rehman, S.M.A. Basra, I. Afzal, M.A. Abbas, M. Naeem and M. Sarwar, 2015. Seed priming improves the performance of late sown spring maize *(Zea mays*) through better crop stand and physiological attributes. *Int. J. Agric. Biol.*, 17: 491–498

Nawaz, A., M. Farooq, S.A. Cheema and A. Wahid, 2013. Differential response of wheat cultivars to terminal heat stress. *Int. J. Agric. Biol.*, 15: 1354–1358

Rice-Evans, C., 2001. Flavonoid antioxidants. *Curr. Med. Chem.*, 8: 797–807

Rincon, M. and J.B. Hanson, 1986. Control of calcium ion fluxes in injured or shocked corn root cells: importance of proton pumping and cell membrane potential. *Physiol. Plant.*, 67: 576–583

Rivero, R.M., M.J. Ruiz, P.C. Garcia, L.R. Lopez-Lefebre, E. Sanchy and L. Romero, 2001. Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and water melon plants. *Plant Sci.*, 160: 315–321

Rosegrant, M.W. and M. Agcaoili, 2010. *Global Food Demand, Supply, and Price Prospects to 2010*. International Food Policy Research Institute, Washington DC, USA

Sakamoto, A. and N. Murata, 2002. The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. *Plant Cell Environ.*, 25: 163–171

Shakirova, F.M., A.R. Sakhabutdinova, M.V. Bezrukova, R.A. Fatkhutdinova and D.R. Fatkhutdinova, 2003. Changes in 734 the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Sci.*, 164: 317–322

Sharkey, T.D. and R. Zhang, 2010. High temperature effects on electron and proton circuits of photosynthesis. *J. Integr. Plant Biol.*, 52: 712–722

Steel, R.G.D., J.H. Torrie and D.A. Dickey, 1997. *Principles and Procedures of Statistics: A Biometrical Approach*, 3rd edition. McGraw Hill Book Inc. Co., New York, USA

Wahid, A., S. Gelani, M. Ashraf and M.R. Foolad, 2007. Heat tolerance in plants: An overview. *Environ. Exp. Bot.*, 61: 199–223

Wang, G.P., F.X. Tian, M. Zhang and W. Wang, 2014. The overaccumulation of glycine betaine alleviated damages to PSII of wheat flag leaves under drought and high temperature stress combination. *Acta Physiol. Plant*, 36: 2743–2753

Wang, L.J. and S.H. Li, 2006. Salicylic acid-induced heat or cold tolerance in relation to Ca^{2+} homeostasis and antioxidant systems in young grape plants. *Plant Sci.*, 170: 685–694

Wardlaw, I.F. and C.W. Wrigley, 1994. Heat tolerance in temperate cereals. An overview. *Aust. J. Plant Physiol.*, 21: 695–703

Waterhouse, A.L., 2001. Determination of total phenolics. *In*: *Current Protocols in Food Analytical Chemistry*, pp: 1–8. Wiley, Hoboken, New Jersey, USA

Wheeler, T.R., G.R. Batts, R.H. Ellis, P. Hadley and J.I.L. Morison, 1996. Growth and yield of winter wheat (*Triticum aestivum L.)* crops in response to CO_2 and temperature. *J. Agric. Sci. Camb.*, 127: 37–48

Yasmeen, A., S.M.A. Basra, A. Wahid, W. Nouman and H.U. Rehman, 2013. Exploring the potential of moringa (Moringaoleifera) leaf extract (MLE) as seed priming agent in improving wheat performance. *Turk. J. Bot.*, 37: 512–520

Zidan, I., H. Azaizeh and P.M. Neumann, 1990. Does salinity reduce growth in maize root epidermal cells by inhibiting their capacity for cell wall acidification? *Plant Physiol.*, 93: 7–11

(Received 26 January 2018; Accepted 04 May 2018)