

## Salicylic Acid and 24-Epibrassinolide Induced Thermotolerance in Bell Pepper through Enhanced Antioxidant Enzyme System and Heat Shock Proteins

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### ABSTRACT

Elevated temperature negatively affects the production of bell pepper (*Capsicum annuum* L.) especially under North Indian plains where the temperature is above 40°C during summers. In the present study, the effect of exogenous application of Plant Growth Regulators (PGRs) viz. Salicylic Acid (SA) and 24-Epibrassinolide (EBR) on biochemical parameters and antioxidant system of bell pepper cv. Royal Wonder was evaluated. PGRs were applied exogenously 30, 60 and 90 Days After Transplantation (DAT). All the concentrations of PGRs i.e SA (0.10, 0.20, and 0.50 mM) and EBR (0.05, 0.10, and 0.20 µM) were effective in ameliorating the heat shock-induced effects, which enhanced thermotolerance in terms of increased proline content, soluble proteins, total phenols, total soluble sugars and starch content, improved antioxidant system (CAT, APX, POX, SOD and GR) with reduced lipid peroxidation and cellulase enzyme activity at high temperature, and, ultimately, improving total fruit yield. Application of 0.20 mM SA improved thermotolerance most efficiently at all growth stages, specifically when spray was done at 30 and 60 DAT. It resulted in a significant enhancement in biochemical parameters and antioxidant enzyme system as compared to the untreated control.

**Keywords:** Bell pepper yield, Biochemical parameters, Heat stress, Plant growth regulators.

### INTRODUCTION

Heat stress alters physiology and biochemistry of the plant by inducing the generation and accumulation of Reactive Oxygen Species (ROS). These are neutralized by antioxidant systems and osmoprotectants. Antioxidant enzymes like Superoxide Dismutase (SOD) convert free superoxide (O<sub>2</sub><sup>-</sup>) radicals to Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and oxygen (Ahmadi and Souri 2019). Catalase (CAT), Ascorbate Peroxidase (APX), and Peroxidase (POX) further detoxify H<sub>2</sub>O<sub>2</sub> by breaking it down into water and oxygen. Glutathione Reductase (GR) recycles oxidized glutathione back to the reduced form that

takes part in neutralizing the ROS, thereby playing an essential role in the maintenance of cellular redox-homeostasis. The accumulation of osmoprotectants such as proline, glycine betaine, and choline O-sulfate help in stabilizing the plant cell metabolic activities, which are disturbed in stress (Souri and Tohidloo, 2019). Synthesis of heat shock proteins is another strategy of the plants to cope up with stressful conditions. This study focuses on understanding of the physiological and biochemical processes of the plants and the mechanisms that are involved in inducing thermotolerance even in sensitive varieties.

Bell pepper is an important vegetable crop. The economic importance and nutritional

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value of its fruits are responsible for its popularity. Bell pepper fruits are an excellent source of vitamins, important antioxidants, flavonoids and phytochemicals (Soare *et al.*, 2017). However, bell pepper is sensitive to temperature fluctuations and optimum temperature for growth and development ranges between 20-25°C. Its fruit setting reduces drastically when the day temperature rises above 32°C and/or night temperature above 20°C (Erickson and Markhart, 2002). Lack of tolerance to high summer temperature acts as a major constraint for pepper production. Elevated temperatures induce the production and accumulation of ROS that can hamper plant growth and development.

Scavenging ROS in plants by antioxidant defense system comprises various enzymes, non-enzymatic metabolites and different phytohormones, among these are SA and 24-EBR. SA plays an important role in stress tolerance by enhancing the biological response against salinity and temperature extremes, and modify antioxidant system (Khan *et al.*, 2015). 24-epibrassinolide upregulates the various physiological and biochemical processes under stressful situations (Kaur and Pati, 2019). This indicates that these phytohormones might have a commercial benefit for the pepper growers in North-Indian plains, where the temperature reaches about 40°C during the summer season. The present study was, therefore, conducted to evaluate the effect of SA and EBR on the tolerance mechanism of bell pepper under the climatic conditions of North-India plains, particularly Punjab, in open field conditions, especially the changes in antioxidant metabolism and expression of heat shock proteins.

## MATERIALS AND METHODS

### Plant Material

The present investigation was conducted in Vegetable Research Farm of Punjab Agricultural University (PAU), Ludhiana,

during 2015-16 and 2016-17. In this experiment, the effect of plant growth regulators viz, SA and EBR were studied on biochemical parameters, antioxidant metabolism, and expression of heat shock proteins in a thermosensitive variety of bell pepper, Royal Wonder (Seminis Seeds Private Limited, India). Seeds were sown in a nursery on 9 November, 2015, followed by transplanting on 7 March, 2016, with planting distance of 90 cm (bed to bed) and 30 cm (plant to plant) on the single side of the bed. The experiment was laid out in randomized complete block design with three replications. There were 10 plants per treatment per replication and all the observations were recorded at an interval of 15 days from 5 representative plants of each replication. Foliar application of SA (0.1, 0.20, and 0.50 mM) and EBR (0.05, 0.10, and 0.20 µM) was done at the following stages 30 (vegetative stage), 60 (reproductive stage), and 90 (post-reproductive stage) Days After Transplantation (DAT). The untreated plants of variety Royal Wonder were taken as the sensitive control and hot pepper variety Punjab Sindhuri was used as a heat tolerant control check and it was kept untreated. The concentrations of PGRs used were standardized in our laboratory in previous studies (unpublished). Stock solutions of SA was prepared by dissolving 138 mg of SA in minimum amount of ethanol and total volume was made to 1 L with distilled water (1 mM stock solution) and EBR solution was prepared by dissolving 4.8 mg of EBR in 1L distilled water (10 µM stock solution) 1,000 mL of distilled water and then the desired concentration was obtained. The standard field management practices for growing the crop were followed as recommended by PAU (Anonymous, 2015). The whole experiment was repeated in the succeeding year (2016-17) for the validation of results. The data on weather parameters such as mean temperature, relative humidity (%) and rainfall (mm) was recorded at the meteorological observatory in PAU for both

**Table 1.** Monthly meteorological data of 2015-16 and 2016-17.

Month	2015-16			2016-2017		
	Mean temp (°C)	Relative humidity (g m <sup>-3</sup> )	Rainfall (mm)	Mean temp (°C)	Relative humidity (g m <sup>-3</sup> )	Rainfall (mm)
November	19.8	64.0	0.0	19.8	60.0	2.0
December	14.3	68.0	16.9	25.3	72.0	0.0
January	12.3	79.0	19.4	12.9	76.0	46.1
February	16.0	68.6	8.8	16.2	68.7	5.2
March	21.3	66.0	41.1	19.9	62.0	40.8
April	28.0	41.8	3.0	28.5	40.0	14.8
May	32.1	42.0	25.2	31.9	38.5	31.6
June	33.7	54.0	86.0	31.6	53.4	127.6
July	30.3	74.0	256.1	30.3	75.0	305.5

years (2015-16 and 2016-17) and is presented in Table 1.

### Observations Recorded

The observations on biochemical parameters were recorded for the third leaf from the top. Biochemical parameters, viz proline (Bates *et al.*, 1973), Total Soluble Sugars (TSS) content and starch content (Dubois *et al.*, 1956), total soluble proteins (Lowry *et al.*, 1951), total phenols (Swain and Hills, 1959), lipid peroxidation (Heath and Packer, 1968) were estimated by taking 100 mg of leaf sample and activity of cellulase (Malik and Singh, 1980) enzyme was measured by taking 100 mg pedicel of flower from the control and treated plants. Activities of CAT (Dhindsa and Motowe, 1981), APX (Nakane and Asado, 1978), POX (Chance and Maehly, 1995), SOD (Marklund and Marklund, 1974), and GR (Esterbauer and Grill, 1987) were recorded for the third leaf from the top of plants at different growth stages. Fruits of the selected five plants from each line were weighed for measuring the fruit yield (kg plant<sup>-1</sup>). Extraction of protein and its profiling was done by using the method of Shevchenko *et al.* (1996). The data presented here represents the mean of both years as well as mean of all the stages.

### Statistical Analysis

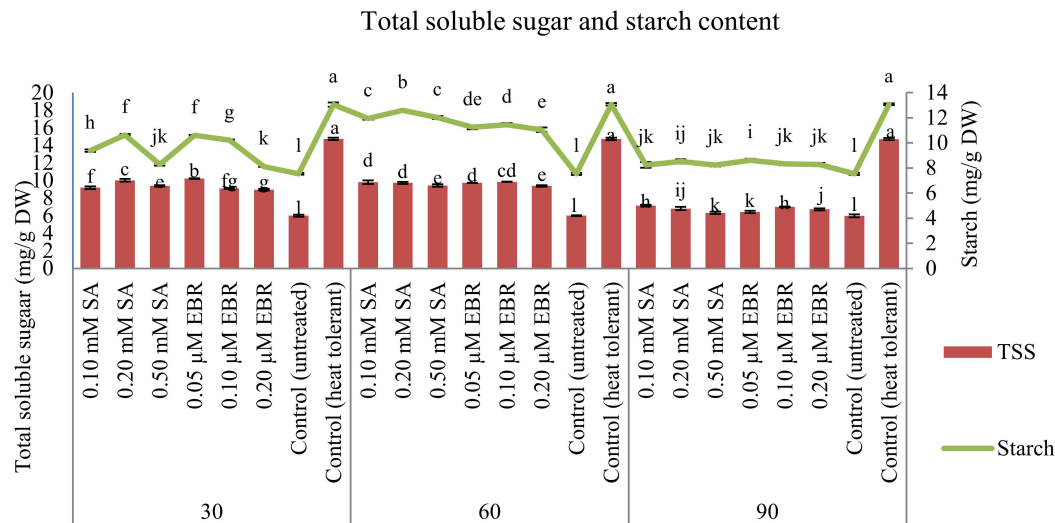
The effects of the treatments on the measured parameters in the three replications were evaluated by using SAS software version 9.3.

## RESULTS

### Biochemical Parameters

**Proline:** The results revealed that application of growth regulators enhanced mean proline content in the treated plants significantly as compared to untreated control (Table 2). Maximum increase in mean proline content at 30 (40.84%), 60 (59.24%) and 90 DAT (17.81%) was observed in the plants treated with 0.20 mM SA, 0.10, and 0.05  $\mu$ M EBR, respectively, over respective control.

**TSS:** Significant enhancement was observed in mean total soluble sugars content of all the treated plants at all the growth stages, thereby imparting thermotolerance (Figure 1). Treating the plants with 0.05  $\mu$ M EBR at 30 DAT resulted in the highest mean TSS content (70.78% more than the control). Whereas, the application of 0.10  $\mu$ M EBR resulted in maximum mean TSS content when spray was done at 60 (64.44%) and 90 (17.09%)



**Figure 1.** Effect of PGRs and time of spray on TSS and starch content of bell pepper cv. Royal Wonder. [Least squares-means with the different letters are significantly different ( $P < 0.05$ )]

DAT in comparison to their respective controls.

**Starch:** Significant differences were noted in starch accumulation of treated and non-treated plants (Figure 1). Exogenous application of 0.20 mM SA caused the highest accumulation of starch content in the plants treated at 30 (41.17%), 60 (67.33%) and 90 (14.48%) DAT, in comparison to their respective controls.

**Soluble Protein Content:** In the present investigation, exogenously applied growth regulators significantly increased the protein content in treated plants (Table 2). Maximum protein content was recorded in the plants treated with 0.20 mM SA at 30 (40.04%) and 60 (42.08%) DAT, in comparison to their respective controls. Foliar application of 0.10 μM EBR was most effective in improving the soluble protein content (11.04% > Control) of plants treated at 90 DAT.

**Phenols:** Our study indicated a significant increase in mean phenols content when spray was done at all the growth stages. Maximum phenol content was observed in

plants treated with 0.10 mM SA when spray was done at 30 (24.29%) and 60 (36.79%) DAT, in comparison to their respective controls. Plants treated with 0.05 μM EBR at 90 DAT showed a maximum increase in total phenols (10.25%) as compared to unsprayed control.

**Lipid Peroxidation:** Application of growth regulators helped in minimizing the lipid peroxidation in plant membrane, thereby maintaining the membrane integrity under elevated temperature stress (Table 2). Spraying the plants with 0.20 mM SA and 0.10 μM EBR at 30 DAT, effectively reduced the lipid peroxidation (13.25% < control). Treating the plants with 0.05 μM EBR reduced the lipid peroxidation when spray was done at 60 (16.06%) and 90 (7.12%) DAT, in comparison to their respective controls.

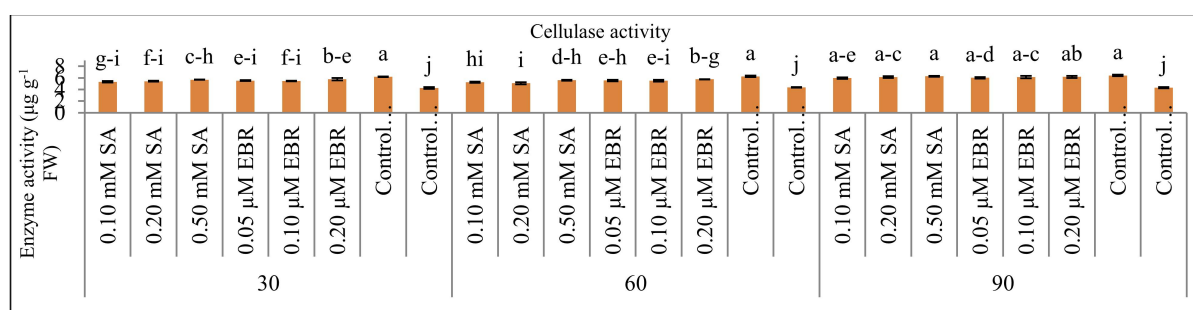
### Enzyme Activities

**Cellulase Activity:** Application of SA decreased the cellulase activity in flower

**Table 2.** Effect of PGRs and the time of spray on biochemical parameters and yield of bell pepper variety Royal Wonder. <sup>a</sup>

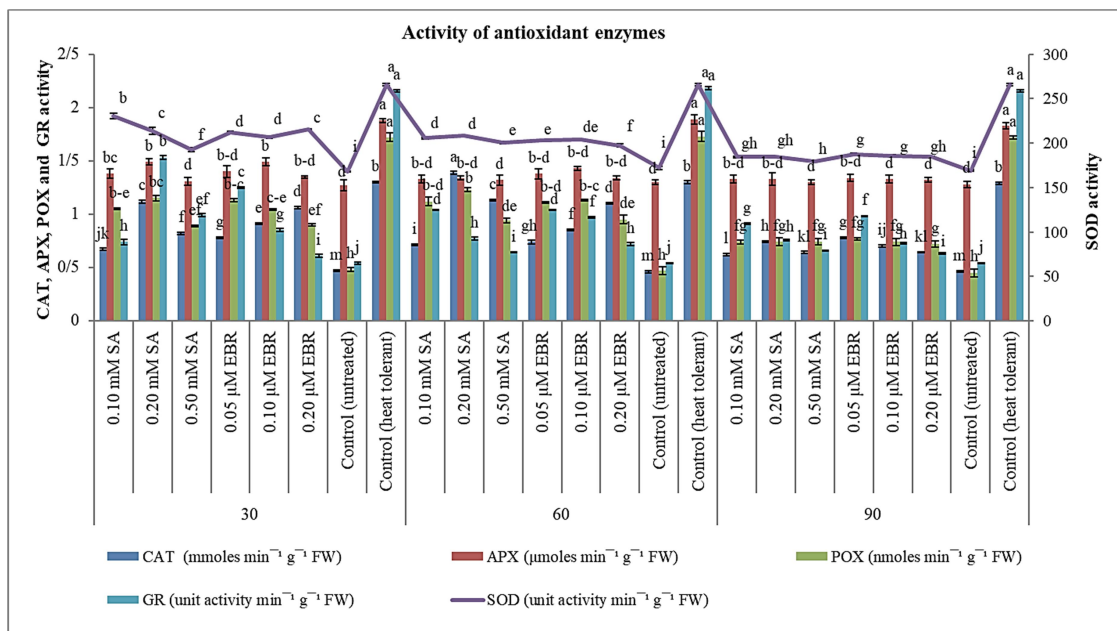
DAT	Treatment	Proline ( $\mu\text{g g}^{-1}$ FW)	Protein ( $\mu\text{g g}^{-1}$ FW)	Phenols ( $\text{mg g}^{-1}$ DW)	Lipid peroxidation (n moles $\text{g}^{-1}$ FW)	Yield ( $\text{kg plant}^{-1}$ )
30	0.10 mM SA	9.82 <sup>g</sup>	11.29 <sup>fg</sup>	12.69 <sup>f</sup>	8.76 <sup>c</sup>	2.13 <sup>b</sup>
	0.20 mM SA	10.76 <sup>e</sup>	12.66 <sup>bc</sup>	11.44 <sup>i</sup>	8.64 <sup>cd</sup>	2.36 <sup>a</sup>
	0.50 mM SA	9.51 <sup>h</sup>	10.99 <sup>h</sup>	10.73 <sup>l</sup>	8.77 <sup>c</sup>	2.19 <sup>b</sup>
	0.05 $\mu\text{M}$ EBR	10.12 <sup>f</sup>	11.48 <sup>ef</sup>	12.03 <sup>h</sup>	8.70 <sup>cd</sup>	1.88 <sup>c</sup>
	0.10 $\mu\text{M}$ EBR	10.52 <sup>e</sup>	11.76 <sup>e</sup>	12.37 <sup>g</sup>	8.64 <sup>cd</sup>	2.28 <sup>a</sup>
	0.20 $\mu\text{M}$ EBR	9.76 <sup>gh</sup>	11.03 <sup>gh</sup>	10.82 <sup>kl</sup>	8.65 <sup>cd</sup>	2.11 <sup>b</sup>
	Control (Unsprayed)	7.64 <sup>l</sup>	9.04 <sup>k</sup>	10.21 <sup>n</sup>	9.96 <sup>a</sup>	1.42 <sup>f-i</sup>
	Control (Heat tolerant)	14.39 <sup>a</sup>	18.38 <sup>a</sup>	18.44 <sup>a</sup>	4.99 <sup>e</sup>	1.35 <sup>h-j</sup>
60	0.10 mM SA	11.10 <sup>d</sup>	12.15 <sup>d</sup>	13.98 <sup>b</sup>	8.40 <sup>d</sup>	1.47 <sup>fg</sup>
	0.20 mM SA	11.75 <sup>e</sup>	12.83 <sup>b</sup>	13.12 <sup>de</sup>	8.50 <sup>cd</sup>	1.69 <sup>d</sup>
	0.50 mM SA	10.66 <sup>e</sup>	12.44 <sup>cd</sup>	12.92 <sup>ef</sup>	8.49 <sup>cd</sup>	1.48 <sup>f</sup>
	0.05 $\mu\text{M}$ EBR	11.78 <sup>c</sup>	12.72 <sup>bc</sup>	13.36 <sup>cd</sup>	8.36 <sup>d</sup>	1.42 <sup>f-h</sup>
	0.10 $\mu\text{M}$ EBR	12.32 <sup>b</sup>	12.49 <sup>c</sup>	13.43 <sup>c</sup>	8.36 <sup>d</sup>	1.59 <sup>e</sup>
	0.20 $\mu\text{M}$ EBR	10.13 <sup>f</sup>	11.75 <sup>e</sup>	12.96 <sup>ef</sup>	8.37 <sup>d</sup>	1.47 <sup>fg</sup>
	Control (Unsprayed)	7.68 <sup>l</sup>	9.03 <sup>k</sup>	10.22 <sup>n</sup>	9.96 <sup>a</sup>	1.42 <sup>f-i</sup>
	Control (Heat tolerant)	14.40 <sup>a</sup>	18.37 <sup>a</sup>	18.38 <sup>a</sup>	4.99 <sup>e</sup>	1.35 <sup>h-j</sup>
90	0.10 mM SA	8.04 <sup>k</sup>	9.93 <sup>ij</sup>	10.57 <sup>lm</sup>	9.28 <sup>b</sup>	1.34 <sup>ij</sup>
	0.20 mM SA	8.71 <sup>j</sup>	10.10 <sup>i</sup>	10.68 <sup>l</sup>	9.30 <sup>b</sup>	1.51 <sup>ef</sup>
	0.50 mM SA	8.07 <sup>k</sup>	9.95 <sup>ij</sup>	10.29 <sup>mn</sup>	9.31 <sup>b</sup>	1.44 <sup>f-i</sup>
	0.05 $\mu\text{M}$ EBR	9.04 <sup>i</sup>	10.16 <sup>i</sup>	11.29 <sup>ij</sup>	9.26 <sup>b</sup>	1.42 <sup>f-i</sup>
	0.10 $\mu\text{M}$ EBR	8.98 <sup>i</sup>	10.04 <sup>ij</sup>	11.02 <sup>jk</sup>	9.27 <sup>b</sup>	1.38 <sup>g-j</sup>
	0.20 $\mu\text{M}$ EBR	7.90 <sup>kl</sup>	9.76 <sup>j</sup>	10.69 <sup>l</sup>	9.27 <sup>b</sup>	1.32 <sup>jk</sup>
	Control (unsprayed)	7.67 <sup>l</sup>	9.15 <sup>k</sup>	10.24 <sup>n</sup>	9.97 <sup>a</sup>	1.30 <sup>k</sup>
	Control (heat tolerant)	14.39 <sup>a</sup>	18.33 <sup>a</sup>	18.36 <sup>a</sup>	4.99 <sup>e</sup>	1.35 <sup>h-j</sup>

<sup>a</sup> Least squares-means with the different letters are significantly different ( $P < 0.05$ )

**Figure 2.** Effect of PGRs and time of spray on cellulase activity of bell pepper cv. Royal Wonder. [Least squares-means with the different letters are significantly different ( $P < 0.05$ )]

pedicel (Figure 2). Significant reduction in cellulase activity was observed when foliar application of 0.10 mM SA done at 30 (13.91% < Control) and 90 (7.01% < Control) DAT showed a significant reduction in

cellulase activity as compared to the unsprayed control. Whereas, 0.20 mM SA caused the maximum reduction in cellulase activity when spray was done at 60 DAT (19.01% < Control).



**Figure 3.** Effect of PGRs and the time of spray on activity of antioxidant enzymes of bell pepper cv. variety Royal Wonder. [Least squares-means with the different letters are significantly different ( $P < 0.05$ ).

**Antioxidant Enzymes:** Application of both growth regulators enhanced the antioxidant enzyme activity (Figure 3) at all the growth stages (30, 60 and 90 DAT). When the foliar application of growth regulators was done at 30 and 60 DAT, maximum mean CAT (138.30 and 202.17%, more than control, respectively), POX (139.58 and 176.74%, more than control, respectively) and GR (183.33 and 92.59%, more than control, respectively) activity was shown by plants treated with 0.20 mM SA. Foliar application of 0.20 mM SA also resulted in maximum APX activity (17.32% > control) when spray was done at 30 DAT and maximum SOD activity (20.99% > control) when spray was done at 60 DAT. However, when growth regulators were applied at 90 DAT, 0.05 μM EBR treatment proved to be the most effective. Both growth regulators improved the activity of antioxidant enzymes.

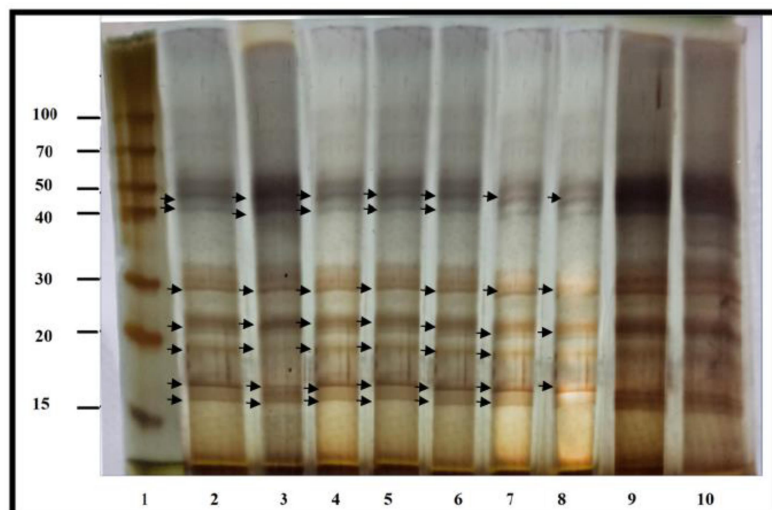
### Total Fruit Yield

The data on the total yield per plant illustrates that maximum mean total fruit

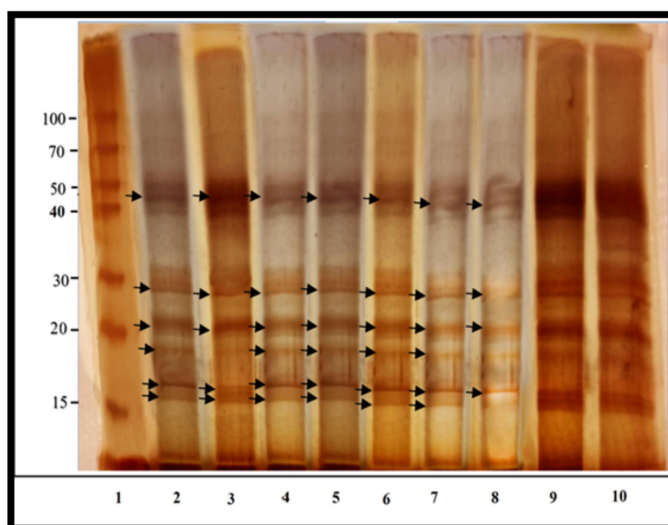
yield increased by 66.20% (2.36 kg), 19.01% (1.69 kg), and 16.15% (1.51 kg) when plants were treated with 0.20 mM SA at 30, 60, and 90 DAT, respectively, as compared to the control plants (Table 2). Under high-temperature stress, SA showed impressive results in enhancing growth and yield by playing an important role in plant metabolism.

### Protein Profiling in Leaves

Figures 4-6 show the silver-stained one-dimensional banding pattern of proteins retrieved from the treated and control plants of bell pepper, indicating the appearance of polypeptides on the application of PGRs at 30, 60, and 90 DAT, respectively. Four polypeptides (55.83, 50.33, 22.32, and 14.99 kDa) were observed in both the control and treated plants, but additional polypeptides of molecular weight 27.83, 19.77 and 15.79 kDa appeared in all the treated plants (Figure 4). When spray was done at 60 DAT, four polypeptides (53.30, 41.98, 22.71 and 16.09 kDa) were noted in the control



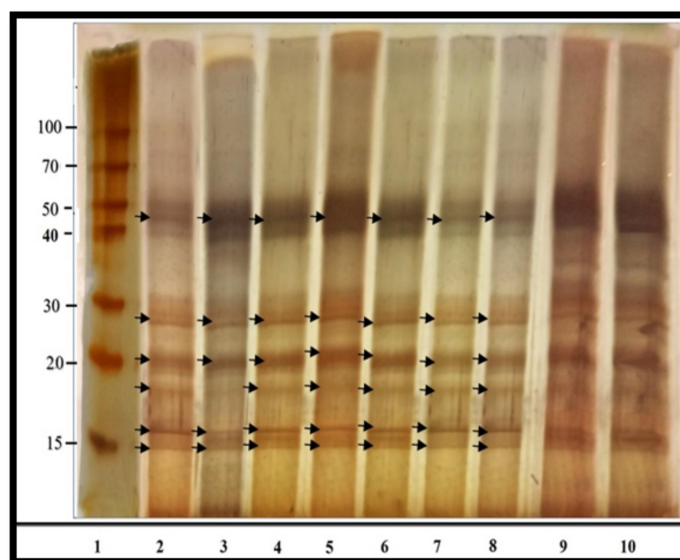
**Figure 4.** Fluorograph of the protein synthesized in the leaves of variety Royal Wonder at 30 DAT (36.8°C). Lane 1: Atandard; Lane 2: 0.10 mM SA; Lane 3: 0.20 mM SA; Lane 4: 0.50 mM SA; Lane 5: 0.05  $\mu$ M EBR; Lane 6: 0.10  $\mu$ M EBR; Lane 7: 0.20  $\mu$ M EBR; Lane 8: Control (untreated); Lane 9: Control (heat tolerant); Lane 10: Control (heat tolerant).



**Figure 5.** Fluorograph of the protein synthesized in the leaves of variety Royal Wonder at 60 DAT (38.6°C). Lane 1: standard; Lane 2: 0.10 mM SA; Lane 3: 0.20 mM SA; Lane 4: 0.50 mM SA; Lane 5: 0.05  $\mu$ M EBR; Lane 6: 0.10  $\mu$ M EBR; Lane 7: 0.20  $\mu$ M EBR; Lane 8: Control (untreated); Lane 9: Control (heat tolerant); Lane 10: Control (heat tolerant).

plants. They were also retrieved from treated plants but with higher intensity. New polypeptides of molecular weight 28.60, 17.10 and 15.60 kDa were noticed in the lanes of all treated plants. Their intensity was high in lane 3 followed by lanes 5, 6,

and 2. The protein bands obtained at 60 DAT were more prominent than the protein bands retrieved at 30 DAT. Six polypeptides (46.17, 27.12, 22.75, 18.06, 16.27, and 14.53 kDa) were noted in the control plants at 90 DAT (Figure 6). These polypeptides were



**Figure 6.** Fluorograph of the protein synthesized in the leaves of variety Royal Wonder at 90 DAT (36.8°C). Lane 1: standard; Lane 2: 0.10 mM SA; Lane 3: 0.20 mM SA; Lane 4, 0.50 mM SA; Lane 5: 0.05  $\mu$ M EBR; Lane 6: 0.10  $\mu$ M EBR; Lane 7: 0.20  $\mu$ M EBR; Lane 8: Control (untreated); Lane 9: Control (heat tolerant); Lane 10: Control (heat tolerant).

also retrieved from treated plants but with higher intensity. Maximum intensity was obtained from lane 5 followed by lanes 2, 4, and 6. Polypeptide of molecular weight 46.32 kDa was observed in lanes 2, 3, and 6. Application of growth regulators at different growth stages also resulted in the appearance of protein bands of low molecular weight at 30 (28.29, 22.32, 19.77, 15.79, and 14.99 kDa), 60 (15.10, 14.60, and 14.09 kDa) and 90 DAT (27.12, 22.75, 18.06, 16.27, and 14.56 kDa).

## DISCUSSION

Heat stress adversely affected biochemical trait and yield in capsicum. Application of PGRs was effective in mitigating heat stress as it significantly reduced the effect of elevated temperature. Proline acts as a cellular osmotic regulator between cytoplasm and vacuole by detoxifying ROS, thereby upregulating the defense mechanism during heat-stressed conditions. Therefore, the increase in the proline in treated plants helped in conferring tolerance against high temperature stress. Our results are in

accordance with previous studies conducted by Kousar *et al.* (2018) in wheat and Hussain *et al.* (2019) in bell pepper with SA and EBR.

Sugars are known to control a wide range of processes in plants, including defense mechanism and hormonal balance, in addition to their role as a supply of energy and carbon backbone (Ahmad *et al.*, 2020). The accumulation of organic solutes like sugars is an important mechanism of stress tolerance in plants (Ibrahim, 2016). EBR-induced increased thermotolerance has been correlated with enhanced sugar supply in wheat genotype (Kumari and Hemantranjan, 2019). Even the sensitive genotypes were able to survive. Heat stress also disrupts starch biosynthesis by down-regulating the activity of enzymes responsible for its synthesis and accumulation (Ruan *et al.*, 2010). Findings of the present study are in coherence with Kaur *et al.* (2019) who suggested that SA helped in easing out the effects of heat stress in spring maize, thereby leading to reduced mobilization of carbohydrate reserves in terms of increased starch content.



High-temperature stress causes the production of ROS that damage and degenerate the proteins (Arif *et al.*, 2020). SA attenuates stress by up-regulating soluble proteins and other defense mechanisms in plants. Application of SA significantly ameliorates abiotic stress by elevating antioxidant activities and soluble protein (Iqbal *et al.*, 2019). Our results are in coherence with Dong *et al.* (2017) who suggested that the induction of transcription and translation by EBR might be the reason behind the enhanced protein content.

Phenolic compounds are involved in plant responses to environmental stresses. The increase in total phenol content in PGRs treated plants in our study is in agreement with the results reported by Mostafa *et al.* (2018) in SA-treated tomato plants. The findings of Mohammadi *et al.* (2020) showed that EBR increased phenol content in common bean helped in providing stability to deal with drastic severities of the environment.

Peroxidation of membrane lipids and pigments by ROS leads to loss of membrane stability. In our study, lipid peroxidation increased with increasing temperature and application of SA and EBR led to a decrease in peroxidation of membrane lipids. Our results are in accordance with Jahan *et al.* (2019) and Ahmed *et al.* (2020), who reported reduced lipid peroxidation in the SA-treated heat-stressed plants of tomato and sweet pepper, respectively. Our results are also in coherence with Wu *et al.* (2014) and Sri *et al.* (2016), who observed pronounced reduction in lipid peroxidation of stressed plants of eggplant and chickpea on treatment with EBR.

Reproductive tissues and organs are highly sensitive to high-temperature stress, leading to significant increment in flower drop (Sivakumar *et al.*, 2018). Cellulase actively takes part in wall loosening at the site of abscission. SA prevents cellulase activity by maintaining the firmness of the cell wall (Abdel-Salam, 2016). Khedr (2018) also noticed a decline in cellulase enzyme on SA

application. These findings are consistent with our results.

Application of SA and EBR enhanced the activity of various antioxidant enzymes, which helped in scavenging of different ROS produced as a result of exposure of plants to higher temperature. Jahan *et al.* (2019) has reported enhanced CAT and SOD activity in SA-treated heat-stressed tomato plants. Li *et al.* (2019) observed that foliar application of SA led to significant increase in APX activity in heat-stressed potato plants. Our results are also in coherence with Wu *et al.* (2014) who reported enhanced antioxidant enzyme activity (CAT, APX, POX and SOD) in EBR treated heat-stressed eggplant. Maia *et al.* (2018) also noted increased CAT and APX activity in tomato plants on application of EBR. da Silva Rodrigues *et al.* (2020) noted 13% increase in the APX activity of EBR-treated soyabean plants. Soylemez *et al.* (2017) recorded an increase in POX and SOD (Mohammadi *et al.*, 2020) activity in EBR-treated stressed plants of tomato. Enhancement in the activity of GR was also reported by Yin *et al.* (2018) in EBR treated heat-stressed tomato plants. All these results are consistent with our studies where SA and EBR application improved heat tolerance in capsicum by regulating antioxidant enzymes.

Plants, when exposed to stresses, induce production of a group of proteins called heat shock proteins. These act as molecular chaperone and regulate protein folding, accumulation and transportation, and removal of damaged protein. Vidya *et al.* (2018) observed that majority of HSPs including HSP22, HSP26.5, and HSP28 were activated at the early stages of heat stress. Our findings are similar to Xue *et al.* (2010), who reported that the enhanced expression of HSP26 in *Arabidopsis thaliana* contributed to the accumulation of defense proteins, free proline and soluble sugars, that function as a stress-protector under high-temperature stress conditions. Similarly, Feng *et al.* (2019) noticed the expression of CaHSP25.9 in response to heat, salt and drought stress. HSP27 is a highly induced heat shock protein under abiotic stresses including heat stress



(Das and Bhattacharya, 2017). A protein, namely, CsHSP45.9 was recognized as heat-shock protein and was found to be responsible for inducing tolerance to heat stress through stimulation of antioxidant system (Kim *et al.*, 2020; Ru *et al.*, 2020). Louis and Roy (2011) also reported the induction of many HSPs in plants of *Solanum tuberosum* including HSPs of low molecular weight *i.e* HSP29, sHSP22.5, sHSP17.8 and sHSP9.5 against heat stress. Yang *et al.* (2020) studied the overexpression of sHSP17.6 in alleviating the heat-stressed inhibited growth and development in *Arabidopsis thaliana*. These results suggest that sHSPs, which appeared due to the application of growth regulators in the present investigation, helped in imparting thermotolerance.

### CONCLUSIONS

Application of PGRs to bell pepper cv. Royal Wonder ameliorated the harmful effects of high-temperature stress in terms of improved biochemical parameters, antioxidant system with reduced lipid peroxidation and cellulase enzyme activity at high temperature, ultimately, resulting in improved total yield. Moreover, Royal Wonder being thermosensitive variety responded more positively when the application of PGRs was done at the vegetative stage than at reproductive and post-reproductive stages. Exogenous application of 0.20 mM SA at vegetative stage (30 DAT) and reproductive stage (60 DAT) were most effective in imparting thermotolerance in bell pepper plants.

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### سالیسیلیک اسید و ۲۴-اپی براسینولید از طریق سامانه تقویت شده آنزیم آنتی اکسیدانی و پروتئین های شوک حرارتی، تحمل حرارت را در فلفل دلمه‌ای القا کرد

ت. پرت، ن. غایی، س. ک. جیندال، و م. کاعورسنگها

#### چکیده

دمای بالا بر تولید فلفل دلمه‌ای (*Capsicum annuum L.*) تأثیر منفی می گذارد، به ویژه در دشت های شمال هند که در تابستان دمای آن به بالای ۴۰ درجه سانتی گراد میرسد. در پژوهش حاضر، اثر کاربرد بیرونی تنظیم کننده های رشد گیاهی (PGRs) یعنی اسید سالیسیلیک (SA) و ۲۴ اپی براسینولید (EBR) بر پارامترهای بیوشیمیایی و سامانه آنتی اکسیدانی فلفل دلمه‌ای رقم رویال واندر (Royal Wonder) ارزیابی شد. PGR ها ۳۰ روز پس از نشاکاری (DAT)، ۶۰ روز DAT، و ۹۰ روز DAT به صورت بیرونی (exogenously) اعمال شد. تمام غلظت‌های PGRs یعنی SA (۰.۱۰ میلی مولار، ۰.۲۰ میلی مولار و ۰.۵۰ میلی مولار) و EBR (۰.۰۵ میکرومولار، ۰.۱۰ میکرومولار و ۰.۲۰ میکرومولار) در بهبود اثرات ناشی از شوک حرارتی مؤثر بودند. این بهبود تحمل باعث افزایش پرولین و محتوای پروتئین های محلول، کل فنل ها، کل قندهای محلول و محتوای نشاسته، سیستم آنتی اکسیدانی بهبود یافته (CAT، APX، POX، SOD، GR) با کاهش پراکسیداسیون لیپیدی و فعالیت آنزیم سلولاز در دمای بالا، و در نهایت، بهبود عملکرد کل میوه شد. کاربرد ۰.۲۰ میلی مولار SA به طور کارآمدی باعث بهبود تحمل حرارتی در تمام مراحل رشد شد، به ویژه زمانی که پاشش یا اسپری در ۳۰ و ۶۰ روز پس از نشاکاری (DAT) انجام شده بود. این امر منجر به افزایش قابل توجهی در پارامترهای بیوشیمیایی و سامانه آنزیم آنتی اکسیدانی در مقایسه با شاهد تیمار نشده گردید.