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COMPARISON OF RESISTANCE TO WATER STRESS OF TWO WHEAT CULTIVARS

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Introduction

Leaf senescence is an organized developmental program. Role for active oxygen species including superoxide, hydrogen peroxide, hydroxyl free radicals and singlet oxygen have been proposed in senescence [THOMPSON et al. 1987]. Presence of these reactive oxygen species leads to oxidative damage in cellular molecules, such as proteins, lipids and nucleic acids. At the cellular level, organelles are disassembled in a precise hierarchy. Chloroplasts are the first to be affected during the senescence program while the nucleus and mitochondria are last [QUIRINO et al. 1999]. Plant cells are protected normally against the destruction effects of reactive oxygen species by a complex of enzymatic and nonenzymatic mechanisms: superoxide dismutase, ascorbate peroxidase, catalase, glutathione reductase, tocopherol, β-carotene and ascorbate form antioxidative defence.

Increasing evidence suggests that drought induces oxidative stress through the production of active oxygen species during stress [SMIRNOFF 1993]. GAMBLE and BURKE [1984] appear to be the first to consider the relationship between antioxidant systems and water deficit, and showed that glutathione reductase activity in wheat leaves was higher in droughted than in irrigated plants.

In the present investigation, changes in the relative water content, in levels of photochemical efficiency and chlorophyll content and in activities of antioxidative enzymes between two wheat cultivars were measured.

Material and methods

Winter wheat (*Triticum aestivum* L.) cvs. Mironovská and Estica were planted in plastic pots in taped sand in the growth chamber with following conditions: 25°/18°C, 80/60 relative humidity day/night, irradiance 200/0 μmol (PAR)$\cdot m^{-2}\cdot s^{-1}$. Plants were supplied twice a week with nutrient solution. The drought stress treatment was started at 11 days of plant age. Sampling of the second leaves was at 18 days of plants age.

Relative water content (RWC) was estimated using the Whetherley method.

Pigment content. Detailed pigment analysis in freeze-dried leaves was carried out using high performance liquid chromatography (HPLC). Leaves were homogenized in a mortar with pestle in 85% acetone with butyl hydroxytoluene, centrifuged, dried and after that 85% acetone was added. Pigments were separated in a HPLC system (Spectra – Physics, San Jose, CA, USA) using a reverse phase column (Sepharon SGX C18, 5μm particle size, 150 x 3 mm, Tessek, Praha). The solvent systems for the combined isocratic and gradient separation were acetonitrile/methanol/water (80 : 12 : 6) followed by 100% methanol, the detection wavelength was 445 nm.

Protein content was determined spectrophotometrically according to the method of BRADFORD [1976].

Photochemical efficiency was measured after a 15 min dark period with PAM Chlorophyll fluorometer (Walz, Effetrich, Germany) at room temperature, measuring irradiance was 0,5 $W \cdot m^{-2}$, actinic irradiance 150 $W \cdot m^{-2}$ and 700 ms saturated flash of 1400 $W \cdot m^{-2}$.

Enzymatic activity. Enzyme extract was prepared at 4°C, by grinding 1 g of leaf material with 10 ml of extraction buffer (0.1 mol(Tris)·dm^{-3}, 0.01 mol(dithiothreitol)·dm^{-3}, 0.01 mol(EDTA)·dm^{-3}, 1% Triton X-100, 4% PVP and 5 mmol(ascorbate)·dm^{-3}, pH 7.0). Ascorbate peroxidase was measured spectrophotometrically at 298 nm using the method of NAKANO and ASADA [1981]. 3ml reaction mixture contained 0.1 HEPES-EDTA (pH 7.0), 30 mmol(ascorbate)·dm^{-3}, 20 mmol(hydrogen peroxide)·dm^{-3} and 0.1 ml of extract. Catalase activity was assayed at 240 nm, with method of CHANCE and MAEHLY [1955]. 3 ml of reaction mixture contained 0.1 mol·dm^{-3} phosphate buffer (pH 7.0), 20 mmol·dm^{-3} hydrogen peroxide, 0.1 ml extract. Glutathione reductase was tested at 340 nm, using the method of SCHAENLE and BASSHAM [1977] in 3 ml of reaction mixture of 0.1 mol(Tris)·dm^{-3} (pH 7.0), 1 mmol(EDTA)·dm^{-3}, 8 mmol(GSSG)·dm^{-3}, 6 mmol(NADPH)·dm^{-3} and 0.1 ml of extract. Peroxidase at 470 nm, according to the method of CHANCE and MAEHLY [1955]: 0.1 mol·dm^{-3} phosphate buffer, 8 mmol·dm^{-3} hydrogen peroxide, 18 mmol·dm^{-3} guaiacol and 0.1 ml extract.

All experiments and treatments were replicated three times. Data were analysed using Student's t-test.

Results

It appears that the cultivar Estica is more resistant to drought stress. In the case of Estica the RWC was higher than in the case of Mironovská. At the end of drought period the decrease of photochemical efficiency was also lower in the case of Estica than in the case of Mironovská.

Chlorophyll decreased in both cultivars after drought treatment. Beta-carotene plays a role of non-enzymatic antioxidant and its content increased in consequence of drought stress.

The contents of antioxidative enzymes increased in both cases. As compared with Mironovská Estica had a two times higher activity of glutathione reductase. Most reports suggest little change in activity of catalase [GAMBLE, BURKE 1984]. The catalase content significantly increased in the case of Mironovská. The main quenching factor in plant cells is ascorbate peroxidase and the increase of APOD was in the case of Estica significant. Asada divides plant

peroxidases into two groups. The first group consists of peroxidases in which oxidation of the substrate by hydrogen peroxide serves a lignification and whose primary function is not hydrogen peroxide scavenging [SMIRNOFF 1993]. Peroxidase increased in both cases and we can assume that lignification increased.

Table 1; Tabela 1

Relative water content (RWC) and leaf photochemical efficiency (Fv/Fm)
(Each value represents of 3 replicates ±SD)

Względna zawartość wody (RWC) i wydajność fotochemiczna liści (Fv/Fm)
(Podane wartości obejmują średnie (±SD) z 3 powtórzeń biologicznych)

Cultivar; Odmiana	RCW (%)	Fv/Fm
Mironovská control; kontrola	98,2 ± 1,77	0,758 ± 0,0093
Mironovská stress; stres	88,3 ± 5,44	0,690 ± 0,0662
Estica control; kontrola	97,1 ± 1,17	0,754 ± 0,0089
Estica stress; stres	94,2 ± 5,42	0,747 ± 0,0235

Table 2; Tabela 2

Chlorophyll a + b content and β-carotene content
(Each value represents of 3 replicates ±SD)

Zawartość chlorofilu a + b i β-karotenu
(Podane wartości obejmują średnie (±SD) z 3 powtórzeń biologicznych)

Cultivar; Odmiana	Chl a + b (μg g–1 DM) Chl a + b (μg·g^{-1} s.m.)	β-carotene (μg·g^{-1} DM) β-karoten (μg·g^{-1} s.m.)
Mironovská control; kontrola	3574 ± 210	539 ± 14,1
Mironovská stress; stres	2454 ± 250	558 ± 13,4
Estica control; kontrola	6647 ± 200	737 ± 11,0
Estica stress; stres	5510 ± 240	852 ± 10,3

Table 3; Tabela 3

Activity of enzymes expressed as $\Delta A \cdot min^{-1} \cdot mg^{-1}$ protein
(Each value represents of 3 replicates ±SD)

Aktywność enzymów wyrażona jako $\Delta A \cdot min.^{-1} \cdot mg^{-1}$ białka
(Podane wartości obejmują średnie (±SD) z 3 powtórzeń biologicznych)

Cultivar; Odmiana	Ascorbate peroxidase Peroksydaza askorbinianowa	Catalase Katalaza	Glutathione reductase Reduktaza glutationowa	Peroxidase Peroksydaza
Mironovská control; kontrola	0,501 ± 0.038	0,0869± 0,008	0,0282 ± 0,001	0,8736 ± 0,102
Mironovská stress; stres	0,568 ± 0,043	0,1672± 0,012	0,0456 ± 0,007	1,6622 ± 0,247
Estica control; kontrola	0,470 ± 0,011	0,1274± 0,009	0,0406 ± 0,003	0,9350 ± 0,016
Estica stress; stres	0,845 ± 0,074	0,1370 ± 0,02	0,0986 ± 0,015	1,3936 ± 0,025

Conclusions

In the case of the Estica cultivar a smaller decrease of relative water content and photochemical efficiency took place as compared with Mironovská. This

signifies that Estica could be more resistant in so far as drought stress is concerned. In the case of Estica the values of catalase, ascorbate peroxidase and glutathione reductase activity were also higher than in the case of Mironovská. It is therefore possible to assume that Estica is capable of dealing with higher levels of active oxygen species created during drought stress. This might enabled it to compensate the effects of drought stress more effectively.

Literature

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Key words: drought, antioxidative enzymes, active oxygen species, *Triticum aestivum* L.

Summary

The effects of drought stress on changes in the relative water content, in levels of photochemical efficiency and chlorophyll content and in activities of antioxidative enzymes of second leaves of winter wheat (*Triticum aestivum* L.) cultivars Mironovská and Estica were compared. In one set, control plants were regularly watered, the other set of plant was subjected to water deficit period since 11th day to 18th day of plants life-span. The activity of all antioxidative enzymes and content of β-carotene increased, relative water content, total chlorophyll content and photochemical efficiency decreased after treatment in both cultivars. In the case of Estica the values of antioxidative enzymes activity was higher than in the case of Mironovská. It is therefore possible to assume that

Estica is capable of dealing with higher levels of active oxygen species created during drought stress. This might enabled it to compensate the effects of drought stress more effectively.

PORÓWNANIE ODPORNOŚCI NA SUSZĘ DWÓCH ODMIAN PSZENICY

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Słowa kluczowe: susza, enzymy antyoksydacyjne, reaktywne formy tlenu, *Triticum aestivum* L.

Streszczenie

W pracy porównano wpływ suszy na względną zawartośc wody, wydajność fotochemiczną PS II i zawartość chlorofilu oraz aktywność enzymów antyoksydacyjnych w 2. liściu pszenicy ozimej odmian Mironowska i Estica. Rośliny grupy kontrolnej regularnie podlewano, drugą grupę poddano działaniu suszy glebowej w okresie od 11. do 18. dnia wegetacji. Zabieg ten zwiększył aktywność enzymów antyoksydacyjnych i zawartość β-karotenu, przy równoczesnym obniżeniu względnej zawartości wody, stężenia chlorofilu i wartości wydajności fotochemicznej PS II liści obydwóch odmian. Aktywność antyoksydantów była wyższa u odmiany Estica w porównaiu z Mironowską. W związku z tym wydaje się możliwe, iż Estica charakteryzuje się zwiększoną wydajnością unieszkodliwiania reaktywnych form tlenu wytwarzanych w zwiększonych ilościach podczas deficytu wody, co może przyczyniać się do tolerowania stresu suszy glebowej przez tę odmianę.

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