J Plant Growth Regul (2011) 30:213–219
DOI 10.1007/s00344-010-9188-2

Influence of Paclobutrazol and Application Methods on High-temperature Stress Injury in Cucumber Seedlings

Bahram Baninasab · Cyrus Ghobadi

Received: 22 May 2010 / Accepted: 21 October 2010 / Published online: 14 December 2010

Abstract Paclobutrazol (PBZ) is a member of the triazole plant growth inhibitor group that is responsible for inducing tolerance to a number of biotic and abiotic stresses. An experiment was therefore conducted to examine whether the application of PBZ at various concentrations (0, 25, 50, and 75 mg l^{-1}) by seed soaking or foliar spray would protect cucumber (*Cucumis sativus*) seedlings subjected to high-temperature stress. Thirty-five-day-old seedlings were exposed to heat stress at 40°C for 4 h per day for 5 days. PBZ improved the majority of the physiological (for example, relative chlorophyll content and chlorophyll fluorescence ratio) and morphological parameters (for example, shoot and root fresh and dry weights) measured in cucumber seedlings subjected to high-temperature stress. PBZ ameliorated the injuries caused by heat stress by increasing leaf proline content and preventing an increase in leaf electrolyte leakage. PBZ was more effective at increasing the heat tolerance of cucumber seedlings when using the seed-soaking method rather than the foliar spray method. The best protection was obtained when seeds were soaked in 50 mg l^{-1} PBZ.

Keywords Application method · *Cucumis sativus* · High-temperature stress · Paclobutrazol

Introduction

Heat stress due to increased temperature is an agricultural problem in many areas in the world. Transitory or constantly high temperatures cause an array of physiological changes in plants such as photosynthesis dark respiration, membrane stability, and mitochondrial respiration, which affect plant growth and development and may lead to a drastic reduction in economic yield (Almeselmani and others 2006; Wahid and others 2007). Due to an excessive excitation of the respiratory and photosynthetic electron transport systems, high temperature increases the production of reactive oxygen species (ROS) such as superoxide and hydrogen peroxide (Mishra and Singhal 1992; Dat and others 1998), which are toxic to plants.

The development of methods to induce stress tolerance in plants is vital and still receives considerable attention. Approaches taken to develop stress-tolerant plants include genetic engineering (McKersie and others 1988), breeding (Vettakkorumakankav and others 1999), in vitro selection, and the use of growth regulators (Senaratna and others 2000). Paclobutrazol (PBZ) [(2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl) pentan-3-ol], a member of the triazole plant growth inhibitor group, is a broad-spectrum gibberellin biosynthesis inhibitor (Davis and Curry 1991). Triazoles have both fungitoxic and plant-growth regulatory effects (Webb and Fletcher 1996). They also increase tolerance of various plant species to biotic and abiotic stresses, including fungal pathogens, drought, air pollutants, and low- and high-temperature stress (Fletcher and others 2000). Therefore, the triazoles have been characterized as plant multiprotectants (Fletcher and others 2000). There is evidence that components of the photosynthetic system and molecules that regulate antioxidant activity constitute some of the principal targets for triazole-induced enhancement of stress tolerance (Kraus and Fletcher 1994). PBZ has been reported to confer protection to plants experiencing stress by reducing oxidative damage via elevation of antioxidants or reducing the

B. Baninasab (✉) · C. Ghobadi
Department of Horticulture, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran
e-mail: bbanin@cc.iut.ac.ir

activity of oxidative enzymes (Lin and others 2006). Kraus and Fletcher (1994) reported that the damage caused by heat stress was due in part to increased generation of active oxygen, and that PBZ protected wheat plants by maintaining increased antioxidant enzyme activity. PBZ normally is applied as a foliar spray or growth medium drench (Still and Pill 2004).

High temperature in summer is common in the regions of Iran where cucumbers are grown extensively; it affects the growth of plants and reduces the yields of marketable fruit. According to our knowledge, there are no reports on the effects of exogenous PBZ enhancing cucumber tolerance to heat stress. Therefore, the objectives of this work were to determine (1) which method of PBZ application would be more effective and (2) the optimum concentration of PBZ that could provide the best protection against high-temperature stress.

Materials and Methods

Plant Material and Cultural Practice

Cucumber seeds (*Cucumis sativus* L. cv Super Aston; Asgrow Vegetable Seeds, Saticoy, CA, USA) were used in this experiment. Seeds were disinfested in a 1% (v/v, active ingredient) sodium hypochlorite solution for 10 min to eliminate possible seed-borne micro-organisms, then rinsed for 1 min under running water prior to drying for 30 min at room temperature.

Eighty seeds were soaked in PBZ at 0 (control), 25, 50, or 75 mg l^{-1} (20 seeds treated at each concentration) for 24 h at room temperature (23 ± 2°C), after which the seeds were washed and immediately planted. Five seeds were sown in 1.5-l plastic pots filled with a 1:1:1 (v/v) mixture of fine sand, leaf mould, and soil. One week after emergence, the emerged ones were thinned to three seedlings per pot. The pots were then transferred to a greenhouse with an average temperature of 25.5/19.5°C (day/night) and natural light.

Before sowing, a second batch of seeds was soaked in distilled water under the same conditions as above to obtain 60 seedlings for the foliar application of PBZ at the concentration above; these seedlings were also raised in a greenhouse under the same conditions. When the seedlings had two true leaves (35 days after sowing), they were sprayed with 0 (control), 25, 50, or 75 mg l^{-1} PBZ until both sides of all the leaves were completely wet.

The layout was a 2 × 4 factorial experiment in a completely randomized design with four replications and three plants per replication (plastic pot).

Stress Imposition

One week after the foliar PBZ application and 5 weeks after the seed treatment, all seedlings (seed soaked + foliar spray) were exposed to heat stress by transferring plants to a growth chamber and increasing the temperature from 25 to 40°C over 1 h, and holding at 40°C for 4 h before returning them to the greenhouse (Still and Pill 2004). The stress period was repeated for 5 days. All plants were assessed 24 h after the end of high-temperature stress to determine the extent of injury (Shi and others 2006), and data were collected.

Relative Leaf Chlorophyll Content (RLCC)

Relative total leaf chlorophyll content of the youngest fully expanded leaf of all three plants per replicate was determined by using a chlorophyll content meter (Hansatech Instrument Ltd, King's Lynn, Norfolk, UK). Relative leaf chlorophyll content is measured using dual-wavelength optical absorbance (620- and 940-nm wavelength) from leaf samples. The chlorophyll meter readings were used as relative values for chlorophyll content (Kapotis and others 2003).

Electrolyte Leakage

Electrolyte leakage was used to assess membrane permeability. This procedure was based on that of Lutts and others (1996). Electrolyte leakage was measured using an electrical conductivity meter (CC-501, Elmetron, Zabrze, Poland). Five leaf discs of one randomly chosen plant per replicate sample were taken from the youngest fully expanded leaf. After three washes with distilled water to remove surface contamination, the five leaf discs were then placed in a test tube containing 10 ml distilled water. The samples were incubated at room temperature, on a shaker, for 24 h. The electrical conductivity (EC) of the solution (EC_1) was then read after incubation. The same samples were then placed in a boiling water bath for 20 min and a second EC reading (EC_2) was taken after cooling the solution to room temperature. Electrolyte leakage was then calculated as EC_1/EC_2 and expressed as a percentage.

Chlorophyll Fluorescence

Measurements of the maximum efficiency of photosystem II photochemistry (F_v/F_m) were performed using a plant efficiency analyzer (Hansatech Instrument Ltd.) after 30 min of dark adaptation. The F_v/F_m ratio was calculated as:

$F_v/F_m = (F_m - F_0)/F_m$

where F_m and F_0 are the maximum and basal fluorescence yields, respectively, of dark-adapted leaves (Abreu and Munne-Bosch 2008).

Proline Determination

Proline was determined according to the method described by Bates and others (1973). Seedlings (0.5 g of fresh leaf material) were homogenized with 10 ml of 3% aqueous sulfosalicylic acid and filtered through Whatman's No. 2 filter paper. Two milliliters of filtrate was mixed with 2 ml of acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100°C. The reaction mixture was extracted with 4 ml of toluene and the chromophore containing toluene was aspirated and cooled to room temperature; then the absorbance was measured at 520 nm with a Shimadzu UV 160A spectrometer (Shimadzu Corporation, Kyoto, Japan). Appropriate proline standards (Sigma Chemical Co., St. Louis, MO, USA) were included for calculation of proline in the sample.

Shoot and Root Characteristics

The shoots of seedlings were cut off at the level of the substrate and their fresh weights (FWs) were recorded. The roots of seedlings were washed carefully under running tap water to remove all traces of growth medium, then dried with paper towels to remove the surface water and their FWs were also recorded. The shoots and roots were dried at 80°C for 72 h and their dry weights (DWs) were determined.

Statistical Analysis

Data were analyzed for significant differences using a factorial analysis of variance, with PBZ application method and PBZ concentration as the main factors. Statistical analysis was performed using the MSTATC v1.4 statistical software program (Michigan State University, East Lansing, MI, USA), and means were compared using the least significant differences (LSD) test at $P = 0.05$.

Results and Discussion

Our results showed that RLCC was affected by both the method of PBZ application and PBZ concentration (Table 1). Plants treated by the seed soak method showed greater RLCC compared with plants treated via the foliar spray method (Table 2). All concentrations of PBZ increased RLCC significantly compared with the control. However, 25 and 50 mg l^{-1} PBZ concentrations were more effective. There was a significant interaction between the method of PBZ application and PBZ concentration. There was an increase in the RLCC when 50 mg l^{-1} PBZ was applied by the seed-soaking method (Tables 1, 2). This result is in agreement with those reported by Zhou and Leul (1999) in winter rape; they found that triazole-treated plants had less of a decrease in chlorophyll concentrations than the non-treated plants following heat stress. The higher RLCC of PBZ-treated cucumber leaves may be related to its influence on the endogenous cytokinin content. Fletcher and others (1982) showed that plants treated with PBZ synthesize more cytokinin that in turn enhances chloroplast differentiation and chlorophyll biosynthesis and prevents chlorophyll degradation. The use of GA biosynthesis inhibitors increased cytokinin content in *Dianthus caryophyllus* (Sebastian and others 2002).

An increase in electrical conductivity indicates elevated leakiness of ions due to a loss of membrane integrity. This is an inherent feature of plants that are exposed to stresses such as extreme heat (Kraus and Fletcher 1994). The method of PBZ application and PBZ concentration, but not the interaction between the two, significantly affected electrolyte leakage (Table 1). Cucumber seedlings treated by the seed-soaking method showed significantly lower percentages of leaf electrolyte leakage than those treated by

Table 1 Analysis of variance (ANOVA) of application method (A), PBZ concentration (P), and their interaction (A × P) for physiological and morphological parameters in cucumber seedlings subjected to high-temperature stress

Source of variance	df	*P* values							
		RLCC	EC_1/EC_2	F_v/F_m	PC	SFW	SDW	RFW	RDW
A	1	0.002	0.001	0.037	0.012	0.0001	0.0001	0.0001	0.0001
P	3	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
A × P	3	0.027	0.82	0.51	0.031	0.0001	0.0001	0.0001	0.0001
Error	24	–	–	–	–	–	–	–	–

RLCC relative leaf chlorophyll content, *EC_1/EC_2* leaf electrolyte leakage, *F_v/F_m* chlorophyll florescence ratio, *PC* proline content, *SFW* shoot fresh weight, *SDW* shoot dry weight, *RFW* root fresh weight, *RDW* root dry weight

Table 2 Effect of PBZ concentration and method of application on physiological parameters of cucumber seedlings subjected to high-temperature stress

	PBZ (mg l^{-1})	RLCC	EC_1/EC_2 (%)	F_v/F_m	Proline content
Application method					
Seed soak	0	2.80e*	40.26a	0.71cd	161.7c
	25	6.23ab	25.71c	0.77ab	193.5b
	50	6.52a	16.24de	0.77ab	215.7a
	75	5.71bc	14.51e	0.79a	208.9a
Foliar spray	0	3.09e	43.62a	0.70d	166.4c
	25	5.44c	30.26b	0.73b–d	191.4b
	50	5.94a–c	17.45de	0.75a–c	193.0b
	75	4.79d	19.55d	0.78a	192.0b
Means for application method					
Seed soak		5.32a	24.18b	0.76a	194.9a
Foliar spray		4.82b	27.72a	0.74b	185.7b
Means for PBZ concentration					
	0	2.95c	41.94a	0.71c	164.0c
	25	5.84a	27.99b	0.75b	192.5b
	50	6.23a	16.84c	0.76ab	204.4a
	75	5.25b	17.03c	0.78a	200.6ab

* Values followed by the same letter within the column are not significantly different at $P < 0.05$

RLCC relative leaf chlorophyll content, EC_1/EC_2 electrolyte leakage, F_v/F_m chlorophyll fluorescence ratio, *PC* proline content (μg g^{-1} FW)

foliar spray application (mean values of 24.18 vs. 27.72%, respectively) (Table 2). The application of PBZ significantly decreased electrolyte leakage in leaf discs, with the largest decrease in leaf electrolyte leakage measured when 50 mg l^{-1} PBZ was applied (Table 2). In agreement with the present results, seedlings of winter rape and tomato treated with uniconazole and PBZ, respectively, had much less electrolyte leakage than the control seedlings following high-temperature stress (Zhou and Leul 1999; Still and Pill 2004). Data also exist showing that triazole compounds could reduce leaf electrolyte leakage and malondialdehyde (MDA) accumulation and consequently decrease heat-induced lipid peroxidation of rape plants. MDA is a product of lipid peroxidation that damages enzymes and plant membranes, and enhanced electrolyte leakage was considered a symptom of stress-induced membrane damage (Senaratna and others 1988).

The chlorophyll fluorescence ratio (F_v/F_m) was used as a noninvasive method to determine the functional state of the photosynthetic machinery. The F_v/F_m ratio was significantly affected by the method of PBZ application and PBZ concentration, but not their interaction (Table 1). PBZ treatment significantly increased the F_v/F_m ratio in cucumber leaves. The highest F_v/F_m ratio was observed in the leaves of seedlings treated with 75 mg l^{-1} PBZ (0.78), which was approximately 10% higher than in the controls (Table 2). Seedlings treated by the seed-soaking method showed a significantly greater F_v/F_m ratio compared with those treated by the foliar spray method (mean values of 0.76 vs. 0.74, respectively) (Table 2). The F_v/F_m ratio in PBZ-treated plants was higher than in control plants during heat stress, indicating that PBZ reduced the heat-induced photoinhibition by protecting photosystem II. The chlorophyll fluorescence ratio (F_v/F_m) is correlated with the efficiency of leaf photosynthesis. A decline in this ratio is an indicator of photoinhibitory damage caused by incident photon flux density when plants are subjected to a wide range of environmental stresses (Bjorkman and Demmig 1987). The maintenance of F_v/F_m in PBZ-treated plants under high temperature stress has been observed in previous studies (Mahoney and others 1998).

The method of PBZ application and PBZ concentration significantly affected the proline content of leaves (Table 1). The PBZ treatment significantly increased proline content. The highest proline content was obtained from leaves of the seedlings treated with 50 mg l^{-1} PBZ (204.4 μg g^{-1} FW), which was 24.6% higher than that of the control (Table 2). Plants that had been treated by the seed-soaking method had higher proline content than those treated by the foliar spray method (mean values of 194.9 vs. 185.7 μg g^{-1} FW, respectively). There was a significant interaction between the method of PBZ application and PBZ concentration. There was an increase in the proline content when 50 mg l^{-1} PBZ was applied by the seed-soaking method (Tables 1, 2). This result is consistent with that reported by Saleh (2007), who showed that PBZ treatment increased the proline content of mung bean seedlings grown under stress conditions. Our results were consistent with previous reports on the effect of triazole compounds on corn (Pinhero and Fletcher 1994), peanut seedlings (Muthukumarasamy and Panneerselvam 1997), and winter rape (Zhou and Leul 1998). Proline has multiple

Table 3 Effect of PBZ and method of application on shoot fresh and dry weights (mg) and root fresh and dry weights (mg) of cucumber seedlings subjected to high-temperature stress

	PBZ (mg l^{-1})	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight
Application method					
Seed soak	0	4,353e*	337e	420g	33f
	25	7,846bc	623bc	751c	60c
	50	8,516a	682a	969a	75a
	75	7,946b	633b	881b	69b
Foliar spray	0	4,754e	357e	405g	32f
	25	6,559d	521	631f	50e
	50	6,227d	506d	682e	53d
	75	7,464c	595c	715d	53d
Means for application method					
Seed soak		7,165a	569a	755a	59a
Foliar spray		6,248b	495b	608b	47b
Means for PBZ concentration					
	0	4,549c	347c	412d	32d
	25	7,202b	572b	691c	55c
	50	7,371b	594ab	826a	64a
	75	7,705a	614a	798b	61b

* Values followed by the same letter within the column are not significantly different at $P < 0.05$

functions in plants, including regulation of osmotic pressure, protection of membrane integrity, stabilization of enzymes/proteins, maintenance of appropriate NADP+/NADPH ratios, and as a scavenger of free radicals (Hare and Cress 1997).The accumulation of proline under stress conditions has been correlated with increased stress tolerance in plants (Misra and Gupta 2005). Our results showed a substantial increase in proline levels following PBZ treatment, which might be attributed to the strategies adapted by plants to cope with stress conditions.

PBZ treatment significantly increased shoot FWs and DWs (Tables 1, 3). However, 75 mg l^{-1} PBZ was more effective than the other concentrations used (Table 3). These data showed that PBZ could effectively improve the growth of high-temperature-stressed cucumber seedlings. Seedlings treated by the seed-soaking method showed significantly higher shoot FWs and DWs than those treated by foliar spraying (Table 3). A significant interaction between PBZ concentration and the method of PBZ application was observed for shoot FWs and DWs (Table 1). Applying 50 mg l^{-1} PBZ by seed-soaking increased shoot FWs and DWs by approximately 2.0-fold compared to controls. On the other hand, 75 mg l^{-1} PBZ applied as a foliar spray resulted in increases in shoot FWs and DWs of 57.0 and 66.7% over controls, respectively (Table 3). From the RLCC results, it can be inferred that the higher chlorophyll contents in leaves, leading to higher photosynthesis, might have increased shoot FWs and DWs in the PBZ-treated seedlings (Table 2). In agreement with our results, Pinhero and Fletcher (1994) observed that PBZ improved the growth rate of corn seedlings subjected to high-temperature stress. Stimulation of shoot growth under chilling stress by PBZ was reported by Baninasab (2009) in watermelon seedlings.

PBZ application also significantly increased both the FWs and DWs of roots, with the largest increase occurring when 50 mg l^{-1} PBZ was applied (Tables 1, 3). Seedlings treated by the seed-soaking method showed significantly greater root FWs and DWs than seedlings treated by the foliar spray method (Table 3). There was a significant interaction between the method of PBZ application and PBZ concentration. The greatest increase in root FWs and DWs occurred when 50 mg l^{-1} PBZ was applied by the seed-soaking method (Tables 1, 3). Stimulation of root growth under salt stress by PBZ was reported by Abdul Jaleel and others (2008) in *Catharathus roseus*. Increased root growth by PBZ is also associated with increased levels of endogenous cytokinin (Fletcher and Arnold 1986).

In this study, the relatedness between and among various physiological indices (for example, RLCC, leaf electrolyte leakage, F_v/F_m, and proline content) and various morphological parameters (for example, shoot and root FWs and DWs) in cucumber seedlings subjected to heat stress was analyzed (Table 4). Our results showed that significant correlations exist between and among these physiological indices and morphological parameters. These correlations suggest that various morphological parameters are positively correlated with RLCC, F_v/F_m ratio, and proline content but negatively correlated with leaf electrolyte leakage. Therefore, we conclude that PBZ ameliorated the negative effects of injury caused by heat stress by preventing decreases in RLCC and the F_v/F_m ratio, by

Table 4 Correlation between physiological and morphological parameters of cucumber seedlings subjected to high-temperature stress

	RLCC	EC_1/EC_2	F_v/F_m	PC	SFW	SDW	RFW	RDW
RLCC	1	−0.80**	0.63**	0.82**	0.85**	0.86**	0.85**	0.86**
EC_1/EC_2	–	1	−0.77**	−0.80**	−0.82**	−0.84**	−0.89**	−0.87**
F_v/F_m	–	–	1	0.64**	0.71**	0.72**	0.74**	0.72**
PC	–	–	–	1	0.87**	0.88**	0.87**	0.87**
SFW	–	–	–	–	1	0.99**	0.94**	0.93**
SDW	–	–	–	–	–	1	0.95**	0.94**
RFW	–	–	–	–	–	–	1	0.99**
RDW	–	–	–	–	–	–	–	1

Pearson correlation coefficient

** Significant at $P < 0.01$

RLCC relative leaf chlorophyll content, *EC_1/EC_2* leaf electrolyte leakage, F_v/F_m chlorophyll florescence ratio, *PC* proline content, *SFW* shoot fresh weight, *SDW* shoot dry weight, *RFW* root fresh weight, *RDW* root dry weight

increasing leaf proline content, and inhibiting increases in leaf electrolyte leakage. Higher RLCC may have caused higher shoot and root FWs and DWs because all these variables are closely related to photosynthetic capacity.

In summary, the response of cucumber seedlings to PBZ treatments outlined in this study suggests that the application of PBZ could partially protect cucumber seedlings against high-temperature stress. PBZ applied either through seed soaking or through foliar spray was most effective at 50 and 75 mg l^{-1} in providing high-temperature tolerance. However, because the seed-soaking method is simpler and more convenient it, would be a more desirable method for PBZ application. The fact that PBZ, readily available, could be used to prevent crop losses due to high-temperature stress may have a significant practical application.

Acknowledgments We thank Mr. M. Baghbanha and Mr. R. Mohammadi for their valuable help with this experiment. This research was supported by the Isfahan University of Technology.

References

Abdul Jaleel C, Gopi R, Kishorekumar A, Manivannan P, Sankar B, Panneerselvam R (2008) Interactive effects of triadimefon and salt stress on antioxidative status and ajmalicine accumulation in *Catharanthus roseus*. Acta Physiol Plant 30:287–292

Abreu ME, Munne-Bosch S (2008) Salicylic acid may be involved in the regulation of drought-induced leaf senescence in perennials: a case study in field-grown *Salvia officinalis* L. plants. Environ Exp Bot 64:105–112

Almeselmani M, Deshmukh PS, Sairam RK, Kushwaha SR, Singh TP (2006) Protective role of antioxidant enzymes under high temperatures stress. Plant Sci 171:382–388

Baninasab B (2009) Amelioration of chilling stress by paclobutrazol in watermelon seedlings. Sci Hortic 121:144–148

Bates LE, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207

Bjorkman O, Demmig B (1987) Photon yield of oxygen evolution and chlorophyll fluorescence characteristics at 77°K among vascular plants of diverse origin. Planta 170:489–504

Dat JF, Lopez-Delgado H, Foyer CH, Scott IM (1998) Parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. Plant Physiol 116:1351–1357

Davis TD, Curry EA (1991) Chemical regulation of vegetative growth. Crit Rev Plant Sci 10:151–188

Fletcher RA, Arnold V (1986) Stimulation of cytokinins and chlorophyll synthesis in cucumber cotyledons by triadimefon. Physiol Plant 66:197–201

Fletcher RA, Kallidumbil V, Steele P (1982) An improved bioassay for cytokinins using cucumber cotyledons. Plant Physiol 69:675–677

Fletcher RA, Gilley A, Sankhla N, Davis TD (2000) Triazoles as plant growth regulators and stress protectants. Hortic Rev 24:55–137

Hare PD, Cress WA (1997) Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regul 21:79–102

Kapotis G, Zervoudakis G, Veltsistas T, Salahas G (2003) Comparison of chlorophyll meter readings with leaf chlorophyll concentration in *Amaranthus vlitus*: correlation with physiological processes. Russ J Plant Physiol 50:395–397

Kraus TE, Fletcher RA (1994) Paclobutrazol protects wheat seedlings from heat and paraquat injury. Is detoxification of active oxygen involved? Plant Cell Physiol 35:45–52

Lin KH, Pai FH, Hwang SY, LO HF (2006) Pre-treating paclobutrazol enhanced chilling tolerance of sweetpotato. Plant Growth Regul 49:249–262

Lutts S, Kinet JM, Bouharmont J (1996) NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Ann Bot 78:389–398

Mahoney SR, Ghosh S, Peirson D, Dumbroff EB (1998) Paclobutrazol affects the resistance of black spruce to high light and thermal stress. Tree Physiol 18:121–127

McKersie BD, Senaratna T, Walker MA, Kendall EJ, Hetherington PR (1988) Deterioration of membranes during aging in plants: evidence for free radical mediation. In: Nooden LD, Leopold AC (eds) Senescence and aging in plants. Academic Press, London, pp 442–464

Mishra RK, Singhal GS (1992) Function of photosynthetic apparatus of intact wheat leaves under high light and heat stress and its relationship with peroxidation of thylakoids. Plant Physiol 98:1–6

Misra N, Gupta AK (2005) Effect of salt stress on proline metabolism in two high yielding genotypes of green gram. Plant Sci 169: 331–339

Muthukumarasamy N, Panneerselvam R (1997) Amelioration of NaCl stress by triadimefon in peanut seedlings. Plant Growth Regul 22:157–162

Pinhero R, Fletcher R (1994) Paclobutrazol and ancymidol protect corn seedlings from higher and low temperature stresses. Plant Growth Regul 15:47–53

Saleh AAH (2007) Amelioration of chilling injuries in mung bean (*Vigna radiata* L.) seedlings by paclobutrazol, abscisic acid and hydrogen peroxide. Am J Plant Physiol 2:318–332

Sebastian B, Alberto G, Emilio AC, Jose AF, Juan AF (2002) Growth, development and colour response of potted *Dianthus caryophyllus* cv. Mondriaan to paclobutrazol treatment. Sci Hortic 94:371–377

Senaratna T, Mackay CE, McKersie BD, Fletcher RA (1988) Uniconazole-induced chilling tolerance in tomato and its relationship to antioxidant content. J Plant Physiol 133:56–61

Senaratna T, Touchell D, Bunn E, Dixon K (2000) Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. Plant Growth Regul 30:157–161

Shi Q, Bao Z, Zhu Z, Ying Q, Qian Q (2006) Effects of differences of salicylic acid on heat tolerance, chlorophyll florescence, and antioxidant enzyme activity in seedlings of *Cucumis sativa* L. Plant Growth Regul 48:127–135

Still JR, Pill WG (2004) Growth and stress tolerance of tomato seedlings (*Lycopersicon esculentum* Mill.) in response to seed treatment with paclobutrazol. J Hortic Sci Biotechnol 79: 197–203

Vettakkorumakankav NN, Falk D, Saxena P, Fletcher RA (1999) A crucial role for gibberellins in stress protection of plants. Plant Cell Physiol 40:542–548

Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. Environ Exper Bot 61:199–223

Webb JA, Fletcher RA (1996) Paclobutrazol protects wheat seedlings from injury due to waterlogging. Plant Growth Regul 16: 201–206

Zhou W, Leul M (1998) Uniconazole-induced alleviation of freezing injury in relation to changes in hormonal balance, enzyme activities and lipid peroxidation in winter rape. Plant Growth Regul 26:41–47

Zhou W, Leul M (1999) Uniconazole-induced tolerance of rape plants to heat stress in relation to changes in hormonal levels, enzyme activities and lipid peroxidation. Plant Growth Regul 27:99–104