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BRIEF COMMUNICATION

Physiological response of paclobutrazol-treated triticale plants to water stress

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Abstract

Gas exchange in paclobutrazol-treated triticale plants during water stress and rehydration was studied. Seed treatments with the retardant (1 and 25 mg dm^{-3}) alleviate negative effect of PEG-induced water stress. Net photosynthetic rate, transpiration rate, stomatal conductance, relative water content, and leaf water potential were higher while peroxidase activity and free proline concentration were lower in the paclobutrazol-treated plants than in control plants. This confirmed our assumption that paclobutrazol possessed a protective effect against water stress.

Additional key words: leaf gas exchange, free proline, *Triticale hexaploide* Lar., water potential.

Water stress is one of the main environmental factors affecting negatively the plants. Investigations showed that natural plant growth regulators take part in the physiological response of plants subjected to water stress (Dicks 1980, Sairam *et al.* 1981, Driessch 1996, Gilley and Fletcher 1997). The applied natural or synthetic plant growth regulators decrease the stress effect (Blum 1988, Stoyanova and Yordanov 2000). In addition, they can induce changes in plant metabolism, which increase plant resistance to subsequent unfavourable factors (Blum 1988). Paclobutrazol (PBZ) [(2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1-H-1,2,4-triazole-1-yl)-pentane-3-ol] is one of plant growth retardants, belonging to the group of triazoles. It reduces the negative effect of water stress, contributing at the same time to the activation of stress-resistance mechanisms (Swietlik and Miller 1983, Latimer 1992, Driessch 1996). The retardant exerts a positive effect on plants under both waterlogging (Webb and Fletcher 1996) and water deficit (Li *et al.* 1994, Gilley and Fletcher 1997).

Irrespective of the progress achieved in the elucidation of the protective role of paclobutrazol against water stress, there are still a number of problems to be solved. There is no information available on the integral physiological response of the treated plants to this kind of stress. The problem related to the mechanism by which the retardant exerts its protective effect is still only partially explained. That was the reason for conducting the present study.

Triticale (*Triticale hexaploide* Lar.) cv. Vihren was grown at an irradiance of 150 μmol m^{-2} s^{-1} (PAR), a 14-h photoperiod, a day/night temperature of 22 ± 2/15 ± 2 °C, and a relative air humidity of 65 %. The seeds were soaked in water or solutions of paclobutrazol (1 and 25 mg dm^{-3}) for 24 h. Then the seedlings were grown hydroponically in 500 cm^3 plastic pots with full-strength Knop solution. Ten-day-old plants were subjected to PEG-induced water stress by replacing the nutrient solution with 15 % polyethylene glycol-6000 (PEG-6000) solution for 24 h. During the recovery period, the plants, which had experienced stress, were grown in the basic nutrient solution. Analyses were made at the beginning of the stress (T_0), at its end (T_1) and 24 h after plant recovery (T_2).

Net photosynthetic rate, transpiration rate and stomatal conductance of the first fully developed intact leaves were determined using a portable infrared gas analyser *LCA-4 (Analytical Development Company*, Hoddesdon, England) with a *PLCB-4* chamber. Measurements were made under irradiance of 800 μmol m^{-2} s^{-1}

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Abbreviations: E - transpiration rate; g_s - stomatal conductance; PA - peroxidase activity, PBZ - paclobutrazol; PEG - polyethylene glycol; P_N - net photosynthetic rate; P_N/E - water use efficiency, RWC - relative water content; Ψ_l - leaf water potential.
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(PAR), temperature of 26 ± 2 °C, an external CO_2 concentration of 400 μmol mol^{-1} and a relative air humidity of 70 %. Leaf water potential (ψ_l) was measured using the pressure chamber *EL 540-305 (ELE-International*, Hemel Hempstead, England) (Turner 1988). To prevent water loss from the leaf, leaves were wrapped in plastic foil prior to excision and the inside of the pressure chamber was lined with wet filter paper. Relative water content (RWC) in leaves was determined according to Morgan (1986). The free proline content was determined by the method of Bates *et al.* (1973). Peroxidase activity (PA) was determined according to Chanda and Singh (1997). One relative PA unit (U) is equal to ΔA_{470} g^{-1} min^{-1}. All results were represented as means ± SE from at least three independent series of experiments (3 - 5 measurements each). The significant differences were determined by the Student's *t*-test.

At the beginning of the experiment (T_0) the paclobutrazol-treated plants were characterized by a higher net photosynthetic rate (P_N) and lower transpiration rate (E) and stomatal conductance (g_s) as compared to the untreated ones (Fig. 1). These data are similar to the results reported by Dicks (1980).

The PEG-induced water stress caused a significant inhibition of leaf gas exchange. After a 24-h stress (T_1) the P_N of untreated plants decreased by 38 %, E by 37 % and g_s by 35 %. In paclobutrazol-treated plants P_N, E and g_s inhibition was lower (20 - 21 %) for both concentrations used.

Stomatal closing is a well-known response of water-stressed plants aiming at limiting water loss. Moreover, the photosynthetic rate decreases mainly for the limited access of carbon dioxide to mesophyllic cells (Ort *et al.* 1994, Yordanov *et al.* 2000). P_N in the paclobutrazol-

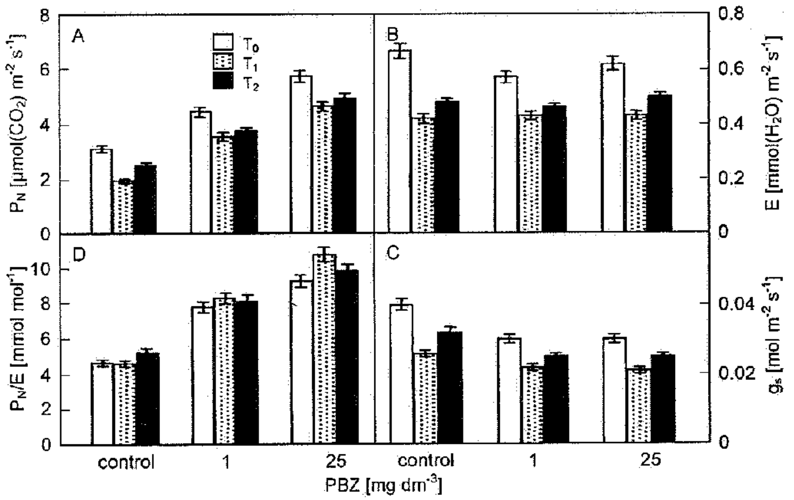

Fig. 1. Effect of PEG-induced water stress on leaf gas exchange in triticale plants treated by PBZ (1 or 25 mg dm^{-3}). P_N - net photosynthetic rate [μmol(CO_2) m^{-2} s^{-1}]; E - transpiration rate [mmol (H_2O) m^{-2} s^{-1}]; g_s - stomatal conductance [mol m^{-2} s^{-1}]; P_N/E - water use efficiency [mmol mol^{-1}]; T_0 - beginning of the experiment; T_1 - 24-h PEG-induced water stress; T_2 - 24-h recovery after PEG-induced water stress.

Table 1. Effect of PEG-induced water stress on water status of triticale plants treated with paclobutrazol. RWC - relative water content [%]; Ψ_l - leaf water potential [MPa]; proline [μmol g^{-1} f. m.]; PA - peroxidase activity [U]; T_0 - beginning of the experiment; T_1 - 24-h PEG-induced water stress; T_2 - 24-h recovery after PEG-induced water stress; * - $P < 0.05$; ** - $P < 0.01$.

Treatments	RWC	ψ_l	Proline	PA
T_0 control	96.30 ± 0.50	-0.21 ± 0.02	228.0 ± 6.7	1240 ± 22
T_1 control	73.19 ± 0.98**	-0.52 ± 0.03**	330.6 ± 12.1*	1390 ± 20*
T_2 control	88.60 ± 0.69*	-0.28 ± 0.01*	296.4 ± 10.1*	1190 ± 18
T_0 PBZ - 1 mg dm^{-3}	98.08 ± 0.58	-0.17 ± 0.01	168.0 ± 10.2	1460 ± 32
T_1 PBZ - 1 mg dm^{-3}	83.82 ± 0.94**	-0.30 ± 0.01*	208.3 ± 10.5*	1520 ± 26
T_2 PBZ - 1 mg dm^{-3}	93.18 ± 0.46*	-0.19 ± 0.01	194.9 ± 11.5	1490 ± 20
T_0 PBZ - 25 mg dm^{-3}	97.80 ± 0.68	-0.18 ± 0.03	144.0 ± 8.6	1510 ± 36
T_1 PBZ - 25 mg dm^{-3}	84.11 ± 0.62**	-0.35 ± 0.02*	169.9 ± 8.2	1540 ± 34
T_2 PBZ - 25 mg dm^{-3}	88.17 ± 0.46*	-0.20 ± 0.02	157.0 ± 9.5	1500 ± 24

treated plants was less suppressed, remaining higher than that in the untreated control plants, with lower g_s. This indicated that stomatal factors for limiting photosynthesis were overcome.

Water use efficiency (P_N/E) in the untreated plants was at the same level throughout the experiment. In the PBZ-treated plants, P_N/E increased in comparison with the beginning of the experiment, which was mainly due to the higher E inhibition, than that of P_N. Water use efficiency is usually closely related to plant resistance to unfavourable conditions. It could be assumed that the P_N/E increase in the treated plants proved the protective properties of paclobutrazol in terms of water stress. The results obtained by us showed good correspondence with those reported by Driessch (1996) for conifer seedlings. 24 h after the water stress, a tendency to restoring the leaf gas exchange parameters was established. P_N in the untreated plants reached 80 % of that at the beginning of the experiment. In the treated plants, this tendency was more strongly expressed and varied within the range of 84 - 86 %. The recovery of the other parameters followed the tendency typical for P_N.

The ability to keep high relative water content (RWC) is one of the main drought resistance factors. It allows maintenance of a number of processes such as stomatal opening, photosynthetic activity, growth, *etc.* (Gorecki and Grzesiuk 1978, Schonfeld *et al.* 1988, Marshall *et al.* 2000). At the beginning of the experiment RWC of the PBZ-treated plants was by about 2 % higher, than RWC in control plants, which was in agreement with the data obtained by Driessch (1996). After 24-h water stress, the RWC decreased by 24 % in the untreated, and by 14 % in the treated plants (Table 1). This confirmed the results obtained by Gniazdowska-Skoczek and Bandurska (1994). Similarly, the PBZ-treated plants had higher leaf water potential (ψ_l) throughout the period of water stress.

The maintenance of total plant water potential under conditions of water stress is achieved by reducing cellular osmotic potential in result of accumulation of osmotically active substances in the cytoplasm. The results presented showed that the proline content of the untreated plants, subjected to water stress, strongly increased (by 45 %). The paclobutrazol-treated plants accumulated less proline than the untreated ones. The changes in the proline content varied from 18 % to 24 % over the control.

It was proved that under stress conditions the activity of the antioxidative enzyme peroxidase increased (Tood 1972). Water stress increased peroxidase activity in the control by 12 % while in the treated plants the peroxidase activity increased slightly (Table 1). These data corresponded to those reported by Nash *et al.* (1982).

In the recovery period, the relative water content increased both in the untreated and the treated plants. After 24-h recovery, the proline content in the untreated plants was by 30 % higher than that at the beginning of the experiment. In the treated plants, these values varied between 9 and 16 %. After stopping the water stress treatment, the peroxidase activity reached a level, similar to that at the beginning of the experiment.

The seed treatment with paclobutrazol at concentrations of 1 and 25 mg dm^{-3} had a positive effect on the physiological status of triticale plants subjected to PEG-induced water stress. The tested parameters demonstrated that PBZ protected triticale plants from water stress. This confirmed the reports for other abiotic stresses (Fletcher and Hofstra 1988, Kraus and Fletcher 1994, Pinhero and Fletcher 1994).

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