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## Effect of foliar application of bioregulators for improving high temperature tolerance of wheat (*Triticum aestivum* L.)

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### Abstract

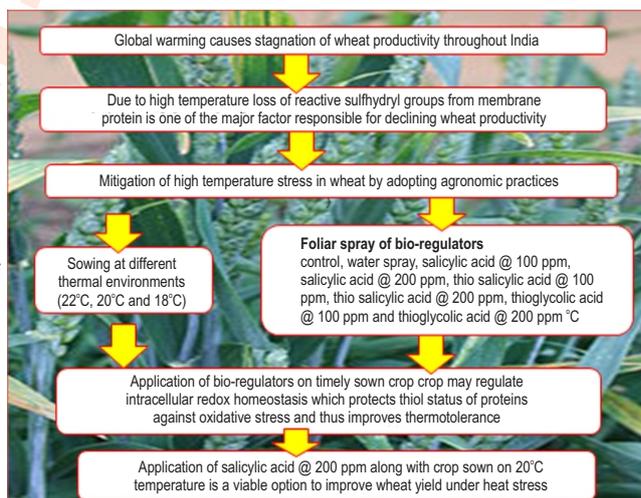
**Aim:** The aim of the present study was to evaluate the effect of sowing at different thermal environments and its interaction with foliar spray of bio-regulators on yield and yield contributing traits in wheat.

**Methodology:** The present study was conducted by using split plot design with four replications consisting of 24 treatments, namely, main plots: three sowing at different thermal environments (22°C, 20°C and 18°C) and sub plots: eight foliar spray of bioregulators (control, water spray, salicylic acid @ 100 ppm, salicylic acid @ 200 ppm, thio salicylic acid @ 100 ppm, thio salicylic acid @ 200 ppm, thioglycolic acid @ 100 ppm and thioglycolic acid @ 200 ppm).

**Results:** The results indicated that sowing at 20°C recorded the maximum yield parameters viz, effective tillers/m row length, length of ear, no. of grains per ear, test weight, days to maturity and grain, straw and biological yield of wheat over sowing at 22°C and 18°C temperature. Sowing at different thermal environment treatments was found to be non-significant with harvest index of wheat. Among the foliar spray of bioregulators, the treatment B<sub>1</sub> (SA @ 200 ppm) significantly increased the yield components and yield, while remaining at par with B<sub>6</sub> (TSA @ 200 ppm) and B<sub>8</sub> (TGA @ 200 ppm) proved superior in comparison to rest of the treatments. However, the significantly higher harvest index was obtained under B<sub>1</sub> (SA @ 200 ppm) treatment and was statistically at par with all other treatments, except B<sub>1</sub> and B<sub>2</sub>.

**Interpretation:** It is concluded that salicylic acid @ 200 ppm at tillering (40-45 DAS) and ear emergence (60-65 DAS) stage may be sprayed on 20°C temperature sown crop is a viable option to improve wheat yield under semi-arid environment.

**Key words:** Bioregulators, Foliar spray, Sowing temperature, Wheat, Yield attributes



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## Introduction

Wheat [*Triticum aestivum* (L.)] is the second most important cereal crop after rice, grown under diverse agro-climatic conditions. It has been described as the 'King of cereals' because of the acreage it occupies, high productivity and the prominent position it holds in the international food grain trade (Khan *et al.*, 2015). India is the second largest producer of wheat next to China in the world as India achieved significant progress in previous four decades. It occupies of about 33.61 Mha area with annual production of 106.21 Mt and productivity of 3160 kg ha<sup>-1</sup> during 2019-20 (IIWBR, 2020). To keep pace with the increasing population, India needs to increase wheat production. Future climate projections show that wheat production and productivity is severely constrained by climate change in the developing countries than developed countries (IPCC, 2014).

Global warming is a new challenge faced by the agriculture sector and mitigating its adverse effects is becoming a challenge. Because, the incoming solar radiations (long wave radiations) being directly raids onto the soil surface without any hindrance. But after striking the soil surface, long wave converted into short wave radiations and global gases *viz.* CH<sub>4</sub>, CO<sub>2</sub> and N<sub>2</sub>O hinders the short wave radiation to emit out of the earth resulting in the net increase in global temperature (Jain *et al.*, 2014). Thus, there is a need to study the effect of sowing at different thermal environments and foliar spray of bioregulators on yield attributes and yield of wheat, which on one side improves the livelihood of farmers while on the other side improves the productivity under abrupt climatic conditions, especially heat stress. High temperatures at both ends of the wheat growing season in arid and semi-arid region of India are the great challenge in agriculture (Sabagh *et al.*, 2019). In early sowing, high temperature during sowing accelerate growth of wheat and thus, reduces tillering phase, prompting the crop to enter the elongation stage, which leads to poor tillering of the crop (Yildirim *et al.*, 2018). In the case of delayed sowing of crops, stages such as flowering and filling of grain coincides with rise in temperature and atmospheric drought in March and April, which causes poor growth and low grain yield (Barutçular *et al.*, 2017; Hossain *et al.*, 2018). Late sown wheat facing heat stress at grain filling stage reduces the grains in ears resulting in 16 percent yield loss as compared to timely sown wheat (Agalodiya *et al.*, 2011; Hussain *et al.*, 2018).

Due to high temperature, loss of reactive sulfhydryl groups from membrane protein is one of the major factors responsible for declining crop productivity (Agarwal *et al.*, 2017). Therefore, application of bioregulators may regulate intracellular redox homeostasis which protects thiol status of proteins against oxidative stress and thus, improves thermotolerance (Pandey *et al.*, 2013). It also play a vital role in maintaining the redox state of membrane proteins as it can quench reactive oxygen species (ROS) generated during heat stress. The oxidized form of sulfhydryl group under heat stress may be responsible for relative leaf water content decrease and

membrane damage, which was significantly reduced with the exogenous use of bioregulators resulting in improved grain growth and yield (Mahatma *et al.*, 2009; Shivran *et al.*, 2019; Singh and Meena, 2020). Therefore, an attempt was made in this study to integrate the effect of sowing at different thermal environments and foliar spray of bio-regulators under heat stress.

## Materials and Methods

**Experimental site, soil and climate:** The present investigation was carried out for two consecutive *rabi* seasons of 2016-17 and 2017-18 at the Agronomy Farm of S.K.N. College of Agriculture, Jobner. The experimental field was ploughed with tractor drawn disc plough followed by cross harrowing and planking to carry the field into fine tilth for appropriate germination and proper crop establishment. The soil of the experimental field was loamy sand in texture. With pH gets value of 8.15, low in available nitrogen (130.3 kg N ha<sup>-1</sup>), available phosphorus (15.2 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) and medium in potassium (149 kg K<sub>2</sub>O ha<sup>-1</sup>). The maximum temperature ranged from 34.8 to 20.4°C during 2016-17 and 34.0 to 23.6°C during 2017-18 and the minimum temperature ranged from 02.8 to 15.1°C during 2016-17 and 01.4 to 13.5°C during 2017-18, respectively.

**Experimental design and treatment detail:** The experiment was carried out in a split plot design, comprising sowing at different thermal environments (D<sub>1</sub>: 22°C, D<sub>2</sub>: 20°C and D<sub>3</sub>: 18°C) as main plot and eight foliar spray of bioregulators (B<sub>1</sub>: control, B<sub>2</sub>: water spray, B<sub>3</sub>: salicylic acid @ 100 ppm, B<sub>4</sub>: salicylic acid @ 200 ppm, B<sub>5</sub>: thiosalicylic acid @ 100 ppm, B<sub>6</sub>: thiosalicylic acid @ 200 ppm, B<sub>7</sub>: thioglycolic acid @ 100 ppm and B<sub>8</sub>: thioglycolic acid @ 200 ppm) as sub plot treatments replicated four time. Bioregulators were sprayed using foot sprayer at tillering and ear emergence stage as per treatment and dissolved in water.

**Plants material:** Seeds @ 100 kg ha<sup>-1</sup> of wheat variety 'Raj-3765' was used for the study. The seeds were treated with Fipronil @ 6 ml kg<sup>-1</sup> seed at the time of sowing. Treated seeds were sown by 'kera' method at 5 cm depth in rows 22.5 cm apart behind the plough. Besides one pre-sowing irrigation, the crop was irrigated at different critical stages of crop growth by sprinkler method. Half dose of nitrogen @ 120 kg ha<sup>-1</sup> through urea and uniform dose of phosphorus @ 40 kg ha<sup>-1</sup> through DAP were applied at the time of sowing. Remaining half dose of nitrogen was top dressed at 20-25 days after sowing.

**Observations recorded:** Standard cultivation practices prescribed for wheat under irrigated conditions were precisely followed. Different observations were recorded on yield attributes, *viz.* effective tillers m<sup>-1</sup> row length, length of ear, number of grains per ear, test weight, days to maturity, yield (grain, straw and biological yields) and harvest index, separately on each plot and average value worked out. The number of tillers bearing productive ears was counted in three randomly selected 1.0 m row length in each plot, averaged and calculated as effective

tillers  $m^{-1}$  row length. From each plot, five productive ears were taken out randomly at harvest and length of each ear was measured. The above five ears were threshed separately and grains were counted and average number of grains per ear was worked out. 1000 seeds were counted from seed samples from each plot separately and weighed as test weight. The number of days was counted from date of sowing to maturity, when the crop plants turned golden colour for days to maturity. Crop was harvested and weighed to record biological yield. The produce was threshed and it was assessed for grain yield. Straw yield was calculated by deducting grain yield from biological yield. The ratio of economic yield to the biological yield was calculated and expressed as harvest index.

## Results and Discussion

Sowing at different thermal environments significantly influenced the yield parameters and yield of wheat. Sowing at 20°C ( $D_2$ ) recorded the maximum effective tillers  $m^{-1}$  row length (61.03), length of ear (11.76 cm), number of grains per ear (43.14), test weight (37.03 g) and days to maturity (123.1) as well as grain (3771  $kg\ ha^{-1}$ ), straw (4880  $kg\ ha^{-1}$ ) and biological (8651  $kg\ ha^{-1}$ ) yield proved significantly superior over  $D_1$  (sowing at 22°C) and  $D_3$  (sowing at 18°C) treatments. Early sown (>20°C) crop faces high soil temperature hamper seed germination which results in unequal plant population and finally decrease the grain yield (Barutçular et al., 2017; Sabagh et al., 2019). The higher yield on 15<sup>th</sup> November (sowing at 20°C temperature) sown crop may be attributed to favorable climatic conditions during the

entire life cycle and thus, the different phases of plant life were completed at appropriate timings, which resulted in production of more number of effective tillers per plant and ultimately longer ear, more number of grains per ear and test weight, which in turn has increased the yield. (Jat et al., 2013; Thorat et al., 2016; Yildirim et al., 2018). The significantly lowest yield attributes and yield were noted with the  $D_3$  (sowing at 18°C) treatment. When the crop was sown late, the temperature was low but February onwards the temperature started rising and plants did not get sufficient favourable environment to express their full potential (Amrawat et al., 2013; Tripathi et al., 2013; Hossain et al., 2018).

The harvest index of wheat was not influenced significantly by sowing at different thermal environment treatments (Shirpurkar et al., 2008). The increase in yield parameters and yield due to the above treatment i.e.,  $D_2$  was 8.15 and 15.21 percent effective tillers  $m^{-1}$  row length, 7.30 and 13.62% length of ear, 5.04 and 9.796% number of grains per ear, 5.98 and 11.10% test weight, 3.01 and 7.13% days to maturity and 5.28 and 9.72% grain, 4.79 and 7.65% straw and 4.99 and 8.54 biological yield, respectively, over  $D_1$  and  $D_3$  treatments. Yield parameters viz., effective tillers/m row length, length of ear, row length, number of grains ear<sup>-2</sup>, test weight, days to maturity and grain, straw and biological yield and harvest index of wheat were significantly influenced by foliar spray of bioregulators. The maximum effective tillers  $m^{-1}$  row length (62.75), length of ear (12.04 cm), number of grains per ear (43.00), test weight (36.87 g) and days to maturity (126.2) were observed under  $B_4$  (salicylic acid @ 200 ppm) treatment, proved significantly superior over

**Table 1:** Effect of sowing at different thermal environments and foliar spray of bioregulators on effective tillers

Treatments	Effective tillers $m^{-1}$ row length		
	2016-17	2017-18	Pooled
<b>Sowing at different thermal environments</b>			
$D_1$ -22°C	57.67	55.20	56.43
$D_2$ -20°C	62.22	59.84	61.03
$D_3$ - 18°C	54.76	51.17	52.97
SEm±	1.43	1.33	0.98
CD (P=0.05)	4.95	4.60	3.01
<b>Foliar spray of bioregulators</b>			
$B_1$ - Control	48.92	46.58	47.75
$B_2$ - Water spray	52.20	49.77	50.99
$B_3$ - Salicylic acid @ 100 ppm	59.35	57.65	58.50
$B_4$ - Salicylic acid @ 200 ppm	64.05	61.46	62.75
$B_5$ -Thiosalicylic acid @100 ppm	58.45	55.94	57.19
$B_6$ -Thiosalicylic acid @ 200 ppm	61.06	58.44	59.75
$B_7$ - Thioglycolic acid @ 100 ppm	57.85	55.46	56.65
$B_8$ - Thioglycolic acid @ 200 ppm	63.85	57.96	60.90
SEm±	2.19	1.92	1.46
CD (P=0.05)	6.19	5.42	4.08
<b>Interaction (D x B)</b>			
SEm±	3.80	3.32	2.52
CD (P=0.05)	NS	NS	NS

**Table 2:** Effect of sowing at different thermal environments and foliar spray of bioregulators on length of ear and number of grains per ear

Treatments	Length of ear (cm)			No. of grains per ear		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
<b>Sowing at different thermal environments</b>						
D <sub>1</sub> -22°C	11.65	10.28	10.96	42.22	39.92	41.07
D <sub>2</sub> -20°C	12.46	11.06	11.76	44.18	42.10	43.14
D <sub>3</sub> - 18°C	11.02	9.68	10.35	40.46	38.12	39.29
SEm±	0.29	0.27	0.20	0.52	0.49	0.35
CD (P=0.05)	0.99	0.92	0.60	1.78	1.69	1.09
<b>Foliar spray of bioregulators</b>						
B <sub>1</sub> -Control	10.82	9.51	10.16	40.76	38.42	39.59
B <sub>2</sub> -Water spray	10.90	9.60	10.25	41.42	39.16	40.29
B <sub>3</sub> -Salicylic acid @ 100 ppm	11.98	10.66	11.32	42.58	39.98	41.28
B <sub>4</sub> -Salicylic acid @ 200 ppm	12.82	11.26	12.04	44.00	42.01	43.00
B <sub>5</sub> -Thiosalicylic acid @100 ppm	11.23	9.93	10.58	41.51	39.53	40.52
B <sub>6</sub> -Thiosalicylic acid @ 200 ppm	12.07	10.58	11.32	42.69	40.39	41.54
B <sub>7</sub> -Thioglycolic acid @ 100 ppm	11.40	10.10	10.75	41.76	39.78	40.77
B <sub>8</sub> -Thioglycolic acid @ 200 ppm	12.46	11.10	11.78	43.59	41.12	42.35
SEm±	0.45	0.42	0.31	0.75	0.76	0.53
CD (P=0.05)	1.29	1.19	0.87	2.13	2.14	1.49
<b>Interaction (D x B)</b>						
SEm±	0.79	0.73	0.54	1.30	1.31	0.92
CD (P=0.05)	NS	NS	NS	NS	NS	2.72

**Table 3:** Effect of sowing at different thermal environments and foliar spray of bioregulators on test weight and days to maturity

Treatments	Test weight (g)			Days to maturity		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
<b>Sowing at different thermal environments</b>						
D <sub>1</sub> -22°C	36.12	33.77	34.94	120.8	118.3	119.5
D <sub>2</sub> -20°C	38.08	35.98	37.03	124.2	122.0	123.1
D <sub>3</sub> - 18°C	34.36	32.31	33.33	116.4	113.3	114.9
SEm±	0.52	0.49	0.35	1.52	1.33	1.01
CD (P=0.05)	1.78	1.69	1.09	5.24	4.60	3.10
<b>Foliar spray of bioregulators</b>						
B <sub>1</sub> - Control	34.66	32.32	33.49	111.1	108.6	109.8
B <sub>2</sub> - Water spray	35.32	33.03	34.18	114.3	111.8	113.1
B <sub>3</sub> - Salicylic acid @ 100 ppm	36.48	33.94	35.21	121.1	118.5	119.8
B <sub>4</sub> - Salicylic acid @ 200 ppm	37.86	35.89	36.87	127.5	125.0	126.2
B <sub>5</sub> -Thiosalicylic acid @100 ppm	35.41	33.28	34.34	120.6	118.0	119.3
B <sub>6</sub> -Thiosalicylic acid @ 200 ppm	36.62	34.24	35.43	123.2	121.3	122.2
B <sub>7</sub> - Thioglycolic acid @ 100 ppm	35.66	33.77	34.72	120.0	118.5	119.2
B <sub>8</sub> - Thioglycolic acid @ 200 ppm	37.49	35.69	36.59	126.0	121.1	123.5
SEm±	0.75	0.76	0.53	2.46	1.92	1.56
CD (P=0.05)	2.11	2.12	1.47	6.97	5.42	4.37
<b>Interaction (D x B)</b>						
SEm±	1.30	1.31	0.92	4.27	3.32	2.71
CD (P=0.05)	NS	NS	NS	NS	NS	NS

rest of the treatments being at par with B<sub>6</sub> (thiosalicylic acid @ 200 ppm) and B<sub>8</sub> (thioglycolic acid @ 200 ppm) treatments. Treatment B<sub>4</sub> represented an increase in yield parameters to the magnitude of 31.41, 23.06 percent in effective tillers m<sup>-1</sup> row length, 18.50,

17.46% in length of ear, 8.61, 6.72% in number of grains per ear, 10.09, 7.87% in test weight and 14.93, 11.58% in days to maturity over B<sub>1</sub> (control) and B<sub>2</sub> (water spray), treatments, respectively. Thus, overall improvement in these parameters seems to be due

**Table 4:** Effect of sowing at different thermal environments and foliar spray of bioregulators on grain and straw yield

Treatments	Yield (kg ha <sup>-1</sup> )					
	Grain			Straw		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
<b>Sowing at different thermal environments</b>						
D <sub>1</sub> -22°C	3667	3498	3582	4742	4572	4657
D <sub>2</sub> -20°C	3860	3681	3771	4940	4820	4880
D <sub>3</sub> - 18°C	3503	3372	3437	4623	4443	4533
SEm±	55	55	39	62	47	39
CD (P=0.05)	189	191	120	213	163	119
<b>Foliar spray of bioregulators</b>						
B <sub>1</sub> - Control	3258	3203	3230	4314	4195	4254
B <sub>2</sub> - Water spray	3398	3318	3358	4470	4326	4398
B <sub>3</sub> - Salicylic acid @ 100 ppm	3538	3327	3432	4623	4312	4467
B <sub>4</sub> - Salicylic acid @ 200 ppm	3991	3758	3874	5045	4952	4998
B <sub>5</sub> -Thiosalicylic acid @100 ppm	3701	3461	3581	4842	4564	4703
B <sub>6</sub> -Thiosalicylic acid @ 200 ppm	3879	3701	3790	4944	4916	4930
B <sub>7</sub> - Thioglycolic acid @ 100 ppm	3724	3575	3649	4910	4697	4804
B <sub>8</sub> - Thioglycolic acid @ 200 ppm	3925	3792	3858	5000	4932	4966
SEm±	87	67	55	94	87	64
CD (P=0.05)	245	189	153	265	247	180
<b>Interaction (D x B)</b>						
SEm±	150	116	95	163	151	111
CD (P=0.05)	NS	NS	265	NS	NS	NS

**Table 5:** Effect of sowing at different thermal environments and foliar spray of bioregulators on biological yield and harvest index

Treatments	Biological yield (kg ha <sup>-1</sup> )			Harvest index (%)		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
	<b>Sowing at different thermal environments</b>					
D <sub>1</sub> -22°C	8409	8070	8240	43.63	43.36	43.50
D <sub>2</sub> -20°C	8801	8501	8651	43.84	43.33	43.58
D <sub>3</sub> - 18°C	8125	7815	7970	43.02	43.13	43.08
SEm±	107	098	073	0.14	0.12	0.12
CD (P=0.05)	370	340	224	NS	NS	NS
<b>Foliar spray of bioregulators</b>						
B <sub>1</sub> - Control	7572	7397	7485	43.03	43.26	43.14
B <sub>2</sub> - Water spray	7868	7644	7756	43.06	43.40	43.23
B <sub>3</sub> - Salicylic acid @ 100 ppm	8160	7639	7900	43.36	43.57	43.47
B <sub>4</sub> - Salicylic acid @ 200 ppm	9036	8710	8873	44.20	43.17	43.68
B <sub>5</sub> -Thiosalicylic acid @100 ppm	8543	8025	8284	43.33	43.22	43.28
B <sub>6</sub> -Thiosalicylic acid @ 200 ppm	8823	8617	8720	44.03	42.98	43.50
B <sub>7</sub> - Thioglycolic acid @ 100 ppm	8633	8273	8453	43.11	43.20	43.15
B <sub>8</sub> - Thioglycolic acid @ 200 ppm	8924	8724	8824	43.85	43.41	43.63
SEm±	153	139	103	0.18	0.16	0.14
CD (P=0.05)	432	392	289	0.51	0.47	0.38
<b>Interaction (D x B)</b>						
SEm±	265	240	179	0.21	0.18	0.17
CD (P=0.05)	NS	NS	NS	NS	NS	NS

to vigorous vegetative growth of plants which ultimately resulted in more leaf area and increased photosynthesis, thereby, forming higher number of effective tillers, number of grains per ear, ear

length and test weight. The results are in agreement with those of Hassanein *et al.* (2012); Meena *et al.* (2014) and Singh *et al.* (2020). However, significantly higher grain, straw and biological

yield were noted under B<sub>4</sub> treatment with the respective values of 3874, 4998 and 8873 kg ha<sup>-1</sup>, being at par with B<sub>6</sub> (thiosalicylic acid @ 200 ppm) and B<sub>8</sub> (thioglycolic acid @ 200 ppm) proved significantly superior over remaining treatments. The treatment B<sub>4</sub> (salicylic acid @ 200 ppm) significantly improved the harvest index over the remaining treatments.

Significant increase in crop yield might be due to delayed senescence of plant organs (particularly leaves and flowers) in response to exogenous bioregulators that will automatically help the plant in extending the duration of photosynthetically active sites by increasing the availability of water, nutrient and more efficient plant metabolic activities (Shivran et al., 2019; Singh et al., 2020), also prevent premature loss of flower and fruits. This subsequently resulted in the observed increase in crop yield (Azimi et al., 2013). The significantly lowest yield parameters and yield were recorded with B<sub>1</sub> treatment (Singh et al., 2013). The quantum increase in yield due to B<sub>4</sub> treatment was 19.94, 15.37% in grain yield, 17.49, 13.64% in straw yield and 18.54, 14.40% in biological yield over B<sub>1</sub> (control) and B<sub>2</sub> (water spray), respectively. These results are in conformity with the findings of Hassanein et al. (2012), Kumawat et al. (2013) and Nathawat et al. (2016). Interaction effect between sowing at different thermal environments and foliar spray of bioregulator treatments failed to bring significant influence on the growth parameters viz., effective tillers m<sup>-1</sup> row length, length of ear, test weight and days to maturity, straw, biological yield and harvest index. While interaction effect of sowing at different thermal environments and foliar spray of bio-regulator treatments on number of grains per ear and grain yield of wheat was found to be significant.

The combined treatment D<sub>1</sub>B<sub>4</sub> (sowing at 22°C along with the application of SA @ 200 ppm) recorded the significantly higher grains per ear (45.54) over remaining treatment combinations, except D<sub>2</sub>B<sub>3</sub>, D<sub>2</sub>B<sub>4</sub>, D<sub>2</sub>B<sub>6</sub> and D<sub>2</sub>B<sub>7</sub>. The minimum number of grains per ear was obtained under D<sub>2</sub>B<sub>5</sub> (38.29). Further, treatment combination D<sub>2</sub>B<sub>4</sub> (sowing at 20 °C along with the application of SA @ 200 ppm) observed the significantly higher grain yield (4078 kg ha<sup>-1</sup>) over other treatment combinations, except D<sub>2</sub>B<sub>2</sub>, D<sub>2</sub>B<sub>6</sub>, D<sub>2</sub>B<sub>8</sub>, D<sub>1</sub>B<sub>4</sub>, D<sub>1</sub>B<sub>6</sub> and D<sub>1</sub>B<sub>8</sub>. Application of bio-regulators on crop sown at different thermal environments showed beneficial effect on plants grown under heat stress by improving water and chlorophyll content, enhanced activities of antioxidants, reduced membrane injury and oxidative stress (Hasanuzzaman et al., 2010; Singh et al., 2020). The lowest grain yield was recorded under D<sub>3</sub>B<sub>5</sub> treatment (2763 kg ha<sup>-1</sup>). The enhanced crop growth in terms of grain yield due to optimum environment obtained by crop has also been reported by Muhal and Solanki (2015).

It is concluded that crop should be sown at prevailing mean temperature of 20°C along with foliar spray of salicylic acid 200 ppm at tillering and ear emergence stages for ameliorating the adverse effects of high temperature stress in wheat.

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## Add-on Information

**Authors' contribution:** H. Lakhran: Conduct/writing of manuscript, O.P. Sharma: Planning, R. Bajiya: Statistical analyses, J.R. Choudhary: Reference setting, S. Kanwar, M. Choudhary: Chemical analyses.

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