The effect of soil drought and rehydration on growth and antioxidative activity in flag leaves of triticale

R. BĄCZEK-KWINTA*[1], W. FILEK*, S. GRZESIAK** and T. HURA**

*Plant Physiology Department, Agricultural University, Faculty of Agriculture and Economics, Podłużna 3, PL-30239 Kraków, Poland**
*Institute of Plant Physiology, Polish Academy of Sciences, Podłużna 3, PL-30239 Kraków, Poland***

Abstract

Changes in the activity of catalase and superoxide dismutase, together with growth parameters were investigated in flag leaves of four winter triticale cultivars. Water stress was applied during shooting (D_s) or heading (D_h) stage. Dry matter of the main shoot and tillers was similarly restricted by both drought periods. The length of the main shoot was more constrained by D_s, in contrast to the number of tillers, restricted strongly by D_h. The resumption of growth after rehydration was clearer in cvs. Bogo and Tewo, and hence these two cultivars were considered more drought-resistant than Presto and Ugo. Neither catalase nor superoxide dismutase activity was influenced by the term of the drought and the genotype.

Additional key words: catalase, superoxide dismutase, *Triticosecale* Wittmack, water stress.

Introduction

Soil drought is a very frequent environmental stress limiting crop production. Dehydration of plant tissues leads to inhibition of various physiological processes, and triggers an increased formation of the superoxide radical ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), which can attack membrane lipids and inactivate thiol-group-containing proteins. This overproduction is both a consequence of the Mehler reaction, providing a pathway for the removal of excess electrochemical energy determined by drought conditions (Biehler and Fock 1996), and, on the other hand, results from restricted antioxidative capacity modulated also by senescence (Sairam *et al.* 2003). Oxidative processes may intensify following rehydration (Sgherri *et al.* 1994) due to the intensification of metabolism. In consequence, the biomass and the final yield are restricted. Such losses may vary upon the physiological phase the drought occurs at.

In this study, we attempt to characterize the influence of soil drought – applied during shooting or heading – on some biomass parameters of various winter triticale cultivars. We also try to establish the involvement of the major two antioxidant enzymes in the adaptive processes of triticale to dehydration. The choice of these two antioxidants was based on their role in the defence system against oxidative stress. Superoxide dismutase (SOD) acts as a "first link" in the chain of reactions scavenging active species of oxygen (Scandalios 1993) during various environmental stresses. This enzyme disproportionates the superoxide radical $O_2^{\bullet -}$, and the toxic product of this reaction, H_2O_2, is then removed by catalase (CAT) in cytosol, mitochondria, peroxisomes and glyoxysomes (Prasad *et al.* 1994), and ascorbate peroxidase (APX) in chloroplasts, mitochondria, peroxisomes and cytosol (Asada 1992, Jimenez *et al.* 1997). CAT pool is more

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Abbreviations: CAT - catalase; C_h - control plants for drought applied during the heading stage; C_s - control plants for drought applied during the shooting stage; D_h - drought applied during the heading stage; D_s - drought applied during the shooting stage; R_h - recovery from the drought applied during the heading stage; R_s - recovery from the drought applied during the shooting stage; SOD - superoxide dismutase.
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[1] Corresponding author; fax: (+48) 12 425 33 20, e-mail: rrbaczek@cyf-kr.edu.pl

abundant in the cell than APX pool, and it was reported that bacterial catalase targeted to transgenic plants was able to remove H_2O_2 instead of chlorophyll APX isoforms, and to protect photosynthetic apparatus from oxidative stress (Miyagawa *et al*. 2000). Catalase activity assay is not expensive and, as the molecule susceptible to unfavourable conditions (Prasad *et al.* 1994a, Streb and Feierabend 1995, Skrudlik *et al.* 2000, Bączek-Kwinta and Kościelniak 2003), this enzyme may be considered as a good marker of stress.

Materials and methods

Winter triticale (*Triticosecale* Wittmack) cultivars popular in Poland: Bogo, Presto, Tewo and Ugo were planted in 7-kg pots with sand, peat and organic soil (3:1:1, v/v). Seedlings were vernalized for 56 d at 4 °C in growth cabinet. Preliminary vegetation was performed in air-conditioned greenhouse (~60 % relative humidity, temperature (day/night) 23/17 °C, 16-h photoperiod. Drought treatment (D, 14 d) was applied by withholding watering. In case of drought applied at the stage of shooting (D_s) the period of preliminary vegetation was 14 d, in case of drought applied during heading (D_h) was 5 weeks.

After both drought periods the soil was rehydrated for 14-d recovery (R_s and R_h). Samples were collected from control and stressed plants at all phases of the experiment.

Assays of antioxidant activity were made spectrophotometrically (*LKB Biochrom Ultrospec II*, Cambridge, UK) in dialysed crude extract from the flag leaf at room temperature (*ca*. 20 °C). Catalase (CAT, EC 1.11.1.6) activity was determined by monitoring the disappearance of H_2O_2 at 240 nm in a reaction mixture containing 50 mmol dm^{-3} phosphate buffer, pH 7.0, and 54 mmol dm^{-3} hydrogen peroxide (Aebi 1984). Superoxide dismutase activity (SOD, EC 1.15.1.1) was quantified by the McCord/Fridovich method (1969). One unit was defined as the amount of enzyme necessary for 50 % inhibition of cytochrome *c* reduction in a coupled system with xanthine and xanthine oxidase. Soluble proteins were determined according to Bradford (1976), using bovine serum albumin as a standard.

For dry mass quantitation, fresh mass of shoots was recorded, and then the tissue was dried at 75 °C for 3 d. All measurements were performed in 5 - 10 replicates. During experiments, observations were conducted to compare results of analyses with visual plants' status. The statistical significance of differences was evaluated by multiple analysis of variance in completely randomized design (*ANOVA*/*MANOVA*) accompanied by Duncan multiple range test.

Results

Dry matter of the main shoot was constrained to a similar degree by drought applied at as shooting or heading (Table 1). Bogo and Tewo cultivars were characterized by bigger dry mass increment following the recovery when compared to Presto and Ugo, irrespectively of the term of drought treatment. Dry matter accumulation in tillers was more decreased by dehydration than that in the main shoot, irrespectively to the drought term (Table 1) (tillers: a mean *ca*. 50 % in all cultivars, main shoots: 16 - 32 % of respective control). The elongation of the

Table 1. The effect of 3-week drought treatment applied at shooting (D_s) or heading (D_h) on the dry mass of shoots and tillers of four winter triticale cultivars: Bogo, Presto, Tewo and Ugo. C_s, C_h - plants of respective control (watered), R_s, R_h - plants after 14-d rewatering. Mean ± SE, n = 8 - 9. Means significantly different from the respective control are marked by different letters (P = 0.05).

Parameter	Cultivar	C_s	D_s	R_s	C_h	D_h	R_h
Shoot dry mass [g plant^{-1}]	Bogo	1.80 ± 0.11a	1.41 ± 0.05b	1.87 ± 0.06a	2.63 ± 0.12a	2.32 ± 0.12b	3.29 ± 0.16c
	Presto	1.57 ± 0.05a	1.29 ± 0.10b	1.38 ± 0.11a	2.22 ± 0.11a	1.87 ± 0.07b	2.26 ± 0.12a
	Tewo	1.43 ± 0.05a	1.12 ± 0.05b	1.60 ± 0.05a	2.07 ± 0.04a	1.93 ± 0.07b	2.71 ± 0.13c
	Ugo	1.37 ± 0.07a	1.02 ± 0.07b	1.15 ± 0.06b	2.19 ± 0.17a	1.58 ± 0.06b	2.10 ± 0.08b
Tiller dry mass [g plant^{-1}]	Bogo	1.43 ± 0.28a	0.78 ± 0.10b	1.71 ± 0.09a	1.90 ± 0.23a	0.80 ± 0.13b	2.06 ± 0.59a
	Presto	1.83 ± 0.26a	0.98 ± 0.09b	1.90 ± 0.13a	2.44 ± 0.38a	1.88 ± 0.17b	2.16 ± 0.27ab
	Tewo	1.54 ± 0.14a	0.70 ± 0.06b	1.57 ± 0.08a	2.10 ± 0.28a	1.29 ± 0.14b	1.82 ± 0.28ab
	Ugo	1.99 ± 0.27a	1.02 ± 0.10b	1.79 ± 0.14a	3.60 ± 0.52a	2.00 ± 0.09b	2.59 ± 0.16b

Table 2. The effect of 3-week drought treatment applied at shooting (D_s) or heading (D_h) on the length of the main shoot and the number of tillers of four winter triticale cultivars: Bogo, Presto, Tewo and Ugo. C_s,C_h - plants of respective control (watered), R_s, R_h - plants after 14-d rewatering. Mean ± SE, n = 8 - 9. Means significantly different from the respective control are marked by different letters (P = 0.05).

Parameter	Cultivar	C_s	D_s	R_s	C_h	D_h	R_h
Length of the main stem [cm]	Bogo	76.9 ± 1.16a	51.7 ± 0.88b	51.9 ± 1.34b	82.0 ± 1.62a	78.2 ± 2.19b	74.6 ± 1.65b
	Presto	87.4 ± 2.28a	54.8 ± 3.93b	54.8 ± 1.98b	96.3 ± 1.8a	87.1 ± 1.84b	86.4 ± 1.06b
	Tewo	59.3 ± 2.37ab	37.5 ± 1.86b	66.5 ± 0.59a	83.7 ± 0.88a	73.8 ± 2.20b	75.1 ± 1.11b
	Ugo	69.8 ± 4.19a	37.8 ± 1.95c	55.4 ± 2.07b	94.8 ± 2.57a	76.4 ± 1.61b	74.8 ± 1.69b
Number of tillers [plant^{-1}]	Bogo	5.78 ± 1.10ab	5.11 ± 0.93b	6.22 ± 0.66a	5.11 ± 0.78a	3.44 ± 0.52b	3.78 ± 0.67b
	Presto	5.67 ± 0.71a	5.22 ± 0.97b	6.11 ± 1.83a	5.22 ± 1.39a	3.22 ± 0.44b	3.33 ± 0.86b
	Tewo	5.89 ± 0.93a	5.00 ± 1.12b	6.44 ± 1.13a	3.77 ± 0.66a	3.33 ± 0.70b	3.33 ± 0.50b
	Ugo	5.22 ± 1.09a	5.11 ± 1.05a	7.44 ± 1.23b	6.44 ± 3.16a	3.22 ± 0.44b	3.00 ± 0.50b

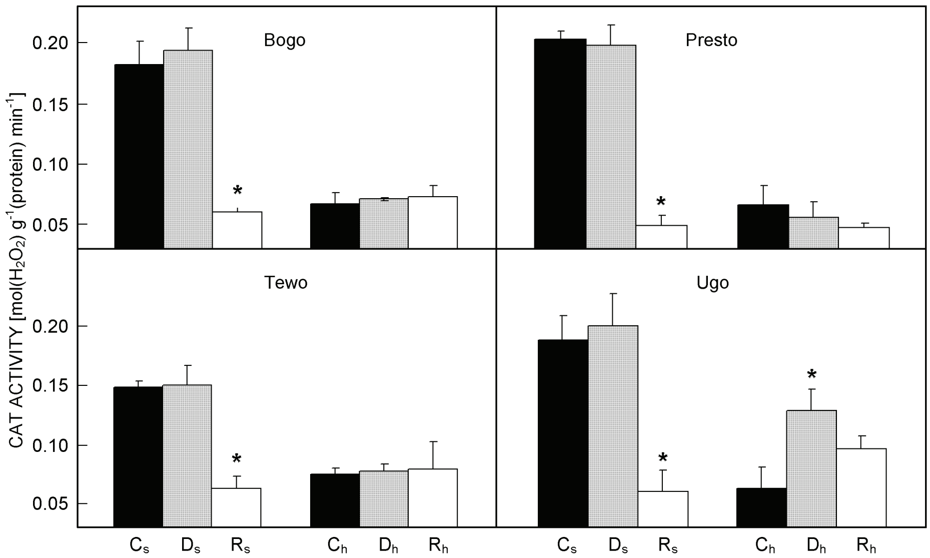

Fig. 1. The effect of 3-week drought treatment applied at shooting (D_s) or heading (D_h) on catalase (CAT) activity in the flag leaves of four winter triticale cultivars: Bogo, Presto, Tewo and Ugo. C_s, C_h - plants of respective control (watered), R_s, R_h - plants after 14-d rewatering. Mean ± SE, n = 5 - 8, * - values significantly different at P < 0.05 from corresponding ones measured on respective control.

main shoot (Table 2) drastically dropped following the dehydration applied in stage of shooting. Tewo and Ugo showed the resumption of growth during rehydration, in contrast to Bogo and Presto. Drought applied during heading only slightly decreased stem length, and this effect was prolonged to subsequent recovery.

D_s slightly restricted the number of tillers, although new tillers were formed after the recovery period (Table 2). At D_h treatment, the restriction in tillers production by plants was stronger than that under D_s, and came up to 50 % of control (in Presto and Ugo). This effect was prolonged to rewatering (R_h).

Activity of CAT in flag leaves was not influenced by D_s in all cultivars, and was strongly inhibited during the subsequent recovery (Fig. 1). CAT activity in cv. Presto showed the decreasing tendency in both drought and recovery treatments. The others cultivars were characterized by a slight increase (significant in Ugo only) in CAT activity following D_h and a slight decrease during the recovery from D_h. During the heading catalase activity was about 30 - 50 % of the values obtained from samples taken on shooting.

Multiple analysis of variance showed that activity of the SOD was neither influenced by the treatment nor related to the genotype (data not shown). However, the differences between means were significant in some cases (Fig. 2). Also the patterns of changes in SOD and CAT activities were similar in some cases only.

The pattern of protein content in leaves on which SOD and CAT activity was measured was usually different than enzymatic activity. The amount of protein strongly decreased following the drought applied during heading, and this effect was prolonged to the R_h recovery (Fig. 3). Protein pool was also genotypically differentiated: Presto and Ugo showed lower values than Bogo and Tewo.

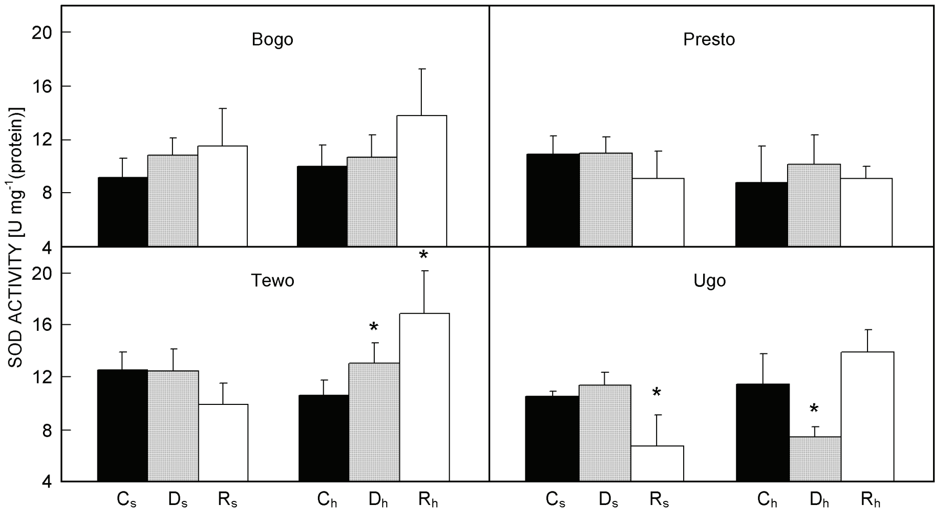

Fig. 2. The effect of 3-week drought treatment applied at shooting (D_s) or heading (D_h) on superoxide dismutase (SOD) activity in the flag leaves of four winter triticale cultivars: Bogo, Presto, Tewo and Ugo. C_s, C_h - plants of respective control (watered), R_s, R_h - plants after 14-d rewatering. Mean ± SE, $n = 5 - 8$, * - values significantly different at $P < 0.05$ from corresponding ones measured on respective control.

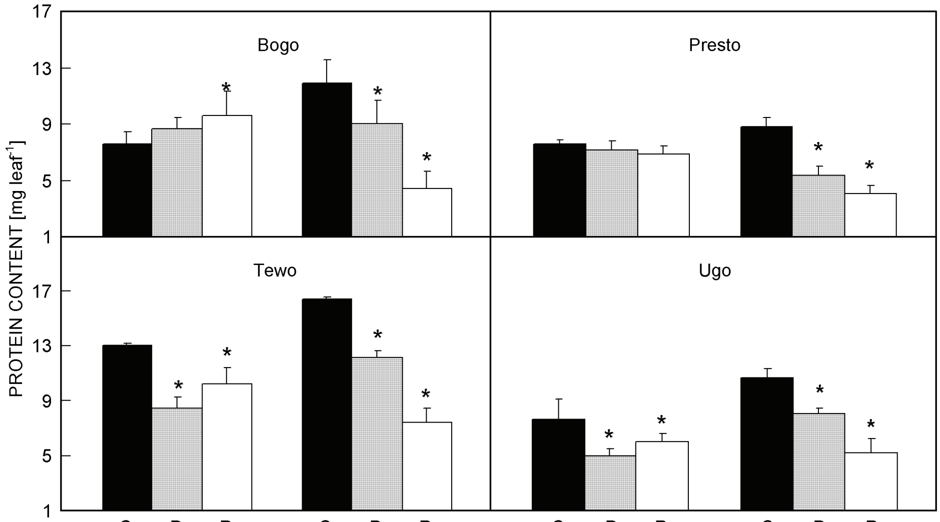

Fig. 3. The effect of 3-week drought treatment applied at shooting (D_s) or heading (D_h) on the soluble protein content in the flag leaves of four winter triticale cultivars: Bogo, Presto, Tewo and Ugo. C_s, C_h - plants of respective control (watered), R_s, R_h - plants after 14-d rewatering. Mean ± SE, $n = 5 - 8$, * - values significantly different at $P < 0.05$ from corresponding ones measured on respective control.

Discussion

The inhibition of growth, both of main shoots and tillers following drought, is the typical response to desiccation, because elongation processes are strictly dependent on pressure potential, elasticity and plasticity of cell walls and membranes (Schulze 1986). The rate of the recovery after rehydration may reflect the resistance of the cultivars to stress. Taking this into account, Bogo and Tewo cultivars seem to be more drought-resistant than Presto and Ugo (Tables 1 and 2).

There is also evidence to support the view that tissue dehydration enhances active oxygen formation (Biehler and Fock 1996, Menconi 1995, Tambussi *et al.* 2000), although it is difficult to state whether free radicals and H_2O_2 are directly responsible for the age-related changes, or whether they reflect degradation processes during plant senescence (Merzlyak and Hendry 1994, Sairam *et al.* 2003). Age-related processes may also occur to different extent in various cultivars and can be accelerated by drought. A drop in expenditure on energy for growth, and its allocation for specific protectants synthesis (*e.g.* SOD and CAT) and repairing processes, was postulated as a prerequisite for survival (Muller *et al.* 1997, Sairam *et al.* 1997, Sgherri *et al.* 1994, İnci *et al.* 1998).

In our studies, the patterns of SOD and CAT activities in flag leaves were not linked with the resistance of the cultivar to dehydration. Moreover, the changes in SOD activity were usually not accompanied with CAT activity (Fig. 1 and 2), and activities of both enzymes were also not clearly related with the protein content (Fig. 3). Moreover, CAT activity was strongly lowered during the ontogenesis (lower values at heading than shooting). Taken together, these facts suggest that ageing- and senescence-related processes dominated those activating antioxidant system during drought and/or rehydration (recovery), and the endogenous mechanism of regulation of SOD and CAT activity outweighed the drought stress-related changes (Hillman *et al.* 1994, Sairam *et al.* 2003).

It is also noteworthy that enzymatic activity results from production of new protein molecules and/or their activation, and both studied enzymes exist as isoforms localized in various organelles (Scandalios 1993, Prasad *et al.* 1994). For this reason one can suggest that they may be independently activated, although this might not play the crucial role in terms of long-term dehydration stress (14 d).

The continuous drop in protein content itself during heading (Fig. 3) may be explained by the optimization of the metabolism to environmental conditions, favouring photosynthates production and their translocation to heads (especially in the main stem), instead of protein synthesis. Alternative explanation is accelerating senescence of flag leaf.

Various patterns of antioxidative enzymes in leaves of cultivars studied in this experiment suggest differentiated susceptibility and response of triticale to drought-related oxidative stress. On the other hand, antioxidative response of flag leaves may not to be linked with dry mass accumulation and elongation process. For this reason the estimation of drought-sensitivity of cultivars of this species with antioxidative activity in flag leaves seems to be not likely.

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