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Expression of *Arabidopsis NDPK2* increases antioxidant enzyme activities and enhances tolerance to multiple environmental stresses in transgenic sweetpotato plants

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Abstract Transgenic sweetpotato (*Ipomoea batatas* L. cv. Yulmi) plants expressing the *Arabidopsis* nucleoside diphosphate kinase 2 (*AtNDPK2*) gene under the control of an oxidative stress–inducible peroxidase (*SWPA2*) promoter (referred to as SN plants) were developed and evaluated for enhanced tolerance of SN plants under various abiotic stress conditions. The level of *AtNDPK2* expression and NDPK activity in SN plants following methyl viologen (MV) treatment was positively correlated with the plant's tolerance to MV. Interestingly, we observed that antioxidant enzyme activities such as peroxidase, ascorbate peroxidase, and catalase increased in MV-treated SN plants. In addition, SN plants showed enhanced tolerance to cold, high salinity, and drought stresses by an increase in the activity of H_2O_2 scavenging enzymes. These results indicate that overexpression of *AtNDPK2* in sweetpotato might efficiently modulate oxidative stress from various environmental stresses.

Keywords H_2O_2 scavenging enzyme · Nucleoside diphosphate kinase 2 · Oxidative stress · Peroxidase promoter · Sweetpotato

Abbreviations

APX	Ascorbate peroxidase
CAT	Catalase
Chl	Chlorophyll
DAB	3,3-Diaminobenzidine
MV	Methyl viologen
NDPK	Nucleoside diphosphate kinase
NT	Non-transgenic
POD	Peroxidase
ROS	Reactive oxygen species
RWC	Relative water content

Introduction

Environmental stress conditions, such as drought, high salinity, and extreme temperatures, are major

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factors affecting plant growth and crop productivity (Boyer 1982). These environmental stressors cause oxidative stress by rapid and excessive accumulation of reactive oxygen species (ROS) in plant cells (Foyer et al. 1994; Asada 1999). A large number of defense genes with a variety of functions are induced by environmental stresses (Mahajan and Tuteja 2005). Many attempts have been made to confer tolerance to stresses to plants of agronomic interest. One of the most widely used strategies is to overexpress plant genes that are induced after stress treatments (Bartels and Sunkar 2005; Umezawa et al. 2006). Many different genes have been overexpressed in plants to improve their tolerance to environmental stresses. However, the engineering of an individual stress response gene has not been effective, because many kinds of stress responses are necessary for plants to survive under various conditions. Thus, a strategy for engineering an upstream key regulatory gene of stress signal transduction pathways is needed. For example, a protein kinase can control the expression of many target genes and transcription factors by activating signal transduction cascades (Tena et al. 2001; Moon et al. 2003; Kobayashi et al. 2004).

Nucleoside diphosphate kinases (NDPKs, EC 2.7.4.6) are ubiquitous housekeeping enzymes that maintain intracellular levels of all nucleoside triphosphates (NTP), with the exception of adenosine triphosphate (ATP). In addition, NDPK is involved in the phytochrome A response (Choi et al. 1999), UV-B signaling (Zimmermann et al. 1999), auxin responses (Escobar Galvis et al. 2001), and oxidative stress signaling (Moon et al. 2003). In previous studies, *AtNDPK2*-overexpressing transgenic plants not only possessed significantly enhanced tolerance to multiple environmental stresses, but also induced numerous genes involved in cellular signal transduction and protection (Moon et al. 2003; Yang et al. 2003).

Sweetpotato [*Ipomoea batatas* (L.) Lam.] ranks seventh in annual production among food crops worldwide. It is not only a good source of energy with its high sugar and carbohydrate content, but also a good source of nutrients, including calcium, iron, and other minerals, as well as antioxidants such as phenolic acids, anthocyanins, and β-carotene (Yoshinaga et al. 1999; Teow et al. 2007). Sweetpotatoes are also used in processed foods and for starch and alcohol production. Moreover, sweetpotato does not require large amounts of fertilizers or other agricultural chemicals and is rather tolerant to environmental stresses. However, conventional breeding programs based on the hybridization of sweetpotato are limited by its sterility and cross-incompatibility (Prakash 1994). To overcome such limitations, we have established regeneration and transformation systems in several cultivars of sweetpotato via somatic embryogenesis (Kwon et al. 2002; Lim et al. 2004).

We previously isolated the oxidative stress–inducible *SWPA2* promoter from cell cultures of sweetpotato and characterized its function in transgenic tobacco plants in terms of environmental stresses, including oxidative stress (Kim et al. 2003). In this study, we show that transgenic expression of *AtNDPK2* in sweetpotato plants under the control of the *SWPA2* promoter confers enhanced tolerance to multiple environmental stresses.

Materials and methods

Plant materials and vector construction

Embryogenic calli were induced from shoot meristems of sweetpotato [*Ipomoea batatas* (L.) Lam. cv. Yulmi] cultured on MS (Murashige and Skoog 1962) medium supplemented with 1 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), 3% sucrose, and 0.4% gelrite. The embryogenic calli were maintained at 25°C in the dark and proliferated by subculture at 4-week intervals in fresh medium (Kwon et al. 2002).

The *AtNDPK2* gene construct was constructed using an oxidative-inducible *SWPA2* promoter and a CaMV *35S* terminator sequence in the pCAMBIA2300 plant expression vector. The full-length cDNA of *AtNDPK2* (accession no. AF017640) was kindly provided by Prof. D. J. Yun (Choi et al. 1999; Moon et al. 2003). The *AtNDPK2* cDNA insert was fused to the 5′-untranslated sequence of the tobacco etch virus (TEV) at the translation initiation codon, which provides highly efficient translational initiation. This cDNA was obtained by PCR using the forward primer with *Nco*I site, 5′-CACCATGGT GGGAGCGACT-3′ and reverse primer with *Xba*I site, 5′-TCTGTCTAGACAAGGATCA-3′. To generate *SWPA2* promoter-*AtNDPK2* vector, the *AtNDPK2*

cDNA was ligated into the corresponding site of pRTL2 vector (*SWPA2p*::*AtNDPK2*/pRTL2). These completed chimeric gene cassettes were inserted into *Hind*III site of the binary vector pCAMBIA2300.

Transformation by particle bombardment

Sweetpotato transformation using particle bombardment was conducted with the PDS-1000/Hg particle delivery system (Bio-Rad, Hercules, CA, USA) as described by Lim et al. (2007). Three days after bombardment, embryogenic calli were transferred to MS selection medium containing 1 mg l^{-1} 2,4-D, 100 mg l^{-1} kanamycin, 3% sucrose, and 0.4% gelrite and subcultured at 3-week intervals on freshly made medium. The calli were transferred to somatic embryo formation medium (MS medium containing 100 mg l^{-1} kanamycin, 3% sucrose, and 0.4% gelrite). Somatic embryos formed from kanamycin-resistant calli were used for regenerating plants by transferring to MS medium containing 100 mg l^{-1} kanamycin, 3% sucrose, and 0.4% gelrite. Regenerated plants were cultured on the same medium and maintained at 25°C under a 16/8 h (light/dark) photoperiod with light supplied at an intensity of 70 μmol m^{-2} s^{-1} using fluorescent lights. The plantlets were then transplanted into pots and grown in the greenhouse.

Southern-blot analysis

For Southern hybridization, genomic DNA of sweetpotato was extracted from leaves according to Kim and Hamada (2005), digested with *Eco*RI (Roche, Manheim, Germany), electrophoresed on 0.8% agarose gel and blotted onto Zeta-probe GT membrane (Bio-Rad, CA, USA). The blots were hybridized to a ^{32}P-labeled probe from the full-length *AtNDPK2* cDNA. Hybridization was carried out in 0.5 M sodium phosphate (pH 7.2), 7% SDS and 1 mM EDTA at 65°C.

RT-PCR analysis

Total RNA was isolated from leaves of sweetpotato using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and treated extensively with RNase-free DNase I in order to remove any contaminating genomic DNA. RT-PCR amplification was conducted using an RT-PCR kit (Promega, Madison, WI, USA) in accordance with the manufacturer's instructions. Total RNA (1 μg) was used for the generation of first-strand cDNA using the MMLV reverse transcriptase. Gene-specific primers used for PCR were as follows: The *AtNDPK2* primer set (5′- GTTGGCC GCATTTCGTCCTCA-3′, 5′- CCACTTGCATAGC TCGCCCTC-3′) was used to amplify a 414 bp product from cDNA coding for *AtNDPK2*. The total synthesized cDNA was also used to amplify *tubulin* as an internal standard using tubulin gene-specific primers (5′-CAACTACCAGCCACCAACTGT-3′, 5′-CAAGATCCTCACGAGCTTCAC-3′).

Enzyme activity assays

For analysis of the NDPK, ascorbate peroxidase (APX), peroxidase (POD), and catalase (CAT) activities, total soluble protein was extracted from the leaves of sweetpotato plants using an extraction buffer, and protein concentrations were determined using the Bio-Rad protein assay (Bradford 1976). The NDPK activity was measured using the coupled reaction method with lactate dehydrogenase and pyruvate kinase (Yano et al. 1995; Tang et al. 2008). NDPK activity was calculated based on the loss of absorbance at 340 nm following the decrease in NADH. One unit of enzyme activity was defined as 1 μmol of ADP production per minute. The POD activity was assayed according to the method described by Kwak et al. (1995) using pyrogallol as a substrate. One unit of POD activity was defined as the amount of enzyme required to form 1 mg of purpurogallin from pyrogallol in 20 s, as measured by absorbance at 420 nm. The APX activity was assayed according to the method by Nakano and Asada (1981) using ascorbic acid as a substrate. The oxidation of ascorbate was initiated by H_2O_2, and the decrease in absorbance at 290 nm was monitored for 1 min 30 s. One unit of APX was defined as the amount of enzyme oxidizing 1 μmol of ascorbate per minute. The CAT activity was assayed according to the method described by Aebi (1984). The activity was determined by the decrease in absorbance at 240 nm for 1 min due to H_2O_2 consumption.

Stress treatment

Methyl viologen damage was analyzed using leaf discs from sweetpotato plants. Seven leaf discs

(16 mm diameter) collected from the third leaves of plants were floated on a solution containing 0.4% (w/v) sorbitol and 5 μM MV, placed in the dark for 12 h to allow diffusion of the MV into the leaf discs, and then subjected to continuous light (150 μmol m^{-2} s^{-1}) at 25°C. For cold treatment of whole plant levels, sweetpotato plants were treated in a plant growth chamber maintained at 4°C for 18 h. Following this 18 h stress treatment, the plants were transferred back to normal culture conditions (25°C) for recovery from the stress. For salt treatment, 2-month-old non-transgenic (NT) and transgenic plants were irrigated with a 200 mM NaCl solution for 14 days and then with water for 4 days in a plant growth chamber at 25°C [60% relative humidity, 16/8-h (light/dark) photoperiod with light supplied at an intensity of 150 μmol m^{-2} s^{-1}]. For dehydration treatment of whole plants, 2-month-old NT and transgenic plants were left unwatered for 12 days and then watered for 6 days.

Ion leakage analysis using leaf discs

Ion leakage was analyzed according to the method by Bowler et al. (1991) with slight modifications. The loss of cytoplasmic solutes following the MV treatment, based on the electrical conductance of the solution, was measured with an ion conductivity meter (model 455C, Istek Co, Seoul, Korea) and compared with the total conductivity of the solution following tissue destruction. The extent of cellular damage was quantified by ion leakage, which is a measure of membrane disruption.

Determination of PSII photosynthetic efficiency and total chlorophyll content

PSII photosynthetic efficiency in leaves was estimated by chlorophyll (Chl) fluorescence determination of photochemical yield (*Fv*/*Fm*), which represents the maximal yield of the photochemical reaction in PSII, using a portable Chl fluorescence meter (Handy PEA, Hansatech, England). Measurement of the Chl content was taken with 0.1 g fresh weight of leaf material that was quick frozen in liquid nitrogen and then extracted with 2 ml methanol. The sample was centrifuged at 12,000*g* for 15 min at 4°C and the Chl content of the supernatant was analyzed using a spectrophotometer according to the methods by Porra et al. (1989).

Qualitative and quantitative analysis of H_2O_2

In order to visualize the H_2O_2, sweetpotato leaves were placed in 1 mg ml^{-1} of 3,3-diaminobenzidine (DAB) solution. The samples were then incubated for 8 h in a growth chamber, and the Chl was cleared at 80°C for 2 h in 80% ethanol (Thordal-Christensen et al. 1997). The H_2O_2 content was assessed with xylenol orange, in which H_2O_2 is reduced by ferrous ions in an acidic solution that forms a ferric product–xylenol orange complex, which is detected by absorption at 560 nm (Bindschedler et al. 2001). H_2O_2 measurements are expressed as relative values.

Analysis of relative water content

The degree of drought stress was assessed by the relative water content (RWC) of leaves after 14 days of withholding water. The RWC was estimated as described by Ma et al. (2006). RWC was measured in the third or fourth leaves from the top of the plant.

Statistical analysis

Data were statistically analyzed with Statistical Package for the Social Sciences (SPSS 12). Means were separated using Duncan's multiple range test at $P = 0.05$.

Results

Molecular and biochemical characterization of *AtNDPK2*-overexpressing transgenic sweetpotato under MV-mediated oxidative stress

Transgenic sweetpotato plants that expressed *AtNDPK2* under the control of an oxidative stress–inducible *SWPA2* promoter (SN plants) were successfully generated by particle bombardment (Fig. 1a). Four independent transgenic lines were established. The integration and gene copy number of the construct in the transformed plants were determined by PCR analysis with *AtNDPK2* and *NPTII* gene-specific primers (data not shown), then analyzed further via Southern-blot analysis. In a genomic DNA

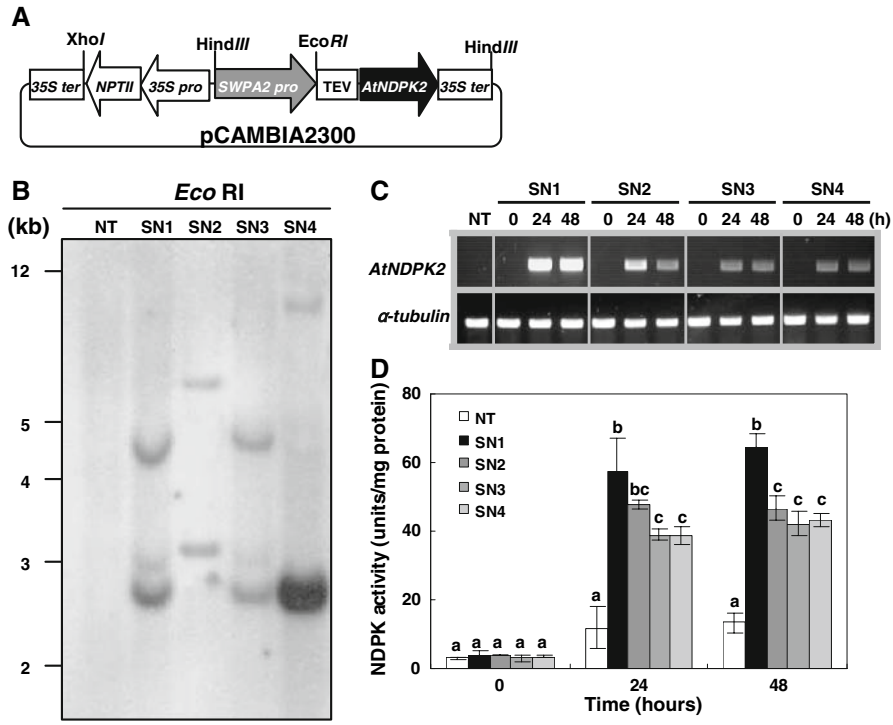

Fig. 1 Molecular and biochemical characterization of *NDPK2* overexpressing transgenic sweetpotato plants (SN plants) under MV-mediated oxidative stress. **a** Diagram of the oxidative stress–inducible *SWPA2* promoter::*AtNDPK2* construct. **b** Southern blot-analysis of transgenic lines. The integration and gene copy number of the construct in the transformed plants were confirmed by *AtNDPK2* gene with *Eco* RI digestion. **c** RT-PCR analysis of SN plants under MV treatment. **d** Specific NDPK activity in SN plants. Data presented are the average of three replicates. *Bars* carrying the *same letter* are not significantly different ($P = 0.05$) according to Duncan's multiple range test

hybridization analysis, over two bands hybridizing with an *AtNDPK2*-specific probe were detected in transgenic plants, and none were detected in non-transgenic (NT) plants (Fig. 1b). This result indicates the presence of multiple copies of the T-DNA insertion in the genomes of the *AtNDPK2* transgenic lines. Four SN transgenic lines were grown in a growth chamber for 6 weeks and utilized to evaluate tolerance against MV-mediated oxidative stress using leaf discs. MV is a typical ROS-generating redox active compound, which has been used as a non-selective herbicide (Babbs et al. 1989). To investigate the level of transgene expression in the SN plants, an RT-PCR analysis was conducted using RNA from MV-treated plant leaves and a *AtNDPK2* gene-specific primer set (Fig. 1c). After induction of MV-stress, transcript expression of *AtNDPK2* was detected in the leaves of all transgenic lines, but not in the NT line. Particularly prominent induction of the *AtNDPK2* expression was detected in the SN1 plants after MV treatment. To further ascertain whether *AtNDPK2* expression is correlated with NDPK enzyme activity, we measured the NDPK activity in soluble extracts from leaves of the SN transgenic lines (Fig. 1d). SN1 plants exhibited 4.8-fold higher NDPK activity than the NT plants.

All the SN plants exhibited reduced symptoms of damage from MV-treatment compared with the NT plants as determined by ion leakage analysis from leaf discs (Fig. 2). The SN1 line exhibited the highest level of *AtNDPK2* expression, consistent with the highest tolerance to MV stress.

NDPK2 regulates the expression of various anti-oxidant genes under stress conditions (Yang et al. 2003). Therefore, to understand the mechanism of the enhanced tolerance of SN plants to MV-mediated oxidative stress, we investigated changes in the activities of H_2O_2-scavenging enzymes, such as POD, APX, and CAT. SN1 plants exhibited approximately 2.3-fold higher POD activity than NT plants (Fig. 3a), and the APX and CAT activities were approximately 1.6-fold higher than NT plants after

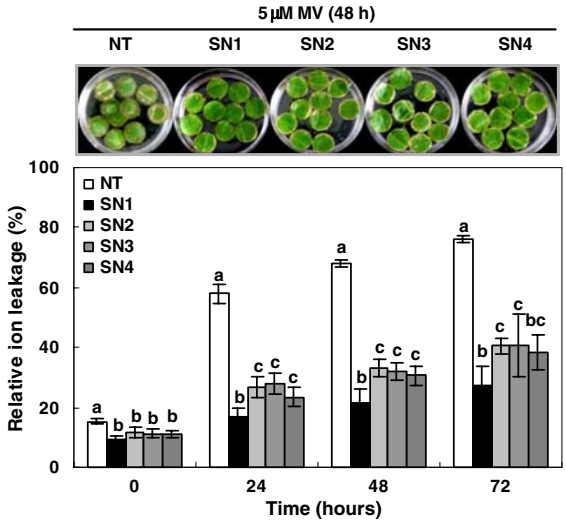

Fig. 2 Ion leakage analysis of independent SN transgenic lines in response to 5 μM MV treatment. The electrical conductivity of the MV solution was compared with the total conductivity of the solution following tissue destruction. Data presented are the average of three replicates. *Bars* carrying the *same letter* are not significantly different ($P = 0.05$) according to Duncan's multiple range test

MV treatment (Fig. 3b, c). Our results suggest that activation of antioxidant enzymes by *AtNDPK2* expression plays an important role in tolerance of SN plants to oxidative stress.

Enhanced tolerance of SN transgenic plants to cold stress is correlated with an increase in the activity of H_2O_2 scavenging enzymes

To assess the effects of *AtNDPK2* expression on cold stress tolerance in soil-grown whole plants, 2-months-old plants were incubated for 18 h at 4°C and then transferred to 25°C. When NT and SN whole plants were exposed to cold for 18 h, the leaves of the NT plants showed severe wilting and curling, whereas those of the SN plants appeared to be only slightly damaged (Fig. 4a). We also determined the photosynthetic activity (Fig. 4a); after 18 h of cold treatment, the photosynthetic efficiency (Fv/Fm) in NT plants was decreased by 39%, whereas that of the SN1 and SN2 plants was only decreased by 8 or 11%, respectively. Furthermore, the photosynthetic efficiency in SN1 plants after a 6 h recovery was almost fully recovered to the initial levels, whereas NT plants exhibited a continued decrease in Fv/Fm. In addition,

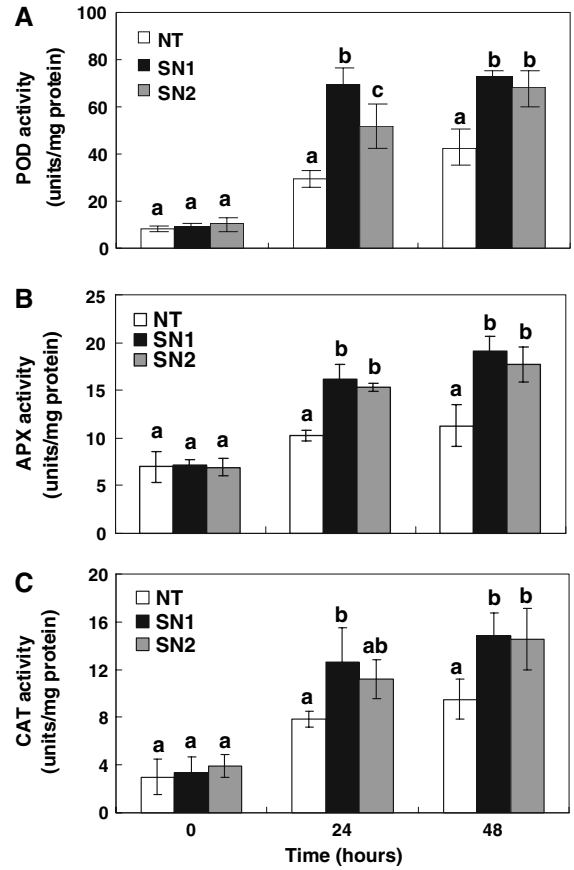

Fig. 3 Changes in the activities of H_2O_2-scavenging enzymes in SN plants after MV treatment. Changes in POD (**a**), APX (**b**), and CAT (**c**) activities in SN plants after MV treatment. Data presented are the average of three replicates. *Bars* carrying the *same letter* are not significantly different ($P = 0.05$) according to Duncan's multiple range test

the SN1 and SN2 plants showed lower levels of H_2O_2, 54 or 48% lower respectively, at 18 h than NT plants exposed to cold stress (Fig. 4b). Cold stress increases H_2O_2 levels in plants and induces expression of various antioxidant enzymes to overcome the oxidative stress (Dat et al. 2000). Direct evidence for accumulation of H_2O_2 during cold treatment has been reported in several plant species (Okuda et al. 1991; Prasad et al. 1994; Fadzillah et al. 1996; O'Kane et al. 1996; Kingston-Smith et al. 1999). Under cold stress, the transcript level of *AtNDPK2* increased in the leaves of the two SN lines, but not in NT plants (Fig. 4c). These results suggest that expression of the *AtNDPK2* gene under cold stress reduced the accumulation of H_2O_2 in SN plants, suggesting that H_2O_2-scavengeing enzymes are regulated by *AtNDPK2*.

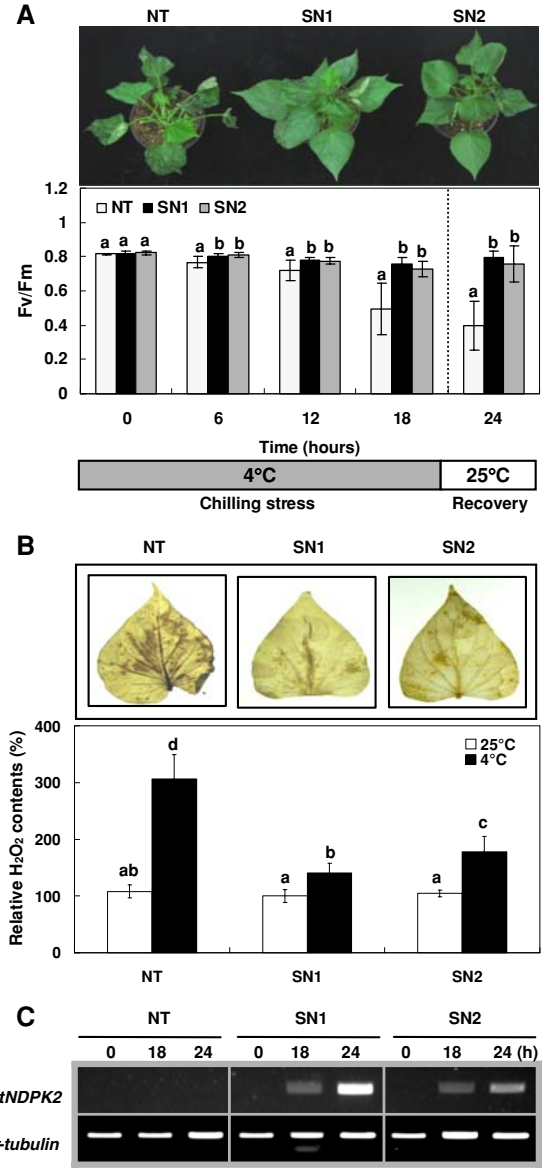

Fig. 4 Effect of cold stress (4°C) on SN plants. **a** Visible damage and PSII photosynthetic efficiency (Fv/Fm) in the leaves of sweetpotato plants after an 18 h cold treatment and 24 h recovery at 25°C. Data presented are the average of three replicates from each of five plants. **b** Qualitative and quantitative analysis of H_2O_2 levels in SN plants after an 18 h cold treatment. **c** RT-PCR analysis of cold-treated SN plants. *Bars* carrying the *same letter* are not significantly different ($P = 0.05$) according to Duncan's multiple range test

We further investigated whether expression of *AtNDPK2* controls the activation of antioxidant enzymes. SN1 plants exhibited 3.7-fold higher NDPK enzyme activity than NT plants at 24 h after initiation of the cold treatment (Fig 5a). In addition, SN1 plants exhibited approximately 4.6-fold higher POD activity than NT plants (Fig. 5b), whereas the APX and CAT activities were approximately 2-fold higher than NT plants at 24 h after cold treatment (Fig. 5c, d). Therefore, we suggest that SN plants exhibited enhanced tolerance to oxidative stress resulting from cold stress through *AtNDPK2*-mediated activation of antioxidant enzymes including POD, APX, and CAT.

Enhanced tolerance of SN plants to high salinity and drought stress

We determined whether SN plants show increased tolerance to various osmotic stresses such as high salt and drought. SN lines recovered significantly better than NT plants with regard to photosynthetic efficiency after 14 days under high salt conditions (200 mM NaCl) (Fig. 6a). The total Chl content of the SN1 and SN2 plants was greater than NT plants by 16 or 11%, respectively, after the 14-day salt stress and 2-day recovery (day 16, Fig. 6c). In addition, leaf discs from SN transgenic lines exhibited enhanced tolerance to the presence of 300 mM NaCl, as judged by assessment of Fv/Fm (data not shown). Similarly, to assess the effects of drought stress tolerance in SN plants, soil-grown sweetpotato plants were not watered for 12 days and then watered for 6 days for recovery. More bleaching and a greater loss of PSII photosynthetic efficiency was observed in NT plants than in SN plants (Fig. 6b). SN plants exhibited higher levels of RWC than NT plants. The RWC of the SN plants was 28% higher than NT plants after 12 days of drought treatment (Fig. 6d). Based on the above results, we suggest that SN plants effectively expressed *AtNDPK2* under multiple stress conditions, including cold, high salinity, and drought stresses. Thus, expression of *AtNDPK2* conferred enhanced tolerance to multiple stresses.

Discussion

We successfully developed transgenic sweetpotato plants expressing *AtNDPK2* under the control of the oxidative stress–inducible *SWPA2* POD promoter. Expression of the *AtNDPK2* gene caused activation of antioxidant enzymes in sweetpotato, thereby conferring increased tolerance to multiple environmental

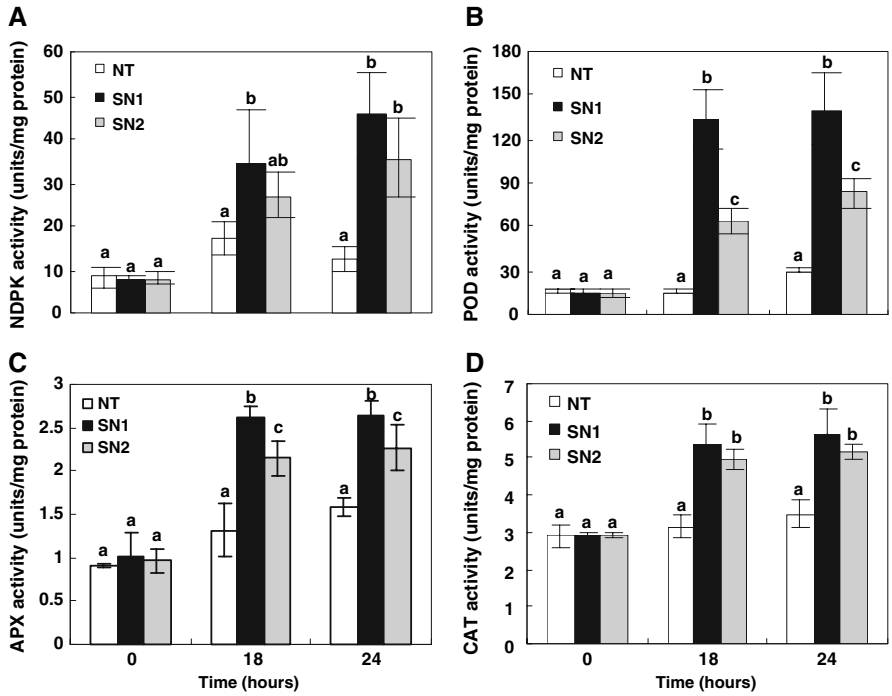

Fig. 5 NDPK, POD, APX, and CAT activity in SN plants after cold treatment. Changes in NDPK (**a**), POD (**b**), APX (**c**), and CAT (**d**) activities in SN plants after cold treatment. Data presented are the average of three replicates. *Bars* carrying the *same letter* are not significantly different ($P = 0.05$) according to Duncan's multiple range test

stresses, including MV-mediated oxidative stress, as well as cold, high salt, and drought stresses.

Plant NDPKs play a prominent role in plant defense mechanisms, and the involvement of NDPK in these phenomena is associated with various stress-defense mechanisms. For example, transgenic Arabidopsis plants overexpressing *NDPK2* showed enhanced tolerance to MV-mediated oxidative stress, freezing, and high salt (Moon et al. 2003). Transgenic potato plants overexpressing *NDPK2* also exhibited enhanced tolerance to MV-mediated oxidative stress, high temperatures, and salt stress (Tang et al. 2008). Sweetpotato is sensitive to low temperatures. Thus, engineering a sweetpotato with an enhanced tolerance to low temperature is important. Therefore, we attempted to determine whether SN plants show an increased tolerance to cold stress (Fig. 4), because previously *AtNDPK2*-overexpressing transgenic *Arabidopsis* exhibit enhanced tolerance to freezing stress (Moon et al. 2003). Since *AtNDPK2* plays a role in regulating osmotic stress due to high salinity (Moon et al. 2003; Tang et al. 2008), we also determined whether SN plants show increased tolerance to various osmotic stresses such as high salt and drought (Fig. 6). As expected, the SN sweetpotato plants showed a significantly enhanced tolerance to various environmental stress conditions, with high levels of NDPK production (Fig. 2, 4, and 6).

Arabidopsis NDPK2 is a component of the H_2O_2-activated MAPK signaling pathway, and overexpression of *AtNDPK2* alters cellular redox conditions in plants (Moon et al. 2003). Using a cDNA microarray analysis, Yang et al. (2003) demonstrated that in transgenic Arabidopsis overexpression of *NDPK2*-induced expression of numerous genes including those involved in signal transduction and protection. Among the induced genes, expression of various antioxidant genes such as POD, APX, CAT, thioredoxin, thioredoxin reductase, and peroxiredoxin were significantly increased in *NDPK2* overexpressing transgenic plants. In addition, overexpression of *NDPK2* in transgenic potato plants increased NDPK and APX activities (Tang et al. 2008). Consistent with these results, we have demonstrated that the SN sweetpotato plants showed increased levels of POD, APX, and CAT activity under stress conditions (Fig. 3, 5). POD, APX, and CAT are the major enzymes responsible for H_2O_2 scavenging during oxidative stress in plants. Plant PODs are involved not only in scavenging H_2O_2 but also in plant growth, development, lignification, suberization, and cross-linking of cell wall compounds (Passardi et al. 2005). APX is also a component of the

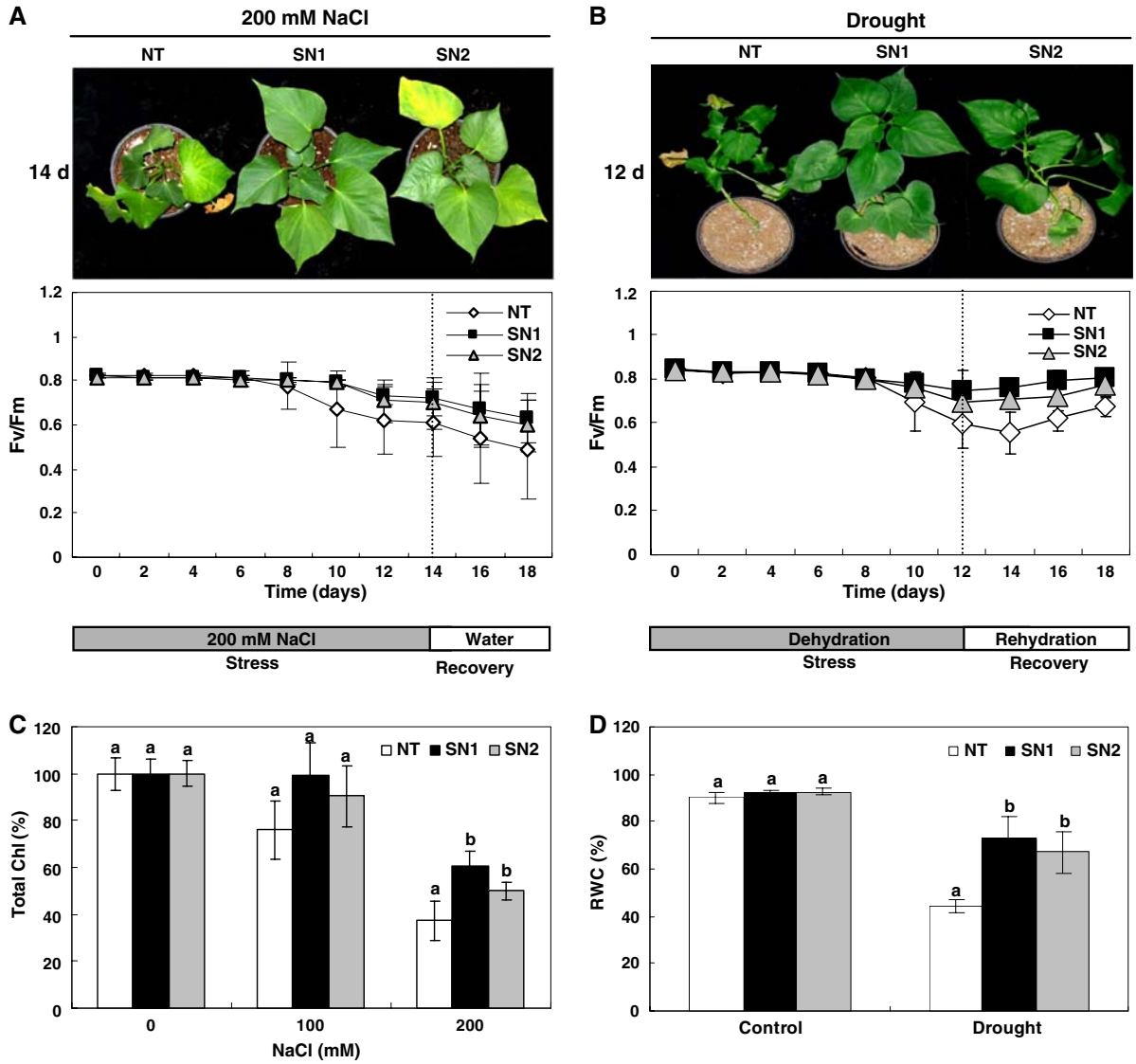

Fig. 6 Enhanced high salt and drought stress tolerance in SN plants. **a** Visible damage and PSII photosynthetic efficiency (Fv/Fm) in the leaves of sweetpotato plants after treatment with salt stress. **b** Visible damage and PSII photosynthetic efficiency (Fv/Fm) in the leaves of sweetpotato plants after treatment with drought stress. Data presented are the average of three replicates from each of five plants. **c** Total Chl content after NaCl treatment. **d** RWC after dehydration treatment. Data presented are the average of five replicates. *Bars* carrying the *same letter* are not significantly different ($P = 0.05$) according to Duncan's multiple range test

ascorbate-glutathione pathway, which plays a key role in H_2O_2 scavenging (Foyer et al. 1994; Mittler 2002). In addition, CAT eliminates H_2O_2 by breaking it down directly to form water and oxygen. Thus, CAT does not require reducing power and has a high reaction rate, but a low affinity for H_2O_2, and can, thereby, only remove H_2O_2 when high concentrations are present (Willekens et al. 1997). These data are consistent with the observations that the increase in the activities of POD, APX, and CAT as the result of *NDPK2* expression in transgenic sweetpotato plants is correlated with environmental stress defense mechanisms involving an H_2O_2-regulated stress response signaling pathway.

The transgene products affect a critical pathway associated with stress resistance, maintenance of redox potential, and cell signaling. Thus, the high levels of protein production and enzyme activity caused by an introduced transgene may disrupt cellular metabolism and lead to negative effects. In addition, ROS can act either as signals to induce protective mechanisms or to accelerate cellular damage under stress conditions (Dat et al. 2000). To allow for these different roles, cellular ROS levels are tightly controlled. Therefore, we expect that the oxidative stress–inducible *SWPA2* promoter may be very useful for developing stress-tolerant plants (Tang et al. 2006, 2008; Lee et al. 2007; Lim et al. 2007). In this study, the expression of *AtNDPK2* and its activity in the transgenic sweetpotato was induced under the control of *SWPA2* promoter by MV- or cold-induced oxidative stress (Fig. 1, 3, 4, and 5). In contrast, the *AtNDPK2* transgene was not expressed in untreated samples, suggesting that expression of the *AtNDPK2* gene is strictly regulated by the *SWPA2* promoter. These results suggest that the *SWPA2* promoter strictly controls expression of the *AtNDPK2* transgene in response to oxidative stress in SN sweetpotato plants.

In conclusion, we generated transgenic sweetpotato plants overexpressing *AtNDPK2* under the control of the oxidative stress–inducible *SWPA2* promoter by particle bombardment using embryogenic calli. The SN plants exhibited enhanced tolerance to multiple environmental stresses, including MV-mediated oxidative stress, as well as cold, high salt, and drought stress, by increasing the activity of H_2O_2-scavenging enzymes such as POD, APX and CAT under the regulation of *NDPK2*. Further characterization of SN sweetpotato plants is under investigation in natural field conditions. Field tests of SN plants under unfavorable growth conditions, such as in dry and cold regions, remain to be conducted. We anticipate that the transgenic sweetpotato plants generated in this study may be useful for sustainable agriculture in marginal soils.

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