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Stress Tolerance in Transgenic Tobacco Seedlings that Overexpress Glutathione S-Transferase/Glutathione Peroxidase

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Overexpression of a tobacco glutathione S-transferase with glutathione peroxidase activity (GST/GPX) in transgenic tobacco (*Nicotiana tabacum* L.) enhanced seedling growth under a variety of stressful conditions. In addition to increased GST and GPX activity, transgenic GST/GPX-expressing (GST+) seedlings had elevated levels of monodehydroascorbate reductase activity. GST+ seedlings also contained higher levels of glutathione and ascorbate than wild-type seedlings and the glutathione pools were more oxidized. Thermal or salt-stress treatments that inhibited the growth of wild-type seedlings also caused increased levels of lipid peroxidation. These treatments had less effect on the growth of GST+ seedling growth and did not lead to increased lipid peroxidation. Stress-induced damage resulted in reduced metabolic activity in wild-type seedlings while GST+ seedlings maintained metabolic activity levels comparable to seedlings grown under control conditions. These results indicate that overexpression of GST/GPX in transgenic tobacco seedlings provides increased glutathione-dependent peroxide scavenging and alterations in glutathione and ascorbate metabolism that lead to reduced oxidative damage. We conclude that this protective effect is primarily responsible for the ability of GST+ seedlings to maintain growth under stressful conditions.

Key words: Glutathione peroxidase — Glutathione S-transferase — Seedling growth — Stress tolerance — Transgenic tobacco.

Abbreviations: APX, ascorbate peroxidase; ASA, ascorbic acid; DPI, days post-imbibition; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione S-transferase; GPX glutathione peroxidase; GR, glutathione reductase; MDA, malondialdehyde; MDHA, monodehydroascorbate; MDHAR, monodehydroascorbate reductase.

Introduction

Exposure of plants to unfavorable environmental conditions such as vicissitudes of temperature, water availability, air pollutants, or salt stress can increase the production of reactive oxygen species (ROS) including singlet oxygen ($^1O_2$), superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\cdot}OH$). To protect themselves against these toxic oxygen intermediates, plants employ defense systems that include the enzymes such as superoxide dismutases, catalases, ascorbate peroxidases (APX), glutathione S-transferases (GST) and glutathione peroxidases (GPX) that catalyze the scavenging of ROS. The activities of APX, GST and GPX depend on the availability of reduced ascorbate (ASA) and glutathione (GSH) that are maintained by enzymes such as glutathione reductase (GR), dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR) using NAD(P)H as an electron donor.

Recently, our understanding of the role of ROS-scavenging systems in plant stress tolerance has increased through the use of gene transfer technology to manipulate the levels of antioxidant enzyme activities in plants. These studies have provided significant insights into the roles of these enzymes in plants (see Foyer 1994, Allen 1995 for reviews). Furthermore, a number of these experiments clearly demonstrated that enhancement of the ROS-scavenging systems in plants can provide partial protection from oxidative damage, indicating that this strategy could be used to improve plant stress tolerance.

While most of the previous work has focused on photosynthetic organs, recent analysis of transgenic tobacco lines that overexpress plant glutathione S-transferase/glutathione peroxidase (GST/GPX) showed substantial improvement in seed germination and seedling growth under stressful conditions (Roxas et al. 1997). Growth of GST/GPX-expressing (GST+) seedlings remained high under chilling or salt-stress conditions that inhibited the growth of control seedlings. Although the mechanisms by which overexpression of GST/GPX enhances seedling growth under stress have not been clearly elucidated, initial studies showed that transgenic GST+ seedlings contained substantially more oxidized glutathione (GSSG) during stress than wild-type seedlings (Roxas et al. 1997). This accumulation of GSSG could indicate increased GPX-dependent peroxide scavenging in these seedlings. Results reported here confirm that overexpression of GST/GPX in seedlings provides enhanced capacity to scavenge ROS. This protective effect appears to prevent cellular damage and allow seedlings to retain high levels of metabolic activity and growth.

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Materials and Methods

Plant materials and growing conditions

Transgenic tobacco (*Nicotiana tabacum* L. cv. Xanthi NN) plants were used that contain a chimeric Nt 107 gene that encodes an enzyme with both GST and GPX activity, under control of an enhanced cauliflower mosaic virus promoter (Roxas et al. 1997). Five independently transformed transgenic plants with high levels of Nt 107 mRNA and GST and GPX specific activities that were elevated by 2- to 3-fold compared to the wild-type plants, were self-pollinated. T_1 seeds from these five independent GST+ seed lines were analyzed together with control seed lines. Untransformed seedlings and non-expressing segregants from the transgenic lines were used as wild-type controls in these experiments. Since they were found to give identical results in all analyses, the results were combined. Seeds were sown on Petri dishes lined with filter paper moistened with deionized water and incubated in the dark under control conditions (25°C) or at stressful temperatures (10°C or 30°C). Seeds were also imbibed with 100 mM NaCl in the dark at 25°C. Seedling growth was evaluated 5 d post-imbibition (DPI) by length measurements of 25 randomly selected seedlings per line.

Enzymology

Transgenic GST+ and wild-type tobacco seedlings (7 DPI) grown under control conditions were ground in 100 μl of buffer (0.2 M Tris-HCl pH 7.8, 1 mM EDTA) and centrifuged at 5,000×*g* for 10 min. GST and GPX specific activities were assayed spectrophotometrically as described by Roxas et al. (1997). For GR activity, seedlings were ground in 0.15 M HEPES-KOH (pH 8.0) containing 1.0 mM EDTA and centrifuged at 12,000×*g* for 5 min. The specific activity of GR was assayed in the supernatant by following the decrease in A_{340} upon the addition of GSSG to a final concentration of 1.0 mM. The reaction contained 50 mM HEPES buffer (pH 8.0), 1.0 mM EDTA, 0.2 mM NADPH, and extract, in a final volume of 1.0 ml (Cakmak et al. 1993). APX specific activity was determined by following the change in A_{290} for 2 min in an assay solution consisting of 50 mM HEPES (pH 7.0), 0.6 mM ASA, 0.1 mM EDTA, 1 mM H_2O_2 and 25 μl extract (Cakmak and Horst 1991). DHAR specific activity was measured by the formation of ASA at 265 nm following the procedure of Nakano and Asada (1981) with some modifications. The reaction mixture consisted of 50 mM KPO_4 (pH 7.6), 2.5 mM GSH, 0.2 mM DHA, 0.1 mM EDTA and 25 μl extract. MDHAR specific activity was assayed by monitoring the change in absorbance at 340 nm for 90 s based on the modified procedure of Cakmak et al. (1993). The assay solution contained 50 mM KPO_4 (7.6), 0.2 mM NADH, 1 unit of ascorbate oxidase, 2.5 mM ASA and 25 μl of extract. Protein concentrations were determined using the dye-binding assay of Bradford (1976).

Glutathione and ascorbate determination

Wild-type and GST+ tobacco seedlings (7 DPI) were grown under control and salt-stress conditions. Tissue samples (0.1 g FW) were frozen in liquid N_2, ground with 0.5 ml of 2.5 M $HClO_4$ and centrifuged for 5 min at 12,000×*g*. The supernatant was neutralized with 5 M K_2CO_3 to obtain pH values between 4–5 and 6–7 for determination of ASA and glutathione, respectively. Glutathione pools were determined according to the methods of Griffith (1980). GSSG was measured after the removal of GSH by 2-vinyl pyridine derivatization. Absorbance was monitored at 412 nm for 15 min. GSH was determined by subtraction of GSSG from the total glutathione content. The glutathione redox ratio was calculated by the formula [GSH]/[GSH]+[GSSG]. For analysis of ASA, 100 μl of the sample was mixed with 0.1 M sodium phosphate buffer (pH 5.6) and the reaction was started with the addition of 4 units of ascorbate oxidase (Arrigoni et al. 1992). Dehydroascorbate was determined by the same method following the reduction to ascorbic acid in a reaction mixture containing 20 mM DTT and 50 mM HEPES-KOH buffer, pH 7.0. The ascorbate redox ratio was calculated using the formula [ASA]/[ASA]+[DHA].

Analysis of lipid peroxidation

Lipid peroxidation is an effective indicator of cellular oxidative damage. Oxidative stress leads to free radical-mediated lipid peroxidation with the potential to damage membranes of seed tissues (Wilson and MacDonald 1986, Willekens et al. 1997). Determination of lipid peroxidation in transgenic GST+ and wild-type seedlings grown under control and stressful conditions was carried out by measurement of malondialdehyde using the 2-thiobarbituric acid (TBA) assay procedure of Draper and Hadley (1990) with some modifications. Briefly, tobacco seedlings (5–7 DPI; 0.1–0.2 g FW) were extracted in a solution of 5% trichloroacetic acid and 0.5 g $liter^{-1}$ methanolic butylated hydroxytoluene. The extracts were boiled for 30 min and reacted with TBA. MDA values were calculated from A_{532} to A_{600} using a molar absorption coefficient of 1.56×10^5 (Gueta-Dahan et al. 1997).

Analysis of metabolic activity

Plant growth generally correlates closely with metabolic activity (Criddle et al. 1991, Hansen et al. 1994). Therefore, calorimetric analysis was used to provide a quantitative measurement of the effects of stress on the metabolic activity of GST+ and wild-type seedlings. Transgenic and control seedlings were grown under control and stressful conditions as described above for 5 DPI. Intact seedling samples (20–40 mg FW) were then loaded into ampoules and analyzed for metabolic heat rates (q_{met}) using a calorimeter (Hart Scientific model CSC 4207, U.S.A.) at 25°C over a period of 3.2 h. All calorimetry experiments were repeated at least twice and duplicate assays were included during each run for each individual sample.

Results

Analysis of the ascorbate-glutathione redox pathway

Overexpression of antioxidant genes can influence the expression of other genes (Sen Gupta et al. 1993). Therefore, we compared the activities of enzymes involved in the ASA-GSH pathway in transgenic GST/GPX expressing and non-expressing seedlings. Transgenic GST+ seedlings had approximately 2.5-fold higher GST and GPX specific activity than wild-type seedlings (Table 1). While GR specific activity was similar in both GST+ and wild-type seedlings, APX specific activity was approximately 25% higher in GST+ seedlings than in wild-type seedling. Though statistically significant ($P \leq 0.05$), it is unlikely that this small increase in activity could substantially affect the overall peroxide-scavenging capacity of the transgenic seedlings. In addition, Roxas et al. (1997) showed that transgenic tobacco seedlings that overexpress cytosolic APX that had APX activity levels more than 3-fold higher than wild-type plants did not show the stress-tolerant phenotype typical of GST+ seedlings. Levels of DHAR specific activity in GST+ seedlings were identical to those in wild-types but MDHAR specific activity in GST+ seedlings was increased by approximately 3-fold relative to wild-type plants (Table 1).

The glutathione and ascorbate content was measured in GST+ and wild-type tobacco seedlings grown under control

Table 1 Specific activity of enzymes for glutathione and ascorbate metabolism in GST/GPX expressing (GST+) and wild-type seedlings

Genotype	Enzyme activities [a]					
	GST [b]	GPX [c]	GR [c]	APX [b]	DHAR [c]	MDHAR [c]
Wild-type [d]	18.45±1.8	22.92±2.7	45.25±1.7	59.78±3.2	59.24±2.2	41.48±3.3
GST+	48.61±2.4	49.91±2.1	46.31±2.6	81.62±3.4	62.86±2.4	124.44±3.7
Relative activity [e]	2.6	2.2	1.0	1.4	1.1	3.0

[a] Data are means±standard deviation from a minimum of three independent experiments.
[b] μmol min^{-1} (mg protein)$^{-1}$.
[c] nmol min^{-1} (mg protein)$^{-1}$.
[d] Seedlings grown in water in the dark at 25°C for 7 DPI.
[e] GST+/wild type.

(water, 25°C, in the dark) and salt-stress (100 mM NaCl, 25°C in the dark) conditions. Under control conditions, the level of GSH in GST+ seedlings was not significantly different than in wild-type seedlings but the mean level of GSSG in GST+ seedlings was about 3-fold higher than in wild types (Table 2). Glutathione content increased substantially in salt-stressed seedlings of both genotypes and the glutathione pool was more oxidized. Mean levels of GSH were about 4-fold higher in both GST+ and wild-type seedlings grown in 100 mM NaCl while levels GSSG increased by about 9-fold. Therefore, under both control and stressful conditions, the glutathione pool in GST+ seedlings was more oxidized than in wild-type seedlings, with redox ratios under salt-stress conditions of 0.40 and 0.61 for GST+ and wild-type seedlings respectively (Table 2). The content of both ASA and DHA also increased in salt-stressed seedlings (Table 3). Under the salt-stress conditions, the mean level of ASA in GST+ seedlings was nearly 2-fold higher than in wild-type seedlings and the ASA pool in GST+ seedlings was more reduced with a redox ratio of 0.67 compared to 0.48 for wild-type seedlings.

Table 2 Analysis of the glutathione content in transgenic GST/GPX expressing (GST+) and wild-type seedlings

Plant line (treatment)	Glutathione content (nmol (g FW)$^{-1}$) [a]		Redox ratio [b]
	GSH	GSSG	
Wild-type (control) [c]	22.45± 7.1	5.93±0.8	0.79
Wild-type (salt stress) [d]	83.43± 6.7	54.45±4.2	0.61
GST+ (control)	24.47± 8.96	19.62±4.8	0.55
GST+(salt stress)	115.42±10.4	172.14±3.1	0.40

[a] Data are means ± standard deviations from two independent experiments.
[b] [GSH]/[GSH]+[GSSG].
[c] Seedlings grown in water in the dark at 25°C for 7 DPI.
[d] Seedlings grown in 100 mM NaCl in the dark at 25°C for 7 DPI.

Growth of transgenic GST+ seedlings

As shown in Fig. 1, GST+ seedlings grew significantly larger than wild-type seedlings at 5 DPI ($P \leq 0.01$) under control conditions (25°C in the dark). As expected, chilling at 10°C reduced the growth of both GST+ and wild-type seedlings. However, growth of wild-type seedlings was reduced by nearly 90% while growth of GST+ seedlings was reduced by only 70% so that by 5 DPI, GST+ seedlings were about 3 times larger than wild-type seedlings. Exposure to elevated temperature (30°C) resulted in a 20% increase in the growth of wild-type seedlings, while growth of GST+ seedlings was nearly 40% greater at 30°C than at 25°C, so that GST+ seedlings were about 1.5 times larger than wild-type seedlings at 5 DPI. Incubation in 100 mM NaCl reduced the growth of wild-type seedlings by approximately 50% compared to those grown in H_2O while growth of GST+ seedlings was not inhibited, resulting in GST+ seedlings that were 2.5-times larger than wild-type seedlings at 5 DPI. By demonstrating that the growth of GST+ seedlings is enhanced at both control and elevated tempera-

Table 3 Analysis of the ascorbate content in transgenic GST/GPX expressing (GST+) and wild-type seedlings

Genotype (treatment)	Ascorbate content (nmol (g FW)$^{-1}$) [a]		Redox ratio [b]
	ASA	DHA	
Wild-type (control) [d]	36.0±27.0	ND [c]	1.00
Wild-type (salt stress) [e]	47.1± 2.5	56.00±5.0	0.48
GST+ (control)	43.0±12.0	ND [c]	1.00
GST+ (salt stress)	88.92±7.9	43.61±1.0	0.67

[a] Data are means±standard deviations from two independent experiments.
[b] [ASA]/[ASA]+[DHA].
[c] Not detected.
[d] Seedlings grown in water in the dark at 25°C for 7 DPI.
[e] Seedlings grown in 100 mM NaCl in the dark at 25°C for 7 DPI.

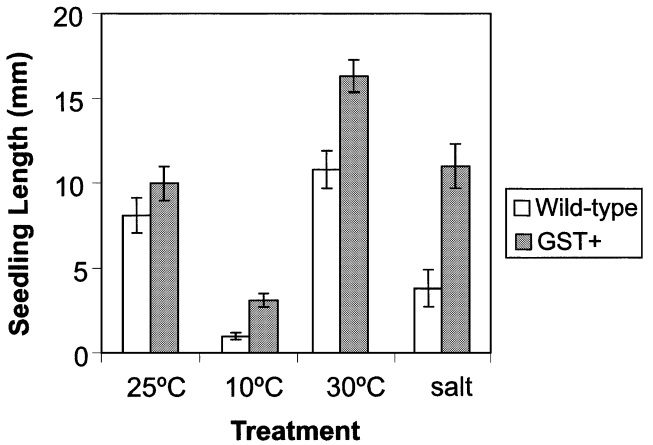

Fig. 1 Seedling growth of GST/GPX-expressing seedlings (GST+) and wild-type seedlings (non-transformed and non-expressing segregants) under various conditions 5 d after imbibition (DPI). Measurements were taken from five independent GST+ lines and two wild-type lines. Data are means ± SD; *n*=25 for each line.

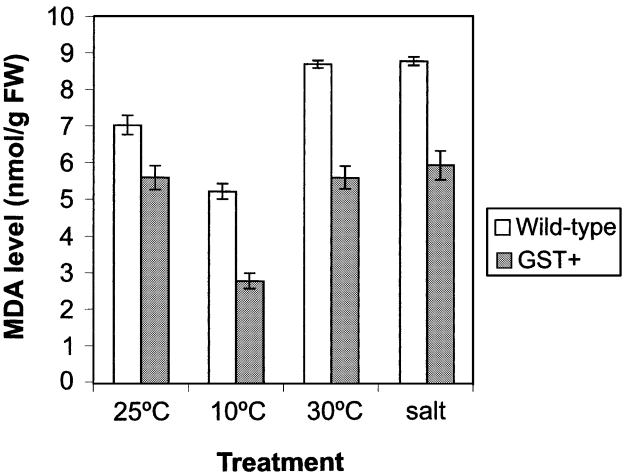

Fig. 2 Lipid peroxidation as indicated by the levels of malondialdehyde (MDA) in GST+ seedlings compared to wild-type seedlings at 5 DPI. Results are the means from three assays of five independent GST+ lines and two wild-type lines (non-transformed and non-expressing segregants). Error bars indicate SD.

tures, these data extend our previous findings that the growth of GST+ tobacco seedlings is less sensitive to low temperatures and salt-stress (Roxas et al. 1997).

Analysis of lipid peroxidation

Since GST+ seedlings have increased glutathione peroxidase activity (Table 1, Roxas et al. 1997), it is possible that the increased growth of these seedlings during stress could be due to reduced levels of oxidative damage compared to wild-type seedlings. To evaluate this possibility, the levels of lipid peroxidation in wild-type and GST+ seedlings were evaluated (Fig. 2). Under control conditions, lipid peroxidation, as indicated by malondialdehyde analysis, in GST+ seedlings was approximately 20% lower than in wild-type seedlings. Exposure to 100 mM NaCl or 30°C caused lipid peroxidation to increase in wild-type seedlings by more than 20% but these stress treatments did not effect lipid peroxidation in GST+ seedlings. While the lower overall level of lipid peroxidation in seedlings grown at 10°C is consistent with their decreased metabolic activity and reduced growth at low temperatures, levels of lipid peroxidation in wild-type seedlings were nearly two times higher than in GST+ seedlings. These results indicate that, even under these highly stressful conditions, lipid peroxidation in the transgenic seedlings was substantially lower than in wild-type seedlings.

Analysis of metabolic activity

The primary phenotype of GST+ seedlings is their ability to maintain growth under adverse conditions. Since levels of metabolic activity closely correlate with plant growth (Criddle et al. 1991, Hansen et al. 1994), we measured the metabolic heat (q_{met}) production in GST+ and wild-type seedlings grown under both control and stressful conditions (Fig. 3). It is important to note that these measurements were performed at 25°C and, therefore represent the residual metabolic activity of the seedlings following stress treatment. Under control conditions (25°C, dark), the metabolic activity of GST+ seedlings was nearly 30% higher than that of wild-type seedlings. This difference correlates with the differential growth of these seedlings (Fig. 1) under control conditions. Salt stress treatments reduced metabolic activity in wild-type seedlings by about 40%

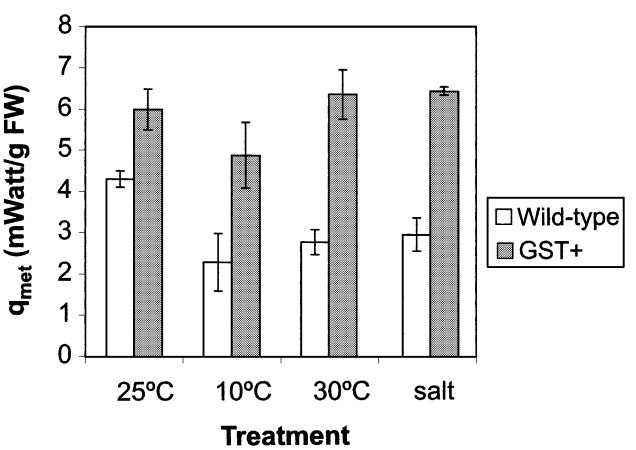

Fig. 3 Metabolic activity of GST+ seedlings compared to wild-type seedlings. Seedlings were grown for 5 DPI under the indicated conditions in the dark and measurements of metabolic heat (q_{met}) were made at 25°C in the dark using a calorimetry. Results are the means from at least two experiments with two replicates per run. Error bars indicate SD.

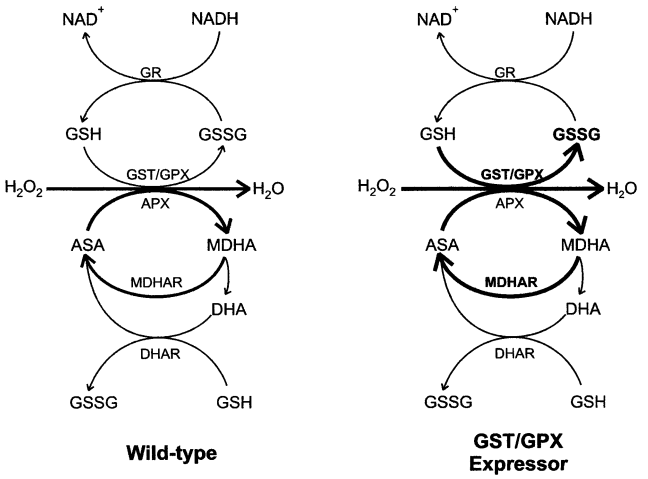

Fig. 4 Proposed model for alterations in peroxide scavenging pathways in tobacco seedling. In the wild-type seedlings, peroxides are scavenged primarily through an ascorbate-dependent pathway, resulting in oxidation of the ascorbate pool. In contrast, the increased peroxidatic capacity of GST+ seedlings leads to increased oxidation of the glutathione pool and reduced demand for ascorbate. The oxidation of the glutathione pool could lead to increased expression of NADH-dependent MDHAR resulting in increased reduction of ascorbate.

while salt-stressed GST+ seedlings retained metabolic activity levels that were comparable to, if not slightly higher than seedlings grown under control conditions, again correlating with the relative growth of these seedlings.

Incubation of seedlings at reduced temperatures will reduce the metabolic activity and, as shown in Fig. 1, inhibit the growth of seedlings directly while growth at elevated temperature can directly increase metabolic activity and growth. Therefore, to measure the effects of chilling- or high temperature-induced damage, seedlings were grown at either 10°C or 30°C for 5 d then calorimetry was performed at 25°C. Under these conditions, changes in q_{met} should represent stress-induced alterations in metabolic activity, not simply the effects of temperature on metabolism whereas changes in growth reflect both the direct effects of temperature and along with the effects of stress. Therefore a direct correlation between changes in metabolic activity and seedling growth is not expected in these experiments. Incubation at 10°C resulted in a nearly 20% decrease in q_{met} in GST+ seedling; while the metabolic activity of wild-type seedlings was reduced by about 50%. These results indicate that, under these conditions, detectable damage does occur in both GST+ and wild-type seedlings, though it is greater in the wild types. Growth of wild-type seedlings increased somewhat at 30°C compared to 25°C yet they showed a decrease in metabolic activity of approximately 35%. Growth of GST+ seedlings increased dramatically at 30°C while the metabolic of these seedlings was unchanged. These results indicate that, in wild-type plants, the increase in metabolic activity afforded by higher temperatures is largely offset by increased oxidative damage, as indicated by reduced q_{met}, resulting in only small increases in growth. This increase in damage is prevented in GST+ plants so that a temperature-dependent increase in growth is realized.

Discussion

The results presented clearly show that transgenic tobacco seedlings that overexpress GST/GPX grow faster than wild-type seedlings under control conditions and this growth difference is accentuated during exposure to a variety of stressful conditions. These seedlings also had reduced levels of lipid peroxidation and maintained higher levels of metabolic activity after stress exposure. These findings indicate that overexpression of GST/GPX in transgenic tobacco seedlings provides protection from oxidative damage during germination and seedling growth. It is likely that the reduced capacity of wild-type seedlings to grow under stressful conditions can be attributed, at least in part, to stress-induced oxidative damage (Wilson and MacDonald 1986, Sung and Jeng 1994).

Seed germination is characterized by a rapid generation of H_2O_2 as a result of high respiratory activity and O_2 consumption following seed imbibition (Cakmak et al. 1993). In addition, the mobilization of storage lipids and iron storage compounds like ferritin and phytate in seedlings leads to increased production of ROS that is responsible for oxidative damage (Halliwell and Gutteridge 1986). Through its peroxidatic activity, GST/GPX is capable of rapidly scavenging products of lipid peroxidation generated as a result of enhanced ROS production (Bartling et al. 1993, Williamson and Beverly 1987) as well as endogenous reactive products of cellular metabolism (see Daniel 1993, Rushmore and Pickett 1993 for reviews). Organic and inorganic peroxides produced during seed germination are detoxified primarily by peroxidases (Puntarulo et al. 1991) and the acclimation of maize seedlings to chilling stress tolerance has been associated with increased expression of peroxidase and catalase activity (Anderson et al. 1995). Therefore, we conclude that protection of seedling tissues from oxidative damage is the most likely mechanism for the increased stress tolerance phenotype of GST+ seedlings. The enhanced growth of GST+ seedlings under control conditions indicates that significant oxidative damage occurs in tobacco seedlings even at optimal temperatures and this damage can be ameliorated by GST/GPX overexpression.

It is possible that the impact of GST/GPX overexpression on glutathione and ascorbate metabolism and on the expression of other enzymes in the ascorbate-glutathione antioxidant pathway could complement the direct effects of increased GST/GPX activity. Most notably, the substantial increase in MDHAR and associated increase in ASA content could have significant effects on seedling stress tolerance. These perturbations could indicate a shift in the peroxide-scavenging pathway of GST+ seedlings. As shown in Fig. 4, peroxide scavenging in

wild-type seedlings appears to be accomplished primarily through an ascorbate-dependent pathway (De Gara et al. 1997). In GST+ seedlings, on the other hand, the enhanced glutathione-dependent pathway leads to increased GSSG accumulation and, perhaps, lower ascorbate utilization. This reduced demand for ASA, along with increased regeneration of ASA from MDHA due to the relatively large increase in MDHAR activity in GST+ seedlings could contribute to the increased accumulation of ASA compared to wild-type seedlings. It is possible that the increase in GSSG in GST+ seedlings acts as signal to induce MDHAR gene expression.

Regeneration of ASA in germinating wheat seeds was found to be catalyzed primarily by MDHAR and not by DHAR (Cakmak et al. 1993). Since DHAR depends on GSH as an electron donor, it is possible that the increase in NAD(P)H-dependent MDHAR in GST+ seedlings could be a response to the relative oxidation of the glutathione pool. One could ask whether it is the increase in GST/GPX activity or the concomitant increase in MDHAR that is primarily responsible for the increased stress tolerance in our transgenic seedlings. The definitive answer to this question awaits thorough analysis of MDHAR-overexpressing tobacco seedlings. These have recently been developed in our laboratory and have 2- to 3-fold higher MDHAR activity than wild-type plants. However, in preliminary analyses, growth of these seedlings appears to be similar to wild-type seedlings under chilling- or salt-stress (Youn and Allen, unpublished data), suggesting that increased levels of MDHAR may not be sufficient to provide increased stress tolerance in tobacco seedlings.

Conclusions

Results presented here confirm that overexpression of GST/GPX in transgenic tobacco seeds leads to enhanced seedling growth under both stressful and non-stressful conditions. This phenotype is associated with maintenance of high metabolic activity and reduced lipid peroxidation during stress exposure. These results indicate that the increased peroxide scavenging capacity provided by GST/GPX could be a critical factor in protecting seedlings from stress-induced oxidative damage.

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