Phyton (Austria) Special issue: "Free Radicals"	**Vol. 37**	Fasc. 3	(13)-(18)	1. 7. 1997

Paraquat Tolerance of Transgenic Tobacco Plants with Altered Activity of Glutathione Reductase

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Key words: Active oxygen, ascorbate, glutathione, glutathione reductase, *Nicotiana tabacum*, ozone, paraquat, transgenic plant.

Summary

AONO M., SAJI H., KONDO N. & TANAKA K. 1997. Paraquat tolerance of transgenic tobacco plants with altered activity of glutathione reductase. - Phyton (Horn, Austria) 37 (3): (13) - (18).

Transgenic tobacco plants which had enhanced activities of glutathione reductase and/or superoxide dismutase showed increased resistance to paraquat. The leaves of these transgenic plants had higher contents of both reduced glutathione (GSH) and ascorbate than those of the control plants during paraquat treatment in the light. These transgenic plants showed no more resistance to an air pollutant ozone than the control in terms of the extent of visible foliar damage, however, the transgenic leaves had higher contents of GSH and ascorbate and slightly lower contents of malondialdehyde than the control leaves during exposure to ozone.

Introduction

Active oxygen species such as superoxide radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) were generated as by-products in many biological reactions. They are toxic to living organisms and, unless removed rapidly by a scavenging system of cells, destroy various cellular components and/or inactivate metabolism. The generation of active oxygen species is promoted under environmental conditions such as drought (SMIRNOFF 1993), low temperature (SCHÖNER & KRAUSE 1990), and exposure to air pollutants (SHIMAZAKI & al. 1980) or some herbicides (DODGE

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1975) in the light, which are thought to cause photooxidative damage to plants. There are enzymes in plants responsible for scavenging active oxygen species and these enzymes are thought to be involved in the photooxidative stress tolerance of plants (SMITH & al. 1990, BOWLER & al. 1994). Glutathione reductase (GR) (EC 1.6.4.2) is one of these enzymes and is postulated to supply reduced form of glutathione (GSH) to the ascorbate-glutathione cycle (FOYER & HALLIWELL 1976).

The role of GR in the plant tolerance to photooxidative stress was previously analyzed using four types of transgenic tobacco plants: 1) those containing the GR of *Escherichia coli* origin in the cytosol (AONO & al. 1991) ; 2) those containing the *E. coli* GR in the chloroplasts (AONO & al. 1993); 3) those having antisense spinach cDNA for chloroplastic GR (AONO & al. 1995a) ; 4) those with the GR of *E. coli* and rice superoxide dismutase (SOD) (EC 1.15.1.1), that is also one of the enzymes for scavenging active oxygen, in the cytosol (AONO & al. 1995b). Transgenic tobacco with high cytosolic [1)] or chloroplastic [2)] GR activity showed enhanced tolerance to photooxidative stress caused by a herbicide paraquat or an air pollutant sulphur dioxide (AONO & al. 1991, 1993). Transgenic tobacco with reduced activity of GR [3)] exhibited enhanced sensitivity to paraquat (AONO & al. 1995a). In addition, transgenic tobacco with simultaneously enhanced activities of GR and SOD [4)] showed extremely high tolerance to paraquat (AONO & al. 1995b). These results indicate that plant tolerance against photooxidative stress is dependent on the activities of these antioxidant enzymes and that co-operative work of these enzymes is important. However, the transgenic plants with enhanced activity of GR showed no more resistance to ozone than the control (AONO & al. 1991, 1993).

In this study, contents of antioxidants, such as GSH and acorbate, and malondialdehyde (MDA) in the leaves of transgenic tobacco which have enhanced activity of GR and/or SOD were measured during photooxidative stress caused by paraquat or ozone.

Materials and Methods

1. Paraquat treatment and measurement of GSH and ascorbate

Tobacco plants (*Nicotiana tabacum* SR1) were grown on a regime of 14 h light (130μE/m^2/s) and 10 h dark at 25°C. Twenty leaf discs were excised from a young-expanded leaf of each of 6-week-old plants. Five individuals were used per one type of tobacco for an experiment. The leaf discs were supplied with 10μM paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride; Sigma) containing 0.1% Tween 20 by vacuum infiltration. They were washed and transferred to deionized water in a Petri dish and were exposed to light (130 μE/m^2/s) at 25 °C for 8 h. Twenty discs (0.4 g) were collected every 2 h and homogenised in 2 volumes (0.8 ml) of 5 % meta-phosphate followed by a centrifugation at 6000 x g for 10 min. Contents of GSH and ascorbate in the supernatants were measured by colorimetric assay using GSH-400 (Bioxyteque, France) and a reflecting photometer (RQflex and Reflectquant Ascorbate test, Merck, Germany).

2. Ozone exposure

Six-week-old tobacco plants were either exposed to 0.3 ppm ozone for 2 h at 25 °C in the light (400 μE/m^2/s) in a growth cabinet (230 x 190 x 170 cm^3) or placed in another growth cabinet without ozone as a control (0 ppm ozone). Ozone exposure was performed as described (AONO & al. 1991). Ten leaf discs were excised from a young-expanded, intact leaf and GSH and ascorbate contents were measured as described above.

3. Measurement of MDA

Seven-week-old tobacco plants were exposed to 0.2 ppm ozone for 3 days as described above. Young-expanded, intact leaves were excised and their fresh weight were measured . Then they were homogenized in 2 volumes (w/v) of 100mM potassium-phosphate buffer (pH 7.8) followed by a centrifugation at 6000 x g for 10 min. MDA contents in the supernatants were measured by the thiobarbituric acid assay as described by BUEGE & AUST 1978.

Results and Discussion

Contents of both GSH and ascorbate decreased during paraquat treatment in the light in leaves of all types of tobacco plants, however, foliar contents of these antioxidants remained higher in the transgenic tobacco with high GR and /or SOD activity than those of the control (Fig. 1).

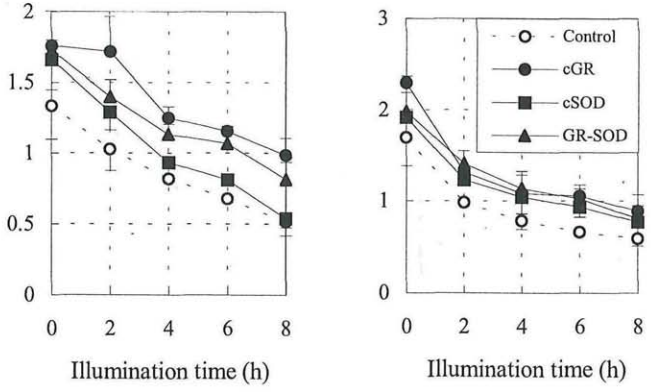

Fig. 1. Changes of the contents of GSH and ascorbate in leaves of the transgenic and the control tobacco plants during paraquat treatment. Each point was the average of results from three independent ements. Vertical bars stand for SE. Control, non-transgenic tobacco; cGR, transgenic tobacco with enhanced cytosolic activity of GR (AONO & al. 1991); cSOD, transgenic tobacco with enhanced cytosolic SOD activity (SAKAMOTO & TANAKA 1993); GR-SOD, transgenic tobacco with enhanced cytosolic activities of both GR and SOD (AONO & al. 1995b).

Hence, increased supplying of GSH by GR is thought to enhance regeneration of ascorbate for raising the capacity of scavenging active oxygen in the cell.

The transgenic and the control plants showed visible damage in older leaves after 2h-exposure to 0.3ppm or 6h-exposure to 0.2ppm ozone (data not shown). The transgenic plants exhibited no more resistance to ozone than the control in terms of the extent of the damage corresponding to the results of previous studies (AONO & al. 1991, 1993). However, GSH and ascorbate contents in the transgenic leaves were higher than those of the controls after the exposure to 0.2ppm ozone (Table 1).

Table 1. Effect of ozone on the contents of GSH and ascorbate in leaves of the transgenic and the control tobacco plants. Control, cGR, cSOD and GR-SOD; see legend for Fig. 1. Shown are average of five individuals and ± SE.

[a]The ratio (%) of the value for 0.3 ppm ozone exposure to that for 0 ppm ozone.

		GSH (μmol/g fr wt)	%[a]	Ascorbate (μmol/g fr wt)	%[a]
Control	0 ppm ozone	1.10 ± 0.04		2.69 ± 0.00	
	0.3 ppm	0.94 ± 0.03	85.5	0.33 ± 0.01	12.3
cGR	0 ppm	1.03 ± 0.00		2.70 ± 0.07	
	0.3 ppm	1.17 ± 0.01	113.6	0.61 ± 0.02	22.6
cSOD	0 ppm	0.96 ± 0.02		1.91 ± 0.04	
	0.3 ppm	1.04 ± 0.00	108.3	0.53 ± 0.07	27.7
GR-SOD	0 ppm	0.95 ± 0.02		1.91 ± 0.06	
	0.3 ppm	1.03 ± 0.03	108.4	0.84 ± 0.05	44.0

MDA contents commenced to increase after one-day exposure to 0.2 ppm ozone both in the transgenic and the control leaves; they were slightly lower in the transgenic leaves before and during the ozone exposure (Table 2). MDA is formed from the breakdown of polyunsaturated fatty acids and it serves as an index of the extent of the peroxidation reaction.

Table 2. MDA contents in leaves of the transgenic and the control tobacco plants during ozone exposure. Control; transgenic tobacco having antisense spinach cDNA for GR, see Introduction (AONO & al. 1995a). The GR activity of these plants was similar to those of non-transgenic plants (data not shown). cGR, cSOD and GR-SOD; see legend for Figure 1. Four individuals were used per one type of tobacco for an experiment.

	MDA (nmol/g fr wt			
	0h	3h	26h	74h
Control	21.1	19.5	26.8	33.9
cGR	14.6	12.3	20.6	22.9
cSOD	14.5	16.4	20.6	25.2
GR-SOD	18.1	15.9	24.1	25.1

The higher contents of GSH and ascorbate in the transgenic leaves may, therefore, have contributed to remove active oxygen species that generated not only during paraquat treatment but also during ozone exposure, however, the capacity of scavenging active oxygen of the transgenic cells may not be high enough to protect the cells from lethal photooxidative damage caused by ozone.

Although several studies besides ours have also indicated the involvement of GR in plant stress tolerance using transgenic plants, the correlation between levels of GR activity and stress tolerance has not always been exhibited (BROADBENT & al. 1995, FOYER & al. 1995). Further studies are required to clarify the mechanism of plant tolerance against photooxidative stress.

Acknowledgements

We thank M. MARUO for technical assistance.

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