The cell wall-bound phenolics as a biochemical indicator of soil drought resistance in winter triticale

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ABSTRACT

The leaf dehydration was accompanied by the highest increase in the content of cell wall-bound phenolics (CPh) during heading (148.3% C; % of control) and in course of drought applied twice during propagation and flowering (130.5% C) of triticale. A statistically significant correlations were obtained only for CPh and parameters of leaf water status and chlorophyll fluorescence. An increase in the content of free phenolics (FPh) under drought conditions was only noticed during the flowering (111.4% C) of plants. Drought application exhibited most spectacular decrease in the ratio of FPh to CPh during propagation (48.5% C) and heading (58.8% C). It was found that the cell wall increases at the expense of free phenolic compounds.

Keywords: photosynthetic apparatus; yield and stress metabolism; drought adaptation; reactive oxygen species; osmotic potential

Under leaf dehydration accompanied by light injuries, phenolic compounds can act as photoprotectors for the photosynthetic apparatus. In our previous studies concerning soil drought we have found that the activity of the photosynthetic apparatus, measured with the chlorophyll fluorescence method, positively correlated with the increase in the level of phenolic compounds in the leaf (Hura et al. 2007, 2009a,b).

Phenolics are also sources of electrons and protons for free radicals, including reactive oxygen species (ROS) (Lachman et al. 2011, 2012). It has been demonstrated that growing conditions, e.g. soil drought, can induce an increase of both phenolics concentration and antioxidant activity in plants (Hamouz et al. 2010, 2011). H_2O_2 is more stable in comparison with other ROS which makes it possible for it to cross cell membranes and penetrate cell structures and the apoplast (Blokhina et al. 2003). It has been found that H_2O_2 can be neutralised by peroxidases and phenolics present in the apoplast as well (Bienert et al. 2006). Phenoxyl radicals produced as a result of phenolics oxidation can be again reduced by monodehydroascorbate reductase, monodehydroascorbate or ascorbic acid, or can be included in the carbohydrate matrix building the structures of the cell wall (Sakihama et al. 2000). The saturation of the cell wall with phenolic compounds results in the cell wall being less stretchable, more compact and tight (Kamisaka et al. 1990), and, therefore, less permeable to water.

The first aim of the studies was to compare changes in the level of the pool of free and cell wall-bound phenolic compounds as potential indicators for the drought resistance in the key phases of growth of triticale. The second aim was to relate contents of both types of phenolic compounds to those physiological features (photosynthetic apparatus activity, water status, leaf biomass) which are indirectly dependent on the level of those types of phenolic compounds.

MATERIAL AND METHODS

Plant material and growth conditions. Measurements were carried out on winter triticale cv. Grenado (resistant to drought at all key growth phases: propagation (p), heading (h), flowering (f)). Seeds were obtained from Danko Plant Breeders Ltd., Choryń, Poland. The vernalization of triticale was performed in cool chambers during 7 weeks at +4°C (±1°C) and illumination of PPFD (photosynthetic photon flux density) of 200 μmol/m^2/s for the photoperiod 10 h light/14 h dark. After the vernalization, plants at the 3 leaves stage were transferred into greenhouse chambers. The cultivation of plants was conducted in Mitscherlich pots, of 5 dm^3 volume, filled with a mixture of soil, peat and sand (1:1:3, v/v/v). The air temperature in the greenhouse was 23/18°C (±2°C) day/night, and relative air humidity about 40%. Plants were additionally illuminated to ensure that the PPFD was about 250 μmol/m^2/s at the level of the flag leaf. The plants were irrigated with full-strength Hoagland's nutrient solution once per week.

Drought conditions. Plants were subjected to 3-week-long soil drought during the propagation (p), heading (h) and flowering (f) phases. Additionally, the additive influence of soil drought applied during the propagation and, then, flowering phase of the plant development was studied. For drought treatments soil humidity was maintained at about 30–35%. For control plants, soil humidity was maintained at the level of about 75%. Soil moisture was controlled gravimetrically every day during experiments.

Measurements. Measurements for all developmental stages were carried out after 3 weeks of drought. For the propagation phase all analyses were completed on the first (from the top), fully developed leaf (Zadoks/Feekes scales: 30/5). Analyses for the heading (Zadoks/Feekes scales: 58/10.5) and flowering (Zadoks scale: 69) stages were done on the flag leaf.

Relative water content (RWC), leaf osmotic potential. Analyses of RWC were done according to the formula:

$$RWC\ (\%) = (fw - dw)/(tw - dw) \times 100$$

Leaves were weighed (fw – fresh weight), and then soaked in distilled water for 24 h in darkness to estimate the turgid weight (tw). The sample was then oven-dried at 80°C for 24 h and weighed (dw – dry weight). The measurements for each growth stage were done in 7 replicates.

The osmotic potential was measured with a psychrometer HR 33T (WESCOR, Inc., Logan, USA). For analyses, six leaf discs were cut from the central part of the flag leaf. The filter paper discs soaked in the leaf sap, squeezed out of leaf discs with a syringe, were placed in the chambers and left for 30 min. The measurements for each growth stage were taken in the dew point mode in 5 replicates.

Chlorophyll fluorescence measurements. Chlorophyll fluorescence analyses were performed with a Handy PEA fluorometer (Hansatech Ltd. Kings Lynn, UK) according to Hura et al. (2012). The measurements for each treatment within growth stage were done in 5 replicates.

Phenolic compounds analysis. For measurements the total pool of free phenolic compounds (FPh) and cell wall-bound phenolics (CPh) the lyophilised material was homogenised in 80% ethanol. The total pool of free phenolic compounds was analysed with Folin-Ciocalteu method (Singleton and Rossi 1965). The absorbance was measured at 760 nm at spectrometer (Ultrospec II, Biochrom, Cambridge, UK). The chlorogenic acid was used as a standard.

Cell wall-bound phenolics were released from insoluble material by basic hydrolysis in 3 mol/L NaOH and incubated 24 h at 25°C. The residues were centrifuged and supernatant was recovered. Then, the supernatant was acidified with an equal volume of 3 mol/L HCl. The content of phenolic compounds released from the cell wall was determined with Singleton and Rossi (1965) method. The content of phenolic compounds, free and cell wall-bound, was counted per unit of surface, and the assays for each treatment were performed in 5 repetitions. For the analysis of free and cell wall-bound phenolic compounds, six discs of 5 mm diameter were cut from the central part of the flag leaf.

The analysis of leaves biomass. The analysis of the increment of the biomass of the flag leaf was carried out on the last day of soil drought. The measurement was carried out in 7 replicates.

Statistical analysis. Duncan's multiple range test at the 0.05 probability level was performed in order to determine the significance of differences between treatments and between growth stages within treatments. The correlation between measured parameters was analysed at a probability of $P < 0.05$.

RESULTS AND DISCUSSION

Phenolics and water status of triticale. The leaf dehydration was accompanied by increase in the content of cell wall-bound phenolics (Table 1)

Table 1. Effect of drought treatment applied during propagation (p), heading (h) and flowering (f) phases of triticale growth (p/f refers to drought conditions applied twice under propagation (p) and flowering (f)) on phenolics content expressed as percentage of control (%C) and ratio FPh (free phenolics)/CPh (cell wall-bound phenolics)

Phenolics		p	h	f	p/f
FPh	%C	72.7	69.6	111.4	100.8
CPh	%C	119.3	148.7	118.3	130.5
FPh/CPh	C	13.4 ± 0.4[a]	16.0 ± 1.0[b]	13.0 ± 0.6[a]	13.0 ± 0.6[a]
	D	6.5 ± 0.3[c]	9.4 ± 0.5[d]	11.1 ± 0.6[d]	10.8 ± 0.4[d]
	%C	48.5	58.8	85.4	83.1

Data are means ± SE of five replicates. Means indicated with the same letters are not statistically significant at the level of α = 0.05 in relation to differences between treatments and between growth stages within treatments. C – control; D – drought

for all analysed sets of soil drought (p: 119.3% C; h: 148.7% C; f: 118.3% C; p/f: 130.5% C). An increase in the content of free phenolics, under drought conditions, was only noticed during the flowering (111.4% C). During the propagation (72.7% C) and heading (69.6% C) phases a decrease in the content of free phenolics was observed which could be a result of their utilisation as components saturating the cell wall. Table 1 presents also data concerning the ratio of free phenolic compounds (FPh) to those bound to the cell wall (CPh). The results show that the pool of phenolic compounds saturating the cell wall increases at the expense of free phenolic compounds in the

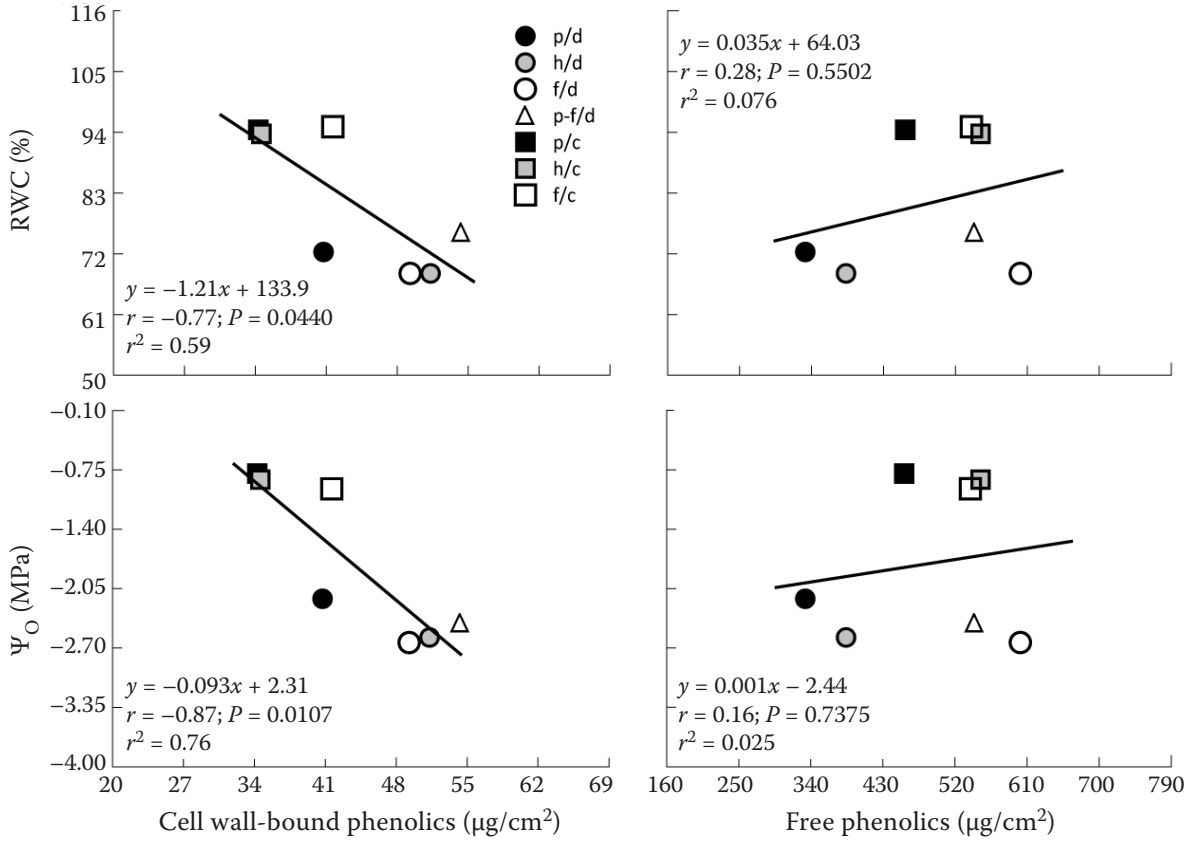

Figure 1. Correlations between leaf water status (RWC, Ψ_o) and phenolics content (free and cell-wall bound phenolics) including means for drought (d) and control (c) treatments under different growth stages of triticale (p – propagation; h – heading; f – flowering; p-f – drought applied twice during propagation and flowering period). Lines represent linear adjustment

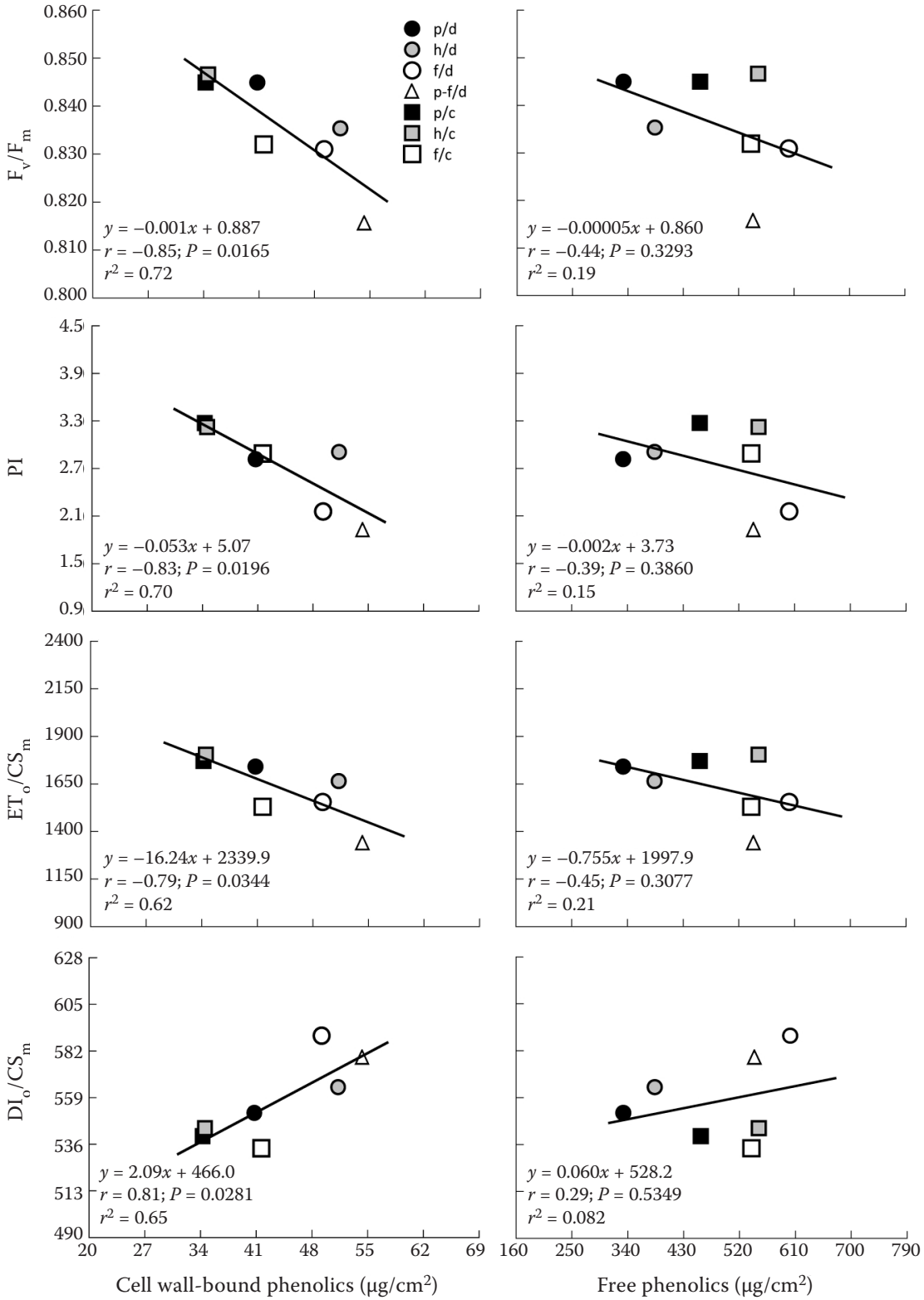

Figure 2. Correlations between chlorophyll fluorescence parameters (F_v/F_m – maximum photochemical efficiency; PI – overall performance index of PSII photochemistry; ET_o/CS_m – amount of energy used for the electron transport; DI_o/CS_m – energy amount dissipated from PSII) and phenolics content (free and cell-wall bound phenolics) including means for drought (d) and control (c) treatments under different growth stages of triticale (p – propagation; h – heading; f – flowering; p-f – drought applied twice during propagation and flowering period). Lines represent linear adjustment

cultivar resistant to drought. Moreover, a statistically significant correlation was obtained only for cell wall-bound phenolics and parameters of leaf hydration (RWC, Ψ_O) (Figure 1). A decrease in leaf hydration correlated with a higher content of phenolic compounds saturating the cell wall for all studied developmental phases of triticale. Therefore, it is possible to suggest that an increase in the content of phenolic compounds in the cell wall, contrary to free phenolic compounds, can be one of essential mechanisms of the adaptation to soil drought and a trustworthy indicator of the resistance to drought.

During studies which did not involve drought, it was shown that the presence of phenolics in the cell wall increased its tightness and the hydrophobicity of the apoplast (Bernards et al. 2000, Graca and Santos 2007). The observed during the presented studies increase in the content of phenolics in the cell wall and, therefore, its saturation with benzene rings, will increase the hydrophobic character of the cell wall. Consequently, it is possible to suppose that such an unfriendly for water apoplast environment will limit water transport from the metabolically active inside of the cell, capillary transport of water in the apoplast and finally the cuticular transpiration.

Relationships between phenolics and plant productivity. The drought stress increases the sensitivity of the photosynthetic apparatus to the photoinhibition of the photosynthesis process (Nogués and Baker 2000). Under water deficit conditions accompanied by the light stress, chlorophyll particles in the assimilative parenchyma absorb more light quanta than they can transform into the chemical energy of assimilates. As a result D_1 protein in PSII is damaged and such a photosystem is excluded from the photosynthetic electron transport till the damaged D_1 protein is synthesized again. Additionally, long-lasting photoinhibition generates reactive oxygen species (ROS) which cause free-radical-induced oxidation of lipids and photosynthetic pigments in the membranes of chloroplasts (Caspi et al. 2000). The xanthophyll cycle and the presence of enzymatic/non-enzymatic antioxidants in chloroplasts enable the adaptation of plants to unfavourable light conditions, but those adaptations may not be sufficient during long-lasting drought stress (Demming-Adams and Adams 1996).

Phenolic compounds can function as photoprotectors and limit the excitation of a chlorophyll particle in unfavourable for the photosynthetic apparatus conditions. Figure 2 shows correlations between the content of phenolic compounds and the parameters of chlorophyll fluorescence (F_v/F_m – maximum photochemical efficiency; ET_o/CS_m – amount of energy used for the electron transport; DI_o/CS_m – energy amount dissipated from PSII; PI – overall performance index of PSII photochemistry). Only for cell wall-bound phenolics were statistically significant correlations obtained which means that the functioning of the photosynthetic apparatus in different leaf hydration conditions (the control, drought) can be related to the content of cell wall-bound phenolics, which can additionally function as photoprotectors of the photosynthetic apparatus. The highest content of cell wall-bound phenolics was noticed in leaves of plants for which the functioning of the photosynthetic apparatus was the most impaired, i.e., in the p-f drought scheme and in the generative phase of development. During propagation the activity of the photosynthetic apparatus was least impaired and the content of cell wall-bound phenolics was significantly lower than the level noticed for the other periods of soil drought. In our previous publications we showed that the activity of the photosynthetic apparatus under leaf dehydration conditions correlates with the content of cell wall-bound phenolics (Hura et al. 2009a, 2011).

The increase in the saturation of the cell wall with phenolic compounds in drought conditions limited the increment of the dry mass of leaves in all studied developmental stages of triticale and the decrease in the dry mass of flag leaves significantly correlated with the increase in the content of cell wall-bound phenolics (Figure 3). The saturation of the cell wall with phenolics may result in the limited utilization of carbohydrates for the increment of leaf biomass (Fry 1979, 1982, Kamisaka et al. 1990, von Ropenack et al. 1998, Schultheiss et al. 2002). Cell wall phenolics may also be factors limiting the utilization of carbohydrates for building processes to benefit protective mechanisms (e.g. the synthesis of cell wall-bound phenolics). The limited utilization of carbohydrates in the process of the increment of leaf biomass may, in turn, increase the pool of carbohydrates acting as osmolites in the cell sap, resulting in the increase in its osmotic potential. Plants can maintain in this way the osmoregulation process which enables to retain water in tissues in order to guarantee a relevant turgor in leaf cells and stomatal apparatuses and, consequently, to run the photosynthesis

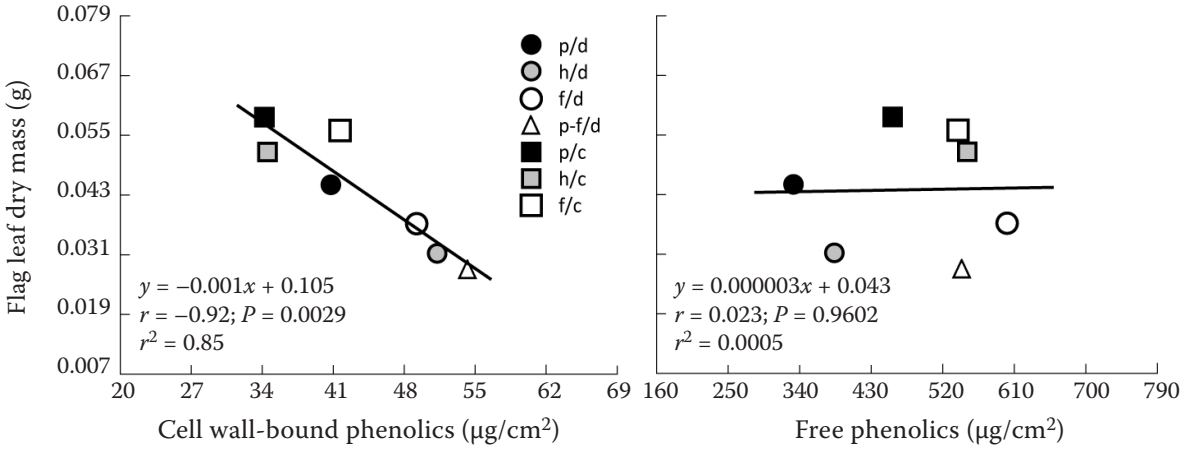

Figure 3. Correlations between flag leaf dry mass and phenolics content (free and cell-wall bound phenolics) including means for drought (d) and control (c) treatments under different growth stages of triticale (p – propagation; h – heading; f – flowering; p-f – drought applied twice during propagation and flowering period). Lines represent linear adjustment

(Hura et al. 2007). In our opinion, an increase in the content of cell wall-bound phenolics enables a better adaptation to drought conditions above all through the limitation of the lost of water which is the basis of, among others, the photosynthesis and, therefore, plant productivity.

For the resistant cultivar Grenado, the full regeneration of plants and crop production occurred after the end of drought and restoration of the optimal hydration. The drought did not cause permanent injuries to the photosynthetic apparatus what could be the results of the protective action of cell wall-bound phenolics. Only was a small, but significant, decrease in grain yield, in comparison with the control, noticed in the case of the double action of drought (p/f) (Figure 4).

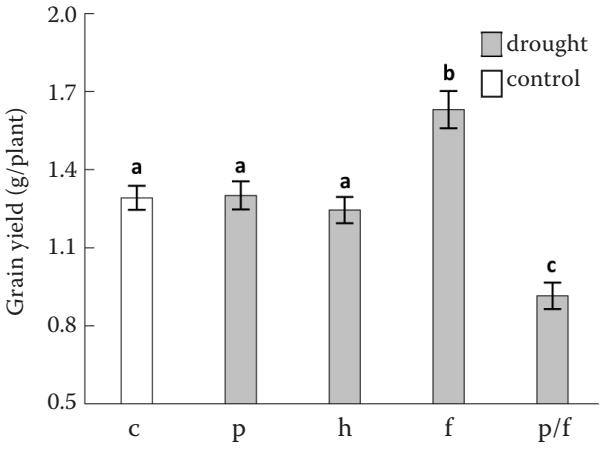

Figure 4. Effect of drought applied at different growth stages of triticale (p – propagation; h – heading; f – flowering, p/f - drought applied twice during propagation and flowering period) on grain yield. Data are means ± SE of eighteen replicates

Our results agree very well with the previous work focused on triticale adaptation to soil drought (Hura et al. 2012). The increase in the saturation of the cell wall with phenolic compounds during soil drought conditions may prevent water loss by metabolically active symplast. In our opinion, protective mechanisms involving the prevention of water loss by the cell decide about the effective adaptation to drought. The effectiveness of the action of cell wall-bound phenolics is not only related to their antioxidative or photoprotective activity, but above all to the blocking of water loss from the cell. As it was shown for the chosen resistant to drought cultivar Grenado and on the basis of our previous several-year-long studies we can conclude that the increase in cell wall-bound phenolics can be a basis of a stable adaptation to drought in all key phases of the growth of triticale. Hence, it is suggested that an increase in the content of phenolic compounds, bound to carbohydrates of the cell wall, can be a trustworthy indicator for the selection of cultivars resistant to drought.

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