

# Improving heat stress tolerance in late planted spring maize by using different exogenous elicitors

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# ABSTRACT

Recent global warming has increased the risk of heat stress which may adversely affect crop productivity worldwide. Higher temperature during reproductive stage is one of major constraint that adversely affects grain filling and seed setting in spring maize (Zea mays L.) The purpose of this study was to evaluate the potential of different elicitors (salicylic acid, CaCl<sub>2</sub>, Moringa oleifera Lam. leaf extract) to improve yield performance of heat stressed spring maize. Seed priming techniques (hydropriming, osmopriming, organic priming and hormonal priming) were used to investigate the impact of exogenous elicitors on physiological, biochemical and yield-related attributes of late planted spring maize. Results revealed that higher temperature at maturity caused membrane leakage, reduced photosynthetic pigments and net assimilation rate which ultimately led to decreased grain yield. However, exogenous elicitors improved emergence characteristics and triggered early seedling development, and exhibited significantly higher seedling chlorophyll contents than control plants. Among elicitors, salicylic acid (SA) exhibited significantly higher photosynthetic pigment (17%), membrane stability index (26%), relative water content (16%), crop growth rate (13%), net assimilation rate (29%), grain yield (27%), biological yield (14%), harvest index (9%) and grain protein (28%) as compared to control in late planted spring maize. The multivariate analysis also indicate that physio-biochemical traits were more pronounced in hormonal priming with SA as compared to other exogenous elicitors. In conclusion, application of exogenous elicitors like SA is most effective and easy approach that may help to improve crop performance with increased grain yield and quality in late planted spring maize that prone to high temperature.

Key words: Global warming, heat stress, maize, Moringa oleifera, priming techniques, salicylic acid.

# **INTRODUCTION**

Maize (*Zea mays* L.) is one of the most significant cereal crop and it is consumed worldwide by both human and animals. Mostly maize grown twice in a year (spring and autumn) and is cultivated on an area of 160 million hectares with the production of 822 million tones around the world (Ashraf et al., 2015). Spring sown crop is much higher in yield as compared to autumn crop but heat stress due to high temperature at reproductive stage reduced its yield potential (Afzal et al., 2008; Ahmad et al., 2017). Plant growth, chlorophyll contents, net photosynthetic rate, pollen kernel setting and seed fillings are severely affected under high temperature in maize (Ramadoss et al., 2004). It has been also observed that increase in day temperature to 38 °C at reproductive stage causes serious damage to pollination and seed setting which ultimately resulting lowers grain yield in spring planted maize (Afzal et al., 2008).

One way of increasing maize productivity in high temperature stress is to breeding the cultivars that are more tolerant to stress but requires long time with limited success. Among several approaches, seed priming is considered an easy, low cost and effective approach for improving plant defense against environmental stresses in numerous crop plants (Afzal et al., 2008; Savvides et al., 2016). Priming is defined as the pre exposure of seeds and young seedlings to chemical agents such as plant growth regulators (PGR), antioxidant, organic compatible solutes, inorganic salts, etc., that makes them more resilient to subsequent stresses (Borges et al., 2014; Hossain et al., 2015; Savvides et al., 2016). Several studies demonstrated that seed priming promotes germination with vigorous stand establishment and also stimulate subsequent growth and metabolic processes that resulting in increased final yield in numerous crops like maize (Rehman et al., 2015; Bakhtavar et al., 2015; Ahmad et al., 2017).

Various priming techniques such as hydropriming, osmopriming, redox priming, halopriming or hormonal priming are employed to ameliorate the deleterious effects of different environmental stresses (Hussain et al., 2016). Various exogenous elicitors such as plant growth regulators (salicylic acid, indole acetic acid (IAA), cytokinin, gibberellin, etc.), signaling molecules (NO,  $H_2O_2$ ), organic solutes (glycine and butane), inorganic salts (CaCl<sub>2</sub>, KCl, KNO<sub>3</sub> and NaCl), different anti-oxidant and some natural extracts like *Moringa oleifera* Lam. leaf extracts (MLE) are used in priming techniques and each of them had different properties with varying level of efficacy. Previously, several studies reported seed priming with different elicitors such as salicylic acid (SA) (Farooq et al., 2008; Ahmad et al., 2015), CaCl<sub>2</sub> (Rehman et al., 2015), H<sub>2</sub>O<sub>2</sub> (Ahmad et al., 2015), moringa leaf extract (Basra et al., 2011; Rehman et al., 2015) and kinetin (Bakhtavar et al., 2015) improves the maize performance under low temperature.

Among PGR, SA was found effective against different abiotic stresses like drought, chilling and heat stress (Ahmad et al., 2015). Ahmad et al. (2017) reported that exogenous application of ascorbic acid (AsA), H<sub>2</sub>O<sub>2</sub> and SA stabilized the membrane damage and mitigate the deleterious effect of high temperature due to enhanced antioxidant activity in maize. Recently among natural resources, moringa gains attention because its leaves are enriched with zeatin, which is a PGR and its leaf extract improved crop performance under stress conditions (Basra et al., 2011; Rehman et al., 2014).

However, the role of these chemicals in late sown spring maize crop is still not clear and the field appraisal of these priming agents under high temperature is need to be tested. Therefore, we hypothesized that exogenous application of these chemicals possibly improves the grain yield and quality by effecting the physiological and biochemical processes of late planted spring maize under high temperature. Thus, present study was designed to evaluate the role of SA, MLE and CaCl<sub>2</sub> on germination, seedling development, physiological attribute, final yield and kernel quality.

# MATERIALS AND METHODS

#### Seed material and experiment details

The present study was carried out at research farm, Department of Crop Physiology, University of Agriculture Faisalabad, Faisalabad (31.25° N, 73.09° E; 184.4 m a.s.l.), Pakistan. Faisalabad falls under arid climate region with higher evapotranspiration rate, and received annually about 200 mm rainfall. The daily weather conditions prevailed during experimentation are presented in Figure 1. Hybrid maize (FH-810) was selected as test variety and its seeds were obtained from Punjab Seed Corporation, Pakistan. The experiment was designed under randomized complete block design (RCBD) with four replicates keeping net plot size of 5 m × 5 m. Recommended seed rate was 25 kg ha<sup>-1</sup> used for sowing done on 14 March. The distance between plants was 20 cm and between rows 75 cm, recommended doses of fertilizer for experimental soil N:P:K (250:120:100 kg ha<sup>-1</sup>) were applied to all treatments. All the P and K with half N were applied during seedbed preparation and remaining N was given at grain filling stage. Irrigations were scheduled as for crop requirement and all other practices (agronomic and plant protection measures) were kept uniform for all treatment. During the experiment period, weather data of experimental site were recorded (Figure 1).

## Seed priming treatments

Present study was carried out with following priming techniques *viz*. hydropriming (seeds soaked in distilled water), osmopriming with CaCl<sub>2</sub> (2.2%) (Rehman et al., 2011), hormonal priming with salicylic acid (SA; 50 mg L<sup>-1</sup>) (Farooq et al., 2008) and organic priming with *Moringa oleifera* Lam. leaf extracts (MLE; 3.3%) (Basra et al., 2011). The non-treated dry seeds and hydropriming were used as controls. For seed priming, maize seeds were soaked in respective



Figure 1. The daily fluctuation in air temperature, evapotranspiration (ET<sub>0</sub>) and rainfall during whole field experimental of late planted spring maize.

treatment solution for 18 h with continuous aeration provided by an aquarium pump and ratio between seeds and working solution was kept 1:5 (kg L<sup>-1</sup>).

#### **Germination indexes**

The field was visited daily to count the emerged seeds until constant germination. From emergence counts, different emergence attributes including coefficient of emergence velocity (CVE), emergence rate index (ERI), time taken to 50% emergence, mean emergence time (MET) and final emergence percentage (FEP) were calculated (Kader, 2005):

$$CVE (\% d^{-1}) = \sum N_i / \sum (N_i T_i) \times 100$$
  

$$ERI (\% d^{-1}) = \sum N_i / I$$
  

$$MET (d) = \sum (N_i T_i) / \sum N_i$$
  

$$E_{so} (d) = ti + (N/2 - ni/nj - ni) (tj - ti)$$
  

$$FEP (\%) = (Total number of emerged seedlings/total number of seeds sown) \times 100$$

where,  $N_i$  is the number of emerged seeds on day i, and  $T_i$  is the number of days from sowing;  $T_i$  is the number of days from sowing; N represent the final emergence count and  $n_i$ ,  $n_j$  are cumulative number of seeds emerged at adjacent days  $t_i$  and  $t_j$  when  $n_i < (N + 1)/2 < n_j$ .

#### **Physiological attributes**

For the determination of Chl *a* and *b* contents, fresh leaves sample (0.2 g) were extracted in 80% acetone for overnight at 0-4 °C and then extract was centrifuged at 8000 g for 10 min. Supernatant absorbance was observed at 645 and 663 optical density (OD's) using UV spectrophotometer (UV 4000, ORI, Ettlingen, Germany) and values were substituted in the following formula:

$$A = [(0.0127(OD \ 663) - 0.00269(OD \ 645)) \ 100]/0.5 = mg \ 100 \ mL^{-1}$$

 $B = [(0.0229(OD 645) - 0.00468(OD663)) 100]/0.5 = mg 100 mL^{-1}$ 

Membrane stability index (MSI) was measured in terms of electrolyte leakage (Sairam, 1994).

For relative water contents, fresh leave sample  $(W_f)$  of 0.5 g was dipped in water until leaves weight was constant. Saturated leaves were weighed  $(W_s)$ , then saturated sample was dried for 24 h at 80 °C. Dried leaves were weighed  $(W_d)$  and RWC were measured by using following formula:

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

#### Crop growth and development

Plant sample (1 m<sup>2</sup> containing four plants) was harvested at knee height from each replicate at fortnight interval. Immediately the fresh weight of harvested sample was recorded and then following parameters were measured. Leaf area was measured by using handheld laser leaf area meter (CI-203, CID Bio Science, Camas, Washington, USA). Leaf area index (LAI) was calculated using following formula:

LAI = Leaf area/Land area

Leaf area duration (LAD), crop growth rate (CGR) and net assimilation rate (NAR) were measured by using following equations (Hunt, 1978):

$$LAD = (LAI_1 + LAI_2) (t_2 - t_1)/2$$

where  $LAI_1$  is leaf area index at first harvest,  $LAI_2$  is leaf area index at  $2^{nd}$  harvest, and  $t_1$  and  $t_2$  are the date of first and second harvest respectively.

$$CGR = (W_2 - W_1)/t_2 - t_1)$$

where,  $W_1$  is total DM at first harvest,  $W_2$  is total DM at second harvest, and  $t_1$  and  $t_2$  are the date of first and second harvest respectively.

## NAR = TDM/LAD

where, TDM is total DM and LAD is leaf area duration.

#### Agronomic and yield related traits

At maturity, agronomic and yield related traits including plant height (PH), number of grains per cob, 1000-grain weight, biological yield (BY) and grain yield (GY) were recorded from unit area. For GY (t ha<sup>-1</sup>), after threshing, grain was air dried to 14% moisture contents. For BY (t ha<sup>-1</sup>), harvested plants were sun dried for 7 d. Harvest index (HI) was computed as ratio of GY to BY and expressed in percentage.

$$HI = GY/BG \times 100$$

#### Grain quality analysis

For grain protein analysis, 1 g oven dried grain sample was taken in Kjeldahl flask, 25 mL concentrated  $H_2SO_4$  added and digested with 5 g digestion mixture ( $K_2SO_4$ :Cu<sub>2</sub>SO<sub>4</sub>:Fe<sub>2</sub>SO<sub>4</sub>) on gas heater. Digested sample was cooled and made the 250 mL volume. For distillation, 10 mL solution was taken and N (ammonia form) was collected in receiver containing (4% boric acid solution). Indicator (bromocresol green) and methyl red was added and then titration was done against standard (0.1 N)  $H_2SO_4$ . After titration, obtained reading was multiplied with 6.25 to get crude protein percentage (AOAC, 1984).

## Statistical analysis

Data were analyzed statistically by using software Statistix 8.1 (Analytical Software, Tallahassee, Florida, USA) and significance between treatments was tested by using least significantly difference (LSD) test 0.05 probability levels. Graphical presentation of data was done with Microsoft Excel 2010. Correlations between traits were analyzed by using Origin Pro 9.1 software (Origin Lab Corporation, Northampton, Massachusetts, USA).

## RESULTS

#### Emergence and seedling stand establishment

The results indicated that seed priming with SA,  $CaCl_2$  and MLE significantly improved the germination indexes that results in vigorous seedling stand establishment. Considerably increased MET and decreased CVE, ERI, MET with reduced FEP was observed in non-treated plants as contrast with primed seeds (Table 1). Among treatments, seeds subjected to hormonal priming with SA took minimum time to reach 50% emergence and reduced MET as compared to control seeds.

Similarly, significantly increased CVE and EI were also observed by SA followed by osmopriming with CaCl<sub>2</sub> and MLE. Likewise, seed priming agents showed higher FEP with higher emergence index as compared to non-primed seeds. Maximum value of ERI was observed in SA followed by CaCl<sub>2</sub> (Table 2). Seed priming markedly improved the seedling growth as compared to non-primed and hydroprimed seeds. Highest value for vigorous seedling development in terms of root length, shoot length, shoot fresh and dry weight and seedling chlorophyll a and b contents were again observed in hormonal priming with SA (Figure 2, Table 2). The seedling vigor was significantly reduced by control treatments as compared to priming treatments, while nonsignificant difference was observed between priming agents.

### **Physiological attributes**

Our study observed late sown spring maize crop face higher temperature at later stages (Figure 1) that causes decreased chlorophyll contents, relative water contents and increased membrane leakage in control treatment as compared to priming treatments (Figure 2, Table 3). However, all the priming treatments significantly improved the above physiological traits

Treatments	E <sub>50</sub>	MET	CVE	ERI	FEP
	d	l	%	d-1	%
Control	8.3a	9.0a	9.2d	2.7c	90d
Hydropriming (DW)	7.5b	8.1b	9.8c	3.6b	94c
Organic priming (MLE)	6.1d	7.4bc	12.4b	3.7b	96b
Osmopriming (CaCl <sub>2</sub> )	6.3c	7.6c	12.2b	3.8ab	100a
Hormonal priming (SA)	5.8d	6.9d	13.7a	4.1a	100a
LSD ( $p \le 0.05$ )	0.46	0.58	0.29	0.40	1.13

Table 1. Time to 50% emergence ( $E_{so}$ ), mean emergence time (MET), coefficient of emergence velocity (CVE), emergence rate index (ERI) and final emergence percentage (FEP) of maize influenced by different priming elicitors.

Values are means of four replicates and different letters indicate significant difference at  $P \le 0.05$ . DW: Distilled water; MLE: *Moringa oleifera* leaf extract; SA: salicylic acid.

Table 2. Root length (RL), shoot length (SL), shoot fresh weight (SFW) and shoot dry weight (SDW) of heat stressed maize influenced by different priming elicitors. Data were collected 30 d after sowing (DAS).

Treatments	RL	SL	SFW	SDW
	c	m		g
Control	16.02b	29.33d	6.80d	1.45e
Hydropriming (DW)	16.37b	33.33c	7.10d	1.61d
Organic priming (MLE)	20.39a	38.67a	8.53c	1.73c
Osmopriming (CaCl <sub>2</sub> )	19.67a	36.00b	9.67b	2.05b
Hormonal priming (SA)	17.73ab	35.67b	10.93a	2.25a
LSD ( $p \le 0.05$ )	1.81	2.82	0.50	0.06

Values are the means of four replicates and different letters indicate significant difference at  $P \le 0.05$ . DW: Distilled water; MLE: *Moringa oleifera* leaf extract; SA: salicylic acid.



Figure 2. Impact of different priming elicitors on shoot length, root length, chlorophyll *a* and *b* contents (30 d old seedlings), membrane stability index and relative water contents (recorded at 90 d after sowing) of heat stressed maize.

 $CK: Control; HP: hydropriming; SA: hormonal priming with salicylic acid; MLE: organic priming with moringa leaf extract; CaCl_2: osmopriming with CaCl_2.$ 

Values are the means of four replicates and different letters indicates significant difference at  $P \le 0.05$ .

Table 3. Impact of different priming elicitors on photosynthetic pigments concentration of heat stressed maize at maturity
(90 d after sowing).

Treatments	Chl a	Chl b	Chl ( <i>a</i> + <i>b</i> )	Chl a/b ratio		
	mg 100 mL <sup>-1</sup>					
Control	0.57d	0.14d	0.71d	0.28d		
Hydropriming (DW)	0.82c	0.28b	1.10c	0.41c		
Organic priming (MLE)	0.88c	0.25c	1.13c	0.44c		
Osmopriming (CaCl2)	1.09b	0.26bc	1.35b	0.54b		
Hormonal priming (SA)	1.54a	0.38a	1.91a	0.77a		
$LSD (p \le 0.05)$	0.13	0.02	0.13	0.07		

Values are the means of four replicates and different letters indicate significant difference at  $P \le 0.05$ . DW: Distilled water; MLE: *Moringa oleifera* leaf extract; SA: salicylic acid.

as compared to control. Higher chlorophyll a and b contents were observed by SA followed by CaCl<sub>2</sub> and MLE and highest value of MSI was also observed by SA followed by MLE and CaCl<sub>2</sub>. Similarly, relative water contents and chlorophyll a and b contents at maturity were also improved by priming treatments and significance was similar as above (Figure 2F, Table 3).

# Crop growth and development

Improved germination, early and vigorous seedling strand establishment due to seed priming also attributed to enhanced crop growth and development. Leaf area index (LAI) and LAD at different crop harvest were found higher by hormonal priming with SA followed by MLE and CaCl<sub>2</sub> (Table 4). Similarly, CGR and NAR at different crop harvest were also significantly increased by priming treatments as compared to control (Figure 3). Higher CGR and NAR were again recorded by SA followed by MLE and CaCl<sub>2</sub>.

	Treatments	45 DAS	60 DAS	75 DAS	90 DAS
CGR, g m <sup>-2</sup> d <sup>-1</sup>	Control	9.14m	15.92e	12.24j	3.57n
	Hydropriming (DW)	9.23c	16.11c	12.54i	3.24q
	Organic priming (MLE)	9.51k	17.94b	13.11h	3.21q
	Osmopriming (CaCl <sub>2</sub> )	9.451	15.97d	13.21g	3.25pq
	Hormonal priming (SA)	9.48kl	18.10a	13.78f	3.290
NAR, g m <sup>-2</sup> d <sup>-1</sup>	Control	2.70gh	3.27d	2.31k	1.91no
	Hydropriming (DW)	2.77g	3.23d	2.33k	1.890
	Organic priming (MLE)	2.96f	4.11b	2.57i	2.03m
	Osmopriming (CaCl <sub>2</sub> )	2.85g	3.86c	2.42j	1.98mn
	Hormonal priming (SA)	3.01e	4.21a	2.63hi	2.161
LAD, d	Control	56.78m	68.10e	57.83j	42.98r
	Hydropriming (DW)	57.231	68.40d	58.35i	43.20q
	Organic priming (MLE)	57.83j	70.58b	60.75g	44.850
	Osmopriming (CaCl <sub>2</sub> )	57.45k	69.90c	60.00h	44.40p
	Hormonal priming (SA)	58.35i	71.18a	61.20f	45.53n
LAI	Control	2.561	3.02i	4.13e	2.18n
	Hydropriming (DW)	2.75k	3.23h	4.38d	2.42m
	Organic priming (MLE)	2.96i	3.67g	4.91b	2.81jk
	Osmopriming (CaCl <sub>2</sub> )	2.93ij	3.64g	4.75c	2.73k
	Hormonal priming (SA)	2.98i	3.86f	5.09a	2.91ij

Table 4. Impact of different priming elicitors on crop growth rate (CGR), net assimilation rate (NAR), leaf area duration (LAD) and leaf area index (LAI) at 45, 60, 75 and 90 d after sowing (DAS) in heat stressed maize.

Values are the means of four replicates and different letters indicate significant difference at  $P \le 0.05$ . DW: Distilled water; MLE: *Moringa oleifera* leaf extract; SA: salicylic acid.

# Agronomic and yield related traits

Priming techniques significantly improved the crop performance and ultimately results in higher final GY of late sown spring maize. Hormonal priming with SA showed maximum plant height and found nonsignificant difference among priming agents except in control which was recorded with minimum plant height (Figure 3). The highest grain rows per cob were recorded for SA followed by MLE. All priming treatments markedly improved the 1000 grain weight, GY, BY and harvest index as contrast to control (Figure 3). However, maximum final yield and yield related traits were recorded for hormonal priming with SA higher followed by MLE except 1000 grain weight which was followed by CaCl<sub>2</sub>.

# Grain quality

Maize grain quality in terms of protein contents were declined by high temperature in control as compared to priming treatments. However all priming agents significantly improved the grain protein contents, maximum value was recorded for SA that was significantly different as compared to osmopriming with MLE and CaCl<sub>2</sub> (Figure 3H).

## Correlation

Correlation among all the studied traits is given in Table 5 and the interrelationships between all parameters and their multivariable response against different priming techniques employing exogenous elicitors are given in Figure 4. Correlation analysis showed that GY and kernel quality of maize is strongly positively correlated with photosynthetic pigment, MSI, CGR, and NAR and leaf relative water contents (Table 5). Biplot of PCA analysis indicates that, hormonal priming technique had higher coefficient of heat stress tolerance as compare to other priming techniques. Principal component analysis (PCA) also indicate that maintaining higher chlorophyll and NAR at maturity were responsible for increased GY in hormonal priming technique (Figure 4).

# DISCUSSION

Seed priming improves germination attributes by early emergence that results in early and uniform crop stand establishment. Primed seeds perform better and makes seedling more resilient to upcoming stresses. Mean emergence time (MET), emergence rate index (ERI), and coefficient of emergence velocity (CVE) are very important indicators



Figure 3. Impact of different priming elicitors on yield, yield related attributes and grain quality (seed protein) of heat stressed maize.

 $CK: Control; HP: hydropriming: SA, hormonal priming with salicylic acid; MLE: organic priming with moringa leaf extract; CaCl_2: osmopriming with CaCl_2.$ 

Values are the means of four replicates and different letters indicates significant difference at  $P \le 0.05$ .

	GY	PH	Chl a <sup>30 DAS</sup>	Chl b <sup>30 DAS</sup>	Chl a <sup>90 DAS</sup>	Chl b90 DAS	RWC	MSI	Seed protein	CGR-75	NAR-75
GY	1.00	0.97	0.92	0.81	0.79	0.89	0.98	0.98	0.99	0.93	0.96
PH		1.00	0.88	0.69	0.66	0.84	0.98	1.00	0.97	0.89	0.87
Chl a <sup>30 DAS</sup>			1.00	0.94	0.92	1.00	0.95	0.92	0.95	1.00	0.87
Chl b <sup>30 DAS</sup>				1.00	1.00	0.95	0.81	0.76	0.83	0.93	0.85
Chl a <sup>90 DAS</sup>					1.00	0.93	0.78	0.73	0.81	0.90	0.86
Chl b90 DAS						1.00	0.93	0.89	0.93	0.99	0.84
RWC							1.00	1.00	1.00	0.96	0.90
MSI								1.00	0.99	0.93	0.89
Seed protein									1.00	0.96	0.93
CGR-75										1.00	0.88
NAR-75											1.00

Table 5. Correlation among all the parameters contributing for heat tolerance in late planted maize crop.

GY: Grain yield; PH: plant height; DAS: days after sowing; RWC: relative water contents; MSI: membrane stability index; CGR: Crop growth rate; NAR: net assimilation rate.

Figure 4. Principal component analysis (PCA) biplot showing correlations among morpho-physiological attributes of heat stressed maize under different priming elicitors.



CK: Control; HP: hydropriming; MSI: membrane stability index; SA: hormonal priming with salicylic acid; MLE: organic priming with moringa leaf extract; CaCl<sub>2</sub>: osmopriming with CaCl<sub>2</sub>; CGR-75: crop growth rate at 75 d after sowing (DAS); NAR-75 DAS: net assimilation rate at 75 DAS; RWC: relative water contents; GY: grain yield.

of uniformity and synchronization of vigorous seedling (Lara et al., 2014). Present study shows that priming agents significantly improved the above attributes and provides an energetic start to maize seedling as compared to control. Early and higher emergence index in treated seeds might be fact that priming triggers the series of biochemical changes like enzyme activation (amylase activity), hydrolysis and breaking seed dormancy (Bakhtavar et al., 2015).

The series of these processes is required for germination process and due to this fact primed seeds emerged earlier and stimulate the seedling vigor which ultimately results in healthy seedling with increased root, shoot lengths and seedling fresh and dry weights in maize as contrast to control. In our study, highest seedling growth was recorded by hormonal priming with SA and this improvement might be due to higher rate of cell division in apical meristem, growth regulated by cell enlargement and cell division in growing seedlings (Vanacker et al., 2001). Moreover, results shows that seed priming with SA, MLE and CaCl<sub>2</sub> also improved the crop growth rate, leaf area index and higher photosynthetic rate in maize as compared to control. Improved performance of maize crop through SA under late sown condition (Ahmad et al., 2017) also supports the findings of our present study. Similarly, increased crop growth with higher photosynthetic rate by seed priming with MLE and CaCl<sub>2</sub> had been reported in early sown spring maize (Bakhtavar et al., 2015; Rehman et al., 2015).

Our study shows that, high temperature significantly reduced the chlorophyll a and b contents in control as compared to primed seeds which were supported by findings of Ahmad et al. (2017) in late sown maize crop. However, hormonal priming with SA markedly increased the chlorophyll a and b contents as evident in early (Rehman et al., 2015) and late sown maize (Ahmad et al., 2017). Salicylic acid improved chlorophyll a and b contents might be due to increased antioxidants which may mitigate the deleterious effect of high temperature stress and protected the chlorophyll from degradation. Some previous studies also justified that exogenous application of antioxidants improved the chlorophyll contents under stress conditions (Sakr and Arafa, 2009).

Moreover, membrane stability index (MSI) and RWC were also improved by SA in late sown spring maize which might be possible due to improved antioxidant system. Senaratna et al. (2000) documented that hormonal priming with SA triggers antioxidant mechanism that reduces oxidative damage and stabilized the membrane integrity to continue cellular functions under stress conditions. Similarly, Ahmad et al. (2017) reported that enhanced antioxidant enzymes

system reduces membrane leakage and increased the MSI under heat stress in spring maize. The control plant shows decreased RWC possibly due to lower metabolites and osmotic concentration within tissue to hold water under high temperature. However, priming with SA significantly improves the water status of late sown spring maize as evident under heat and chilling stress by Farooq et al. (2008).

Additionally, all priming agents significantly improves the physiological attributes (MSI, RWC, chlorophyll a and b contents) in maize under heat stress as compared to non-primed seeds. Among, priming agents hormonal priming with SA was ranked highest followed by MLE and CaCl<sub>2</sub> as evident by PCA analysis (Figure 4). Therefore, our results suggested that priming induced enhancement in physiological attributes may enabled the plant to cope with heat stress that ultimately results in higher plant growth and yield in late sown spring maize. In control plants, high temperature (Figure 1) significantly reduced the GY possibly due to poor seed setting and reduce grain filling as evident from less NAR in present study (Figure 3) However, all the enhancement in germination indexes, uniform seedling stand establishment, physiological attributes, crop growth and development due to seed priming were harvested in the form of higher GY under heat stress. Increased GY with improved yield related traits in present study could be attributed to mitigate the deleterious effects of high temperature at reproductive stage in late sown maize due to seed priming with potential priming elicitors (SA, MLE and CaCl<sub>2</sub>).

Among priming treatments, SA followed by MLE gives higher GY including maximum number of grains per cob, 1000 grain weight and enhanced BY with higher harvest index (Figure 3). Increased GY through seed priming possibly attributed to stabilized membrane integrity and enhanced antioxidant activity with SA application as evident in wheat (Sharma and Bhardwaj, 2014) that may enabled the maize plant to maintain photosynthesis due to higher chlorophyll contents at maturity even under high temperature which increases NAR (Figure 3) and ultimately resulted in higher yield.

Moreover, correlation analysis also indicated that higher chlorophyll contents and NAR at maturity were strongly positively correlated with GY and kernel quality in present study (Figure 4, Table 5). Enhanced yield performance through hormonal priming with SA and MLE had been reported in wheat, rice, linseed and maize under different abiotic stress conditions (Farooq et al., 2008; Basra et al., 2011; Rehman et al., 2011; 2014; 2015).

Furthermore, grain quality traits such as protein content plays central role in kernel quality and hormonal priming with SA or MLE markedly improved the protein contents of maize seed in present study. This improvement in protein contents under heat stress might be the results of higher NAR and increased LAD that provides better and uniform distribution of photo assimilates.

# CONCLUSIONS

Different priming elicitors used in present work, salicylic acid and *Moringa oleifera* leaf extracts (MLE) seems to be more effective in ameliorating the deleterious effect of heat stress in late sown spring maize. Early seedling stand establishment, enhanced crop growth, increased chlorophyll contents and higher membrane stability index due to salicylic acid and MLE ultimately improve grain yield and quality in spring maize. Our study suggests that delayed plantation of spring maize can be improved by employing these exogenous elicitors trough seed priming techniques.

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