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Enhanced oxidative-stress defense in transgenic potato expressing tomato Cu,Zn superoxide dismutases

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Summary. The two cDNAs coding for the cytosolic (*cyt*) and the chloroplast-located (*chl*) Cu,Zn superoxide dismutases (SODs) of tomato (Perl-Treves et al. 1988) were cloned into respective binary vectors and mobilized into *Agrobacterium* strains. Potato tuber discs were infected with either of the two agrobacterial strains and cultured on selective medium containing kanaymcin. The integration of either of the *cyt* or the *chl* SOD transgenes was verified by Southern-blot hybridization. The enzymatic activity of the additional tomato *chl* Cu,Zn SOD could be distinguished from endogenous SOD activity since the latter isozyme migrated faster on SOD-activity gels. Several transgenic potato lines harboring either the *cyt* or the *chl* SOD genes of tomato showed elevated tolerance to the superoxide-generating herbicide paraquat (methyl viologen). After exposure of shoots to paraquat, tolerance was recorded either by scoring symptoms visually or by measurements of photosynthesis using the photoacoustic method. Root cultures from transgenic lines that harbored the additional *cyt* Cu,Zn SOD gene of tomato were tolerant to methyl viologen up to 10^{-5} M; a lower tolerance was recorded in roots of transgenic lines that expressed the additional *chl* Cu,Zn SOD of tomato.

Key words: *Lycopersicon esculentum* – Oxygen toxicity – Paraquat – Photoacoustic spectroscopy – *Solanum tuberosum* – Transformation

Introduction

While oxygen is vital for all aerobic life forms, its reduced derivatives, such as singlet oxygen, superoxide and peroxide, are very reactive oxidants that may cause lethal reactions (e.g., Asada and Takahashi 1987; Monk et al. 1987). The discovery of superoxide dismutases (SODs) by McCord and Fridovich (1969) led to the unraveling of scavenging systems that protect aerobic cells against noxious oxygen radicals (see: Frank 1985). Monk et al. (1989) reviewed evidence in plants that correlated high SOD levels with resistance to drought (Burke et al. 1985), ozone (Decleire et al. 1984), sulphur dioxide (Heiska and Huttunen 1987), calcium deficiency (Monk and Davies 1989), and hypoxia (Monk et al. 1987). We recently found that ethylene application (by ethephon), drought, and wounding, enhanced SOD levels in tomato (Perl-Treves and Galun 1991). Furusawa et al. (1984) showed that tobacco calli with resistance to the superoxide-generating herbicide paraquat (methyl viologen, MV) also had vastly enhanced levels of Cu,Zn SOD activity. Jansen et al. (1989, 1990) reported that a *Conyza bonariensis* biotype that is resistant to MV contained higher SOD levels than sensitive biotypes and that drought-tolerant maize lines were cross-resistant to MV. When the bipyridinium herbicide MV is photoreduced univalently in photosystem I (PSI) it generates a labile cation radical, that interacts with molecular oxygen and generates superoxide and peroxide radicals (Halliwell 1984; Asada and Takahashi 1987). A series of reactions is thus initiated causing short-range and long-range damage by trapping photoreducing equivalents in MV, and particularly by the peroxidation of unsaturated fatty acids, causing irreversible damage to the thylakoids and other lipid membranes. In turn, when plants were treated with MV their SOD activity was elevated (e.g., Matters and Scandalios 1986; Perl-Treves and Galun 1991).

Damage to the photosynthetic apparatus can be evaluated conveniently by the photoacoustic method which results in comparative measurements of microphone sig-

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nals related to photosynthetic oxygen evolution (Bults et al. 1982; Poulet et al. 1983). Photoacoustic measurements were previously used to monitor chilling stress (Yakir et al. 1986), drought (Havaux et al. 1986), and heat tolerance (Havaux et al. 1987). The correlations between elevated SOD activity and stress tolerance suggested that the regulation of SOD levels provides plants with a stress-defense capability but a more direct proof was lacking. Increasing the expression of SOD genes in a given plant by molecular-genetic manipulations could furnish the required evidence. Moreover, the molecular-genetic approach could lead to a specific increase of the constitutive levels of either the cytosolic (*cyt*) or the chloroplast-located (*chl*) Cu,Zn SODs. This approach became feasible following the isolation of cDNAs that encode plant SOD genes. The cDNAs for tomato (Perl-Treves et al. 1988), pea (Scioli and Zilinskas 1988), and petunia (Tepperman et al. 1988) *chl* Cu,Zn SODs were isolated, sequenced and found to be rather conserved. The cDNAs for *cyt* Cu,Zn SOD were also isolated, from maize (Cannon et al. 1987), tomato (Perl-Treves et al. 1988), and recently from pea (White and Zilinskas 1991) and *Nicotiana plumbaginifolia* (Tsang et al. 1991). The cDNAs encoding the Mn SOD and the Fe SOD, which reside in mitochondria and chloroplasts, respectively, were isolated and cloned from *N. plumbaginifolia* and *Arabidopsis thaliana* (Bowler et al. 1989; Van Camp et al. 1990). A comprehensive review of superoxide dismutases was recently provided by Beyer et al. (1991). In the present study we used the cDNAs of tomato coding for the *chl* or the *cyt* Cu,Zn SOD to obtain the respective transgenic potato plants by *Agrobacterium*-mediated transformation. These plants were then utilized to examine whether there is a causal relation between the expression of the SOD transgenes and tolerance to MV.

Materials and methods

Cloning of tomato SOD cDNAs into transformation vectors

The two plasmids pP31 and pT1 contained, respectively, full-length cDNA copies of the *cyt* and the *chl* Cu,Zn SODs from tomato as described previously (Perl-Treves et al. 1988, 1990). As a first stage, fragments of these plasmids, comprising all the protein-coding region and some of the 5′ and 3′ non-coding sequences, were sub-cloned into the pUC-derived vector pBI221 ("Clonetech"). For sub-cloning pT1, pBI221 was linearized with *Sac*I and rendered blunt with T_4 DNA polymerase, then cut with *Bam*HI to remove the β-glucuronidase (GUS) gene from the vector. A *Bam*HI site was added at the 5′ end of the pT1 insert by a suitable linker and a 920-bp *Bam*HI-*Hin*cII fragment was isolated and cloned into pBI221, between its CaMV 35*s* promoter and the nos terminator. For sub-cloning pP31A the pBI221 vector was first double-digested with *Bam*HI and *Sac*I to remove the GUS gene. T_4 DNA polymerase treatment rendered it blunt and allowed self-ligation. A 637-bp *Spe*I fragment from pP31A was then cloned into the *Xba*I site of the GUS-less pBI221 vector, between its CaMV 35*s* promoter and its nos terminator. Plasmids having the cDNA in the forward ("sense") orientation were identified and used in this study. In a second stage, each of the two whole expression cassettes from engineered pBI221 clones were removed by *Hin*dIII digestion, followed by partial *Eco*RI digestion. Each was cloned into a pGA492 binary vector (An 1986) digested with *Eco*RI and *Hin*dIII. This resulted in two constructs, p492T1 and p492P31A (Fig. 1). These constructs were then mobilized into *Agrobacterium* strain EHA101 via tri-parental mating (An 1987).

Agrobacterium-mediated transformation

Tubers of *S. tuberosum* cv "Desiree" were surface-sterilized in 1% sodium hypochlorite for 20 min and then washed three times in sterile distilled water. We followed the transformation procedure of Sheermann and Bevan (1988) with some modifications. Three millimetre-thick tuber discs were incubated for 20 min in overnight cultures of *Agrobacterium* strains that contained either p492T1 or p492P31A. After blotting on sterile paper the discs were cultured on solidified (1% agar) MS (Murashige and Skoog 1962) medium supplemented with 2 mg/l of zeatin riboside and 1 mg/l of indole-3-aspartic-acid (Research Organics).

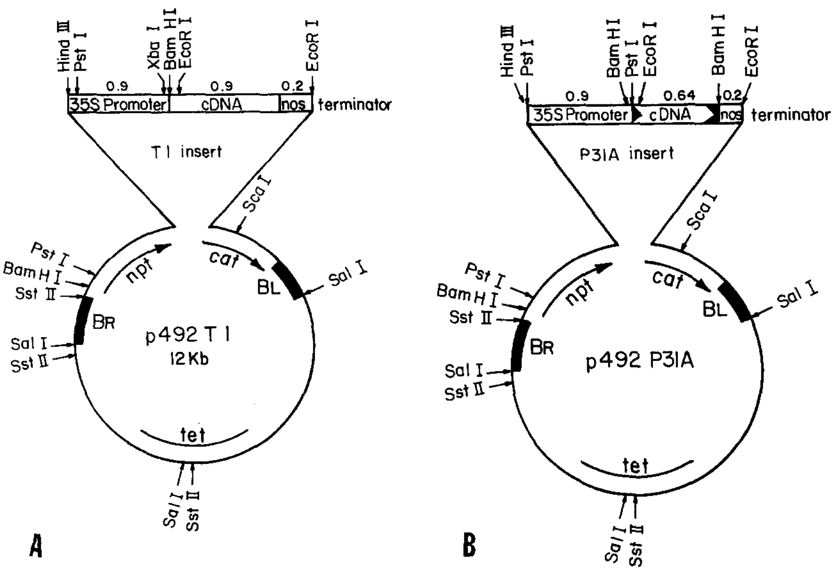

Fig. 1 A, B. Constructs for expressing tomato SOD sequences in transgenic plants. Plasmid p492T1 (**A**) and p492P31 (**B**) contain, respectively, the chloroplast Cu,Zn SOD and the cytosolic Cu,Zn SOD cDNAs from tomato, under the control of the CaMV 35*s* promoter, in a binary vector for *Agrobacterium* transformation

After 48 h of co-cultivation the discs were transferred to the same medium but with the addition of 500 mg/l of carbenicillin and 100 mg/l of kanamycin. After about 1 month of culture (24 °C, 40 µE m^2 s^{-1} light fluence), shoots were regenerated. These were further rooted on solidified (0.8% agar) Nitsch (1969) medium supplemented with 100 mg/l of kanamycin, 1 mg/l of indole-3-butyric acid (IBA) and 250 mg/l of carbenicillin. Regenerated shoots that rooted in the presence of kanamycin were transferred to the greenhouse for further observations, propagation and analyses.

SOD activity-gels

Leaf samples (0.5–1.0 g fresh weight) were frozen in liquid nitrogen and ground to powder in precooled mortars. The powder was homogenized with extraction buffer (2 ml/g tissue) consisting of 0.2 M phosphate buffer and 2 mM EDTA (pH 7.8) as well as 14 mg/ml of isoascorbate. The resulting slurry was transferred to microfuge tubes and centrifuged (25 min, 10,000 g, 4 °C). The supernatant was assayed for protein content (Bradford 1976) and samples (60 µg of protein per slot) were loaded on a non-denaturing 10% polyacrylamide gel (Laemmli 1970). The gel was run at 4 °C (6 V cm^{-1}) and stained by the riboflavin – nitroblue tetrazolium method (Beauchamp and Fredovich 1971).

Application of methyl viologen to shoots

Stem cuttings from greenhouse-grown untransformed "Desiree" and transgenic plants, with three fully expanded mature leaves, were placed in tubes containing 50 ml of 10^{-4} M MV (1,1 dimethyl-4,4-bipyridinium chloride, Sigma). After overnight incubation the MV was discarded and the test-tubes were washed and filled with tap water. Each experiment was repeated at least three times. For photoacoustic spectroscopy, in vitro cultured potato shoots were used. Shoots were maintained on solidified (0.8% agar) Nitsch (1969) basal medium supplemented with 2 mg/l of silver thiosulphate. For MV application, shoots of transgenic plants, as well as of non-transformed "Desiree", were cut above the agar level and the stems were infiltrated for 10 min with 3×10^{-5} M MV followed by 4 h light (70 µE m^{-2} s^{-1}) incubation in the same solution. The shoots were then transferred to tap water before analysis. Control shoots (of "Desiree" and of each of the transgenic lines) were water-infiltrated.

Root cultures

Shoot cultures of "Desiree" and of the transgenic potato plants were cultured in solidified (0.8% agar) Nitsch medium. Root sections (1–2 cm) of the former plants were excised and cultured (500 mg fresh weight per dish) in 9 cm Petri dishes containing 6 ml of MS medium supplemented with 2 mg/l of IBA and either of several MV concentrations. After 10 days at 25 °C on a gyratory shaker (100 rpm), the roots were blotted on filter paper and their fresh weight was determined.

Photoacoustic measurements

The instrumentation for photoacoustic spectroscopy was as previously described (Bults et al. 1982). A leaf, or a leaf disc, was placed in a small enclosure in communication with a microphone. As a result of the absorption of periodically modulated light in the leaf, sound waves are generated within the enclosure, which are then sensed by the microphone. A part of the microphone signal is contributed by modulated oxygen evolution (photobaric signal) and another part is related to the conversion of light to heat (photothermal signal). The first part can be eliminated by the addition of strong, photosynthetically saturating, but non-modulated (background), light. The signal difference in the presence and the absence of the background light defines the relative extent of the contribution of oxygen evolution to the signal. The remaining signal, in the presence of the background light, defines the photothermal contribution. The ratio O/T between the oxygen evolution component (O) to the photothermal component (T) is used as a relative measure of the quantum yield of photosynthesis. The analysis of the microphone signals was done according to Poulet et al. (1983) and Jansen et al. (1989), except for the following modifications. The modulated measuring beam, provided from a monochrometer, was set at 680 nm; the modulation frequency was 18–20 Hz and had an intensity of about 50 µE m^{-2} s^{-1}; background light was white (about 540 µE m^{-2} s^{-1}).

Neomycin phosphotransferase II activity in transformed plants

Leaves (0.5–1.0 g) from transformed potato plants, as well as from control "Desiree" plants, were extracted and assayed for neomycin phosphotransferase according to Reiss et al. (1984).

Results

Derivation of transgenic potato plants

Potato tuber discs were infected with either of the two *Agrobacterium* strains harboring, respectively, constructs p492T1 or p492P31A (see Materials and methods). These constructs contained sequences that encode respectively the mRNAs for the *chl* and the *cyt* Cu,Zn SODs of tomato under the control of the CaMV 35*s* promoter and a marker gene (*nptII*) that confers resistance to kanamycin.

After culture in the presence of kanamycin, as detailed in the experimental procedures, we isolated 40 shoots from each of the two transformation types. Most of these shoots did regenerate roots when planted in kanamycin-containing rooting medium. There was a clear-cut difference between the rooting capability of control shoots from untransformed "Desiree" plants and putative transgenic shoots; only the latter showed profused rooting. To verify the presence of active *nptII* genes in the rooted plants the latter were checked for neomycin phosphotransferase activity; all tested plants had this activity (data not shown).

The presence of the tomato Cu,Zn SOD cDNAs in the transformed potato plants was verified by Southern-blot hybridization of the *Eco*RI-digested DNA from these plants with the tomato *cyt* and *chl* Cu,Zn SOD probes. The DNA samples from transgenic plants, transformed with p492P31A or with p492T1, showed the expected hybridization signals of 0.9 and 1.1 kbp, respectively, (data not shown).

Isozyme pattern of transformed potato plants

Enzymatic activity of a protein coded by an alien gene indicates that the respective gene was not only intro-

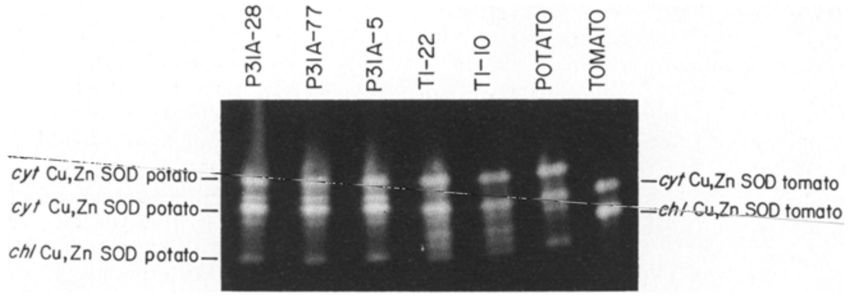

Fig. 2. SOD activity gel. SOD isozyme patterns of protein extracts from potato, tomato, and transgenic potato plants that showed tolerance to MV. The non-denaturing polyacrylamide gel was loaded with 60 µg protein per slot and (negatively) stained with riboflavin-nitroblue tetrazolium. *Lanes* marked *P31A-28*, *P31A-77* and *P31A-5* were loaded with proteins from transgenic plants with the tomato *cyt* Cu,Zn SOD; *lanes T1-10* and *T1-22* were loaded with proteins from transgenic plants with the *chl* Cu,Zn SOD

duced into this transgenic plant but also expressed a functional product. We examined whether or not the transgenic potato plants indeed express extra isozymes of tomato Cu,Zn SOD. Protein extracts were run on SOD activity gels, together with extracts of tomato plants and of untransformed "Desiree" plants. The identification of the potato and tomato isozymes in this gel system was previously reported (Perl-Treves et al. 1990). About 50 transgenic plants displayed additional SOD bands (data not shown) out of which five were chosen for further study. These latter plants overproduced tomato Cu,Zn SODs without any apparent reduction in the expression level of the endogenous SODs. Figure 2 exemplifies the migration of SOD activity bands from these five plants. The *chl* Cu,Zn SOD isozyme of potato migrated faster than the respective tomato SOD. Consequently, the two representative transgenic lines, transformed with the p492T1 plasmid, could be analyzed for the presence of the tomato *chl* Cu,Zn SOD isozyme (Fig. 2). Both the latter plants displayed an additional band that co-migrated with the *chl* Cu,Zn SOD of tomato. The extracts of these transformed plants also revealed a "novel" band, at an intermediate position between the potato and the tomato *chl* Cu,Zn SOD bands. This novel band could represent a heterodimer enzyme. The fact that the additional enzyme is active and migrates to an identical gel-position, suggests that the tomato SOD transit peptide has been recognized and processed by the potato chloroplasts. In contrast with tomato *chl* isozyme, the tomato *cyt* isozyme co-migrated with one of the two potato *cyt* isozymes. Therefore the three representatives of the potato plants, transformed with the p492P31A plasmid, were not expected to reveal a pattern different from the untransformed potato control. Nevertheless, an additional band appeared in all these three transgenic plants (Fig. 2). This band was sensitive to pre-incubation with 2 mM KCN, (data not shown), suggesting a heterodimer between the tomato Cu,Zn SOD and the slower migrating potato *cyt* Cu,Zn SOD. It should be noted that the endogeneous *chl* and *cyt* isozymes were not diminished in the transgenic plants. Therefore, their heterodimers may be considered as an over-expression of Cu,Zn SOD in these transgenic potato plants.

The effect of MV on transformed potato plants

Elevated SOD activity was correlated, in several studies, with plants having elevated defense capability against oxidative stress (see Introduction). We therefore investigated whether transgenic potato plants will show increased tolerance to superoxide radicals generated by paraquat (MV). Our first approach was to evaluate this tolerance by scoring visual symptoms.

We dipped 30 untransformed shoots, as well as transformed shoots with plasmids that do not contain SOD genes, in 10^{-4} M MV. After 16 h exposure to MV the shoots were transferred to tap water. This treatment resulted in very clear paraquat-damage symptoms. The leaves turned chlorotic 2 days after paraquat treatment and then wilted irreversibly. We then applied this MV treatment to test the five representative transgenic plants that expressed either the *cyt* (p492P31A) or the *chl* (p492T1) Cu,Zn SOD gene of tomato. The tests were repeated three times and provided the same result: all five plants consistently lacked any symptoms of damage. Figure 3 demonstrates the difference between two paraquat-tolerant transgenic plants (T1-22 and P31A-28) and non-transformed "Desiree" plants. Three other transgenic plants, T1-10, P31A-77 and P31A-5 (data not shown), were also tolerant to this paraquat treatment. Thus we could establish a two-sided correlation: a positive one, between MV tolerance and extra bands of tomato SOD, and a negative one: transgenic plants without prominent additional (tomato) SOD bands do not show MV tolerance.

Root cultures of transgenic potato

Paraquat toxicity is not limited to cells active in photosynthesis; it was also reported in mammalian cells (e.g.,

Fig. 3. Response of shoots from greenhouse-grown potato plants to paraquat (methyl viologen, MV). Shoots with three to four unfolded leaves were dipped (16 h) in 10^{-4} M MV, then transferred to water and photographed 48 h after release from MV exposure. *A*, two shoots from non-transformed potato ("Desiree"); *B*, shoot of a plant (T1-22) that was transformed with the *chl* Cu,Zn SOD coding sequence of tomato; *C*, shoot of a plant (P31A-28) that was transformed with the *cyt* Cu,Zn SOD coding sequence of tomato

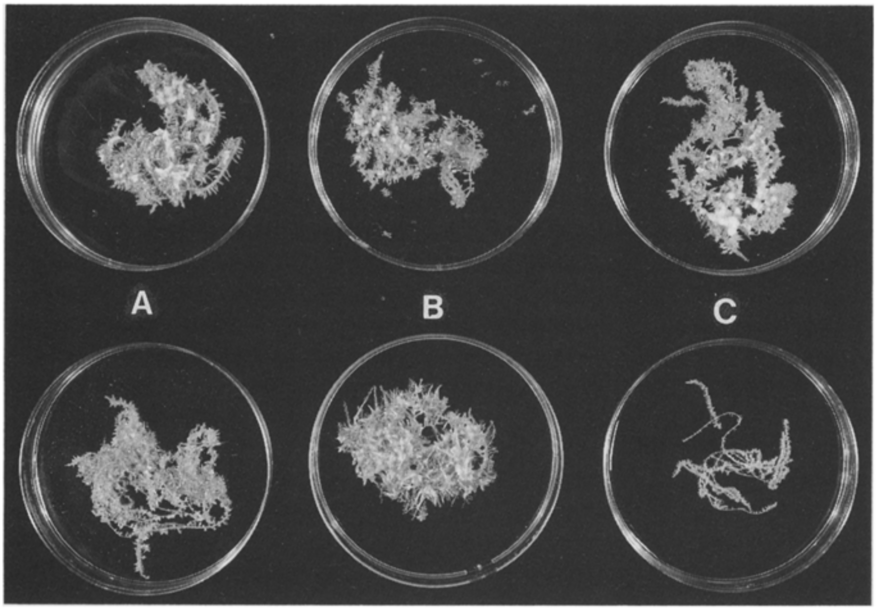

Fig. 4. Response of root cultures to paraquat (methyl viologen, MV). Root sections were cultured in Petri dishes in medium devoid of MV (upper dishes) or with 10^{-5} M MV. *A*, roots from a plant (T1-22) transformed with the tomato *chl* Cu,Zn SOD sequence; *B*, roots from a plant (P31A-28) transformed with the tomato *cyt* Cu,Zn SOD sequence; *C*, roots from a non-transformed "Desiree" plant. Dishes were photographed 10 days after start of culture

Elroy-Stern et al. 1986). Similarly, we found potato root cultures to be very sensitive to MV. We therefore compared the MV tolerance of transgenic potato roots and roots of non-transformed "Desiree". Out of the five transgenic plants that showed shoot-tolerance to MV we chose four to serve in the following two experiments. First, roots of "Desiree" and of two transgenic potato lines, harboring either the *cyt* (P31A-28) or the *chl* (T1-22) Cu,Zn SOD genes of tomato, were cultured with or without 10^{-5} M MV. The roots of these three plants grew at similar rates in the control medium while the growth of untransformed "Desiree" roots was arrested in culture medium containing MV (Fig. 4). To quantitatively evaluate the paraquat tolerance of such root cultures we compared the growth of roots from untransformed "Desiree" to that of roots from the four transgenic plants when cultured in several concentrations of MV (Fig. 5). The roots of transgenic plants with the tomato *cyt* Cu,Zn SOD gene, were highly tolerant to MV. This tolerance was statistically significant for both plants as compared to controls. Interestingly, the roots of line T1-22 harboring the *chl* Cu,Zn SOD of tomato also showed a statistically significant tolerance when cultured in the presence of MV. Roots of line T-10 showed some tolerance although this was not significant in all MV concentrations.

Photoacoustic measurements

When in vitro cultured shoots of untransformed "Desiree" plants were exposed to 3×10^{-5} M MV for 4 h and their leaves were subjected to photoacoustic analysis, it was found that the relative quantum yield of oxygen

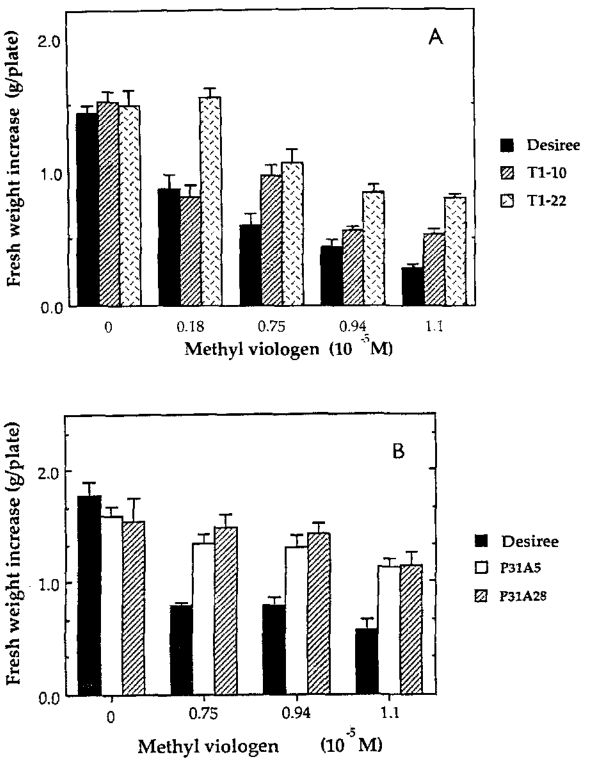

Fig. 5. Fresh-weight increase of roots derived from a non-transformed "Desiree" plant and from plants transformed with either the *chl* Cu,Zn SOD (**A**) or the *cyt* Cu,Zn SOD (**B**) coding sequence of tomato. Roots were exposed to MV for 10 days. *Vertical bars* represent the standard error of the mean for triplicate determinations

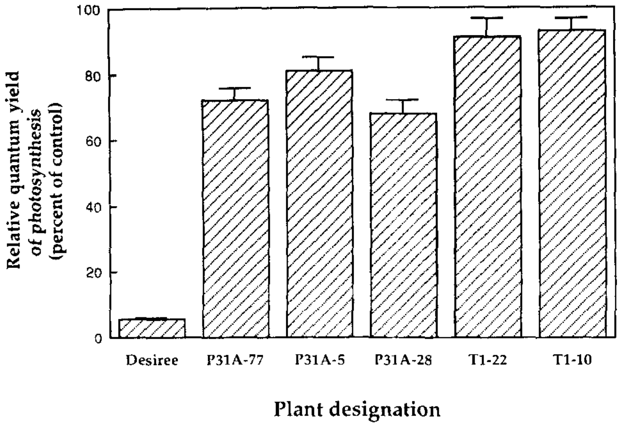

Fig. 6. Relative quantum yield of photosynthetic oxygen evolution in the presence of MV. In vitro shoots were infiltrated for 10 min with 3×10^{-5} M MV followed by 4 h incubation in the same solution. Incubation in the light (light fluence: 70 µE m^{-2} s^{-1}). The relative values are expressed as a percentage of the control quantum yield (i.e., in the absence of MV) for each plant. The first two fully expanded leaves from in-vitro transformed shoots of the same developmental stage were used for all determinations. *Vertical bars* represent the standard error of the mean for triplicate determinations

evolution was drastically reduced, to about 5% of the control level. In contrast, the five representative plants harboring either the *cyt* or the *chl* Cu,Zn SOD gene of tomato showed high levels of tolerance to this concentration of MV. The reduction of oxygen evolution was mild; oxygen evolution reached 70–90% of the control (Fig. 6). It is noteworthy that unlike the reaction of root cultures from the same transgenic plants, the leaves of the transgenic plants that harbored the *chl* Cu,Zn SOD gene of tomato (i.e., T1-10, T1-22) were significantly more resistant to MV than leaves of the transgenic plants harboring the *cyt* Cu,Zn SOD transgenes (i.e., P31A-5, P31A-28, P31A-77). Nevertheless both groups were significantly much more resistant to MV than the control "Desiree", corroborating the reaction of root-cultures of these two transgenic plants to MV (Fig. 5). It should be noted that without MV treatment the quantum yield of oxygen evolution was similar in transformed and in control plants.

Discussion

Elevated SOD activity, imposed by an enhanced expresson of alien SOD genes in transgenic plants, could protect these plants from oxidative toxicity if two requirements are met. First, the level of SOD activity should be high enough, in the susceptible plant tissue or organelles, to dismutase the toxic levels of superoxide. Then, the resulting H_20_2 should be quickly scavenged, by other enzymes, to reduce H_20_2 toxicity and to minimize the production of the highly toxic hydroxyl radical (see: Halliwell 1987; Imlay and Linn 1988; and Beyer et al. 1991 for reviews and detailed discussions). It seems that the second requirement is the more complicated one: we lack information in plants that clearly indicates whether or not the up-regulation of enzymes, such as ascorbate peroxidase, glutathion reductase and catalase, will cope with the elevated H_20_2 levels that result from enhanced SOD activity. Indeed a very high over-expression of SOD in transgenic plants can be detrimental, as discussed by Bowler et al. (1991). Such a high over-expression, of 30 to 50 fold over control, was found in transgenic tobacco plants with an alien *chl* Cu,Zn SOD gene (Tepperman and Dunsmuir 1990). In contrast to our transgenic potatoes, (that had only a modest increase in Cu,Zn SOD expression), the transgenic tobacco plants did not show tolerance to paraquat.

We found previously (Perl-Treves 1990; Perl-Treves et al. 1991) that upon exposure of tomato plants to MV the transcripts of the *cyt* and the *chl* Cu,Zn SOD genes were increased several fold within 1–4 h. But the respective increase of SOD activities was much delayed: little or no increase was detected within 4 h of MV treatment. This lag in defense-response could be critical to plants treated with MV. A constitutively expressed SOD activity, imposed by transgenes, under the control of an appropriate promoter, could thus provide the required interim protection against superoxide radicals, by shifting the O_2^-, H_2O_2 equilibrium, until the endogenous SOD genes are induced and a higher SOD activity is reached. The latter increased SOD activity from the endogenous SOD genes may then provide the long-range protection. The Cu,Zn SODs are inactivated by H_2O_2. Transgenic plants expressing high levels of Cu,Zn SOD and producing high levels of H_2O_2, may inactivate the Cu,Zn SOD, leading to the presence of both O_2^- and H_2O_2 and repromoting hydroxyl formation. Indeed, Tepperman and Dunsmuir (1990) could not find any enhanced oxidative resistance to MV in transgenic tobacco plants expressing 50-fold more Cu,Zn SOD. The same plants were used by Pitcher et al. (1991) who found that these plants were not protected against ozone toxicity. It was suggested by these authors that elevating SOD without co-elevating enzymes that remove H_2O_2 does not provide protection against oxygen toxicity. On the other hand, a small increase of the Cu,Zn SOD was able to provide resistance against MV in human and mouse cells (Elroy-Stein et al. 1986). This information may provide an answer to the question: why are transgenic plants, having only a modest increase in SOD activity, tolerant to MV. We suggest that the constitutively expressed Cu,Zn SOD transgenes actually provide a "first-aid" protection against MV by shifting the O_2^-, H_2O_2 equilibrium. In addition, it cannot be ruled out that in tobacco, but not in potato and some other organisms, ascorbate peroxidase and/or glutathion reductase are the limiting factors in scavenging toxic oxygen species. Mn SOD is not inactivated by H_2O_2. Transgenic tobacco plants over-expressing this isozyme were tolerant to the cellular damage caused by oxyen radicals (Bowler et al. 1991). MV transiently inhibits CO_2 fixation in vivo even in MV-resistant plants (Shaaltiel and Gressel 1987; Polos et al. 1988; Szigeti et al. 1988) and it was previously suggested that this resistance is due to detoxification of the active oxygen species produced by MV (Shaaltiel and Gressel 1986). Based on these observations and suggestions we hypothesize that transgenic plants with constitutively elevated levels of SODs, should be more tolerant to photooxidative damage. Indeed our results support this hypothesis: oxygen evolution as measured by photoacoustic spectroscopy, as well as the response of greenhouse plants and of root cultures to MV treatment, indicated an increased level of tolerance to MV toxicity in transgenic potato plants. We may therefore assume that in potato an enhanced radical scavenging system with elevated levels of SOD, can supply protection against photooxidative damage. It is interesting to note that either of the two SOD constructs (containing either the *cyt* or the *chl* SOD) were able to confer this protection in at least some of the respective transgenic plants. The chloroplast was suggested to be the main target of MV action (Jansen et al. 1990) but MV was also found to produce superoxide radicals in other cellular components (e.g., the endoplasmic reticulum) in non-green plant tissues (Rich and Bonner 1978; Phillips et al. 1989). It is also possible that the SOD activity in the cytoplasm acts as a sink for superoxide diffusing out from the chloroplast. This may explain why transgenic potato plants having elevated levels of the cytosolic Cu,Zn SOD showed tolerance to MV damage. A notable finding of this study was that root cultures provided a convenient method to evaluate the response of a plant organ, that has no photosynthetic activity, to elevated superoxide. The very same transgenic plants that showed high MV tolerance by the root culture method also showed high tolerance by other means of evaluation. The MV tolerance of a plant tissue that harbors added SOD genes, but is devoid of chloroplasts, is in line with the report (Elroy-Stern et al. 1986) that transgenic mammalian cells with high SOD activity were found to be MV-tolerant.

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