Research article

SCOF-1-expressing transgenic sweetpotato plants show enhanced tolerance to low-temperature stress

Yun-Hee Kim[1], Myoung Duck Kim[1], Sung-Chul Park, Kyoung-Sil Yang, Jae Cheol Jeong, Haeng-Soon Lee, Sang-Soo Kwak*

Environmental Biotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), 111 Gwahangno, Yusong-gu, Daejeon 305-806, Republic of Korea

ABSTRACT

Low-temperature stress represents one of the principal limitations affecting the distribution and productivity of many plant species, including crops such as sweetpotato. Transgenic sweetpotato (*Ipomoea batatas* L. cv. Yulmi) plants expressing the soybean cold-inducible zinc finger protein (SCOF-1) under control of an oxidative stress-inducible peroxidase (*SWPA2*) promoter (referred to as SF plants), were developed and evaluated for enhanced tolerance to low-temperature conditions. Following 4 °C treatment of SF plants, *SCOF-1* expression correlated positively with tolerance to low-temperature stress at the leaf disc level. Increased *SCOF-1* expression also correlated with enhanced tolerance to different low-temperature treatments at the whole plant level. SF plants treated with low-temperature stress (4 or 10 °C for 30 h) exhibited less of a reduction in photosynthetic activity and lipid peroxidation levels than non-transgenic (NT) plants. Furthermore, the photosynthetic activity and lipid peroxidation levels of SF plants recovered to near pre-stress levels after 12 h of recovery at 25 °C. In contrast, these activities remained at a reduced level in NT plants after the same recovery period. Thus, this study has shown that low-temperature stress in sweetpotato can be efficiently modulated by overexpression of *SCOF-1*.

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1. Introduction

Low-temperature stress is a major environmental factor that affects plant growth and crop productivity. In temperate regions, many plants have developed natural adaptive mechanisms to tolerate low and freezing temperatures. Cold acclimation involves changes at the cellular level, including alterations to lipid, protein and metabolome compositions [1]. The majority of these changes are regulated by cold via alterations in gene expression, and low-temperature-inducible genes have been isolated and characterized in several plant species [1,2]. Many attempts have been made to confer low-temperature stress tolerance to plants of agronomic interest. One of the most widely-used strategies is to overexpress plant genes that are induced by low-temperature stress treatments [2,3]. Although many different genes have been overexpressed in plants in order to improve low-temperature tolerance, no individual stress-response gene has been effective, since many kinds of response are necessary for plants to survive under such conditions.

However, a strategy that employs an upstream regulatory gene required for low-temperature stress signal transduction pathways could be successful. Transcription factors can control the expression of many target genes by activating signal transduction cascades [4–6]. The soybean zinc finger protein (SCOF-1) is a candidate transcription factor for modulating cold tolerance, since *SCOF-1* transcription is induced by cold- and ABA-treatment [5]. In addition to constitutive overexpression of *SCOF-1* in *Arabidopsis*, the induction of *COR* expression enhances tolerance to cold- and freezing-stress in transgenic tobacco and *Arabidopsis* plants [5].

Sweetpotato [*Ipomoea batatas* (L.) Lam] is important food crop in the world. It is used not only as a staple food, but also as an important industrial raw material for animal feed and alcohol production. In addition, sweetpotato is rich in secondary metabolites, including antioxidant compounds such as anthocyanins, carotenoids and vitamin C [7,8]. Moreover, sweetpotato does not require large amounts of fertilizers or other agricultural chemicals. Although sweetpotato is relatively tolerant to environmental stresses, it is sensitive to low-temperature conditions. Thus, it is very important to engineer a sweetpotato with enhanced tolerance to low temperatures.

Previously, we isolated the oxidative stress-inducible *SWPA2* promoter from sweetpotato cell cultures and characterized its

function in transgenic tobacco plants with respect to environmental stresses such as oxidative stress [9]. In this study, we show that enhanced tolerance to different low-temperature stresses can be conferred to sweetpotato plants by transgenic expression of SCOF-1 under control of the SWPA2 promoter.

2. Results

2.1. Molecular characterization of SCOF-1-overexpressing transgenic sweetpotato under low-temperature conditions

Transgenic sweetpotato plants expressing SCOF-1 under control of the oxidative stress-inducible SWPA2 promoter (SF plants) were generated successfully via Agrobacterium-mediated transformation (Fig. 1). Two independent transgenic lines were established and grown in a growth chamber for 6 weeks. Low-temperature stress tolerance (4 °C) was evaluated using leaf discs. To investigate the level of transgene expression in SF plants, RT-PCR analyses were conducted using RNA from low-temperature-treated plant leaf discs and a SCOF-1 gene-specific primer set (Fig. 2A). After induction of low-temperature stress, SCOF-1 transcript was detected in leaf discs from both transgenic lines, but not from NT plants. Particularly strong induction of SCOF-1 expression was detected in SF2 plants after low-temperature treatment. To ascertain whether SCOF-1 expression correlated with low-temperature stress tolerance, the ion leakage in leaf discs was analyzed after 4 °C treatment (Fig. 2B). Compared to NT plants, all SF plants exhibited reduced symptoms of damage from low-temperature treatment. SF2 leaf discs exhibited the highest levels of SCOF-1 expression and showed slightly better tolerance to low-temperature stress than the SF1 line.

2.2. SCOF-1-overexpressing transgenic sweetpotato showed enhanced tolerance to different low-temperature conditions

To assess the effects of SCOF-1 expression on low-temperature stress tolerance in soil-grown whole plants, 2-month-old plants were incubated for 30 h under different temperature conditions (25, 15, 10 and 4 °C) and then transferred to 25 °C for recovery. Following exposure to 10 or 4 °C for 30 h, the leaves of NT plants showed severe wilting and curling, whereas SF leaves showed only slight damage (Fig. 3A). After 30 h of 4 °C treatment, the photosynthetic efficiency (Fv/Fm) of NT plants had decreased by 35.4%, whereas it only decreased by 20.8 and 16.6% in SF1 and SF2 plants, respectively (Fig. 3B). After a 12 h recovery period, the photosynthetic efficiency of SF1 plants had returned to near pre-treatment levels, whereas NT plants continued to show reduced Fv/Fm. Low-temperature stress increases degradation of plant lipid membranes and thus, lipid peroxidation is induced during low-temperature conditions [10]. Plants were treated with different low temperatures for 24 h and then transferred to 25 °C for 12 h to allow recovery. In comparison to NT plants exposed to the same conditions, SF1 and SF2 plants showed 60.2 and 63.1% lower levels
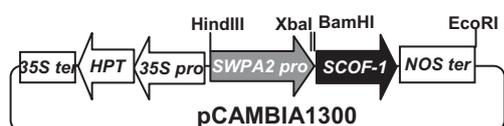

Fig. 1. Schematic representation of expression vectors used for sweetpotato transformation. SWPA2 pro, oxidative stress-inducible sweetpotato peroxidase promoter; SCOF-1, soybean cold-inducible zinc finger protein; NOS ter, nopaline synthesis terminator; 35S pro, cauliflower mosaic 35S promoter; HPT, hygromycin phosphotransferase; 35S ter, cauliflower mosaic 35S terminator.
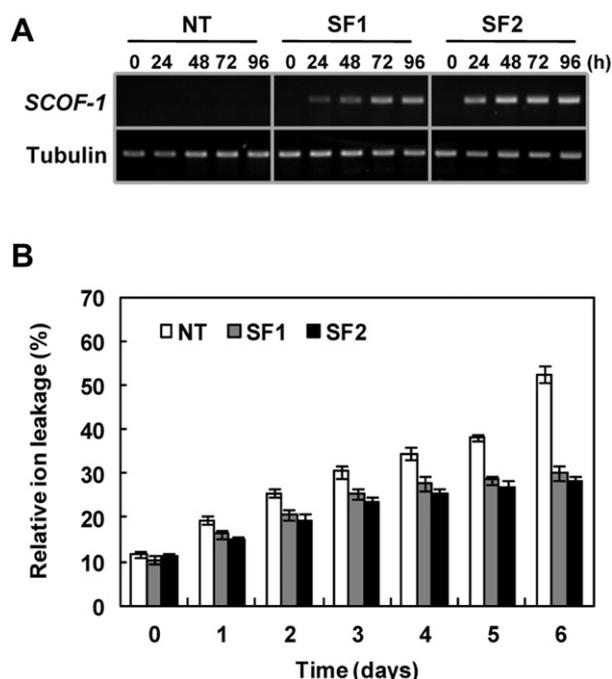

Fig. 2. Analysis of transcript expression and ion leakage in SCOF-1-overexpressing transgenic sweetpotato plants (SF plants) under low-temperature stress. RT-PCR analysis of SF plants under 4 °C treatment (A). Ion leakage analysis of independent SF transgenic lines in response to 4 °C treatment (B). The electrical conductivity of leaves from low-temperature-treated plants was compared with the total conductivity of the solution following tissue destruction. Data represent the means of three replicates.

of lipid peroxidation, respectively (Fig. 4). In whole plants treated with low-temperature stress, SCOF-1 transcript levels increased in the leaves of both SF lines, but not in the leaves of NT plants (Fig. 5). These results suggest that under low-temperature stresses such as 10 and 4 °C, SCOF-1 gene expression reduces the accumulation of lipid peroxidation and damage to photosynthetic efficiency in SF plants.

2.3. Acclimation of SCOF-1-overexpressing transgenic sweetpotato to severe cold stress during a periods of moderate cold treatment

In order to determine whether SCOF-1 expression is involved in the acclimation of sweetpotato plants to severe cold stress during a period of moderate cold treatment, the plants were first exposed to 15 °C for 24 h and then to 4 °C for 36 h. After cold treatments, sweetpotato plants were allowed to recover at 25 °C. Following exposure to 15 °C for 24 h, the leaves of NT and transgenic plants showed only slight damage (Fig. 6A and B). However, after 36 h of 4 °C, the photosynthetic efficiency (Fv/Fm) of NT plants decreased by 40.8%, whereas it only decreased by 30.6 and 25.9% in SF1 and SF2 plants, respectively (Fig. 6B). After a 12 h recovery period, the photosynthetic efficiency of both NT and transgenic plants returned to near pre-treatment levels. These results suggest that SCOF-1 expression acclimates sweetpotato to severe cold stress at 4 °C during a period of moderate cold treatment at 15 °C.

3. Discussion

SCOF-1 expression is induced by cold- and ABA-treatment conditions, and its induction is associated with cold stress defense mechanisms [5]. In this study, we have successfully developed transgenic sweetpotato plants expressing SCOF-1 under the control
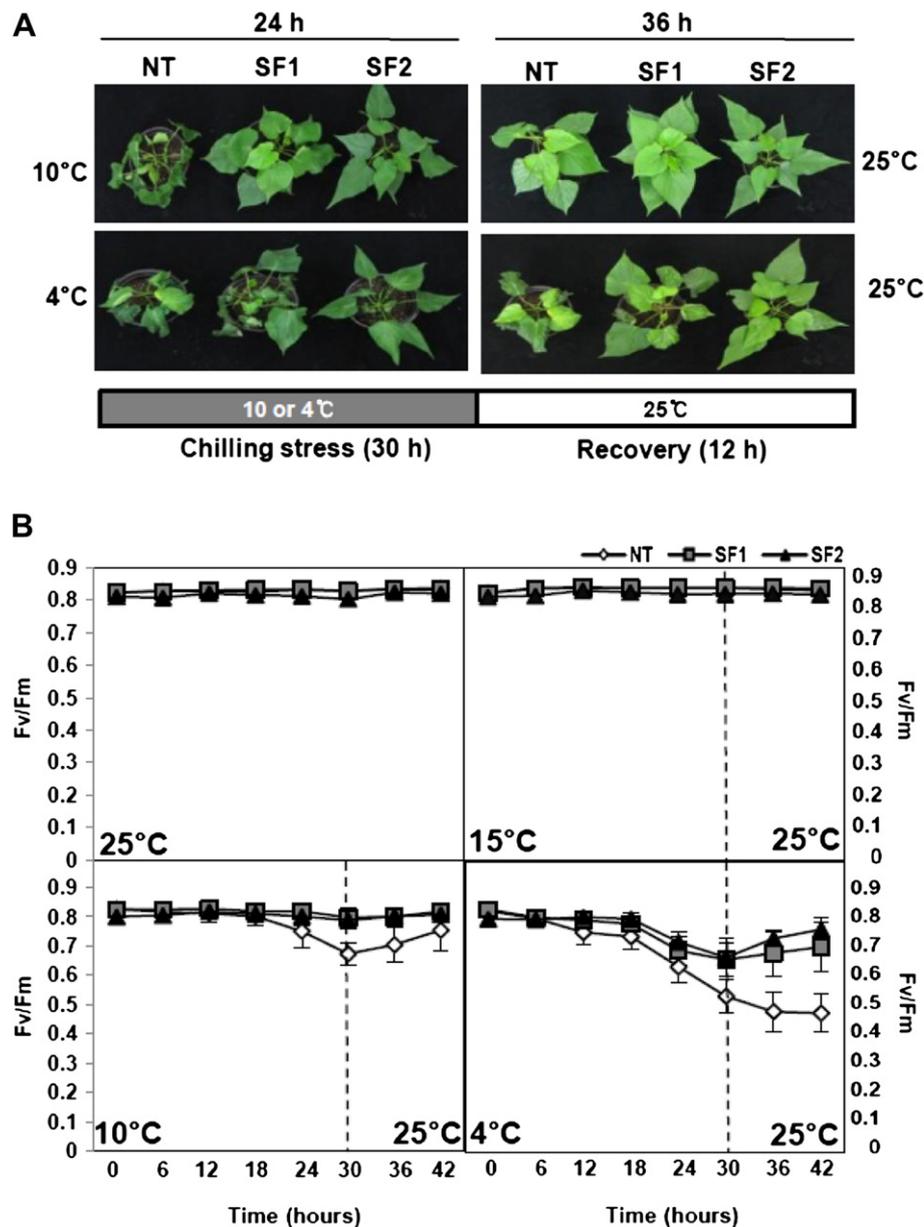
Fig. 3. Effect of different low-temperature stresses on SF plants. Visible damage in the leaves of sweetpotato plants after a 30 h low-temperature treatment and 12 h recovery at 25 °C (A). PSII photosynthetic efficiency (*Fv/Fm*) in the leaves of sweetpotato plants after a 30 h low-temperature treatment and 12 h recovery at 25 °C (B). Data represent the means of three replicates from each of five plants.

of the oxidative stress-inducible *SWPA2* promoter. Expression of *SCOF-1* resulted in increased tolerance to low-temperature stress in sweetpotato. During recovery from low-temperature stress, remarkable differences exist in the chilling- or freezing-tolerance of NT plants, transgenic tobacco and *Arabidopsis* plants constitutively overexpressing *SCOF-1* [5]. As expected, this study showed that SF transgenic sweetpotato plants had enhanced tolerance to low-temperature stress, especially under recovery conditions, and that this cold-tolerance correlated with high levels of *SCOF-1* expression (Figs. 3–5).

We also checked whether *SCOF-1* expression was involved in acclimation to severe cold stress during a period of moderate cold treatment (Fig. 6). Interestingly, during the recovery period, the photosynthetic efficiency of both NT and transgenic plants returned to near pre-treatment levels. The set of biochemical and physiological changes that increases plant tolerance to low-temperature stress is termed cold acclimation [11,12]. In cold-tolerant crop

plants, cold acclimation involves adjustments in various functions, such as changes in cellular metabolism, function to the biophysical and kinetic constraints imposed by decreased temperature, and the induction of tolerance to freezing conditions [13]. Under non-freezing low-temperature conditions that promote cold acclimation, many plant species also exhibit sensitive changes in gene expression that appear to be different from the responses induced by other types of environmental stresses [13,14]. Therefore, these results suggest that increased expression of the cold-responsive genes under moderate cold treatment conditions may be caused by enhanced cold tolerance in NT sweetpotato plants.

Previously transgenic *Arabidopsis* expressing *SCOF-1* exhibited strong induction of various cold-responsive gene expressions such as *cor15a*, *cor47* and *rd29B*, which is associated with a concomitant improvement in freezing-tolerance [5]. This correlation suggests that *SCOF-1* functions as a positive regulator of the expression of low-temperature-inducible downstream
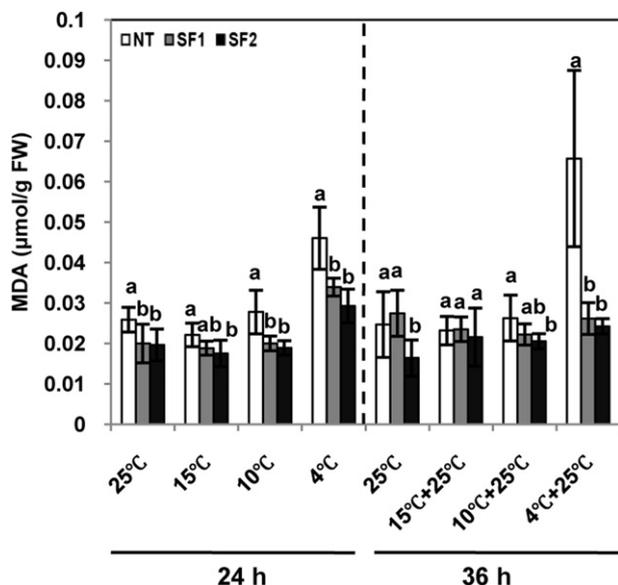
Fig. 4. Changes in lipid peroxidation of SF plants under low-temperature stress conditions. Lipid peroxidation in the leaves of sweetpotato plants was determined by MDA level. Plants were treated with different low temperatures for 24 h and then allowed to recover at 25 °C for a further 12 h. Data represent the means of five replicates. No significant difference ($P \geq 0.05$) was found between bars carrying the same letter, according to Duncan's multiple range test.

oxidative stress-inducible *SWPA2* promoter has proved useful for developing stress-tolerant plants, since it only up-regulates gene expression under stress conditions [15–17]. In this study, the *SWPA2* promoter increased *SCOF-1* expression in the transgenic sweetpotato in response to low-temperature-induced oxidative stress, and *SCOF-1* was not expressed under control conditions (Figs. 2 and 5). These results suggest that the *SWPA2* promoter provides strict control over the expression of *SCOF-1* in the sweetpotato and that it elevates *SCOF-1* expression in response to low-temperature-induced oxidative stress.

Although sweetpotato is recognized as a relatively low-temperature stress-sensitive crop plant, the molecular mechanisms underlying low temperature are not well defined. Moreover, no reports have been published on genetic engineering using low temperature-inducible transcription factor in sweetpotato. It is known that a protein kinase and transcription factors can control the expression of many target genes by activating signal transduction cascades [3,18]. The transgenic sweetpotato plants expressing the *Arabidopsis* nucleoside diphosphate kinase 2 (AtNDPK2) gene exhibited enhanced tolerance to multiple environmental stresses, including cold stress by increasing the activity of H_2O_2-scavenging antioxidant enzymes under the regulation of NDPK2 [15]. In case of transcription factor, previously DRE-binding (DREB) transcription factor was isolated from dehydration-treated fibrous roots sweetpotato, and their expression levels were characterized in response to various environmental stresses such as dehydration, low temperature, and stress-related chemicals [19]. However, the functional study of sweetpotato DREB genes to drought and low-temperature stress have not yet been characterized in detail. Therefore, our present study is the first report on generation of low temperature-tolerant transgenic sweetpotato plants by genetic engineering using low temperature-inducible transcription factor.

In conclusion, this study has shown that *SWPA2* promoter-controlled expression of *SCOF-1* confers enhanced tolerance to low-temperature stress in SF sweetpotato plants. Currently, SF sweetpotato plants are being characterized under natural field conditions; however, field tests for tolerance to unfavorable growth conditions such as low-temperature, remain to be conducted. Changes in the responses of genes that function downstream of
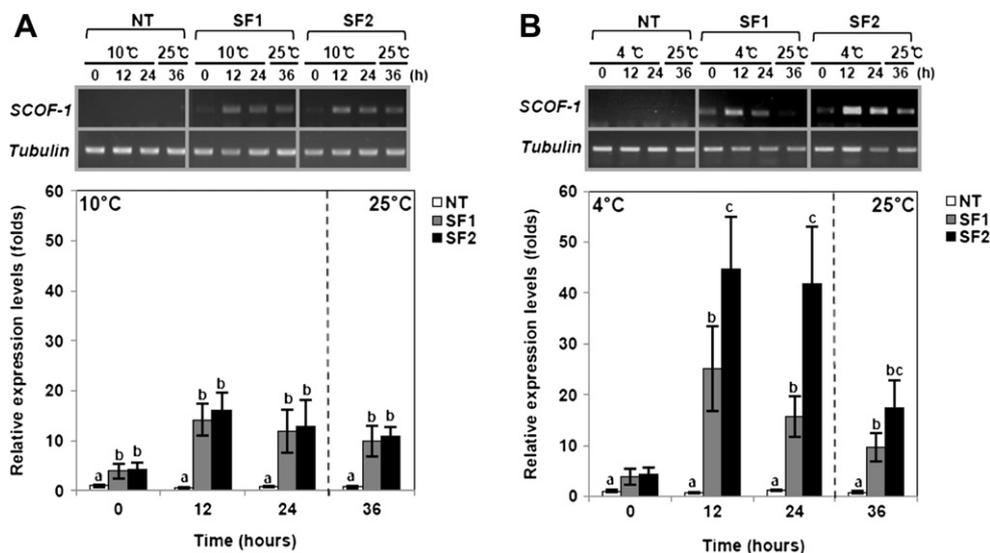
genes in the sweetpotato and that overexpression of *SCOF-1* results in enhanced low-temperature tolerance. However, in this study, we were not able to check the expression analysis of *SCOF-1* downstream genes such as, *cor15a*, *cor47* and *rd29B* in sweetpotato plants because we could not clone these genes from sweetpotato using the *Arabidoposis* sequence. These genes show high sequence homology to other genes in the genus Brassica, such as *Brassica napus* in the NCBI database.

Since *SCOF-1* expression can affect critical pathways associated with stress tolerance, maintenance of redox potential and cell signaling, artificially elevated expression of this transgene could disrupt cellular metabolism and lead to negative effects. The

Fig. 5. Analysis of *SCOF-1* expression in sweetpotato plants under low-temperature and recovery conditions. Expression patterns of *SCOF-1* under 10 °C treatments for 24 h and recovery conditions for 12 h (A). Expression patterns of *SCOF-1* under 4 °C treatments for 24 h and recovery conditions for 12 h (B). Data represent the means of three replicates. No significant difference ($P \geq 0.05$) was found between bars carrying the same letter, according to Duncan's multiple range test.
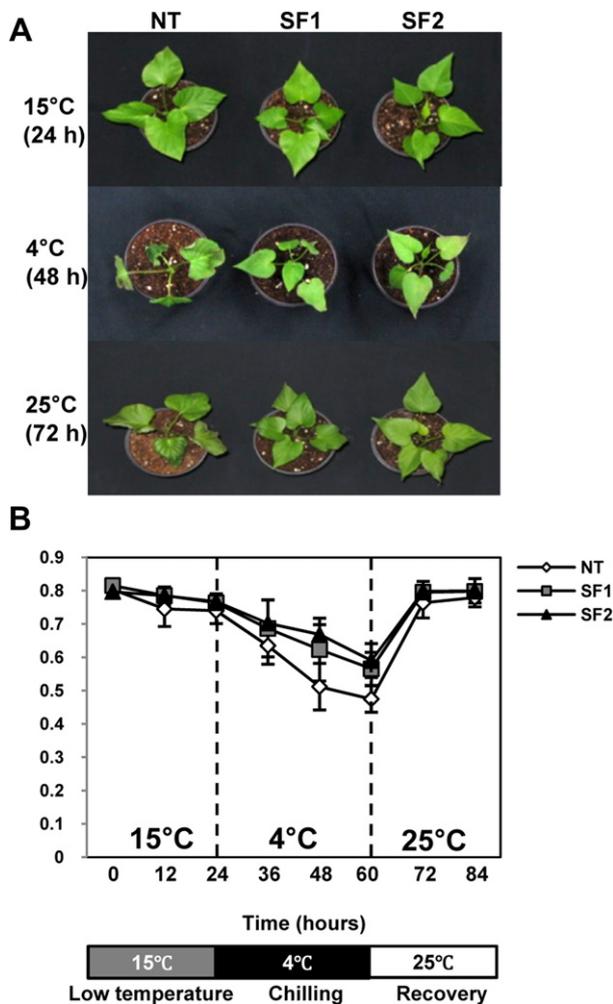
Fig. 6. Effects of acclimation to severe cold stress during a period of moderate cold treatment in SF plants. Visible damage to the leaves of sweetpotato plants after a 24 h low-temperature (15 °C) treatment, 24 h cold (4 °C) treatment and 12 h recovery at 25 °C (A). PSII photosynthetic efficiency (*Fv/Fm*) in the leaves of sweetpotato plants after a 24 h low-temperature (15 °C) treatment, 36 h cold (4 °C) treatment and 24 h recovery at 25 °C (B). Data represent the means of three replicates from each of six plants.

SCOF-1 in transgenic sweetpotato under different low-temperature conditions also remain to be studied. We anticipate that the transgenic sweetpotato plants generated in this study could be useful for sustainable agriculture on marginal soils. Further study of the effects of changes in the regulation of *SCOF-1* on downstream gene expression in sweetpotato plants should help understand how plants deal with low-temperature stress.

4. Materials and methods

4.1. Plant materials and vector construction

Embryogenic calli were induced from shoot meristems of sweetpotato [*I. batatas* (L.) Lam. cv. Yulmi] cultured on MS [20] 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 into fresh medium at 4-week intervals [21].

The *SCOF-1* gene construct was generated in the plant expression vector pCAMBIA2300 using an oxidative-inducible *SWPA2*

promoter and a *NOS* terminator sequence. The pCAMBIA1300 vector, which contains the full-length cDNA of *SCOF-1* (accession no. U68763), was kindly provided by Prof. S. H. Lee [5]. To generate the *SWPA2* promoter::*SCOF-1* vector, *SCOF-1*::*NOS* terminator cassettes from pCAMBIA1300 were inserted into the *Bam*HI and *Eco*RI sites of the binary vector pCAMBIA1300, which contains the *SWPA2* promoter.

4.2. Agrobacterium-mediated transformation

SWPA2 pro::*SCOF-1* plasmids were introduced into *Agrobacterium tumefaciens* EHA 105, which was then transformed into embryogenic calli of sweetpotato via an *Agrobacterium*-mediated transformation method, as described by Lim et al. [22]. After 3 days of co-culture, 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 then subcultured onto freshly-prepared medium at 3-week intervals. The calli were transferred to somatic embryo formation medium (MS medium containing 100 mg l^{-1} kanamycin, 3% sucrose and 0.4% gelrite) and somatic embryos were transferred to MS medium containing 100 mg l^{-1} kanamycin, 3% sucrose and 0.4% gelrite, in order to regenerate plants. 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.

4.3. Gene expression analysis

Total RNA was isolated from leaves of sweetpotato using TRIzol reagent (Invitrogen, Carlsbad, CA). Samples were 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), in accordance with the manufacturer's instructions. MMLV reverse transcriptase was used to generate first-strand cDNA from total RNA (1 μg). The *SCOF-1* primer set (5′-TTGCCACAAATCCTTCCCCA-3′, 5′-ATGTTGAGATCGAA GTCGCGGT-3′) was used to amplify a 177 bp product from cDNA encoding *SCOF-1*. As an internal standard, α-tubulin gene-specific primers (5′-TTGGTGCCCAACTGGATTCAA-3′, 5′-ACGAAAGCACGCTT GCAGTACA-3′) were used to amplify a 180 bp product from total cDNA. For quantitative RT-PCR, fragments of interest were amplified by real-time PCR using gene-specific primers and EverGreen fluorescent dye.

4.4. Ion leakage analysis using leaf discs

The extent of cellular damage can be quantified by ion leakage, which is a measure of membrane disruption. Ion leakage was analyzed according to Bowler et al. [23], with slight modifications. Following 4 °C treatment, the electrical conductance of the solution, i.e. the loss of cytoplasmic solutes, was measured with an ion conductivity meter (model 455C, Istek Co, Seoul, Korea) and then compared with the total conductivity of the solution following tissue destruction.

4.5. Low-temperature stress treatment of whole plants

Sweetpotato plants were grown at 25 °C in a growth chamber for 6 weeks and then transferred to a plant growth chamber maintained at 15, 10 or 4 °C [60% relative humidity, 16/8-h (light/dark) photoperiod, with light supplied at an intensity of 320 μmol m^{-2} s^{-1}] for 30 h. Following this stress treatment, plants were transferred back to normal culture conditions (25 °C) for recovery.

4.6. Determination of PSII photosynthetic efficiency

The PSII photosynthetic efficiency of leaves was estimated from the chlorophyll (Chl) fluorescence determination of photochemical yield (Fv/Fm), using a portable Chl fluorescence meter (Handy PEA, Hansatech, England). PSII efficiency represents the maximal yield of the photochemical reaction in PSII.

4.7. Analysis of lipid peroxidation

Lipid peroxidation was measured in the leaves of sweetpotato using a modified thiobarbituric acid method [24]. The specific absorbance of extracts was recorded at 532 nm. Non-specific absorbance was measured at 600 nm and then subtracted from the 532 nm readings. The concentration of malondialdehyde (MDA) was calculated as a measure of lipid peroxidation.

4.8. Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS 12). Means were separated using Duncan's multiple range test and statistical significance was set at $P \leq 0.05$.

Acknowledgments

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