Leaf Dehydration Induces Different Content of Phenolics and Ferulic Acid in Drought-Resistant and -Sensitive Genotypes of Spring Triticale

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Analyses of the total pool of phenolic compounds and ferulic acid, as a photoprotector of the photosynthetic apparatus, and the activity of L-phenylalanine ammonia-lyase (PAL), as a key enzyme in phenolics synthesis, were carried out. Measurements were performed on drought-resistant (CHD 12, CHD 147) and -sensitive (CHD 220, CHD 247) genotypes of spring triticale during flowering under increasing leaf water deficit. Additionally, the emission of blue and red fluorescence from leaves were estimated.

The exclusively in the resistant triticale genotype CHD 247 observed simultaneous increase in the content of ferulic acid and the total pool of phenolic compounds as a response to the leaf water deficit seems to be a promising biochemical indicator for a reliable selection of genotypes most resistant to drought stress. For the other genotypes, an increase in the total pool of phenolic compounds is accompanied by a decrease in the content of ferulic acid. An increase in the emission of red fluorescence, correlated with the high content of phenolic compounds, indicates the possibilities of these substances participating in the mechanisms of adaptation of the photosynthetic apparatus to water deficit in leaf tissues.

Key words: Triticale, Drought, Phenolics

Introduction

Although triticale is a species with a short history, its genetical features facilitate a quick biological progress in improving its production on a large scale through studies of its biochemical and physiological responses to drought stress. In our previous experiments it has been shown, that water deficit increases the emission of blue fluorescence from leaf tissues (Hura et al., 2006, 2007a). Phenolic compounds, mainly ferulic acid, belong due to their chemical structure to chemically active substances, and are the source of such fluorescence (Schweiger et al., 1996; Lichtenhalter and Schweiger, 1998). The activity of phenolics depend on the presence of a benzene ring, which interacts with UV light and visible radiation of short wavelength or can also be involved in the scavenging of reactive oxygen species (ROS) (Morales et al., 1996; Blokhina et al., 2002; Kikutaki et al., 2002; Solovchenko and Merzlyak, 2003). Through absorption phenolic compounds change the short wavelength, high energy and highly destructive radiation into the blue one with longer wavelength and consequently lower destructive potential (Bilger et al., 2001). It should be mentioned here, that both radiation and ROS most frequently cause injuries to the photosynthetic apparatus during drought stress (Barber and Andersson, 1991; Loggini et al., 1999; Nogués and Baker, 2000; Khanna-Chopra and Selote, 2007; Mohsenzadeh et al., 2006; Tahkokorpi et al., 2007). Therefore, the protection of the photosynthetic apparatus during drought stress may additionally depend on the high concentration of phenolic compounds in the leaf tissue (Sullivan and Teramura, 1990; Hura et al., 2007b). Moreover, it can be an additional biochemical factor used in the selection of drought-resistant genotypes. Studies concerning soil drought led by Hura et al. (2007a) showed a statistically significant increase in the content of ferulic acid of genotypes resistant to drought in comparison with sensitive ones.

Our study had two objectives. Firstly, to determine whether increasing the leaf water deficit...
provokes induction of the synthesis of phenolics, ferulic acid and activity of L-phenylalanine ammonia-lyase (PAL) in sensitive and resistant genotypes of spring triticale. Secondly, to study correlations between the content of phenolics, as photoprotectors and the emission of red fluorescence, as an indicator of injuries to the photosynthetic apparatus.

**Materials and Methods**

**Plant material**

Four strains of spring triticale (× *Triticosecale* Wittmack), obtained from Polish Breeding Station Choryn, were included in this study. Each of the chosen strains (CHD 12, CHD 147, CHD 220, CHD 247) had a different drought susceptibility index (DSI) calculated by Grzesiak et al. (2003) according to Fischer and Maurer (1978). The genotypes CHD 12 (DSI = 0.562) and CHD 147 (DSI = 0.553) were classified as drought-sensitive, while CHD 220 (DSI = 0.390) and CHD 247 (DSI = 0.391) as drought-resistant genotypes.

**Plant growth conditions**

Plants were grown in a greenhouse chamber at a temperature of (23/18 ± 2) °C day/night during a 16-h photoperiod, (40 ± 5)% relative humidity and at a photosynthetic photon flux density (PPFD) of 350 μmol m–2 s–1. Plants were grown in Mitscherlich pots filled with a mixture of soil, peat and sand (1:1:3, v/v/v).

Drought treatment was initiated by the withdrawal of the water supply during flowering. To control plants 70% of the FWC (field water capacity) and to water-stressed plants 30–35% of the FWC for 2 weeks were applied. The pots were weighed every day, and the amount of the water loss through transpiration was refilled to keep the appropriate weight of pots for each treatment. Drought treatment consisted of 15 pots within each genotype and in total there were 90 plants on one studied genotype. After 8, 11 and 14 d of stress, fragments from the middle part of the flag leaf were taken for all measurements. The plants were irrigated with a full-strength Hoagland’s nutrient solution once a week. The analyses of both physiological and biochemical parameters for each genotype were completed during flowering in flag leaves in five or seven replicates.

**Leaf water and osmotic potential**

The measurements were taken with a dew point microvoltmeter (model HR-33T with C-52 sample chambers; Wescor Inc., Logan, Utah, USA). To measure the water potential, the leaf discs (⌀ = 0.5 cm) were cut from the middle part of the expanded leaves, immediately placed inside the psychrometer chamber, and left to balance the temperature and water vapour equilibrium for 60 min before the water potential measurements.

Samples for osmotic potential measurements were taken as leaf discs (⌀ = 0.5 cm) from the middle part of leaves, stored in an Eppendorf tube, frozen in liquid nitrogen, and kept at –70 °C. Directly before this, the measurements of leaf samples were thawed at room temperature, and the sap from the leaf discs was extracted and quickly transferred to a leaf chamber for 30 min before the osmotic potential measurements.

**Spectrofluorescence**

Fluorescence spectra were measured using a Perkin-Elmer LS 50B spectrofluorometer (Perkin Elmer, Norwalk, CT, USA). The emission spectra of the red fluorescence were recorded between 650 and 800 nm. The leaf samples were excited at 450 nm and excitation and emission slit widths were set at 10 nm.

Emission spectra of the blue fluorescence were recorded between 380 and 600 nm. The excitation wavelength was set at 350 nm. The slit widths for excitation were set at 15 nm and for emission at 20 nm.

**L-Phenylalanine ammonia-lyase (PAL) activity**

The PAL activity was measured according to Peltonen and Karjalainen (1995). All procedures were carried out at +4 °C. The reaction mixture contained 2.5 ml of 0.2% L-phenylalanine solution in 50 mM Tris-HCl (2-amino-2-hydroxymethyl-propane-1,3-diol hydrochloride) (pH 8.5) and 0.5 ml of supernatant. The incubation of the reaction mixture was set for 24 h at 38 °C. The absorbance at 290 nm was measured. The enzyme activity was expressed as ng of cinnamic acid produced during 1 min per 1 mg of protein. In enzyme assays, protein contents were determined according to Bradford (1976).
Fig. 1. Correlations between both osmotic and leaf water potential for strains of spring triticale with different drought tolerances. * Statistically significant correlations between measured parameters at a probability of $P < 0.05$.

Fig. 2. Correlations between emission of blue fluorescence and leaf water potential for strains of spring triticale with different drought tolerances. * Statistically significant correlations between measured parameters at a probability of $P < 0.05$. 
Phenolics analysis

For both total phenolics and ferulic acid measurements the lyophilized material was homogenized in 80% ethanol. The total content of phenolics was determined according to Singleton and Rossi (1965). The absorbance was measured at 760 nm. Chlorogenic acid was used as a standard.

Free ferulic acid contents were analyzed with a spectrofluorometer LS 50B (Perkin-Elmer). Before measurements chlorophyll was removed by several extractions with n-hexane until no green colour was visible. The samples were excited at 243 nm and the detection was at 434 nm. The slit widths for both excitation and emission monochromators were adjusted to 10 nm.

Statistical analysis

Statistica 5.0 software for Windows was used. Correlations between measured parameters were tested at a probability of $P < 0.05$.

Results and Discussion

Plant water status, leaf water and osmotic potential

Statistically significant correlations between the osmotic potential and leaf water potential were found for all tested triticale strains (Fig. 1). Low values of the water potential were correlated with low scores from the osmotic potential for both sensitive (CHD 12, CHD 147) and resistant (CHD 220, CHD 247) to drought genotypes. The above results exclude the presence of the osmoregulation mechanism which enables, under reduced leaf water potential, the maintenance of a relatively high volume of the leaf protoplast (Nayyar, 2003) and is recognized as an important factor in the selection of drought-resistant genotypes (Cushman, 2001). Our findings are not the same as observed during the previous study. We have found that drought-resistant genotypes of winter triticale during the same flowering period demonstrated osmoregulation and maintained photosynthetic activity due to the high capacity of the photosynthetic apparatus (Hura et al., 2007b).

Emission of blue fluorescence

A decrease of leaf water potential provoked an increase in the emission of blue fluorescence from leaves of all tested genotypes (Fig. 2). Similarly results were observed in other investigations (Schweiger et al., 1996; Hura et al., 2006, 2007a; Grzesiak et al., 2007). It has been proven, that an increase in the emission of blue fluorescence under stress conditions is related to the accumulation of phenolic compounds, which can be involved in the protective and adaptation mechanisms (Lang et al., 1996; Kikuzaki et al., 2002; Hura et al., 2007b).

Total pool of phenolic compounds and ferulic acid content

Drought-sensitive CHD 147 and -resistant CHD 247 strains exhibited high total phenolics accumulation as the effect of leaf water deficit (Fig. 3a). For the two other genotypes, a decrease in the leaf water potential was accompanied by the low content of phenolic compounds.

As the result of leaf desiccation, a rise in the content of ferulic acid for CHD 12, CHD 220 and CHD 247 was observed (Fig. 3b). However, only for CHD 247 an increase in the ferulic acid content positively correlated with the content of the total pool of phenolic compounds (Fig. 4). The obtained results point to the fact that accumulation of ferulic acid as effective photoprotector and/or as a ROS scavenger may occur at the cost of the synthesis of the other phenolics. In the case of the resistant genotype, CHD 247, a simultaneous increase in the ferulic acid content and total phenolic compounds content, discerned under weak hydration of leaf tissues, could be a reliable indicator of the resistance to drought stress. Since phenolic compounds, due to their chemical structure, are capable of light absorption as well as neutralization of ROS (Lang et al., 1996; Kikuzaki et al., 2002; Meyer et al., 2003), they seem to be useful molecules in preventing injuries from the photosynthetic apparatus, which under drought stress is more sensitive to short wavelengths radiation (Sullivan and Teramura, 1990; Nogués and Baker, 2000; Bilger et al., 2001).

PAL activity

For both drought-sensitive CHD 12 and CHD 147 genotypes, an increase in the PAL activity was correlated with the low leaf water potential (Fig. 5), whilst a weak activity of PAL in desiccated leaves of drought-resistant genotypes (CHD 220, CHD 247) was found. The result noted for CHD 247 suggests the predominance of the accu-
Fig. 3. Correlations between (a) total phenolics content, (b) ferulic acid content and leaf water potential for strains of spring triticale with different drought tolerances. * Statistically significant correlations between measured parameters at a probability of $P < 0.05$. 
Fig. 4. Correlations between ferulic acid content and total phenolics content for strains of spring triticale with different drought tolerances. * Statistically significant correlations between measured parameters at a probability of $P < 0.05$.

Fig. 5. Correlations between PAL activity and leaf water potential for strains of spring triticale with different drought tolerances. * Statistically significant correlations between measured parameters at a probability of $P < 0.05$. 
Fig. 6. Correlations between emission of (a) red fluorescence, (b) far-red fluorescence and leaf water potential for strains of spring triticale with different drought tolerances. * Statistically significant correlations between measured parameters at a probability of $P < 0.05$. 

Leaf water potential [MPa]
Fig. 7. Correlations between emission of red fluorescence and (a) ferulic acid content and (b) total phenolics content for strains of spring triticale with different drought tolerances. * Statistically significant correlations between measured parameters at a probability of $P < 0.05$. 
mulation processes of phenolics and ferulic acid, as e.g. photoprotectors, in dehydrated leaf tissues (Fig. 3) over their utilization (observed decrease in PAL activity at low leaf water potential). Results for the other resistant genotype, CHD 220, point to the accumulation of only ferulic acid as an effective photoprotector. In the drought-sensitive genotypes CHD 12 and CHD 147, the high activity of PAL at low values of water potential could be provoked by the utilization of phenolics as effective ROS scavengers. It has been found that water deficit in leaf tissues induces an increase in the PAL activity in some drought-resistant and -sensitive genotypes of winter triticale (Hura et al., 2007b).

Photosynthetic apparatus. Emission of red and far-red fluorescence

The analysis of the emission of red fluorescence generally allows an estimate of the functioning of the photosynthetic apparatus (Buschmann and Lichtenthaler, 1998; Buschmann et al., 2000), although without the possibility of diagnosing its individual elements. The genotypes CHD 12, CHD 220 and CHD 247 exhibited an increase in the emission of red fluorescence as a response to low leaf water potential (Fig. 6a), although for CHD 247, the correlation was not statistically significant. Surprisingly, the drought-sensitive genotype CHD 147 showed a low intensity of red fluorescence in dehydrated leaves. It can be explained by an alternative pathway of energy dissipation, such as photorespiration, under drought conditions (Flexas et al., 1998; Flexas and Medrano, 2002).

Similar courses as mentioned above were also found for the far-red fluorescence (Fig. 6b). The low intensity of red fluorescence at a low level of leaf hydration for drought-sensitive CHD 147 and simultaneously the high intensity of far-red fluorescence can be explained by a partial transfer of the excitation energy to PS I (Kitajima and Butler, 1975; Agati et al., 2000). Transfer of the excitation energy from PS II to PS I could be the result of serious injuries to PS II or could involve a protective mechanism, in which PS I takes over the function of deactivating the excitation energy through the emission of far-red fluorescence (Katona et al., 1992; van Heerden et al., 2007).

The high emission intensity of red fluorescence during drought stress either point to injuries of the photosynthetic apparatus or to protective mechanisms, involving the scattering of energy as fluorescence outwards from the photosynthetic apparatus (Grzesiak et al., 2007; Hura et al., 2007a). It has been shown, that an increase in the emission of red fluorescence occurs at the cost of the photosynthetic light conversion and is caused by disorganization of the photosynthetic apparatus, due to disturbances in migration of the excitation energy (Lichtenthaler, 1996; Schweiger et al., 1996; Hura et al., 2007b).

Through absorption of short wavelength radiation, which reaches the leaf, phenolic compounds can function as photoprotectors of the photosynthetic apparatus (Demmig-Adams and Adams, 1992; García-Plazaola and Becerril, 2000; Cerovic et al., 2002). In Fig. 7, correlations between the ferulic acid content (a), the total pool of phenolic compounds (b) and the emission of red fluorescence are shown. The increase in the emission intensity of red fluorescence positively correlated with the content of ferulic acid for all studied genotypes. A similar relationship regarding the total pool of phenolic compounds, just in the resistant genotype CHD 247, was observed. Such correlations may indicate the participation of phenolic compounds in the acclimatization/protection of the photosynthetic apparatus to/ from the low leaf water content (Caldwell et al., 1983; Cerovic et al., 2002).

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