Drought stress led to a decrease in PSII photochemical activity in tobacco leaves and the blockage of electron transfer, especially from $Q_A$ to $Q_B$ on PSII acceptor side. In addition, drought stress caused a dramatic increase in H$_2$O$_2$ and MDA contents. However, the overexpression of 2-Cys Prx significantly reduced the H$_2$O$_2$ accumulation in tobacco seedlings and alleviated the degree of oxidative damage under drought stress. It also improved stomatal limitation and net photosynthetic rate of tobacco seedlings and reduced its PSII photoinhibition under drought stress which promoted the ability of PSII electron transfer.

**ABSTRACT**

Drought stress led to a decrease in PSII photochemical activity in tobacco leaves and the blockage of electron transfer, especially from $Q_A$ to $Q_B$ on PSII acceptor side. In addition, drought stress caused a dramatic increase in H$_2$O$_2$ and MDA contents. However, the overexpression of 2-Cys Prx significantly reduced the H$_2$O$_2$ accumulation in tobacco seedlings and alleviated the degree of oxidative damage under drought stress. It also improved stomatal limitation and net photosynthetic rate of tobacco seedlings and reduced its PSII photoinhibition under drought stress which promoted the ability of PSII electron transfer.
and Arnell 2016). If overexpression of 2-cys Prx can effectively reduce ROS content in plant cells, it may improve the photosynthetic capacity of tobacco plants. There are few studies on the overexpression of 2-Cys Prx gene in plants. Therefore, in this paper, the effects of 2-Cys Prx overexpression on gas exchange parameters, PSII electron transfer, energy distribution, and reactive oxygen metabolism in tobacco seedlings under drought stress were studied using tobacco plants overexpressing 2-Cys Prx to explore its role in protecting plant photosynthetic apparatus under drought stress.

1. Materials and methods

1.1. Test materials and treatments

The experiment was carried out at the Plant Physiology Laboratory of Northeast Forestry University in March 2013. Tobacco plants overexpressing 2-Cys Prx were generated using the gene amplified from the leaves of tobacco variety 'Longjiang 911' by RT-PCR method described by Zhang et al. (2017). Gene expression analysis by RT-PCR showed that 2-Cys Prx was expressed in all 15 tobacco plants (6–20 plants in Figure 1) with less expression in OE-6 and OE-15. No expression of the exogenous 2-Cys Prx gene was detected in wild-type tobacco plants (CK) and these were used as negative control (Lane 3–5; Figure 1).

Selection of PCR banded plants with relatively high brightness (OE-12 and OE-19), i.e. plants with relatively higher expression of 2-Cys Prx, seeds from non-transgenic tobacco 'Longjiang 911' were used as the control (CK). The seeds of OE-12, OE-19, and CK were sown in a soil mixture consisting of peat soil and quartz sand (V:V = 2:1) and cultured in an artificial growth chamber at temperature of 25/23°C (light/dark), light intensity of 400 μmol m⁻² s⁻¹ using an in-built light source. The net photosynthetic rate (Fₘ), stomatal conductance (Gₛ), transpiration rate (Tᵣ), and intercellular CO₂ concentration (Cᵢ) of tobacco seedlings grown under drought stress for different days were measured. Each measurement was repeated five times.

1.3. Measurements of physiological parameters

1.3.1. Determination of gas exchange parameters

The penultimate unfolded leaves of tobacco seedlings treated with different drought days were selected. Carbon dioxide concentration was maintained at 400 μL L⁻¹ by using CO₂ pressure cylinders and the light intensity PFD was set at 1000 μmol m⁻² s⁻¹ using an in-built light source. The net photosynthetic rate (Fₘ), stomatal conductance (Gₛ), transpiration rate (Tᵣ), and intercellular CO₂ concentration (Cᵢ) of tobacco seedlings grown under drought stress for different days were measured.

1.3.2. Determination of PSII electron transport rate (ETR) and energy allocation

The penultimate unfolded leaves of tobacco seedlings under different drought days were adapted to darkness using dark-adaptation leaf clips for 0.5 h, and its initial fluorescence (Fᵢ) and maximum fluorescence (Fₘ) were measured using a portable pulse modulated chlorophyll fluorometer FMS-2 (Hansatech Instrument Ltd., UK). The determination of photosynthetic efficiency (Fᵥ) and maximum steady-state fluorescence (Fₘ') treated at light intensity (PFD) of 1000 μmol m⁻² s⁻¹ for light adaptation, the PSII ETR was calculated using the following equation:

\[
ETR = PFD \times 0.84 \times 0.5 \times \Phi_{PSII}
\]

Here, PFD is the 1000 μmol m⁻² s⁻¹, 0.84 is the empirical absorption coefficient, 0.5 is a hypothetical value that the energy absorbed by antenna chlorophyll is evenly distributed into two optical systems, and \( \Phi_{PSII} \) is the quantum yield of PSII calculated as PSII = (Fₘ'/Fᵢ)/Fₘ'. The distribution of absorbed light energy in PSII reaction center was calculated according to the method proposed by Hendrickson and Zhou et al. (Hendrickson et al. 2004; Zhou et al. 2007). The parameters of quantum efficiency of photochemical energy dissipation (\( \Phi_{PSII} \)), light-dependent and ΔpH – and xanthophyll – mediated thermal dissipation (\( \Phi_{NPQ} \)), the combined quantum efficiency of fluorescence and constitutive thermal dissipation (\( \Phi_{TD} \)), and quantum efficiency of thermal dissipation associated with photooxidated, nonfunctional PSII (\( \Phi_{NP} \)) were calculated using the following formulas:

\[
\Phi_{PSII} = [1 - (Fᵢ/Fₘ')] [(Fᵢ/Fₘ)/(Fᵢ/Fₘ')] ;
\]

\[
\Phi_{NPQ} = [(Fᵢ/Fₘ') - (Fᵢ/Fₘ)] [(Fᵢ/Fₘ)/(Fᵢ/Fₘ')] ;
\]

\[
\Phi_{TD} = (Fᵢ/Fₘ)(Fᵢ/Fₘ)/(Fᵢ/Fₘ') ;
\]

Figure 1. RT-PCR detection of 2-Cys Prx in transgenic tobacco plants.
\[
\Phi_{\text{NF}} = 1 - \left(\frac{F_v}{F_m}/\left(\frac{F_v}{F_{mM}}\right)\right)
\]

The sum of each of these parameters is 1 (i.e. \(\Phi_{\text{PSII}} + \Phi_{\text{NPQ}} + \Phi_{\text{f,D}} + \Phi_{\text{NF}} = 1\)), where \(F_v/F_{mM}\) is \(F_v/F_m\) of nonphoto-inhibited leaves. Each measurement was repeated five times.

1.3.3. Determination of chlorophyll fluorescence kinetics

The penultimate unfolded leaves of tobacco seedlings under different drought days were adapted to darkness using dark-adaptation leaf clips for 0.5 h, and the OJIP curve of each treatment was recorded using a multifunctional plant efficiency analyzer (Handy-PEA, Hansatech Instrument Ltd., UK). Each treatment was repeated five times, and the OJIP curves were plotted with the average values of those repetitions. The points O, J, I, and P corresponds to the time points 0.01, 2, 30, and 1000 ms, respectively, and their relative fluorescence intensities (RFIs) were expressed as \(F_O, F_J, F_I,\) and \(F_P\), respectively. The OJIP curve was analyzed using JIP-test to obtain the chlorophyll fluorescence parameters such as the maximum photochemical efficiency of PS II (\(F_v/F_m\)) and the photosynthetic performance index (\(\text{PI}_{\text{ABS}}\)) based on the absorption of light energy. The JIP-test method by Strasser and coworkers (1995). In order to analyze the relative variable fluorescence (\(V_j\)) at J point, the OJIP curve was standardized according to \(V_{O-P} = (F_i - F_O)/(F_P - F_O)\) to obtain the \(V_{O-P}\) curve in which \(F_i\) is the RFI at each time point on OJIP curve. The difference in \(V_{O-P}\) curve of OE-12, OE-19, and CK tobacco seedlings under different drought days was compared and expressed as \(\Delta V_{O-P}\).

Chlorophyll content was determined using CCM-200 chlorophyll meter (Opti-Sciences, USA), and the SPAD readings of chlorophyll meter were used as relative leaf chlorophyll contents. Leaf water content was measured by drying method, \(\text{H}_2\text{O}_2\) content was detected using titanium tetrachloride (Lin et al. 1988), and MDA content was determined using thiobarbituric acid (TBA) method (Wang et al. 2003). The determination of each parameter was repeated three times.

1.4. Data processing

The measured data were statistically analyzed using Excel and SPSS (22.0) software. The data represented are mean and standard deviation (SE) of three independent experiments.

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**Figure 2.** Effects of 2-Cys Prx overexpression on leaf water content (A) and SPAD value (B) of tobacco seedlings under drought stress.

**Figure 3.** Effects of 2-Cys Prx overexpression on net photosynthetic rate (A), stomatal conductance (B), transpiration rate (C), and intercellular \(\text{CO}_2\) concentration (D) in tobacco seedlings under drought stress.
The differences between different data groups were compared using one-way ANOVA and LSD.

2. Results

2.1. Leaf water content and SPAD value of transgenic and control tobacco seedlings under drought stress

The leaf water content and SPAD value of CK tobacco seedlings were slightly lower than those of OE-12 and OE-19 transgenics at 0th and 5th day of drought, but the difference was not significant (Figure 2(A,B)). With the prolongation of drought stress, leaf water content and SPAD value of tobacco seedlings showed a decreasing trend starting on 10th day of drought. However, the decrease in leaf water content and SPAD values of OE-12 and OE-19 tobacco seedlings was much less than CK, and both values of OE-12 and OE-19 were significantly higher than those of CK on 10th and 15th day of drought. There was no significant difference in leaf water content and SPAD values between OE-12 and OE-19 tobacco seedling during the drought treatment.

2.2. Photosynthetic gas exchange parameters of transgenic and control tobacco seedlings under drought stress

The photosynthetic gas exchange parameters ($P_n$, $G_i$, and $T_i$) of OE-12 and OE-19 tobacco seedlings were slightly higher than those of CK under normal water condition (0th day of drought), but the difference was not significant (Figure 3(A–C)). During the first 5–15 days of drought, $G_i$ in the leaves of tobacco seedlings decreased, accompanied by a decrease in $T_i$ and $P_n$. Whereas, the decrease in OE-12 and OE-19 tobacco seedlings was significantly lower than those of CK. (On 15th day of drought, the leaves of CK tobacco seedlings got severely dehydrated and were not used for the determination of photosynthetic gas exchange parameters). $G_i$ in the leaves of CK, OE-12, and OE-19 tobacco seedlings on 5th day of drought did not change significantly compared with that at 0 days (Figure 3(D)). On 10th and 15th day of drought, $C_i$ of OE-12 and OE-19 tobacco seedlings exhibited a significant increase, while that of CK was similar to 10th day of drought.

2.3. Chlorophyll fluorescence parameters of transgenic and control tobacco seedlings under drought stress

2.3.1. Electron transport rate

ETR of CK was slightly lower than that of OE-12 and OE-19 transgenics on the 0th and 5th day of drought, while the difference was not significant (Figure 4). ETR of tobacco seedlings decreased rapidly on 10th and 15th day of drought. But the decrease in ETR was significantly greater than that of OE-12 and OE-19, and there was no significant difference in ETR between OE-12 and OE-19 tobacco seedlings during the drought treatment.

2.3.2. Relative fluorescence intensity (RFI) of OJIP curve and its characteristic points (O, J, I, and P)

As shown in Figure 5(A,B), the OJIP curve of OE-12 and OE-19 tobacco seedlings was slightly different from CK on 0th and 5th day of drought. However, the OJIP curves of tobacco seedlings changed significantly on 10th and 15th day of drought (Figure 5(C,D)). The RFI of O point in tobacco seedlings increased, and the RFI of O point in CK tobacco seedlings decreased little. The RFI of J, I, and P points in CK, OE-12, and OE-19 tobacco seedlings decreased significantly. The OJIP curve became relatively flat, but the OJIP curve of OE-12 and OE-19 tobacco seedlings was significantly lower than that of CK. Quantitative analysis of RFI showed that $F_O$ of CK varied insignificantly, but that of OE-12 and OE-19 increased significantly on 10th and 15th day of drought compared with that on 0th day (Figure 6(A)). $F_J$, $F_I$, and $F_P$ of CK, OE-12, and OE-19 showed minor changes on 5th day of drought, but those of CK tobacco seedlings on 10th and 15th day were significantly lower than those on the 0th day when the decrease in $F_P$ was maximum (Figure 6(B–D)). Whereas, the decrease in $F_J$, $F_I$, and $F_P$ in OE-12 and OE-19 was significantly lower than that of CK.

2.3.3. PSII photochemical efficiency

$F_v/F_m$ and $PI_{ABS}$ of tobacco seedling leaves showed no significant change on 5th day of drought compared with that on 0th day, and the $F_v/F_m$ and $PI_{ABS}$ of OE-12 and OE-19 showed no significant difference from those of CK (Figure 7(A,B)). However, they both decreased significantly on 10th and 15th day of drought, and the reduction in $PI_{ABS}$ was greater than that of $F_v/F_m$. However, $F_v/F_m$ and $PI_{ABS}$ of OE-12 and OE-19 tobacco seedlings were significantly higher than that of CK on 10th and 15th day of drought, and there was no significant difference in those values between OE-12 and OE-19 under different drought treatments.

2.3.4. Normalized OJIP curve and relative variable fluorescence of characteristic points

The OJIP curves of tobacco seedlings grown under drought stress for different periods were standardized according to the formula $V_{O-P}=(F_F-F_O)/(F_P-F_O)$. The $V_{O-P}$ curves of CK, OE-12, and OE-19 tobacco seedlings varied little, and there was no significant difference in $V_{O-P}$ curve between OE-12 and OE-19 tobacco seedlings (Figure 8(A,B)). But on 10th and 15th day of drought (Figure 8(C,D)), the $V_{O-P}$ curve of CK was significantly different from that of OE-12 and OE-19, and the $V_I$ of OE-12 and OE-19 showed in $V_{O-P}$ and $\Delta V_{O-P}$ curves were significantly lower than that of CK. Quantitative analysis showed that $V_I$ of tobacco seedlings varied insignificantly on 5th day of drought compared with...
that on 0th day, and there was no obvious difference in $V_J$ of CK, OE-12, and OE-19 tobacco seedlings (Figure 9). But $V_J$ of tobacco seedlings began to increase rapidly on day 10 and continued on 15th day of drought. There was no significant difference between CK, OE-12, and OE-19 tobacco seedlings on 10th day, while $V_J$ of CK was higher than OE-12 and OE-19 by 32.16% ($P < 0.05$) and 31.99% ($P < 0.05$), respectively, on 15th day.

2.3.5. Energy allocation parameters

With the prolongation of drought stress, $\Phi_{PSII}$ of tobacco seedlings showed a decreasing trend (Figure 10). However, $\Phi_{NF}$ increased, $\Phi_{NPQ}$ first increased and then decreased, and $\Phi_{FD}$ increased slightly on 10th and 15th day of drought, but the change was less. On 5th day of drought, $\Phi_{NF}$ of CK, OE-12, and OE-19 tobacco seedlings increased slightly, while $\Phi_{PSII}$ decreased significantly.

**Figure 5.** Effect of 2-Cys Prx overexpression on OJIP curve of tobacco seedlings under drought stress.

**Figure 6.** Effects of 2-Cys Prx overexpression on $F_o$ (A), $F_i$ (B), $F_r$ (C), and $F_p$ (D) of tobacco seedlings under drought stress.
and $\Phi_{NPQ}$ increased significantly compared with day 0 of drought. In addition, there was little difference in the energy distribution parameters of CK, OE-12, and OE-19 tobacco seedlings on 0th and 5th day of drought. On 10th and 15th day of drought, $\Phi_{PSII}$ of tobacco seedlings decreased rapidly while $\Phi_{NF}$ increased significantly, and the changes in $\Phi_{PSII}$ and $\Phi_{NF}$ of OE-12 and OE-19 were significantly lower than those of CK. $\Phi_{NPQ}$ in CK tobacco seedlings reached maximum on 5th day of drought, but decreased sharply on 10th and 15th day. While $\Phi_{NPQ}$ of OE-12 and OE-19 tobacco seedlings remained at a higher level on 5th and 10th day of drought, but slightly decreased on 15th day which was significantly higher than that of CK.

2.4. Hydrogen peroxide and malondialdehyde contents of transgenic and control tobacco seedlings under drought stress

The contents of $\text{H}_2\text{O}_2$ and MDA in the leaves of tobacco seedlings showed an increasing trend under drought stress (Figure 11). On 0th of drought, $\text{H}_2\text{O}_2$ and MDA contents
in leaves of OE-12 and OE-19 tobacco seedlings were slightly different from those of CK. The H2O2 and MDA contents of OE-12 and OE-19 tobacco seedlings were significantly lower than those of CK throughout the drought treatment, except that the MDA content was not significantly different from those of CK on day 5 of drought. No significant difference in H2O2 and MDA contents was found between OE-12 and OE-19 tobacco seedlings during drought treatment.

2.5. Plant morphology of transgenic and control tobacco seedlings and its response to drought stress

The leaves of CK tobacco seedlings exhibited obvious water loss and wilting, and the leaves were chlorotic on 10th and 15th day of drought with the aged leaves showing more significant changes (Figure 12). However, under the same drought conditions, the leaf growth in OE-12 and OE-19 transgenic tobacco seedlings was more vigorous and the symptoms of leaf injury were lesser than CK.

3. Discussion

So Far, many researchers have studied the limiting factors of plant photosynthesis under stress, but there is no unified conclusion (Lawson et al. 2003). The decrease in photosynthesis under drought stress have been attributed to stomatal factors (reduced CO2 supply caused by stomatal closure) (Kano et al. 2011) or non-stomatal factors (decreased photosynthetic activity of mesophyll cells) (Liu and Chen 1990), while others

Figure 10. Response of energy allocation parameters of PSII reaction center to drought stress in control and transgenic tobacco seedlings [CK (A), OE-12 (B), and OE-19 (C)].

Figure 11. Effects of 2-Cys Prx overexpression on the contents of hydrogen peroxide (A) and malondialdehyde (B) in tobacco seedlings under drought stress.
believe that it is a consequence of both stomatal and non-stomatal factors (Zhang et al. 2010; Xu et al. 2018). In this experiment, $P_m$, $G_s$, and $T_1$ values of tobacco seedlings decreased with increase in number of drought days, which indicate that stomatal factors play an important role in limiting photosynthetic capacity of tobacco seedlings under drought stress. However, the decrease in $P_m$, $G_s$, and $T_1$ values of OE-12 and OE-19 transgenic tobacco seedlings were significantly lower than those of CK seedlings, suggesting that overexpression of 2-Cys Prx can maintain relatively high photosynthetic capacity in tobacco seedlings under drought stress. This is related to higher water content and chlorophyll content in transgenic tobacco seedlings under drought stress (Figure 1). The $C_i$ value of CK, OE-12, and OE-19 tobacco seedlings on day 5 of drought was similar to that at the start, indicating that stomatal factors were responsible for the decrease in photosynthetic capacity of tobacco seedlings. On day 10 and day 15 of drought, the $C_i$ value of OE-12 and OE-19 tobacco seedlings increased significantly, suggesting that the decrease in photosynthetic capacity of transgenic tobacco seedlings was also influenced by non-stomatal factors. However, there was no significant change in $C_i$ value of CK tobacco seedlings on day 10 of drought. This may not be due to the absence of non-stomatal constraints. This was mainly due to the significant reduction in $G_s$ of CK tobacco seedlings, which limits the resistance of CO₂ entry into cells.

The decrease in chlorophyll under drought stress often leads to a reduction in the ability of PSII and/or PSI to capture and utilize light energy. PSII is one of the most sensitive to stress, and drought stress, in particular, can lead to decrease in plant PSII photochemical efficiency, blockage of electron transfer, and occurrence of photoinhibition (Lima et al. 2018; Zhang et al. 2018b). In this study, ETR, $F_v/F_m$, and $PI_{ABS}$ values of tobacco seedlings did not change on day 5 of drought compared to the start, and the change was significantly lower than that of $P_m$, $G_s$, and $T_1$. This indicates that tobacco seedlings in the early stage of drought adapted to drought stress mainly by stomatal closure instead of causing a decrease in PSII photochemical activity and ETR. However, with the prolongation of drought stress (day 10 and day 15 of drought), the ETR of tobacco seedlings decreased significantly and the PSII activity of leaves was inhibited. Under severe drought stress, ETR, $F_v/F_m$, and $PI_{ABS}$ values in the leaves of OE-12 and OE-19 tobacco seedlings were significantly higher than those of CK, indicating that overexpression of 2-Cys Prx can improve the tolerance of PSII photochemical activity and the ETR in tobacco seedlings under drought stress. In order to further analyze the relatively high ETR in transgenic tobacco seedlings under drought stress, the OJIP curves of tobacco seedlings at different drought days were standardized. The results showed that there was no significant difference in the relative variable fluorescence ($V_f$) of point J at 2 ms on the standardized OJIP curve ($V_{o-p}$) of transgenic tobacco seedlings and CK on day 0 and day 5 of drought. But the $V_f$ values on day 10 and day 15 of drought increased significantly, while the $V_f$ values of OE-12 and OE-19 tobacco seedlings were significantly lower than that of CK. The increase in $V_f$ indicates that the electron transfer from $Q_A$ to $Q_B$ in photosynthetic electron transfer chain was blocked, resulting in an increased accumulation of $Q_A$ (Zhang et al. 2016, 2018c). This suggests that the decrease in ETR in tobacco seedlings under severe drought stress was mainly related to the blockage of electron transfer from $Q_A$ to $Q_B$ in its PSII receptor side. Overexpressed 2-Cys Prx can increase the PSII ETR in tobacco seedlings and alleviate PSII photoinhibition (decrease in $F_v/F_m$ and $PI_{ABS}$) was mainly related to the relatively improved stability of electron transfer on PSII acceptor side of tobacco seedlings under drought stress.

If the light energy absorbed by plants under adversity cannot be fully utilized, the unutilized excitation energy must be dissipated in the form of heat or it will cause light suppression or light destruction (Ort 2001). In order to adapt to stress, plants will reduce the damage of excess light energy via absorption of light energy and changes in light distribution. Stress often leads to a decrease in the proportion of light energy absorbed by plants for photochemical reactions and an increase in the proportion of light used for other non-photochemical reactions (Kramer et al. 2004). With the prolongation of drought stress, $\Phi_{PSII}$ of tobacco seedlings decreased significantly in this study. However, $\Phi_{PSII}$ of tobacco seedlings overexpressing 2-Cys Prx was higher than CK, which was consistent with the change in ETR. Tobacco seedlings overexpressing 2-Cys Prx had less leaf damage under drought stress. In addition to driving electrons for photochemical reactions, the excess excitation energy absorbed by PSII antenna chlorophyll is dissipated in the form of light (Govindjee 2002), while a small proportion of the energy is dissipated in the form of increasing chlorophyll fluorescence (Maxwell and Johnson 2000). With the prolongation of drought stress, $\Phi_{D,D}$ of tobacco seedlings increased slightly on day 10 and day 15 of drought in this study, and there was no significant difference in $\Phi_{D,D}$ between CK and OE-12 and OE-19 tobacco seedlings. This indicates that tobacco seedlings dissipate excess excitation energy through heat energy under severe drought stress. But this protection mechanism is not dominant. On day 5 of drought, $\Phi_{NPQ}$ of tobacco seedlings increased significantly. $\Phi_{NPQ}$ is positively correlated to heat dissipation dependent on Lutein cycle, which is an important quenching mechanism of excess excitation energy (Li et al. 2000). Therefore, tobacco seedlings under mild drought stress may mainly dissipate the excess excitation energy by initiating energy dissipation mechanism dependent on Lutein cycle. However, with increase in days of drought stress (day 10 and day 15 of drought), $\Phi_{NPQ}$ of CK tobacco seedlings began to decrease and that of OE-12 and OE-19 remained relatively high. Therefore, this is an important reason for the relatively high PSII activity and ETR of tobacco seedlings overexpressing 2-Cys Prx. $\Phi_{NPQ}$ of tobacco seedlings showed an increasing trend with the prolongation
of drought stress, but the increase in $\Phi_{NF}$ of OE-12 and OE-19 tobacco seedlings was significantly lower than that of CK. Although studies have shown that the deactivation reaction center and the excess light energy distributed to the deactivation reaction center play an important role in the protection of photosynthesis (Zhang et al. 2013b), the inactivation of reaction center is a suicidal protective mechanism. The proportion of light energy allocated to the inactivated reaction center in transgenic tobacco seedlings under drought stress was significantly lower than that of CK, indicating that the inactivation ratio of PSII reaction center was significantly lower.

Under stress, the light energy absorbed by plants and the excess electrons in ETC will cause ROS outbreak (Zhou et al. 2016). Excess ROS can oxidize the plant cell membrane causing electrolyte leakage (Chen et al. 2005; Venkatesh et al. 2012). ROS also attack photosynthesis-related proteins, thereby inhibiting photosynthesis (Mittler 2002; Zhang et al. 2018d). Further, the reduction in photosynthesis aggravates the formation of ROS resulting in a vicious cycle. Therefore, effective reduction of ROS such as $H_2O_2$ plays an important role in protecting the normal functioning of plant photosynthetic apparatus. In this study, the contents of $H_2O_2$ and MDA in tobacco seedlings increased significantly with the aggravation of drought, but their contents in the seedlings overexpressing 2-Cys Prx were significantly lower than those in CK besides drought 0th d. It has been reported that 2-Cys Prx can efficiently convert $H_2O_2$ into water and alcohols to reduce the oxidative damage of $H_2O_2$ (Tripathi et al. 2009; Hong et al. 2017). Therefore, the findings of this study indicate that overexpression of 2-Cys Prx can effectively reduce the $H_2O_2$ content in tobacco seedlings under drought stress, protect cell membrane from lipid peroxidation, and alleviate oxidative damage under drought stress.

In addition, thylakoid membrane, one of the main cell membrane structures for $H_2O_2$ oxidation in chloroplast (Khorobrykh et al. 2015), may destroy the function of electron transporters attached to it when its structure is peroxidized, resulting in the blockage of photosynthetic electron transport (Long et al. 2017; Guo et al. 2018). The analysis of chlorophyll fluorescence parameters demonstrated that overexpression of 2-Cys Prx can protect PSIII function and promote electron transfer in tobacco seedlings under drought stress, which may be related to the effective protection of thylakoid membrane.

4. Conclusion

Tobacco seedlings overexpressing 2-Cys Prx maintain relatively high chlorophyll content and water content, and have higher photosynthetic capacity and PSII ETR under drought stress. Furthermore, they can effectively protect PSI function and maintain PSII photochemical activity by initiating dissipative mechanism of NPQ-dependent energy. NPQ of non-transgenic tobacco seedlings reduced significantly under severe drought stress, resulting in decreased activity of PSII reaction center and increased inactivation rate. Overexpression of 2-Cys Prx can also reduce cellular $H_2O_2$ content and alleviate ROS-induced membrane peroxidation injury, which is of great importance in maintaining photosynthetic capacity in the leaves of tobacco seedlings under drought stress.

Disclosure statement

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