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ARTICLE

Effect of abiotic stress on antioxidants in maize

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ABSTRACT Changes in the level of reactive oxygen species and antioxidants were investigated in maize (*Zea mays* L.) subjected to abiotic stress. Abscisic acid and direct oxidative stress induced an increase in the hydrogen peroxide and lipid peroxide concentrations. The level of the antioxidants examined was increased by nearly all the treatments. The glutathione concentration was higher in the stress-tolerant line Z7 than in the stress-sensitive Penjalinan after treatment with hydrogen peroxide or polyethylene glycol and lower after the application of abscisic acid or NaCl. The activity of glutathione reductase, ascorbate peroxidase, catalase and glutathione S-transferase was greater after almost all the treatments in Z7. A comparison of the antioxidants in two maize lines having different levels of stress tolerance corroborated the importance of the effective removal of reactive oxygen species in defence against abiotic stresses.
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KEY WORDS

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Antioxidants play an important role in preventing the stress-induced accumulation of toxic concentrations of reactive oxygen species (Foyer and Noctor 2005). Among the various types of reactive oxygen species, the amount of superoxide radical was increased by drought stress in wheat (Price et al. 1989) and the concentration of hydrogen peroxide and lipid peroxides in tobacco (Hideg et al. 2003). The increase in ROS concentration in turn activates antioxidants, as reported in several publications (Foyer and Noctor 2005). The components of the ascorbate-glutathione cycle were induced by various stress treatments. GR activity was greater following drought and salt stress in tolerant poplar and cotton genotypes, respectively, compared to sensitive ones (Gossett et al. 1994; Edjolo et al. 2001). The relationship between stress tolerance and antioxidant levels was also demonstrated in transgenic plants (Noctor et al. 1998). It can thus be concluded that antioxidants contribute to improved stress tolerance due to the detoxification of ROS.

The aim of the present investigations was to find out whether various abiotic stress treatments have specific effects on free radical and antioxidant levels in maize lines differing in their tolerance to low temperature and osmotic stress (Kocsy et al. 2001; Kellős et al. 2008). Glutathione synthesis and reduction was compared at both the transcriptional and post-transcriptional levels. In addition, the effect of abscisic acid on the antioxidants was studied.

Materials and Methods

Two maize (*Zea mays* L.) cultivars, Z7 and Penjalinan, were studied, originating from the temperate zone and the tropical regions, respectively, and having contrasting tolerance to extreme temperatures and osmotic stress (Kocsy et al. 2004; Kellős et al. 2008). The plants were cultivated as described previously (Kocsy et al. 2004). Eleven-day-old seedlings having two completely developed leaves were subjected to the following treatments: further cultivation under unchanged conditions (control), or in nutrient solution supplemented with 0.1 mM ABA, 1 mM H_2O_2, 200 mM NaCl or 15% polyethylene glycol 4000 (PEG) for 7 days.

The amount of H_2O_2 and lipid peroxides and the activity of ascorbate peroxidase, glutathione reductase, glutathione S-transferase and catalase were determined spectrophotometrically from leaves, as described previously (Kellős et al. 2008). Glutathione and its precursor were measured by HPLC (Kellős et al. 2008). The transcript level of the enzymes involved in glutathione synthesis and degradation was determined by semiquantitative RT-PCR (Kellős et al. 2008). The statistical analysis was done using two-component (treatments, genotypes) analysis of variance (Microsoft Excel 2000).

Results and Discussion

The previously reported difference in the stress tolerance of the two maize lines (Kocsy et al. 2004) was confirmed, since the tolerant Z7 suffered less damage and had a smaller reduction in growth as a consequence of the stress treatments. Z7 accumulated less H_2O_2 and lipid peroxide during the various treatments, indicating less severe oxidative damage (Table 1). The more effective detoxification of ROS in this genotype could be the result of a more active antioxidant system, demonstrated by the higher ratio of reduced (GSH) to oxidised glutathione (GSSG) after non-ionic osmotic stress

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Table 1. Effect of the various treatments on the amount of H_2O_2 and lipid peroxides, on the ratio of reduced to oxidised glutathione (GSH/GSSG) and on the activity of glutathione reductase (GR) in Penjalinan (stress-sensitive) and Z7 (stress-tolerant). SD: significant difference.

	H_2O_2 [nmol (g FW)$^{-1}$]		Lipid peroxides		GSH/GSSG		GR [nkat (g protein)$^{-1}$]	
	Penjalinan	Z7	Penjalinan	Z7	Penjalinan	Z7	Penjalinan	Z7
Control	190.6	135.4	107.8	60.8	30.3	1386.0	0.1	0.2
ABA	374.5	249.7	568.1	134.4	1441.3	21.8	0.3	0.9
H_2O_2	315.3	261.9	589.1	222.2	1481.2	1385.5	0.2	0.7
NaCl	19.6	11.8	130.4	36.9	3014.8	497.0	1.3	1.0
PEG	226.0	123.2	184.4	43.1	557.9	1074.8	0.2	0.4
SD	73.9		162.8		785.5		0.56	

(polyethylene glycol) and higher glutathione reductase (GR) activity following all the treatments except for NaCl (Table 1). Consistently with the present findings, the accumulation of H_2O_2 was observed following various abiotic stresses in maize (Prasad et al. 1994) and tobacco (Hideg et al. 2003). Interestingly, ABA and direct oxidative stress increased the ROS levels in maize, as in the case of H_2O_2 in ABA-treated wheat (Agarwal et al. 2005), while osmotic stress caused no change or even a decrease. The role of GSH and GR in stress tolerance was demonstrated by chemical manipulation of their levels (Kocsy et al. 2001a). From these studies it is evident that there is a relationship between the level of these antioxidants and stress tolerance. It is interesting to note that ionic osmotic stress induced by NaCl resulted in changes in GSH synthesis similar to those caused by ABA.

In the present study the activity of GR and three other antioxidant enzymes (ascorbate peroxidase, glutathione S-transferase and catalase) was significantly higher in the stress-tolerant variety Z7 compared to the sensitive variety Penjalinan. A relationship between stress tolerance and enzyme activity was also found in the case of GR following drought in poplar and salt stress in cotton (Edjolo et al. 2001; Gossett et al. 1994) and for superoxide dismutase in salt-treated cotton (Gossett et al. 1994).

Since the observed changes in the amount of GSH or the activity of GR did not always coincide with the alterations in the corresponding transcript levels, it can be assumed that the differences in GSH synthesis and reduction after the various treatments are the result of post-transcriptional regulation. For instance, ABA increased the γ*ECS* transcript level but reduced the amount of γEC and GSH, while NaCl did not change the expression of the GR gene but increased the activity of GR in Z7.

The stress-induced accumulation of ABA may affect antioxidants through a signalling pathway involving ROS (Foyer and Noctor 2005). However, H_2O_2 is also thought to regulate GSH levels independently of ABA (Kocsy et al. 2004). It is anticipated that ROS may participate in stress signalling both directly and indirectly through their interactions with stress hormones.

In summary, the treatment- and genotype-specific differences in GSH level and GR activity appear to be determined at the post-transcriptional level. ABA influences GSH levels due to its effect on GSH synthesis.

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