Enhancement of photo-oxidative stress tolerance in transgenic tobacco plants overexpressing *Synechocystis* PCC 6803 glutathione peroxidase (GPX-1)

(Received: 20.09.2008; Accepted: 05.10.2008)

Ahmed Gaber
Department of Genetics, Faculty of Agriculture, Cairo University, El-Gamaa street, P.O. Box 12613, Giza, Egypt.

ABSTRACT

Glutathione peroxidases (GPX) (EC 1.11.1.9) are a family of multiple isozymes that catalyze the reduction of H_2O_2, organic and lipid hydroperoxides by reduced glutathione, thus helping to protect the cells against the oxidative damage. To analyze the potential of the Reactive Oxygen Species (ROS) scavenging system, the Synechocystis PCC 6803 glutathione peroxidase–like protein was introduced into tobacco cytosol (TGPX-1) using the Agrobacterium as a gene mediated transformation. The stable integration of the transgene into plant genome was detected by polymerase chain reaction (PCR) analysis and the expression of the GPX-1 gene was detected by the enzyme activity and western blotting analyses. The enzyme activities of transgenic plants were measured using NADPH as an electron donor and linolenic acid hydroperoxide as electron acceptor (19 ~ 59 nmol/min/mg protein), while no activity was detected in wild-type plants. The transgenic plants showed increased tolerance to oxidative stress caused by the application of methyl viologen (MV: 1 μM) under moderate light intensity (200 $\mu E/m^2/sec^1$). At 24 hr after the MV application, chlorophyll was destroyed in the wild-type plants, but not in the transgenic plants. The data indicate that the modification of ROS scavenging systems could lead to considerable changes in oxidative stress tolerance.

Key words: *Synechocystis PCC 6803, glutathione peroxidase, methyl viologen, oxidative stress.*

INTRODUCTION

Aerobic organisms use molecular oxygen (O_2) for respiration or oxidation of nutrients to obtain energy. Reactive by-products of oxygen, such as superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), and the highly reactive hydroxyl radicals (^-OH), are generated continuously in cells grown aerobically. Rates of these reactive oxygen species (ROS) generation and cellular ROS levels both increase greatly when plants are subjected to environmental or biotic stresses (Elstner, 1991; Foyer and Noctor, 2000). Generally, ROS have been proposed to affect stress responses in two different ways. They react with a large variety of biomolecules, such as lipids, proteins, and nucleic acids that are essential to maintain the integrity of cellular structures and thus may cause irreversible damage that can lead to tissue necrosis and ultimately may kill the plant (Rebeiz *et al.*, 1988; Girotti, 2001). On the other hand, ROS have been shown to influence the expression of a number of genes and signal transduction pathways. These observations have been interpreted to suggest that cells have evolved strategies to use ROS

as biological stimuli and signals that activate and control various genetic stress-response programs (Dalton *et al.*, 1999). Methyl viologen (MV: 1,1'-dimethyl-4, 4'-bipyridyliumion) is a widely used herbicide that causes lethal intoxication in plant cells. Because MV easily undergoes redox cycling with subsequent production of ROS, it has been proposed that MV causes injury by producing ROS following induction of oxidant stress (Bus *et al.*, 1975). Plants have developed several nonenzymatic and enzymatic systems to withstand the oxidative damage caused by ROS. While, the major non-enzymatic antioxidant compounds include carotenoids, tocopherols, reduced glutathione (GSH) and ascorbate, the enzymatic systems rely on superoxide dismutases, catalases, ascorbate peroxidases, thioredoxin (Trx) peroxidases and glutathione peroxidases (GPXs) (Foyer and Noctor, 2000; Inze and van Montagu, 1995; Milla *et al.*, 2003; Noctor and Foyer, 1998). In mammals, GPXs that form a group of enzymes with an important role in protecting cells against ROS, using GSH as the reducing substrate, have been studied extensively (Roy *et al.*, 2005). In plants, different isoforms of GPX are found to be expressed in various subcellular compartments. Typical plant enzymes display sequences similar to those of animal GPX enzymes, except that the selenocystiene (SeCys) in the catalytic site of most animals GPX is replaced by cystiene (Cys) in plant GPX, which in general show reduced catalytic efficiency (Holland *et al.*, 1993; Jung *et al.*, 2002; Kang *et al.* 2004; Navrot *et al.*, 2006; Sugimoto and Sakamoto, 1997). In previous studies Gaber *et al.* (2001 and 2004) found that the *Synechocystis* PCC 6803 (*S.* PCC 6803) GPX-1 enzyme utilize NADPH as an electron donor to reduce unsaturated fatty acid hydroperoxides. Interestingly, the steady-state transcript level of *gpx-1* gradually increased under oxidative stress conditions imposed by high light intensity, high salinity, or application of methyl viologen or *t*-butyl hydroperoxide in the wild-type cells of *Synechocystis* PCC 6803 (Gaber *et al.*, 2004).

In this paper, the question of whether *S.* PCC 6803 GPX-1 plays a role in protection of tobacco plants against oxidative stress was addressed. Therefore, transgenic tobacco plants expressing the *S.* PCC 6803 GPX-1 in cytosol were generated and the degradation of chlorophyll content and increased tolerance to oxidative stress caused by MV application was demonstrated.

MATERIALS AND METHODS

The DNA encoding the *S.* PCC 6803 GPX-1 was originally cloned into plasmid pBluescript SK (Gaber *et al.*, 2001). Linolenic acid hydroperoxides was prepared according to the method described previously by Gaber *et al.* (2001). Tobacco *(Nicotiana tabacum* L. var Xanthi) seeds were a kind gift from the laboratory of Prof. Dr. Shigeoka (Department of Advanced Biosciences, Faculty of Agriculture, Kinki University, Japan). All other chemicals were of the highest grade commercially available.

Vector construction and transformation of tobacco

The DNA encoding the S. PCC 6803 GPX-1 was ligated into plant binary vector pBI121 containing linker derived from Ti-plasmid, in which the DNA was placed under the control of the CaMV 35S promoter in the sense orientation (Fig. 1). The recombinant plasmid was introduced into *Agrobacterium tumefaciens* strain LBA 4404 (Hoekema *et al.*, 1983), and transformation of tobacco plants by *A. tumefaciens* was performed as described by Miyagawa *et al.* (2000). Transgenic tobacco and wild-type plants were cultured for 8 weeks in a growth chamber under a 12 hr photoperiod

with moderate light intensity (200 - 300 μE/m^2/sec^1), 60% relative humidity, and day/night temperature of 25/20 °C. No difference could be seen in growth or morphology between wild-type and transgenic plants.

Detection of the transgene by polymerase chain reaction (PCR)

Leaves [1.0 g fresh weight (FW)] were collected from wild-type and transgenic plants. Genomic DNA (10 ng) extracted from leaves as described previously (Yoshimura *et al.* 2004) was used to amplify the transgene with PCR, using primers as follows: *S.* PCC *gpx-1* N-terminal, 5`-GCTAAATCATATGACTGCCC-3` and *S.* PCC*gpx-1* C-terminal, 5`-AGAAAATTACAACAATTTCT-3`. The reaction mixture (50 μl) contained 1 μM of N-terminal primer, 1 μM C-terminal primer, 200 μM dNTPs, 1.5 units of ExTaq DNA polymerase (Takara Shuzo, Kyoto, Japan) and 2 μl plant DNA as a template. PCR amplification was performed for 30 cycles of denaturation at 94 °C for 60 s, annealing at 58 °C for 60 s and elongation at 72 °C for 60 s, followed by 72 °C for 10 min. The amplified bands were photographed under UV light after electrophoresis.

SDS-PAGE and Western blot analysis

To measure the protein level of GPX-1enzyme, 1.1 cm^2 of leaf tissues (about 20 discs were ground to a fine powder in liquid N_2 and then homogenized with 1 ml of SDS-loading buffer (150 mM Tris-HCl (pH 6.8), 4% SDS, and 10% 2-Mercaptoethanol). The homogenates were boiled for 5 min and centrifuged at 10,000 *g* for 10 min. The supernatant (30 μl) was separated on a 15% slab gel, according to the method of Leammli (1970). The gels were stained with Coomassie Brilliant Blue R-250. For Immunolotting, the proteins were transferred to PVDF membranes at 13 V for 1 hr. The membranes were treated with the polyclonal antibody raised against GPX-1 protein (Gaber *et al.*, 2004).

Enzyme extraction and assay

NADPH-dependent GPX activity in tobacco plants was assayed spectrophotometrically with NADPH and linolenic acid hydroperoxide as described previously (Gaber *et al.*, 2001). Leaf tissues (0.5 g FW) were ground to a fine powder in liquid N_2 and homogenized in 1 mL of 100 mM Tris-HCl (pH 8.2), 10% sorbitol, and 1 mM EDTA using a mortar and pestle. The homogenate was centrifuged for 15 min at 12 000 *g*. The supernatant was used for the assay of NADPH-dependent GPX activity. The reaction mixture contained 100 mM Tris-HCl, pH 8.2, 0.4 mM NADPH, 0.2 mM linolenic acid hydroperoxide, 0.1% Triton X-100, and the supernatant, in a total volume of 1 mL.

MV application to leaf discs

Leaf discs (1-cm diameter) punched out from the third leaves of 8 weeks-old plants using a cork borer, were subjected to MV application. The leaf discs from wild-type and transgenic plants were infiltrated on a solution with different concentrations of MV and 0.1% Tween-20, placed in the dark for 1 hr, and then illuminated at moderate light intensity (200 μE/m^2/sec^1) for 24 hr at 25 °C.

Determination of chlorophyll content

Chlorophyll content was determined spectrophotometrically at 663 and 645 nm after the extraction of fresh leaves with 80% acetone (Arnon, 1949).

RESULTS AND DISCUSSION

Expression of *S.* PCC 6803 GPX-1 in transgenic tobacco plants

Many analyses of transgenic plants in which the levels of antioxidant enzymes have

been manipulated through gene transfer technology have provided significant insights into the role of these enzymes in higher plants. However, only a few studies of the scavenging systems of lipid hydroperoxide in higher plants have been reported. Transgenic tobacco plants expressing glutathione-S-transferase (GST) with GPX activity had elevated levels of GSH and ascorbate (AsA) and monodehydroascorbate reductase (MDAR) activity compared to wild-type plants, as well as enhanced seedling growth under thermal and salt-stress conditions that caused an increased level of lipid hydroperoxidation (Roxas *et al.*, 1997). In addition, it was found that tobacco plants expressing *Chlamydomonas* GPX-like proteins in the cytosol or chloroplasts showed increased tolerance to oxidative stress caused by application of MV under moderate light intensity and chilling stress under high light intensity (Yoshimura *et al.*, 2004). Moreover, Glutathione peroxidase-like protein of *Synechocystis* PCC 6803 (GPX-2) confers tolerance to oxidative and environmental stresses in transgenic *Arabidopsis* (Gaber *et al.*, 2006). These results suggest that alterations in the expression levels of enzymes involved in lipid hydroperoxide-scavenging systems, like GPX, might provide a strategy for developing transgenic plants with increased tolerance against the oxidative membrane damage caused by a variety of stressful conditions.

Two genes (*gpx-1* and *gpx-2*) with significant similarity to the cDNAs of GPX-like proteins from higher plants and GPX from animals were present in *Synechocystis* PCC 6803 cells. Interestingly, enzymes encoded by both genes showed activities with NADPH and unsaturated fatty acid hydroperoxides or alkyl hydroperoxides (Gaber *et al.*, 2001).

In the present study, the *S.* PCC 6803 *gpx-1* gene encoding glutathione peroxidase was cloned in the binary plant pBI121 vector and the gene expression was controlled under the strong constitutive promoter 35S CaMV promoter (Fig. 1). Ten independent transgenic lines were obtained by selection on kanamycin-MS medium. There were no phenotypic differences between transgenic lines and wild-type plants. To determine the stable integration of the *gpx-1* gene into the transgenic tobacco plants genome, genomic DNA of wild-type and transgenic plants were isolated and used as a template in PCR technique. PCR analysis using primers specific to *gpx-1* gene amplified the expected 0.6 Kb DNA fragment from 10 lines of T0 plants and three lines of T1 (Fig. 2A and B). This fragment was not detected in wild-type and non transformant lines (Fig. 2A and B). These transgenic lines of T1 were selected for further study. Transgenic lines were tested for GPX-1 protein accumulation by western blot analysis in the extracts prepared from the leaves of transgenic and wild-type plants. All transgenic lines but not wild-type plants exhibited accumulation of the GPX-1 protein with a molecular mass of 18.4 kDa (Fig. 2C). The enzyme activities of GPX-1 were determined in T0 and T1 of transgenic plants using NADPH as electron donor and linolenic acid hydroperoxide as electron acceptor and it were 19 ~ 59 nmol/min/mg protein (Table 1). There was no enzyme activity in wild-type plants. It seems likely that the relatively low activities of GPX-1 detected in this study as well as GPX-like proteins from higher plants compared to those of mammalian phospholipid hydroperoxide GPXs (PHGPXs) are due to the presumed presence of a Cys catalytic residue in these enzymes in contrast to the SeCys residue in the mammalian GPXs. In fact, it has been demonstrated that the replacement of the catalytic SeCys by Cys in pig heart PHGPX by a point mutation in the cDNA resulted in a drastic decrease in the enzyme

activity to a value of only 0.4% of that of the native enzyme (Maiorino *et al.*, 1995). Therefore, the present results confirmed that the *gpx-1* gene was introduced and functionally expressed at various levels in tobacco plants.

Table (1): GPX-1 activity in wild-type (Xi) and transgenic plants in T0 (T0-GPX1) and T1 (T1-GPX1).

	Activity (nmol/ min/ mg protein)
Xi	0
T0-GPX1-1	59
T0-GPX1-2	44
T0-GPX1-3	27
T0-GPX1-4	21
T0-GPX1-5	26
T0-GPX1-6	19
T0-GPX1-7	0
T0-GPX1-8	49
T0-GPX1-9	22
T1-GPX1-1	30
T1-GPX1-4	19
T1-GPX1-8	22

Activity is towards linolenic acid hydroperoxide.

Effect of MV on visible bleach and chlorophyll content

The herbicide MV can readily penetrate into leaf tissue through the cuticula. Shortly after spraying or floating leaf discs on MV, photosynthetic activity decreases, membranes rupture, and the treated parts of the plant wilt due to the loss of turgor within hours (Lehoczki *et al.*, 1992; Chang and Kao, 1997). In the final stage, necrotic lesions or bleach appear on the leaf surface and the plant dies.

To assess the tolerance to oxidative stress caused by MV, the leaf discs from wild-type and three T1 transgenic plants were incubated with different concentrations of MV (0, 0.5 and 1 μM) under moderate light intensity (200 $\mu E/m^2/sec$). At 6 hr after floating, no detectable damage was observed in wild-type and transgenic plants. However, at 24 hr after the MV treatment, destruction of chlorophyll became apparent in wild-type leaf discs, but not in the transgenic leaf discs (Fig. 3A). Neither the wild-type nor transgenic plants treatment with MV in the dark showed degradation of chlorophyll (data not shown).

The chlorophyll content in all lines of transgenic plant (90 - 145 mg/m^2) was higher than that in wild-type plants (20 mg/m^2) at 24 hr of incubation (Fig.3 B). These results demonstrated that photooxidative damage is caused by the excess generation of ROS via O_2^- in chloroplasts exacerbated by MV under continuous illumination and that transgenic tobacco plants had increased tolerance to photooxidative damage imposed by ROS. These results were in agreements with the data previously reported (Yoshimura *et al.*, 2004; Kwon *et al.*, 2002; Gaber *et al.*, 2006).

Resistant mechanism of transgenic plant to oxidative stress caused by MV stress

Oxidative damage poses a great risk to the survival of cells because it can undermine cellular structures, including those of DNA, proteins, and lipids (Frankenberg *et al.*, 1993; Levine *et al.*, 1994; Morados-Ferreira *et al.*, 1996; Steels *et al.*, 1994). It has been reported that the steady-state levels of *gpx* mRNA and/or GPX protein in photosynthetic organisms, including eukaryotic algae and

higher plants, increase in response to various types of stress conditions, high osmolarity, MV, H_2O_2, high salinity and high light (Criqui *et al.*, 1992; Holland *et al.*, 1993; Sugimoto and Sakamoto, 1997; Depège *et al.*, 1998; Roeckel-Drevet *et al.*, 1998; Leisinger *et al.*, 1999). Furthermore, *E. coli* cells expressing the *Chlamydomonas* W80 GPX-like protein gene showed increased resistance against the oxidative stress caused by MV treatment or NaCl treatment (Takeda *et al.*, 2003). Similar results were observed in *E. coli* cells expressing GPX-like protein gene of *Citrus sinensis* (Holland *et al.*, 1994).

In previous work, Gaber *et al.* (2004) found that the transcript level of *gpx-1* gradually increased under oxidative or environmental stresses in the wild-type cells of *Synechocystis* PCC 6803. Therefore, to examine the tolerance of the tobacco transgenic plants to photooxidative stress by foreign GPX-1, leaf disk from wild-type and transgenic plants were incubated with 0, 0.5 and 1 μM MV under illumination at 200 $\mu E/m^2/sec$. The MV is a redox-active compound that is photo-reduced by photosystem I (PS I) and subsequently re-oxidized by transfer of its electrons to oxygen, forming O_2^- and H_2O_2 that cause lipid peroxidation and membrane destruction (Asada and Takahashi 1987; Bowler *et al.*, 1992). The visible bleach of wild-type leaf disk by MV treatment demonstrated that ROS generated by MV under illumination caused the oxidative damage of plant cells and that the expression of *S.* PCC GPX-1 in transgenic lines resulted in increased antioxidant capacity and improved tolerance to photooxidative stress (Fig. 3). Based on the data presented here, a mechanism for resistance of transgenic plants to oxidative stress could be proposed. During the early stages of photooxidative stress, O_2^- generated by MV is disproportionated to H_2O_2 by superoxide dismutase (SOD), which is then scavenged by the resident enzyme systems, resulting in a low level of H_2O_2. However, under stress conditions, it is possible that the ROS production far exceeds the endogenous ROS-scavenging capacity and the level of accumulated H_2O_2 increases to a level in which the cell cannot efficiently reduce it to water. The excess of H_2O_2 undergoes Fenton reaction with Fe^{+2}, which eventually leads to the formation of hydroxyl radical (^-OH), and subsequently ^-OH can extract *bis*-allylic hydrogen atoms of unsaturated fatty acid (LH) to form lipid alkyl radical (L^-). The L^- is oxidized by molecular oxygen to generate a lipid peroxy radical (LOO^-), and the LOO^- thus formed reacts with LH to give lipid hydroperoxide (LOOH) and L^-. The LOOH in biological membranes may be one of the major oxidative damages to the cells. In accordance with enzymatic specificity for lipid radicals such as *S.* PCC 6803 GPX-1, the harmful hydroperoxides of lipids may be reduced and thus eliminated by GPX-1, which is over-expressed in transgenic tobacco plants.

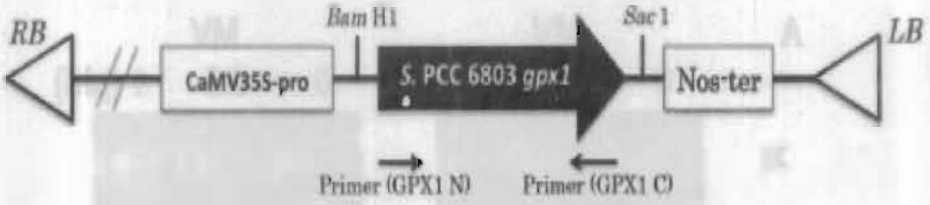

Fig. (1): Construction of the S. PCC 6803 GPX-1 expression vector used for transformation. The drawing is not to scale. Arrows indicate the locations of primers as described in Materials and Methods section.

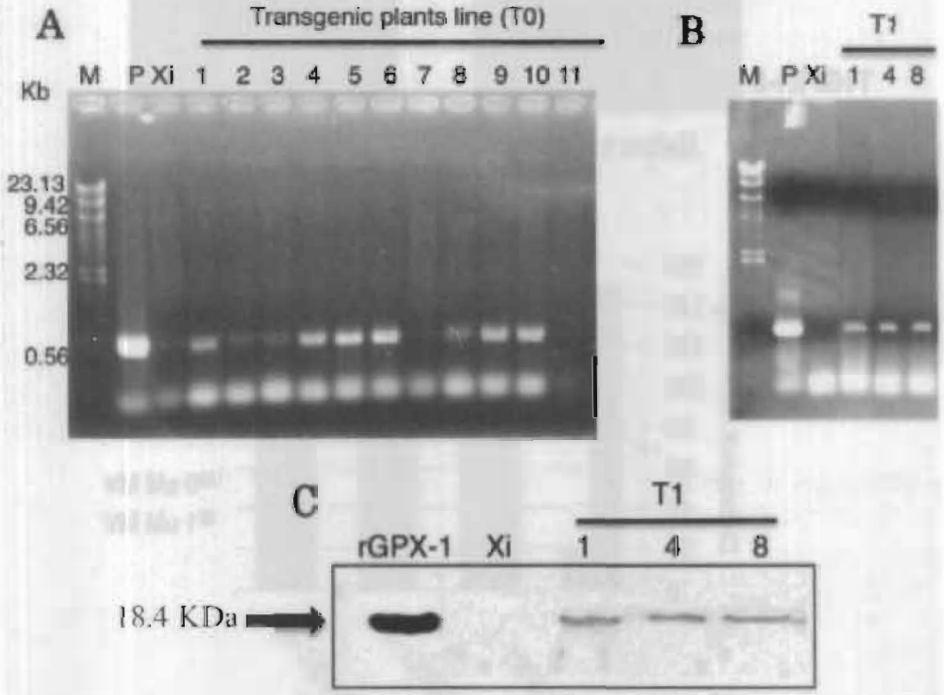

Fig. (2): Examination analysis of the transgenic plants. A and B, detection of the transgene encoding S. PCC GPX-1 in transformed tobacco plants. Genomic DNAs were isolated from the positive control (P), and leaves of wild-type (Xi) and transgenic plants (T0 and T1 lines) and were used to amplify the transgene by PCR using primers as shown in Fig. 1. C, western blot analysis. Total proteins (50 µg each) from the leaves of wild-type (Xi) and transgenic plants were subjected to SDS-PAGE followed by Western blot analysis with a mouse antiserum against S. PCC GPX-1 [1]recombinan GPX; [1]rGPX. in E.Coli used as a positive control. Detailed procedures are described in the Materials and Methods section.

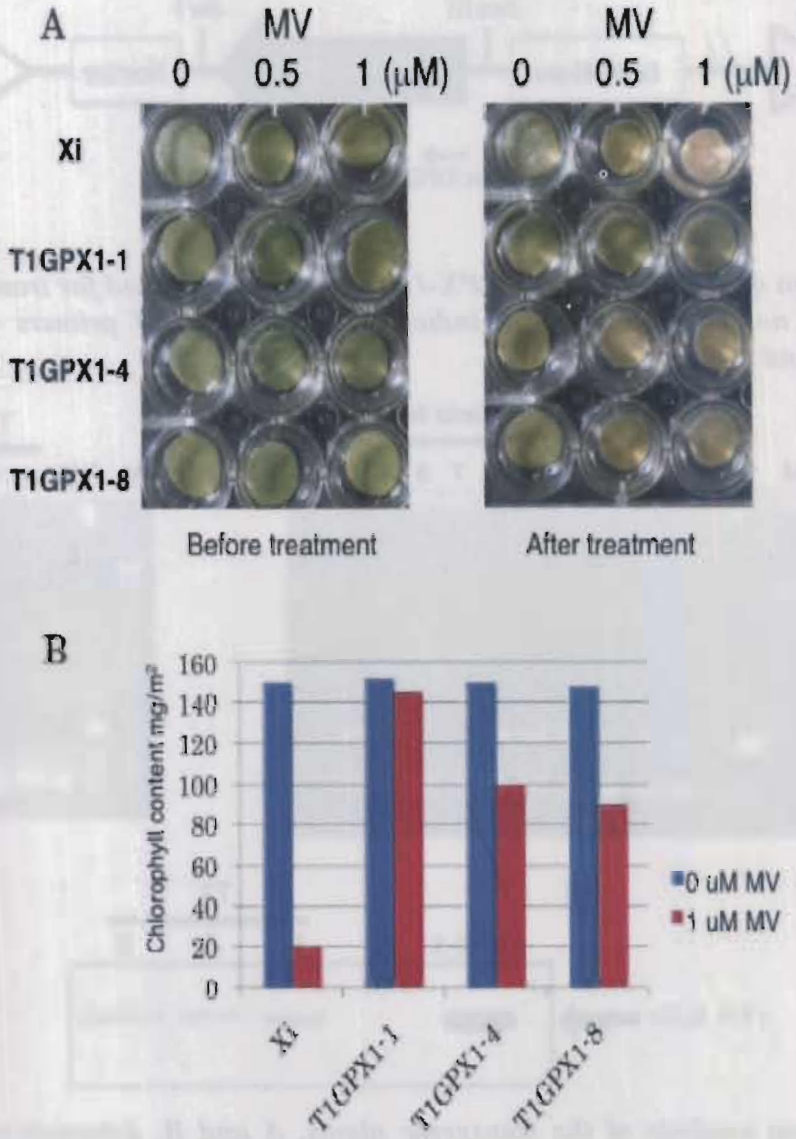

Fig. (3): Effect of MV application on leaf discs of wild-type (Xi) and Transgenic GPX-1 tobacco lines (T1GPX1). The leaf discs from Xi and transgenic plants floated on a solution containing 0, 0.5 and 1 μM MV and 0.1 %Tween-20, placed in the dark for 1 h, and then illuminated at moderate light intensity (200 μE/m2/sec) for 24 h at 25 °C. (A), photograph of the leaf discs before and after MV treatment. (B), the chlorophyll contents in the leaf discs before and after MV treatment (0 and 1 μM, respectively).

If the plant's defense against oxidative stress could be reinforced with a new gene and coordinated to maintain the appropriate physiological balance of all components, photooxidative stress tolerance would be improved. The expression of a single gene, *S.* PCC6803 *gpx-1*, provides significant protective effects against photooxidative stress

caused by MV under moderate light intensity. However, it should be noted that the stress regimes use are designed to produce detectable stress damage in a short period of time in the laboratory. It is not clear whether these increases in tolerance could have substantial effects in nature, such as plant exposed to frequent periods of sever stress throughout a growing season period. Field tests of transgenic plants will surely provide the answer to these and other questions about the utility of enhancing the ROS-scavenging system of crop plants.

ACKNOWLEDGEMENTS

I thank Dr. Shigeru Shigeoka, Department of Advanced Bioscience, Faculty of Agriculture, Kinki University, Japan, for the generous help and gifts of plasmids, antibody, and bacterial strains.

REFERENCES

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts: polyphenol oxidase in *Beta vulgaris*. Plant Physiol., 24: 1–15.

Asada, K. and Takahashi, M. (1987). Production and scavenging of active oxygen in photosynthesisi. *In* Photoinhibation (Kyle, D.J., Osmond, C.B. and Arntzen, C.J., eds). New York: Elsevier Science Publishers, pp. 227-287.

Bowler, C; Van Montagu, M. and Inze, D. (1992). Superoxide dismutase and stress tolerance. Annu. Rev. Plant Physiol. Plant Mol. Biol., 43: 83-116.

Bus, J. S.; Aust, S. D. and Gibson, J. E. (1975). Lipid peroxidation: a possible mechanism for paraquat toxicity. Res Commun. Chem. Pathol. Pharmacol., 11: 31-38.

Chang, C. J. and Kao, C. H. (1997). Paraquat toxicity is reduced by polyamines in rice leaves. Plant Growth Regul., 22: 163–168.

Criqui, M. C.; Jamet, E.; Parmentier, Y.; Marbach, J.; Durr, A. and Fleck, J. (1992). Isolation and characterization of a plant cDNA showing homology to animal glutathione peroxidase. Plant Mol. Biol., 18: 623-627.

Dalton, T. P.; Shertzer, H. G. and Puga, A. (1999). Regulation of gene expression by reactive oxygen. Annu. Rev. Pharmacol. Toxicol., 39: 67–101.

Depége, N.; Drevet, J. and Boyer, N. (1998). Molecular cloning and characterization of tomato cDNAs encoding glutathione peroxidase-like proteins. Eur. J. Biochem., 253: 445-451.

Elstner, E. F. (1991). Mechanisms of oxygen activation in different compartments of plant cells. In Active Oxygen/Oxidative Stress in Plant Metabolism, E.J. Pelland and K.L. Steffen, eds (Rockville, MD: American Society of Plant Physiologists), pp. 13-25.

Foyer, C. H. and Noctor, G. (2000). Oxygen processing in photosynthesis: regulation and signaling. New Phytol., 146: 359–388.

Frankenberg, D.; Frankenberg-Schwanger, M. and Harbich, R. (1993). Mechanisms of oxygen radiosensitization in irradiated yeast. I. DNA double-strand breakage. Int. J. Radiat. Biol., 64: 511-521.

Gaber, A.; Tamoi, M.; Takeda. T.; Nakano, Y. and Shigeoka, S. (2001). NADPH-dependent glutathione peroxidase-like proteins (Gpx-1, Gpx-2) reduce unsaturated fatty acid hydroperoxides in *Synechocystis* PCC 6803. FEBS Lett., 499: 32–36.

Gaber, A.; Yoshimura, K.; Tamoi, M.; Takeda, T.; Nakano, Y. and Shigeoka, S. (2004). Induction and functional analysis of two NADPH-dependent glutathione peroxidase-like proteins in *Synechocystis* PCC 6803 during the progression of

oxidative stress. Plant Physiol., 136: 2855–2861.

Gaber, A.; Yoshimura, K; Yamamoto, T.; Yabuta, Y.; Takeda, T.; Kanaboshi, H.; Miyasaka, H.; Nakano, Y. and Shigeoka, S. (2006). Glutathione peroxidase-like protein of *Synechocystis* PCC 6803 confers tolerance to oxidative and environmental stresses in transgenic *Arabidopsis*. Physiologia Plantarum., 128: 251–262.

Girotti, A. W. (2001). Photosensitized oxidation of membrane lipids: Reaction pathways. cytotoxic effects and cytoprotective mechanisms. J. Photochem. Photobiol., B 63: 103–113.

Hoekema, A.; Hirsch, P. R.; Hooykaas, P. J. J. and Schilperoort, R. A. (1983). A binary plant vector strategy based on separation of vir- and T-region of the *Agrobacterium tumefaciens* Ti-plasmid. Nature, 303: 179–180.

Holland, D.; Ben-Hayyim, G.; Faltin, Z.; Camoin, L.; Strosberg, A. D. and Eshdat, Y. (1993). Molecular characterization of salt-stress-associated protein in citrus: protein and cDNA sequence homology to mammalian glutathione peroxidases. Plant Mol. Biol., 21: 923–927.

Holland, D.; Faltin, Z.; Perl, A.; Ben-Hayyim, G. and Eshdat, Y. (1994). A novel plant glutathione peroxidase-like protein provides tolerance to oxygen radicals generated by paraquat in *Escherichia coli*. FEBS Lett., 337: 52-55.

Inze, D. and van Montagu, M. (1995). Oxidative stress in plants. Curr. Opin. Biotechnol., 6: 153-158.

Jung, B. G.; Lee, K. O.; Lee, S. S.; Chi, Y. H.; Jang, H. H.; Kang, S. S.; Lee, K.; Lim, D.; Yoon, S.C.; Yun, D.J.; Inoue, Y.; Cho, M.J. and **Lee, S.Y. (2002).** A chinese cabbage cDNAwith high sequence identity to phospholipids hydroperoxide glutathione peroxidases encodes a novel isoform of thioredoxin-dependent peroxidase. J. Biol. Chem., 277: 12572–12578.

Kang, S.-G.; Jeong, H. K. and Suh, H. S. (2004). Characterization of a new member of the glutathione peroxidase gene family in Oryza sativa. Mol. Cells, 17: 23–28.

Kwon, S. Y.; Jeong, Y. J; Lee, H. S.; Kim, J. S.; Cho, K. Y.; Allen, R. D. and Kwak, S. S. (2002). Enhanced tolerances of transgenic tobacco plants expressing both superoxide dismutase and ascorbate peroxidase in chloroplasts against methyl viologen-mediated oxidative stress. Plant Cell Environ., 25: 873–882.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680–685.

Lehoczki, F.; Laskay, G. and Gaal, I. (1992). Mode of action of paraquat in leaves of paraquat-resistant Conyza nandensis (L.). Plant Cell Environ., 15 (5): 531–539.

Leisinger, U.; Rüfenacht, K.; Zehnder, A. J. B. and Eggen, R. I. L. (1999). Structure of a glutathione peroxidase homologous gene involved in the oxidative stress response in *Chlamydomonas reinhardtii*. Plant Science, 149: 139-149.

Levine, A.; Tenhaken, R.; Dixon, R. and Lamb, C. (1994). H_2O_2 from oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell, 79: 583-593.

Maiorino, M.; Aumann, k. D.; Brigelius-Flohé, R.; Doria, D.; van den Heuvel, J.; McCarthy, J.; Roveri, A.; Ursini, F. and Flohé, L. (1995). Probing the presumed catalytic tried of selenium-containing peroxidases by mutational analysis of phospholipid hydroperoxide glutathione peroxidase (PHGPx). Biol. Chem. Hoppe-Seyler, 376: 651-660.

Milla, M. A. R.; Maurer, A.; Huete, A. R. and Gustafson, J. P. (2003). Glutathione peroxidase genes in *Arabidopsis* are ubiquitous and regulated by abiotic stresses

through diverse signalling pathways. The Plant J., 36: 602–615.

Miyagawa, Y.; Tamoi, M. and Shigeoka, S. (2000). Evaluation of the defense system in chloroplasts to tobacco plants expressing catalse from *Escherichia coli*. Plant Cell Physiol., 41: 311-320.

Morados-Ferreira, P.; Costa, V.; Piper, P. and Mager, W. (1996). The molecular defences against reactive oxygen species in yeast. Mol. Microbiol., 19: 651-658.

Navrot, N.; Collin, V.; Gualberto, J.; Gelhaye, E.; Hirasawa, M.; Rey, P. Knaff, D.B.; Issakidis, E.; Jacquot, J.P. and Rouhier, N. (2006). Plant glutathione peroxidases are functional peroxiredoxins distributed in several subcellular compartments and regulated during biotic and abiotic stresses. Plant Physiol., 142: 1–17.

Noctor, G. and Foyer, C. H. (1998). Ascorbate and glutathione: keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Biol., 49: 249–279.

Rebeiz, C. A.; Montazer-Zouhoor, A.; Mayasich, J. M.; Tripathy, B. C.; Wu, S. M. and Rebeiz, C. (1988). Photodynamic herbicides: Recent developments and molecular basis of selectivity. CRC Crit. Rev. Plant Sci., 6: 385–436.

Roeckel-Drevet, P.; Gagne, G.; de Labrouhe, D.; Dufaure, J.; Nicolas, P. and Drevet, J. (1998). Molecular characterization, organ distribution and stress-mediated induction of two glutathione peroxidase-encoding mRNAs in sunflower (*Helianthus annuus*). Physiol. Plant., 103: 385-394.

Roxas, V.P.; Smith, Jr; R.K.; Allen, E.R. and Allen, R.D. (1997). Overexpression of glutathione S-transferase/glutathione peroxiedase enhances the growth of transgenic tobacco seedlings during stress. Nature Biotec., 15: 988-991.

Roy, G.; Sarma, B. K.; Phadnis, P. P. and Mugesh, G. (2005). Selenium-containing enzymes in mammals: chemical perspectives. J. Chem. Sci., 117: 287–303.

Steels, E. L.; Learmonth, R. P. and Watson, K. (1994). Stress tolerance and membrane lipid unsaturation in *Saccharomyces cerevisiae* grown aerobically or anaerobically. Microbiology, 140: 569-576.

Sugimoto, M. and Sakamoto, W. (1997). Putative phospholipids hydroperoxide glutathione peroxidase gene from *Arabidopsis thaliana* induced by oxidative stress. Genes Genet. Syst., 72: 311–316.

Sugimoto, M.; Furui, S. and Suzuki, Y. (1997). Molecular cloning and characterization of a cDNA encoding putative phospholipid hydroperoxide glutathione peroxidase from spinach. Biosci Biotechnol.Biochem., 61: 1379-1381.

Takeda, T.; Miyao, K.; Tamoi, M.; Kanaboshi, H.; Miyasaka, H. and Shigeoka, S. (2003). Molecular characterization of glutathione peroxidase-like protein in halotolerant *Chlamydomonas* sp. W80. Physiol. Plant., 117: 467-475.

Yoshimura, K.; Miyao, K.; Gaber, A.; Takeda, T.; Kanaboshi, H.; Miyasaka, H. and Shigeoka, S. (2004). Enhancement of stress tolerance in transgenic tobacco plants overexpressing *Chlamydomonas* glutathione peroxidase in chloroplasts or cytosol. Plant J., 37: 21–33.

الملخص العربي

زيادة المقاومة لضغوط الأكسدة الضوئية في نباتات الدخان المحولة وراثيا بزيادة تعبير بروتين الجلوتاثيون بيروكسيديز -١ المفصول من خلايا السيانوباكتيريا سلالة السينيكوسيستيس PCC6803

•••• •••• •••••

قسم الوراثة – كلية الزراعة – جامعة القاهرة- الجيزة – مصر

تعتبر إنزيمات الجلوتاثيون بيروكسيديز من ضمن عائلة تقوم بتحليل أنواع ايونات الأكسيجين النشطة مثل فوق أكسيد الهيدروجين و الهيدروبروكسيدات العضوية و الدهنية وذلك عن طريق اختزال الجلوتاثيون مما يؤدي إلى حماية الخلايا من التلف. لدراسة امكانية تثبيط أيونات الأكسيجين النشطة هذه تم الحصول على نباتات دخان محولة وراثيا بزيادة تعبير جين الجلوتاثيون بيروكسيديز-١ عن طريق استخدام تكنيك الاجروباكتيريم. تم التأكد من دخول و ثبات الجين داخل جينوم نبات الدخان عن طريق استخدام تكنيك تفاعل البلمرة التسلسلي ، هذا إلى جانب التأكد من تعبير الجين عن طريق قياس نشاط الإنزيم ، كذلك التهجين مع أجسام مضادة لنفس البروتين. يتراوح نشاط الإنزيم في النباتات المحولة وراثيا ما بين (19~59 nmol/min/mg protein) في حين لم يكن هناك أي نشاط للإنزيم في نباتات الكنترول غير المحولة وراثيا. أظهرت النباتات المحولة وراثيا زيادة في مقاومة ضغوط الأكسدة الناتجة من التعرض إلى مادة الميثايل فيولوجين بمقدار واحد ميكرومول تحت كثافة متوسطة من الإضاءة وهي (200 $\mu E/m^2/sec$) حيث وجد انه بعد ٢٤ ساعة من المعاملة تحلل كلوروفيل نباتات الكنترول على عكس ظهور النباتات المحولة وراثيا بصورة جيدة. وعليه فإن النتائج المتحصل عليها هنا تعبر عن إمكانية زيادة المقاومة لضغوط الأكسدة بزيادة كفاءة الإنزيمات المقاومة لايونات الأكسيجين النشطة مثل إنزيم الجلوتاثيون بيروكسيديز-١ .