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Superoxide radical production in wheat plants differently sensitive to drought

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Introduction

There is increasing evidence to support the view that during dehydration oxygen radicals play a role in mediating the degradative reactions that precede loss of membrane integrity (Price *et al.* 1989; Quartacci & Navari-Izzo 1992; Sgherri *et al.* 1993).

Oxygen radicals are normally produced in biological tissues as a consequence of oxidative metabolism and electron transport. In normal conditions these reactions are tightly controlled, but during stress radicals become cytotoxic and cause an oxidative injury to cellular components. The electron transport systems of the chloroplasts may leak electrons to oxygen when the availability of $NADP^+$ is reduced (Thompson *et al.* 1987; Sgherri *et al.* 1993).

Once the capacity of the cell to scavenge and detoxify free radicals is exceeded, radicals will degrade cellular components such as membrane lipids and proteins (Duxbury 1991; Quartacci & Navari-Izzo 1992). The superoxide radical ($O_2^{\cdot -}$), or its derivatives, induces membrane rigidity as well as peroxidation of membrane lipids and can also directly attack certain sulphydryl-containing enzymes. The inactivation of membrane-bound enzymes and the separation of membrane lipid phases as a consequence of lipid compositional changes may then contribute to the loss of membrane integrity and selective permeability, as observed following stress.

The aim of this paper is to determine whether the difference in drought susceptibility of two wheat cultivars is related to differences in the rate of $O_2^{\cdot -}$ production by thylakoids under water deficit conditions, as well as to changes in the lipid composition of thylakoid membranes.

Materials and methods

Seedlings of two wheat cultivars (*Triticum durum* L.), one drought-tolerant (cv. Ofanto) and the other drought-sensitive (cv. Adamello), were grown under field irrigation and dryland conditions. In one set, control plants from both cultivars were regularly watered, whereas the other set of plants was subjected to water deficit by withholding water for 14 days, starting 30 days after sowing.

Leaf water status was determined by analyses of the pressure-volume curves performed as previously described by Navari-Izzo *et al.* (1990). Chlorophyll (Chl) and protein contents of isolated thylakoid membranes were determined as reported by Sgherri *et al.* (1993).

Lipid contents and composition were determined using TLC and GLC techniques according to Navari-Izzo *et al.* (1992).

Electron spin resonance (ESR) spectra were recorded using a Varian E-112

Table 2. Protein mobility and protein sulphydryl groups of two wheat cultivars (S, and T as Table 1) differently sensitive to drought subjected to 14 days of water deficit conditions. Means in rows followed by different letters are significantly different at $P=0.01$. T, spin label rotational correlation time (ns); Cl, mobile proteic portion (%); -SH, reduced sulphydryl groups (%).

	cv. Adamello (S)		cv. Ofanto (T)	
	Control	Stressed	Control	Stressed
T	0.13a	0.22b	0.16a	0.19b
Cl	6b	4a	2a	2a
-SH	100b	50a	100b	69a

duction in the control plants of both cultivars (Table 1). This might indicate that the photosynthetic electron transport rate was reduced under stress. Because of the altered membrane structure, which expose the normally unaccessible chlorophyll to oxygen, $O_2^{\cdot-}$ formation under water deficit conditions was higher in the drought-sensitive cultivar in comparison with the tolerant cultivar.

The examination of the main polar lipid components of thylakoid membranes shows different molar ratios of monogalactosyldiacylglycerol (MGDG) to digalactosyldiacylglycerol (DGDG) in the two cultivars. Whereas in cv. Adamello this ratio increased from 1.9 to 2.1 after water depletion, in cv. Ofanto it decreased from 2.0 to 1.4 due to MGDG decrease. Because of the different arrangements within the membranes, a change in their proportion is likely to be correlated with a change in the structure of thylakoids.

In the drought-tolerant wheat, free fatty acid contents did not change following stress. The higher free fatty acid contents in the stressed plants of cv. Adamello, produced as consequence of polar lipid de-esterification, may disturb bilayer organization and PSII activity (Hirayama & Nushida 1978).

Drought did not induce any changes in the total fatty acid unsaturation level in either cultivar. This suggests that no peroxidation of the acyl chains occurred, and also that changes in the unsaturation level, which are considered to be the only result of the oxidative stress, are, in part, a comparatively minor response to the attack of free radicals.

ESR data of spin-labelled proteins indicate (Table 2) that thylakoids of the drought-sensitive cultivar underwent a more pronounced oxidation of their sulphydryl groups under water stress in comparison with those of the tolerant cultivar.

In comparison with the controls of both cultivars the increase in the spin label rotational correlation time and the decrease in the mobile proteic portion were higher in the thylakoids of the unwatered plants of cv. Adamello. These data show that water-stressed thylakoid membranes were more rigid in comparison with the controls, especially in the drought-sensitive cv. Adamello. This higher thylakoid membrane rigidity can be attributed to differences in protein-surrounding lipids and/or to changes in protein conformation.

Conclusions

In comparison with the drought-sensitive cv. Adamello, thylakoids of the drought-tolerant wheat (cv. Ofanto) subjected to 14-days of water deficit conditions showed:

spectrometer equipped with a Varian variable temperature accessory. The thylakoid preparations were diluted to 0.2 mg Chl ml^{-1} with a buffer following the procedure of Sgherri *et al.* (1993). Kinetic measurements of the production of $O_2^{\cdot -}$ by illuminated thylakoids were determined by measuring the increase in amplitude with time of the low-field spectral line of the Tiron (1,2-dihydroxybenzene-3,5-disulphonic acid) semiquinone radical, which is formed when the spin trap Tiron reacts with $O_2^{\cdot -}$.

Protein sulphydryl groups of isolated thylakoids were labelled using the spin label 3-maleimido-proxyl in a reaction mixture containing 4 mg membrane proteins, and then analysed by ESR (Duxbury 1991).

Results and discussion

A water-deficit condition imposed for fourteen days determined similar declines in leaf water potential in both cultivars (1.1 MPa in cv. Adamello and 1.3 MPa in cv. Ofanto), but in the drought-tolerant wheat it resulted in a higher relative water content value.

In the stressed cv. Ofanto, the levels of Chl *a*, Chl *b* and lipids remained unchanged, and protein degradation was limited: these facts allowed the maintenance of an efficient photosynthetic activity in comparison with cv. Adamello, in which Chl *a*, proteins and, in lesser amounts, lipids were severely degraded. The decreased Chl *a*/Chl *b* ratio in the stressed sensitive wheat (from 3.1 to 1.5), which can be regarded as an index of PSII alteration, might have disturbed light absorption and conveyance and also electron supply to photosystems.

In stressed drought-sensitive wheat, lipid degradation of thylakoid membranes can induce changes in the lipid–protein interaction and protein conformation, and initiate proteolysis (Duxbury *et al.* 1991). The illuminated thylakoids of the drought-sensitive cultivar increased the production of $O_2^{\cdot -}$ by about 40% following water depletion (Table 1), whereas the thylakoids of the stressed drought-tolerant cultivar did not show any difference in the production of $O_2^{\cdot -}$ in comparison with the control. In the latter cultivar it seems likely that the antioxidants (carotenoids, tocopherols) present in the thylakoid membranes may have scavenged $O_2^{\cdot -}$ radicals and limited their level under stress.

The superoxide radical formation rate indicates a higher efficiency in $O_2^{\cdot -}$ pro-

Table 1. Kinetic production of $O_2^{\cdot -}$ radicals by illuminated thylakoids in two wheat cultivars (S, drought-sensitive; T, drought-tolerant) differently sensitive to drought subjected to 14 days of water deficit conditions. Means in columns followed by different letters are significantly different at $P=0.01$. A_o, maximum amplitude of ESR signal (arbitrary units); K_f, superoxide formation rate (s^{-1}); K_d, superoxide decay rate (s^{-1}).

	cv. Ademello (S)		cv. Ofanto (T)	
	Control	Stressed	Control	Stressed
A_o	33.9a	47.8b	83.2a	83.5a
K_f	$3.8/10^{-2}$b	$3.1/10^{-2}$a	$3.5/10^{-2}$b	$1.7/10^{-2}$a
K_d	$1.2/10^{-4}$a	$1.7/10^{-4}$b	$5.9/10^{-4}$b	$5.0/10^{-4}$a

unchanged production of $O_2^{\cdot-}$;
maintained chlorophyll and lipid contents;
limited protein degradation;
reduced MGDG/DGDG molar ratio due to MGDG decrease;
no accumulation of free fatty acids nor lipid de-esterification;
limited membrane rigidity and -SH group oxidation.

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Defence mechanisms against production of free radicals in cells of 'resurrection' plants

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Introduction

The ability of protoplasm to revive following severe water deficit is at its greatest in desiccation-tolerant or 'resurrection' plants. *Boea hygroscopica* is a resurrection plant that is able to survive air-dryness following slow dehydration (80% RH) in a physiological state called anabiosis (Schwab & Gaff 1990). However, this plant loses the ability to recover complete physiological activity following rapid water loss (0% RH).

The ability to recover complete physiological activity following repeated protoplasmic dehydration of fully differentiated tissues is an adaptation mechanism unique to resurrection plants.

Drought may be regarded as an oxidative stress (Burke *et al.* 1985). In response to dehydration, the stomata close and CO_2 fixation becomes low, while photosynthetic electron transport still operates at normal rates. Under these conditions, limited quantities of $NADP^+$ are able to accept electrons, and oxygen can function as an alternative electron acceptor, which results in the production of toxic oxygen species such as superoxide anion radicals ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) (Anderson *et al.* 1990). Several endogenous protective mechanisms involving glutathione, ascorbate and related enzymes such as glutathione reductase (GR) (EC 1.6.4.2), ascorbate peroxidase (EC 1.11.1.11) and dehydroascorbate reductase (DHAR) (EC 1.8.5.1), scavenge and remove those toxic products in the ascorbate/glutathione cycle (Fig. 1) before damage occurs. The possibility that desiccation tolerance may be dependent on the ability to process species of activated oxygen was investigated in *Boea hygroscopica* subjected to either slow or rapid desiccation and rehydration. Knowledge of the changes in the substrates and the enzymes of the ascorbate/glutathione cycle might give us a better comprehension of the molecular aspects of metabolic reactivation following desiccation.

Materials and methods

Plants of *Boea hygroscopica* were grown at 27 °C, with a 16-h photoperiod and 85–95% air humidity. Control leaves were detached from plants grown in well-watered conditions and then subjected to: (i) rapid dehydration on activated silica gel (0% RH), and (ii) slow dehydration on NH_4NO_3-saturated solution (80% RH), both for 144 h. Slowly dried leaves were then rehydrated for 36 h in 20 ml distilled water. Rapidly dried leaves were not able to revive, whereas slowly dried leaves recovered their normal metabolic status.

Hydrogen peroxide contents were evaluated following the method of Mondal & Choudhuri (1981). Changes in GR, ascorbate peroxidase and DHAR activities were