# SALICYLIC ACID MEDIATED HEAT STRESS TOLERANCE IN SELECTED BREAD WHEAT GENOTYPES OF PAKISTAN

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#### Abstract

Wheat is one of the most important cereal crops significantly affected by continual and terminal heat stress. Phytohormones are recognized as strong tool for sustainably alleviating adverse effects of abiotic stress in crop plants. The impact of externally applied salicylic acid was investigated on physiological and agronomical traits of five wheat varieties under heat stress in pot and field study. Salicylic acid was applied exogenously through two ways, 1) pre-soaking seeds in  $10^{-4}$  M solution of salicylic acid for 24 hours, and 2) foliar application of salicylic acid to the adult wheat plants three days prior to heat stress, while untreated seeds were used as control. In pot experiment, high temperature stress was imposed to plants at post anthesis stage, whereas, in field study one set was sown late so that plants can receive high temperature at grain filling stage. Both field and pot study revealed that heat stress reduced the grain yield and thousand kernel weights. Heat stress also reduced leaf chlorophyll content by 34% and brought about an increase of 40% in proline content, 20% in leaf soluble proteins and 17% in soluble sugars as compared to the control plants. Application of salicylic acid through seed treatment and foliar application increased the chlorophyll content in leaves by 18% and 24%, enhanced soluble protein by 21%, proline by 40% and 47% and sugar accumulation by 81% and 88% respectively, and enhanced the net yield by 19% in pot experiment and by 13% in field experiment in selected wheat genotypes of Pakistan under heat stress. Based on results, it can be concluded that exogenous application of salicylic acid through seed priming or foliar spray led to stimulate heat stress tolerance in local wheat genotypes.

Key words: Abiotic stress, Foliar application, Heat stress, Phytohormones, Seed priming, Wheat genotypes.

#### Introduction

Wheat (Triticum aestivum L.) is one of the major staple crops of the world including Pakistan, with approximately 720 million tons being produced globally (Mwadzingeni et al., 2016). Wheat yields are reported to be drastically reduced due to abiotic stresses that are an imminent threat to the agricultural production all over the world. It has been reported that heat stress is the most damaging for agriculturally important cereal crops (Bita & Gerats, 2013). Wheat yields have been predicted to decrease approximately 10% for every one-degree increase in temperature (Hemantaranjan et al., 2014). Rise in temperature reduce average grain number per spike in wheat due to sterility and abortion of grains, this results in a non-recoverable reduction in crop yield (Barlow et al., 2015). High temperature stress in wheat particularly during reproductive stage is detrimental; post-anthesis heat stress results in significant reduction in the individual kernel weight and kernel number (Pradhan et al., 2012). High temperature during the grain development in wheat cause a decline in photosynthesis rate and leaf area, decrease in shoot and grain mass, and reduction in individual weight and sugar content of the kernel (Bita & Gerats, 2013). Grain filling duration is shortened by elevated temperatures during anthesis to grain maturity, which leads to considerable reduction in the grain yield (Farooq et al., 2011, Lobell et al., 2012). Heat stress brings about irreversible changes in plant growth and development. It damage proteins causing them to denature and aggregate, increases fluidity of lipids membrane, inactivate enzymes, and cause loss of membrane integrity (Zhao et al., 2011; Li et al., 2017; Zhao et al., 2017). Heat changes the stability of proteins, enzymes, nucleic acids, bio membranes, and cytoskeletal structures (Asthir, 2015).

Phytohormones are recognized as a strong tool for mitigating the adverse effects of abiotic stresses in crop plants owning to their growth promoting and antioxidant capacity (Ahammed et al., 2016). Exogenous application of phytohormones have shown beneficial effect on yield of rice, wheat, sorghum and cicer plants grown under heat stress, (Hasanuzzaman et al., 2017). The significance of salicylic acid (SA) in particular has been well recognized in improving plant's abiotic stress-tolerance through controlling plant's major metabolic processes (Khan et al., 2015). Exogenous application of salicylic acid notably improved all aspects of plant's growth and development under stress, a significant increase in net CO<sub>2</sub> assimilation and increased chlorophyll content has been observed (Martel & Qaderi, 2016). SA regulates important plant physiological processes including photosynthesis and proline (Pro) metabolism under stress conditions thereby providing protection to plant (Miura & Tada, 2014). The role of SA in thermo tolerance is well reported. According to Li et al., (2017), it improves heat tolerance in a wide range of plant species including potato, mustard, tobacco, tomato, bean, and Arabidopsis thaliana (Ahammed et al., 2016), salinity tolerance in lentil (Lens esculenta) through increasing proline accumulation, maintaining membrane stability, and biosynthesis of amino acids and carbohydrates in bent grass.

Exogenously applied SA to stressed plants, through seed soaking or by addition to nutrient medium, or by irrigation, or by foliar application through spraying was reported to induce heat stress tolerance-mechanisms in maize (Kaur & Gupta 2016). It has been reported that SA treated drought stressed wheat plants had an increased level of sugars, protein and mineral contents (Sharma *et al.*, 2017). Foliar application of SA mitigated the salinity induced adverse effects on fenugreek plants by improving growth parameters, and chlorophyll contents (Babar *et al.*, 2014). SA was reported to improve soluble sugars and proline content, higher grain yield, spikelet number per panicle and higher setting rate in rice under heat stress (Zhang *et al.*, 2017). This indicates that salicylic acid has positive effects on plants growth and development (Hemantaranjan *et al.*, 2014). SA seed treatment or foliar application increase crops yield owing to reduction in stress induced inhibition of plant growth (Fahraji *et al.*, 2014).

Owning to the reported role of SA in ameliorating the deleterious effects of heat stress on plants, a study was conducted to test the hypothesis that exogenous application of SA through seed treatment or foliar application may have the potential to mitigate heat stress in wheat.

#### **Material and Methods**

Field and pot experiments were designed to study the effect of salicylic acid seed priming and foliar application on selected wheat cultivars under heat stress. Seeds of five wheat genotypes viz., Pak-2013, NARC-2009, GA-2002, Inqilab-91 and Baj were brought from National Agricultural Research Center, Islamabad. These were surface sterilized in 3% (v/v) sodium hypochlorite solution for 30 minutes and then washed thoroughly with distilled water to remove the residue and dried. Two strategies were adopted for the hormonal application in both experiments i.e. seeds presoaking and foliar application. For pre-soaking, seeds were soaked in aerated SA (10<sup>-4</sup> M) solution for 24 hand control, seeds were soaked in sterilized water for equal period of time. Primed seeds were washed with distilled water, blotted and sown in plastic pots measuring 24x30 cm<sup>2</sup> (filled with 10 kg soil). Six caryopses were sown in three holes per pot. In field, the wheat varieties were planted under normal (November-20) and late (December-25) at the National Agricultural Research Centre, Islamabad (31° 49' N latitude and 70° 55' E longitude) during the year 2015-16 in RCBD fashion in pots experiment. In the field, early and late sowing was done so that effect of terminal high temperature on the yield can be studied in late sown plants, while early sown set acted as control. In pots, foliar SA was applied at post anthesis stage, 3 days prior to heat stress; while in field experiment, plants were sprayed with the hormonal solution at post anthesis stage. Approached at post anthesis stage, plants were shifted to glass house for heat stress maintained at  $36-40^{\circ}$  C during the day for 15 consecutive days. Flag leaf was sampled at the onset of visual symptoms of heat stress i.e. white veins, leaf discoloration, and leaf tip necrosis. Leaf was replicated trice for the measurement of electrolyte leakage according to Lutts et al., (1996), Chlorophyll content by Arnon (1949), Leaf proline content following Bates et al., (1973), soluble sugar content by DuBois et al., (1956) and soluble proteins by Bradford (1976). At physiological maturity, the data regarding yield attributes viz., days to maturity, 1000 kernel weights and grain yield (g/plant) was recorded from randomly selected plants. The data was analyzed statistically by applying ANOVA by using statistical software Statistix10.

### Results

The effect of salicylic acid seed treatment and foliar application was investigated on the yield and physiological parameters of wheat subjected to heat stress. Heat stress significantly affected the yield of wheat varieties under study ( $p \le 0.01$ ). In the pot experiment, Heat stress shortened the growth period (139.13±1.5days) of wheat plant as compared to control (146.1±1.5days) so maturity duration was reduced by 7.6 days on an average (Table 1). However, SA application significantly increased the days to maturity (146.9±1.7days) in stressed plant compared to the heat stressed control plants alone. There was a 6% increase in days to maturity in the plants that developed from SA presoaked seeds for SA foliar application (Table 2). Heat stress at post anthesis stage significantly affected the 1000 kernel weight and grain yield (g plant<sup>-1</sup>) as shown in Table 1. Under heat stress TKW was 33.10±0.6g that showed a significant ( $p \le 0.01$ ) reduction by 23% in the thousand 1000 kernel weight (TKW) and hence 21% reductions in grain with an average of 7.4±0.34g (Table 1&2). SA vield application significantly ( $p \le 0.01$ ) enhanced the TKW and grain yield of wheat under heat stress. SA seed priming caused 17% increase in TKW (38.6±0.69g) and 19% increase in grain yield (8.77±0.43g) per plant under heat stress as compared to their controlled stress plants. While SA foliar application caused an increase of 10% in TKW (36.4±0.58g) and 19% in yield (8.05±0.36g) of wheat genotypes under stress (Tables 1 & 2). SA pre-soaking was found more effective in increasing the wheat yield than SA foliar, while among varieties used GA-2002 and NARC-2009 were better performing and more responsive varieties to heat stress and SA application (Table 1; Fig. 1).

In the field experiment, heat stress significantly ( $p \le 0.05$ ) affected days to maturity, TKW and wheat yield. A 19% reduction was observed in days to maturity in late sown plants (Table 3). SA application was able to extend the grain filling duration by 1-3%. Similarly, the yield per plant was reduced in the order of Inqilab-91 > GA-2002 > NARC-2009 > Pak-13 and > Baj (Fig. 1). Overall, SA seed priming improved the TKW and yield by 13% on the average compared to the heat stressed plants, while its foliar application enhanced TKW by 11% (Table 3).

Table 1. Effect of seed priming and foliar application of SA on yield and physiology of wheat under heat stress.

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Treatment	DMT	TKW	Yield	Sugars	Proline	Protein	Chlorophyll	ELL
Treatment	(days)	( <b>g</b> )	(g plant <sup>-1</sup> )	$(mg g^{-1} fwt)$	(µmol g <sup>-1</sup> fwt)	$(mg g^{-1} fwt)$	$(mg g^{-1} fwt)$	(%)
Control	146.1±1.5 <sup>b</sup>	43.1±0.77 <sup>a</sup>	$9.4\pm0.46^{a}$	$8.08 \pm 0.17^{d}$	2.92±0.1 <sup>d</sup>	$0.57 \pm 0.02^{\circ}$	13.10±1.03 <sup>a</sup>	$66.89 \pm 3.6^{a}$
Control+ Stress	139.13±1.5°	$33.10\pm0.6^{a}$	$7.4\pm0.34^{b}$	12.5±0.22 <sup>c</sup>	$3.52 \pm 0.1^{\circ}$	$0.63 \pm 0.02^{b}$	9.99±0.69 <sup>c</sup>	$86.99 \pm 1.2^{a}$
Stress + SA Pre- soaking	146.9±1.7 <sup>ab</sup>	$38.6\pm0.69^{b}$	$8.77 \pm 0.43^{a}$	14.6±0.27 <sup>b</sup>	$4.08 \pm 0.2^{b}$	$0.69{\pm}0.02^{a}$	11.79±0.73 <sup>b</sup>	$73.17 \pm 2.5^{b}$
Stress+SA Foliar	$147.57 \pm 1.6^{a}$	$36.4 \pm 0.58^{\circ}$	$8.05 \pm 0.36^{b}$	$15.02 \pm 0.35^{a}$	$4.29 \pm 0.15^{a}$	$0.69 \pm 0.02^{a}$	12.36±0.91 <sup>ab</sup>	$69.30 \pm 2.5^{\circ}$
Significance level	<i>p</i> ≤0.01	<i>p</i> ≤0.01	<i>p</i> ≤0.01	<i>p</i> ≤0.05	<i>p</i> ≤0.01	<i>p</i> ≤0.01	<i>p</i> ≤0.01	<i>p</i> ≤0.01

Legends: DMT= Days to maturity, TKW= thousand kernel weight, ELL= Electrolyte Leakage, SA= Salicylic Acid; All means which share a common letter in the column are similar otherwise differ significantly at p < 0.05.

Treatment	DMT (days)	TKW (g)	Yield (g plant <sup>-1</sup> )	Sugars (mg g <sup>-1</sup> fwt)	Proline (µmol g <sup>-1</sup> fwt)	Protein (mg g <sup>-1</sup> fwt)	Chlorophyll (mg g <sup>-1</sup> fwt)	ELL (%)
Stress	-5%	-23%	-21%	55%	21%	11%	-24%	30%
Stress+SA Pre-soaking	6%	17%	19%	81%	40%	21%	18%	-16%
Stress+SA Foliar	6%	10%	9%	86%	47%	21%	24%	-20%

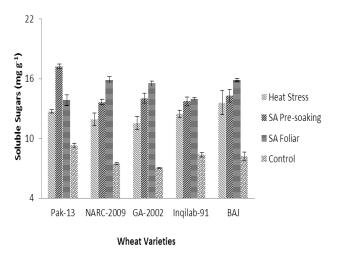
Table 2. Effect of seed priming and foliar application of SA on yield and physiology of wheat under heat stress in terms of % change

Legends: DMT= Days to maturity, TKW= thousand kernel weight, ELL= Electrolyte leakage, SA= Salicylic acid

 Table 3. Field experiment data of seed priming and foliar application of SA on the yield of wheat under heat stress.

Treatment -	Early Sown			Late Sown			Percent Change (compared to control)		
	DMT (days)	TKW (g)	Yield (g plant <sup>-1</sup> )	DMT (days)	TKW (g)	Yield (g plant <sup>-1</sup> )	DMT (days)	TKW (g)	Yield (g plant <sup>-1</sup> )
Control	$157.07 \pm 0.84^{\circ}$	$36.60 \pm 1.50^{b}$	137.66±11 <sup>b</sup>	$127.07 \pm 0.84^{\circ}$	32.35±1.47 <sup>b</sup>	$104.78 \pm 9.43^{b}$	-19%	-12%	-24%
SA Pre- soaking	$158.93{\pm}1.11^{\text{b}}$	$41.47{\pm}1.60^{a}$	151.69±13 <sup>a</sup>	$128.93{\pm}1.11^{\text{b}}$	$36.65{\pm}1.57^a$	$118.51 \pm 10^{a}$	1%	13%	135
SA Foliar	$160.73 \pm 0.86^{a}$	$39.35 \pm 1.67^{a}$	$146.49 \pm 12^{a}$	$130.73 \pm 0.86^{a}$	$35.77{\pm}1.54^{a}$	98.55±13 <sup>b</sup>	3%	11%	-6%

Legends: DMT= Days to maturity, TKW= thousand kernel weight, SA= Salicylic Acid; All means which share a common letter in the column are similar otherwise differ significantly at p < 0.05.



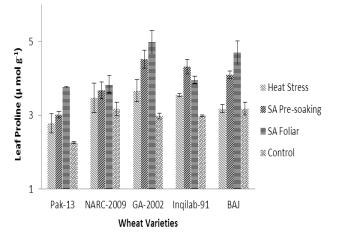


Fig. 1. Effect of exogenous application of SA on the yield of Pak-2013, NARC-2009, GA-2002, Inqilab-91 and Baj under heat stress.

**Electrolyte leakage:** High temperature stress significantly  $(p \le 0.01)$  increased electrolyte leakage  $(86.99\pm1.2)$  by 30% in the leaves of the tested stressed wheat genotypes as compared to their control plant (Table 1 & 2). Exogenous application of SA lowered the electrolyte leakage as compared to heat stressed plants alone the SA seed priming reduced electrolyte leakage  $(73.16\pm2.5)$  significantly  $(p \le 0.01)$  by 16%. The reduction was higher in Pak-13, followed by NARC-2009, Baj, GA-2002, and least for Inqilab 91 (Fig. 2). The foliar application reduced the electrolyte leakage  $(69.30\pm2.5)$  by 20% on the average (Table 1&2). The reduction was highest in stressed Pak-13, followed by GA-2002, while Inqilab-91 was least responsive variety (Fig. 2).

**Proline content:** Proline content of the wheat genotypes was significantly ( $p \le 0.01$ ) increased ( $3.52 \pm 0.02 \mu$ mol g<sup>-1</sup> fwt) under heat stress conditions compared to the control ( $2.92 \pm 0.02 \mu$ mol g<sup>-1</sup> fwt) the increase was 21% (Table 1&2). While, SA application through seed soaking and foliar

Fig. 2. Effect of exogenously applied SA on electrolyte leakage of Pak-13, NARC-2009, GA-2002, Inqilab-91 and Baj under heat stress.

application further enhanced the leaf proline content of wheat genotypes compared to the non-stressed control plants (Table 1). SA seed priming caused 40% increase in proline content ( $4.08\pm0.02\mu$ mol g<sup>-1</sup> fwt) in stressed plants compared to control. Under SA seed priming and heat stress, a significant highest increase was observed for GA-2002 and Inqilab-91 while least for Pak-13 (Fig. 3). On the other hand, SA foliar spray under heat stress caused an increase in proline content ( $4.29\pm0.15\mu$ mol g<sup>-1</sup> fwt) in the stressed plants by 47% (Table 1 & 2). GA-2002 and Pak-13 were the most responsive cultivars while Inqilab-91 showed least response to SA application under heat stress (Fig. 3).

**Soluble sugars:** Sugar content was significantly ( $p \le 0.05$ ) increased in all the wheat germplasm under heat stress (Table 1). The results revealed that different genotypes responded differently to different modes of SA application. Pak-13 was found more responsive to SA seed soaking, while in rest of genotypes, foliar application of SA was more effective GA-2002 and NARC-2009 showed higher

response to SA application (Fig. 4). SA seed priming significantly increased leaves sugar content (14.6±0.27mg g<sup>-1</sup> fwt) by 81% as compared to the control (Tables 1 & 2), while foliar application of SA caused an increase in sugar content (15.02±0.35 mg g<sup>-1</sup> fwt) which was 88% higher than the control plants (Tables 1 & 2).

Significant differences ( $p \le 0.01$ ) were observed in the interaction between the genotypes and different treatments of salicylic acid. Wheat plants accumulated higher protein content in their leaves under heat stress compared to controlled plants (Table 1). SA seed priming and SA foliar application caused 21% increase in soluble protein content ( $0.69\pm0.02$ mg g<sup>-1</sup> fwt) in stressed plants as compared to the control ( $0.57\pm0.02$ mg g<sup>-1</sup> fwt) (Tables 1 & 2). Pak-13 and Inqilab-91 accumulated more proteins in their leaves under SA seed priming (Fig. 5) compared to the stressed plants alone. The GA-2002 and Baj were more responsive to SA foliar application under heat stress condition. NARC-2009 showed equal response to both types of applications (Fig. 5).

**Soluble proteins:** There was significant difference  $(p \le 0.01)$  in the interaction between the genotypes and different treatments of salicylic acid. Wheat plants accumulated higher protein content in their leaves under heat stress than those of controlled plants (Table 1). SA seed priming and SA foliar application caused 21% increase in soluble protein content with an average of  $0.69\pm0.02$ mg g<sup>-1</sup> fwt in stressed plants compared to the control (Tables 1 & 2). Pak-13 and Inqilab-91 accumulated more proteins in their leaves under SA seed priming (Fig.

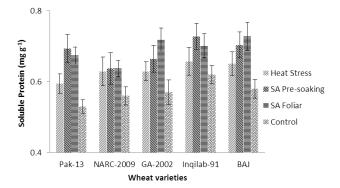


Fig. 3. Effect of exogenously applied SA on leaf proline content of Pak-13, NARC-2009, GA-2002, Inqilab-91 and Baj under heat stress.

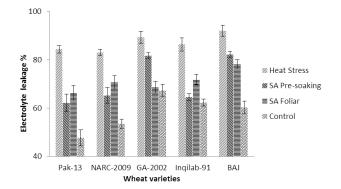


Fig. 5. Effect of exogenously applied SA on leaf total soluble protein content of Pak-13, NARC-2009, GA-2002, Inqilab-91 and Baj under heat stress.

5) compared with the stressed plants. There was trend for GA-2002 and Baj as tose of soluble sugar in response to SA foliar application under heat stress, while NARC-2009 showed equal response to both types of application (Fig. 5).

**Chlorophyll content:** Heat stress significantly  $(p \le 0.01)$ reduced leaf chlorophyll content (9.99±0.69 mg g<sup>-1</sup> fwt) compared to the control  $(13.10\pm1.03 \text{mg g}^{-1} \text{ fwt})$  with 24% reduction (Tables 1 & 2). The highest reduction was observed in Inqilab-91 and GA-2002; whereas, NARC-2009 was least affected by heat stress (Fig. 6). Application of SA under heat stress caused significant ( $p \le 0.01$ ) increase in Chlorophyll content of wheat genotypes under study compared to the stressed plants (Table 1). SA seed priming caused 18 % higher chlorophyll content with an average of 11.79±0.73 mg g<sup>-1</sup> fwt in the stressed plants compared to stress plants. Ingilab-91 and Baj showed higher response to SA seed priming in terms of Chlorophyll content (Fig. 6), while foliar application caused an increase of 24% in chlorophyll content (12.36±0.91 mg g<sup>-1</sup> fwt) compared to control stressed plants (Tables 1 & 2). NARC-2009 and Baj were found to contain higher amounts of chlorophyll content compared to the stressed plants alone. Pak-13 and GA-2002 showed very less response to SA seed priming under heat stress in accumulating leaf chlorophyll content. GA-2002 and Baj showed highest chlorophyll content under SA foliar application; while, NARC-2009 showed least response to hormonal application under heat stress (Fig. 6)

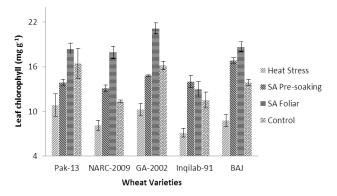


Fig. 4. Effect of exogenously applied SA on leaf soluble sugar content of Pak-13, NARC-2009, GA-2002, Inqilab-91 and Baj under heat.

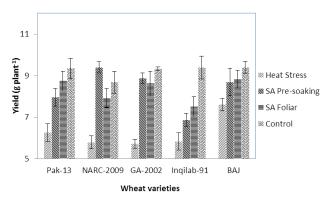


Fig. 6. Effect of exogenously applied SA on leaf chlorophyll content of Pak-13, NARC-2009, GA-2002, Inqilab-91 and Baj under heat stress.

#### Discussion

Heat stress adversely affected yield and biochemical traits of selected wheat varieties (Table 1). The exogenous application of SA either through seed soaking or foliar spray was found effective in field as well as pot experiment in mitigating heat stress in wheat (Tables 1 & 3) since it helps in adapting the plant to heat stress and significantly reduces the effect of high temperature on the selected wheat varieties. According to Hasanuzzaman *et al.*, (2014), the exogenous application of SA reduces the damaging effect of abiotic stress in plants.

Heat stress has an adverse effect on the plants yield, its due to the negative effect of heat on grain development as translocation of assimilate; duration and the rate of grain filling. It hastens the crop development thus resulting in smaller and shrinkage and light weight kernels and adversely affects the yield (Hasanuzzaman et al., 2014). Our results indicated that heat stress reduced thousand kernel weight by 23% and 13% in pots and field experiment (Table 2 & 3) due to reduction in weight of individual kernel. It is due to the fact that development of spike is hastened by the heat stress (Porter & Gawith, 1999), which is also shown in our results that heat stress reduces the wheat maturity period by 19% (Table 3) leading to reduced seed setting rate. It was also observed that enhanced proline content (40-47%) is accompanied with improved grain yield (19%) (Table 1), which is an effective selection tool for stress tolerant genotypes (Mwadzingeni et al., 2016). Our results were in accordance to previous study who found that positive effect of SA on yield and TKW is due to the delayed maturity which expands the grain filling duration (Arshad et al., 2017).

Chlorophyll content in leaves was decreased in heatstressed plants compared to the control of all wheat germplasm under study (Fig. 6). It was observed that there was 24% decrease in chlorophyll content under heat stress (Table 2) that led to decline in photosynthesis rate, and in return cause reduced grain yield per plant and decreased thousand kernel weight (Babar et al., 2014). Bita & Gerats (2013) also reported a decline in photosynthesis rate and leaf area, leading to decrease in grain mass, and reduction in individual kernel weight. Increase in chlorophyll content was observed under SA application during heat stress (Babar et al., 2014) which is similar to our findings that SA seed priming led to increased chlorophyll content by 18% under heat stress and in kernel weight and grain yield per plant by 19% in pots and 13% in the field experiment (Tables 2 & 3). The SA foliar application increased chlorophyll content by 24% and grain yield of the plant by 19% (Table 2). Results were in accordance to the reported increase in chlorophyll content under SA and stress treatment in pea plants (Martel & Qaderi, 2016), and emancipating the salt-induced reduction in chlorophyll contents by the foliar application of SA in wheat crops (Babar et al., 2014). Reduced yield might be due to reduction in chlorophyll content that leads to less photosynthetic activity which results in loss of yield (Arshad et al., 2017). Improvements in the chlorophyll content under high temperatures can be an indication of heat stress tolerance (Ahmad et al., 2016) as revealed by the stabilization of heat stress sensitive important physiological processes (Bita and Gerats 2013).

The results further revealed accumulation of soluble proteins in response to heat stress (Table 1). It is previously being reported that protein synthesis is enhanced in plants during stress (Hayat et al., 2005). Accumulation of soluble proteins is increased with increasing levels of salinity in barley (Ahmed et al., 2011). Another study has shown that exogenous application of SA stimulated the accumulation of soluble protein in response to salinity stress in broad beans (Azooz et al., 2013). Noreen et al., (2017) reported that SA foliar application caused 22.8% increase in soluble proteins in wheat leaves under drought stress, while our results indicate 21% increase in protein content in response to SA application (Table 2). Significant interaction was found between hormones and heat treatment in terms of protein content showing a positive effect of hormones on the protein content of plants resultantly there was higher protein content in heat-stressed plants subjected to SA (Tables 1 & 2). Nimir et al., (2015) reported the similar results stated that the application of SA significantly raised soluble protein levels in salt-stressed plants. An increase in sugar content of leaves was observed under heat stress and SA application similar to Sharma et al., (2017), who reported higher level of sugars, protein and mineral contents in SA treated water-stressed wheat plants. The accumulation of organic solutes such as sugars, is an important mechanism of stress tolerance in plants (Ibrahim, 2016).

Electrolyte leakage was increased by 30% during heat stress and SA application effectively reduced the effect of heat on plants (Table 1). Electrolyte leakage was associated with sugar, proline, protein and chlorophyll content, increased levels of these compatible solutes accompanied decreased electrolyte leakage (Zheng *et al.*, 2016). It confirms that seed priming enhances stress tolerance by inducing numerous metabolic changes in plants (Jisha *et al.*, 2013; Kubala *et al.*, 2015). Another study reported that SA enhanced accumulation of proline, total carbohydrate and total protein with  $\beta$ -amino butyric acid seed priming in green gram (Jisha & Puthur, 2016).

### Conclusion

The study concludes that exogenous application of SA improved the physiological and yield attributes of wheat through conferring tolerance against temperature stress. SA seed priming effectively enhanced tolerance against two heat susceptible germplasm such as GA-2002 and Inqilab-91 and successfully increased yield. Besides, both the strategies further augmented the two heat tolerant genotypes like NARC-2009 and Baj. This study also suggests the benefit of SA foliar application over SA seed priming in terms of physiological attributes. Future research is required to understand molecular and metabolic process of SA seed priming under heat stress required for a successful breeding program.

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