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Comparison of the Drought Stress Responses of Tolerant and Sensitive Wheat Cultivars During Grain Filling: Changes in Flag Leaf Photosynthetic Activity, ABA Levels, and Grain Yield

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Abstract Water status parameters, flag leaf photosynthetic activity, abscisic acid (ABA) levels, grain yield, and storage protein contents were investigated in two drought-tolerant (*Triticum aestivum* L. cv. MV Emese and cv. Plainsman V) and two drought-sensitive (cvs. GK Élet and Cappelle Desprez) wheat genotypes subjected to soil water deficit during grain filling to characterize physiological traits related to yield. The leaf water potential decreased earlier and at a higher rate in the sensitive than in the tolerant cultivars. The net CO_2 assimilation rate (P_N) in flag leaves during water deficit did not display a strict correlation with the drought sensitivity of the genotypes. The photosynthetic activity terminated earliest in the tolerant cv. Emese, and the senescence of flag leaves lasted 7 days longer in the sensitive Cappelle Desprez. Soil drought did not induce characteristic differences between sensitive and tolerant cultivars in chlorophyll *a* fluorescence parameters of flag leaves during post-anthesis. Changes in the effective quantum yield of PSII (Φ_{PSII}) and the photochemical quenching (qP) depended on the genotypes and not on the sensitivity of cultivars. In contrast, the levels of ABA in the kernels displayed typical fluctuations in the tolerant and in the sensitive cultivars. Tolerant genotypes exhibited an early maximum in the grain ABA content during drought and the sensitive cultivars maintained high ABA levels in the later stages of grain filling. In contrast with other genotypes, the grain number per ear did not decrease in Plainsman and the gliadin/glutenin ratio was higher than in the control in Emese during drought stress. A possible causal relationship between high ABA levels in the kernels during late stages of grain filling and a decreased grain yield was found in the sensitive cultivars during drought stress.

Keywords Abscisic acid (ABA) · Chlorophyll fluorescence · Drought stress · Flag leaves · Grain filling · Grain yield · Photosynthesis · Storage proteins · Wheat (*Triticum aestivum* L.)

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Introduction

The sensitivity of crop plants such as wheat (*Triticum aestivum* L.) to soil drought is particularly acute during the grain-filling period because the reproductive phase is extremely sensitive to plant water status. It was demonstrated by several authors that post-anthesis drought stimulated senescence of the whole plant in wheat and enhanced the remobilization of pre-anthesis stored carbohydrates from the straw and leaves to the grains (Yang and others 2001a, 2003). The primary signs of leaf senescence are the breakdown of chlorophyll (Chl) and the decline of photosynthetic activity (Yang and others 2001b; Gregersen and Holm 2007). It is generally accepted that genotypes that are able to sustain photosynthesis in the flag leaf for a longer time tend to yield more.

Because photosynthesis is one of the main metabolic processes determining grain yield in wheat (Araus and others 2002), Chl *a* fluorescence is frequently used to monitor the function of the photosynthetic apparatus in response to water stress (Flexas and others 2002;

Fracheboud and Leipner 2003). A number of authors considered that water-limiting conditions probably have no significant effects on the primary photochemistry of photosystem II (PSII) in wheat or durum wheat (Lu and Zhang 1999; Hura and others 2007), but other experiments indicated that the maximal quantum efficiency of PSII in dark-adapted leaves (F_v/F_m) decreased in flag leaves during severe drought stress (Paknejad and others 2007). Contradictory results were also presented concerning the effects of drought on other fluorescence induction parameters such as the actual quantum yield of PSII electron transport (Φ_{PSII}), the nonphotochemical quenching (NPQ) and the photochemical quenching coefficient (qP), or the ground-state fluorescence (F_0) (Lu and Zhang 1999; Czövek and others 2006; Hassan 2006; Tari and others 2008). Although chlorophyll fluorescence is considered a useful tool for screening wheat cultivars under dry conditions (Flagella and others 1995), its combination with other methods may provide a more accurate assessment of drought tolerance.

The plant hormone abscisic acid (ABA) plays a major role in the acclimation to water deficit during different types of stress facilitating survival by changing the physiology and growth of the plants (Zhang and others 2006; Christmann and others 2007). Our knowledge is far from complete as concerns the relationship between plant senescence and remobilization of assimilates into grains, but ABA is believed to be one of the major regulators of these processes (Tadas and others 1999). However, the involvement of ABA in the regulation of senescence and assimilate remobilization is controversial: it was demonstrated that exogenous application of ABA at anthesis increased the level of soluble carbohydrates in the shoots, which were transported to the grains at maturity (Travaglia and others 2007).

Soil drying increased ABA accumulation not only in the leaves but also in the grains, and peak values significantly correlated with maximal grain-filling rates. Exogenous ABA increased the chlorophyll loss, enhanced the remobilization of prestored carbon from the stem to the endosperm, and increased grain weight (Yang and others 2001a). There was also evidence of the inhibitory effect of ABA on grain growth (King 1976). In wheat the reduction in grain weight may be due to the reduction in endosperm cell number, that is, to a limited maximal storage capacity of the kernels (Setter and Flannigan 2001). ABA inhibited endosperm cell division (Myers and others 1990) and exogenous ABA applied after the cell division period inhibited the reserve deposition process. Continuously high ABA levels inhibited the total sucrose uptake by the grains, and hence the grain growth (Ahmadi and Baker 1999). Activities of the enzymes participating in starch biosynthesis in the endosperm, sucrose synthase, soluble starch synthase, and starch branching enzyme, were substantially enhanced during drought stress and showed strong positive correlations with ABA contents (Yang and others 2004). In contrast, wheat genotypes selected for ABA accumulation during drought stress were found to be no better or even worse than the average with respect to the yield during drought stress (Read and others 1991). Other studies demonstrated that drought decreased the kernel size by shortening the duration but not the rate of the grain-filling process (Kobata and others 1992; Altenbach and others 2003).

Environmental conditions may affect the accumulation of storage proteins (Daniel and Triboi 2000). It has been suggested that enhanced ABA levels were negatively related to starch content but favored storage protein accumulation (Xie and others 2003). The rates of accumulation of the different protein fractions were not significantly modified by drought during post-anthesis, but the duration of accumulation was significantly reduced (Panozzo and others 2001; Triboï and others 2003).

Yield is the most important economic trait, and grain production is the main selection criterion for drought resistance of wheat. There are several physiologic traits that are related to water stress, and considerable effort has been devoted to finding direct correlations between these parameters and grain yield to facilitate the screening and selection of cultivars for drought tolerance.

Despite its importance, very few studies have provided a complete comparison of the responses to soil drought of vegetative and reproductive organs of wheat genotypes with different degrees of tolerance to drought. Our aim was to combine investigations of flag leaf photosynthetic activity with studies of changes in ABA levels of flag leaves and grains during grain filling in two drought-tolerant and two drought-sensitive wheat genotypes to characterize the complex responses of the genotypes with high yield stability during water deficit. CO_2 fixation and Chl *a* fluorescence induction parameters were compared in drought-tolerant and drought-sensitive cultivars in well-watered and drought-stressed conditions, and it was observed how these water stress-induced changes in ABA contents affect the yield, storage protein fractions, and contents of the genotypes. We suggest that besides the sensitivity of the physiologic parameters of the vegetative organs, the sensitivity of the reproductive organs should also be taken into consideration because this is what ultimately determines crop production.

Materials and Methods

Plant Material and Water Stress Treatment

Two drought-tolerant (*Triticum aestivum* L. cv. Plainsman V and MV Emese) and two drought-sensitive (cv. GK Élet

and cv. Cappelle Desprez) winter wheat cultivars were investigated under well-watered and drought stress conditions. GK Élet and MV Emese were bred in Hungary (central Europe), Plainsman in the USA, and Cappelle Desprez in France. The experiments were carried out during the grain-filling period in a temperature-controlled greenhouse. The plants were grown in 5-dm^3 plastic pots (3 plants per pot) containing a mixture of soil (type Terra, Hungary) and sand (1:1 v/v) at a minimum light intensity of 300 μmol m^{-2} s^{-1}, under a 12/12-h day/night cycle, at a continuous day/night temperature of 25/20°C, respectively, and at 55-60% air humidity. Plants were exposed to water stress (WS) 4 days before the booting stage (the stage when the ligule becomes visible) (Zadoks and others 1974) by withholding irrigation. Plants were irrigated every 2 days to achieve 60 or 25% of the total soil water-holding capacity for the control and the water-stressed plants, respectively. Samples were prepared and in vivo measurements were made at the booting stage, on the day of anthesis, and on the 4th, 9th, 12th, 18th, 21st, and 24th days post-anthesis (DPA). At maturity, ears were harvested to determine the kernel weight, the number of kernels per spike, and the thousand-kernel weight. The experiments were carried out in 2005, 2006, and 2007. The data for 2007 are reported here.

Water Potential

The leaf water potential (Ψ_w) was measured on the penultimate leaf with a pressure chamber (PMS Instrument Co., Corvallis, WA, USA).

Pigment Analyses

The fully expanded flag leaves were homogenized in ice-cold 100% (v/v %) acetone (1.5 mL for 250-mg sample) and extracted for 24 h. Samples were centrifuged at 5,000*g* for 15 min at 4°C. The pellet was extracted again with 80% (v/v %) acetone (1.5 mL for 250-mg sample) for 24 h. After centrifugation (5,000*g*, 15 min, 4°C), the supernatants were collected. The pigment composition was measured with a double-beam spectrophotometer using the method of Lichtenthaler and Wellburn (1983). This method involves measurement of the light absorbed in the plant extract at 470, 646.8, and 663.2 nm.

Measurement of Chlorophyll Fluorescence and Photosynthesis

Chl *a* fluorescence and the net photosynthetic rate (P_N) were measured on flag leaves using a portable photosynthesis system (LI-6400, LI-COR, Inc., Lincoln, NE, USA). The leaves were maintained within a leaf chamber during the measurements. External air was scrubbed of CO_2 and mixed with a supply of pure CO_2 to create a reference concentration of 360 μmol m^{-2} s^{-1}. The flow rate was set to 500 μmol m^{-2} s^{-1}, and the external humidity was 60-70%. The temperature inside the leaf chamber was maintained at 25°C. After 20 min of dark adaptation, the ground-state fluorescence level (F_0) was determined with modulated measuring light which was sufficiently low to not induce photochemical reaction. The maximal fluorescence level (F_m) was measured after 20 min of dark adaptation by applying a 0.2-s saturating pulse at 8,000 μmol m^{-2} s^{-1}. The leaves were then continuously illuminated with white actinic light at an intensity of 500 μmol m^{-2} s^{-1}. After 20 min the steady-state value of fluorescence (F_s) was recorded and the maximal fluorescence level (F_m') in the light-adapted state was determined with a second saturating pulse (8,000 μmol m^{-2} s^{-1}). Next, the actinic light was turned off and the minimal fluorescence level in the light-adapted state (F_0') was determined by illuminating the leaf with far-red light for 3 s (5 μmol m^{-2} s^{-1}). The CO_2 assimilation rate was determined together with the chlorophyll fluorescence parameters after 20 min of light adaptation. The maximal quantum yield of PSII photochemistry, F_v/F_m, the photochemical quenching coefficient $qP = (F_m' - F_s)/(F_m' - F_0')$ (Bilger and Schreiber 1986), the nonphotochemical quenching $NPQ = (F_m/F_m' - 1)$ (Bilger and Björkman 1990), and the actual quantum yield of PSII electron transport in the light-adapted state [$\Phi_{PSII} = (F_m' - F_s)/F_m'$] (Genty and others 1989) were determined.

ABA Extraction and Quantification

Samples were purified and quantified and recovery was determined as described by Yang and others (2003). Briefly, samples of 500 mg of leaf tissue or 30-500 mg of grain tissue were ground in a mortar (at 0°C) in 2.5 ml of 80% (v/v) methanol extraction medium containing 1 mg of butylated hydroxytoluene as an antioxidant. Samples were dissolved in Tris-buffered saline (TBS) (25 mM Tris, 100 mM NaCl, 1 mM $MgCl_2$ hexahydrate, and 3 mM imidazole, pH 7.5) (1,500 μL/100 mg sample). ABA was analyzed by indirect enzyme-linked assay (ELISA), using a Phytodetek Assay Kit (Idetek, supplied by Sigma Ltd.). The color absorbency following reaction with the substrate was read at 405 nm using a plate autoreader (Dynatech MR 4000). The percentage binding was calculated by established procedures (Weiler and others 1981). For the recovery test, 200 pmol of ABA (Sigma-Aldrich, St. Louis, MO, USA) was added to 1 g of fresh plant sample before purification. The method used in this study for ABA extraction, purification, and quantification in flag leaves and in kernels by ELISA led to a recovery of more than 70%.

Determination of Storage Proteins

The quantitative analysis of gliadins and glutenins was performed by reversed-phase high-performance liquid chromatography (RP-HPLC) after extraction. The extraction of gliadins and glutenins, the sample preparation, and the separation of these proteins by RP-HPLC were carried out according to the method of Abonyi and others (2007).

Statistical Analysis

Ψ_w and photosynthetic parameters were measured on the leaves of five randomly chosen plants, and the pigment and ABA contents were determined in three parallel samples. Data presented in the figures are the means ± standard deviation (SD) of three to five replications. Data from each sampling date were analyzed separately by Student's t test using SigmaStat 3.1 software. Means denoted by *, **, or *** proved significant at levels $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Results

Plant Water Status

Withholding irrigation resulted in reduction in Ψ_w in both drought-sensitive and -tolerant genotypes; the reduction was more pronounced in the drought-sensitive cultivars Élet and Cappelle Desprez. During drought stress, Ψ_w was lower, between 4 and 12 DPA, in Élet and Cappelle Desprez than in the tolerant Emese. In the sensitive cultivars, Ψ_w decreased and remained near −1.5, whereas in the more tolerant Emese, Ψ_w reached −1.3 just at 12 DPA. In Plainsman, there was a slight decrease in Ψ_w at 12 DPA during water stress, but Ψ_w of control plants also decreased up to 24 DPA (Fig. 1).

Pigment Content

Chl $a + b$ contents did not decrease markedly in response to WS until 12 DPA, and no significant changes were found between the Chl $a + b$ contents until the late measuring days (Fig. 2). However, the lower Chl contents were measured in well-watered flag leaves of the drought-sensitive Cappelle Desprez. Carotenoid (Car) content was decreased significantly only at 24 DPA in all genotypes (data not shown). The senescence process started earlier in control plants of Plainsman than in those of Emese and Élet.

Photosynthetic Rate and Chlorophyll *a* Fluorescence

P_N and fluorescence parameters were measured on the flag leaves during grain filling. The last measuring day, scheduled for day 24 after flowering, was moved to days 18 and 21 for the stressed plants of Emese and Élet, respectively, due to their earlier senescence. It was found that the carbon assimilation activity of flag leaves ceased earlier in the tolerant Emese and in the sensitive Élet during drought; P_N exhibited a more moderate decline in both control and water-stressed plants in the other two genotypes.

F_v/F_m remained at around 0.8 until 12 DPA in all varieties and decreased only afterward during water deficit (data not shown). During drought stress, there were no significant decreases in Φ_{PSII} and qP until 12 DPA in all four genotypes compared to controls, and, with the exception of Plainsman, the decline in the stressed plants became significant only at the end of the grain filling. NPQ was increased significantly in Élet and Cappelle Desprez at 21 and 24 DPA, and in Emese it remained similar to the control. In Plainsman, the values of F_v/F_m and all of the other fluorescence induction parameters changed in control leaves parallel with those in stressed plants (Fig. 3).

Endogenous ABA Levels in Flag Leaves and Grains

Relative to the control, endogenous ABA levels in the flag leaves increased significantly after water withdrawal and remained high at anthesis in all genotypes; later, the hormone concentrations decreased markedly. ABA levels in Emese, Élet, and Plainsman exhibited a second maximum at the end of the experiment, whereas in Cappelle Desprez the content remained as low as in the control plants. The tendencies were similar in three varieties (Emese, Plainsman, and Élet), but higher ABA concentrations were determined in Emese and Plainsman than in Élet during water deficit (Fig. 4).

The ABA level in the grains was increased significantly in all genotypes exposed to water stress at 9 DPA. In drought-tolerant cultivars Emese and Plainsman, the highest levels were measured at 9 DPA during the experimental period; the hormone levels then decreased rapidly until full maturity. In contrast, in sensitive cultivars Élet and Cappelle Desprez, the ABA concentration remained high up to the end of grain filling. ABA accumulated during the second half of the grain-filling period in the grains of well-watered controls of Emese, Plainsman, and Cappelle Desprez (Fig. 5).

Yield Parameters and Storage Protein Contents

The number of kernels per spike, the kernel weight per spike, and the thousand-kernel weight decreased during

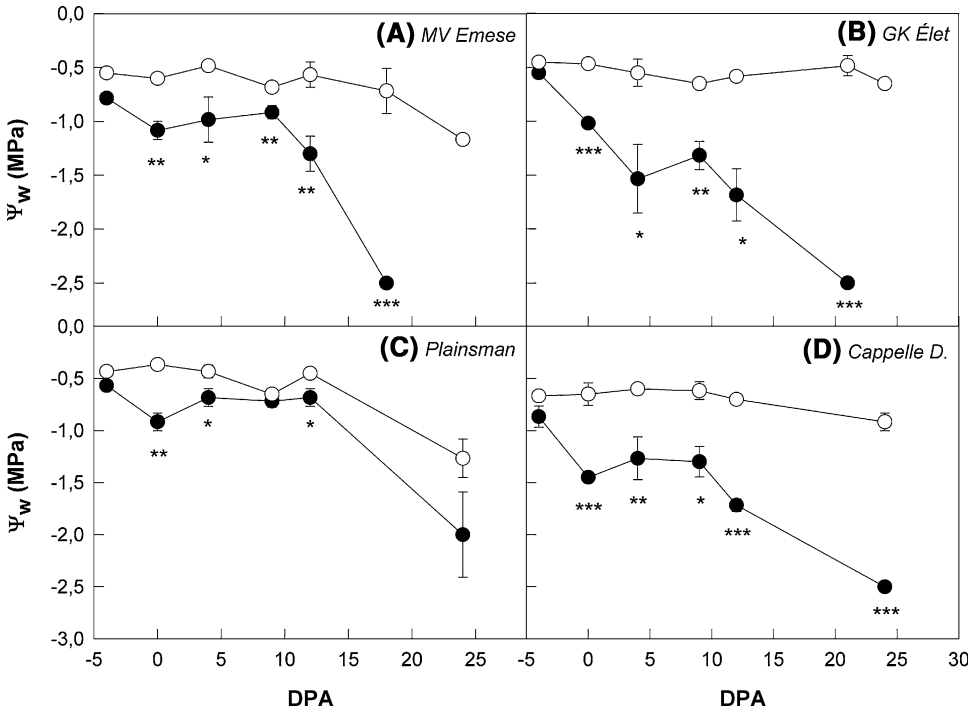

Fig. 1 Changes in water potential (Ψ_w) in well-watered (○) and water-stressed (●) leaves during grain filling in MV Emese (**a**), GK Élet (**b**), Plainsman (**c**), and Cappelle Desprez (**d**) wheat cultivars. Data are means ± SD of three to five independent samples. SD bars are not shown where they are smaller than symbols. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

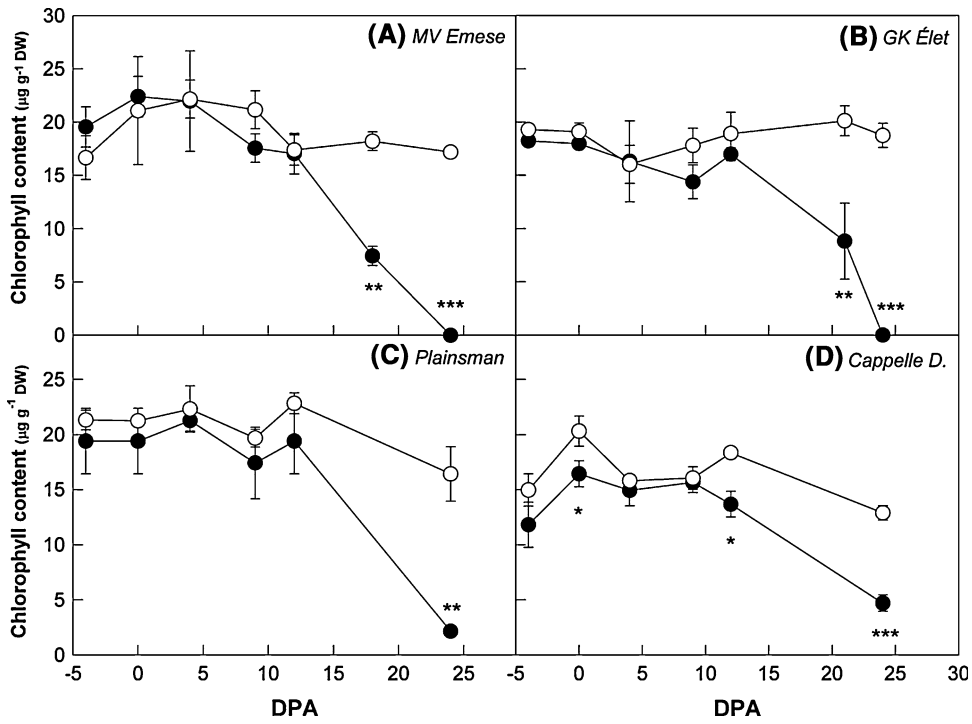

Fig. 2 Changes in chlorophyll ($a + b$) content expressed on a dry weight basis in well-watered (○) and water-stressed (●) flag leaves during grain filling in MV Emese (**a**), GK Élet (**b**), Plainsman (**c**), and Cappelle Desprez (**d**) wheat cultivars. Data are means ± SD of three to five independent samples. SD bars are not shown where they are smaller than symbols. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

water deficit in Emese, Élet, and Cappelle Desprez. Differences between the tolerant Emese and the sensitive varieties were significant, and the yield parameters of Élet and Cappelle Desprez declined at a higher rate than those for Emese. In Plainsman, the crop yield did not decrease significantly during drought stress (Table 1).

Amounts of gliadins and high-molecular-weight (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS) in the mature seeds did not change markedly during drought stress, although considerable differences were detected between genotypes. A significant difference could be observed only in relative amounts of LMW-GSs in Emese

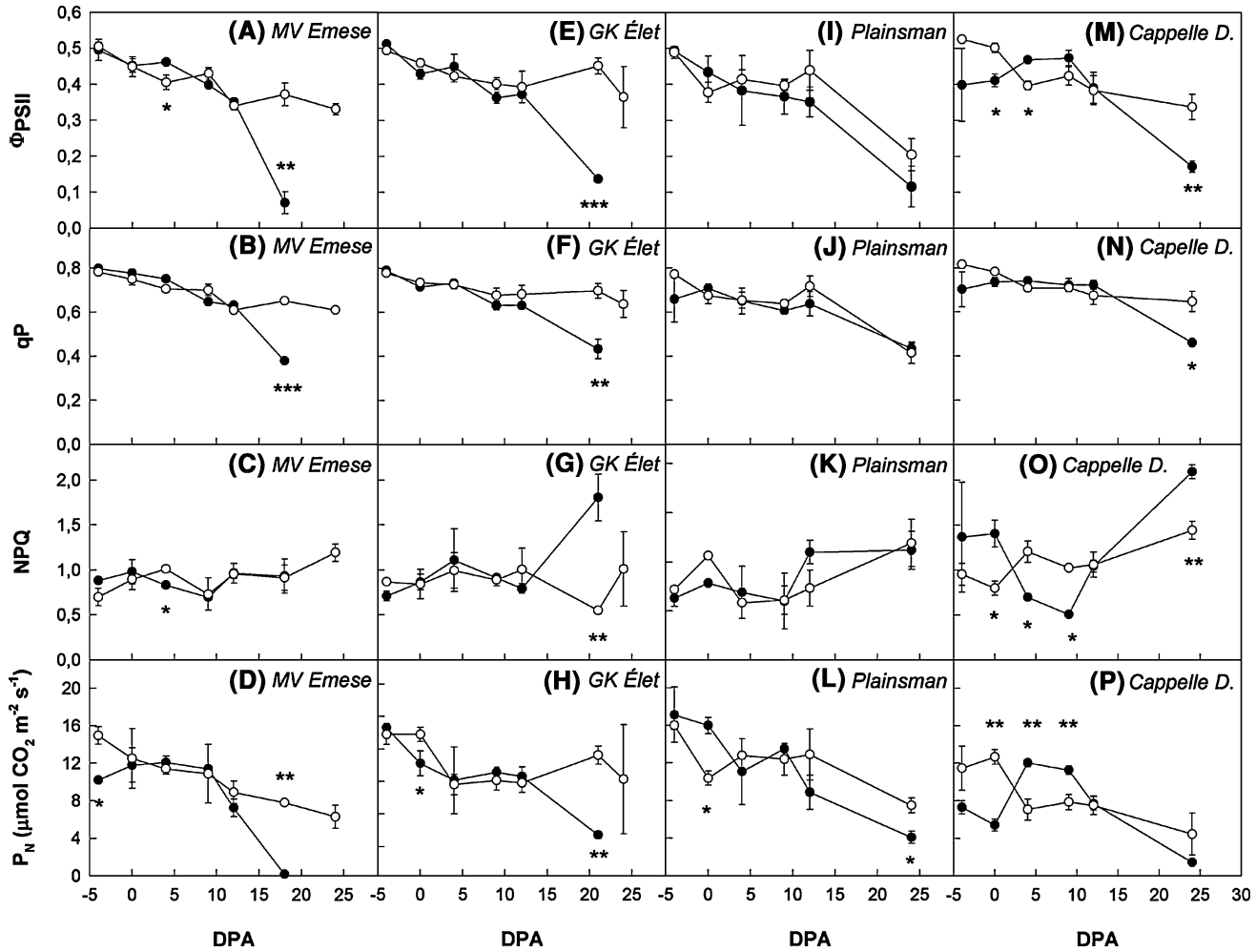

Fig. 3 Changes in the actual quantum yield of PSII photochemistry (Φ_{PSII}), photochemical quenching parameter (qP), nonphotochemical quenching (NPQ), and net photosynthetic rate (P_N) in well-watered (○) and water-stressed (●) flag leaves during grain filling in MV Emese (**a**–**d**), GK Élet (**e**–**h**), Plainsman (**i**–**l**), and Cappelle Desprez (**m**–**p**) wheat cultivars. Data are means ± SD of three to five independent samples. SD bars are not shown where they are smaller than symbols. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

and Plainsman, but the gliadin/glutenin ratio increased in the kernels of the stressed Emese plants (Table 2).

Discussion

In this work we monitored two sensitive and two tolerant wheat cultivars exposed to water stress during the grain-filling period by comparing the physiologic parameters used most frequently for screening drought resistance, namely, photosynthetic performance and ABA accumulation of flag leaves, as well as ABA changes and storage protein accumulation in the grains.

The yield parameters of the sensitive Élet and Cappelle Desprez declined much more than those of the tolerant Emese and Plainsman genotypes after irrigation withdrawal. While the environmental conditions were optimal, Élet and Cappelle Desprez performed well or even better than Plainsman; during severe drought Plainsman exhibited very high yield stability.

Ψ_w in the leaves of the tolerant Emese and of the two sensitive genotypes decreased significantly in response to water deficit, but Élet and Cappelle Desprez reached lower Ψ_w values much earlier after water withdrawal, indicating that these plants respond to soil drought with a faster decrease in Ψ_w than do tolerant genotypes. In Plainsman, however, drought affected the plant water status less significantly.

The significant changes in P_N, Φ_{PSII}, qP, and NPQ during drought stress can be explained by earlier senescence, as drought may promote whole-plant senescence in monocarpic plants (Yang and Zhang 2006). Earlier senescence during water deficit in sensitive and tolerant varieties was indicated by the earlier decline in pigment content, CO_2 fixation, and decline of the photosynthetic electron transport in the chloroplast, revealed by decreased Φ_{PSII}

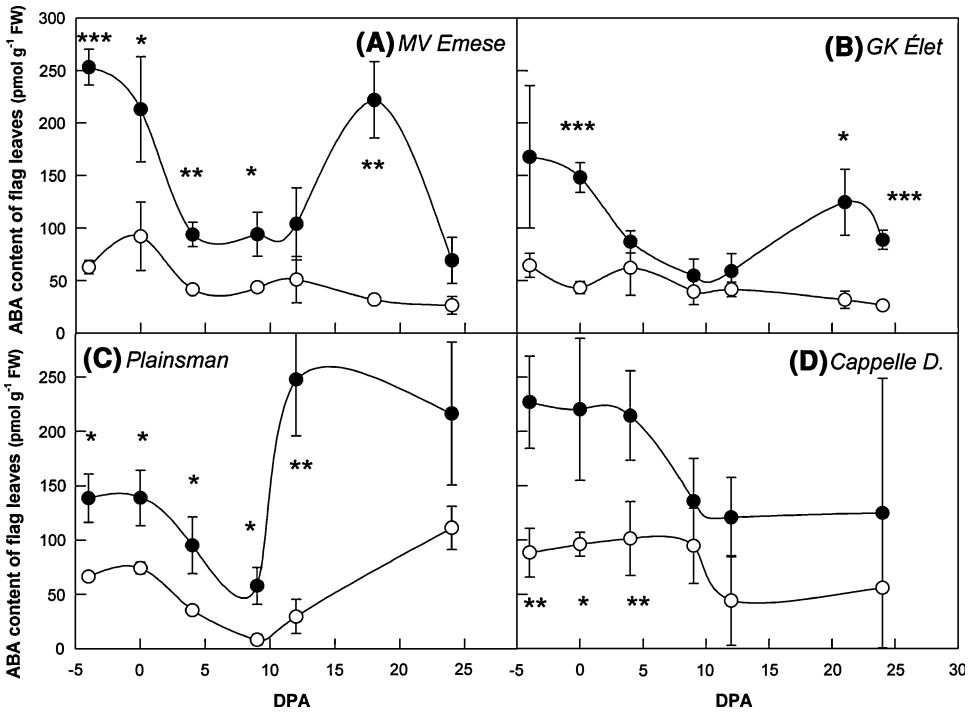

Fig. 4 Changes in the ABA content in well-watered (○) and water-stressed (●) flag leaves during grain filling in MV Emese (**a**), GK Élet (**b**), Plainsman (**c**), and Cappelle Desprez (**d**) wheat cultivars. Data are means ± SD of three to five independent samples. SD bars are not shown where they are smaller than symbols. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

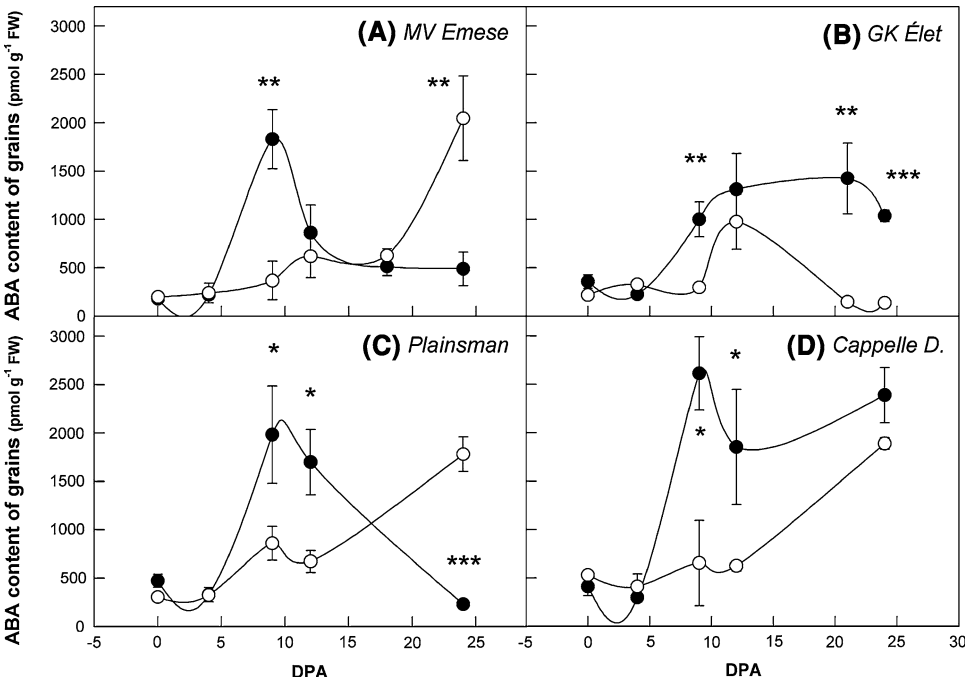

Fig. 5 Changes in the ABA content in well-watered (○) and water-stressed (●) grains during grain filling in MV Emese (**a**), GK Élet (**b**), Plainsman (**c**), and Cappelle Desprez (**d**) wheat cultivars. Data are means ± SD of three to five independent samples. SD bars are not shown where they are smaller than symbols. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

and qP. The control plants of the tolerant Plainsman also demonstrated symptoms of faster senescence.

In spite of the sustained CO_2 fixation activity of flag leaves, the filling of grains was incomplete and led to severe shriveling of the grains in the sensitive cultivars during water stress. This may be a consequence of the limited utilization of stem reserves. In contrast with several other findings (Hassan 2006; Li and others 2006; Paknejad and others 2007), these data suggest that the Chl *a* fluorescence induction parameters changed continuously in flag leaves during grain filling and did not characterize the sensitivity of the genotypes to drought.

ABA contents of the flag leaves in water-stressed plants in the booting stage were markedly higher than in the controls in all genotypes, indicating that this hormone is a signal in response to an extreme soil water status (Davies

Table 1 Effect of soil drought on the final number of kernels per spike, the kernel weight per spike, the thousand-kernel weight, and the total yield of the experiment (yield of 10 ears) of MV Emese, GK Elet, Plainsman, and Cappelle Desprez wheat cultivars

Variety	Treatment	Number of grains per ear	Mass of grains per ear (g)	1,000 grain dry mass (g)	Total grain yield (g)
MV Emese	Control	27.73 ± 8.0	1.90 ± 0.6	62.36 ± 1.4	19.0
	Drought	19.33 ± 4.3** (−30.29%)	0.75 ± 0.1*** (−60.52%)	47.74 ± 6.4** (−23.44%)	7.5 (−60.52%)
GK Élet	Control	31.42 ± 5.1	1.68 ± 0.3 49	30 ± 9.54	16.8
	Drought	16.18 ± 6.9*** (−48.50%)	0.37 ± 0.1*** (−77.97%)	27.46 ± 7.7** (−44.30%)	3.7 (−77.97%)
Plainsman V.	Control	17.34 ± 8.4	0.64 ± 0.4	38.01 ± 8.9	6.4
	Drought	15.80 ± 4.5 (−8.91%)	0.58 ± 0.1 (−9.41%)	36.48 ± 3.9 (−4.01%)	5.8 (−9.41%)
Cappelle D.	Control	27.71 ± 7.6	1.94 ± 0.7	74.36 ± 5.4	19.4
	Drought	15.85 ± 4.3*** (−42.78%)	0.87 ± 0.4** (−55.10%)	36.78 ± 13.3*** (−50.53%)	8.7 (−55.10%)

Numbers in parentheses indicate the percentage of decrease compared to control

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 2 Relative amount of storage proteins and the gliadin/glutenin ratio in mature grains of MV Emese, GK Élet, Plainsman, and Cappelle Desprez wheat cultivars

Triticum aestivum L. cultivar	Treatment	Gliadin[a]	HMW-GS[a]	LMW-GS[a]	Gliadin/glutenin
cv. MV Emese	Control	46.36 ± 5.1	11.52 ± 1.6	17.05 ± 0.6	1.61 ± 0.1
	Drought	52.21 ± 1.1	13.78 ± 0.4	13.78 ± 0.4**	2.10 ± 0.2*
cv. GK Élet	Control	38.66 ± 4.7	20.28 ± 3.6	16.80 ± 0.7	1.05 ± 0.2
	Drought	41.26 ± 4.6	19.01 ± 1.27	19.01 ± 1.2	1.11 ± 0.1
cv. Plainsman	Control	29.65 ± 8.0	13.49 ± 0.6	17.82 ± 1.6	0.95 ± 0.2
	Drought	38.50 ± 4.9	16.34 ± 2.3	23.55 ± 1.8*	0.96 ± 0.0
cv. Cappelle D.	Control	45.30 ± 2.4	7.93 ± 0.8	12.59 ± 1.4	2.22 ± 0.2
	Drought	37.04 ± 8.9	12.59 ± 1.4	12.21 ± 1.0	1.75 ± 0.3

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

[a] Indicates 10^6 absorbance units (AU) corresponding to 500 mg of flour

and Zhang 1991), but a hydraulic signal can precede ABA signaling (Christmann and others 2007).

ABA levels in flag leaves of Emese and Plainsman during drought stress, at the end of the grain-filling period, were higher than in the sensitive Élet and Cappelle, which can explain better yield parameters as ABA can regulate assimilate remobilization into the grains (Tadas and others 1999; Travaglia 2007).

Lower ABA concentrations in the flag leaves coincide with ABA accumulation in the grains; this can probably be explained by the transport of the hormone from flag leaves to the ears. High ABA levels at the end of the experiment imply that this hormone is also responsible for senescence acceleration in the tolerant genotypes (Noodén and others 1997; Yang and others 2002). Our data clearly show that ABA contents can vary from day to day during post-anthesis drought stress. This result contrasts with the data of Read and others (1991) who found that there was a negative correlation with the bulk-leaf ABA content and grain yield during drought stress in field experiments.

In our experiment, obvious differences between the sensitive and tolerant genotypes were found in the changes of ABA content of grains during drought stress. ABA in the grain may result from de novo synthesis within the grain and from translocation from leaves and/or roots (Ober and others 1991). Because water stress increases the ABA level of the flag leaf and ABA is highly mobile in the phloem, translocation of ABA in the phloem could contribute to the higher ABA content of grains in all varieties (Ahmadi and Baker 1999). During water deficit in Emese and Plainsman, a maximal ABA content was measured at 9 DPA, and the rapid rise was followed by a rapid decline. In contrast, in the sensitive Élet and Cappelle Desprez, the ABA content was increased at 9 DPA too, but it remained high throughout the following phases of grain filling, allowing it to influence the accumulation of food reserves in the endosperm and hasten grain dormancy and programmed death of the starchy endosperm cells.

Grain crops display a sensitivity to drought during floral initiation and the premeiotic differentiation of floral parts, and water deficit can additionally cause damage in the male gametophyte (Saini 1997; Barnabás and others 2008), resulting in a lower number of kernels per spike. ABA can also cause a reduction in grain growth, possibly through a

reduction in endosperm cell numbers, limiting the maximal storage capacity (Myers and others 1990), or through its inhibitory effect on the transport of ^{14}C-sucrose (Borkovec and Prochazka 1992; Tietz and others 1981). Other studies indicated that continuously high ABA levels after the cell division period exerted an inhibitory effect on grain filling and grain growth (Ahmadi and Baker 1999). In our experiment, significantly lower grain masses in Élet and Cappelle Desprez may be caused by the constantly high ABA levels in the grains from 9 to 24 DPA.

The LMW-GS level increased significantly in Emese and Plainsman as did the gliadin/glutenin ratio in Emese. High temperature and nitrogen fertilizers were reported to increase the gliadin/glutenin ratio, which correlated with poorer dough quality (Dupont and Altenbach 2003). The different protein fractions were not significantly modified by drought, which confirms earlier results (Panozzo and others 2001; Triboï and others 2003). The enhanced ABA levels at 9–12 DPA in each genotype may favor the accumulation of storage proteins, which may explain the similar storage protein contents in the grains of the control and stressed plants.

Our results indicate that for an accurate assessment of the sensitivity of a genotype to drought stress, the responses of the whole plant, including the vegetative and reproductive organs must be taken into consideration. Conclusions drawn from the physiologic parameters of the vegetative parts offer only limited information concerning the sensitivity of the genotypes in terms of agriculture. We found that the sensitivity of reproductive organs and the continuously high ABA levels in the kernels coincided and may be responsible for a reduced grain yield, whereas the earlier ABA peak in the grains and senescence of flag leaves led to better yield components.

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