Phyton (Austria) Special issue: "D. Grill"	Vol. 45	Fasc. 3	(39)-(44)	1.9.2005

Enhanced Inducibility of Glutathione S-Transferase Activity by Paraquat in Poplar Leaf Discs in the Presence of Sucrose

By

G. GULLNER[1], G. GYULAI[2], A. BITTSÁNSZKY[2], J. KISS[2]
L. HESZKY[2] & T. KOMIVES[1]

Key words: Glutathione S-transferase, inducibility, oxidative stress, paraquat, poplar, sucrose

Summary

GULLNER G., GYULAI G., BITTSÁNSZKY A., KISS J., HESZKY L. & KOMIVES T. 2005. Enhanced inducibility of glutathione S-transferase activity by paraquat in poplar leaf discs in the presence of sucrose. - Phyton (Horn, Austria) 45 (3): (39)-(44).

Leaf discs of the wildtype poplar hybrid *Populus canescens* and its two transgenic lines overexpressing γ-glutamylcysteine synthetase in the cytosol or in the chloroplasts were exposed to the herbicide paraquat (4×10^{-9} to 4×10^{-6} M). Leaf discs were incubated on tissue culture media containing 0.2, 1.0 or 2.0 % sucrose. Exposure to paraquat led to a concentration-dependent decrease of GST activities at 0.2 % sucrose. However, at higher external sucrose supplies, paraquat brought about marked inductions of GST activities in all poplar lines. The GST induction was light-dependent. Fluorescence measurements showed that the efficiency of the photosynthetic apparatus was inhibited by high paraquat concentrations (4×10^{-7} - 4×10^{-6} M) in all poplar clones. The photosynthetic efficiency (F_v/F_m) decreased also in leaf discs incubated in continuous darkness in the presence of paraquat.

Introduction

A wide range of plant species are capable of removing and/or degrading toxic organic substances and heavy metals from polluted soils. Phytoremediation is an emerging new technology based on these phenomena (SALT & al. 1998,

[1] Plant Protection Institute, Hungarian Academy of Sciences, 1022 Budapest, Herman Ottó u. 15, Hungary, e-mail: ggull@nki.hu
[2] HAS-SIU Research Group, Department of Genetics and Plant Breeding, Szent István University, 2103 Gödöllő, Páter K. u. 1, Hungary.

KOMIVES & GULLNER 2000). Poplar trees are good candidates for phytoremediation purposes due to their extensive root system, high water uptake, rapid growth and large biomass production (BURKEN & SCHNOOR 1998, DI BACCIO & al. 2003, KOMIVES & al. 2003, BITTSÁNSZKY & al. 2005). The remediative capacity of poplars can be significantly increased by genetic manipulations. Some years ago poplar plants were transformed to overexpress the bacterial gene encoding γ-glutamylcysteine synthetase (γ-ECS, EC 3.2.3.3), which is the rate-limiting regulatory enzyme in the biosynthesis of the ubiquitous tripeptide thiol compound glutathione (GSH, γ-L-glutamyl-L-cysteinyl-glycine). The transformed poplars contained higher levels of GSH and its precursor γ-L-glutamyl-L-cysteine (γ-EC) than the wildtype. The increased production of GSH improved the detoxification capacity of poplars against various environmental pollutants (NOCTOR & al. 1998), but the transgenic poplar lines did not show enhanced tolerance to photo-oxidative stress caused by the herbicide paraquat (WILL & al. 2001).

The glutathione S-transferase isoenzymes (GSTs, E.C. 2.5.1.18.), which catalyze conjugation reactions between GSH and a number of xenobiotics, play crucial roles in the degradation of toxic substances. GST is inducible by a wide range of stress effects (GULLNER & al. 2001, WAGNER & al. 2002). To gain a deeper insight into the stress resistance and detoxification capacity of transgenic poplar plants, GST activities were measured in leaf discs of the wildtype poplar hybrid *Populus canescens* and two of its transgenic lines overexpressing γ-ECS in the cytosol (11ggs) or in the chloroplasts (6LgI) following light- and dark exposures to paraquat.

Material and Methods

The untransformed poplar hybrid *Populus canescens* (*Populus tremula x Populus alba*, INRA clone 717-1-B4), and its two genetically transformed lines overexpressing the *Escherichia coli gshI* gene encoding γ-ECS in the cytosol (11ggs) or in the chloroplasts (6LgI) were used. Vector construction, transformation, identification and characterization of transformants were published earlier (NOCTOR & al. 1998).

Poplar clones were micropropagated in aseptic in vitro shoot culture. Leaf discs (8 mm in diameter) were cut and placed on aseptic woody plant media (WPM) supplemented with a concentration series (4×10^{-9} to 4×10^{-6} M) of paraquat (methyl viologen or 1,1'-dimethyl-4,4'-bipyridinium dichloride, purchased from Sigma, St. Louis, MO, U.S.A.) as described earlier (GYULAI & al. 1995). The tissue culture media contained 0.2, 1.0 or 2.0 % sucrose. Discs were incubated under 16/8 h light (40 μmol m^{-2} s^{-1}) /dark photoperiods or in continuous darkness. Photosynthetic activities and GST enzymatic activities of leaf discs were measured after 21 days exposure to paraquat.

Photosynthetic activity was characterized by chlorophyll fluorescence measurements. Maximum PSII efficiency (F_v/F_m) was determined by a laser induced (635 nm) two-wavelength spectrofluorometer (CFM-636973) detecting chlorophyll fluorescence at 690 nm (LICHTENTHALER & RINDERLE 1988, BAROCSI & al. 2000). GST activities were determined spectrophotometrically by measuring the formation of the reaction product conjugate molecule at 340 nm using 1-chloro-2,4-dinitrobenzene as substrate (GULLNER & al. 2001).

At least three independent parallel experiments were carried out in each case. The significant differences between mean values were evaluated by Student's t-test. Differences were considered to be significant at P = 0.05.

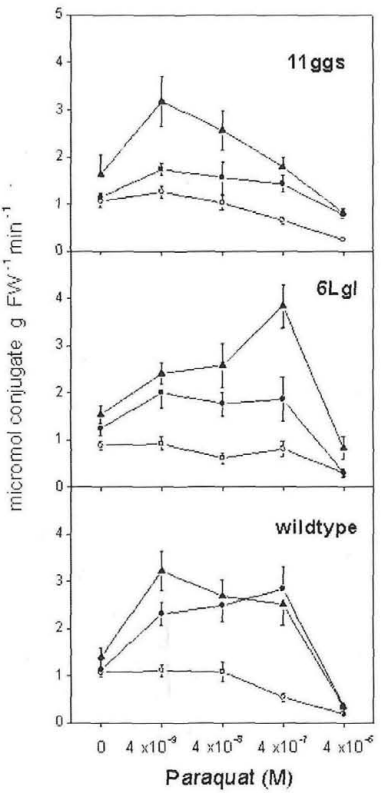

Fig. 1. Induction of glutathione S-transferase (GST) activity in leaf discs of the wildtype poplar hybrid *P. canescens* and its two genetically transformed lines (11ggs and 6LgI) by different paraquat concentrations. The leaf discs were incubated in a 16 h light/8 h dark regime at three different external sucrose supplies in the solid medium. Symbols: -○- 0.2 % sucrose; -●- 1.0 % sucrose; -▲- 2.0 % sucrose. Means of three independent parallel experiments ± standard deviations are shown.

Results

Phytotoxic effects of paraquat

Paraquat at 4 x 10^{-6} M concentration led to bleaching in illuminated leaf discs of all clones, while at 4 x 10^{-7} M caused chloroplast sublethality with a mixture of bleached and green spots on leaf discs. No visual symptoms were observed at lower paraquat concentrations. In darkness no leaf bleaching was

observed at any paraquat concentration used. The sucrose content of the medium did not influence the visual toxic symptoms of paraquat exposure.

GST induction

In accordance with an earlier report (GULLNER & al. 2001) poplar leaf crude extracts showed a marked GST activity. Exposure of leaf discs to paraquat led to a gradual, concentration-dependent decrease of GST activities at 0.2 % sucrose in the medium. However, at higher external sucrose supplies, paraquat brought about marked inductions of GST activities in the concentration range of 4 x 10^{-9} - 4 x 10^{-7} M. The GST induction was significantly higher at 2.0 % than at 1.0 % sucrose level (Fig. 1). This GST induction at high external sucrose supply was observed in both transgenic and wildtype poplar lines, although the rate and extent of induction differed considerably between different clones (Fig. 1). The GST induction was light-dependent: no induction was observed in leaf discs incubated in darkness neither at 1.0 % nor at 2.0 % sucrose concentration (data not shown).

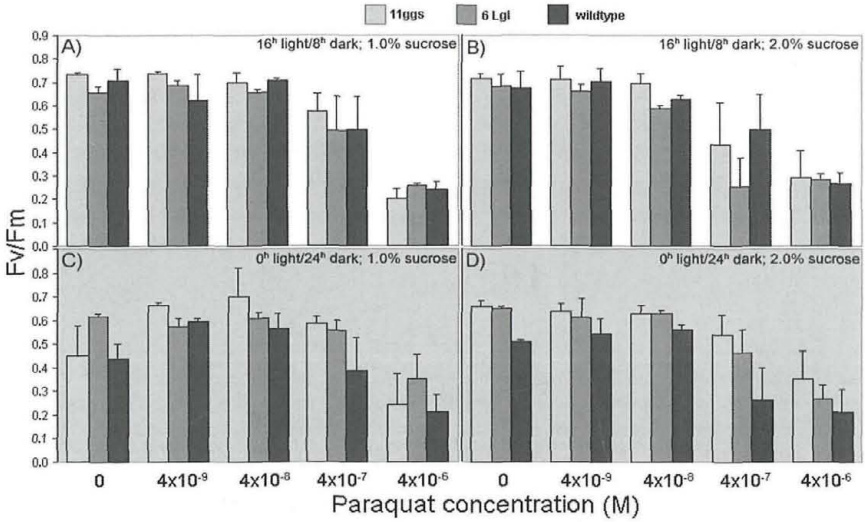

Fig. 2. Changes in the photosynthetic activity as characterized by chlorophyll fluorescence (F_v/F_m) in leaf discs of the wildtype poplar hybrid *P. canescens* and its two genetically transformed lines (11ggs and 6LgI) by different paraquat concentrations. The leaf discs were incubated in a 16 h light/8 h dark regime or in continuous darkness at 1.0 and 2.0 % external sucrose supplies in the solid medium. Means of 10 independent parallel experiments ± standard deviations are shown.

Fluorescence measurements

The efficiency of the photosynthetic apparatus was characterized by fluorescence measurements. Paraquat at high concentrations (4 x 10^{-7} - 4 x 10^{-6} M)

markedly decreased the photosynthetic activity in all poplar clones (Fig. 2). At the moderately toxic 4 x 10^{-7} M paraquat concentration the F_v/F_m value decreased stronger in the presence of 2.0 % than of 1.0 % sucrose probably because the photosynthetic electron transport chain (PETC) switches off at higher sucrose concentrations (2.0 or 3.0 %). Interestingly, the fluorescence signal decreased by paraquat treatment also in leaf discs incubated in continuous darkness indicating that the paraquat toxicity was not strictly light-dependent.

Discussion

The major target of the non-selective contact herbicide paraquat is the chloroplast. In the light the divalent paraquat cation is reduced via photosystem I to the blue, monocationic paraquat radical, which is rapidly reoxidized by molecular oxygen forming superoxide radical and regenerating dicationic paraquat. Repeated redox cycles lead to sustained oxidative stress, membrane damage, chlorophyll loss and desiccation. Paraquat inhibits the reduction of NADP and inactivates Calvin cycle enzymes (DODGE 1994). Paraquat is also toxic in the dark by an unknown mechanism. Paraquat treatments induce various antioxidative defense reactions in plants including GST enzyme activity (GARRETON & al. 2002). Paraquat led to a slight induction of GST activity in leaves of a paraquat-tolerant tobacco mutant, but no induction was found in leaves of the sensitive wildtype (GULLNER & al. 1991). Overexpression of GST in transgenic cotton plants led to an elevated resistance to paraquat (YU & al. 2003). Our present results showed that none of the transgenic poplar lines with elevated GSH content exhibited higher tolerance to paraquat than the wildtype hybrid, in accordance with an earlier report (WILL & al. 2001). Interestingly, the GST activity was strongly inducible by paraquat in all poplar lines in the presence of 1.0 or 2.0 % sucrose in the culture medium. Sugar-induced tolerance was observed also in *Arabidopsis* seedlings exposed to the herbicide atrazine (SULMON & al. 2004). The effect of exogenous sucrose on GST induction probably can not be attributed to metabolic sugar compensation of photosynthesis inhibition at very low paraquat concentrations. The protective effect of sucrose may be the result of an improved NADPH synthesizing capability of poplar leaf tissues by the induction of glucose-6-phosphate dehydrogenase activity (HAUSCHILD & VON SCHAEWEN 2003). Altenatively, the sucrose supply may elicit sugar-signaling pathways (SULMON & al. 2004), which results in enhanced GST inducibility. Via the improved GST inducibility the external sucrose supply leads to a better detoxification capacity of poplar leaf discs.

Acknowledgements

Grants from the Hungarian Scientific Research Fund (OTKA T 43476), the Research and Technology Division of the Hungarian Ministry of Education (Biotechnology 2001 Programme), and the Hungarian Ministry of Economy and Transport (GVOP - 2004 - 3 .1.1) are gratefully acknowledged.

References

BAROCSI A., KOCSANYI L., VARKONYI S., RICHTER P., CSINTALAN Z. & SZENTE K. 2000. Two-wavelength, multipurpose, truly portable chlorophyll fluorometer and its application in field monitoring of phytoremediation. - Meas. Sci. Technol. 11: 717-729.

BITTSÁNSZKY A., KOMIVES T., GULLNER G., GYULAI G., KISS J., HESZKY L., RADIMSZKY L. & RENNENBERG H. 2005. Ability of transgenic poplars with elevated glutathione content to tolerate zinc(2+) stress. - Environ. Int. 31: 251-254.

BURKEN J.G. & SCHNOOR J.L. 1998. Predictive relationships for uptake of organic contaminants by hybrid poplar trees. - Environ. Sci. Technol. 32: 3379-3385.

DI BACCIO D., TOGNETTI R., SEBASTIANI L. & VITAGLIANO C. 2003. Responses of *Populus deltoides* x *Populus nigra* (*Populus* x *euramericana*) clone I-214 to high zinc concentrations. - New Phytol. 159: 443-452.

DODGE A.D. 1994. Herbicide action and effects on detoxification processes. - In: FOYER C.H. & MULLINEAUX P.M. (Eds.), Causes of photoactive stress and amelioration of defense systems in plants, pp. 219-236. - CRC Press. Boca Raton.

GARRETON V., CARPINELLI J., JORDANA X. & HOLUIGUE L. 2002. The as-1 promoter element is an oxidative stress-responsive element and salicylic acid activates it via oxidative species. - Plant Physiol. 130: 1516-1526.

GULLNER G., KOMIVES T. & KIRÁLY L. 1991. Enhanced inducibility of antioxidant systems in a *Nicotiana tabacum* L. biotype results in acifluorfen resistance. - Z. Naturforsch. 46c: 875-881.

— , — & RENNENBERG H. 2001. Enhanced tolerance of transgenic poplar plants overexpressing γ-glutamylcysteine synthetase towards chloroacetanilide herbicides. - J. Exp. Bot. 52: 971-979.

GYULAI G., JEKKEL Z., KISS J. & HESZKY L.E. 1995. A selective auxin and cytokinin bioassay based on root and shoot formation in vitro. - J. Plant Physiol. 145: 379-382.

HAUSCHILD R. & VON SCHAEWEN A. 2003. Differential regulation of glucose-6-phosphate dehydrogenase isoenzyme activities in potato. - Plant Physiol. 133: 47-62.

KOMIVES T. & GULLNER G. 2000. Phytoremediation. - In: WILKINSON R.E. (Ed.), Plant-environment interactions, pp. 437-452. - Marcel Dekker. New York.

— , — , RENNENBERG H. & CASIDA J.E. 2003. Ability of poplar (*Populus* spp.) to detoxify chloroacetanilide herbicides. - Water Air Soil Poll. Focus 3: 277-283.

LICHTENTHALER H.K. & RINDERLE U. 1988. The role of chlorophyll fluorescence in the detection of stress conditions in plants. - Crit. Rev. Anal. Chem. 19: 29-85.

NOCTOR G., ARISI A.C.M., JOUANIN L. & FOYER C.H. 1998. Manipulation of glutathione and amino acid biosynthesis in the chloroplast. - Plant Physiol. 118: 471-482.

SALT D.E., SMITH R.D. & RASKIN I. 1998. Phytoremediation. - Ann. Rev. Plant Physiol. Plant Mol. Biol. 49: 643-668.

SULMON C., GOUESBET G., COUÉE I. & EL AMRANI A. 2004. Sugar-induced tolerance to atrazine in *Arabidopsis* seedlings: interacting effets of atrazine and soluble sugars on psbA mRNA and D1 protein levels. - Plant Sci. 167: 913-923.

WAGNER U., EDWARDS R., DIXON D.P. & MAUCH F. 2002. Probing the diversity of the *Arabidopsis* glutathione S-transferase gene family. - Plant Mol. Biol. 49: 515-532.

WILL B., JOUANIN L. & RENNENBERG H. 2001. Protection from paraquat-mediated photo-oxidation stress by glutathione in poplar (*Populus tremula* x *P. alba*) plants. - Plant Biol. 3: 272-278.

YU T., LI Y.S., CHEN X.F., HU J., CHANG X. & ZHU Y.G. 2003. Transgenic tobacco plants overexpressing cotton glutathione S-transferase (GST) show enhanced resistance to methyl viologen. - J. Plant Physiol. 160: 1305-1311.