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# Enhanced tolerance of transgenic potato plants expressing both superoxide dismutase and ascorbate peroxidase in chloroplasts against oxidative stress and high temperature

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**Abstract** Oxidative stress is a major damaging factor for plants exposed to environmental stresses. In order to develop transgenic potato plants with enhanced tolerance to environmental stress, the genes of both Cu/Zn superoxide dismutase and ascorbate peroxidase were expressed in chloroplasts under the control of an oxidative stress-inducible *SWPA2* promoter (referred to as SSA plants). SSA plants showed enhanced tolerance to 250 μM methyl viologen, and visible damage in SSA plants was one-fourth that of non-transgenic (NT) plants that were almost destroyed. In addition, when SSA plants were treated with a high temperature of 42°C for 20 h, the photosynthetic activity of SSA plants decreased by only 6%, whereas that of NT plants decreased by 29%. These results suggest that the manipulation of the antioxidative mechanism of the chloroplasts may be applied in the development of industrial transgenic crop plants with increased tolerance to multiple environmental stresses.

**Keywords** Antioxidant enzyme · Environmental stress · Molecular breeding · Potato · Transgenic plant

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## Introduction

Oxygen is essential for the existence of aerobic life, but toxic reactive oxygen species (ROS), which include the superoxide anion radical ($O_2^{-\bullet}$), hydroxyl radical ($OH^{\cdot}$), and hydrogen peroxide ($H_2O_2$), are generated in all aerobic cells during the processes of metabolism (Foyer et al. 1994; Asada 1999). Oxidative stress caused by these ROS is one of the major damaging factors in plants exposed to environmental stresses. The damaging effects of ROS have caused plant cells to develop complex redox homeostatic mechanisms to cope with oxidative stress by using ROS-scavenging enzymes. These ROS-scavenging enzymes include superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD), catalase (CAT), and low molecular weight antioxidants such as ascorbic acid, glutathione, and phenolic compounds (Noctor and Foyer 1998; Asada 1999).

The chloroplast, which is the compartment associated with the high-energy photosynthetic electron transport system and a generous supply of oxygen, is a rich source of ROS (Asada 1999). Two key enzymes for ROS detoxification in the chloroplast are SOD and APX. SOD catalyzes the dismutation of two molecules of superoxide anion radical into oxygen and hydrogen peroxide. Utilizing ascorbate as an electron donor, APX reduces hydrogen peroxide to water. The formation of most toxic hydroxyl radicals by superoxide anion radical and hydrogen peroxide can be controlled by combinational reactions of both SOD and APX. The water–water cycle has been proposed to explain the role of these antioxidant mechanisms at the onset of oxidative stress in chloroplasts (Asada 1999). The most

important function of the water–water cycle is the rapid scavenging of superoxide radicals and hydrogen peroxide at the sites of their generation, prior to their interaction with target molecules. To maintain the productivity of plants under the stress conditions, it is possible to fortify the antioxidative mechanism of the chloroplasts by manipulating the antioxidant enzymes present in chloroplasts. As we have previously reported, transgenic tobacco plants expressing both Cu/Zn SOD and APX in chloroplasts under the control of CaMV 35S promoter provided strong protection to methyl viologen (MV)-induced oxidative stress (Kwon et al. 2002).

Generally, a strong constitutive promoter, such as CaMV 35S promoter, is used for expression of foreign genes in plants. The use of an inducible promoter which provides a more precise regulation of expression might be useful for the development of stress-tolerant plants (Yoshida and Shinmyo 2000). We recently isolated a strong oxidative stress-inducible POD (*SWPA2*) promoter from cultured cells of sweetpotato and characterized its function in transgenic tobacco plants and cultured cells in terms of environmental stresses (Kwak et al. 1995; Kim et al. 1999, 2003). We anticipate that the *SWPA2* promoter will be biotechnologically useful for the development of environmental stress-tolerant transgenic plants.

The potato (*Solanum tuberosum* L.) is a major food crop in many parts of the world and ranks fourth in world production after wheat, maize, and rice (Ross 1986). To our knowledge, many efforts have been made to increase the pathogen resistance of potato plants, but few research studies have been conducted to improve its tolerance to environmental stresses using molecular breeding technology (Perl et al. 1993; Jeong et al. 2001). Atlantic potato cultivar is widely grown for making chips and French fries. However, tubers may have internal brown spots or hollow hearts under severe high-temperature conditions.

Previously, we have generated transgenic potato plants expressing both Cu/Zn SOD (GenBank accession no. AF170297; Lee et al. 1999) and APX (GenBank accession no. X62077; Allen et al. 1997) genes in chloroplasts under the control of an oxidative stress-inducible *SWPA2* promoter (Kim et al. 2003), referred to as SSA plants (Tang et al. 2004). When leaf discs of SSA plants were subjected to 10 μM MV, SSA plants showed the reduced visible damage against MV-mediated oxidative stress compared to NT plants at 36 h (Tang et al. 2004). Among 11 SSA plants, SSA9 and SSA11 plants with high tolerance activity and good growth were selected for further study. In this report, we evaluated SSA plants for protection against in terms of oxidative stress induced by MV and high-temperature stress.

## Materials and methods

### Plant materials and growth conditions

Two transgenic potato lines (SSA9 and SSA11) with tolerance to methyl viologen (MV, 10 μM) at the level of leaf discs (Tang et al. 2004) were used as plant materials. Plants were propagated under sterile conditions in Petri dishes containing MS (Murashige and Skoog 1962) basal medium supplemented 100 mg $L^{-1}$ kanamycin. Plants for MV and heat tolerance assays were transferred in pots and grown in a growth chamber under a 16-h photoperiod with light intensity (100 μmol photons $m^{-2}$ $s^{-1}$), 60% (w/v) relative humidity at 25°C.

One chimeric gene construct, named pSSA-K, expressing Cu/Zn SOD (Lee et al. 1999) and APX (Allen et al. 1997) in chloroplasts using an oxidative stress-inducible *SWPA2* promoter (Kim et al. 2003) was used for transformation (Tang et al. 2004). The cDNA insert was fused at the translation initiation codon within the 5′ untranslated sequence of the tobacco etch virus (TEV) that provided highly efficient translational initiation. For chloroplast-targeted expression, the tobacco Cu/Zn SOD transit peptide sequence was translationally fused adjacent to the APX or SOD cDNA in the transgene (Allen et al. 1997). The completed chimeric gene cassette was inserted into the binary shuttle vector, pCAMBIA2300, and mobilized to *Agrobacterium tumefaciens* strain EHA105.

### Methyl viologen (MV) treatment

MV (0, 150, 200, or 250 μM) dissolved in 0.1% (w/v) Tween-20 solution was sprayed on the leaves of plants grown in a greenhouse for 4 weeks using a spray booth (Model SB-6; DeVries Manufacturing, Hollandale, MN, USA). The MV solution (70 mL) was applied to five potato plants using an 8001 VS type nozzle, 1.3 cm $s^{-1}$, 0.22 MPa. The tests for visible damage analysis by MV applications were repeated in triplicate. The percentage of leaf damage that appeared on the leaves after MV spraying was evaluated at 5 days after treatment (0% indicated no damage to the leaves; 100% meant fully damaged leaves). The percentage of the dry weight of non-damaged leaf from the first to fourth leaves of non-transgenic plants (NT) and SSA plants at 5 days after MV spraying was measured on the basis of the dry weight of untreated plants under the same conditions. The chlorophyll contents were determined from discs (1.3 cm in diameter) from central tissue of the fifth leaves of plants at 5 days after MV spraying.

### Reverse transcriptase-polymerase chain reaction (RT-PCR)

For analysis of the transcriptional levels of Cu/Zn SOD and APX genes, the fourth to fifth leaves from the top of both NT and SSA plants sprayed with 150 μM MV were sampled over a time period. All plant materials were immediately frozen in liquid nitrogen and stored at −70°C until further experiments.

Total RNA was extracted from leaves of potato (NT, SSA) plants with cetyltrimethylammonium bromide (CTAB) method (Kim and Hamada 2005). Total RNA samples were treated extensively with RNase-free DNase I to remove any

contaminating genomic DNA. First-strand cDNA was then synthesized using MMLV Reverse Transcriptase (Stratagene, USA, http://www.stratagene.com) from 1 μg of total RNA in a 20 μL reaction volume, and 1 μL of the reaction mixture was subjected to subsequent PCR in a 50 μL reaction volume. Cu/Zn SOD (5′-GTG AAG GCT GAA GCT GTT CTT-3′ and 5′-TCC TCG CAA ACC AAT AAT ACC G-3′), APX (5′-GGA AAA TCT TAC CCA ACT GTT A-3′ and 5′-GGC TTC AGC AAA TCC AAG CTC-3′), and actin (5′-TGG ACT CTG GTG ATG GTG TC-3′ and 5′-CCT CCA ATC CAA ACA CTG TA-3′), were amplified using the indicated primers for 30 cycles according to the following conditions for each cycle: 94°C for 0.5 min, 58°C for 0.5 min, and 72°C for 1 min. This was followed by a final cycle at 72°C for 5 min to allow completion of the polymerizations. Actin mRNA served as an internal control.

### High-temperature treatment

Four-week-old potato plants growing at 25°C growth chamber were transferred to a 42°C growth chamber for 20 h. The treated plants were transferred to normal conditions (25°C, 100 μmol photons $m^{-2}$ $s^{-1}$) for recovery from the stress. The tolerance in SSA plants treated with high-temperature stress for 20 h was estimated as the percentage of initial fresh weight that remains after 42°C treatment.

### Photosynthetic activity

The photosynthetic activity was estimated by chlorophyll fluorescence determination of photochemical yield ($F_v/F_m$), which represented the maximum quantum yield of photosystem II, using a portable chlorophyll fluorescence meter (Handy PEA, Hansatech, England, http://www.hansatech-instruments.co.uk) after 30 min of dark adaptation. Measurements were performed at room temperature (25°C) using saturating light flashes, on the fifth leaves of plants determined at 0, 10, and 20 h after high-temperature treatment.

## Results

### Enhanced tolerance to MV-mediated oxidative stress in whole plants

To investigate oxidative stress tolerance, SSA and NT plants were evaluated for visible damage 5 days after spraying with solutions containing 0, 150, 200, or 250 μM (Fig. 1). NT plants with 150 μM MV showed visible damage on 18% of leaf area, while SSA plants showed much less leaf damage (2–5%). Visible leaf damage on NT plants became more severe with increased concentration. When 200 and 250 μM MV treatment was applied, NT plants showed 53 and 60% damage, respectively, whereas SSA11 plants exhibited only 10 and 17% leaf damage (Fig. 2A). The young leaves of SSA plants remained unaffected (Fig. 1). In addition to visible damage, the effect of MV spray was also investigated at 5 days post-MV treatment by determining the dry weight of non-damaged leaf tissues from the first to fourth leaves. The leaves of SSA plants appeared to have a stronger tolerance against MV-induced oxidative stress than NT plants (Fig. 2B). At 150 and 200 μM MV treatment, no reduction in the dry weight of SSA11 plants was detected, although NT plants showed 26 and 50% reductions in dry weight at 200 and 250 μM MV treatment, respectively. Chlorophyll contents were also evaluated in the central portion of fifth leaves from the plants treated with MV at various concentrations (Fig. 2C). There were no significant differences in chlorophyll content between NT and SSA plants in untreated conditions. The chlorophyll contents of SSA plants were not significantly affected by high MV concentration (250 μM), but these were significantly reduced in

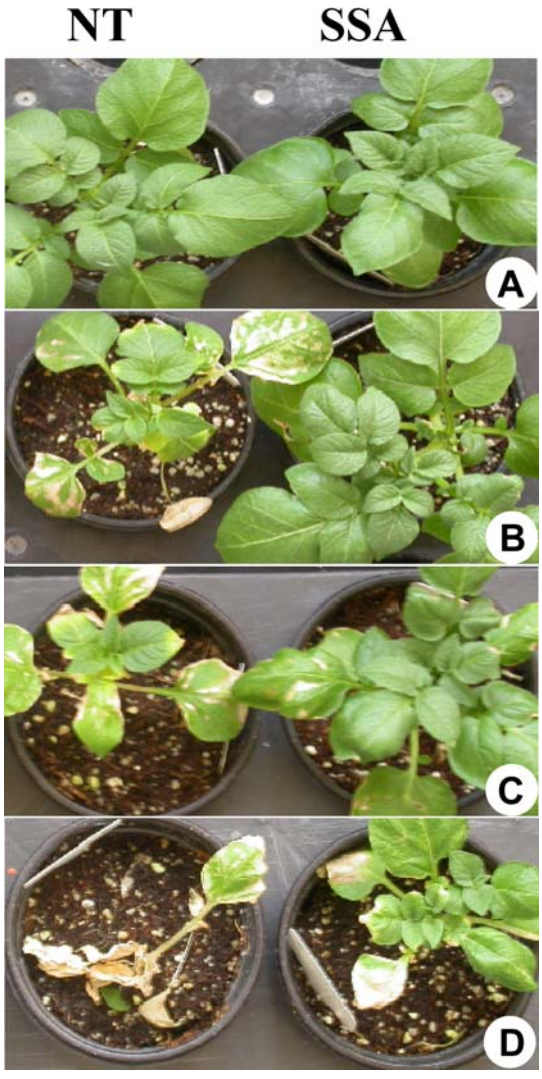


**Fig. 1** Differential visible damages in the leaves of NT and SSA (SSA11) plants at 5 days after treatment with 0 (**A**), 150 (**B**), 200 (**C**), or 250 μM MV (**D**). NT, non-transgenic potato plants; SSA, transgenic potato plants

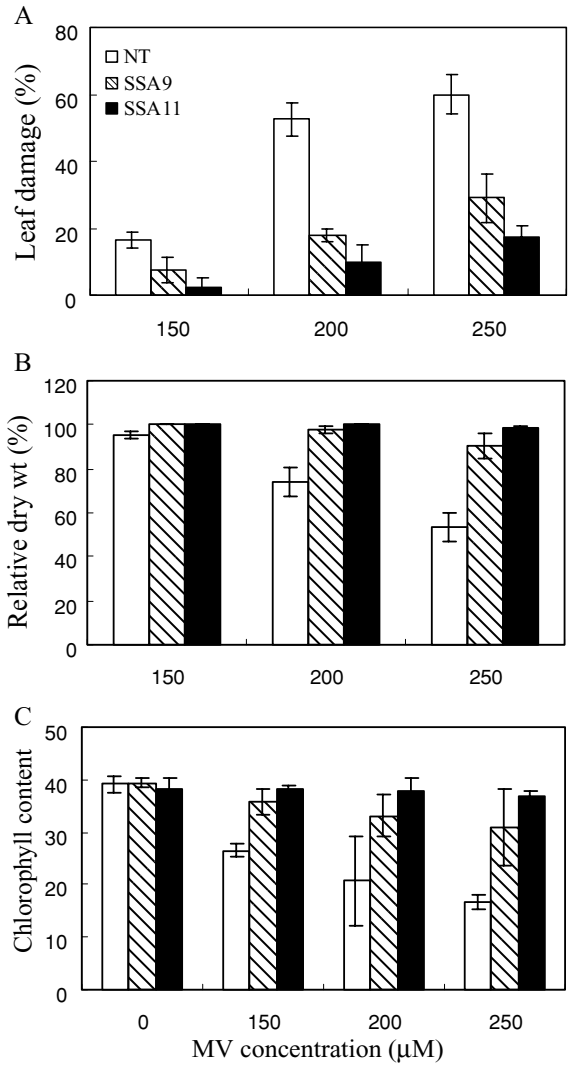


**Fig. 2** Effect of MV on the NT and SSA plants at 5 days after treatment with 0, 150, 200, or 250 μM MV. **A** Quantitative estimate of visible damage that appeared on leaves from NT and SSA plants. **B** Relative dry weight of leaves from non-damaged parts in NT and SSA plants. The percentage of dry weight was calculated on the basis of the content of untreated plants (0 μM MV) under the same light conditions. **C** The chlorophyll contents ($mg \cdot cm^{-2}$) in the fifth leaves. Measurements were made on the central tissue ($d = 1.3$ cm) of the fifth leaves from NT and SSA plants. Data are means ± SE of three independent measurements. NT, non-transgenic potato plants; SSA, transgenic potato plants

NT plants. When NT plants were treated with 150, 200, or 250 μM MV, the chlorophyll contents were significantly reduced to 32, 47, and 57% compared to the untreated NT plants, respectively. However, SSA11 plants showed less chlorophyll content loss following MV treatment, showing 96% chlorophyll content at 250 μM MV treatment.

To understand the tolerance mechanism in SSA plants to MV-mediated oxidative stress, we investigated changes in gene expression of introduced Cu/Zn SOD and APX after 150 μM MV treatment with time course. As shown in Fig. 3, gene expression of Cu/Zn SOD and APX was induced at low level after 12 h of MV treatment and then increased gradually until 72 h, after which a slight decrease was observed. However, transcripts of Cu/Zn SOD and APX genes were not detected in NT and non-treated SSA plants. Interestingly, expression of those genes in SSA11 plants was higher than SSA9 plants. These results indicate that SOD and APX transcript levels in SSA plants well reflected tolerance to MV. In addition, the elevated SOD and APX gene expression in SSA plants in response to MV treatment markedly increased enhanced tolerance to oxidative stress.

### Enhanced tolerance to high temperature in whole plants

When whole plants of SSA and NT were exposed to high temperature at 42°C for 20 h, NT plants were wilted from heat shock after 10 h, whereas SSA plants appeared to remain healthy (Fig. 4A). The fresh weights in both NT and SSA plants treated with heat stress for 20 h were measured on the basis of the control plants grown at 25°C (Fig. 4B). NT plants showed a 32.7% decrease in fresh weight. However, SSA plants remained at similar fresh weights compared with the SSA plants grown at 25°C, showing only a decrease of 1.5% (SSA11).

Using the fluorescence parameter ($F_v/F_m$), the photosynthetic activity was determined in the fifth leaf of each plant (Fig. 4C). The $F_v/F_m$ of NT plants was decreased by 14 and 29% at 10 and 20 h after high-temperature treatment, respectively, while the activity from SSA plants only decreased 4 and 6%. Furthermore, after 3 h of recovery following heat stress, the $F_v/F_m$ of SSA plants almost fully recovered to the initial levels, while that of NT plants remained low (data not shown).

## Discussion

ROS has been implicated in a variety of environmental stresses in plants and the chloroplast appears to be a main location affected by conditions of stress in plant cells. The protection of plants against ROS is achieved by partial suppression of ROS production and scavenging of previously produced ROS. Direct modification of the expression of SOD or APX isoenzyme in chloroplasts has been assigned a key role in the protection of plants against various oxidative stresses (Van Camp et al. 1997; Badawi et al. 2004). However, some studies have found no protection from stress in transgenic plants (Payton et al. 1997). These differences are often attributed to the complexity of the ROS detoxification system, as changing one enzyme may not change the capacity of the pathway as a whole. This suggests that the expression of combinations of antioxidant enzymes in transgenic plants might have synergistic effects on stress tolerance. However, only a few reports have been conducted to address scavenging systems expressing two enzymes in the chloroplasts of higher plants (Aono et al. 1995; Allen et al. 1997; Kwon et al. 2002).

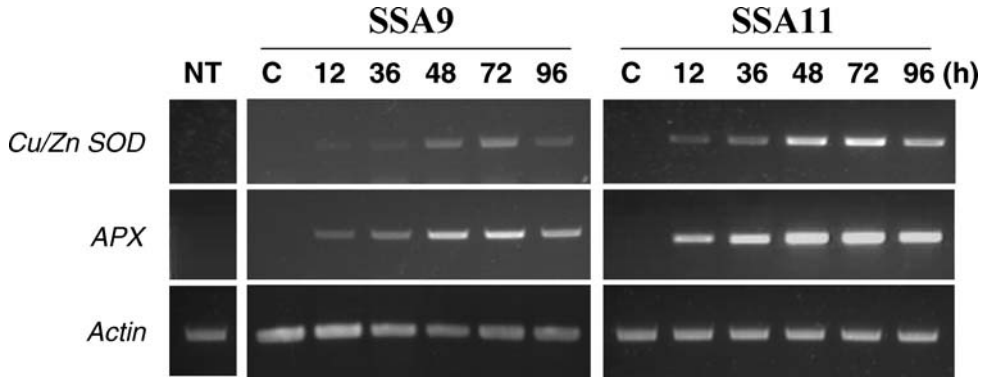


**Fig. 3** RT-PCR analysis of the expression of Cu/Zn SOD and APX genes in leaves from NT and SSA plants subjected to 150 μM MV spray. Total RNA was extracted from leaves 12, 24, 36, 48, 72, and 96 h after treatment with MV. First-strand cDNA synthesis and PCR were performed according to the instructions of the manufacturer. Actin was used to control for equal loading. Reaction products (10 μL) were analyzed by gel electrophoresis. NT, non-transgenic potato plants; SSA, transgenic potato plants; C, non-treated SSA plants

Since the transgene products affect a critical pathway associated with stress tolerance, maintenance of redox potential and cell signaling, a high level of activity of an introduced transgene may cause too much disruption and lead to a negative overall effect. In addition, under stress conditions, ROS may act either as signals to induce protection mechanisms or to accelerate injury (Dat et al. 2000). To allow for these different roles, cellular levels of ROS should be tightly controlled. Thus an oxidative stress-inducible *SWPA2* promoter could be very useful for development of stress-tolerant plants (Kim et al. 2003). Reduced expression of foreign genes might avert losses in phenotypic performance.

The present work describes transgenic potato plants (SSA plants) expressing both Cu/Zn SOD and APX in chloroplasts under the control of *SWPA2* promoter, and their enhanced tolerance to oxidative stress induced by MV and high temperature on the level of whole plants. Visible damage of SSA plants was one-fifth that of NT plants at 5 days after spraying at various concentrations of MV (Figs. 1 and 2). Chlorophyll contents in NT plants following MV spray were dramatically decreased, but SSA plants retained their initial chlorophyll levels. Therefore, we predicted that SSA plants expressing transgenes for both SOD and APX could show synergistic effects which lead to increased stress tolerance. These results indicate that SSA plants expressing both SOD and APX are able to rapidly scavenge superoxide anion radicals and hydrogen peroxide at the site of generation, as well as prevent the formation of hydroxyl radicals, the most toxic ROS, prior to their interaction with target molecules. Similar results were observed in a few reports. Following MV treatment, transgenic tobacco plants expressing both glutathione reductase (GR) and Cu/Zn SOD in cytosol exhibited less damage than plants expressing individual GR or Cu/Zn SOD transgenes (Aono et al. 1995).

Transgenic plants with elevated levels of chloroplast-targeted antioxidant genes such as SOD, APX, or GR have exhibited increased protection of oxidative stress (Sen Gupta et al. 1993; Allen et al. 1997; Payton et al. 1997, 2001). In the SSA plants of this study, it seems that the transcript levels of foreign Cu/Zn SOD and APX genes were parallel to that of the tolerance to MV-induced oxidative stress (Fig. 3). Elevated expression of foreign genes in SSA plants could protect these plants from oxidative toxicity. It is expected that Cu/Zn SOD and APX proteins in SSA plants were also correctly targeted into chloroplast because the sequence for transit peptide were same as that of chimeric APX constructs (Allen et al. 1997). It is likely that the sequential reactions of Cu/Zn SOD and APX in chloroplasts of SSA plants may play an important role in enhanced tolerance to MV-induced oxidative stress.

Next, we checked the effects of the SSA plants on the tolerance to oxidative stress caused by high-temperature stress. It was known that temperature fluctuations could induce the formation of ROS. Potato plants, specially, cv. Atlantic is sensitive to high temperature. NT plants were wilted after 10 h of heat shock at 42°C, whereas SSA plants appeared to remain healthy (Fig. 4). Fluorescence measurements ($F_v/F_m$) showed that photosynthesis of SSA was transiently reduced during treatment at 42°C, while in NT plants the reduction was higher. There was increasing evidence for considerable correlation between the responses to heat stress and oxidative stress. There was also evidence of a relationship between heat-induced oxidative stress and expression of antioxidant enzymes in plants (Gong et al. 1998; Storozhenko et al. 1998). Cu/Zn SOD was strongly induced by heat shock in cassava plants (Lee et al. 1999) and even during the recovery period after heat stress at 37°C in tobacco plants (Tsang et al. 1991). In transgenic *Arabidopsis* plants overexpressing heat shock transcription factor 3, APX activity was positively affected following heat stress (Panchuk et al. 2002). This enzyme was present in additional to thermolabile cytosolic APX1, the prevalent isoform in unstressed cells. Here, our result demonstrated that transgenic SSA plants expressing both SOD and APX could lead to increased thermotolerance, confirming that the roles of SOD and APX were related to high-temperature stress.

In conclusion, we successfully developed SSA transgenic potato plants expressing both SOD and APX in chloroplasts

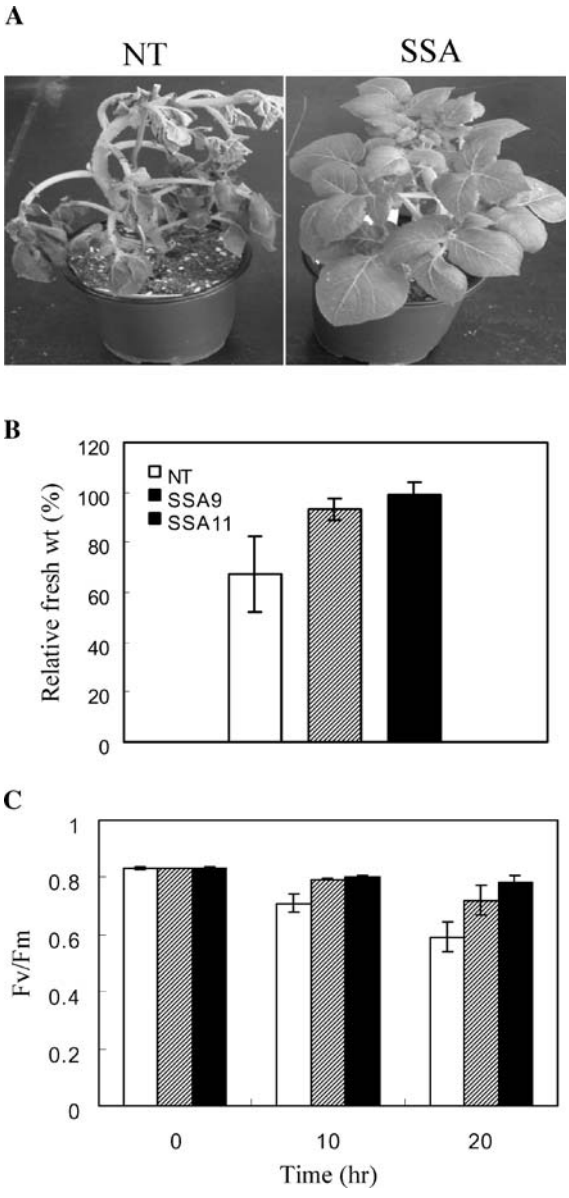


**Fig. 4** Effects of high temperature (42°C) on NT and SSA plants. **A** Visible differential damages in the leaves of NT and SSA (SSA11) plants at 20 h after treatment. **B** Fresh weight of plants at 20 h after treatment. **C** Photosynthetic activity ($F_v/F_m$) in the leaves of NT and SSA plants for 20 h after treatment. The percentage of fresh weight was calculated on the basis of untreated plants grown at 25°C. Data are means ± SE of three independent measurements. NT, non-transgenic potato plants; SSA, transgenic potato plants

under the control of an oxidative stress-inducible *SWPA2* promoter. SSA potato plants exhibited a strong tolerance to MV-mediated oxidative stress and high temperature. Further characterization of SSA potato plants is under investigation in terms of multiple stresses including drought and salinity. The function of SOD and APX genes and the field tests of these transgenic plants for the purposes of agricultural cultivation remain to be conducted. To develop transgenic potato plants with more enhanced tolerance to multiple environmental stress, SSA plants will be further transformed with other stress-tolerant genes, such as nucleoside diphosphate kinase 2 (Moon et al. 2003) or peroredoxin (Jang et al. 2004), in cytosol in a gene-stacking manner. In addition, SSA potato plants in this study could be useful breeding materials for production of valuable components in the tuber.

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## References

Allen RD, Webb RP, Schake SA (1997) Use of transgenic plants to study antioxidant defenses. Free Radic Biol Med 23:473–479

Aono M, Saji H, Sakamoto A, Tanaka K, Kondo N (1995) Paraquat tolerance of transgenic *Nicotiana tabacum* with enhanced activities of glutathione reductase and superoxide dismutase. Plant Cell Physiol 36:1687–1691

Asada K (1999) The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annu Rev Plant Physiol Plant Mol Biol 50:601–639

Badawi GH, Kawano N, Yamauchi Y, Shimada E, Sasaki R, Kubo A, Tanaka K (2004) Over-expression of ascorbate peroxidase in tobacco chloroplasts enhances the tolerance to salt stress and water deficit. Physiol Plant 121:231–238

Dat J, Vandenabeele S, Vranova E, Van Montagu M, Inze D, Van Breusegem F (2000) Dual action of the active oxygen species during plant stress responses. Cell Mol Life Sci 57:779–795

Foyer CH, Descourvierse P, Kunert KJ (1994) Protection against oxygen radicals: an important defense mechanism studied in transgenic plants. Plant Cell Environ 17:507–523

Gong M, Li Y, Chen S (1998) Abscisic acid-induced thermotolerance in maize seedling is mediated by calcium and associated with antioxidant systems. J Plant Physiol 153:488–496

Jang HH, Lee KO, Chi YH, Jung BG, Park JH, Lee JR, Moon JC, Yun JW, Choi YO, Kim WY, Kang JS, Cheong GW, Yun DJ, Rhee SG, Cho MJ, Lee SY (2004) Two enzymes in one; two yeast peroxiredoxins display oxidative stress-dependent switching from a peroxidase to a molecules chaperone function. Cell 28:625–635

Jeong MJ, Park SC, Byun MO (2001) Improvement of salt tolerance in transgenic potato plants by glyceraldehydes-3 phosphate dehydrogenase gene transfer. Mol Cells 12:185–189

Kim KY, Hur KH, Lee HS, Kwon SY, Hur Y, Kwak SS (1999) Molecular characterization of two anionic peroxidase cDNAs isolated from suspension cultures of sweetpotato. Mol Gen Genet 261:941–947

Kim KY, Kwon SY, Lee HS, Hur Y, Bang JW, Kwak SS (2003) A novel oxidative stress-inducible peroxidase promoter from sweetpotato: molecular cloning and characterization in transgenic tobacco plants and cultured cells. Plant Mol Biol 51:831–838

Kim SH, Hamada T (2005) Rapid and reliable method of extracting DNA and RNA from sweetpotato, *Ipomoea batatas* (L.). Lam Biotech Lett 27:1841–1845

Kwak SS, Kim SK, Lee MS, Jung KH, Park IH, Liu JR (1995) Three acidic peroxidases from suspension-cultures of sweetpotato. Phytochemistry 39:981–984

Kwon SY, Jeong YZ, Lee HS, Kim JS, Cho KY, Allen RD, Kwak SS (2002) Enhanced tolerance 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

Lee HS, Kim KY, You SH, Kwon SY, Kwak SS (1999) Molecular characterization and expression of a cDNA encoding copper/zinc superoxide dismutase from cultured cells of cassava (*Manihot esculenta* Crantz). Mol Gen Genet 262:807–814

Moon H, Lee B, Choi G, Shin D, Prasad DT, Lee OS, Kwak SS, Kim DH, Nam JS, Bark J, Hong JC, Lee SY, Cho MJ, Lim CO, Yun DJ (2003) NDP kinase 2 interacts with two oxidative stress-activated MAPKs to regulate cellular redox state and enhances multiple stress tolerance in transgenic plants. Proc Natl Acad Sci USA 100:358–363

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 15:473–497

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

Panchuk, II, Volkov RA, Schoffl F (2002) Heat stress- and heat shock transcription factor-dependent expression and activity of ascorbate peroxidase in Arabidopsis. Plant Physiol 129:838–853

Payton P, Allen R, Trolinder N, Holaday A (1997) Over-expression of chloroplast-targeted Mn Superoxide dismutase in cotton does not alter the reduction of photosythesis after short exposures to low temperature and high light intensity. Photosynth Res 52:233–244

Payton P, Webb R, Kornyteyev D, Allen RD, Holaday AS (2001) Protecting cotton photosynthesis during moderate chilling at high light intensity by increasing chloroplastic antoxidant enzyme activity. J Exp Bot 52:2345–2354

Perl A, Perl-Treves R, Dalili S (1993) Enhanced oxidative stress defence in transgenic potato expressing Cu/Zn superoxide dismutase. Theor Appl Genet 85:568–576

Ross H (1986) Potato breeding: problems and perspectives. J Plant Breed Suppl 13. Adv Plant Breed. Parey, Berlin and Hamburg, 132 pp

Sen Gupta A, Heinen JL, Holaday AS, Burke JJ, Allen RD (1993) Increased resistance in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase. Proc Natl Acad Sci USA 90:1629–1633

Storozhenko S, De Pauw P, Van Montagu M, Inze D, Kushnir S (1998) The heat-shock element is a functional component of the Arabidopsis APX1 gene promoter. Plant Physiol 118:1005–1014

Tang L, Kwon SY, Kwak SS, Sung CK, Lee HS (2004) Selection of transgenic potato plants expressing both CuZnSOD and APX in chloroplasts with enhanced tolerance to oxidative stress. Korean J Plant Biotechnol 31:109–113

Tsang EW, Bowler C, Herouart D, Van Camp W, Villarroel R, Genetello C, Van Montagu M, Inze D (1991) Differential regulation of superoxide dismutases in plants exposed to environmental stress. Plant Cell 3:783–792

Van Camp W, Inze D, Van Montagu M (1997) The regulation and function of tobacco superoxide dismutases. Free Radic Biol Med 23:515–520

Yoshida K, Shinmyo A (2000) Transgene expression systems in plants, a natural bioreactor. J Biosci Bioeng 90:353–362