INFLUENCE OF NUTRIENT AVAILABILITY ON DROUGHT-INDUCED CHANGES IN THE ACTIVITY OF ANTIOXIDANT ENZYMES IN SUNFLOWER LEAVES

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ABSTRACT

We aimed to evaluate if plants with different nutrient availability evidenced a different capacity to overcome drought-induced oxidative stress. Membrane peroxidative damages as MDA concentration and the activity of several antioxidant enzymes were determined in leaves of well watered (WW) and water stressed (WS) *H. annuus* plants grown either with adequate (Adeq Nutr) or limited (Limit Nutr) nutrient regimes. Constitutive capacity to eliminate ROS was not overall changed by growing plants with different nutrient supply regimes, but a diverse enzyme-dependent response was observed. In response to drought SOD and DHAR activity increased only in plants with limited nutrient supply, while in plants with adequate nutrient supply the activity of these enzymes did not change and were constitutively higher, but the activity APX increased by 50%. The subtle observed changes in the activity of the antioxidant enzymes are discussed, given that no oxidative damage was observed.

INTRODUCTION

Reactive derivatives of oxygen (ROS), such as hydrogen peroxide (H_2O_2), the superoxide radical anion ($O_2^{\bullet -}$) and hydroxyl radical ($OH^{\bullet}$), are inevitable by-products of biological redox reactions (Foyer *et al*. 1994; Apel and Hirt, 2004). ROS accumulation may inactivate enzymes and damage important cellular components (Azzi *et al.* 2004), and adequate protection against ROS is provided by antioxidative defence systems, including enzymes as superoxide dismutase (SOD) that catalyzes the dismutation of $O_2^{\bullet -}$ radicals to molecular oxygen and H_2O_2, while H_2O_2-scavenging is accomplished by catalase (CAT), various peroxidases and the ascorbate-glutathione cycle, a series of coupled redox reactions involving four enzymes, ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) (Apel and Hirt, 2004).

It is well documented that water deficits may strongly limit both photosynthesis (Flexas *et al*. 2004), and nitrate reduction (Correia *et al.* 2005) inducing the generation of ROS (e.g., Jiang and Zhang 2002; Reddy *et al.* 2004). The extent of cellular damage by ROS may be limited or reduced by the up-regulation of the activities of several antioxidant enzymes. However, under mild to moderate water stress conditions, the degree to which the activities of antioxidant enzymes increase is extremely variable (Reddy *et al.* 2004), and in some cases no effect, or a negative effect, has been reported (Schwantz and Polle 2001; Turkan *et al.* 2005).

Low availability of nutrients is another factor that may also decrease photosynthetic CO_2 fixation, leading to over reduction of the photosynthetic electron transport (ETC) components and hence increasing the demand for oxidative protection. In accordance, nutrient deficiencies have been found to affect the activities of antioxidant enzymes, the response depending on the enzyme and the nutrient (e.g., Logan *et al.* 1999; Schmitz-Eiberger *et al.* 2002; Kandlbinder *et al*. 2004; Tewari *et al.* 2004). These studies assessed the changes in the activity of several antioxidant enzymes in response to the depletion of individual nutrients in well-watered plants, but under field conditions, plants may be subjected to low availability of several nutrients. So far there is a lack of information on the influence of nutrient availability on the response of antioxidant enzymatic system to drought stress. In the present work we aimed to study the influence of low nutrient supply on drought-induced changes in lipid peroxidation levels of membranes and in the activity of several antioxidant enzymes in sunflower potted plants.

MATERIAL AND METHODS

Plants of *Helianthus annuus* L. (var. "Giant") were grown in 3 L pots (peat and vermiculite 1v:1v) in a naturally lit greenhouse (light intensity 35% the outside levels, min and max temperatures averaging 15ºC and 29ºC). The pots were regularly brought to field capacity and watering was done using either an Hoagland modified, full strength nutrient solution (Adequate Nutr) or half strength nutrient solution (Limited Nutr). The onset of water stress imposition (WS) took place 26 days after sowing by replacing partially the water lost by evapotranspiration (determined gravimetrically) for 3 weeks and, at the same time, control plants (WW), of both nutrient treatments, were watered to full capacity. In order to assure similar environmental conditions between treatments in each measurement day, on the evening before, the plants were transferred to a growth chamber (Fitoclima, Aralab, Lisboa) with daytime temperature of 22ºC, 70% relative humidity and 200 μmol m^{-2} s^{-1} photosynthetic photon flux density. Samples were collected in two fully expanded, non-senescent leaves, except pre-dawn leaf water potential that was determined in an older leaf. The photosynthetic rate at saturating light and CO_2 (A_{max}) and at 25ºC was determined in a Clark type leaf disc oxygen electrode (Hansatech, Kings Lynn, U.K.) according to Delieu and Walker (1981) and soluble protein quantitation as in David *et al* (1998) with the BioRad Protein Assay Dye (BioRad, Hercules, California, USA). Lipid peroxidation level of plant membranes was determined as malondialdehyde (MDA) concentration as described by Hodges *et al*. (1999). Enzyme extracts were obtained as in Polle *et al.* (1993) modified in accordance to Nakano and Asada (1987). APX, DHAR, MDHAR and GR activities were measured as in Polle and Morawe (1995). SOD activity was assayed according to Polle *et al.* (1989) and CAT as in Aebi (1984).

RESULTS

By the end of the drought period WS plants experienced a moderate water stress presenting a pre-dawn leaf water potential (Ψ_{PD}) of -0.6 MPa, whereas WW plants always presented values higher than -0.2 MPa. No significant differences in Ψ_{PD} were observed between plants with different nutrient supplies (data not shown). Water deficits did not negatively affect either A_{max} or MDA (Table 1), nor there was any significant difference between nutrient treatments. Changing the availability of nutrients

did not affect protein content, but water deficits induced a significant decrease in leaf soluble protein content (Table 1).

Table 1 Photosynthetic rate at saturating light and CO_2 (A_{max}), malondialdehyde (MDA) and soluble protein (P_{sol}) determined in leaves of well watered (WW) and water stressed (WS) *H. annuus* plants grown with adequate (Adeq Nutr) or limited (Limit Nutr) nutrient supply regime (mean ± standard error, n = 5).

	WW		WS	
	Adeq Nutri	Limit Nutr	Adeq Nutr	Limit Nutr
A_{max} (μmol O_2 s^{-1} g^{-1}Chl)	61.7 ± 5.8	61.7 ± 3.9	57.0 ± 0.7	49.4 ± 1.3
MDA (nmol g^{-1}DM)	281 ± 37	274 ± 31	330 ± 32	289 ± 16
P_{sol} (mg g^{-1} DM)	318 ± 14	299 ± 12	265 ± 15	265 ± 14

In the absence of water deficit, the plants adequately supplied with nutrients exhibited SOD and DHAR activities two and four-fold higher, respectively, than those from plants subjected to a nutrient deficient supply regime (Fig. 1). An opposite, but less intense trend was evidenced for GR and MDHAR. Regardless of the nutrient supply regime, water deficit did not induce any significant response in MDHAR activity whereas the activity of CAT decreased in water-stressed plants. As to the response of the other enzymes to water deficit, they were dependent on the availability of nutrients (Fig. 1). The activities of SOD and DHAR increased in WS plants with limited nutrient supply, contrasting with a decrease in WS plants with adequate nutrient supply. In response to water deficit, APX activity was unchanged in plants with low nutrient supply but increased by 50% in plants adequately supplied with nutrients. In the later the GR activity was unaffected by drought stress, but in leaves of plants subjected to nutrient deficiency the activity of this enzyme decreased by 35% in response to water deficit.

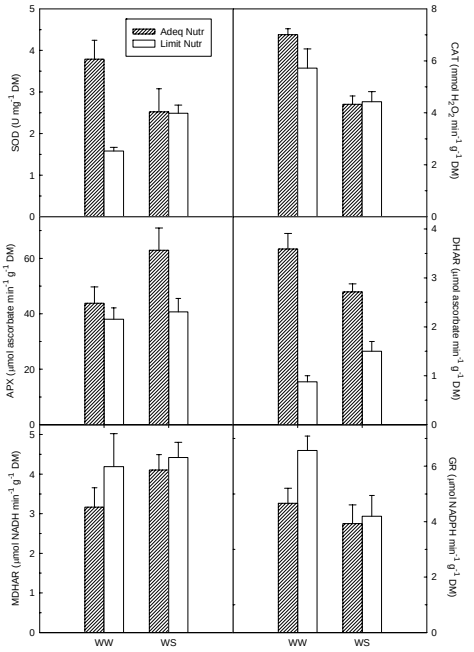

Figure 1 Antioxidant enzymes activity (see text for details) in leaves of well watered (WW) and water stressed (WS) *H. annuus* plants grown with adequate (filled bars) or limited (open bars) nutrient supply regime (mean ± standard error, n = 5)

DISCUSSION

Previous studies indicate that the differential water stress tolerance of plants may be related with differential protection against drought-induced oxidative stress due to differences in constitutive levels and/or responsiveness of the antioxidant enzymes to water stress (Sairam *et al.* 1998, Lima *et al.* 2002; Türkan *et al*. 2005). Our study show that the constitutive capacity to eliminate ROS is not overall changed

by growing plants with adequate nutrient supply, since only SOD and DHAR activities were enhanced in response to increased availability of nutrients.

Our results concerning plants with an adequate nutrient supply generally agree with those of Zhang and Kirkham (1996) who observed that mild and moderate water deficits did not affect total activities of SOD, APX, CAT, MDHAR, DHAR and GR in sunflower seedlings, the exception is made, in our case, for CAT activity that, under mild water deficit, decreased irrespective of the nutrient supply regime. In response to water deficits, SOD and DHAR activity increased only in plants with limited nutrient supply. The adaptive meaning of enhanced SOD protection against over reduction processes, often associated with depleted intercellular CO_2 situations (Arisi *et al.* 1998), would only be effective if the H_2O_2-scavenging capacity is also enhanced (Foyer *et al.* 1994). In WS plants with adequate nutrients, higher APX activity indicates a higher potential to eliminate H_2O_2, on the other hand, in nutrient deficient plants, the elevated GR and MDHAR activities might increase $NADP^+$/NADPH ratio, ensuring $NADP^+$ availability to accept electrons from the ETC, and thereby minimizing $O_2^{\bullet-}$ production.

Apparently the subtle changes observed in the activity of the enzymes involved in the ascorbate-glutathione cycle were sufficient to prevent oxidative damage, as shown by the lack of increase of MDA in leaves either from moderate droughted or nutrient limited sunflower plants. The contrasting patterns of drought-induced changes in SOD, APX, DHAR and GR activities between plants grown with different nutrient supplies may also contribute to explain some diversity of drought-induced responses in antioxidant enzymes reported in the literature.

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