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Increasing Resistance to Oxidative Damage in Cucumber (*Cucumis sativus* L.) Plants by Exogenous Application of Salicylic Acid and Paclobutrazol

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Abstract: Pot experiments were conducted on cucumber (*Cucumis sativus* L.) plants in the green house of the National Research Center. Dokki, Giza, Egypt to assess the impacts of low temperature on cucumber plants cultivated in January 2007 and 2008 to achieve early production during April. To alleviate the harmful effect of low temperature, salicylic acid (2, 4mM) and paclobutrazol (25, 50 mg/l) were investigated. The work concerned to study the effect of salicylic acid or paclobutrazol separately on growth criteria (plant height, number of leaves, fresh and dry weights of leaves and stems and leaf area of cucumber/ plant), plant survival, photosynthetic pigments, antioxidant enzyme (CAT, POX, APX and GR) activities, lipid peroxidation, electrolyte leakage and yield. Obtained results revealed that plants grown under low temperature and foliarly treated after transplanting with SA at the concentration of 4mM followed by 2mM and paclobutrazol at the concentration of 25 mg/l mitigated the harmful effects of low temperature stress through the enhancement of their protective parameters, such as antioxidant enzymes activity and caratenoids. SA at 4mM recorded the highest increments in GR and APX activities, survival percentage and carotenoids contents. Meanwhile, PBZ at 25mg/l recorded the highest increments in POX and CAT activities, chl. a and b. Remarkable decreases were also recorded in MDA and electrolyte leakage (EL) with SA and PBZ treatments. The results also, showed that the highest value of yield per plant was recorded with plants received Salicylic acid at the concentration of 4mM. Based on the obtained results, it could be suggested that the protection mechanism had helped the plants to increase their tolerance against low temperature stress, through mainly the decrease in membrane damage symptoms leading to intercellular osmotic adjustment.

Key words: Cucumber % Low temperature % Salicylic acid % Paclobutrazol % Antioxidant enzymes % Lipid peroxidation

INTRODUCTION

Cucumber plant (*Cucumis sativus L.*) is one of the important vegetable crops in Egypt. Plantation area in 2008 reaches 70635 ha [1]. Cucumber is usually cultivated in the open field in Egypt during March and April where the prevailing temperatures are suitable for growth and development of cucumber plant. Maximum yield is obtained at the end of May and June. Promotion of early production of cucumber is an attractive approach to satisfy the local need and to increase export. If the plants are to be cultivated in December, January and February, they will be subjected to low temperature. Minimum level of night temperature during these months drops several times below 10°C. Cucumber is a heat-loving plant and will suffer from cold stress which causes chilling injury when exposed during their lifecycle to temperatures below 10-15°C and down to 0°C. Chilling induces oxidative stress [2, 3], where it has been suggested that during exposure to low temperatures the normal mitochondria electron transport might be disrupt, causing the production of reactive oxygen species (ROS) [2, 4], including hydrogen peroxide (H_2O_2) in concentration higher than necessary for normal metabolism [5] leading to several biochemical and physiological dysfunctions. Those active oxygen species are highly cytotoxic and can react with unsaturated fatty acids to cause peroxidation of essential membrane lipids in plasmalemma and intercellular organelles [6], leakage of solutes from cells and the bleaching of chlorophyll [7]. These changes lead to reduction of growth and poor quantity and quality of the yield. ROS are scavenged by plant antioxidant defense systems, comprising both enzymatic and non-enzymatic components [8].

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It can be seen the importance to alleviate the harmful effect of low temperature on cucumber plants cultivated early (Decembers-April) by using some specific treatments through increasing protective enzymes activity, as one of the protection mechanisms by inhibiting or quenching free radicals [9] where salicylic acid and paclobutrazol have protective role in this respect [10, 11].

Salicylic acid (SA) acts as a potential non-enzymatic antioxidant as well as a plant growth regulators, which plays an important role in regulating a number of plant physiological processes including photosynthesis [12-14]. Exogenous SA could ameliorate the damaging effects of heavy metals in rice [15], drought stress in wheat [13, 16] and salt stress in wheat [14]. These observations suggest that SA being an oxidant could be linked to oxidative stress [17]. Paclobutrazol (PBZ) increased cold hardness of different plants and protected plants from exposure to extreme temperature on cucumber seedlings [18], wheat and corn [19].

MATERIALS AND METHODS

Pot experiments were carried out in the greenhouse unit of the National Research Centre, Giza, Egypt during two successive growth seasons (2006/2007 and 2007/2008) to study the effect of salicylic acid or paclobutrazol separately on growth, yield and some biochemical constituents of cucumber (*Cucumus sativa L.*, cv. Alpha beta). Cucumber seedlings obtained from protected greenhouse of the Ministry of Agriculture and Land Reclamation (MALR) were used in this experiment. Seedling (one true leaves) were transplanted carefully with the surrounding soil to 40 cm diameter pots at the beginning of second week of January. Each pot filled with 13kg of a mixture of loam clay soil and sand soil at the ratio of 1:1 (w/w) mixed with 6.7g of super phosphate and 3.25g of ammonium nitrate. The seedlings were cultivated at a density of 5 seedlings per pot. The plants were supplied with water according to their requirements which was governed by climatic conditions. Each pot received 3.25g ammonium nitrate weekly for a period of 4 weeks. The pots were arranged in complete randomized block design with three replicates for each treatment. The replicates were represented by five pots. The plants were sprayed twice, one day after transplanting and one week later with solutions of either salicylic acid (SA) at the rate of 2 or 4mM or paclobutrazol (PBZ) at the rate of 25 or 50mg/l, while the control plants were sprayed with distilled water.

Growth Criteria Determinationl: Two samples were collected for the estimation of fresh and dry weights of leaves, stems, area of leaves/plant (cm^2) and number of leaves/plant. The first sample was collected at vegetative growth (45 days after sowing) and second one at flowering stage (60 days after sowing). Survival percentage was recorded every week starting from the 1st week of transplanting.

Biochemical Constituents Determination: Fresh leaves were collected for estimation the activity of antioxidant enzymes (CAT, POX, GR and APX), photosynthetic pigments and electrolyte leakage and lipid peroxidation. At the same time the extraction of the antioxidant enzymes CAT, POX, GR and APX were determined. 5g of frozen leaves tissues were homogenized in pre-chilled mortar in presence of 10ml of 50mM potassium phosphate buffer (pH7) with 1% (w/v) insoluble polyvinyl pyrolidone (PVP) and 0.1mM EDTA. The extraction procedures were repeated twice and supernatants were pooled, raised to a certain volume, referred as crude enzyme extract, all operation were carried out at -4°C for further analysis. The activity of CAT was determined according to Aebi [20], POX and APX activities according to Nakano and Asada [21] and GR activity was determined according to Zanetti [22]. The activity was expressed as change in the optical density per gram fresh weight per minute under the experimental conditions. Photosynthetic pigments (chlorophyll a, b and carotenoids) were determined according to the method described by Metzner *et al* [23]. Lipid peroxidation was determined by measuring Malondialdehyde (MDA) content as described by Dhindsa *et al*. [24] and leakage of electrolyte measurements according to Gilley and Fletcher [25]. At harvest time, the fruits were collected at time intervals when they were about 10 cm in length, weighed and then the fruit yield was determined.

Statistical Analysis: The data obtained were subjected to standard analysis of variance procedure according to Snedecor and Cochran [26]. The values of L.S.D. were calculated whenever F values were significant at 5% level.

RESULTS AND DISCUSSION

Vegetative and Flowering Growth Characteristics: Data presented in Table 1 show that foliar application of salicylic acid at the rate of 2 or 4mM on cucumber plants mostly led to significant increase in the values of all the studied growth parameters i.e. plant height, number of

Table 1: Mean values for vegetative growth characters of cucumber plants grown under low temperature and treated with salicylic acid or paclobutrazol

	45 days after transplanting							60 days after transplanting						
Treatments	Plant height cm	No of leaves	Leaves fresh weight G	Leaves dry weight g	Stem fresh weight g	Stem dry weight g	Leaf areacm2	Plant heightcm	No of leaves	Leaves fresh weightg	Leaves dry weightg	Stem fresh weight g	Stem dry weightg	leaf area cm2
Control	9.50	4.00	1.63	0.37	1.84	0.22	117.4	12.67	5.56	3.19	0.75	2.88	0.36	124.6
2mM SA	11.00	5.00	2.29	0.38	2.13	0.23	134.2	15.50	9.00	4.21	1.04	3.82	0.48	167.3
4mM SA	12.66	5.67	2.36	0.42	2.18	0.24	161.5	17.83	9.33	5.06	1.19	4.58	0.57	197.4
25 mg/l PBZ	7.33	4.56	1.48	0.33	1.88	0.18	85.5	10.67	7.56	2.86	0.73	2.61	0.26	129.9
50 mg/l PBZ	6.83	4.33	1.35	0.29	1.66	0.17	75.1	10.50	6.33	2.70	0.68	2.36	0.24	114.8
LSD 5%	1.37	N.S.	0.24	0.05	0.07	0.01	13.08	1.43	0.47	0.36	0.05	0.21	0.04	9.8

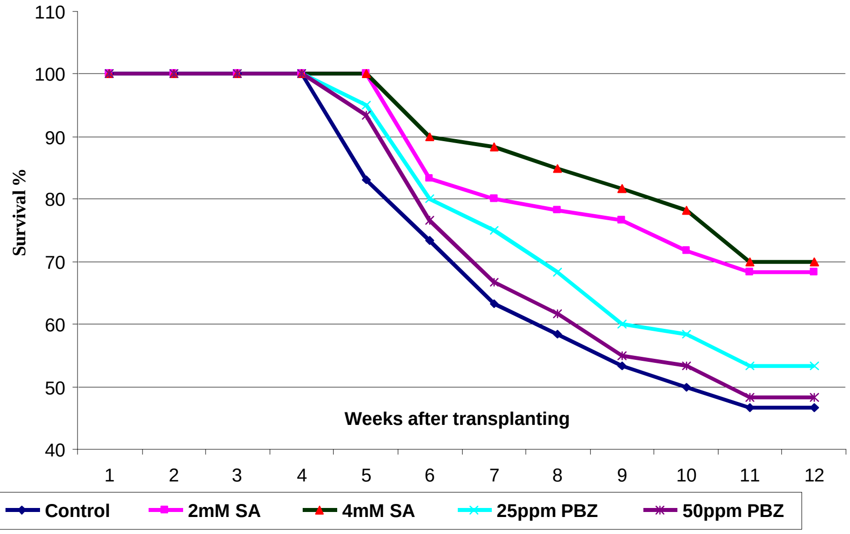

Fig. 1: Effect of salicylic acid or paclobutrazol on the percentage of survival of cucumber plants grown under low temperature

leaves per plant, fresh and dry weights of leaves or stems and leaf area/plant as compared to the corresponding control. Meanwhile, foliar application of paclobutrazol at 25 or 50 mg/l mostly led to significant decrements except number of leaves and leaf area/plant which recorded slight increments compared with the corresponded control highest significant decrements was recorded at 50mg/l PBZ. Previous studies have shown the involvement of SA in increasing shoot growth for many plants [16, 27]. Regulation of some photosynthetic reactions may be due to the known effects of SA on stomata function, transpiration rate and respiratory pathways as suggested by Khan *et al*. [28]. Kord and Hathout [29] mentioned that application of SA at 0.01mM increased leaf number and leaf area. In addition El-Mergawi *et al*. [30] pointed that the highest values for growth characters in *Cathranthus roses* plants grown under water shortage with 4mM SA. Moreover, in salt stressed wheat, improvement of growth was attained by increasing shoot dry weight of seedlings [31], whereas in salt stressed sunflower, SA induced enhancement in growth and that might be due to SA induced increase in antioxidant capacity [17]. The reduction of growth and dry weights in cucumber plants treated with paclobutrazol (Table 1) might be attributed to decrease of IAA and gibberellins revealed by previous studies on triazoles [32, 33]. Decreases in fresh and dry weight of plants treated with triazoles were also reported by Imam *et al*. [34] and Khalil and Uikreem [35].

Survival Percentage: Fig. 1 illustrated that plants at five, six, seven and eight - weeks old, survived under low temperature, at nine-weeks old, all plants treated with salicylic acid still survived whereas, untreated plants as well as plants treated with paclobutrazol began to perish. At ten-week to fourteen weeks old, plants treated with salicylic acid, paclobutrazol and untreated plants began to perish. Best results of survival were obtained with salicylic acid followed by paclobutrazol especially at 4mM and 25 mg/l, respectively. In this concern Dat et *al*. [10] mentioned that SA increased survival percentage during thermo tolerance inducing in mustard seedlings.

Table 2: Chlorophyll a, b and caratenoids contents (mg/g/fresh weight) of cucumber plants grown under low temperature and treated with salicylic acid or paclobutrazol

Treatments	chlorophyll a	chlorophyll b	carotenoids
Control	1.29	0.53	0.45
2mM SA	1.64	0.73	0.55
4mM SA	1.69	0.78	0.60
25 mg/l PBZ	1.97	0.80	0.56
50 mg/l PBZ	1.79	0.81	0.53
LSD 5%	0.13	0.09	0.05

Table 3: Mean values of activity (unit/g.f.w) of CAT, POX, APX, GR, MDA (µmole/g. f.w) and electrolyte leakage percentage of cucumber plants grown under low temperature and treated with salicylic acid paclobutrazol

Treatments	CAT µmole	% of cont	POX µmole	% of cont	APX µmole	% of cont	GR nmole	% of cont	MDA µmole	% of cont	E.L.%	% of cont
Control	71.35±2.61	100	2.78±0.17	100	1.39±.09	100	241.13±7.73	100	7.94±0.17	100	58.50±3.80	100
2mM SA	78.36±3.44	109.82	3.41±0.11	122.66	3.21±0.11	230.94	417.56±3.76	173.17	5.78±0.20	72.80	41.31±1.87	70.62
4mM SA	74.86±4.18	104.92	3.96±0.13	142.45	3.71±0.12	266.91	513.57±14.64	212.98	5.11±0.11	64.36	38.11±1.51	65.15
25 mg/l PBZ	87.92±3.98	123.22	4.77±0.21	171.58	2.93±0.10	210.79	381.83±10.23	158.35	6.20±0.06	78.09	47.71±2.05	81.56
50 mg/l PBZ	80.59±2.23	112.96	5.61±0.15	201.80	2.12±0.04	152.52	355.04±17.72	147.24	6.83±0.11	86.02	46.02±1.71	78.67

Values are means of three replicates ±SE

Several investigators declared that triazoles increased the survival of different plants under low temperature stress. Paclobutrazol was found to increase the survival of different crops grown outdoors during winter [36] on cereal [37] on peas [38] on herbaceous plants [39] on winter rape. Triadimefon was reported to protect barley plants from freezing injury and increased the survival of the seedling subjected to -6°C [40]. Prasad [41] reported that when pretreated 3 days old *Zea mays* seedlings with 3mM aminotriazol and exposed the seedling to chilling stress (-6°C), an increase of survival ratio was recorded. The decrease in plant survival could be partially attributed to the induction of oxidation of proteins and lipids, this inter predation was confirmed by Prasad [42] on *Zea mays* seedling.

Biochemical Constituents: Data in Table 2 reveled that all applied concentrations of salicylic acid or Paclobutrazol increased significantly chl. a, b and carotenoids. Maximum increase was obtained by application of 4mM for salicylic acid and 50 mg/l for paclobutrazol. Also, salicylic acid had an important role in photosynthesis [12-14, 43]. In bean plants, foliar spray with salicylic acid, increased chl. a, band carotenoids under normal field conditions, Türky?lmaz *et al.* [44] reported that photosynthetic pigments were increased with SA application. Moreover, Khan *et al.* [28] showed that SA increased photosynthetic rate in corn and soybean. However, in salt stressed wheat, salicylic acid recorded increments in chl. a, b and caratenoids [31].Our results are also in agreement with the findings of many investigators who reported that triazole compounds were effective in increasing chlorophylls and carotenoids in treated plants [35, 45, 46]. The obvious high level of carotenoids (Table 2) might lead to a protective mechanism for plants grown under low temperature, since, carotenoids are important antioxidant for eliminating singlet oxygen [47].

Antioxidant Enzyme Activities: Table 3 shows that CAT activity as well as POX activity recorded higher response in plant treated with paclobutrazol. The highest value for POX activity was attained by 50 mg/l paclobutrazol. Meanwhile, remarkable increments were obtained in APX and GR activities due to salicylic acid treatments especially at 4mM. The little increments change in CAT activity according to salicylic acid treatments was confirmed by the findings of Noreen *et al*. [17] on salt stressed sunflower plants. Ananieva *et. al.* [48] reported that SA treatment resulted in an increase of peroxidase and catalase by 17 and 20% compared to the control plants, respectively. Salicylic acid application was also increased in CAT and POX in heat stress [49]. Contrastingly salicylic acid treatment decreased the activities of catalease in tomato [50] and catalease and peroxidase in pea seedlings [51]. It suggested that salicylic acid enhancing the AOS such as H_2O_2 [10, 52, 53], whereas one of the reactive oxygen species that accumulate in plant tissues during cold stress is hydrogen peroxide [5]. SA tends to increase the activities of POX and APX for decomposing the harmful H_2O_2 [5].

Results attained by the present study showed obvious enhancement of APX and GR activity followed by POX activity as a result of salicylic acid treatments (Table 3). These treatments led to the proper protection of cells against photo inhibition. This view is supported by experimental evidence with transformed plants showing enhanced GR activities. Higher GR activities in these plants stabilize their ascorbat pools and increase their tolerance to active oxygen species [54].

Higher GR and APX activities resulted in tolerant plants against salt stress [55] on onion and [56] on sugar beet. The marked increase in the activities of these enzymes helped the plant to destroy H_2O_2 accumulated by cold stress [5] and maintained the ascrobate pool which in turn led to elevate the plant tolerance to low temperature stress.

Increase of antioxidant enzyme activities by paclobutrazol treatments (25 and 50 mg/l) could be supported by Upadhyaya *et al*. [57], Zhou and Leul [58], who reported increase in the enzyme activities due to triazole treatments under both normal and stress conditions. Moreover, Kraus and Fletcher [59] explained the role of paclobutrazol to enhance detoxification of active oxygen species through increasing CAT, POX and GR enzymes.

Other studies have shown that exogenous SA can regulate the activities of antioxidant enzymes and increase plant tolerance to abiotic stress [60, 61]. This pronounced increase of the activities of enzymes at vegetative stage (Table 3) might be attributed to the sudden exposure of the plants, grown under suitable temperature for 4 weeks, to low temperature shock in the open field, such treatments promoted high enzyme activities required to overcome the low temperature stress where salicylic acid appear to be a defense-mediating signal in cucumber [62, 63].

Lipid Peroxidation and Electrolyte Leakage: Treatment with salicylic acid or paclobutrazol (Table 3) showed remarkable decreases in the content of MDA and electrolyte leakage as compared with the control. These decreases showed the minimal values at 4mM followed by 2mM salicylic acid and 25mg/l paclobutrazol. The remarkable decreases in lipid peroxidation and electrolyte leakage as indicators of reduction of membrane damage, increased membranes stability and tolerance of plants [55, 64]. In agreement with this view SA involved in thermal tolerance [49] through inducing decrements in lipid peroxidation in pea leaves [65]. It also decreased electrolyte leakage under salt stress [66]. Paclobutrazol also provides maximum protection from cold by decreasing MDA content [57] and from heat by decreasing electrolyte leakage in wheat seedlings [59] and during drought stress from loss of membrane integrity and ion leakage on wheat seedlings [25]. These results might be attributed to the obvious increases in antioxidant enzymes activities [55] which in turn enhanced scavenging of harmful free radicals.

Table 4: Mean values for fruit weight (g/plant) of cucumber plants grown under low temperature and treated with salicylic acid or paclobutrazol

Treatments	Fruit weight g/plant	Percentage%
Control	144.86	100
2mM SA	270.36	186.6
4mM SA	296.07	204.4
25 mg/l PBZ	159.81	110.3
50 mg/l PBZ	129.11	89.1
LSD 5%	15.47	--

Fruit Weightl: Table 4 shows that significant increases in fruit weight were recorded in SA treatments followed by the low concentration of paclobutrazol (25mg/l). These increments surpassed untreated plants collectively according to the lowest measured survival ratio of those later plants. It could be stated that the beneficial effect of SA on improving fruit weight may due to the translocation of more photoassimilates to fruits thereby increasing fruit weight. These findings are in agreement with those reported by Arfan *et al*. [14] and Singh and Usha [43] on wheat, Gunes *et al*. [67] on maize and Elwan *et al*. [68] on pepper. Triazoles application showed increases in the yield of several plants [69] on rice and [70] on wheat.

In conclusion, SA is reported recently to induce multiple abiotic stress tolerance to plants such as chilling tolerance of maize [11], drought tolerance in wheat [43] and salt tolerance in sunflower [17]. In addition, paclobutrazol was consistently the most effective for protection under stress conditions between other triazoles such as propiconazole and tetraconazole [25].

These views are confirmed by our results, where cucumber plants grown under low temperature and foliar treated with SA or paclobutrazol after transplanting could alleviate the harmful impacts of low temperature through the enhancement of their protective parameters, such as antioxidant enzymes activity and caratenoids. This could suggest that the protection mechanism had helped the plants to increase their tolerance against low temperature stress, through mainly the decrease in membrane damage symptoms leading to intercellular osmotic adjustment.

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