Physiological Responses of Two Wheat Cultivars to Soil Drought

Radoslav R. Chipilski[a,b], Konstantina V. Kocheva[b,*], Veselina R. Nenova[b], and Georgi I. Georgiev[b]

[a] Institute of Plant Genetic Resources, Agricultural Academy, Sadovo 4122, Bulgaria
[b] Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, 'Acad. G. Bonchev' str., block 21, Sofia 1113, Bulgaria. Fax: +359–2-8739952.
E-mail: konstvk@abv.bg

* Author for correspondence and reprint requests

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Young plants of the two wheat cultivars Katya and Prelom, differing in their reaction to drought in the field, were grown in soil in pots, and their water status was assessed as well as the intensity of gas exchange, chlorophyll fluorescence, and accumulation of compatible solutes and hydrogen peroxide after 7 days of dehydration. It was established that cv. Katya displayed markedly better tolerance to soil drying in comparison with cv. Prelom. This was partly due to the more effective control of water balance, activity of the photosynthetic apparatus, and metabolic activity of leaves under stress. Consequently, lower amounts of hydrogen peroxide were accumulated and a lower membrane injury index was determined.

Key words: Chlorophyll Fluorescence, Injury Index, Oxidative Stress

Introduction

Plants possess various mechanisms for tolerating, avoiding or resisting water deficit stress (Griffiths and Parry, 2002; Kramer and Boyer, 1995). Maintenance of a better leaf water content under stress correlates with certain metabolic changes in the cytosol leading to osmoregulation in cells (Handa *et al*., 1983), changes in the plant phytohormone balance (Mittler, 2002), reorganization of the photosynthetic apparatus (Lawlor, 2002; Cornic and Fresneau, 2002), and various morpho-anatomical changes (Pankhurst and Loucks, 1972). The limitation of transpiration under stress is associated with reduced CO_2 absorption from the atmosphere by the leaf and leads to certain disturbances in the activity of the photosynthetic apparatus. Among them are the destructive role of excess light on the activity of photosystems I (PSI) and II (PSII), disturbed regulation of photochemical reactions of photosynthesis, and development of oxidative stress (Lawlor, 2002; Mittler, 2002; Bartosz, 1997). The reason for this is the decreased utilization of NADPH due to reduced CO_2 consumption. Directing the excess of electrons generated by PSII and I to other acceptors such as oxygen may be regarded as a protective mechanism for the photosynthetic apparatus against the excess energy when it is accompanied by scavenging of reactive oxygen species (ROS) (Lawlor, 2002; Cornic and Fresneau, 2002). Another mechanism is the dissipation of this energy in the form of heat from the light-harvesting complexes of PSI and II by means of the xanthophyll cycle (Neubauer and Yamamoto, 1992) and eventually by activation of photoinhibition processes (Cornic, 1994; Biehler and Fock, 1996; Cornic and Fresneau, 2002; Lawlor, 2002). Some of these photoprotective mechanisms may be assessed through changes in the chlorophyll fluorescence parameters. An important element of plants' protection mechanism against water stress is the metabolic turnover and synthesis of osmotically active substances (compatible solutes) that influence the water retention capacity of the cells (Stewart and Larher, 1980). Another mechanism counteracting stress is the reduction of the cell area and changes in the leaf morphology which confine the absorption of excessive light from the leaf. Cereal plants in general have drought tolerance mechanisms which may interrelate all of the above-mentioned mechanisms (Cattivelli *et al*., 2008). Hence, the determination of differences in the physiological tolerance to water deficit stress

Abbreviations: PS, photosystem; ROS, reactive oxygen species; RWC, relative water content; SLA, specific leaf area.

within a certain species requires the selection of parameters measured at a definite stress level in a distinct indicative plant organ. The establishment of such a physiological model could be of great practical value for the use in crop breeding.

The aim of the present research was to study the relationship between growth reaction, water status, osmoregulatory characteristics, photosynthesis, and degree of oxidative stress in two wheat cultivars differing widely in their drought tolerance in the field.

Material and Methods

The two wheat (*Triticum aestivum* L.) cultivars Katya and Prelom, selected in the Institute of Plant Genetic Recourses, Sadovo, Bulgaria, and differing in their reaction to desiccation in the field, were used in the experiments. Ten seeds were superficially sterilized with 4% sodium hypochlorite (NaOCl) and then soaked in tap water for 6 h prior to sowing in 1-L pots with dry alluvial meadow soil. The soil contained easily hydrolyzable nitrogen (4.06 mg N/100 g dry soil), 4.90 mg P_2O_5/100 g dry soil, 18 mg K_2O/100 g dry soil, and 1.4% total organic matter, with pH 6.5 of the water extract. Experiments were conducted in a greenhouse with the soil moisture kept at 60% of the full soil moisture capacity, under natural light conditions, and at average day/night temperatures of 26/20 °C. At the 20th day after seed germination, watering of half of the pots was suspended for 7 d in order to obtain permanent wilting of the plants. The second fully developed leaf was used for the analyses.

The relative water content (*RWC*) of the second leaf was calculated according to Turner (1981) using the following formula: $RWC\ (\%) = (FW-DW)/(TW-DW) \cdot 100$, where *FW* represents leaf fresh weight, *DW* is leaf dry weight obtained after drying the leaves for 24 h at 80 °C, and *TW* is the turgid weight after soaking the leaves in water for 24 h.

The phenol-sulfuric acid assay of Ashwell (1966) and the acid ninhydrin method of Yemm and Cocking (1955) were followed for determination of reducing sugars and free amino acids, respectively. Ethanol extracts (1 g *FW*/21 mL ethanol) of leaf tissue were evaporated to dryness and subsequently dissolved in water (1:1, w/v) with respect to the fresh weight of the sample. Plant leaf contents of these cellular osmolytes were determined spectrophotometrically. Fifteen leaf pieces from stressed and control plants were immersed in 20 mL distilled water for 24 h for determination of cell membrane injury. Conductivity of the solutions was measured with an Elwro 5721 (Wroclaw, Poland) conductometer. The leaf injury index (*I*) was estimated from the formula: $I\ (\%) = [1-(1-T_1/T_2)/(1-C_1/C_2)] \cdot 100$, where T_1 and T_2 represent the conductivity of treated samples after 24 h of incubation and after tissue killing, respectively; C_1 and C_2 are the corresponding values for the controls.

The hydrogen peroxide content was measured spectrophotometrically according to Alexieva *et al.* (2001). The photosynthetic rate (*A*), transpiration rate (*E*), stomatal conductance (g_s), and internal leaf CO_2 concentration (C_i) of the second fully expanded leaf were measured using a portable gas-exchange system with a leaf chamber LCpro+ (ADC, BioScientific Ltd., Herts, UK) at a light intensity of 900 μmol m^{-2} s^{-1}. The leaf area of plant samples was measured by the method described by Tsonev and Sergiev (1993).

Chlorophyll fluorescence was measured in leaf discs by a pulse modulation chlorophyll fluorometer (PAM 101; H. Walz, Effeltrich, Germany), using actinic light at 330 μmol m^{-2} s^{-1} and saturating light at 3500 μmol m^{-2} s^{-1} photon flux density. The minimum chlorophyll fluorescence yield in the dark-adapted state (after 5 min of dark adaptation) and in the light-adapted state (F_0 and F_0', respectively), maximum chlorophyll fluorescence yield in the dark-adapted state and in the light-adapted state (F_m and F_m', respectively), and steady-state chlorophyll fluorescence (F_s) were recorded. The following parameters were calculated according to Roháček (2002): maximum variable chlorophyll fluorescence yield in the dark-adapted state ($F_v = F_m - F_0$); potential maximum quantum yield of PSII (F_v/F_m); actual quantum yield of PSII [$\Phi_{PSII} = (F_m' - F_s)/F_m'$]; photochemical quenching of the variable chlorophyll fluorescence [$q_P = (F_m' - F_s)/(F_m' - F_0')$]; effective quantum yield of PSII photochemistry [$\Phi_{exc} = (F_m' - F_0')/F_m'$]; non-photochemical chlorophyll fluorescence quenching [$NPQ = (F_m - F_m')/F_m'$]. The fluorescence decrease ratio [$R_{Fd} = (F_m - F_s)/F_s$] was calculated according to Lichtenthaler and Babani (2004).

Two independent experiments were conducted and all parameters were measured in at least 3

replicates each time. Data are presented as mean values ± SD.

Results and Discussion

Table I presents changes in fresh and dry weight of plants after 7 days of dehydration in soil pots. The fresh weight of the plants of cv. Prelom was 28% of the control value, while in cv. Katya it was 31% of the respective control value. Dry biomass in Prelom was 75% while in Katya it was 68% of the control value. These changes were related to a greater water loss from the leaves of Prelom – *RWC* being 32.9% –, whereas in Katya it was as high as 61.5%, indicating a better water status under dehydration. The lower *RWC* in Prelom leaves was consistent with a higher membrane injury index (Table I). Greater membrane injury due to dehydration was evident in leaves of Prelom (53%), while in Katya this parameter was 37% after 7 days of drought stress. Twenty-eight-day-old plants of cv. Prelom had a higher area of the second fully developed leaf in comparison with cv. Katya (Table II). After 7 days of soil drying, the leaf area (*LA*) in cv. Katya was more strongly reduced (51% of the control) than that of the same leaf in cv. Prelom, which at the same time had reached 80% of the respective control. The latter parameter was related to changes in specific leaf area (*SLA*). In cv. Prelom, *SLA* showed a tendency to increase in response to desiccation (107% of control), while in cv. Katya it declined to 66% of the control (Table II). Plant *LA* plays an essential role in the regulation of transpiration and direction of the light flow towards the photosynthetic apparatus. Thus cv. Katya's ability to greatly reduce its leaf area under desiccation could reflect an important adjustment reaction (Pankhurst and Loucks, 1972).

The changes in fresh and dry plant biomass are related to the gas-exchange status of the leaves experiencing water deficit (Table III). Under normal water supply, both cultivars displayed similar leaf gas-exchange rates (CO_2 absorption and H_2O transpiration). After 7 days of dehydration photosynthetic rate (*A*), stomatal conductance (g_s) and transpiration (*E*) were significantly reduced in both cultivars. The internal leaf CO_2 concentration in the leaves of the two cultivars was increased after dehydration, with cv. Katya showing lower values (137% of control) than cv. Prelom (252% of control). It is possible that the increase in the internal CO_2 concentration is due to enhanced photo- and dark respiration which could be more pronounced in cv. Prelom (Noctor *et al.*, 2002), or alternatively, this could be due to an altered regulation of *Rubisco* activity under stress and a reduced CO_2 fixation (Demirevska

Table I. Fresh (FW) and dry weight (DW) of shoots, leaf relative water content (*RWC*), and leaf injury index (*I*) of two wheat cultivars subjected to soil drought for 7 days.

Cultivar/treatment	Shoot fresh weight		Shoot dry weight		*RWC* (%)	*I* (%)
	[g FW plant^{-1}]	(%)	[g DW plant^{-1}]	(%)		
Katya control	0.338 ± 0.136	100	0.038	100	99.3 ± 0.3	
Katya stressed	0.106 ± 0.071	31	0.026	68	61.5 ± 1.9	37.2 ± 4.2
Prelom control	0.325 ± 0.062	100	0.044	100	98.4 ± 0.3	
Prelom stressed	0.090 ± 0.029	28	0.033	75	32.9 ± 3.1	53.4 ± 5.0

Data are means of 6 replicates ± SD.

Table II. Changes in total and specific leaf area and dry matter of second leaf of two wheat cultivars subjected to soil water deprivation for 7 days.

Cultivar/treatment	Leaf area		Leaf dry weight		Specific leaf area	
	[mm^2 leaf^{-1}]	(%)	[mg DW leaf^{-1}]	(%)	[mm^2 mg^{-1} DW]	(%)
Katya control	13.12 ± 2.88	100	14.50 ± 0.23	100	0.90 ± 0.07	100
Katya stressed	6.65 ± 1.46	51	11.31 ± 0.42	78	0.59 ± 0.07	66
Prelom control	21.85 ± 2.15	100	13.50 ± 0.26	100	1.62 ± 0.21	100
Prelom stressed	17.41 ± 3.40	80	10.01 ± 0.11	74	1.74 ± 0.55	107

Data are means of 5 replicates ± SD.

Table III. Leaf gas-exchange rate characteristics of two wheat cultivars subjected to soil water deprivation for 7 days.

Cultivar/treatment	A [μmol CO_2 m^{-2} s^{-1}]	E [mol H_2O m^{-2} s^{-1}]	g_s [μmol CO_2 m^{-2} s^{-1}]	C_i [μmol CO_2 m^{-2} s^{-1}]
Katya control	28.52 ± 2.52	5.84 ± 0.82	0.805 ± 0.150	280.0 ± 6.4
Katya stressed	1.21 ± 0.05	0.45 ± 0.16	0.023 ± 0.006	383.5 ± 7.4
Prelom control	26.46 ± 2.70	4.50 ± 0.73	0.540 ± 0.180	250.0 ± 9.4
Prelom stressed	1.18 ± 0.23	0.14 ± 0.09	0.012 ± 0.002	629.8 ± 18.3

Data are means of 5 replicates ± SD.
A, photosynthetic rate; E, transpiration rate; g_s, stomatal conductance; C_i, internal leaf CO_2 concentration.

et al., 2009; Parry *et al.*, 2002). In the cited studies, it was shown that upon dehydration of wheat plants, including cv. Katya, down-regulation of the *Rubisco* activity was observed with probable participation of protease inhibitors and lowered *Rubisco* regeneration activity. The relative ratio between cytochrome a and alternative, *i.e.* cyanide-insensitive, respiration in leaf mitochondria in cv. Katya has already been demonstrated to be higher than in cv. Prelom (Vassileva *et al.*, 2009).

On the other hand, chlorophyll fluorescence data (Table IV) showed that the functional state of the photosynthetic apparatus under desiccation of cv. Katya was better than that of cv. Prelom. The actual quantum yield of PSII (Φ_{PSII}) in cv. Katya after dehydration was reduced by only 6%, while in cv. Prelom a reduction of 19% was recorded. Moreover, the observed decrease was not due to a change of the effective quantum yield of PSII photochemistry (Φ_{exc}), which showed a slight tendency to increase in both genotypes, but rather to the reduction of the photochemical quenching of the variable chlorophyll fluorescence (q_P). The latter parameter, which indicates the fraction of open PSII reaction centres, was reduced by 11% in cv. Katya and 27% in cv. Prelom with respect to the controls. Therefore, under dehydration the fraction of light absorbed in PSII antennae utilized in subsequent PSII photochemistry was more significantly reduced in cv. Prelom, and this is supposed to be associated with the photochemical energy conversion by the charge separation in the reaction centres of PSII (Roháček, 2002). The fluorescence decrease ratio (R_{Fd}) which, according to Lichtenthaler and Babani (2004), correlates with the potential photosynthetic rates of leaves, was reduced by only 11% in cv. Katya and by 39% in cv. Prelom. Under lowered transpiration conditions, heat energy dissipation plays a substantial role in the regulation of the electron flow at PSII. It can be measured by non-photochemical chlorophyll fluorescence quenching (*NPQ*), which reveals the activation of processes mostly leading to non-radiative energy dissipation to heat in the PSII antenna complexes. Under dehydration this parameter was more significantly lowered in cv. Prelom (37% of control) than in cv. Katya (8%). In this regard, drought-stressed plants of cv. Prelom had a lower capacity of protection from the absorbed excess energy by emitting it in the form of heat. The potential maximum quantum yield of PSII (F_v/F_m) under dehydration in cv. Katya was

Table IV. Changes in chlorophyll fluorescence parameters of two wheat cultivars subjected to soil water deprivation for 7 days.

Parameter	Katya			Prelom		
	Control	Stressed	% Control	Control	Stressed	% Control
F_v/F_m	0.76 ± 0.01	0.77 ± 0.01	101	0.77 ± 0.02	0.75 ± 0.02	97
F_v/F_0	3.21 ± 0.14	3.35 ± 0.16	104	3.40 ± 0.31	3.01 ± 0.38	89
Φ_{PSII}	0.33 ± 0.02	0.31 ± 0.05	94	0.32 ± 0.04	0.26 ± 0.06	81
q_P	0.66 ± 0.03	0.59 ± 0.07	89	0.63 ± 0.09	0.46 ± 0.09	73
Φ_{exc}	0.49 ± 0.01	0.52 ± 0.03	106	0.52 ± 0.09	0.57 ± 0.03	110
NPQ	1.30 ± 0.15	1.19 ± 0.16	92	1.28 ± 0.43	0.80 ± 0.15	63
R_{Fd}	2.42 ± 0.11	2.16 ± 0.09	89	2.36 ± 0.63	1.45 ± 0.09	61

Data are means of 4 replicates ± SD.

not changed, while in cv. Prelom a slight tendency towards reduction was observed. Differences between the two cultivars were more clearly manifested if the ratio F_v/F_0, which is very sensitive to changes in F_v/F_m in the vicinity of the 'optimum' value (Roháček, 2002), was used as an indicator of maximum efficiency of photochemical processes in PSII. These data are interpreted to be indicative of photoinhibition or another type of injury of the PSII complexes in cv. Prelom.

The discrepancy between the strongly reduced CO_2 assimilation rate in stressed plants and a less affected Φ_{PSII} could be explained by an increased partitioning of electrons to competing processes such as photorespiration, Mehler reaction, and/or nitrogen metabolism (Lawlor, 2002). The increased levels of H_2O_2 and free amino acids measured under stress (Table V) could be regarded as an evidence for this situation.

An important part of cellular reaction to water stress is the ability to accumulate compatible osmolytes and ions contributing to the processes of osmoregulation. The level of cellular free amino acids in cv. Katya subjected to dehydration was elevated 10-fold, while in cv. Prelom their content was increased 5.7 times compared with the respective control (Table V). At the same time the content of reducing sugars in leaves of stressed Katya plants was increased significantly, whereas in cv. Prelom their level was not changed. Changes in the functional condition of the leaf photosynthetic apparatus and cell membrane stability under desiccation are often connected with the accumulation of ROS and the development of oxidative stress. Analysis of H_2O_2 accumulation in leaves of drought-stressed plants showed that in cv. Prelom the peroxide content was raised 10-fold, but only 7.7-fold in cv. Katya. Changes in the ROS content may be associated with the action of a number of photoprotective mechanisms in the cells among which photorespiration and the Mehler reaction are probably the most significant (Noctor *et al.*, 2002; Cornic, 1994). In cv. Katya a lower peroxide content was sustained in leaves, implying that in this genotype the mechanisms for control of excessive light absorption and for generation and degradation of H_2O_2 were more effective. A lowered effect of oxidative stress in cv. Katya allowed plants to better the maintain the membrane stability and a better leaf water status, which ensures lesser disturbance of the metabolic activities (Cattivelli *et al.*, 2008; Jones, 1985). On the other hand, this state is connected with a greater accumulation of osmotically active substances such as amino acids and sugars capable of affecting the cellular osmotic potential (Passioura, 1996; Stewart and Larher, 1980). Furthermore, the level of the cytochrome pathway of mitochondrial respiration of young dehydrated plants of cv. Katya was found to be higher than that of cv. Prelom (Vassileva *et al.*, 2009).

In conclusion, it could be affirmed that cv. Katya clearly displayed a better tolerance to soil dehydration in comparison with cv. Prelom partly due to the escape from an excess of light through the regulation of the leaf area as well as to the reduction of water loss by a more effective control of photosynthetic and metabolic activities in leaves and a lower accumulation of hydrogen peroxide.

Table V. Changes in leaf levels of free amino acids, reducing sugars, and hydrogen peroxide of two wheat cultivars subjected to soil water deprivation for 7 days.

Cultivar/treatment	Free amino acids		Reducing sugars		Hydrogen peroxide	
	[μmol g^{-1} DW]	(%)	[mmol g^{-1} DW]	(%)	[μmol g^{-1} DW]	(%)
Katya control	10.50 ± 1.56	100	0.32 ± 0.04	100	2.46 ± 0.11	100
Katya stressed	101.0 ± 9.23	962	1.23 ± 0.08	384	18.87 ± 1.24	767
Prelom control	10.00 ± 0.89	100	0.87 ± 0.05	100	2.87 ± 0.09	100
Prelom stressed	57.11 ± 0.34	571	0.82 ± 0.06	94	31.62 ± 1.98	1102

Data are means of 3 replicates ± SD.

Alexieva V., Sergiev I., Mapelli S., and Karanov E. (2001), The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ. **24**, 1337–1344.

Ashwell G. (1966), New colorimetric methods of sugar analysis, VI. The phenol-sulfuric acid reaction for carbohydrates. Methods Enzymol. **8**, 93–95.

Bartosz G. (1997), Oxidative stress in plants. Acta Physiol. Plant. **19**, 47–64.

Biehler K. and Fock H. (1996), Evidence for the contribution of the Mehler-peroxidase reaction in dissipating excess electrons in drought-stressed wheat. Plant Physiol. **112**, 265–272.

Cattivelli L., Rizza F., Badeck F.-W., Mazzucotelli E., Mastrangelo A. M., Francia E., Marè C., Tondelli A., and Stanca M. (2008), Drought tolerance improvement in crop plants: An integrated view from breeding to genomics. Field Crops Res. **105**, 1–14.

Cornic G. (1994), Drought stress and light effects on leaf photosynthesis. In: Photoinhibition of Photosynthesis (Baker N. R. and Bower J. R., eds.). Bios Scientific Publishers, Oxford, pp. 297–313.

Cornic G. and Fresneau C. (2002), Photosynthetic carbon reduction and carbon oxidation cycles are the main sinks for photosystem II activity during a mild drought. Ann. Bot. **89**, 887–894.

Demirevska K., Zasheva D., Dimitrov R., Simova-Stoilova L., Stamenova M., and Feller U. (2009), Drought stress effects on *Rubisco* in wheat: changes in the *Rubisco* large subunit. Acta Physiol. Plant. **31**, 1129–1138.

Griffiths H. and Parry M. A. J. (2002), Plant response to water stress. Ann. Bot. **89**, 801–803.

Handa S., Bressan R. A., Handa A. K., Carpita N., and Hasegawa P. M. (1983), Solutes contributing to osmotic adjustment in cultured plant cells adapted to water stress. Plant Physiol. **73**, 834–843.

Jones H. G. (1985), Partitioning stomatal and non-stomatal limitation to photosynthesis. Plant Cell Environ. **8**, 95–104.

Kramer P. J. and Boyer J. S. (1995), Water Relations of Plants and Soils. Academic Press, New York.

Lawlor D. W. (2002), Limitation to photosynthesis in water-stressed leaves: stomata vs. metabolism and the role of ATP. Ann. Bot. **89**, 871–885.

Lichtenthaler H. and Babani F. (2004), Light adaptation and senescence of the photosynthetic apparatus. Changes in pigment composition, chlorophyll fluorescence parameters and photosynthetic activity. In: Advances in Photosynthesis and Respiration, Vol. 19. Chlorophyll a Fluorescence. A Signature of Photosynthesis (Papageorgiou G. C. and Govindjee, eds.). Springer, Dordrecht, pp. 713–736.

Mittler R. (2002), Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. **7**, 405–410.

Neubauer C. H. and Yamamoto H. (1992), Mehler-peroxidase reaction mediates zeaxanthin formation and zeaxanthin fluorescence quenching in intact chloroplasts. Plant Physiol. **99**, 1354–1361.

Noctor G., Veljovic-Jovanovic S., Driscoll S., Novitskaya L., and Foyer C. H. (2002), Drought and oxidative load in the leaves of C3 plants: a predominant role for photorespiration? Ann. Bot. **89**, 841–850.

Pankhurst D. L. and Loucks D. L. (1972), Optimal leaf size in relation to environment. J. Ecol. **60**, 505–537.

Parry M. A., Andralojic P. J., Khan S., Lea P. J., and Keys A. J. (2002), *Rubisco* activity: Effects of drought stress. Ann. Bot. **89**, 833–840.

Passioura J. (1996), Drought and drought tolerance. Plant Growth Regul. **20**, 79–83.

Roháček K. (2002), Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. Photosynthetica **40**, 13–29.

Stewart G. R. and Larher F. (1980), Accumulation of amino acids and related compounds in relation to environmental stress. In: The Biochemistry of Plants: a Comprehensive Treatise, Vol. 5 (Mihlin B. J., ed.). Academic Press, New York, pp. 609–635.

Tsonev T. and Sergiev I. (1993), Leaf area measurement using hand scanner. Photosynthetica **29**, 625–630.

Turner N. C. (1981), Techniques and experimental approaches for the measurement of plant water status. Plant Soil **58**, 339–366.

Vassileva V., Simova-Stoilova L., Demirevska K., and Feller U. (2009), Variety-specific response of wheat (*Triticum aestivum* L.) leaf mitochondria to drought stress. J. Plant Res. **122**, 445–454.

Yemm E. W. and Cocking E. C. (1955), The determination of amino-acids with ninhydrin. Analyst **80**, 209–213.