Silencing of fatty acid desaturase (FAD7) gene enhances membrane stability and photosynthetic efficiency under heat stress in tobacco (Nicotiana benthamiana)

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Maintenance of cell membrane stability under heat stress is considered as an important adaptive mechanism. In this study, an attempt was made to improve membrane stability under high temperature stress through downregulation of fatty acid desaturase (FAD7) gene by post-transcriptional gene silencing (PTGS) approach using virus-induced gene silencing (VIGS) system in order to decrease trienoic fatty acids. Endogenous FAD7 gene fragment from Nicotiana benthamiana was cloned to construct VIGS vector (pTRV2: FAD7). The Agrobacterium strain harboring the pTRV2: FAD7 was infiltrated into 4-leaf stage N. benthamiana. The downregulation of FAD7 gene in VIGS plants were confirmed through semi quantitative RT-PCR. The FAD7 gene downregulated plants were exposed to normal and 35 and 42°C temperature stress. In FAD7 silenced plants, the content of linolenic acid (18:3) decreased significantly, while linoleic acid (18:2) increased under high-temperature stress. FAD7 silenced plants showed higher net photosynthetic rate and PS-II efficiency under high-temperature stress as compared to control and empty vector transformed plants. Conversely, lower H2O2 content, membrane damage, electrolyte leakage and antioxidative enzyme activities were observed in silenced plants as compared to control. The FAD7 silenced plants exhibited better tolerance to high temperature than wild type due to higher dienoic to trienoic fatty acids ratio imparting membrane stability. The study concludes that alteration in fatty acid composition in thylakoid membranes can lower oxidative stress, improve photosynthetic rate, PS-II efficiency in plants under high temperature stress.

Keywords: Abiotic stress, Fatty acid desaturase (FAD7) gene, Gene silencing, Heat stress, Photosystem-II, Post-transcriptional gene silencing (PTGS), Thylakoid membranes, Virus-induced gene silencing (VIGS)

High temperature or heat stress is one of the abiotic stresses that can affect plant growth and development. Under present climate change scenario, global mean temperatures may increase between 1.6ºC and 6.9ºC by the end of this century.1 High temperature stress often leads to oxidative stress2 and causes various physiological and biochemical detrimental changes in plants.3 Photosynthesis is considered highly sensitive to high temperature4 and often inhibited before other cell functions are impaired5,6. Impaired photosynthetic response induces cellular energy imbalance causing alteration in redox state associated with thylakoid membranes.7 Photosystem-II (PS-II) is most thermosensitive component of light reaction of photosynthesis8. Increased fluidity of thylakoids membranes causes dislodging of PS-II light harvesting complexes and affect electron transport and phosphorylation under high temperature stress9.

A defined proportion of saturated and unsaturated fatty acids in membrane lipids is required for photosynthetic thermostability10 and maintaining appropriate membrane fluidity11. Fatty acid desaturase 7 (FAD7) plays important role in maintaining the composition of membrane lipids12,13. Plants can modify their lipid composition through regulation of by ω-3 fatty acid desaturases14. Various studies have shown that the accumulation of saturated fatty acids in polar lipids contributes to the maintenance of membrane fluidity under adverse environment12,15. High temperature stress is associated with production of reactive oxygen species (ROSs) which can damage photosynthetic membranes and reduce photosynthetic yield1. Excessive accumulation of ROSs under stress leads to lipid peroxidation and membrane damage and affects various cellular activities2,16. To counter oxidative damage caused by ROS production, plants

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have developed various enzymatic and non-enzymatic antioxidant systems. Increased antioxidative enzyme activities facilitate the removal of excessive ROS and check lipid peroxidation. Various approaches have been used to impart tolerance for high temperature stress. Most prominent are engineering chaperone activity through synthesis of HSPs, levels of compatible osmolytes, antioxidation and detoxification pathways. Membrane engineering for increased level of saturated fatty acids in membrane lipids determining high temperature tolerance in plants have been suggested. Virus induced gene silencing (VIGS) is based on RNA-mediated post-transcriptional gene silencing (PTGS), which targets viral RNA in a sequence-specific manner, is one of the most widely used tools for plant functional genomics during the last decade. Although the precise VIGS mechanisms still being studied, a clear outline of the VIGS processes has been defined. In a recent study, RNA silencing was induced in nontransgenic tobacco scions after grafting onto silenced rootstocks, and virus resistance was conferred to the nontransgenic scions.

In this study, we downregulated FAD7 gene in Nicotiana benthamiana Domin and described the effect of FAD7 silencing on membrane composition, underlying the detoxification mechanism of ROS and photosynthetic activity under high-temperature stress using PTGS/VIGS approach.

Materials and Methods

Plant material

The seeds of Nicotiana benthamiana were germinated in trays containing soilrite and later transplanted to pots containing the potting mixture, FYM and sand (2:1:1 ratio). Plants were irrigated twice a day using hoagland solution and soil water status was maintained at 90-100% field capacity. Four leaf staged plants were used as plant material for the study.

Cloning of FAD7 gene homolog from Nicotiana benthamiana

Total RNA was isolated from the leaves of Nicotiana benthamiana plants. About 5µg of total RNA was reverse transcribed to cDNA with oligodT primer using Molony Murine Leukemia Virus Reverse Transcriptase (MMLV-RT). PCR-based cloning approach was followed to clone partial FAD7 gene fragment from Nicotiana benthamiana cDNA. Primers FAD-7F (5’-GTTCACGTCTCTGGTGGGACA-3’) and FAD-7R (5’-GTTCACGTCTCTGGTGGGACA-3’) were designed based on the sequence information from Nicotiana tabacum with the help of DNA STAR software (Primer Select, DNA Star Inc.).

The PCR amplified products were fractionated on agarose (0.8% w/v) gel and eluted using GenElute™ Gel Extraction kit (Sigma, USA). The eluted fragments were cloned with T/A cloning vector using InsT/A clone PCR product cloning kit (MBI, Fermentas Life Sciences), and mobilized into E. coli cells. The recombinant colonies were selected, plasmids isolated and confirmed by restriction profile, sequencing and BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST).

Off targets prediction for FAD7 gene

RNA silencing/interference for suppressing gene expression rely on specific and efficient silencing of particular gene/s or gene families. The understanding of off-target silencing is crucial for accurate interpretation of gene function by PTGS. The web-based computational tool siRNA Scan (http://bioinfo2.noble.org/RNAiScan.htm) was used to identify potential off-targets for FAD7 gene homolog to design better construct for PTGS.

Construction of silencing vector

FAD7 gene fragment was released from pTZ57R/T cloning vector and cloned into VIGS vector, pTRV2 at Xbal and BamHI sites. VIGS silencing construct (pTRV2: PDS) containing a fragment of phytene desaturase (PDS) gene from N. benthamiana was used to silence the endogenous PDS gene and treated as positive control in all silencing experiments. All constructs (pTRV1; pTRV2: PDS; pTRV2: FAD7) were mobilized into Agrobacterium cells (strain GV2260) and recombinant clones were identified.

Agrobacterium infiltration to induce FAD7 gene silencing

The Agrobacterium strains harboring the pTRV1; pTRV2: FAD7; pTRV2: PDS and pTRV2 without insert (mock) were infiltrated into 4-leaf stage N. benthamiana plant. Agrobacterium harboring the pTRV2 carrying the gene of interest and pTRV1 was mixed in equal proportion (1:1) and the mixture was used to infiltrate the test plants. The treatment included (i) pTRV2: PDS (positive control); (ii) pTRV2: FAD7; and (iii) pTRV2 alone (mock). Infiltrated plants were maintained under controlled environmental condition for effective viral infection and systemic silencing.
Semi-quantitative RT-PCR

Plants were subjected to semi-quantitative RT-PCR after 15 days of post infiltration (dpi) for analyzing FAD7 gene transcript levels. The total RNA was extracted from the leaves above the infiltrated leaves and the transcript level was analyzed by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) using the gene specific primers. Under standardized reaction condition, the house-keeping gene actin was amplified with primer pair actin F (5’-TCCATAATGAAGTGTGATGT-3’) and actin R (5’-GGACTGACTGCTCATACTC-3’) and treated as a positive control.

High temperature stress imposition

Fifteen days post infiltration (dpi), the plants were transferred to growth chambers with set temperatures of 35 and 42°C for one week, while one set of plants was retained at temperature 26°C as control. Fresh leaf samples were harvested on 7th day of temperature exposure for physio-biochemical analysis and all observations were completed within 25th dpi.

Net photosynthetic rate and PSII efficiency

Net photosynthetic rate was measured using portable photosynthesis system LI-6400 (LI-COR Inc., Lincoln, NE, USA) for topmost fully expanded leaves. These measurements were performed at ambient CO₂ (360 µmol mol⁻¹) using LI-COR CO₂ injection system and photosynthetic photon flux density (PPFD) of 500 µmol m⁻² s⁻¹ from the LI-COR LED light source. Air temperature of the leaf chamber was maintained at 28°C±0.5°C. Before recording the data, the leaves were kept in the leaf chamber for at least 2 min to attain a steady state.

Chlorophyll a fluorescence from PS-II (PS-II efficiency) in leaves was measured with Li-6400-40 LCF, LI-COR, USA. To ensure maximum photochemical efficiency of PS-II, leaves were dark-adapted for at least 30 min. The minimum (Fo) and maximum fluorescence (Fm) were measured in the dark-adapted leaves. The maximum photochemical efficiency of PS-II in the dark-adapted leaves was calculated as the ratio of variable fluorescence (Fv = Fm - Fo) to Fm (Fv/Fm = Fm-Fo/Fm)³².

Hydrogen peroxide (H₂O₂) content

Leaf samples (0.2 g) were homogenized in mortar and pestle with 2 mL of 0.1% trichloro acetic acid (TCA). The homogenate was centrifuged at 10000 rpm for 30 min at 4°C. Aliquots of 0.5 mL were taken to which 0.5 mL of 0.1M potassium phosphate buffer (pH=7.6) and 2 mL of 1M potassium iodide were added. The reaction mixture was incubated at room temperature (25°C) in dark for 1 h and the absorbance was taken at 390 nm. Amount of H₂O₂ (µmol g⁻¹ f.w.) was calculated by plotting standard curve of H₂O₂³³.

Thio barbituric acid reacting substance (TBARS)

Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) content produced by the thiobarbituric acid reaction³⁴. Fresh leaf sample (0.2 g) was homogenized in 3 mL 0.1% TCA and centrifuged at 10000 rpm for 10 min. 1.2 mL of 0.5% thio barbituric acid in 20% TCA was added to 0.3 mL supernatant and incubated in water bath at 95°C for 30 min. Reaction was terminated in ice and centrifuged at 10000 rpm for 10 min. Absorbance was determined at 532 and 600 nm. After subtracting the non-specific absorbance at 600 nm, the MDA concentration (µmol g⁻¹ f.w.) was determined using the extinction coefficient of 155 mM⁻¹ cm⁻¹.

Membrane stability index

Conductivity tests were carried as described by Onwueme³⁵. Fresh leaf material (100 mg) from pooled leaves (3 or 4 fully expanded) of three plants was weighed and placed in a test tube containing 10 mL of double distilled deionized water. Three replicates were prepared for each treatment. These tubes were incubated at 45°C for half an hour in a water bath. Electrical conductivity (C₁) of this solution was measured with the help of conductivity meter (CM 82T, ELICO Pvt. Ltd., Hyderabad). These tubes were kept in boiling water (100°C) for 10 min and cooled at room temperature and conductivity (C₂) was measured again. Percent conductivity was used to calculate membrane stability index using the formula: Membrane stability index = (1-C₁/C₂) × 100; Where, C = conductivity in m or µ mhos.

Antioxidant enzymes

Enzyme extract for total superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidases (GPX) and catalase (CAT) was prepared by grinding fresh leaf sample (1 g) in liquid nitrogen to prevent proteolytic activity followed with 10 mL extraction buffer (0.1 M phosphate buffer, pH 7.5, containing 0.5 mM EDTA in case of SOD, CAT, and POX, and 1 mM ascorbic acid in case of APX). Extract was passed through 4 layers of cheesecloth
and filtrate was centrifuged for 20 min at 15000 × g and the supernatant was directly used as enzyme source.

SOD activity was estimated by recording the decrease in optical density of formazone made by superoxide radical and nitro-blue tetrazolium dye by the enzyme. CAT activity was assayed by measuring the disappearance of H₂O₂ (extinction coefficient, ε = 39.4 mM⁻¹ cm⁻¹)³⁷. Absorbance was measured at 240 nm. Enzyme activity expressed as µmol of H₂O₂ consumption min⁻¹ g⁻¹ f.w. APX activity was assayed by recording the decrease in optical density due to ascorbic acid at 290 nm. The initial and final contents of ascorbic acid are calculated by comparing with a standard curve drawn with known concentrations of ascorbic acid. Enzyme activity was calculated as concentration of ascorbic acid oxidized min⁻¹ g⁻¹ f.w. GPX activity was assayed as increase in optical density due to the oxidation of guaiacol to tetra-guaiacol³⁹. Enzyme activity is expressed as µmol tetra-guaiacol formed min⁻¹ g⁻¹ f.w.

Estimation of fatty acids
Methyl ester preparation from fresh powdered seeds was done using one step lipid extraction and fatty acid methyl esters preparation method⁴⁰. Methanol (analytical grade), Conc. sulphuric acid and hexane were used for esterification.

The samples were transferred to GC vials and loaded for detection of fatty acid esters using Perkin Elmer GC (Model- Clarus 500, USA). From the chromatogram, the retention times of fatty acids were compared with the retention time of standard. The percentage of fatty acids was calculated.

Statistical analysis
The data were statistically verified with analysis of variation (ANOVA) and the significance of differences between the means was tested using Tukey post-hoc test at the significance level P ≤ 0.05 using SPSS v.10 for Windows (SPSS Inc., Chicago, USA).

Results
Cloning of FAD7 gene from Nicotiana benthamiana
PCR based cloning approach was carried out to clone FAD7 gene from N. benthamiana cDNA for expression and silencing experiments. Database (NCBI, TIGR, N. benthamiana) search was carried out and FAD7 gene sequence from N. tabacum. Gene specific primers were used to amplify 360 bp amplification. This amplicon was cloned and sequenced. The cloned FAD7 sequence showed >90% homology with FAD7 gene from N. tabacum (97%), Capsicum annum (93%), Solanum tuberosum (92%) and Lycopersicon esculentum (92%). The cloned gene fragment designated as NbFAD7 was submitted to NCBI database (accession number GW992807.1, DBESTID 69732818).

Prediction of off targets for FAD7 gene
Off target prediction was carried out with the help of siRNA scan software with the parameters as siRNA of length 21 nucleotides, 5’ end of antisense strand must be A or U. The first 7 bases of antisense strand (5’ - 3’) have at least 5 A or U bases and the 5’ end of sense strand must be G or C with a percentage GC content of 30 to 70. The output data showed that 360 bp of NbFAD7 sequence produced 35 siRNA’s and did not have any off target gene hits to available target dataset.

FAD7 mRNA silencing and evaluation of silenced plants
The plants infiltrated with Agrobacterium carrying pTRV2: PDS showed photo bleached phenotype within 10-12 days post infiltration (dpi). Ten dpi, the infiltrated plants were characterized by semi-quantitative RT-PCR to examine the extent of endogenous FAD7 gene silencing. The template concentrations used for the RT-PCR was normalized with the actin gene. Significant reduction in endogenous FAD7 gene transcript was observed in pTRV2: FAD7 infiltrated plants (Fig. 1). There was no reduction in transcript level in mock and PDS gene silenced plants. There was no phenotypic differences in tobacco plants grown at control (26°C) and 35 and 42°C growth temperature and all three types of plants showed yellowing and senescence of leaves at 42°C temperature stress (Fig. 2).

Fig. 1 — Semi-quantitative RT-PCR showing down regulation of FAD7 gene in N. benthamiana 15 days post infiltration (dpi) and exposed to normal (26°C) and high temperature (35 and 42°C) for one week duration. [Control = normal wild type plants; mock = plants infected with virus without insert; FAD (1, 2, 3) = FAD7 gene silenced plants]
Net photosynthetic rate ($P_N$) and PS II efficiency ($F_v/F_m$)

Control and mock plants showed significant decrease in $P_N$ (17.9%, 53.9%) and $F_v/F_m$ (1.4%, 25.0%) as the temperature increased from 26 to 35 and 42°C. Conversely, the FAD7 silenced plants showed very less reduction in $P_N$ (3.5%) and $F_v/F_m$ (0.9%) at 35°C, while a significant decrease of 23.0% and 4.6% was observed at 42°C as compared to that at 26°C (Fig. 3).

Membrane stability index (MSI), TBARS and $H_2O_2$ content

The $H_2O_2$ and TBARS content were significantly more influenced under high temperature compared to control and mock plants. The $H_2O_2$ content significantly increased when the temperature increased from 26 to 35 and 42°C in control (93.2%, 229.5%) and mock plants (113.3%, 250.6%) compared to that of FAD7 gene silenced plants (27.4%, 119.1%). Similarly, TBARS contents were significantly increased in control (77.8%, 186.8%) and mock plants (67.7%, 161.3%) as the temperature increased from 26 35 and 42°C. Conversely, in FAD7 gene silenced plants increase in TBARS content was lesser at 35°C (53.1%) and 42°C (96.3%) as compared to that at 26°C (Table 1). At high temperature conditions, membrane stability index (MSI) decreased more in control (27.4%) and mock plants (31.5%) as compared to FAD7 silenced plants at 42°C (15.6%) (Table 1).

Antioxidant enzymes activity

The activity of antioxidant enzymes was significantly influenced at both the levels of high temperature stress (35 and 42°C). The SOD activity significantly increased at 42°C in control (71.6%) and mock plants (73.3%) as compared to that at 26°C. In contrast, no difference was noted in SOD activity...
Table 1 — Hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) and malondialdehyde (TBARS) content and membrane stability index (MSI) of control, mock and \textit{FAD7} gene silenced plants exposed to normal (26°C) and high temperature (35 and 42°C) for one week duration

<table>
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<tr>
<th>Temperature</th>
<th>Treatment</th>
<th>H\textsubscript{2}O\textsubscript{2} (µmol g\textsuperscript{-1} f.w.)</th>
<th>TBARS (µmol g\textsuperscript{-1} f.w.)</th>
<th>MSI (%)</th>
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<tr>
<td></td>
<td>Control</td>
<td>0.88±0.03</td>
<td>24.97±1.36</td>
<td>94.13±0.36</td>
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<td>Mock</td>
<td>0.83±0.03</td>
<td>26.97±0.26</td>
<td>95.38±0.47</td>
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<td>26°C</td>
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<td>0.87±0.04</td>
<td>27.16±0.71</td>
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<td>FAD7 (2)</td>
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<td>FAD7 (3)</td>
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<td>26.46±1.81</td>
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<td>Mock</td>
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<td>42°C</td>
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CD at \(P \leq 0.05\)

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[Values represent the mean ± SE of three replicates. Control = normal wild type plants; mock = plants infected with virus without insert; FAD (1, 2, 3) = \textit{FAD7} gene silenced plants]

at 35°C while a significant increase was at 42°C, in \textit{FAD7} silenced plants. Similar to SOD, activity of CAT, APX and GPX was significantly increased in control (60.1, 63 and 34.8%) and mock plants (65.9, 66.3 and 38.2%) as the temperature increased from 26 to 42°C. In \textit{FAD7} silenced plants the increment in the activity of these enzymes was 16, 25.7 and 16%, respectively for CAT, APX and GPX activity at 42°C. No considerable changes occurred in the activities of these enzymes in \textit{FAD 7} silenced plants at 35°C, while, in control and mock plants the increase in the activities of these enzymes was significant as compared to plant grown at 26°C (Fig. 4 A-D).

**Fatty acids**

Changes in the composition of fatty acids were measured in control, mock and \textit{FAD7} silenced plants grown under 26, 35 and 42°C. No significant changes occurred in palmitic (16:0), stearic (18:0) and oleic (18:1) acid contents in the leaves under temperature stress. In contrast, linoleic acid (18:2) content increased significantly with increasing temperature in all plants with the highest levels recorded at 42°C. \textit{FAD7} silenced plants showed considerably higher proportion of linoleic acid, 30.7 and 36.34 mol% at 35 and 42°C, respectively. Percent increase of linoleic acid in \textit{FAD7} silenced plants (14.3 and 32%) was quite lower than that of control and mock plants (48.8 and 64.3%) at high temperatures. In contrast, the linolenic acid (18:3) levels were found to decrease with increasing temperature. A minor decline was observed in linolenic acid levels with increase in temperature (26 to 42°C) in control (12.8%) and mock (17.7%) plants. However, the proportion of decline was comparatively higher as the temperature increased from 26 to 35°C. In \textit{FAD7} silenced plants linolenic acid content at 26°C was 36.65, which was reduced to 19.59 (42°C) as compared to that of control and mock plants. Overall, all \textit{FAD7} silenced plants showed higher linoleic acid (18:2) as 27.85 (26°C), 30.7 (35°C) and 36.34 (42°C) and lower linolenic acid (18:3) as 36.65 (26°C), 32.27 (35°C) and 28.85 (42°C) as compared to that of control and mock plants (Figs. 5 and 6).
Fig. 6 — Line graphs represent ratio of dienoic to trienoic fatty acids in leaves of control, mock and FAD7 gene silenced plants exposed to normal (26°C) and high temperature (35 and 42°C) for one week duration. [Control = normal wild type plants; mock = plants infected with virus without insert; FAD (1, 2, 3) = FAD7 gene silenced plants]

Discussion

Linolenic acid (18:3) is the major fatty acid in plants which is synthesized from the desaturation of linoleic acid (18:2) by omega-3 fatty acid desaturases (FAD). The modification of membrane fluidity is mediated by changes in unsaturated fatty acid levels, a function provided in part by the regulated activity of fatty acid desaturases (FAD). Adjustment of membrane fluidity maintains an environment suitable for the functioning of critical integral proteins during stress environment. The FAD7 is a gene for a chloroplast ω-3 fatty acid desaturase which is located in the inner membrane of the chloroplast envelope. In our study, silencing of FAD7 gene resulted in the reduction of trienoic fatty acids and increase in dienoic fatty acids in the FAD7 silenced plants.
reduction in dienoic to trienoic fatty acid ratio enhanced membrane stability and temperature tolerance in silenced plants. Similar reductions in trienoic fatty acids and increases in dienoic fatty acids with enhanced high temperature tolerance in antisense expression of tomato chloroplast omega-3 fatty acid desaturase gene (LeFAD7) were observed in transgenic tomato plants. Co-suppression of the chloroplast ω-3 fatty acid desaturase (FAD7) in the transgenic rice led to reduction in 18:3 and increase in 18:2 content as compared to the wild type plants. Similar results were also found in co-suppression of ω-3 fatty acid desaturase in transgenic tobacco.

Physiological and biochemical changes like water deficit and oxidative stress which affects the cellular metabolism, membrane integrity and basic physiological processes are detrimental to plants survival under high temperature stress. Under heat stress, sensitivity of photosynthesis and PS-II efficiency is more amongst several physiological process as the photosystem-II (PS-II) is highly thermo-labile and heat stress can inhibit the oxygen evolution. In this study, control and mock plants showed significant decrease in Pn and Fv/Fm as the temperature increased from 26 to 35 and 42°C. Conversely, FAD7 silenced plants maintained higher photosynthetic rate and PSII efficiency under high temperature stress as compared to control plants (Fig. 3). The better photosynthetic response of FAD7 silenced plants was driven by stable proportions of saturated and unsaturated fatty acids in membrane lipids required for photosynthetic thermostability under elevated temperatures. Similar results were found in transgenic tomato plants silenced for FAD7 gene which could retain higher photosynthetic rate, chlorophyll fluorescence and O2 evolution at higher temperatures.

Several reports suggested that increase in saturated fatty acid levels in membranes resulted in maintenance of relatively higher photosynthesis and growth rate. High temperature stress is often associated with the oxidative stress. Excessive accumulation of reactive oxygen species (ROS) under stress is a typical plant response of plants which can cause potential damage unless effectively detoxified by antioxidant system. Prevention of oxidative damage by strengthening the antioxidative system of plants is considered as one of the mechanisms of their thermotolerance. Antioxidation and detoxification pathways for increased level of saturated fatty acids in membrane lipids could determine the high temperature tolerance in plants. In our study, FAD7 silenced plants exhibited significantly lower electrolyte leakage and lipid peroxidation (TBARS) as compared with that of control/mock under high-temperatures. The electrolytes leakage, an indication of decreased cell membrane thermostability, has been used as an indirect measure of high temperature stress tolerance in diverse plant species.

ROS scavenging mechanisms have an important role in protecting plants against temperature stresses or combination of high light and temperature stress. In our experiments, activities of antioxidant enzymes were increased with a parallel increase in H2O2 content. However, lesser increase in SOD, GPX, CAT and APX activity was observed in the FAD7 silenced plants as compared to control and mock plants which might be due to lower production of superoxide radicals. This may be due to the reasons for high membrane integrity in addition to decrease in trienoic and increase in dienoic fatty acids in the FAD7 silenced plants. Our results are consistent with other studies reporting the increase in antioxidant enzyme activity in response to high temperature stress in tomato, rice, wheat and maize.

Conclusion

Here, we investigated the effect of high temperature stress on membrane fatty acids alteration, detoxification mechanism and its contributory role in photosynthetic activity in model plant tobacco through using PTGSVIGS approach. The findings of the study, confirmed that FAD7 gene silencing using VIGS reduces trienoic fatty acids and enhances the ratio of saturated to unsaturated fatty acids and improve high-temperature tolerance in N. benthamiana plants in terms of lesser H2O2 production and also improved detoxification of ROS, membrane stability and photosynthetic activity.

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