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# Virus induced *PhFTRv* gene silencing results in yellow-green leaves and reduced cold tolerance in petunia

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## Abstract

Ferredoxin-thioredoxin reductase (FTR) is an iron-sulfur protein that supplies electrons from photochemically reduced ferredoxin (Fd) to thioredoxin (Trx) in the ferredoxin/thioredoxin system in chloroplasts. The FTR is a heterodimer with a variable subunit (FTRv) and a catalytic subunit (FTRc). The function of *FTRv* is not well known. In petunia (*Petunia hybrida*), *FTRv* is a single-copy gene, which is named *PhFTRv*. In this study, the spatio-temporal expression of *PhFTRv* in petunia was analyzed, and *PhFTRv* transcription was found to be high in leaves and stems. A tobacco rattle virus gene silencing was used in this study. Virus induced gene silencing-mediated *PhFTRv* silencing resulted in large yellow-green leaves, delayed flowering, and reduced cold tolerance in petunia plants.

*Additional key words*: flowering, chloroplasts, chlorophyll, leaf development, *Petunia hybrida*, photosynthesis, temperature stress.

## Introduction

Thioredoxins (Trxs) serve as a key factor in the redox system that regulates the activity of a series of target proteins through dithiol-disulfide exchange reactions in plants. Chloroplasts, which serve as the site of photosynthesis, contain at least two systems of thiol-disulfide reductases that function in the control of redox-dependent protein stability and/or activity (Geigenberger and Fernie 2014, Nikkanen *et al.* 2016). The ferredoxin/thioredoxin (Fd/Trx) system plays important roles in chloroplasts (Buchanan *et al.* 2002). Ferredoxin-thioredoxin reductase (FTR), an iron-sulfur protein, supplies electrons from photochemically reduced ferredoxin to thioredoxin in chloroplasts under light conditions and plays an important role in the Fd/Trx regulatory chain (Dai *et al.* 2000). The FTR could efficiently reduce chloroplast f- and m-type Trxs. The reduced thioredoxins activate target enzymes, thereby activating anabolic pathways in metabolism (Dai *et al.* 2000). In addition, plastids contain an NADPH-dependent thioredoxin reductase C (NTRC), which is a key redox-mediator protein activated by NADPH both under light and dark conditions and responsible for regulatory functions that is able to catalyse thiol-disulfide exchange reactions in target enzymes (Serrato *et al.* 2004, Yoshida and Hisabori 2017). Both FTR and NTRC act as key regulators of ATP synthase by regulating the γ subunit under limited light conditions (Luo *et al.* 2012, Yoshida *et al.* 2013). In plants, studies of the Trx system have mainly focused on stress resistance (Lim *et al.* 2010, Dietz and Pfannschmidt 2011, Chae *et al.* 2013, Belin *et al.* 2015).

Ferredoxin-thioredoxin reductase is a heterodimer with a variable subunit (FTRv) and a catalytic subunit (FTRc). In plants, both subunits are encoded in the nucleus (Schurmann and Jacquot 2000). FTRc contains a redox-active disulfide and a [4Fe-4S] centre (Schurmann and Jacquot 2000). FTR has been crystallized, and its structure has been solved. The variable subunit has an open β-barrel structure, and the catalytic subunit is essentially α-helical (Dai *et al.* 2000). In *Solanum lycopersicum*, *SlFTRc* silencing induces the expression of pathogenesis-related genes and pathogen resistance, and SlFTRc acts as a regulator of programmed cell death (PCD) and pathogen resistance (Lim *et al.* 2010). In *A. thaliana*, the young inner leaves of *FTRc*-silenced plants exhibit a sectorial chlorotic leaf phenotype and chloroplasts in *FTRc*-silenced leaves are not fully developed (Wang *et al.* 2014).

In *A. thaliana,* there are two FTRv isoforms, AtFTRA1

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*Abbreviations*: $c_i$ - internal $CO_2$ concentration; Fd - ferredoxin; FTR - ferredoxin-thioredoxin reductase; $g_s$ - stomatal conductance; NTRC - NADPH-dependent thioredoxin reductase C; $P_N$ - net photosynthetic rate; TRV - tobacco rattle virus; Trx - thioredoxin; VIGS - virus induced gene silencing.
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and AtFTRA2, and only single *AtFTRA1* mutant plants exhibit subtle developmental changes when compared to wild-type plants (Keryer *et al.* 2004). Plants with disrupted *AtFTRA* were found to be significantly more sensitive to oxidative stress (Keryer *et al.* 2004). There is only one *FTRv* gene in some species of *Solanaceae*, such as petunia (*Petunia hybrida*), tomato, and pepper. Thus, it is necessary to clearly define the biological function of *FTRv*.

The aim of this study was to analyze the spatiotemporal expression of *PhFTRv* in petunia as affected by a low temperature and virus induced gene silencing.

## Materials and methods

**Plant materials and growth conditions:** Petunia (*Petunia hybrida* J.D. Hooker Vilmorin) cv. Ultra was grown in a culture chamber with a 14-h photoperiod, day/night temperatures of 24/12 ℃, a relative humidity of 60 - 70 %, and an irradiance of 250 μmol $m^{-2}$ $s^{-1}$. Flower, leaf, stem, and root tissues were collected from 4- to 8-week-old petunia plants. Samples from the corollas were harvested at anthesis.

**RNA extraction and real-time quantitative PCR assays:** Total RNA was extracted and reverse-transcribed according to previous methods (Liu *et al.* 2011). Total RNA was extracted using *TRIzol* according to the manufacturer's instructions and treated with DNase using amplification grade DNase (*Sangon Biotech*, Guangzhou, China). The sequences of *PhFTRv* were obtained from the petunia genome (https://solgenomics.net/organism/Petunia_axillaris/genome). The kit (*TSE202*, *Tsingke*, Guangzhou, China) was used for quantitative PCR (qPCR) analysis. The qPCR procedure is set according to the instructions of the kit: 95 ℃/60 s, 95 ℃/10 s, 55 ℃/5 s, 72 ℃/10 s, 40 cycles. The petunia *Actin* (accession No. FN014209) was used as internal reference gene to quantify the cDNA abundances. Melting curve analysis is referred to Chang *et al*. (2016), data analysis is carried out by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). The sequences of all primers used for qPCR analysis are in Table 1 Suppl.

**Sequence analysis:** Alignments were performed using *DNAMAN* software, and a phylogenetic tree was generated using *ETE3 v3.1.1* software (https://www.genome.jp/tools-bin/ete). Searches of nucleotide and translated amino acid sequences were performed using the National Center for Biotechnology Information (*NCBI*) *BLAST* network server (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

**Virus induced gene silencing (VIGS) assay:** A tobacco rattle virus (TRV)-based VIGS system described previously was used to silence the *PhFTRv* gene (Chen *et al*. 2017). The TRV based cDNA clone was placed between the CaMV 35S promoter with duplicated enhancer region and the nopaline synthase terminator in a T-DNA vector. The pTRV1 vector (AF406990, GenBank) includes RNA1 part of T-DNA construct and pTRV2 vector (AF406991, GenBank) includes RNA2 part of T-DNA construct, pTRV1 and pTRV2 must be used at the same time in an infection (Liu *et al*. 2002). To construct the pTRV2-FTRv vectors, the coding region of *PhFTRv* was amplified by real time qPCR from a petunia cDNA library with forward primer 5'CGGGATCCCTAACAACCCTTTATCCC3' and reverse primer 5'CGGAATTCATACTGTTTCAATGTCCC3'. The method of VIGS was described previously (Chen *et al.* 2017). The pTRV2 vector or its derivatives and the pTRV1 vector were simultaneously introduced into *Agrobacterium tumefaciens* L. strain EHA105. The *A. tumefaciens* strains containing plasmid constructs were grown overnight at 28 ℃ in Luria-Bertani medium with 50 mg $dm^{-3}$ kanamycin and 200 mM acetosyringone. The cells were harvested and resuspended in sterile 10 mM MES and 10 mM $MgCl_2$ buffer with 200 μM acetosyringone. The bacteria containing pTRV1 were mixed with bacteria containing the pTRV2 derivatives in a 1:1 ratio, and the absorbances ($A_{600}$) were measured before the infiltration of petunia plantlets. The expressions of *PhFTRv* and *PhFTRc* in *PhFTRv*-silenced plants were determined by qPCR.

**Photosynthesis measurements and analyses:** Photosynthesis in leaves was measured according to a method described in a previous study (He *et al.* 2018). Three healthy leaves were selected from each plant from among the 3rd to 4th leaves on the middle-upper branches. A *GFS-3000* portable photosynthetic system (*Walz*, Effeltrich, Germany) was used to monitor the response of photosynthesis to irradiance in the selected leaves at 11:00 every day. We then obtained values for net photosynthetic rate ($P_N$), stomatal conductance ($g_s$), and intercellular $CO_2$ concentration ($c_i$). The measurements were repeated three times for each leaf, and three biological replicates were performed.

**Determination of chlorophyll content:** Chlorophyll content was determined according to our previously described protocol (Liu *et al.* 2019). Approximately 1.5 g of leaves were harvested and freeze-dried, and three replicate methanol extractions were prepared. The absorbance of the solution was measured at 646.8, 663.2, and 470.0 nm and compared to that of the solvent (methanol) blank. The content of total chlorophyll was measured with a spectrophotometer and calculated according to Lichtenthaler (1987).

**Statistical analysis:** The data were analyzed using *SPSS v. 11.09* (*IBM Corporation*, Armonk, NY, USA) and are presented as the means of three replicates. A least significant difference (LSD) test was used to analyze the differences between the means. The significance level was set at $\alpha < 0.05$.

## Results

To obtain the sequences of the *PhFTRv* cDNAs in petunia, the amino acid sequence of the *AtFTRv* gene from *A. thaliana* (AtFTRA1, locus BAB09560) (Keryer

*et al.* 2004) was used as a query for *BLAST* searches of the *Petunia axillaris* draft genome sequence (v1.6.2); only one *FTRv* sequence from petunia was recovered. *PhFTRv* was predicted to encode a protein containing 155 amino acids. The multiple sequence alignments of petunia PhFTRv, *A. thaliana* AtFTRA1 (AT5G23440) and AtFTRA2 (At5g08410), *Solanum lycopersicum* SlFTRv (XP_004229646), *Nicotiana tomentosiformis* NtFTRv (XP_016508304), *Oryza sativa* OsFTRv1 (XP_015634256) and OsFTRv2 (XP_015623472) are presented in Fig. 1*A*. The protein alignment showed that the carboxyl-terminus of FTRv in different species showed high sequence similarity, while the sequence similarity of the amino-terminus was very low (Fig. 1*A*). In addition, FTRv contained a conserved EDEFE motif in its carboxyl-terminus. PhFTRv shared 78.1 and 74.5 % amino acid sequence similarity with NtFTRv and SlFTRv, respectively (Table 2 Suppl.).

To elucidate the evolutionary relationships among FTRv proteins in dicot and monocot species with genome sequences that have not been published, we examined FTRv-like sequences in 14 dicot and 4 monocot species that were randomly selected, and a phylogenetic tree was generated using *MEGA* software with amino acid sequences derived from GenBank (Fig. 1*B*, Fig. 1 Suppl.). The phylogenetic tree showed that the FTRv family in dicot species included one or two members, and three members were found only in *Brassica rapa*. In the monocot species, the FTRv family generally included two members, and only three members were found in *Ananas comosus* (Fig. 1 Suppl.).

The expression of *PhFTRv* was examined in different plant organs by real time qPCR. The *PhFTRv* transcription was high in leaves and stems and low in roots (Fig. 2*A*). A previous study demonstrated the role of *S. lycopersicum* SlFTRc in plant stress responses, and the lesion mimic phenotypes related to defense in *S. lycopersicum slftrc* mutants are dependent on temperature (Lim *et al*. 2010). The effects of different temperatures on the expression of *PhFTRv* were examined, and the results showed that low temperatures increased the transcription of *PhFTRv* (Fig. 2*B*).

Four to five weeks old petunia seedlings were infected with a mixture of *Agrobacterium* transformed with pTRV2-PhFTRv or pTRV2 vectors and *Agrobacterium* transformed with the pTRV1 vector. The expressions of *PhFTRv* and *PhFTRc* (Peaxi162Scf00129g00821.1) were examined in plants inoculated with *Agrobacterium* containing pTRV2-FTRv and pTRV2 by real time qPCR. Although *PhFTRv* transcripts were detected in the *PhFTRv*-silenced plants, the expression of *PhFTRv* was significantly reduced compared with control plants that were inoculated with *Agrobacterium* containing the empty pTRV2 vector (Fig. 2*C*). The expression of *PhFTRc* was not affected by *PhFTRv* silencing (Fig. 2*D*).

The newly emerged leaves of plants infected with *Agrobacterium* containing pTRV2-PhFTRv exhibited a yellow-green leaf phenotype, while those infected by the

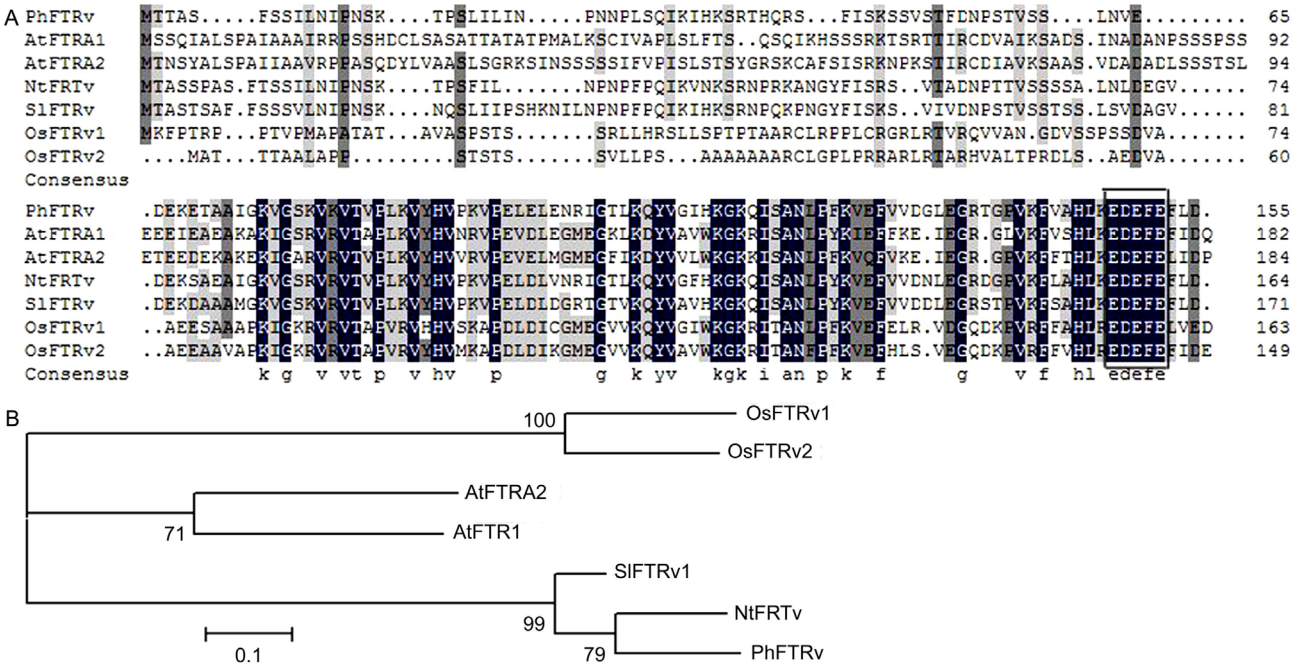


Fig. 1. Predicted amino acid sequence alignments and neighbour-joining trees of ferredoxin-thioredoxin reductase variable subunit (FTRv). *A* - predicted amino acid sequence alignments of petunia PhFTRv with *Arabidopsis thaliana* AtFTRA1 (BAB09560) and AtFTRA2 (At5g08410), *Solanum lycopersicum* SlFTRv (XP_004229646), *Nicotiana tomentosiformis* NtFTRv (XP_016508304), and *Oryza sativa Os*FTRv1 (XP_015634256) and *Os*FTRv2 (XP_015623472), respectively. Conserved residues are shaded in *black*. *Grey* shading indicates similar residues in six out of seven of the sequences. *Light grey* shading indicates similar residues in four out of seven of the sequences. The carboxyl-terminal EDEFE is *boxed*. *B* - neighbour-joining trees among proteins encoded by the FTRv-like genes using the *ETE3 v3.1.1* software (https://www.genome.jp/tools-bin/ete).

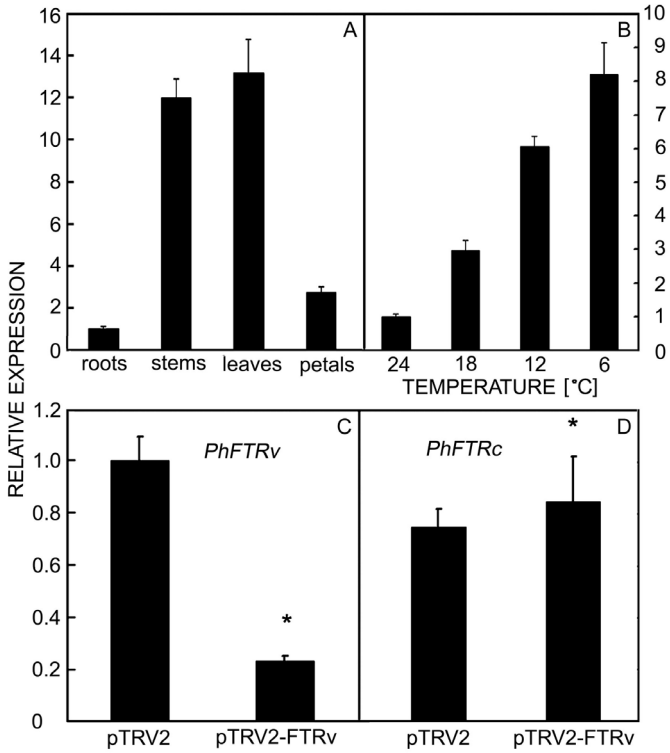


Fig. 2. The expression patterns of *Petunia hybrida ferredoxin-thioredoxin reductase variable subunit* (*PhFTRv*) and *Petunia hybrida ferredoxin-thioredoxin reductase catalytic subunit* (*PhFTRc*) determined by real time quantitative PCR (qPCR). *A* and *B* - the expression patterns of *PhFTRv* in different organs (*A*) and in leaves (*B*) in response to different temperatures. *C* and *D* - the effects of pTRV2-PhFTRv treatment on the expression of *PhFTRv* (*C*) and *PhFTRc* (*D*) in the fifth leaves under the buds from five-week-old plants as determined by real time qPCR. *Actin* was used as an internal reference gene to quantify the cDNA abundance. The relative expressions are presented as fold-change values. Means ± SDs, $n = 3$, * - significant differences at $P < 0.05$.

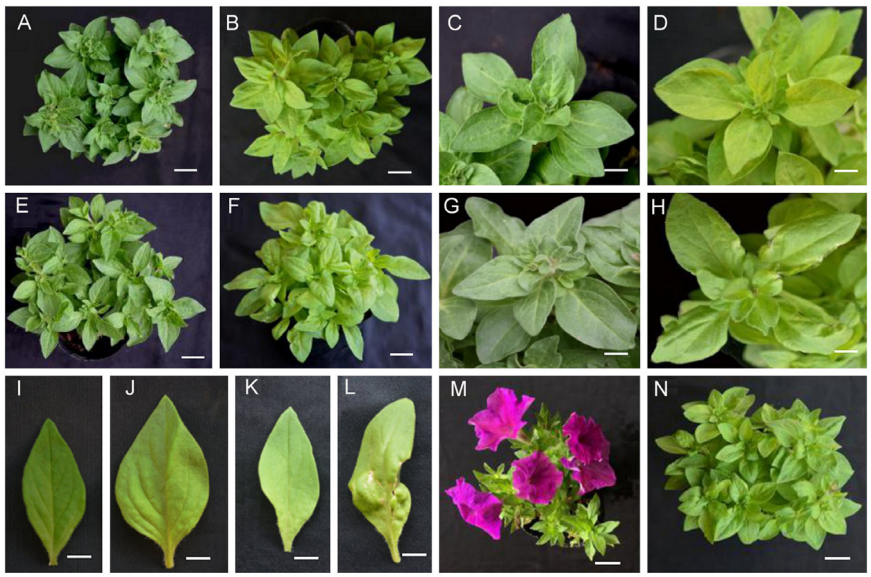


Fig. 3. The phenotypes of *Petunia hybrida ferredoxin-thioredoxin reductase variable subunit* (*PhFTRv*)-silenced plants in response to different temperatures. *A-D* - different leaf colors in four-week-old plants infected with *Agrobacterium* with *plasmid tobacco rattle virus 2* (*pTRV2*) (*A* and *C*) or *pTRV2-PhFTRv* (*B* and *D*) and grown at 24 °C; *E-H* - different leaf colors in four-week-old plants with *pTRV2* (*E* and *G*) or *pTRV2-PhFTRv* (*F* and *H*) grown at 12 °C; *I-J* - leaves of control (*I*) and *PhFTRv*-silenced (*J*) plants grown at 24 °C, and leaves of control (*K*) and *PhFTRv*-silenced (*L*) plants grown at 12 °C; *M-N* - differences in flowering in seven-week-old plants with *pTRV2* (*M*) and *pTRV2-PhFTRv* (*N*) grown at 24 °C.

Table 1. Effects of *Petunia hybrida ferredoxin-thioredoxin reductase variable subunit* silencing on plant growth. Means ± SEs from 15 to 20 samples. Statistical analysis was performed using Student's *t*-test; *asterisks* mean significant differences at $P < 0.05$. pTRV2 - plasmid tobacco rattle virus 2, pTRV2-FTRv - pTRV2- ferredoxin-thioredoxin reductase variable subunit.

| | pTRV2 (control) | pTRV2-FTRv | pTRV2-FTRv/pTRV2 [%] |
|---|---|---|---|
| Length of leaf [cm] | 4.38 ± 0.33 | 5.39 ± 0.43* | 123.1 |
| Width of leaf [cm] | 2.52 ± 0.21 | 3.14 ± 0.26* | 124.6 |
| Diameter of abaxial epidermal cells of leaf [μm] | 91.28 ± 5.29 | 113.39 ± 6.52* | 122.3 |

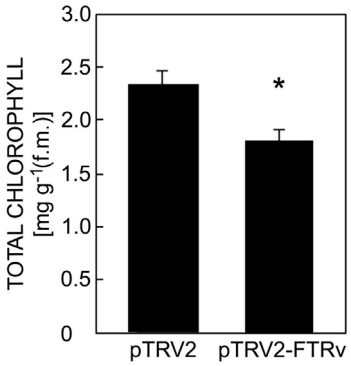


Fig. 4. Effects of *Petunia hybrida ferredoxin-thioredoxin reductase variable subunit* silencing on the total chlorophyll content in leaf mesophyll cells from five-week-old plants. pTRV2 - plasmid tobacco rattle virus 2, pTRV2-FTRv - plasmid tobacco rattle virus 2-ferredoxin-thioredoxin reductase variable subunit. Means ± SDs, $n = 3$, * - significant differences at $P < 0.05$.

*Agrobacterium* containing pTRV2 (control plants) showed a green leaf phenotype (Fig. 3*A-D*). The leaves of plants containing pTRV2-PhFTRv maintained a yellow-green leaf phenotype until they became old. Compared with the control plants, the size of the leaves in the plants containing pTRV2-PhFTRv was larger (Fig. 3*I,J*, Table 1), the content of chlorophyll in the leaves was significantly reduced (Fig. 4), and the epidermal cells on the abaxial surface were larger (Fig. 2 Suppl., Table 1).

The effect of *PhFTRv* silencing on leaf physiological parameters was further analyzed. The internal $CO_2$ concentration increased significantly in *PhFTRv*-silenced leaves, while *PhFTRv* silencing significantly decreased $P_N$ and $g_s$ ($\alpha = 0.05$) (Fig. 5).

In variegated mutants, most leaves have pale green areas with abnormally small mesophyll cells and reduced numbers of chloroplasts (Sakamoto *et al.* 2003). To determine the effect of *PhFTRv* suppression on the number of chloroplasts, the protoplasts in mature leaves were extracted and observed under an optical microscope. The numbers of chloroplasts in mesophyll cells in *PhFTRv*-silenced plants were reduced compared with those in control plants (Fig. 2 Suppl.).

In addition, a delay of two weeks for the emergence of the floral buds was observed in *PhFTRv*-silenced plants, and *PhFTRv* silencing delayed flowering by approximately ten days (Fig. 3*M,N*). Moreover, even when blooming, the number of flowers in *PhFTRv*-silenced plants was less than in control plants.

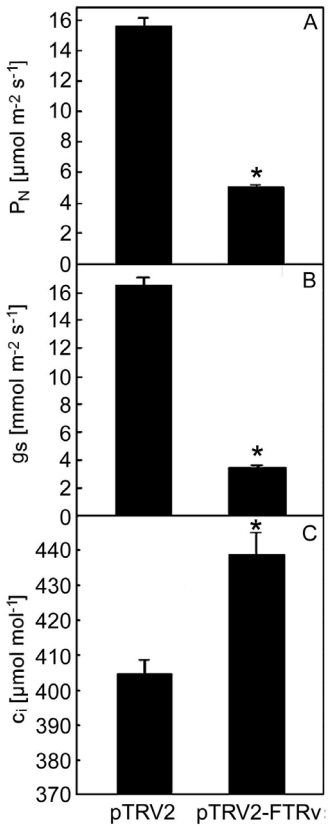


Fig. 5. Effect of *Petunia hybrida ferredoxin-thioredoxin reductase variable subunit* silencing on photosynthesis characteristics in leaves of petunia grown in pots in a greenhouse. $P_N$ - net photosynthetic rate, $g_s$ - stomatal conductance, $c_i$ - intercellular $CO_2$ concentration. pTRV2 - plasmid tobacco rattle virus 2, pTRV2-FTRv - plasmid tobacco rattle virus 2-ferredoxin-thioredoxin reductase variable subunit. Means ± SEs, $n = 3$, * - significant differences at $P < 0.05$.

A previous study suggested that *FTRv* is involved in stress response (Keryer *et al.* 2004). After infection with *Agrobacterium* containing pTRV2-FTRv and pTRV2, the plants were subjected to low temperatures (12 °C). Compared with control plants, the young leaves in the *PhFTRv*-silenced plants were wrinkled, and distinctive necrotic lesions appeared after 3 weeks in mature leaves in *PhFTRv*-silenced plants grown at 12 °C (Fig. 3*E-H,K,L*), which was not observed at 24 °C (Fig. 3*A-D*). In addition, the electrolyte leakage from cells of the *PhFTRv*-silenced leaves was significantly increased compared with that

in control leaves (Fig. 3 Suppl.). These results showed that *PhFTRv* silencing resulted in the reduction of cold tolerance in the plants.

## Discussion

The protein sequence alignments of the FTRvs showed that the carboxyl-termini had very high homology, which suggested that the carboxyl-terminus of FTRv might play an important role. Phylogenetic analysis showed that the small FTRv family has different copy numbers in different plant species. Petunia and other species of the *Solanaceae* family possess one copy of FTRv, while most monocot species contain two members. The present work involved the isolation of PhFTRv from petunia and the identification of its function.

Previous studies have shown that *FTR* functions in chloroplasts (Yoshida and Hisabori 2017). *PhFTRv* transcription was high in leaves and stems, where there are many chloroplasts in the cells, showing that the expression of *FTRv* genes is consistent with their function and characteristics.

In *A. thaliana,* there are two FTRv isoforms, AtFTRA1 and AtFTRA2, and the single *AtFTRA1* mutant exhibited phenotypic perturbations when compared with wild-type plants (Keryer *et al.* 2004). There is only one *FTRv* gene, named *PhFTRv*, in petunia. In petunia, *PhFTRv* silencing reduced chlorophyll content in leaves and delayed flowering. FTR converts a light-activated electron signal into a thiol signal that is further transmitted by Trxs. The Trx system regulates carbon flow and other biochemical processes (Schurmann and Buchanan 2008). Reduced activity of the FTR system down-regulated $CO_2$ fixation (Buchanan 2016, Nikkanen *et al.* 2016). Due to the reduced $CO_2$ fixation, the whole photosynthetic activity including photochemical reactions can be affected. In addition, a variegated leaves have been observed in the young *FTRc*-silenced *A. thaliana* and *inap1* (*ftrc*) mutant plants, and the sectors near the petioles of young leaves in *FTRc*-silenced plants appeared to be conspicuously chlorotic (Wang *et al.* 2014), although the characterized T-DNA insertional *ftrc* mutant showed no apparent physiological abnormalities due to the leaky FTR protein expression (Yoshida and Hisabori 2017).

In *A. thaliana inap1* (*ftrc*) mutants, there are pale green areas in leaves that have abnormally small mesophyll cells and reduced numbers of chloroplasts (Hashida *et al.* 2018). Similarly, in this study, *PhFTRv* silencing resulted in the reduction of the number of chloroplasts in mesophyll cells (Fig. 2 Suppl.). Wang *et al.* (2014) suggested that *A. thaliana* FTRc probably participates in the regulation of chloroplast development. In addition, in *Pisum sativum*, a phenotype characterized by pale-green leaves was observed in plants with VIGS-mediated silencing *TRX-M* and *TRX-F*, and TRX-F regulates the ATPase activity of the magnesium chelatase CHLI subunit (Luo *et al*. 2012). Overall, the decrease in chlorophyll content in *PhFTRv*-silenced plants may be attributed to the change in the number and development of chloroplasts and/or the ATPase activity of the magnesium chelatase CHLI subunit. In addition, *PhFTRv* silencing significantly decreased the $P_N$ and $g_s$ and significantly increased the $c_i$ in leaves, indicating the significance of chloroplast thioredoxin systems in maintaining photosynthetic efficiency in petunia.

In tomato, distinctive necrotic lesions appear after 3 weeks in *SlFTRc*-silenced plants cultured at 20 °C, while necrotic lesions in *SlFTR*c-silenced plants are completely suppressed at 26 °C (Lim *et al.* 2010). Similarly, in this study, *PhFTRv* silencing resulted in distinctive necrotic lesions in leaves under low temperature conditions at 12 °C, while there were no necrotic lesions in *PhFTRv*-silenced leaves at 24 °C. It is worth noting that petunia is more resistant to cold than tomato. In addition, the expression of *PhFTRv* was increased under low temperature (Fig. 2*B*), and *PhFTRv* silencing resulted in wrinkled, distinctive necrotic lesions and higher electrolyte leakage from cells leaves. It seems that PhFTRv functions as a regulator of low temperature resistance in petunia plants. In addition, *PhFTRv* silencing could decrease the activity of Calvin cycle (Balsera *et al.* 2013, Buchanan 2016, Nikkanen *et al*. 2016), which results in lower production of sugars that can affect the response to low temperature. In *A. thaliana*, the overexpression of *NTRC* resulted in enhanced tolerance to heat shock, whereas NTRC knockout mutant plants exhibited a temperature-sensitive phenotype (Chae *et al.* 2013). Core chloroplastic TRXs (TRXf and TRXm) exhibit a slight tendency towards decreased expression in response to almost all stressors, including cold stress (Belin *et al.* 2015). Previous studies suggested that SlFTRc acts as a regulator of programmed cell death and pathogen resistance in tomato plants (Lim *et al.* 2010). Moreover, previously characterized oxidative-stress-sensitive *A. thaliana* plants with *FTRA* mutations appear normal until they are exposed to stress conditions (Keryer *et al.* 2004). These results show that the TRX system is associated with many forms of stress.

In this study, *PhFTRv* silencing resulted in larger leaves and epidermal cells compared with the control (Fig. 3*I,J* and Fig. 2 Suppl.) and lower activity of Calvin cycle also can indirectly effect developmental processes including the size of leaves. Together with the effects of *PhFTRv* silencing on leaf pigments and division of chloroplast, these results show that PhFTR activity affects leaf development. However, there were no obvious differences in leaf morphology between the control and *SlFTRc*-silenced plants in tomato (Lim *et al.* 2010). These results could indicate the different functions of PhFTRv, the variable subunit, and SlFTRc, the catalytic subunit.

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