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# Lipid Composition and Protein Dynamics in Thylakoids of Two Wheat Cultivars Differently Sensitive to Drought[1]

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**Two wheat (*Triticum durum* Desf.) cultivars with different sensitivities to drought were either grown under regular irrigation or subjected to water deficit by withholding water for 14 d. Water-stressed plants of both cultivars underwent similar decreases in leaf water potential, but the drought-tolerant cultivar showed higher relative water content and turgor. Neither osmotic nor elastic adjustment mechanisms appeared to be active under the conditions described here. Thylakoids isolated from the stressed, drought-tolerant wheat showed an increase in lipid-to-protein ratio, in comparison with the control, whereas this ratio remained unchanged in the sensitive wheat. In both cultivars, water deficit determined different rearrangements in the composition of the thylakoid individual polar lipids, but their unsaturation level remained unaffected with the exception of monogalactosyldiacylglycerol. In the drought-sensitive cultivar, an accumulation of free fatty acids together with a reduction in polar lipid amount was observed. Electron paramagnetic resonance measurements of spin-labeled proteins of stressed plants from the sensitive cv Adamello showed a higher spin label rotational correlation time together with lower sulphydryl group and mobile proteic portion levels, in comparison with the control. In the tolerant cv Ofanto, the first two parameters changed to a lesser extent following water depletion, and the mobile proteic portion was not altered.**

Within the thylakoids, the membrane lipids have an important role to play in stabilizing the structural arrangement and, via the lipid-protein interactions, in integrating the protein complexes and possibly in maintaining their spatial distribution (Li et al., 1989b). The finding of lateral asymmetry in the distribution of acyl lipids in thylakoid membranes may imply that there are specific associations between certain lipids and the heterogeneously distributed protein complexes. Pick et al. (1985) suggested a tight association between SQDG and the protein-translocating $CF_o$-$CF_1$ ATP synthase complex. A nonspecific association between MGDG and the light-harvesting complex of PSII was found by Quinn and Williams (1983).

Spin-label EPR spectroscopy has been found to be particularly useful in the investigation of lipid-protein interactions (Quinn and Williams, 1990). In the case of thylakoids, Li et al. (1989b) demonstrated that nitroxide-labeled MGDG, phosphatidylcholine, and PG of the boundary layer surrounding proteins all show appreciable motional restriction on the EPR time scale in comparison with the bulk lipid environment and that PG expresses a selectivity of lipid-protein interaction in both of the photosystems.

In response to perturbed environmental conditions such as drought, the adaptation shown by many plants could partly be due to changes in membrane composition and phase behavior, which optimizes the fluidity (Navari-Izzo et al., 1993). Indeed, models for thylakoid membrane function require mobility of protein components and redox carriers, and this mobility would be strictly limited at the lipid/protein interface if the lipid acyl chain motion is restricted in this region (Li et al., 1989a). It also has been proposed that alterations in bulk membrane lipids, such as those caused by dehydration, perturb cell function by inducing changes in the structure and function of several intrinsic membrane protein complexes (Caldwell and Whitman, 1987; Horvath et al., 1989).

In this study, two cultivars of wheat with different sensitivities to drought were grown under normal and water-deficit conditions. To try to clarify membrane variations, we investigated thylakoid lipid composition and its influence on the molecular dynamics of membrane proteins spin labeled using 3-maleimido proxyl.

## MATERIALS AND METHODS

### Plant Material

Seedlings of two wheat (*Triticum durum* Desf.) cultivars, one drought tolerant (cv Ofanto) and the other drought sensitive (cv Adamello), were grown under field irrigation and dryland conditions. Seeds were provided by the Istituto Sperimentale per la Cerealicoltura (Foggia, Italy). The different sensitivities of the two cultivars to drought had been determined by their grain yield during a 5-year period in different areas of southern Italy (Mariani and Novaro, 1993). In one set, control plants from both cultivars were regularly watered, and another set of plants from both cultivars was subjected to water deficit by withhold-

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Abbreviations: DGDG, digalactosyldiacylglycerol; EPR, electron paramagnetic resonance; $\epsilon$, bulk modulus of elasticity; FFA, free fatty acids; FS, free sterols; MGDG, monogalactosyldiacylglycerol; MP, mobile proteic portion; PG, phosphatidylglycerol; $\psi_p$, pressure potential; $\psi_s$, osmotic potential; $\psi_w$, leaf water potential; RWC, relative water content; SH, reduced sulphydryl groups; SQDG, sulfoquinovosyldiacylglycerol; $\tau$, spin label rotational correlation time.

ing water for 14 d, starting 30 d after sowing. Plants from both treatments and cultivars were harvested 44 d after sowing. Roots and shoots were separated, fresh weights were recorded, and samples were taken for dry weight measurements.

### Leaf Water Status

Leaf water status was determined by pressure-volume curves using a pressure chamber. Curve analyses were performed and analyzed as previously described by Navari-Izzo et al. (1993).

### Isolation of Thylakoid Membranes

Thylakoid membranes were isolated by homogenizing the leaves in an ice-cold isolation medium (1:3, w/v) containing 0.5 M Suc, 75 mM Hepes-KOH, pH 7.5, 10 mM diethyldithiocarbamic acid, 5 mM ascorbic acid, 3.6 mM Cys, and 5 mM EDTA following the procedure of Sgherri et al. (1993).

### Lipid Analysis

The isolated thylakoids were first boiled in isopropanol for 5 min. Lipids then were extracted at 4°C with chloroform:methanol (2:1, v/v), containing butylhydroxytoluol as an antioxidant. The combined lipid extracts were washed and fractionated into individual polar lipid components, FFA and FS, on activated silica gel plates as reported by Navari-Izzo et al. (1992). The individual phospho- and glycolipids were assayed and quantified, as previously described, by their phosphorus and Gal contents, respectively (Navari-Izzo et al., 1991). FFAs were quantified on the basis of their fatty acid contents and relative conversion factors (Navari-Izzo et al., 1991). The fatty acid methyl ester derivatives were obtained by transmethylation with a mixture containing methanol:benzene:sulfuric acid (100:5:5, v/v/v) after heating at 70°C for 1 h (Douce et al., 1990). The qualitative and quantitative analyses of fatty acid methyl esters were carried out by GLC under the condition previously defined (Navari-Izzo et al., 1991) using heptadecanoic acid as the internal standard. FSs scraped off of the TLC plates were identified and quantitatively assayed according to the method of Zlatkis and Zak (1969).

### Protein Spin Labeling

Isolated thylakoid membranes were labeled with the paramagnetic probe 3-maleimido proxyl, essentially as described by Lynch et al. (1987). Briefly, the tube containing the membrane suspension (5.6 mg protein $mL^{-1}$) in 10 mM Mes, pH 6.6, and 4 mM 3-maleimido proxyl was capped under nitrogen, vortexed for 2 min, and incubated in the dark for 16 h at 4°C. Following incubation, thylakoid membranes were washed eight times in 50 mM Tris-HCl buffer, pH 7.4, by centrifugation at 10,000*g* for 20 min. Free spin label could not be detected in the supernatant following the eighth centrifugation. The protein content of isolated thylakoid membranes was determined as reported by Sgherri et al. (1993).

### EPR Measurements

For EPR analysis, the concentrated membrane suspension was taken up into a 100-$\mu$L capillary tube, which was then sealed at one end and inserted into a quartz sample holder for placement in the microwave cavity of the spectrometer. Spectra were recorded in a temperature range of −50 to +30°C using a Varian (Palo Alto, CA) E-112 spectrometer that was equipped with a Varian variable-temperature control accessory. The spectrometer was interfaced to an AST (Irvine, CA) Premium 486/25-mHz Extended Industry Standard Architecture computer by means of a homemade data acquisition system. This consisted of an acquisition board (Ambrosetti and Ricci, 1991) and a software package especially designed for EPR and electron nuclear double resonance experiments (Pinzino and Forte, 1992).

At each temperature, spectra were recorded at a field setting of 3270 G, a microwave power setting of 5 mW, a frequency of 9.2 GHz, a time constant of 0.125 s, and a modulation amplitude of 1.25 G. 2,2-Diphenyl-1-picrylhydrazyl powder was used as a *g* value standard ($g$ = 2.0037). The rotational correlation time of the protein-bound spin label, which physically characterizes the protein motions, can be obtained by computer simulation of the EPR line shape if the elements of the nitrogen hyperfine (**A**) and electron Zeeman (**g**) tensors are known (Freed, 1976). These values can be determined from the simulation of the rigid-limit spectrum, which was obtained at −50°C.

Spectra were simulated by using the previously determined magnetic parameters according to the Freed algorithm (Schneider and Freed, 1989), which includes axially symmetric rotational reorientation about a principal axis of the tensors **A** and **g**. Spectral titrations of data were performed according to the method of Klopfenstein et al. (1972). The number of spin labels bound to SH groups was calculated from the second integral of the EPR spectrum shown in Figure 1A, which represents the derivative absorption spectrum of the spin label (Griffith and Jost, 1976). The spectrum shown in Figure 1B was obtained by computer simulation, whereas the spectrum shown in Figure 1C represents the difference between the experimental EPR spectrum and the contribution from the more mobile spin probe.

## RESULTS

Dryland conditions induced similar decreases in $\psi_w$ in the two cultivars, but the $\psi_p$ and the RWC of the drought-tolerant cv Ofanto were higher in comparison with the drought-sensitive cv Adamello (Table I). Water-deficit conditions caused no significant changes either in the $\psi_s$ at full turgor ($\psi_s^{100}$) or in the $\epsilon$ of both cultivars.

As shown in Table II, water deficit caused different rearrangements in the lipid composition of thylakoids in the two cultivars but caused an increase in the lipid-to-protein ratio only in the tolerant wheat. Whereas the

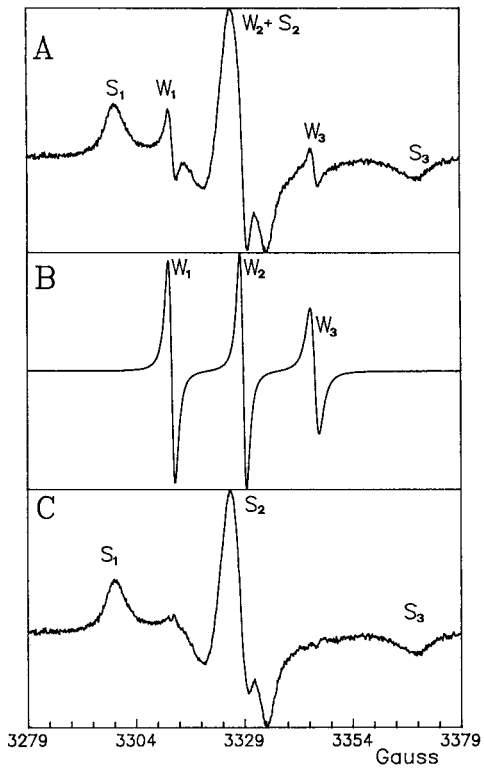


**Figure 1.** Electron spin resonance spectra of thylakoid membranes isolated from wheat (cv Adamello) and labeled with 3-maleimido proxyl. Spectra were recorded at 20°C. The letters W and S denote weakly and strongly immobilized components, respectively, of the low-field line. A, Experimental first derivative absorption EPR spectrum. B, Computer simulation of the weak component of the spectrum (enlarged 3 times). C, Spectrum of the strong component obtained by subtraction of the experimental and simulated curves.

DGDG level was unaffected by water deficit in both cultivars, MGDG, SQDG, and PG amounts showed an opposite behavior in the two cultivars following water depletion (Table II). Following stress, the relative amounts of FS were reduced in both cultivars. Water-deficit conditions increased the proportion of FFA by 75% in the sensitive wheat, whereas the level was unchanged in the tolerant plants (Table II). Following water deficit, MGDG and SQDG unsaturation levels showed a shift toward higher levels in cv Adamello (Table III), whereas in the tolerant wheat, thylakoid unsaturation was unaffected by stress conditions with the exception of MGDG, in which the unsaturation decreased (Table IV). It is worth noting the opposite behavior of PG-16:1 *trans*, which increased in cv Ofanto and decreased in cv Adamello following water depletion.

The effects of water-deficit conditions on membrane proteins were evaluated by spin labeling them with 3-maleimido proxyl, a paramagnetic probe that binds to free and accessible protein SH groups. The procedure used to spin label membrane proteins does not label membrane lipids and has no effect on the fatty acid composition or fluidity of the membranes, nor does the procedure alter the thylakoid protein composition during the incubation period required for labeling (Lynch et al., 1987).

Typical EPR spectra for thylakoid-labeled membranes are illustrated in Figure 1A. The spectrum low-field line (Fig. 1A) contains peaks referable to a strongly immobilized component ($S_{1-3}$) and to a more freely rotating component ($W_{1-3}$). The weakly immobilized component (Fig. 1B) indicates that the spin label is bound to SH groups located mainly at sites that allow the label to retain a high degree of rotational freedom, i.e. SH groups present on the surfaces of protein molecules and/or groups that belong to proteins embedded in a fluid matrix. The strongly immobilized component of the spectrum (Fig. 1C) is due to the label bound to SH groups located in crevice-like regions of the proteins or to SH groups of proteins surrounded by a more rigid environment (Cornell et al., 1981). Thus, the relative proportions of the W and the S peaks are sensitive parameters of protein conformation and/or lipid matrix surrounding proteins.

We must point out that, in contrast to other reports (Lynch et al., 1987; Duxbury et al., 1991a, 1991b), our study of spin-labeled membrane proteins is based on changes in the relative proportions of the $W_{1-3}$ and $S_{1-3}$ peak intensities, which are not influenced by analysis temperature (Fig. 1A). The above-mentioned authors considered the relative amplitudes of the peaks $W_1$ and $S_1$, which do not represent

**Table I.** *Water status and growth parameters of two wheat cultivars differently sensitive to drought subjected to 14 d of water-deficit conditions*

Results are the means ± SE of three replicates of 10 plants each. For each cultivar, means followed by * are significantly different from control by an analysis of variance test ($P \leq 0.01$). $\Psi_s^{100}$, Full tugor.

| Parameter | cv Adamello[a] | | cv Ofanto[b] | |
|---|---|---|---|---|
| | Control | Stressed | Control | Stressed |
| $\Psi_w$ (MPa) | −0.6 ± 0.1 | −1.7 ± 0.3* | −0.5 ± 0.1 | −1.8 ± 0.2* |
| $\Psi_s$ (MPa) | −2.0 ± 0.1 | −2.4 ± 0.2* | −1.9 ± 0.3 | −2.8 ± 0.2* |
| $\Psi_p$ (MPa) | 1.4 ± 0.2 | 0.7 ± 0.3* | 1.4 ± 0.1 | 1.0 ± 0.2* |
| $\Psi_s^{100}$ (MPa) | −1.6 ± 0.1 | −1.6 ± 0.2 | −1.6 ± 0.1 | −1.7 ± 0.1 |
| RWC (%) | 95.4 ± 1.3 | 78.2 ± 2.1* | 95.8 ± 1.5 | 84.8 ± 2.4* |
| $\epsilon$ (MPa) | 2.5 ± 0.2 | 2.8 ± 0.2 | 2.1 ± 0.1 | 2.4 ± 0.3 |
| Fresh wt (g/plant) | 1.4 ± 0.1 | 1.0 ± 0.2* | 1.9 ± 0.1 | 1.7 ± 0.2 |
| Dry wt (%) | 12.9 ± 0.5 | 20.1 ± 0.3* | 14.8 ± 0.4 | 20.8 ± 0.4* |

[a] Drought sensitive. [b] Drought tolerant.

**Table II.** *Lipid composition and lipid-to-protein ratio of isolated thylakoids of two wheat cultivars differently sensitive to drought subjected to 14 d of water-deficit conditions*

Results are the means ± SE of three replicates that were each analyzed twice. The significance of * is the same as in Table I.

| Lipid | cv Adamello[a] | | cv Ofanto[b] | |
|---|---|---|---|---|
| | Control | Stressed | Control | Stressed |
| | | *mol%* | | |
| MGDG | 43.8 ± 2.1 | 50.4 ± 3.0* | 44.5 ± 1.9 | 35.6 ± 2.7* |
| DGDG | 23.0 ± 1.5 | 24.4 ± 0.9 | 22.3 ± 1.3 | 24.7 ± 0.8 |
| SQDG | 16.4 ± 1.7 | 11.7 ± 1.1* | 16.1 ± 2.1 | 22.8 ± 1.6* |
| PG | 13.9 ± 1.9 | 10.4 ± 0.6* | 6.1 ± 1.2 | 8.2 ± 1.0 |
| FFA | 0.8 ± 0.2 | 1.4 ± 0.3* | 2.0 ± 0.3 | 1.8 ± 0.3 |
| FS | 2.1 ± 0.2 | 1.7 ± 0.1* | 9.0 ± 0.8 | 6.9 ± 0.4* |
| Sum[c] | 10.1 ± 0.6 | 7.8 ± 0.9* | 6.9 ± 0.2 | 6.8 ± 0.5 |
| Lipid/protein[d] | 0.63 ± 0.03 | 0.67 ± 0.04 | 0.46 ± 0.02 | 0.57 ± 0.03* |

[a] Drought sensitive. [b] Drought tolerant. [c] Expressed as $\mu$mol $g^{-1}$ dry weight. [d] Expressed as mg/mg.

by themselves all of the weakly and strongly immobilized components of the spectrum. Moreover, the $W_1$ and $S_1$ peak amplitudes change with peak width, which is related to the analysis temperature.

As shown in Table V, thylakoid proteins decreased in both cultivars following water-deficit conditions, but drought-sensitive plants suffered a more marked reduction. At 20°C the sulfydryl-bound $\tau$ values were higher in thylakoids from stressed leaves in both cultivars (Table V), but the increase in comparison with the control was lower in the drought-tolerant wheat. As far as the MP is concerned, namely the membrane portion containing freely rotating spin labels (Fig. 1B), its percentage decreased in stressed cv Adamello and remained constant in cv Ofanto (Table V). The variations of these two parameters with temperature are shown in Figures 2 and 3. It is evident that at physiological temperatures the differences in $\tau$ and MP between control and drought-affected thylakoid membranes are more marked in the drought-sensitive wheat in comparison with the tolerant one. At decreasing temperatures, the $\tau$ and MP curves tend to meet because of the rigid system caused by the frozen environment in which the proteins are immersed.

Water-deficit conditions, which caused in both cultivars a decrease in the total amount of SH groups available for binding by the spin label (Table V), resulted in a decrease of 31% of thiol groups in the drought-tolerant cultivar, whereas it produced a reduction of 50% of the thiol groups in the sensitive wheat.

## DISCUSSION

The higher water content and its better distribution in the stressed cv Ofanto permitted the plants to retain a higher turgor in comparison with cv Adamello, which resulted in maintained growth (Table I). The greater dehydration tolerance of cv Ofanto cannot be ascribed to either osmotic adjustment or cell-wall elasticity regulation.

Water deficit caused significant changes in the composition of thylakoid lipids of both cultivars (Table II). With regard to the main glycolipid, it is known that MGDG, on isolation, forms a cylindrical inverted hexagonal configuration instead of the bilayer configuration adopted by the other thylakoid lipids (Quinn and Williams, 1983). Stabilization of thylakoids can be achieved by reducing the tendency of MGDG to form nonbilayer arrangements.

**Table III.** *Fatty acid composition of polar lipids and FFA of the thylakoids of the drought-sensitive wheat cv Adamello subjected to 14 d of water-deficit conditions*

Results are the means ± SE of three replicates that were analyzed twice. The significance of * is the same as in Table I.

| Lipid | Treatment | FFA | | | | | | | | | Unsaturation |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 14:0 | 16:0 | 16:1 | 16:1t | 18:0 | 18:1 | 18:2 | 18:3 | Others[a] | |
| | | | | | | *mol%* | | | | | |
| MGDG | Control | 1 ± 0.2 | 5 ± 0.7 | 3 ± 1.3 | | 2 ± 0.6 | 2 ± 0.7 | 3 ± 0.3 | 82 ± 2.6 | 1 ± 0.2 | 92 ± 2.4 |
| | Stressed | Trace | 2 ± 0.5* | 1 ± 0.7 | | 1 ± 0.4 | 1 ± 0.3 | 3 ± 0.5 | 91 ± 3.0* | 1 ± 0.3 | 97 ± 1.8* |
| DGDG | Control | 1 ± 0.2 | 9 ± 1.1 | 2 ± 0.4 | | 2 ± 0.5 | 2 ± 0.2 | 2 ± 0.2 | 81 ± 2.5 | 1 ± 0.2 | 88 ± 2.5 |
| | Stressed | 1 ± 0.3 | 8 ± 1.3 | 2 ± 0.2 | | 1 ± 0.5 | 2 ± 0.4 | 2 ± 0.3 | 83 ± 1.6 | 1 ± 0.5 | 90 ± 1.1 |
| SQDG | Control | 2 ± 0.5 | 32 ± 2.0 | 3 ± 0.5 | | 5 ± 0.6 | 5 ± 0.7 | 3 ± 0.4 | 47 ± 2.4 | 3 ± 0.4 | 61 ± 1.4 |
| | Stressed | Trace | 24 ± 1.8* | 2 ± 0.5 | | 4 ± 0.4 | 4 ± 0.4 | 4 ± 0.4 | 60 ± 3.2* | 2 ± 0.3 | 72 ± 2.6* |
| PG | Control | 2 ± 0.4 | 28 ± 1.7 | 3 ± 0.4 | 23 ± 1.2 | 5 ± 0.5 | 5 ± 0.6 | 6 ± 0.3 | 24 ± 1.9 | 4 ± 0.5 | 65 ± 1.5 |
| | Stressed | 3 ± 0.6 | 25 ± 1.5 | 4 ± 0.6 | 18 ± 0.9* | 4 ± 0.5 | 7 ± 1.4 | 6 ± 0.5 | 29 ± 2.5* | 4 ± 0.5 | 68 ± 3.0 |
| FFA | Control | 8 ± 0.9 | 26 ± 0.9 | 14 ± 1.1 | | 6 ± 0.7 | 14 ± 1.5 | 14 ± 1.3 | 13 ± 1.1 | 5 ± 0.3 | 60 ± 2.1 |
| | Stressed | 5 ± 0.5* | 29 ± 1.6 | 13 ± 0.6 | | 8 ± 1.4 | 11 ± 1.2* | 10 ± 2.0* | 21 ± 1.9* | 3 ± 0.2 | 63 ± 1.1 |

[a] 14:1 + 20:1 + 20:2.

**Table IV.** *Fatty acid composition of polar lipids and FFA of the thylakoids of the drought-tolerant wheat cv Ofanto subjected to 14 d of water-deficit conditions*

Results are the means ± SE of three replicates that were analyzed twice. The significance of * is the same as in Table I.

| Lipid | Treatment | FFA | | | | | | | | | Unsaturation |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 14:0 | 16:0 | 16:1 | 16:1t | 18:0 | 18:1 | 18:2 | 18:3 | Others[a] | |
| | | | | | | *mol%* | | | | | |
| MGDG | Control | 1 ± 0.3 | 10 ± 0.7 | 4 ± 1.5 | | 4 ± 0.6 | 5 ± 1.2 | 6 ± 0.4 | 69 ± 2.5 | 1 ± 0.5 | 85 ± 1.3 |
| | Stressed | 1 ± 0.2 | 13 ± 1.1 | 2 ± 0.6 | | 6 ± 1.5 | 3 ± 0.9 | 7 ± 1.0 | 67 ± 2.0 | 1 ± 0.3 | 80 ± 2.0* |
| DGDG | Control | 1 ± 0.1 | 29 ± 1.3 | 3 ± 0.5 | | 9 ± 1.8 | 5 ± 0.6 | 4 ± 0.5 | 48 ± 1.4 | 1 ± 0.5 | 61 ± 2.5 |
| | Stressed | 5 ± 0.5* | 24 ± 0.9* | 3 ± 0.4 | | 6 ± 1.2 | 4 ± 0.4 | 4 ± 0.3 | 52 ± 2.3* | 2 ± 0.6 | 65 ± 1.6 |
| SQDG | Control | 2 ± 0.4 | 35 ± 1.5 | 5 ± 0.7 | | 6 ± 0.5 | 5 ± 0.5 | 5 ± 1.0 | 40 ± 1.4 | 2 ± 0.4 | 57 ± 2.3 |
| | Stressed | 2 ± 0.3 | 39 ± 1.3* | 2 ± 0.3* | | 7 ± 0.6 | 4 ± 0.5 | 5 ± 0.3 | 37 ± 1.9 | 4 ± 1.0* | 52 ± 2.9 |
| PG | Control | 1 ± 0.3 | 21 ± 1.8 | 5 ± 0.8 | 15 ± 0.9 | 12 ± 0.8 | 9 ± 0.9 | 9 ± 0.8 | 23 ± 1.4 | 5 ± 1.5 | 66 ± 2.4 |
| | Stressed | 2 ± 0.7 | 24 ± 1.4 | 7 ± 1.3 | 20 ± 1.5* | 8 ± 1.0* | 7 ± 1.3 | 7 ± 0.6 | 22 ± 0.8 | 3 ± 0.6 | 66 ± 1.5 |
| FFA | Control | 7 ± 0.3 | 27 ± 0.9 | 10 ± 0.7 | | 7 ± 0.6 | 14 ± 1.2 | 5 ± 1.1 | 30 ± 2.5 | Trace | 59 ± 1.1 |
| | Stressed | 7 ± 0.6 | 26 ± 1.1 | 7 ± 0.4* | | 9 ± 1.5 | 16 ± 0.9 | 5 ± 0.7 | 30 ± 1.6 | Trace | 58 ± 1.3 |

[a] 14:1 + 20:1 + 20:2.

Gounaris et al. (1983) showed that increasing the extent of saturation reduces the tendency of MGDG to form nonbilayer structures. It should be kept in mind that the more MGDG present in the lipid mixture the larger the tendency to form nonlamellar phases (Selstam et al., 1990).

The lesser MGDG level and its lower unsaturation in the tolerant wheat (Tables II and IV) may maintain membrane fluidity, as shown by the smaller increase in $\tau$ (Table V), although the potential of acyl lipids to form lamellar or nonlamellar configurations is dependent on several other factors, such as temperature, water content, pH, and cation type and concentration (Brentel et al., 1985).

Changes in fatty acid saturation are required to preserve an appropriate balance of bilayer- and nonbilayer-forming lipids in the membrane. It has been suggested (Gounaris et al., 1983) that in drought adaptation it is probably the occurrence of bilayer/nonbilayer transformations and their influence on the packaging of proteins that are of primary importance. Unsaturation changes would, in the absence of these transformations, be expected to lead to minimal changes in membrane fluidity.

It is known that charged lipids interact closely with integral proteins. Indeed, EPR studies have shown that spin-labeled PG has a specificity of interaction for the light-harvesting Chl protein complexes of both PSI and PSII (Li et al., 1989b). A strong correlation among the level of PG-16:1 *trans* and the degree of oligomerization of the light-harvesting complex and the ability to perform state II-state I transition was also found (Tremolieres et al., 1989). A tight association between SQDG and the $CF_o$-$CF_1$ ATP synthase complex was found by Pick et al. (1985). Thus, in our study the maintained PG level and the increased PG-16:1 *trans* and SQDG amounts in the stressed cv Ofanto (Tables II and IV) may have maintained membrane protein complex configurations. The larger capacity of the

**Table V.** *Protein mobility (detected at 20°C), protein sulphydryl groups, and the protein content of thylakoids of two wheat cultivars differently sensitive to drought that were subjected to 14 d of water-deficit conditions*

Results are the means ± SE of three replicates. The significance of * is the same as in Table I.

| Parameter | cv Adamello[a] | | cv Ofanto[b] | |
|---|---|---|---|---|
| | Control | Stressed | Control | Stressed |
| Proteins[c] | 31.4 ± 1.5 | 16.3 ± 0.6* | 45.8 ± 1.6 | 33.2 ± 1.2* |
| $\tau$ (ns) | 0.13 ± 0.01 | 0.22 ± 0.02* | 0.16 ± 0.01 | 0.19 ± 0.01* |
| MP (%) | 6 ± 0.4 | 4 ± 0.2* | 2 ± 0.2 | 2 ± 0.1 |
| SH (%) | 100 | 50 ± 3* | 100 | 69 ± 2* |

[a] Drought sensitive. [b] Drought tolerant. [c] Expressed as mg $g^{-1}$ dry weight.

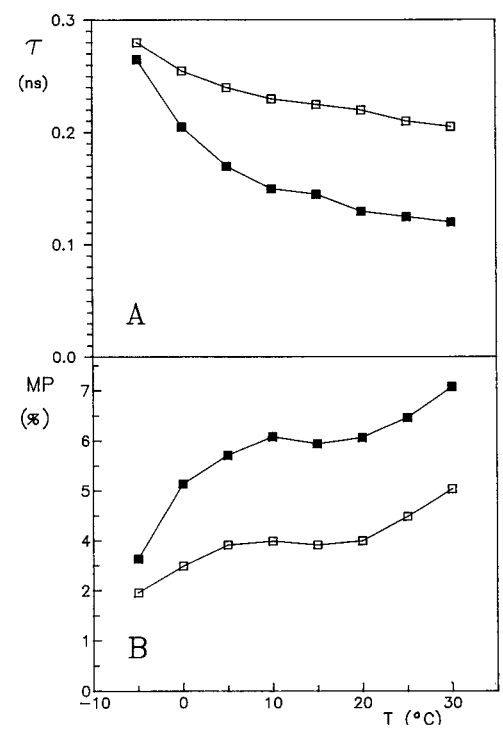


**Figure 2.** Temperature profiles for the $\tau$ (A) and for the MP (B) derived from EPR spectra of 3-maleimido proxyl-labeled thylakoids isolated from the drought-sensitive wheat cv Adamello. ■, Control; □, stressed.

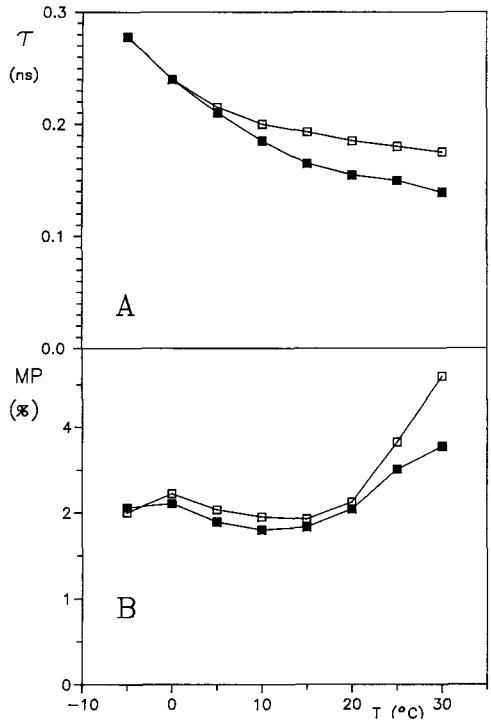


**Figure 3.** Temperature profiles for the $\tau$ (A) and for the MP (B) derived from EPR spectra of 3-maleimido proxyl-labeled thylakoids isolated from the drought-tolerant wheat cv Ofanto. ■, Control; □, stressed.

charged lipids to swell with water (Selstam et al., 1990) may also have increased the ability to bind water in the tolerant cultivar.

Although there was only a minor change in the lipid unsaturation in response to drought (Tables III and IV), there was a change in the lipid-to-protein ratio in cv Ofanto thylakoids (Table II). This may be important, since the lipid-to-protein ratio could be one of the factors that contributes to the regulation of fluidity in this particular membrane. It has been shown that an increase in this ratio leads to a more fluid membrane (Chapman et al., 1983).

The FFA that are liberated in the stressed sensitive wheat (Table II), which are hydrophobic and remain in the membrane, may also alter phase properties (Shinitzky and Inbar, 1976; Kendall and McKersie, 1989). The higher $\tau$ value that is observed in cv Adamello following water deficit (Table V) is probably due to the increase in thylakoid membrane microviscosity and to changes in the lipid-protein interactions and protein conformation induced by FFA accumulation. Furthermore, it must be considered that alterations in protein composition that occur during periods of water deficit (Sgherri et al., 1993) may also influence the EPR parameters as previously hypothesized by Lynch et al. (1987). The average change in $\tau$ in both cultivars did not reflect the effects of protein aggregation because the FS amounts (Table II) were below 33 mol%, the value invoked as giving rise to large aggregates of intramembraneous particles (McKersie and Thompson, 1979; Duxbury et al., 1991a).

In the stressed, drought-sensitive cultivar, we have previously found a higher superoxide radical production in comparison with the tolerant cultivar, in which no increase was observed following water depletion (Quartacci et al., 1994). Thus, enhanced superoxide production would have caused protein cross-linking (Roberts et al., 1991) as evidenced by the increased $\tau$ and the SH group decrease (Table V). The reduction in SH group levels was probably due both to SH group oxidation and to the conformational changes of proteins, which may have made thiol groups inaccessible to the spin label.

The plot of the MP parameter versus temperature displayed discontinuities or inflections in the slopes in both cultivars (Figs. 2 and 3). Lynch et al. (1987) proposed that the changes in protein conformation are not in response to physical changes in the bilayer that are directly related to protein denaturation but to more subtle and less dramatic changes in molecular ordering of lipids than lipid phase transition (Dickens and Thompson, 1980).

In conclusion, in the drought-affected, tolerant wheat the lower degree of conformational changes in membrane proteins, in comparison with the sensitive cultivar, is probably due to the presence of a more fluid bilayer, since neither nonbilayer-forming lipids nor FFA accumulated following stress.

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