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EFFECT OF PACLOBUTRAZOL ON WHEAT SEEDLINGS UNDER LOW TEMPERATURE STRESS

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Summary. Wheat (*Triticum aestivum* L., cv. Beloslava) seeds were imbibed for 24 h in a water solutions containing 0, 25 and 50 mg.l^{-1} of paclobutrazol. The seedlings were grown as a substrate culture under controlled climatic conditions. Seven-day-old plants were exposed to low temperature stress by placing them in a cold room at a temperature of 2±1ºC for 10 days – the first phase of hardening, -4±1ºC for 3 days – the second phase of hardening and freezing, -10±1ºC for 1 day – the third phase of hardening and freezing. After exposure to stress, the seedlings were returned to a climatic chamber with controlled climatic conditions. Under stress conditions the growth rates of the PBZ-treated seedlings measured by height, fresh and dry weights were greater than the control. Low temperature stress (LTS) induced lipid peroxidation and increased peroxidase activity. It was also found that LTS decreased the chlorophyll and carotenoid levels. A decrease in fluorescence ratio (F_v/F_m) indicated lower photosynthetic efficiency. These deteriorative symptoms in the control seedlings were ameliorated by the PBZ treatment. Based on the results of triazoles studies, we presume that the stress protection caused by PBZ probably contributes to some extent to the enhanced activity of the free-radical scavenging systems.

Key words: low temperature stress, paclobutrazol, protection, wheat

Abbreviations: ABA – abscisic acid, F_v/F_m – variable to maximal fluorescence ratio, LTS – low temperature stress, MDA – malondialdehyde, PA – peroxidase activity, PAR – photosynthetic activity radiation, PBZ – paclobutrazol, PFD – photon flux density, TBA – thiobarbituric acid

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Introduction

Cultivated plants are often subjected to different types of environmental stress during their growth in the field, which could result in reduction of their yield. Low temperature stress induces considerable changes in biochemistry and physiology of plants. The main known disturbances are reduction of chlorophyll levels, blocking of photosynthetic electron transport, reduction of photosynthetic enzyme activity and stomatal conductance. Cold stress can affect photosynthesis rates by inhibiting the light and dark reactions of photosynthesis (Berry and Björkman, 1980, Graham and Patterson, 1982, Öquist, 1983, Huner et al., 1989, Kacperska, 1989, Li, 1989, Katterman, 1990).

The triazoles are the largest and most important group of systemic compounds, developed in 1960s for the control of fungal diseases in plants and animals. Commercial triazole derivatives (such as paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazolyl)-pentan-3-ol]) have been recommended for use as either fungicides or plant growth regulators (Fletcher et al., 1986). Characteristic of morphological and anatomical effects of the triazole include reduced shoot elongation and trichome length, increased epicuticular wax, larger chloroplasts and increased root growth (Fletcher and Hofstra, 1988, Gao et al., 1988, Grossmann, 1990). Biochemical effects of the triazole include detoxification of active oxygen (Upadhyaya et al., 1989, Kraus and Fletcher, 1994), increased levels of proline (Mackay et al., 1990), antioxidants (Senaratna et al., 1988) and chlorophyll content (Fletcher and Hofstra, 1988).

More recently, it was found that triazole compounds are able to protect plants from the environmental stress conditions, e.g. drought, extreme temperature, gaseous sulphur dioxide and fungal infections (Wang, 1985, Fletcher and Hofstra, 1988, Davis and Curry, 1991, Pinhero and Fletcher, 1994). The triazole mediated stress protection is often explained in terms of hormonal changes such as an increase in cytokinins, a transient rise in ABA and a decrease in ethylene (Asare-Boamah and Fletcher, 1986, Fletcher and Hofstra, 1988, Mackay et al., 1990). Enhanced chilling tolerance in triazole-treated cucumber (Upadhyaya et al., 1989) and tomato (Senaratna et al., 1988) was associated with increased antioxidant enzyme concentrations. In treated tomatoes, apart from the increase in the antioxidants α-tocopherol and ascorbate, free fatty acids were higher and there was a reduction in the loss of membrane phospholipids, as compared to the untreated controls. Triazole-induced tolerance to low temperature stress has been associated with increased levels of endogenous ABA (Fletcher et al., 2000), which has been reported to trigger the genetic processes for hardening (Zeevaart and Creelman, 1988). In field studies, winter survival of peas and cereal crops (Davis et al., 1988) and resistance to frost damage in corn and tomatoes (Fletcher and Kraus, 1995) were enhanced by triazoles.

In spite of the increasing number of studies on PBZ-induced stress protection, a little is known about its effect on the physiological state of low temperature-stressed

plants. Thus, the aim of the present study was to determine whether the paclobutrazol (PBZ) could protect wheat seedlings from injuries caused by low temperature stress.

Materials and methods

Plant growth and low temperature treatment

Wheat (*Triticum aestivum* L., cv. Beloslava) seeds were imbibed for 24 h in a water solution containing 0, 25 and 50 mg.l^{-1} of paclobutrazol at 20±1ºC. The seedlings were grown as a substrate culture in a Knop nutrient solution, under controlled climatic conditions: by irradiance of 150 µmol.m^{-2}.s^{-1} (PAR), day/night temperatures of 18±1/14±1ºC, 16-h photoperiod and 70% relative humidity. Seven-day-old plants were exposed to low temperature stress by placing them in a cold room at a temperature of +2±1ºC, in the dark, for 10 days – the first phase of hardening, -4±1ºC, in the dark, for 3 days – the second phase of hardening and freezing, -10±1ºC, darkness, for 1 day – the third phase of hardening and freezing (method of artificial freezing of seedlings according to Zagnanska and Rybka, 1988). After exposure to stress, the seedlings were left for unfreezing at a temperature of +2ºC, in the dark, for 16 hours. After that, for 24 h, they were exposed to irradiance of 30 µmol m^{-2}.s^{-1}. Then the plants were brought back to the climatic chamber, where they have recovered for 10 days.

Growth parameters

The fresh and dry weight of the shoots were measured immediately prior to and 10 days after the stress treatment. The dry weights were measured by drying the shoot and root at 75ºC, to give a constant weight.

Photosynthetic pigments

Total chlorophyll and carotenoids in the second leaf of the wheat seedlings were extracted in 80% water acetone. The pigments were determined spectrophotometrically after centrifugation of extract at 3000 rpm for 5 min (Welschen and Bergkotte, 1994) and calculated according to the Lichtenthaler and Wellburn (1983) formulae.

Fluorescence ratio

Variable (F_v) to maximal (F_m) fluorescence ratio was measured by the Pulse Modulated Fluorimeter MINI-PAM (H. Walz, Germany) after 30 min of dark adaption in the second leaf of the wheat seedlings before stress and 10 days after stress. The values of the ground (F_0) and maximal fluorescence (F_m) were recorded at measuring light 0.15 µmol.m^{-2}.s^{-1} and saturating light pulse (SLP) – 5000 µmol.m^{-2}.s^{-1}, respectively, for 0.8 s.

Determination of the malondialdehyde (MDA) content

For the measurement of lipid peroxidation in roots, the thiobarbituric acid (TBA) test, which determines malondialdehyde (MDA) level was applied (Heath and Packer, 1968). The amount of MDA-TBA complex (red pigment) was calculated from the extinction coefficient 155 $mM^{-1}.cm^{-1}$.

Peroxidase activity

Peroxidase activity (PA; EC 1.11.1.7) was determinated according to Herzog and Fahimi (1973). The material was homogenized with 0.05 M Tris-glycine buffer, pH 8.3, containing 17% sucrose (m/v). One relative PA unit (U) is equal to $\Delta A\ 470\ g^{-1}.min^{-1}$.

Two independent experiments, each with 3–5 replications per treatment were conducted. The results showed similar tendencies. Data from one representative experiment are given in this work. The significance of the differences between control and each treatment was analyzed by Student's *t*-criterion.

Results and discussion

Immediately prior to the stress treatment the wheat seedlings exhibited typical characteristics of triazole treatment (Fletcher and Hofstra, 1988, Davis and Curry, 1991, Webb and Fletcher, 1996). There was a significant reduction in length (44–49%) and fresh weight of shoots (15–23%), and an increase in root production, leading to an increased root to shoot ratio (21–32%). (Table 1). The leaves appeared thicker and greener with higher chlorophyll and carotenoid levels, although these values were not significantly different from the controls. The changes in chlorophyll fluorescence ratio F_v/F_m were relatively slight. PBZ-treated seedlings were characterized with higher activity of peroxidase – typical for triazole treatment (Davis et al., 1988).

It is known that low temperature stress alone can inhibit plant growth. Increase in growth rate such as shoot length, fresh and dry weights after the temperature stress was higher in PBZ-treated seedlings compared to the controls (Fig. 1.). The differences between the variants were significant and mathematically proven ($P<0.05$–0.01). It was established that paclobutrazol and ancymidol protect corn plants from chilling injury and a similar increase in plant growth parameters was observed (Pinhero and Fletcher, 1994).

An increase of MDA accumulation following low temperature stress was observed in both the control and the treated variants, i.e. a state of oxidative stress is induced related to membrane damage (Fig. 2.). The MDA content in PBZ-treated seedlings was reduced to 77–79% of its value in controls ($P<0.05$). According to Fletcher et al., (2000), inhibition of lipid peroxidation may be one of the mechanisms responsible

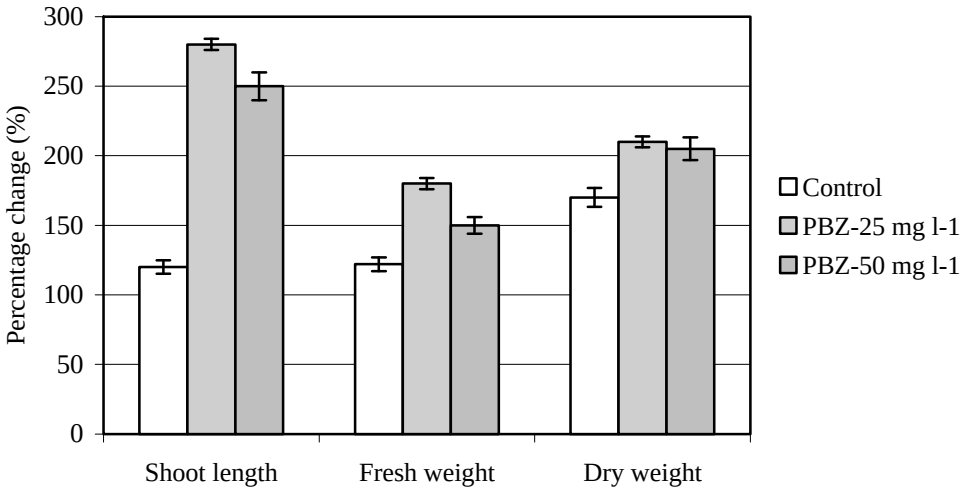

Fig.1. Percentage change in shoot length, fresh and dry weights of control and PBZ-treated wheat seedlings under low temperature stress followed by 10 days recovery. For initial values, see Table 1. Values are means ±SE (n=5)

for the anti-senescence effects of the triazoles. Lipid peroxidation mediated by activated toxic oxygen species should be accompanied by changes in activities of enzymes involved in oxygen metabolism (Cakmak and Horst, 1991). Peroxidase is one of the major systems for the enzymatic removal of H_2O_2 in plants. The increased activity

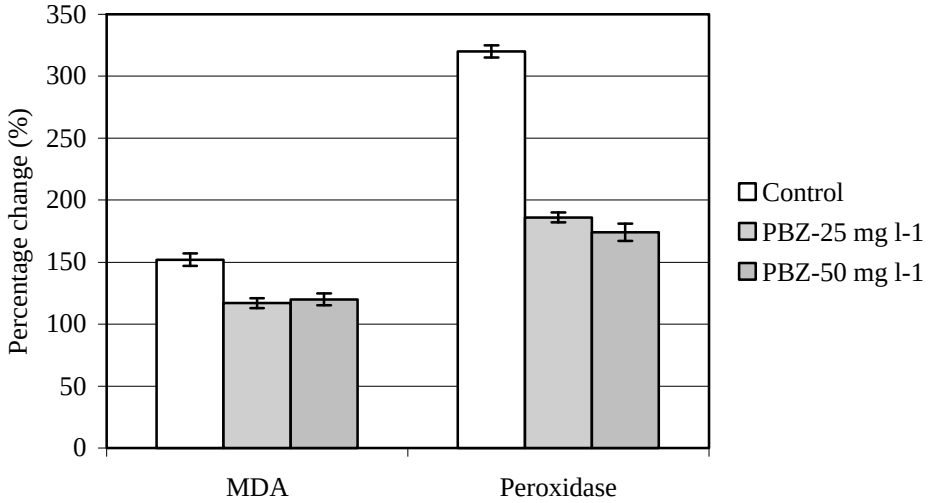

Fig. 2. Percentage change in malondialdehyde (MDA) level and peroxidase activity in control and PBZ-treated wheat roots under low temperature stress followed by 10 days recovery. For initial values, see Table 1. Values are means ±SE (n=5)

of peroxidase in plants suggests the protective role of the enzyme in different stress situations, such as acid rain (Velikova et al., 2000), Fe (Hendry et al., 1985) and Al toxicity (Cakmak and Horst, 1991). After exposure to stress, the peroxidase activity increased in both the control and treated seedlings (Fig. 2.). At the same time the differences between control and PBZ-treated plants were considerable. Treatment with PBZ led to a reduction of peroxidase activity amounting to 54–58% of the control ($P<0.01$). The higher activity of peroxidase in the PBZ-treated seedlings immediately prior to the stress treatment (Table 1) suggests, that paclobutrazol probably prepares the cell to meet and overcome stress by stabilizing membranes and forming a potential of higher antioxidant capacity.

Chlorosis of leaves is the first visual symptom of stress leading to senescence (Fletcher and Hofstra, 1988) and is associated with a concomitant decline in concentration of photosynthetic pigments (Fletcher and Hofstra, 1990). The leaves of control plants after low temperature stress were chlorotic and the photosynthetic pigments – chlorophylls and carotenoids markedly decreased ($P<0.01$). Paclobutrazol significantly reduced the severity of this damage (Fig. 3.). Ten days after stress, the PBZ treatment was 15–18% more effective than the control in preventing the decline in chlorophyll levels. It was established that paclobutrazol and ancymidol prevent the decline in total chlorophyll content in corn plants after exposure to chilling temperatures (Pinhero and

Table 1. Growth and some physiological parameters of seven-day-old wheat seedlings before the low temperature stress treatment. The seedlings were germinated in a controlled growth box at an irradiance of 150 μmol.m^{-2}.s^{-1} (PAR), a day/night temperatures of 18±1/14±1°C, a 16-h photoperiod and a relative air humidity of 70%. Means marked by * and ** were significantly different at P = 0.05 and P = 0.01 levels respectively.

Parameters	Control	PBZ – 25 mg.l^{-1}	PBZ – 50 mg.l^{-1}
Shoot length [cm]	6.20±0.10	3.50±0.12**	3.15±0.09**
Fresh wt of shoots [g plant^{-1}]	0.145±0.009	0.124±0.006*	0.112±0.008*
Dry wt of shoots [g plant^{-1}]	0.015±0.001	0.013±0.002	0.013±0.001
Fresh wt of roots [g plant^{-1}]	0.096±0.012	0.099±0.010	0.097±0.005
Dry wt of roots [g plant^{-1}]	0.010±0.002	0.011±0.002	0.010±0.002
Root/shoot ratio [fresh wt]	0.66±0.02	0.80±0.01*	0.87±0.02*
Total chlorophyll (a + b) [mg.g^{-1} dry wt]	9.20±0.57	10.29±0.59	10.87±0.62
Carotenoids [mg g^{-1} dry wt]	2.63±0.20	3.25±0.24	3.40±0.39
Fluorescence ratio (F_v/F_m)	0.808±0.03	0.810±0.05	0.817±0.05
Peroxidase activity [U g^{-1} FW]	1245±28	1375±32*	1540±34*
Lipid peroxidation [nmol MDA g^{-1} FW]	10.07±0.42	9.82±0.24	9.59±0.36

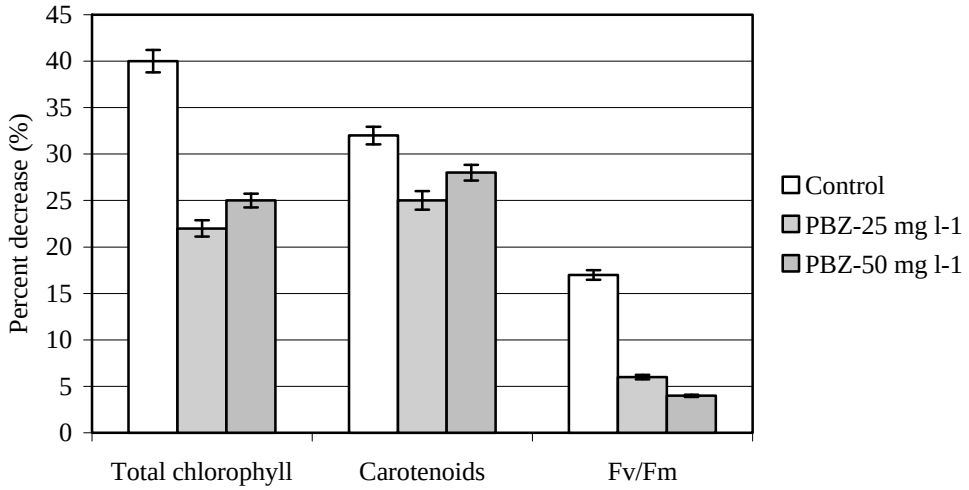

Fig. 3. Percent decrease in total chlorophyll and carotenoid levels, and variable (F_v) to maximal (F_m) fluorescence ratio in control and PBZ-treated wheat seedlings under low temperature stress followed by 10 days recovery. For initial values, see Table 1. Values are means ± SE (n=5).

Fletcher, 1994). Carotenoid levels decreased in both the control and the treated seedlings although the decrease in the treated plants was less than the controls. Similar results were obtained with PBZ when wheat plants were subjected to waterlogging (Webb and Fletcher, 1996).

The chlorophyll fluorescence ratio F_v/F_m is correlated with the efficiency of leaf photosynthesis and a decline in this ratio is a good indicator of photoinhibitory damage caused by incident PFD when plants are subjected to a wide range of environmental stresses (Bjorkman and Demming, 1987). A fluorescence ratio of 0.8 is indicative of healthy leaf tissue capable of maximum photosynthesis (Webb and Fletcher, 1996). This value was obtained for leaves from both control and PBZ-treated seedlings before low temperature stress (Table 1). After exposure to stress, the chlorophyll fluorescence ratio differed between control and PBZ-treated wheat plants (Fig. 3.). The decrease in the treated seedlings was from 4 to 6% while the control – exhibited a decrease of 17% during the same period. Thus, the plants treated with PBZ were found to be from 11 to 13% more efficient photosynthetically. The maintenance of relatively high fluorescence ratio in PBZ-treated plants under temperature stress has been observed in previous studies (Pinhero and Fletcher, 1994).

It has been reported that several environmental factors such as drought, low and high temperature can cause an excess highly of toxic oxygen-free radicals (Scandalios, 1993). Some of the free radical scavenging enzymes are reported to increase in wheat (Kraus and Fletcher, 1994), corn (Pinhero and Fletcher, 1994) and cucumber (Upadhyaya et al., 1989) plants after triazole treatments and their activities are conserved even after exposure to extreme temperature. The triazole compounds enhance the free

radical scavenging capacity of treated plants including the levels of carotenoids, ascorbate, superoxide dismutase and ascorbate peroxidase (Senaratna et al., 1988, Kraus et al., 1995). In this study we suggest that the protection caused by paclobutrazol was due to a similar mechanism of enhanced free-radical scavenging systems.

References

Asare-Boamah, N., R. Fletcher, 1986. Protection of bean seedlings against heat and chilling injury by triadimefon. Physiol Plant., 67, 353–358.

Berry, J., O. Björkman, 1980. Photosynthetic response and adaptation to temperature in higher plants. Annu. Rev. Plant Physiol., 31, 491–543.

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

Cakmak, W., J. Horst, 1991. Effects of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root of soybean (Glycine max). Physiol. Plant., 83, 463–468.

Davis, T., E. Curry, 1991. Chemical regulation of vegetative growth. Critic Rev. Plant Sci., 10, 151–188.

Davis, T., G. Steffens, N. Sankhla, 1988. Triazole plant growth regulators. Hort. Rev., 10, 63–105.

Fletcher, R., A. Gilley, T. Davis, N. Sankhla, 2000. Triazoles as plant growth regulators and stress protectants. Hort. Rev., 24, 55–138.

Fletcher, R., G. Hofstra, 1988. Triazoles as potential plant protectants. In: Sterol Synthesis Inhibitors in Plant Protection. Eds D. Berg, M. Plempel, Cambridge, Ellis Horwood Ltd, 321–331.

Fletcher, R., G. Hofstra, 1990. Improvement of uniconazole-induced protection in wheat seedlings. J. Plant Growth Regul., 9, 207–212.

Fletcher, R., G. Hofstra, J. Gao, 1986. Comparative fungitoxic and plant growth regulating properties of triazole derivatives. Plant and Cell Physiol., 27, 367-371.

Fletcher, R., T. Kraus, 1995. Triazoles: Protecting plants from environmental stress. In: Agrifoot Research, Ed. R. Meerveld, Ontario, Queens Printer, 18, 15–19.

Gao, J., G. Hofstra, R. Fletcher, 1988. Anatomical changes induced by triazoles in wheat seedlings. Can. J. Bot., 66, 1178–1185.

Graham, D., B. Patterson, 1982. Responses of plants to low, nonfreezing temperatures: proteins, metabolism and acclimation. Annu. Rev. Plant Physiol., 33, 347–372.

Grossmann, K., 1990. Plant growth retardants as tools in physiological research. Physiol. Plant., 78, 640–648.

Heath, R., L. Packer, 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys., 125, 189–198.

Hendry, G., K. Brocklebank, 1985. Iron-induced oxygen radical metabolism in waterlogged plants. New Phytol., 101, 199–206.

Herzog, V., H. Fahimi, 1973. A new sensitive colorimetric assay for peroxidase using 3,3´-diaminobenzidine as hydrogen donor. Anal. Biochem., 55, 554–562.

Huner, N., J. Williams, E. Maissan, E. Myscich, M. Krol, A. Laroche, J. Singh, 1989. Low temperature-induced decrease in trans-Δ^3-hexadecenoic acid content is correlated with freezing tolerance in cereals. Plant Physiol., 89, 144–150.

Kacperska, A., 1989. Metabolic consequences of low temperature stress in chilling-intensitive plants. In: Low temperature stress physiology in crops, Ed. P. H. Li. CRC Press Inc., Boca Raton, Florida, 27–40.

Katterman, F., 1990. Environmental Injury to Plants. Academic Press, New York.

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

Kraus, T., B. McKersie, R. Fletcher, 1995. Paclobutrazol-induced tolerance of wheat leaves to paraquat may involve increased antioxidant enzyme activity. J. Plant Physiol., 145, 570–576.

Li, P., 1989. Temperature Stress Physiology in Crops. CRC Press, Boca Raton, FL.

Lichtenthaler, H., A. Wellburn, 1983. Determination of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. Biochem. Soc. Trans., 603, 591–592.

Mackay, C., J. Hall, G. Hofstra, R. Fletcher, 1990. Uniconazole-induced changes in abscisic acid, total amino acids and proline in *Phaseolus vulgaris*. Pesti. Biochem. Physiol., 37, 74–82.

Öquist, G., 1983. Effects of low temperature on photosynthesis. Plant Cell Environ., 6, 281–300.

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

Scandalios, J., 1993. Oxygen stress and superoxide dismutases. Plant Physiol., 101, 7–12.

Senaratna, T., C. Mackay, B. McKersie, R. Fletcher, 1988. Uniconazole-induced chilling tolerance in tomato and its relationship to antioxidant content. J. Plant Physiol., 133, 56–61.

Upadhyaya, A., T. Davis, R. Walser, A. Galbraith, N. Sankhla, 1989. Uniconazole-induced alleviation of low temperature damage in relation to antioxidant activity. Hort. Sci., 24, 955–957.

Wang, C., 1985. Modification of chilling susceptibility in seedlings of cucumber and zucchini squash by the bioregulator paclobutrazol. Sci. Hort., 26, 293–298.

Webb, J., R. Fletcher, 1996. Paclobutrazol protects wheat seedlings from injury due to waterlogging. Plant Growth Regul. 18, 201–206.

Welschen, R., M. Bergkotte, 1994. Ecophysiology. Handbook of methods. Department of Plant Ecology and Evolutionary Biology, The Netherlands.

Velikova, V., I. Yordanov, A. Edreva, 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. Plant Sci., 151, 59–66.

Zagnanska, B., Z. Rybka, 1984. Laboratory method of frost resistance estimation of Triticale Biul. IHAR, 155, 51–55.

Zeevaart, J., R. Creelman, 1988. Metabolism and physiology of abscisic acid. Annu. Rev. Plant Physiol., 39, 439–475.