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Antioxidative Defense System, Pigment Composition, and Photosynthetic Efficiency in Two Wheat Cultivars Subjected to Drought[1]

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We analyzed antioxidative defenses, photosynthesis, and pigments (especially xanthophyll-cycle components) in two wheat (*Triticum durum* Desf.) cultivars, Adamello and Ofanto, during dehydration and rehydration to determine the difference in their sensitivities to drought and to elucidate the role of different protective mechanisms against oxidative stress. Drought caused a more pronounced inhibition in growth and photosynthetic rates in the more sensitive cv Adamello compared with the relatively tolerant cv Ofanto. During dehydration the glutathione content decreased in both wheat cultivars, but only cv Adamello showed a significant increase in glutathione reductase and hydrogen peroxide-glutathione peroxidase activities. The activation states of two sulfhydryl-containing chloroplast enzymes, $NADP^+$-dependent glyceraldehyde-3-phosphate dehydrogenase and fructose-1,6-bisphosphatase, were maintained at control levels during dehydration and rehydration in both cultivars. This indicates that the defense systems involved are efficient in the protection of sulfhydryl groups against oxidation. Drought did not cause significant effects on lipid peroxidation. Upon dehydration, a decline in chlorophyll *a*, lutein, neoxanthin, and β-carotene contents, and an increase in the pool of de-epoxidized xanthophyll-cycle components (i.e. zeaxanthin and antheraxanthin), were evident only in cv Adamello. Accordingly, after exposure to drought, cv Adamello showed a larger reduction in the actual photosystem II photochemical efficiency and a higher increase in nonradiative energy dissipation than cv Ofanto. Although differences in zeaxanthin content were not sufficient to explain the difference in drought tolerance between the two cultivars, zeaxanthin formation may be relevant in avoiding irreversible damage to photosystem II in the more sensitive cultivar.

A consequence of the drought-induced limitation of photosynthesis is the exposure of plants to excess energy, which, if not safely dissipated, may be harmful to PSII because of overreduction of reaction centers (Demmig-Adams and Adams, 1992) and increased production of reactive oxygen species in the chloroplasts (Smirnoff, 1993).

To counteract the toxicity of active oxygen species, a highly efficient antioxidative defense system, including both nonenzymic and enzymic constituents, is present in plant cells. A relevant defense system is represented by glutathione, which protects many cellular components and the thiol status of proteins against oxidative stress (Gilbert et al., 1990). GSH may also metabolize hydrogen peroxide by participating in the ascorbate/glutathione cycle or in the reaction catalyzed by GP (EC 1.11.1.9) (Drotar et al., 1985). Hydrogen peroxide is especially toxic in the chloroplasts because even at low concentrations it inhibits the Calvin-cycle enzymes possessing exposed sulfhydryl groups, such as G3PDH (EC 1.2.1.13) and FBPase (EC 3.1.3.11), hence reducing the photosynthetic carbon dioxide assimilation (Takeda et al., 1995). The glutathione system is efficient provided that GSSH is rapidly reduced to GSH by GR (EC 1.6.4.2). Furthermore, GP and GT (EC 2.5.1.18) reduce organic peroxides, thus protecting cell proteins and membranes from oxidation (Bartling et al., 1993; Navari-Izzo and Izzo, 1994).

It is now well documented that carotenoids are involved in the protection of the photosynthetic apparatus against photoinhibitory damage by singlet oxygen ($^1O_2$), which is produced by the excited triplet state of chlorophyll. Carotenoids can directly deactivate $^1O_2$ and can also quench the excited triplet state of chlorophyll, thus indirectly reducing the formation of $^1O_2$ species (Sieferman-Harms, 1987; Foyer and Harbinson, 1994). On the other hand, it is now widely accepted that zeaxanthin is involved in the de-excitation of excess energy via nonradiative dissipation in the pigment bed (Demmig-Adams and Adams, 1992). This process is associated with the de-epoxidation of violaxanthin to zeaxanthin and with the development of a transthylakoidal ΔpH. There is increasing evidence that zeaxanthin, and perhaps the intermediate antheraxanthin, may be responsible for the development of nonradiative energy dissipation (Demmig-Adams and Adams, 1992; Pfündel and Bilger, 1994; Horton et al., 1996; Gilmore, 1997; Niyogi et al., 1998).

Among crop plants, durum wheat (*Triticum durum*), which is often grown in water-limited conditions, is an attractive study system because of the natural genetic variations in traits related to drought tolerance (Labhilili et al., 1995).

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Abbreviations: FBPase, Fru-1,6-bisphosphatase; GP, glutathione peroxidase; GR, glutathione reductase; GT, glutathione transferase; G3PDH, $NADP^+$-dependent glyceraldehyde-3-phosphate dehydrogenase; Ψ_w, water potential.

The objective of this study was to investigate possible mechanisms responsible for differential drought tolerance in two wheat cultivars, Adamello and Ofanto. The effects of drought were studied to elucidate the mechanisms that confer protection from oxidative stress and photoinhibition and to analyze the recovery after rehydration. For these purposes we studied the hydrogen peroxide content and the glutathione system upon dehydration and rehydration. We also tested the hypothesis that GSH may protect the sulfhydryl groups of proteins, as was previously found in *Boea hygroscopica* (Navari-Izzo et al., 1997), by monitoring the activities of the chloroplast sulfhydryl enzymes G3PDH and FBPase. The effects of dehydration on photosynthetic efficiency, energy utilization and dissipation, and pigment composition were also studied. Special emphasis was given to the role of zeaxanthin in photoprotection under conditions of drought.

MATERIALS AND METHODS

Plant Material

Seedlings of two durum wheat (*Triticum durum* Desf.) cultivars, Adamello, which is more sensitive to drought, and Ofanto, which is less sensitive to drought (Quartacci et al., 1995), were grown under fully irrigated conditions and under drought conditions. Seeds were provided by the Istituto Sperimentale per la Cerealicoltura (Foggia, Italy).

Wheat seedlings were grown in 10-L pots containing a mixture of garden soil and sand (1:1, v/v) from January to March in a greenhouse. The mean daily fluence on the upper leaves was 38 mol photons m^{-2}, with the peak of PPFD around 1700 μmol m^{-2} s^{-1}. The mean air temperature was 10°C and the mean RH was 70%. Starting from 30 d after sowing, two watering treatments were applied: one group of plants was continuously maintained under optimal irrigation (control) and a second group was subjected to drought by omitting irrigation for 35 d (until 65 d after sowing). Subsequently, droughted plants were re-irrigated for 4 d and the recovery was studied. Plants were harvested at 65, 66, and 69 d after sowing. Plants were harvested and samples were collected at 9 AM, with a PPFD of about 200 μmol m^{-2} s^{-1}. Roots and shoots were separated, the fresh weights of shoots were recorded, and samples were taken for dry weight measurements and pigment analysis. Plants (30 for each treatment) were randomly collected. Ψ_w was determined on leaves using a pressure chamber.

Hydrogen Peroxide Determination

Hydrogen peroxide concentration was evaluated as described by Sgherri et al. (1994a). This method is very sensitive and reproducible, and excludes the interference of other peroxides (except for a small effect of lipid peroxide). A standard curve in the 0- to 350-μM range was used.

GSH and GSSH

Fresh leaf tissue (0.5 g) was homogenized in ice-cold 5% sulfosalicylic acid (w/v), centrifuged at 12,100*g* for 15 min, and the supernatant was used for total and GSSH determinations by the 5,5′-dithio-bis-(2-nitrobenzoic acid)/GSSH reductase recycling procedure, as described previously (Sgherri and Navari-Izzo, 1995). GSSH was determined after removal of GSH by 2-vinylpyridine derivatizations. Changes in absorbance of the reaction mixture were measured at 412 nm at 25°C, and the contents of total glutathione and GSSH were calculated as previously described (Sgherri et al., 1994b). GSH was determined by subtraction of GSSH (as GSH equivalents) from the total glutathione content.

Enzyme Extraction and Activity Determination

The overall procedure was carried out at 0°C to 4°C. Leaf tissue was ground in a chilled mortar using different specific buffers and pH values for each enzyme. Homogenates were squeezed through two layers of muslin, and centrifuged at 12,100*g* for 20 min. Supernatants obtained were used for enzyme determination. Conditions for all assays were chosen so that the rate of reaction was constant for the entire experimental period and proportional to the amount of enzyme added. Proteins were determined according to the method of Bensadoun and Weinstein (1976), using BSA as a standard.

GT

The extraction buffer was 100 mM potassium phosphate, pH 7.0, containing 1 mM Na_2EDTA and 4% (w/v) Polyclar AT (BDH Chemicals Ltd., Poole, UK). The GT activities were assayed as previously described (Navari-Izzo and Izzo, 1994). The formation of the conjugate between GSH and 1-chloro-2,4-dinitrobenzene was monitored at 340 nm, and the activity was calculated using an extinction coefficient of the conjugate of 9.6 mM^{-1} cm^{-1}.

GP

Extraction of GP was with the same extraction buffer used for GTs. The GP activity was assayed according to the method described by Navari-Izzo et al. (1997), but modified by using lower peroxide concentrations (0.22 mM). Reaction was initiated by the addition of hydrogen peroxide or *t*-butyl hydroperoxide, and the decrease in A_{340} was monitored. The enzyme activity was calculated using the extinction coefficient of 6.2 mM^{-1} cm^{-1}. Blank values, obtained without the addition of samples, were subtracted from the assay values.

GR

Extraction of GR was with the same extraction buffer used for GTs, and its activity was determined following the procedure of Sgherri et al. (1994b), measuring the decrease in A_{340} and using the extinction coefficient of 6.2 mM^{-1} cm^{-1}. The assay mixture was maintained at 30°C and contained 0.2 M potassium phosphate, pH 7.5, 0.2 mM Na_2EDTA, 1.5 mM $MgCl_2$, 0.25 mM GSSH, 25 μM NADPH, and 50 μL of enzyme extract in a 1-mL final volume. The

reaction was initiated by the addition of NADPH, and corrections for GSSH-independent NADPH oxidation were not necessary.

G3PDH

Extraction of G3PDH was in 100 mM Tris-HCl, pH 8.1, containing 0.1 mM Na_2EDTA and 4% (w/v) Polyclar AT. To avoid oxidation of the sulfhydryl group, the solution was depleted in oxygen under vacuum and all extractions were carried out under a nitrogen atmosphere. The activity was determined according to the method of Harten and Eickmeier (1986), with slight modifications. The decrease in A_{340} was measured, and the enzyme activity was calculated using an extinction coefficient of 6.2 mM^{-1} cm^{-1}. Initial activity was assayed immediately after homogenization. Total activity was assayed on aliquots of enzyme extract incubated for 20 min with 20 mM DTT. The assay mixture for initial and total activities was maintained at 25°C, and consisted of 100 mM Tris-HCl, pH 8.1, containing 5 mM $MgCl_2$, 2 mM ATP, 1 mM 3-phosphoglyceric acid, 0.06 unit of 3-phosphoglyceric phosphokinase from bakers' yeast (Sigma), 0.14 mM NADPH, and 20 μL of enzyme extract in a 1-mL final volume. The reaction was initiated by the addition of NADPH.

Chloroplast FBPase

The extraction and assay conditions utilized were specific for the chloroplastic isoform of the enzyme (Hurry et al., 1995). The extraction of FBPase was performed in 100 mM Tris-HCl, pH 8.0, containing 1 mM Na_2EDTA, 10 mM $MgCl_2$, and 4% (w/v) Polyclar AT. To avoid oxidation of the sulfhydryl group during the extraction and determination, the same conditions outlined for G3PDH were used. The activity was determined by monitoring the increase in A_{340} using an extinction coefficient of 6.2 mM^{-1} cm^{-1} (Takeda et al., 1995). Initial activity was assayed immediately after homogenization. Total activity was assayed on aliquots of enzyme extract incubated for 20 min with 20 mM DTT. The assay mixture for initial and total activities, maintained at 25°C, consisted of 100 mM Tris-HCl, pH 8.0, containing 0.5 mM Na_2EDTA, 10 mM $MgCl_2$, 0.3 mM $NADP^+$, 0.6 mM Fru-1,6-bisP, 0.6 unit of Glc-6-P dehydrogenase from bakers' yeast (Sigma), 1.2 units of Glc-P isomerase from bakers' yeast (Sigma), and 100 μL of enzyme extract in a 1-mL final volume. The reaction was initiated by the addition of enzyme extract.

Lipid Extraction and Peroxide Analysis

Leaf tissue was boiled in isopropanol and lipids were extracted immediately, as described by Navari-Izzo et al. (1991). Two milliliters of lipid extract in chloroform was added to a solution of 5 mL of ethanol, 0.2 mL of 1 M HCl, and 0.1 mL of 1% (w/v) ammonium ferrous sulfate (Droillard et al., 1987). After 30 s, 1 mL of 20% (w/v) ammonium ferrous thiocyanate was added, and the A_{480} was read 3 min later. A calibration curve with *t*-butyl hydroperoxide (0.6–12 μM) was used for quantification.

Pigment Analysis

Pigment extraction and HPLC analysis were conducted on 0.85-cm^2 leaf discs taken with a cork borer from fully expanded, exposed leaves, as described by Brugnoli et al. (1994). The degree of de-epoxidation of xanthophyll-cycle components was expressed as (Z+A)/(V+A+Z), where Z is zeaxanthin, A is antheraxanthin, and V is violaxanthin.

Photosynthesis Measurements and Chlorophyll Fluorescence

At the end of the drought period, chlorophyll fluorescence measurements were taken on fully expanded, exposed leaves using a modulated fluorometer (PAM 101, Walz, Effeltrich, Germany), as previously described (Brugnoli and Björkman, 1992). Droughted and control leaves were predarkened for 30 min before starting experiments. The PPFD of the saturation pulses to determine the maximal fluorescence emission in the presence (F_m') and in the absence (F_m) of quenching on the upper surface of the leaf was 9500 μmol m^{-2} s^{-1}, whereas the actinic light was 900 μmol m^{-2} s^{-1}. The photon yield of PSII (Φ_{PSII}) in the light was determined as $\Phi_{PSII} = (F_m' - F)/F_m'$ (Genty et al., 1989) after about 45 min of illumination, when steady state was achieved. Stern-Volmer nonphotochemical quenching was determined as $F_m/F_m' - 1$ (Bilger and Björkman, 1990). Fluorescence nomenclature is according to van Kooten and Snel (1990).

Gas-exchange measurements were taken on the same leaves used for fluorescence measurements using an open-flow gas-exchange system, as previously reported by Brugnoli and Lauteri (1991).

Table I. *Effects of dehydration and rehydration on Ψ_w and growth parameters in the wheat cvs Adamello and Ofanto*

Results are the means of 10 repetitions from three independent experiments. SE of the means was always less than 10%. For comparisons between the means, one-way analysis of variance was used. Values in each row followed by different letters are significantly different at $P \leq 0.01$. C, Control; D, drought-stressed; R_1, rehydrated for 1 d; R_2, rehydrated for 4 d.

	cv Adamello				cv Ofanto			
	C	D	R_1	R_2	C	D	R_1	R_2
Ψ_w (MPa)	−0.50bc	−2.32a	−0.66b	−0.37c	−0.48b	−1.95a	−0.49b	−0.30c
Fresh wt (g)	6.4a	2.2b	3.2b	4.1b	5.7a	3.1b	4.2ab	5.3ab
Dry wt (g)	0.79a	0.49b	0.49b	0.53b	0.71a	0.54a	0.54a	0.63a

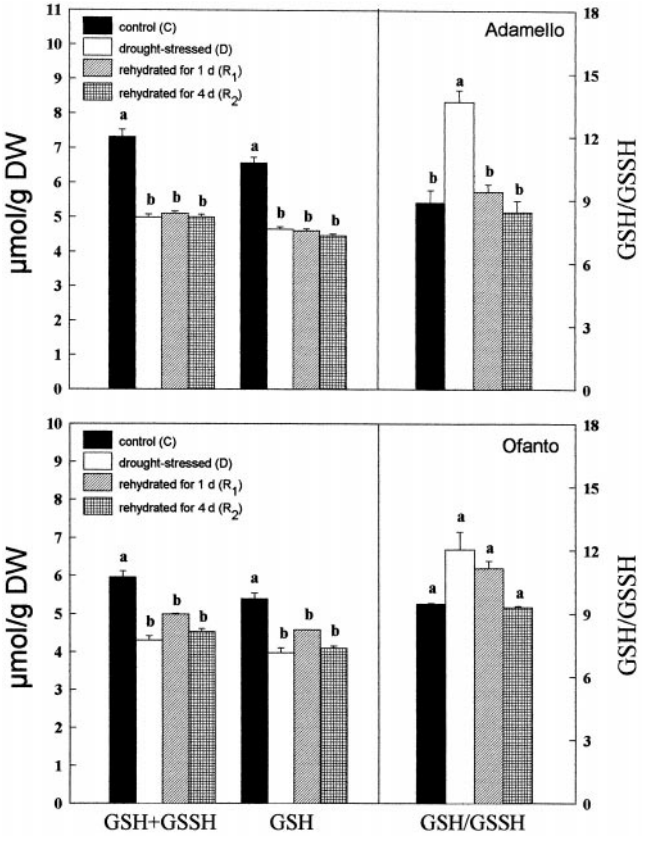

Figure 1. Effects of dehydration and rehydration on GSSH and GSH contents in the wheat cvs Adamello and Ofanto. Bars represent the SE (n = 10 repetitions from three independent experiments). One-way analysis of variance was used for comparisons between the means. Bars with different letters are significantly different at $P \leq 0.01$.

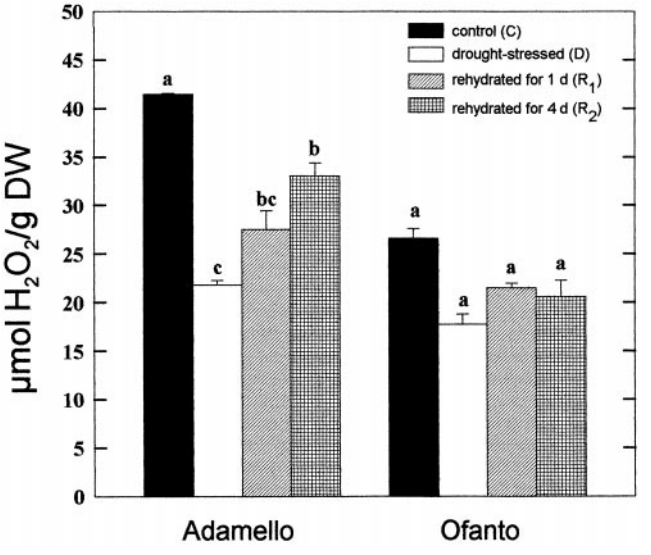

Figure 2. Effects of dehydration and rehydration on hydrogen peroxide content in the wheat cvs Adamello and Ofanto. Bars represent the SE (n = 10 repetitions from three independent experiments). One-way analysis of variance was used for comparisons between the means. Bars with different letters are significantly different at $P \leq 0.01$.

Statistical Analysis

The significance of differences between mean values obtained from 10 samples produced in three independent experiments was determined by one-way analysis of variance. Fluorescence and gas-exchange results are the means of four independent repetitions on different plants. Comparison among means was performed using the Newman-Keuls test. An analysis of variance test among control values on d 65, 66, and 69 showed no significant variation in all of the parameters analyzed. Therefore, analysis of variance between treatments was performed using control data on d 65.

RESULTS

Drought conditions induced a slightly larger decrease in Ψ_w in the more sensitive cv Adamello than in the more tolerant cv Ofanto. However, during rehydration a full recovery of leaf water status was achieved in both cultivars (Table I). Table I also shows that cv Adamello plants had a reduction in dry weight following dehydration. Four days of rehydration were not sufficient to recover the growth rate of control cv Adamello plants, whereas cv Ofanto showed a smaller, insignificant reduction in dry weight.

Drought caused a 30% decrease in GSH+GSSH and GSH contents. Full recovery of these contents could not be achieved after 4 d of rehydration in either cultivar (Fig. 1). In plants subjected to drought, net oxidation of GSH did not occur, as was evident from the lack of significant differences in the GSH/GSSH ratio in cv Ofanto and the

Table II. *Effects of dehydration and rehydration on GR, GTs, and GP activities (nkat/mg protein) and lipid peroxides (nmol/mg dry weight) in the wheat cvs Adamello and Ofanto*

Results are the means of 10 repetitions from three independent experiments. SE of the means was less than 10%. For comparisons between the means, one-way analysis of variance was used. Values in each row followed by different letters are significantly different at $P \leq 0.01$. C, Control; D, drought-stressed; R_1, rehydrated for 1 d; R_2, rehydrated for 4 d; BUT, butyl hydroperoxide.

	cv Adamello				cv Ofanto			
	C	D	R_1	R_2	C	D	R_1	R_2
GR	2.05bc	2.79a	2.31b	1.88c	1.93a	2.02a	1.80a	1.82a
GTs	1.32a	1.42a	1.61a	1.44a	1.11a	1.06a	0.95b	1.16a
Hydrogen peroxide-GP	1.06b	2.03a	1.18b	1.18b	1.08a	1.14a	0.98a	1.17a
BUT-GP	0.18a	0.15a	0.17a	0.16a	0.17a	0.12a	0.15a	0.13a
Lipid peroxides	156a	149a	137a	156a	192a	181a	201a	194a

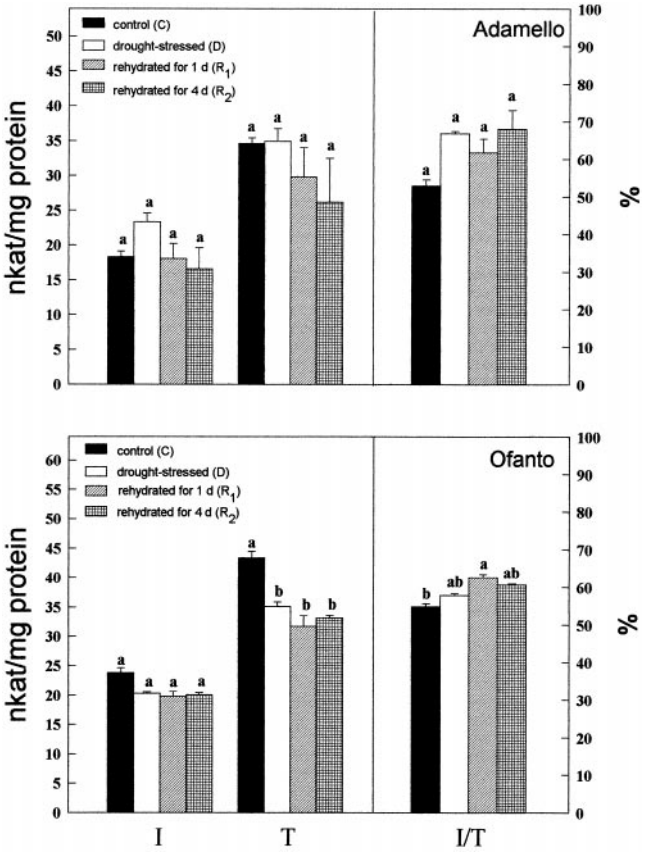

Figure 3. Effects of dehydration and rehydration on the initial (I) and total (T) activities and activation state (I/T) of G3PDH in the wheat cvs Adamello and Ofanto. Bars represent the SE ($n = 10$ repetitions from three independent experiments). One-way analysis of variance was used for comparisons between the means. Bars with different letters are significantly different at $P \leq 0.01$.

increase in this ratio in cv Adamello compared with the control (Fig. 1).

In cv Adamello the GR and hydrogen peroxide-GP activities increased in stressed plants, reaching 136% and 200% of the control, respectively; however, after 1 d of rehydration the activities of these enzymes recovered and were not significantly different from those of the control (Table II). In cv Ofanto drought did not cause changes in the specific activities of GR and GP (Table II), whereas the specific activity of GTs was about 80% of the control value after 1 d of rehydration (Table II). Lipid peroxide contents did not change during dehydration and rehydration in either cultivar (Table II).

According to the trend of hydrogen peroxide-GP activity, the hydrogen peroxide content (Fig. 2) showed significant changes only in cv Adamello, with a decrease of about 50% compared with control during drought, and with a partial recovery of 80% of the control value in plants rehydrated for 4 d.

The sulfhydryl plus disulfide groups, sulfhydryl levels, and percentage of sulfhydryl groups of soluble and membrane proteins did not show significant variations during dehydration and rehydration in either cultivar (data not shown).

The activity of G3PDH was significantly affected by drought in cv Ofanto but not in cv Adamello. In drought-stressed and rehydrated cv Ofanto leaves the total activity was about 70% to 80% of the control value, whereas the initial activity was maintained at control levels. However, in drought-stressed plants the activation state of this enzyme (percentage of initial activity on total activity) did not change (Fig. 3). On the contrary, FBPase enzyme activity changed only in cv Adamello during drought, with a further decrease during rehydration, when initial and total activities were about 60% to 70% of control values (Fig. 4). During drought and recovery the activation state of FBPase was maintained at the control level in both cultivars (Fig. 4).

In cv Adamello drought caused a general decrease in pigment contents, including chlorophyll *a*, lutein, neoxanthin, and β-carotene. However, all pigments recovered to control values after 4 d of rehydration (Table III). This pattern of change was not evident in cv Ofanto, in which all pigments did not change statistically. The chlorophyll *a/b* ratio decreased significantly during drought in cv Adamello, whereas the difference was not significant in cv Ofanto. Drought did not induce significant changes in the pool size of xanthophyll-cycle components expressed on

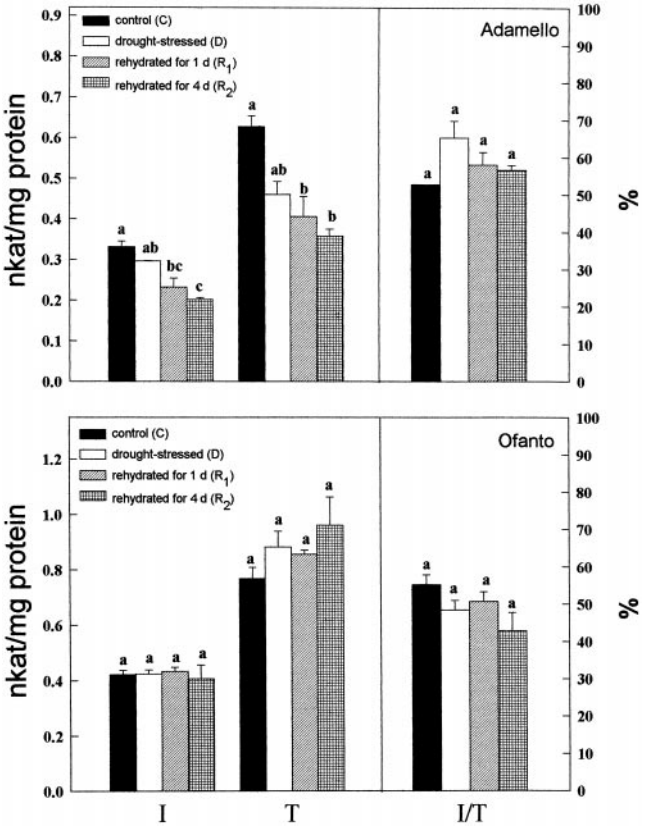

Figure 4. Effects of dehydration and rehydration on the initial (I) and total (T) activities and activation state (I/T) of FBPase in the wheat cvs Adamello and Ofanto. Bars represent the SE ($n = 10$ repetitions from three independent experiments). One-way analysis of variance was used for comparisons between the means. Bars with different letters are significantly different at $P \leq 0.01$.

Table III. *Pigment content of the wheat cvs Adamello and Ofanto during dehydration and rehydration*

Results are the means of 10 repetitions from three independent experiments. SE of the means was always less than 10%. For comparisons between the means, one-way analysis of variance was used. Values in each row followed by different letters are significantly different at $P \leq 0.01$. C, Control; D, drought-stressed; R_1, rehydrated for 1 d; R_2, rehydrated for 4 d; Chl, chlorophyll.

	cv Adamello				cv Ofanto			
	C	D	R_1	R_2	C	D	R_1	R_2
	μmol g⁻¹ dry wt							
Chl *a*	7.82a	4.96b	5.77b	7.07a	7.70a	7.21a	7.66a	6.88a
Chl *b*	3.61a	3.06ab	2.70b	3.40a	3.59a	3.63a	3.79a	3.41a
Chl *a/b*	2.17a	1.62b	2.13a	2.08a	2.14a	1.99a	2.02a	2.02a
Lutein	1.57a	1.25b	1.20b	1.50a	1.48a	1.54a	1.65a	1.49a
Neoxanthin	0.26a	0.20b	0.20b	0.25a	0.27a	0.27a	0.29a	0.25a
β-Carotene	0.69a	0.44b	0.48b	0.62a	0.63a	0.63a	0.67a	0.59a
V+A+Z	0.59a	0.41a	0.41a	0.51a	0.55a	0.50a	0.52a	0.52a
(Z+A)/(V+A+Z)	0.09b	0.35a	0.11b	0.11b	0.12a	0.16a	0.10a	0.12a

the basis of dry weight in either cultivar. There was only a slight drought-induced increase in V+A+Z expressed on the basis of chlorophyll *a* in cv Adamello. The content of Z+A on the basis of the V+A+Z pool increased by about 74% in cv Adamello under drought conditions compared with control plants (Table III). However, the recovery after 1 d was complete, returning to control levels. The Z+A content did not change in cv Ofanto.

The rate of carbon dioxide assimilation and the photon yield of PSII showed a drought-induced decrease in both cultivars. However, this decrease was more pronounced in cv Adamello than in cv Ofanto (Fig. 5). Accordingly, nonphotochemical quenching increased in drought-stressed plants compared with fully irrigated controls: by about 48% in cv Ofanto and 70% in cv Adamello (Fig. 5).

DISCUSSION

Exposure to drought caused a significant decrease in dry mass accumulation in cv Adamello, whereas it had no significant effects in cv Ofanto, even though both cultivars showed a significant drop in Ψ_w (Table I). Variation in drought tolerance and in the various parameters analyzed were not explained by the observed slight differences in Ψ_w, since similar results were obtained with the same genotypes maintained at a similar Ψ_w but with different soil water contents (F. Navari-Izzo and A. Scartazza, unpublished results).

The GSH+GSSH and GSH contents under drought conditions were maintained at relatively high levels (Fig. 1) with respect to those of other species (Buckland et al., 1991; Sgherri et al., 1994b; Schwanz et al., 1996). The drought-induced reduction in GSH content may indicate that both cultivars can rely on a large amount of constitutive GSH to counteract the potentially harmful effects of drought. In agreement with the present results, a partial degradation of the constitutive GSH was reported in *Sporobolus stapfianus* leaves subjected to dehydration (Sgherri et al., 1994b) and in sunflower plants under a severe drought (Sgherri et al., 1995). On the other hand, it has been reported that other species, such as the resurrection plant *Boea hygroscopica*, respond to drought by increasing antioxidant synthesis (Sgherri et al., 1994a; Navari-Izzo et al., 1997).

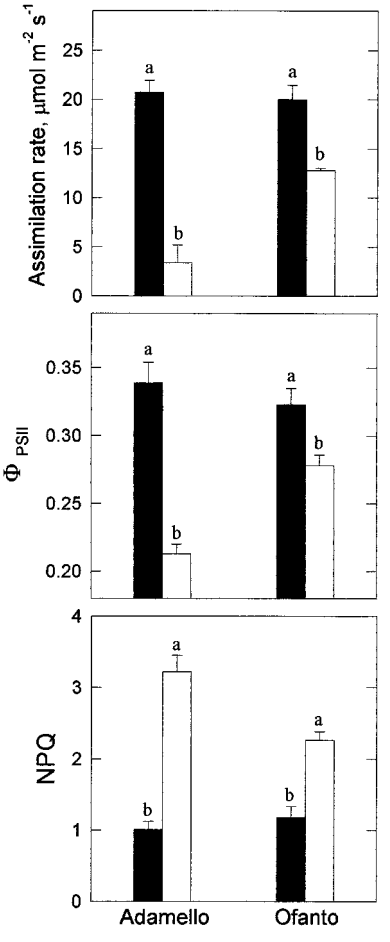

Figure 5. Effects of dehydration on carbon dioxide assimilation rate, photochemical efficiency of PSII, and nonphotochemical quenching (NPQ) in the wheat cvs Adamello and Ofanto. Bars represent the SE from four independent experiments. One-way analysis of variance was used for comparisons between the means. Bars with different letters are significantly different at $P \leq 0.01$. Black bars, Control; white bars, drought-stressed.

Adaptation to drought may depend on different mechanisms, including the capacity to maintain high levels of antioxidants and to regenerate them through the induction of GR activity. A similar effect was evident in cv Adamello (Table II; Fig. 2). Furthermore, the increased capacity to metabolize hydrogen peroxide, which was evident from the observed drought-induced 2-fold increase in hydrogen peroxide-GP activity in cv Adamello (Table II), may confer tolerance to oxidative stress due to the increase in $O_2\cdot^-$ production under drought conditions (Quartacci et al., 1994).

Levels of GR and hydrogen peroxide-GP activities sufficient to maintain the balance of cellular components were present in cv Ofanto, so there was no further increase in these enzyme activities. In cv Ofanto it is also possible that ascorbate peroxidase and monodehydroascorbate reductase (Moran et al., 1994) and other antioxidant enzymes (Zhang and Kirkham, 1994) may play a role in maintaining low levels of hydrogen peroxide in the cells.

In previous experiments cv Ofanto plants subjected to two water-deficit cycles did not show changes in hydrogen peroxide content and, after the second cycle, the $O_2\cdot^-$ production decreased in parallel with the activities of the glutathione-ascorbate-cycle enzymes (Menconi et al., 1995). In other experiments with maize plants (Brown et al., 1995) subjected to gradual drought imposition (and hence involving a possible acclimation), GR activity and hydrogen peroxide levels were unaffected by drought. It has been suggested that when drought is imposed slowly so that plants can acclimate, an increase in the $O_2\cdot^-$ level may be avoided (Sgherri et al., 1993). In the present experiment, 35 d to reach a rather severe drought probably represents a period long enough to allow acclimation. Therefore, it is possible that plants of the more tolerant cv Ofanto had the chance to acclimate to drought, avoiding exposure to oxidative stress. In such conditions there was no induction of antioxidative defenses.

Hydrogen peroxide, even at low concentrations, inhibits chloroplast sulfhydryl-containing enzymes by readily oxidizing their sulfhydryl groups. Therefore, it is important for plant cells to keep the levels of hydrogen peroxide low or to scavenge it efficiently. A low hydrogen peroxide content (Fig. 2) and a high GSH/GSSH ratio (Fig. 1) enabled both wheat cultivars to maintain the sulfhydryl groups of soluble and membrane proteins in the reduced state (data not shown) during dehydration and rehydration. Moreover, the activation state of the enzymes containing essential sulfhydryl groups, G3PDH (Fig. 3) and FBPase (Fig. 4), was not significantly affected by drought. Previous studies on *Selaginella lepidophylla* showed drought-induced decreases in sulfhydryl-containing enzyme activities (Harten and Eickmeier, 1986). In contrast, in the resurrection plant *B. hygroscopica* the GSH/GSSH ratio, the G3PDH specific activity, and the percentage of sulfhydryl groups of thylakoid proteins remained at control levels, whereas the percentage of sulfhydryl groups of soluble proteins increased (Navari-Izzo et al., 1997).

During drought conditions maintaining low levels of hydrogen peroxide would also be reflected in a low rate of the Haber-Weiss reaction, which is involved in the production of hydroxyl radicals responsible for lipid peroxidation. Accordingly, during dehydration and rehydration in both cultivars, the unsaturation of total lipids (data not shown) and their content and the lipid hydroperoxide levels (Table II) did not change. Therefore, the maintenance of a low hydrogen peroxide level in both cultivars explains the lack of increase in the activities of GTs and GP on lipid hydroperoxide and *t*-butyl hydroperoxide (Table II).

The more drought-sensitive cv Adamello showed a decrease in chlorophyll *a*, the chlorophyll *a/b* ratio, and carotenoid pigments during dehydration (Table III). A drought-induced reduction in pigment contents was previously reported in several species, including pea (Moran et al., 1994) and *Nerium oleander* (Demmig-Adams et al., 1988). Photoinhibition and photodestruction of pigments may contribute to such changes. In addition, the photosynthetic apparatus may show acclimation responses such as changes in the relative proportion of stacked and unstacked membrane domains (Anderson and Aro, 1994).

In cv Ofanto the lack of changes in pigment content and composition under drought conditions (Table III) indicates the capacity to preserve the photosynthetic apparatus. In agreement with this hypothesis, the drought-induced decline in the actual PSII photon yield was more marked in cv Adamello than in cv Ofanto (Fig. 5). Similarly, the net carbon dioxide assimilation rate at the end of the drought period decreased by only 36% in cv Ofanto, whereas it declined by about 84% in cv Adamello (Fig. 5).

Although neither cultivar showed significant changes in the pool size of xanthophyll-cycle components, a drought-induced increase in (Z+A)/(V+A+Z) was evident in cv Adamello (Table III). This may reflect an increased excessive energy in the pigment bed and a consequent increased need for radiationless dissipation. Accordingly, after exposure to drought, cv Adamello showed a higher increase in nonradiative energy dissipation, estimated as nonphotochemical quenching, than cv Ofanto (Fig. 5). The fact that cv Ofanto did not show significant changes in the pool size and composition of V+A+Z in response to drought and subsequent rehydration (Table III) may be explained by its higher total rate of electron transport compared with cv Adamello. Therefore, in cv Ofanto the photosynthetic electron transport was probably sufficient to preclude the buildup of excess energy in PSII. Zeaxanthin seems to be involved in the development of nonphotochemical quenching and nonradiative energy dissipation, according to numerous previous reports (for reviews, see Demmig-Adams and Adams, 1992; Pfündel and Bilger, 1994; Horton et al., 1996; Gilmore, 1997), and may be important in preventing irreversible damage in cv Adamello. However, zeaxanthin does not seem to play a crucial role in determining differences in drought tolerance between these two wheat cultivars.

The link between the xanthophyll cycle, ascorbate content, and redox state via ascorbate ← GSH ← NADPH is well known (Boch et al., 1994); therefore, in cv Adamello the drought-induced GR induction and the consequent increase of the GSH/GSSH ratio may be related to zeaxanthin formation and nonradiative energy dissipation.

In conclusion, the more drought-sensitive cv Adamello responded to a period of stress by reducing photosynthetic efficiency and biomass accumulation. However, it is remarkable that all defense mechanisms assayed returned to control levels rapidly upon rehydration. This indicates that in this cultivar the defense mechanisms prevent plants from suffering irreversible damages during the drought period. On the other hand, the more drought-tolerant cv Ofanto seems able to avoid drought stress by maintaining a high photosynthetic activity, and does not suffer an oxidative stress high enough to trigger the defense mechanisms active in cv Adamello. Consequently, cv Ofanto under drought may maintain a growth rate similar to that of well-watered control plants.

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