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Identification of a putative stearoyl acyl-carrier-protein desaturase gene from *Saussurea involucrata*

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Abstract

Saussurea involucrata Kar. *et* Kir. tolerates severe abiotic stresses including cold, and the level of membrane fatty acid desaturation is associated with its cold acclimation. We discovered and characterized a full-length cDNA of stearoyl acyl-carrier-protein desaturase (*sikSACPD*) which encodes a protein consisted of 396 amino acids. A sequence alignment of the SikSACPD protein showed that it shares 91 and 86 % identity with the *SACPDs* of *Carthamus tinctorius* and *Helianthus annuus*, respectively. Semi-quantitative RT-PCR showed that the expression of *sikSACPD* increased in *S. involucrata* leaves as the temperature decreased from 20 to -10 °C. *Agrobacterium tumefaciens* was used to transform fatty acid biosynthesis 2 (FAB2):SikSACPD and FAB2:FAB2 constructs into tobacco to investigate resistance to a freezing stress and fatty acid composition of the transgenic plants. The FAB2:SikSACPD transgenic plants showed a slightly more resistance to the freezing stress than the FAB2:FAB2 transgenic plants and the wild-type. The proportion of oleic acid (C18:1) in the leaves of SikSACPD transgenic tobacco increased from approximately 5 to 20 % compared with the leaves of non-transgenic tobacco when both were exposed to cold stress treatments. This study demonstrates that the *SikSACPD* transgene, when expressed in tobacco, conferred a higher cold tolerance in comparison with that observed in non-transgenic tobacco. Thus, this gene may be a candidate for enhancing cold tolerance in other crop plants.

Additional key words: *Agrobacterium tumefaciens*, cold resistance, fatty acids, *Nicotiana tabaccum*, *sikSACPD*, transgenic plants.

Introduction

Low temperature stress is a prevalent worldwide problem that causes substantial reductions in food and cash crop production. Therefore, the study of plant tolerance to cold stress and improving the plant capacity for cold stress tolerance are extremely important (Wada *et al*. 1990, Thomashow 1999, Chen *et al*. 2001, Hirayama and Shinozaki 2010). Plastidic fatty acids are important components of membrane lipids synthesized by the fatty acid synthase complex with most acyl intermediates being esterified to the acyl carrier protein (ACP). Stearoyl-acyl-carrier-protein desaturase (*SACPD*) mediates the conversion of stearic acid (18:0) to oleic acid (18:1), a key step that regulates the amount of unsaturated fatty acids in cells and fluidity of biological membranes (MeKeon *et al*. 1982, Kodama *et al*. 1995, Moellering *et al*. 2010).

The first step in C-18 fatty acid desaturation is catalyzed by *SACPD*. This enzyme introduces a *cis* double bond at position 9/10 of the C-18 chain, converting stearoyl-ACP to oleoyl-ACP (Los *et al*. 1993). Several plant Δ^9-desaturases have been identified and characterized, *e.g.*, in safflower (Thompson *et al*. 1991),

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Abbreviations: FA - fatty acid; F_v/F_m - variable to maximum chlorophyll fluorescence ratio; MDA - malondialdehyde; PS II - photosystem II; RT-PCR - reverse transcriptase - polymerase chain reaction; SACPD - stearoyl acyl-carrier-protein desaturase.
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cucumber (Shanklin *et al*. 1991), castor bean (Shanklin *et al*. 1991), potato (Taylor *et al*. 1992), spinach (Nishida *et al*. 1992), canola (Slocombe *et al*. 1992), turnip (Knutzon *et al*. 1992), jojoba (Sato *et al*. 1992), *Jatropha curcas* (Tong *et al*. 2006), and soybean (Zhang *et al*. 2011). In these plants, the Δ^9-desaturase genes encode soluble plastidial stearoyl-ACP desaturases. Kodama *et al*. (1994) transferred a *SACPD* gene encoding a yeast stearoyl acyl coenzyme A desaturase into tobacco, and Los *et al*. (1993) transferred a spinach *SACPD* gene into tobacco. These genes significantly improve the tobacco cold resistance. Also Ma *et al*. (1996a,b) showed that the expression of *SACPD* in tobacco significantly enhances its cold resistance, and that the antisense-expression of the *SACPD* gene significantly reduces its cold resistance. This indicates that the fatty acid desaturase can improve cold resistance, however, the relationship is not yet conclusive.

Saussurea involucrata grows in the high-altitude mountains in Xinjiang, China, and it is able to tolerate severe cold (Chen *et al*. 1999, Huang *et al*. 2002). The majority of the species is located above the snow line where there are year round snowfalls and the highest average monthly temperature is below 10 °C. After a long exposure to these extreme environmental conditions, natural selection in *S. involucrata* has led to the formation of stable physiological and biochemical mechanisms which may form a general cold tolerance (Guo *et al*. 2012, Qiu *et al*. 2014). Therefore, it is important to understand how *S. involucrata* maintains the integrity of membrane structures and normal physiological functions in such extreme conditions. In this study, we obtained a putative *SACPD* with unique characteristics that could improve the cold resistance of transgenic tobacco.

Materials and methods

Saussurea involucrata Kar. *et* Kir. was cultured under day/night temperatures of 21/19 °C, a relative humidity of 60/70 %, a 16-h photoperiod, and an irradiance of 60 μmol m^{-2} s^{-1}. Seeds of *Nicotiana nudicaulis* S. Watson (ecotype NC98) were germinated on Murashige and Skoog (MS; *Sigma-Aldrich*, St. Louis, MO, USA) medium plates solidified with 0.8 % (m/v) *Phytagel*. Plants were grown at a temperature of 25 °C, a 16-h photoperiod and an irradiance of 60 μmol m^{-2} s^{-1}. DNA gel extraction kits, Taq polymerase, and restriction enzymes were purchased from *Sangon Biological Engineering Technology & Services Co*. (Shanghai, China).

The degenerate primers (SikSACPD P1and P2) used in this experiment are shown in Table 1 and were synthesized by *Sangon Biological Engineering Technology & Services Co*. DNA sequencing was performed by *Sangon Biotech Company* (Shanghai, China). The *PGEM-T* vector was purchased from *TaKaRa* (Dalian, China). All the other chemicals used were of analytical grade.

For isolation of total RNA and genome DNA, *S. involucrata* was pretreated at 4 °C for 1 d and at 0 °C for 2 d. Young leaves were powdered in liquid nitrogen using a mortar and pestle. Total RNA was extracted using a *RNeasy*® plant mini kit (*Qiagen*, Shanghai, China) following the manufacturer's instructions. Genomic DNA was extracted using the plant DNA preparation procedure described previously (Dellaporta *et al*. 1983, Doyle *et al*. 1990).

The RT-PCR experiment was performed using a *TaKaRa* RNA PCR kit (*AMVv*.3.0), taq polymerase, and two degenerate primers (Table 1). The PCR reaction consisted of 30 cycles at 94 °C for 0.75 min, 55 °C for 0.75 min, and 72 °C for 1 min followed by a final extension at 72 °C for 10 min. The RT-PCR and PCR amplified products were separated by 1.0 % agarose gel electrophoresis containing ethidium bromide and visualized by UV-radiation.

Table 1. Degenerate primers used to obtain a specific segment of stearoyl-acyl carrier protein desaturase (SACPD). GSP primers used for obtaining the 5′ and 3′ regions of the *sikSACPD* gene. GSP 5′ and 3′ primers used to obtain the full-length cDNA CDS sequence of *sikSACPD* from *Saussurea involucrata*. GAPDH primers used for semi-quantitative RT-PCR analysis. **R** = A/G; **Y** = C/T; **N** = A/G/C/T.

Primer name	Primer sequence (5′-3′)
SikSACPD P1	GTTGGAGATATGATCCA**N**GA**R**GAAGC
SikSACPD P2	TTCCAGTATATC**N**GC**R**TA**R**TC**Y**TT
sikSACPDa GSP1	CTGTCTGGACTAGGGCTTGGACG
sikSACPDa GSP2	GCACAAAGACTTGGCGTTTAC
sikSACPD5′GSP3	CCATGGATGGCTCTTCGGATCAGTCC
sikSACPD3′GSP4	CACGTGTTCAGAGCTTCACCTGTCTAT
GAPDH (KF563904.1) forward	GTTGCTAGAGTTGCACTTCAGAGAG
GAPDH (KF563904.1) reverse	TTCCTGAAGCCGAAAACAGC

According to the sequence of a specific segment of the *sikSACPD* gene from DNA sequencing, a pair of primers, 5′-*sikSACPD*a GSP1 and 3′-*sikSACPD*b GSP2, were designed and synthesized (Table 1).

A full-length cDNA library from *S. involucrata* was constructed using the *Creator*™ *SMART*™ cDNA library construction protocol (*Clontech*, Mountain View, CA, USA; Zhu *et al*. 2006). Using the *sikSACPDa* GSP1 and M13-forward sequencing primers (a library vector), the 5′ region of the *sikSACPD* gene was obtained. Using the *sikSACPDb* GSP2 and M13-reverse sequencing primers (a library vector), the 3′ region of the *sikSACPD* gene was obtained. By comparing and aligning the sequences of the *sikSACPD* 5′ and 3′ regions, the full-length cDNA sequence of the *sikSACPD* CDS was deduced. The CDS was subsequently amplified by PCR using a pair of GSP primers in which *Nco*I and *Pml*I sites existed at the 5′ ends (Table 1).

Total RNA was extracted from *S. involucrata* using a *RNeasy* mini kit (*Qiagen*) and treated with RNase free DNase (*Promega*, Madison, WI, USA) under temperatures ranging from normal to low (20 →10 →4 →0 →-2 →-4 →-6 →-8 →-10 °C). After being exposed to the different temperatures, RNA was extracted from these plants. Reverse transcription of 2 μg of total RNA was performed with *SuperScript*™ *II* reverse transcriptase (*Invitrogen*, Grand Island, NY, USA). The cDNA samples were diluted to 2 and 8 ng cm^{-3}. All reactions were performed in triplicate. GADPH (acc. No. KF563904.1, the forward primer: 5′-GTTGCTAGAG TTGCACTTCAGAGAG-3′, the reverse primer: 5′-TTC CTGAAGCCGAAAACAGC-3′, Qiu *et al*. 2014) was included in each assay as loading control.

For the ProFAB2:SikSACPD and ProFAB2:FAB2 constructs, the fatty acid biosynthesis 2 (FAB2) promoter was first amplified from *Arabidopsis thaliana* using the forward primer 5′-AAGCTTGTGAAATGATATATG ACATGTTTGTTTG-3′ (underlining indicates the *Hind*III site) and the reverse primer 5′-CCATGGT TCTGGATATGATGAATGAATCCTTC-3′ (underlining indicates the *Nco*I site) and cloned into the pCAMBIA1301 vector which was digested with the same enzymes to replace the CaMV35S promoter. The construct was then digested with *Nco*I and *Bst*E II and ligated to the *SikSACPD* and *Fab2* PCR product digested with the same enzymes. The *GUS* gene was replaced with the *SikSACPD* and *Fab2* genes, and the identity of the clone insert was confirmed by sequencing.

These two constructs were introduced into *N. nudicaulis* wild-type plants (ecotype NC98) through *Agrobacterium tumefaciens* (strain GV3101) mediated T-DNA transformation as described previously (Severin and Schöffl 1990). Transformants were selected on a MS medium containing 20 μg cm^{-3} of hygromycin B and then transferred to soil to set seeds. Homozygous T2 plants were used for subsequent analyses.

Cold treatment and freezing tolerance assays consisted of transferring whole plants to a chamber (*Percival Scientific*, Perry, IA, USA) set at 20, 10, 5, 0, or -2 °C for 2 h. Relative conductivity assays after the treatment were carried out essentially as described by Ishitani *et al*. (1998). Briefly, for each treatment, one excised leaf was placed in a test tube containing 0.1 cm^3 of deionized H_2O, and the tube was placed in a circulating freezing bath (*VWR Scientific*, San Francisco, CA, USA) set at 0 °C. For each temperature treatment, three replicates were measured. The temperature of the bath was programmed to decrease to -2 °C at 2 °C h^{-1}. When the designated temperature was reached, the tubes were removed and placed immediately on ice to allow gradual thawing. The leaves were then transferred carefully to another tube containing 25 cm^3 of deionized H_2O and shaken overnight before the conductivity was measured. The tubes with the leaves were then autoclaved. After cooling to room temperature, the conductivities of the solutions were measured again. The percentage of electrolyte leakage was calculated as the ratio of the conductivity before autoclaving to that after autoclaving. The relative conductivity experiment was repeated twice with three replicates in each experiment. Representative results from an experiment are presented here.

Plant survival was monitored by whole plant freezing tests based on the protocol of Xin and Browse (1998). Although this protocol was developed for seedlings grown in Petri dishes, Guo *et al*. (2002) and Gong *et al*. (2002) have also successfully used it for assessing the tolerance of soil-grown *Arabidopsis* plants in pots.

MDA content in the leaves was analyzed following Carmak and Horst (1991). Fresh leaves (1.0 g) were ground completely in 20 cm^3 of a 0.1 % (m/v) trichloroacetic acid (TCA) solution and centrifuged at 12 000 *g* for 10 min. A sample (1 cm^3) of the supernatant was added to 4 cm^3 of a 20 % TCA solution containing 0.5 % (m/v) thiobarbituric acid, heated in a 95 °C water bath for 30 min and immediately cooled on ice. After centrifugation at 12 000 *g* for 10 min, the absorbances of the supernatant was measured at 532 and 600 nm. The MDA content was determined using the coefficient of absorbance 155 mM^{-1} cm^{-1} with the formula: MDA content = $(A_{532} - A_{600})/1.56 \times 105$.

The ratio of variable to maximum chlorophyll fluorescence (F_v / F_m) was used to quantify the sensitivity of leaf tissue to freezing. F_v / F_m represents the maximum quantum efficiency of photosystem (PS) II (Maxwell and Johnson 2000). F_v / F_m is calculated as $(F_m - F_0) / F_m$, in which F_0 is the minimum fluorescence of a dark-adapted leaf and F_m is the maximum fluorescence of the same leaf after application of a saturating flash (Maxwell and Johnson 2000). Chlorophyll fluorescence was measured using a pulse-modulated fluorometer (*OS5-FL*, *Opti-Sciences*, Hudson, NH, USA). Measurements were taken before preparing leaf samples for the freezing tests (BF), and again 3 h and 24 h after they were removed from the

freezer. Measurements were also taken from leaf segments kept in a refrigerator (4 °C) during the experiment.

Fatty acids were extracted by mixing 200 mg of leaf tissue with 4 cm^3 of 1 M KOH-CH_3OH under N_2 at 75 °C for 10 min. The residues were re-extracted twice and the combined extracts were methylated in 12 cm^3 of 2 M HCl-CH_3OH at 75 °C for 10 min. Then, 4 cm^3 of hexane was added to the cooled suspension, the separated fatty acid esters were dried under N_2, and redissolved in 1 cm^3 of hexane. C17:0 (*Sigma*, St. Louis, MO, USA) was added as internal standard. Fatty acid esters were analyzed by a gas chromatograph (*GC-17A*, *Shimadzu*, Kyoto, Japan) with a flame-ionization detector and a *Carbowax* capillary column (15 m × 0.53 mm). The initial column temperature was set at 165 °C for 1 min and then increased to 235 °C at 10 °C min^{-1}. The detector and injector were maintained at 235 °C. Samples of 2 mm^3 were injected under a split mode (a split ratio, 1:10). Fatty acid methyl esters were identified based on retention times that were referenced against standard samples. The quantities of fatty acids were calculated on the basis of the known amount of the internal standard.

All statistical analyses were performed with the *SPSS v. 16.0* statistical software (*SPSS Inc*., Chicago, IL, USA), and the data were evaluated by one-way *ANOVA*. Data were expressed as mean ± SD, $P < 0.05$ was taken as significant, and $P < 0.01$ was taken as highly significant.

Results

To amplify parts of the *sikSACPD* genes by RT-PCR and PCR, degenerate primers were constructed based on conserved amino acid regions of fatty acid desaturases from several higher plants. The RT-PCR product was 614 bp, and the PCR product was 1 011 bp. Using specific and vector PCR primers, the 5′ and 3′ regions of the *SACPD* gene were obtained, which were subsequently confirmed by sequencing. The full-length cDNA of the *sikSACPD* gene (GenBank acc. No. DQ516384) was also obtained. The full-length cDNA was 1 555 bp containing an open reading frame of 1 191 bp (http://us.expasy.org/tools/protparam.html or *DNAMAN* tool) which had a deduced relative molecular mass of approximately 45 kDa. A transmembrane helix prediction (*TMpred*, http://www.ch.embnet.org/software/TMPRED_form.html) indicated no trans-membrane helices in the deduced SikSACPD protein implying that the SikSACPD protein did not function on the membrane and might function in the cytosol or nucleus. Homologous analyses of the deduced amino acid sequences were performed by *Phyre* (http://www. sbg.bio.ic.ac.uk/phyre/). The results indicate that the 396 residues of SiKSACPD shared the highest identities with *Carthamus tinctorius* and *Helianthus annuus.*

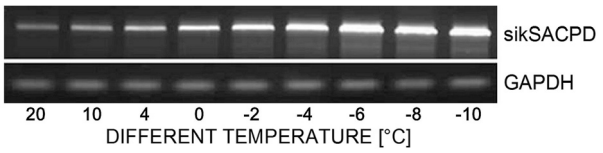

Fig. 1. The quantitative RT-PCR detection of stearoyl acyl-carrier-protein desaturase from the *Saussurea involucrata* (*sikSACPD*) gene expression in tobacco under different temperatures [°C]. GAPDH was used as internal reference.

Semi-quantitative RT-PCR was employed to confirm the expression of the *sikSACPD* gene in *S. involucrata* during different temperature treatments. The expression of *sikSACPD* increased with decreasing temperatures. In particular, if the temperature was below zero, then the expression was very high (Fig. 1).

Transgenic tobacco plants were generated to examine the effects of the *sikSACPD* gene under Fab2 promoters on freezing stress tolerance. PCR and RT-PCR methods were used to identify transgenic plants (Fig. 1 Suppl.) and the seedling progeny was sprayed with 20 μg cm^{-3} hygromycin B to screen for positive plants.

Both transgenic and wild-type tobacco displayed normal growth at room temperature (20 °C; Fig. 2*A*,*B*), and even when the temperature decreased to 0 °C, there was little difference between the transgenic and wild-type plants in the phenotype (Fig. 2*D*). However, when the temperature was lowered to -2 °C for 2 h, the wild-type tobacco leaves showed slight curling (Fig. 2*E*). Finally, when the temperature was returned to room temperature for 1 week, the wild-type tobacco leaves became wilted and yellow, and the transgenic leaves showed relatively normal appearance (Fig. 2*F*).

Measurements of the MDA content showed an upward trend in both wild-type and transgenic tobacco (Fig. 3*A*). When the temperature dropped to 0 °C, the wild-type and transgenic tobacco leaves showed a different MDA content, and this difference gradually increased as the temperature dropped further. The MDA content in ProFAB2:FAB2 tobacco was always higher than in ProFAB2:SikSACPD tobacco, but this difference was only significant when the temperature was -2 °C.

A relative conductivity assay was performed to quantify the freezing tolerance of the wild-type and transgenic tobacco leaves. This assay indicates that the conductivity increased for all plant leaves with a decreasing temperature (Fig. 3*B*), but the injury was in order: wild-type > transgenic ProFAB2:FAB2 > transgenic ProFAB2:SikSACPD. The difference in conductivity between them also increased over time (data not shown).

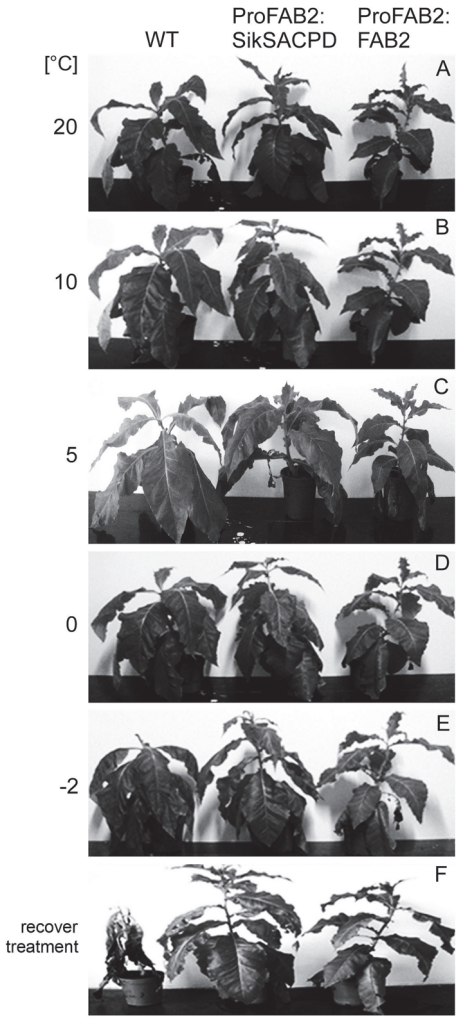

Fig. 2. Morphology of wild-type (WT), and transgenic ProFAB2:SikSACPD and ProFAB2:FAB2 tobacco plants grown at 20, 10, 5, 0, and -2 °C for 2 h (*A* - *E*), and recovering after -2 °C for 7 d (*F*).

When the temperature dropped to -2 °C, the wild-type tobacco leaf relative conductivity was almost twice of that in the transgenic tobacco indicating that the wild-type tobacco could not tolerate low temperatures.

The F_v/F_m values of wild-type and transgenic tobacco decreased under the cold stress (Fig. 3*C*). The F_v/F_m values of the transgenic ProFAB2:FAB2 tobacco declined less than in the transgenic ProFAB2:SikSACPD plants and much less than in the wild-type tobacco. This indicates that the PS II injury rate in the transgenic ProFAB2:SikSACPD tobacco was lower than in the transgenic ProFAB2:FAB2 plants, and that the injury rates in both the transgenics were lower than in the wild-type tobacco.

Because *SACPD* mediated the conversion of stearic acid (18:0) to oleic acid (18:1), which is a key step that regulates the content of unsaturated fatty acids in cells, the compositions of fatty acids were measured in both transgenic and wild-type tobacco (Table 2). When plants grown at 20 °C for 6 d were shifted to 10, 5, 0, and -2 °C, the proportion of oleic acid (C18:1) in the leaves of *sikSACPD* transgenic tobacco increased more compared with the leaves of non-transgenic tobacco (Fig. 4). The proportions of total desaturation products in the transgenic ProFAB2:SiKSACPD and ProFAB2:FAB2 plants increased by 26.3 and by 14.9 %, respectively, whereas in wild-type, they increased by about 10.3 % when the temperature decreased from 20 °C to -2 °C (Fig. 5).

Discussion

Our long-term goal is to reveal how *S. involucrata* maintains the integrity of its membrane system and its normal physiological functions under extreme cold. Low temperatures influence membrane lipids by changing the fatty acid composition and their relative content. The latter is especially of great significance. The stearoyl-ACP desaturation enzyme is the first enzyme in the conversion of stearic acid to oleic acid. As a key step, it determines the saturated and unsaturated fatty acid content and proportions. In this study, we isolated a *SACPD* from a *S. involucrata* cDNA library. This library was screened using a PCR-amplified fragment, *SACPD*, corresponding to the middle portion of the *S. involucrata* *SACPD* cDNA.

In this paper, *SikSACPD* has the highest homology to the *SACPDs* of *C. tinctorius* and *H. annuus* with a 91 and 86 % identity, respectively; however, although this gene shares characteristics common to *SACPD*, it may also have unique features. A large number of genes functioning in *S. involucrata* have evolved under an extreme environment and these need to be studied further.

Low temperature stress can cause membrane peroxidation leading to an increased membrane permeability and an increased electrolyte leakage (Gao *et al*. 1999). MDA is membrane lipid peroxidation product whose production is related to the degree of plant stress. Under low temperature stress, intracellular reactive oxygen species that degrade polyunsaturated lipids to MDA are produced. Similarly, when cells are exposed to drought stress, membrane oxidation and decomposition results in a massive accumulation of MDA (Yan *et al*. 2007).
Such oxidation causes massive cell membrane damage

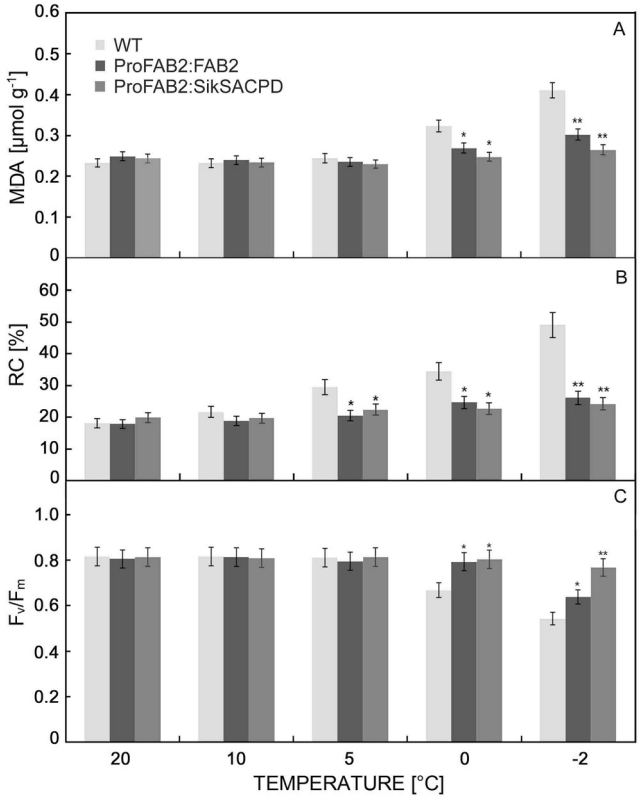

Fig. 3. The physiological analysis of wild-type (WT), and transgenic ProFAB2:SIKSACPD and ProFAB2:FAB2 tobacco plants after cold stress treatments: *A* - MDA content, *B* - relative conductivity, and *C* - F_v/F_m (the maximum efficiency of photosystem II photochemistry). Means of three replicates ± SE, *, ** - significant differences at $P \leq 0.05$ and 0.01, respectively.

Table 2. The analysis of fatty acid composition [%] and the content of desaturation products, DSP [%] in wild-type and ProFAB2:FAB2 and ProFAB2:SikSACPD transgenic tobacco under different temperatures. Means ± SE, $n = 3$.

Temperature	Plant	16:0	18:0	18:1	18:2	18:3	DSP
20 °C	wild-type	24.80±0.12	20.73±0.15	14.83±0.15	17.50±0.25	22.13±0.12	54.46
	ProFAB2:FAB2	24.63±0.26	18.93±0.20	16.60±0.25	17.60±0.15	22.23±0.03	56.43
	ProFAB2:SikSACPD	28.47±0.12	14.07±0.22	18.60±0.12	18.87±0.38	20.00±0.10	57.47
10 °C	wild-type	23.30±0.49	20.37±0.07	15.60±0.17	18.50±0.21	22.23±0.41	56.33
	ProFAB2:FAB2	23.83±0.09	17.87±0.19	17.57±0.22	17.93±0.18	22.80±0.29	58.31
	ProFAB2:SikSACPD	27.67±0.15	13.83±0.33	19.53±0.20	18.40±0.35	20.57±0.29	58.50
5 °C	wild-type	20.80±0.20	19.50±0.15	17.17±0.19	19.43±0.32	23.10±0.15	59.70
	ProFAB2:FAB2	19.97±0.26	17.87±0.26	19.43±0.18	19.53±0.35	23.20±0.12	62.16
	ProFAB2:SikSACPD	25.83±0.22	13.80±0.21	20.50±0.15	19.30±0.25	20.57±0.31	60.37
0 °C	wild-type	18.90±0.15	18.87±0.40	17.80±0.15	20.77±0.23	23.67±0.28	62.24
	ProFAB2:FAB2	17.17±0.23	16.77±0.18	20.13±0.24	21.37±0.03	24.57±0.18	66.07
	ProFAB2:SikSACPD	18.80±0.40	11.33±0.30	25.60±0.40	20.63±0.34	23.63±0.19	69.86
-2 °C	wild-type	20.17±0.15	19.10±0.15	15.27±0.12	22.67±0.52	22.80±0.40	60.74
	ProFAB2:FAB2	17.87±0.09	15.80±0.15	20.70±0.26	21.43±0.34	24.20±0.21	66.33
	ProFAB2:SikSACPD	10.97±0.22	11.10±0.26	31.77±0.18	21.90±0.35	24.27±0.03	77.94

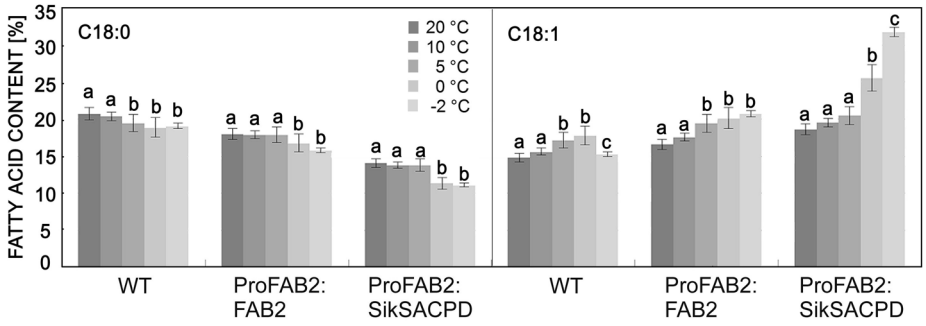

Fig. 4. The fatty acid content of wild-type (WT) and transgenic plants ProFAB2:FAB2 and ProFAB2:SIKSACPD under different temperatures. Means of three replicates ± SE, different letters indicate significant differences at $P \leq 0.05$.

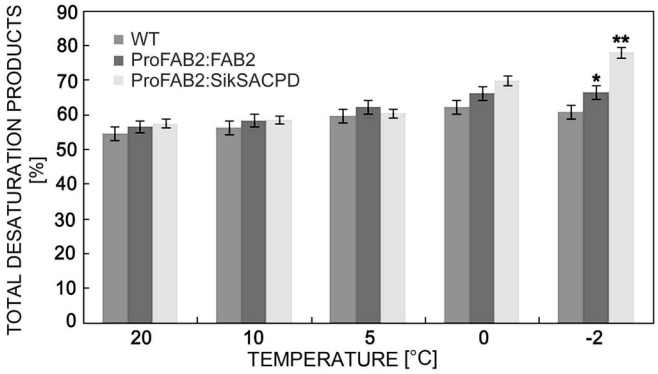

Fig. 5. Proportions of total desaturation products in wild-type (WT), and transgenic ProFAB2:SIKSACPD and ProFAB2:FAB2 tobacco plants. Means of three replicates ± SE, *, ** - significant differences at $P \leq 0.05$ and 0.01, respectively.

(He *et al*. 2007). Here, we found that under the low temperature stress, the MDA content and relative electrical conductivity in the transgenic leaves were lower than in the wild-type, and the difference gradually increased with the prolonged stress. This indicates that the expression of the *sikSACPD* gene resulted in the protection of the cell membrane. PS II photochemical efficiency (F_v/F_m) changes are also indicators of abiotic stress (Chen *et al*. 2006). Mauro *et al*. (1997) showed that low temperature affects the PS II antenna, reaction center, and electron transport activity in maize, finally resulting in the photodamage of the reaction center. Our study shows that the low temperature stress led to reduced F_v/F_m values in the tobacco leaves, and the F_v/F_m decrease in the transgenic tobacco leaves was lower than in the wild-type indicating that the *sikSACPD* gene could effectively protect the PS II reaction center.

De novo fatty acid (FA) synthesis occurs exclusively in plastids and leads to the accumulation of palmitic acid (16:0)-ACP and oleic acid (18:1)-ACP (Ohlrogge *et al*. 1995). Desaturation of stearic acid (18:0)-ACP to 18:1-ACP catalyzed by SSI2/FAB2 encoded *SACPD* is a key step in the FA biosynthesis pathway that regulates unsaturated FA content in the cell. We also know that the freezing tolerance of plants correlates with the degree of unsaturation of membrane lipids. In this study, we found that the correlation between the freeze tolerance and the *SACPD* gene expression is greater in *S. involucrata* than in other plants, and greater than between freeze tolerance and the *Fab2* gene in *Arabidopsis*. By comparing the functional difference in freeze tolerance between these two stearoyl-ACP desaturation enzymes, sikSACPD and Fab2, it was revealed that sikSACPD has unique characteristics.

With further research and the use of new technology and methods, the structure and function of *SACPD* will be revealed. Meanwhile, this sikSACPD can be used to improve plant frost resistance.

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