EFFECTS OF CADMIUM STRESS ON PHOTOSYNTHETIC APPARATUS OF TOBACCO

CAI, Y.[1†] – QI, Y.[2†] – ZHU, S. Q.[1] – NIAN, H.[1] – HE, G. Q.[3*] – XU, N.[4*]

[1]*College of Horticulture, Jilin Agricultural University, Changchun 130118, China*

[2]*Jilin economic management cadre institute, Changchun 130118, China*

[3]*Mudanjang Tobacco Science Research Institute, Mudanjang, Heilongjiang 157011, China*

[4]*Key Laboratory of Heilongjiang Province for Cold-Regions Wetlands Ecology and Environment Research, and School of Geography and Tourism, Harbin University, Harbin 150086, China*

[†]*These authors contributed equally to this work*

[*]*Corresponding author*
e-mail: zzdjlnd@126.com

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Abstract. The effects of cadmium (Cd) stress on the photosynthetic apparatus of the tobacco to Cd stress were analyzed. The results showed that the chlorophyll content in the tobacco leaves declined as the Cd concentration increased. In Cd-challenged tobacco leaves, the performance index on absorption basis (PI_{ABS}), which is based on light absorption, could better reflect Cd-induced toxicity to PSII reaction centers than the maximum photochemical efficiency of PSII (F_v/F_m). As the concentration of Cd increased, the relative variable fluorescence at the J step (V_J) was significantly increased in the tobacco leaves, and a V_J of 50 μmol·L^{-1} Cd was significantly different from that of the control (CK). As Cd concentration increased, the quantum yield for electron transport (φE_o) was significantly reduced in the tobacco leaves, while maximum quantum yield for non-photochemical quenching (φD_o) was markedly increased. Moreover, absorption flux per reaction center (*ABS/RC*) significantly increased when Cd concentration reached 100 mol·L^{-1}. The above results suggest that the tobacco leaves adapt to Cd stress by lowering the electron transmission rate and the activity of PSII reaction centers and increasing thermal dissipation.
Keywords: *tobacco, cadmium stress, photosystem II, OJIP curve*

Introduction

Over the past 50 years, a total of 2.2×10^4 tons of cadmium (Cd) were disposed of globally (Singh et al., 2003). Cadmium is a non-essential and hazardous element to living organisms. Excessive exposure to Cd can cause renal injury and osteoporosis in human bodies (Bah et al., 2010), and it also exhibits carcinogenicity, teratogenicity, and mutagenicity (Qian et al., 2009). Humans are usually exposed to Cd via Cd-contaminated air or water, or via a food chain containing Cd-contaminated animals or plants. Cadmium in tobacco is an important source of Cd exposure (Sheetal et al., 2016). It has been found that Cd absorbed from cigarette smoke composes about 40% of Cd intake from the diet in the smokers who have smoked for 20 years (Cai et al., 1998), and the Cd exposure from 20 cigarettes is equivalent to 1 μg Cd intake (Jarup et al., 2009). Calculation of cadmium uptake from smoking was based on the assumption that 3% of the cadmium content in the tobacco smoked was absorbed, which is the usual estimate for cigarette smoking. Because Cd did not increase if smokers used more than

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0.5 kg of tobacco per month, the influence of tobacco usage on intake was maximized at 0.5 kg per month (Cai et al., 1998). Thus, understanding the toxicology of Cd in tobacco is of great significance for improving tobacco safety.

Cadmium can negatively influence a variety of physiological processes in plants. Cadmium inhibits seed germination, causes root injur and leaf necrosis, inhibits nutrient absorption, alters mitochondrial structure and function, impedes synthesis of carbohydrates and proteins, induces hormone metabolism disorder (Masood et al., 2012), increases reactive oxygen species and membrane peroxidation, and leads to ion leakage (Wagner, 1993; Hall, 2002; Zhang et al., 2006, 2007; Rodrıguez-Serrano et al., 2006; Dong et al., 2006; Kieffer et al., 2008; López-Millán et al., 2009). Cadmium stress also affects the photosynthetic capacity in plants (Kola et al., 2005). In the leaves of (*Populus deltoides*×*P. nigra*) hybrids, Cd stress results in damage of chloroplast structure, a reduced number of chloroplasts, expansion of chloroplast membrane, derangement, fusion or even disappearance of thylakoids (Zhang et al., 2014) In addition, Cd can inhibit utilization of solar energy (Krantev et al., 2008) and carbon assimilation in plants (Deng et al., 2014). Cd can also inhibit the activity of certain enzymes by binding to the functional domain of the enzyme (Tukaj et al., 2007). In *Populus tremula* L., for example, the content and activity of Rubisco are significantly reduced in response to Cd stress (Marmiroli et al., 2013). In other studies; however, a low concentration of Cd was demonstrated to increase the level of both large and small subunits of Rubisco and enhance the activity of Rubisco in the leaves of *Typha angustifolia* (Bah et al., 2010). Moreover, in *Glycine max* L. (Hossain et al., 2012) the activities of the enzymes in photosynthetic carbon assimilation are shown to increase in response to a low concentration of Cd.

Tobacco exhibits a relatively high Cd tolerance, and is one of the Cd-enriched plants. Accumulation of Cd in tobacco from Cd-rich ground may pose a threat to human health (Cao et al., 2015). Photosystem II(PSII) is one of the major protein complexes in the photosynthetic machinery in higher plants (Jiang et al., 2006), and it is sensitive to environmental stress (Dewez et al., 2005). Previous studies have shown that low Cd stress has insignificant effects on the electron transmission rate and photochemical efficiency but stimulates the activity of the PSII reaction center (Sebastian et al., 2014), which is inhibited by a high concentration of Cd (Sebastian et al., 2014; Bayçu et al., 2017). Studies on the effects of Cd on photosynthesis in tobacco mainly focuses on photosynthetic carbon assimilation and the photochemical activity of PSII (Wu et al., 2011; Zhang et al., 2020), yet the underlying mechanisms for Cd-induced reduction of the photosynthetic capacity in tobacco are seldom studied. Hence, in this study, we investigate the photochemical efficiency of PSII, the electron transmission at the electron acceptor side of PSII, the function of the oxygen-evolving complex (OEC), and the state of thylakoids in the leaves of tobacco that was treated with different concentrations of Cd. Our study aims to reveal the toxicological effect of Cd on the photosynthesis of flue-cured tobacco, and to provide fundamental data for the understanding of the mechanisms underlying the anti-Cd response in tobacco.

Materials and Methods

Test materials and treatment

The experiment was conducted in the laboratory of soil science of Jilin Agricultural University (Changchun, Jilin Province, China) from March to June 2019. The seeds

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were provided by the Mudanjiang Tobacco Research Institute. The seeds were sown in peat and vermiculite (1:1 in volume) in early March, and the seedlings were cultivated indoors at 25±2 ℃ under an artificial light with an intensity of 200 μmol·m^{-2}·s^{-1} and a light/dark cycle of 12 h. When the seedlings grew four leaves and one shoot, the seedlings were carefully removed out of the culture medium, and the adherent medium was rinsed off the roots. Each seedling was planted into a hole of a black foam that was floating on Hoagland complete culture solution in a light-proof container that was 25 cm in diameter and 30 cm in height. The size of the foam exactly fit the opening of the container to prevent light penetration through the interface. Each container contained 10 L Hoagland complete culture solution (0.75×10^{-3} mmol·L^{-1} K_2SO_4, 0.65×10^{-3} mmol·L^{-1} $MgSO_4$, 0.1×10^{-3} mmol·L^{-1} KCl, 2.0×10^{-3} mmol·L^{-1} $Ca(NO3)_2$, 0.25×10^{-3} mmol·L^{-1} KH_2PO_4, 1.0×10^{-5} mmol·L^{-1} H_3BO_3, 1×10^{-6} mmol·L^{-1} $MnSO_4$, 1×10^{-7} mmol·L^{-1} $CuSO_4$, 1×10^{-6} mmol·L^{-1} $ZnSO_4$, 5×10^{-9} mmol·L^{-1} $(NH4)_6Mo_2O_4$, 1.0×10^{-4} mmol·L^{-1} Fe-EDTA, pH 7, adjusted with KOH or H_2SO_4). Five seedlings were cultivated in one container. Air was pumped into the solution with an electric air pump. The culture solution was refreshed every five days, and the inner surfaces of the container were washed when replacing the solution. The plants were subjected with treatment after 30-days in this culture. Cd $(NO_3)_2$ was added into the culture solution to a final solution of 0 (control / CK), 25, 50, 75, and 100 μmol·L^{-1}. The chlorophyll fluorescence parameters in the tobacco leaves were measured on the fifth day of the treatment.

Determination parameters and methods

Chlorophyll fluorescence parameters were measured: The electron transfer rate (ETR) and non-photochemical quenching (NPQ), the maximum photochemical efficiency (F_v/F_m) and the actual photochemical efficiency (Φ_{PSII}) of PSII reaction center under light acclimation were measured by FMS-2 (Hansatch company, UK) for 5 times.

The chlorophyll fluorescence kinetic curve and its parameters were determined: After 30 min dark adaptation, the OJIP curve of leaves after dark adaptation was measured by handy pea (Hansatech compan, UK). According to Strasser et al.'s method (1995), the OJIP curves were standardized by $V_{O\text{-}P}=(F_t\text{-}F_o)/(F_m\text{-}F_o)$ and $V_{O\text{-}J}=(F_t\text{-}F_o)/(F_J\text{-}F_o)$, respectively. The relative variable fluorescence (V_J and V_K) of J point at 2 ms and K point at 0.3 ms were obtained, respectively. The differences between the standardized $V_{O\text{-}P}$ and $V_{O\text{-}J}$ curves of different treatments and the control were calculated, expressed as $V_{O\text{-}P}$ and $V_{O\text{-}J}$, respectively. The measured OJIP curve was analyzed by JIP test. The maximum photochemical efficiency (F_v/F_m) of PSII and the photosynthetic performance index (PI_{ABS}) based on absorbed light energy were measured.

The OJIP curve analyze were determined: The OJIP curve was analyzed using the JIP test method reported by Strasser et al. (1995) to obtain the following: maximum photochemical efficiency of PSII (F_v/F_m), performance index on absorption basis (PI_{ABS}), the quantum yield for electron transport (φE_o), maximum quantum yield for non-photochemical quenching (φD_o), absorption flux per reaction center (*ABS/RC*), the trapped energy flux per reaction centers (TR_o/RC), the electron transport flux pe reaction centers (ET_o/RC), and the dissipated energy flux per reaction centers (DI_o/RC).

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Statistical analysis

Excel and SPSS software (Version. 22) were used to conduct statistical analyses on the measured data. The data in the figure was denoted as mean ± standard deviation (SE). One way ANOVA and least significant difference (LSD) were used to compare the differences among different data groups.

Results and analysis

Chlorophyll content of tobacco leaves in response to Cd stress

As shown in *Figure 1*, Cd stress significantly reduced the chlorophyll content in the tobacco leaves. The content of chlorophyll a was reduced by 5.92% ($P>0.05$), 20.71% ($P<0.05$), 33.14% ($P<0.01$), and 47.93% ($P<0.01$) in the leaves treated 25, 50, 75, and 100 μmol·L^{-1} Cd, respectively. The content of chlorophyll a dropped with the increase of Cd concentration. Treatment with of 25 and 50 μmol·L^{-1} Cd did not alter the content of chlorophyll b in the tobacco leaves, but chlorophyll b content significantly decreased when the concentration of Cd reached 75 μmol·L^{-1}. The change in total chlorophyll content was similar with the change in chlorophyll a. As chlorophyll a decreased to a greater extent than chlorophyll b, the ratio of chlorophyll a/b in the tobacco leaves declined as Cd concentration increased, but the ratio was significantly different from that of CK only at 100 μmol·L^{-1} Cd.

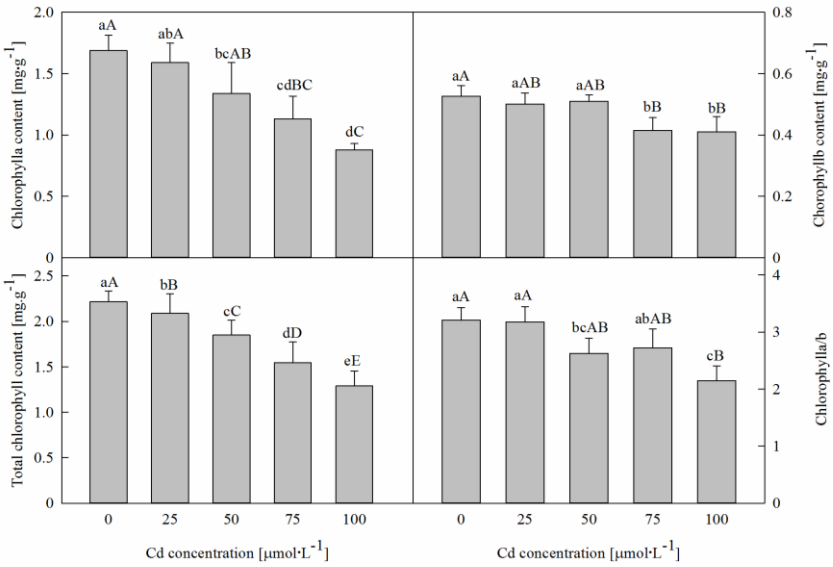

Figure 1. *Chlorophyll content in leaves of tobacco under cadmium stress. Note: Bar graphs depict mean ± SD, values followed by different small letters mean significant difference ($p<0.05$), values followed by different capital letters mean very significant difference ($p<0.01$).*

OJIP curve in tobacco leaves under Cd stress

The relative fluorescence intensities at all time points on the OJIP curve of the tobacco leaf treated with 25, 50, and 75 μmol·L^{-1} Cd were reduced compared to CK, and the differences became greater with time (*Fig. 2*). The relative fluorescence intensities between the OJIP curves of these three concentrations were not significantly

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different. The relative fluorescence intensity between O and J (i.e., 0-2 ms) in the tobacco under 100 μmol·L^{-1} Cd was slightly higher than that of CK. However, the relative fluorescence intensity gradually dropped below that of CK after 2 ms, and the relative fluorescence intensity was further reduced with time.

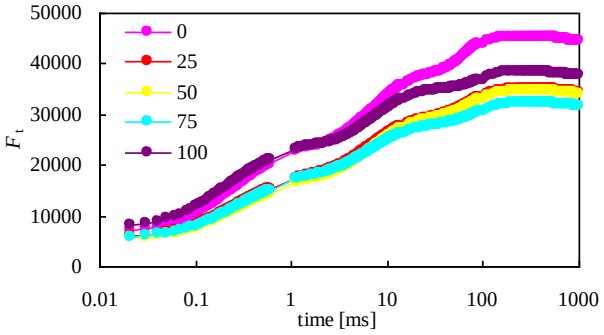

Figure 2. *OJIP curve in leaves of tobacco under cadmium stress. Note: Bar graphs depict mean ± SD, values followed by different small letters mean significant difference (p<0.05), values followed by different capital letters mean very significant difference (p<0.01)*

Standardized O-P curve and relative viable fluorescence in tobacco leaves under Cd stress

After standardizing the O step and P step of the OJIP curves of various treatments. The relative viable fluorescence around the J step (2 ms) showed the biggest variance among all the curves, and the relative fluorescence intensity at the J step increased with the increase of Cd concentration. In addition, the relative viable fluorescence at the I step (30 ms) showed significant variance: the values of 25 and 50 μmol·L^{-1} Cd were not significantly different from that of CK, whereas the values of plants treated with 75 and 100 μmol·L^{-1} Cd were significantly higher than that of CK. The relative viable fluorescence at the J step (V_J) and I step (V_I) was quantitatively analyzed. V_J in the tobacco leaves treated with 25, 50, 75, and 100 μmol·L^{-1} Cd increased by 5.22% ($P>0.05$), 7.83% ($P<0.05$), 14.38% ($P<0.01$), and 28.89% ($P<0.01$), respectively, compared with CK. In contrast, V_I only increased by 2.30% ($P>0.05$) and 8.72% ($P<0.01$) in response to 75 and 100 μmol^{-1} Cd treatment, and the amount of increase of V_I was significantly smaller than that of V_J (*Fig. 3*).

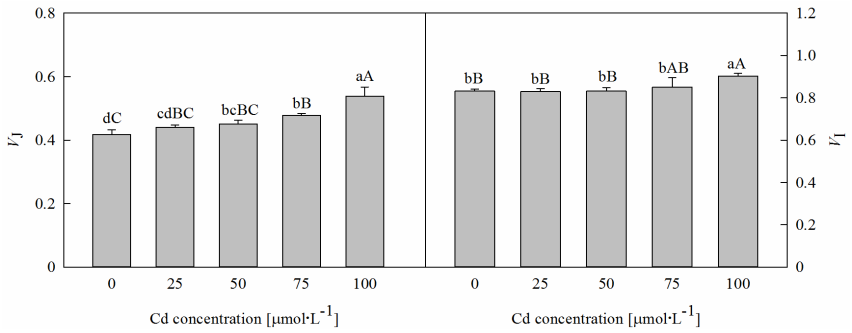

Figure 3. *V_{O-P}, V_J and V_I in leaves of tobacco under cadmium stress. Note: Bar graphs depict mean ± SD, values followed by different small letters mean significant difference (p<0.05), values followed by different capital letters mean very significant difference (p<0.01)*

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Standardized O-J and O-K curves and relative viable fluorescence in tobaccoleaves under Cd stress

To specifically analyze the relative viable fluorescence at the K and L step on the OJIP curve, standardized O-J and O-K curves were generated (*Fig. 4*). As shown in the figure, significant variances appeared at the K step (0.3 ms) and the L step (0.15 ms) on the O-J and O-K curves of tobacco leaves in response to different concentrations of Cd. By comparing between the O-J or O-K curves of various Cd treatments and that of CK, the differences at the K step and L were the largest between the treatments and CK, and the change in the relative variable fluorescence at the K step was greater than that at the L step. The quantitative analysis showed that V_K in the leaves of tobacco treated with 25, 50, 75, and 100 μmol·L^{-1} Cd increased by 2.36% ($P>0.05$), 5.55% ($P>0.05$), 6.92% ($P>0.05$), and 19.21% ($P<0.01$), compared with CK. In contrast, V_L was only increased by 8.52% ($P>0.05$) in the 100 μmol·L^{-1} Cd treatment compared with CK, and the difference was insignificant.

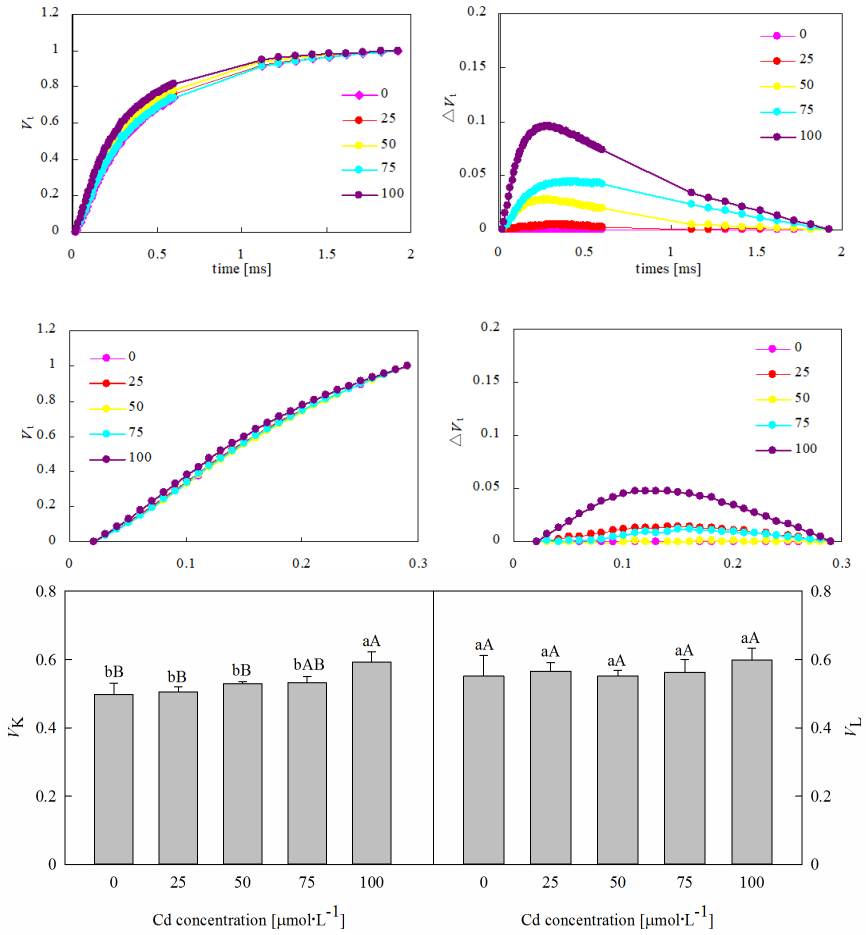

Figure 4. *V_{O-J}, V_{O-K}, V_K and V_L in leaves of tobacco under cadmium stress. Note: Bar graphs depict mean ± SD, values followed by different small letters mean significant difference ($p<0.05$), values followed by different capital letters mean very significant difference ($p<0.01$)*

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Radar plot of fluorescence data in leaves of tobacco seedlings under Cd stress

As shown in *Fig. 5*, F_v/F_m was minimally changed in the leaves of tobacco when treated with Cd, with a 7.29% reduction when treated with 100 μmol·L^{-1} Cd compared with CK. In contrast, PI_{ABS} in the leaves of tobacco treated with 25, 50, 75, and 100 μmol·L^{-1} Cd was reduced by 16.89%, 15.74%, 25.57%, and 61.36%, respectively. This displayed a greater amplitude of change compared to F_v/F_m. Cadmium stress also altered the parameters of light energy distribution in the PSII reaction centers and in each PSII reaction center. As Cd increased, φE_o was markedly reduced, whereas ET_o/RC did not show significant change. Moreover, Cd stress led to significant increases in φD_o and DI_o/RC in tobacco leaves, with the greatest changes at 100 μmol·L^{-1} Cd. When plants were treated with 100 μmol·L^{-1} Cd, φD_o and DI_o/RC increased by 35% and 73%, respectively, when compared with CK. *ABS*/*RC* in tobacco leaves was minimally changed when Cd concentration was between 0 and 75 μmol·L^{-1}, and *ABS*/*RC* increased by 28.81% compared with CK when the Cd concentration was 100 μmol·L^{-1}.

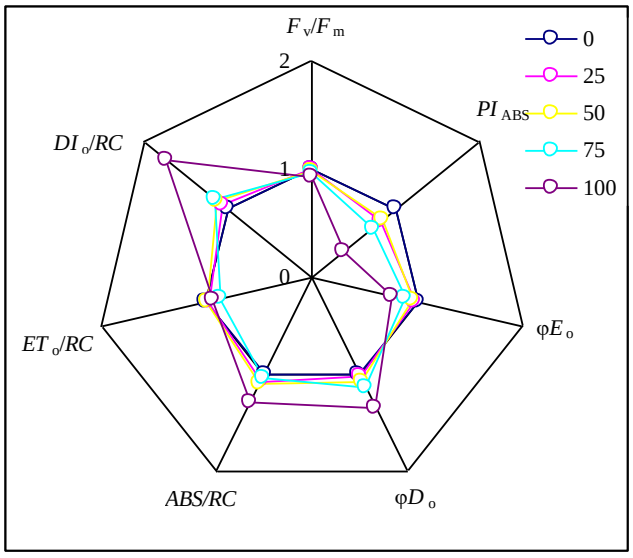

Figure 5. *Radar plot of fluorescence data in leaves of tobacco seedlings under cadmium stress. Note: Bar graphs depict mean ± SD, values followed by different small letters mean significant difference ($p<0.05$), values followed by different capital letters mean very significant difference ($p<0.01$)*

Discussion

Chlorophyll, the primary executor of photosynthesis in the leaves of plants, is responsible for the absorption, transmission, and conversion of solar energy. An adequate amount of chlorophyll in the plant is the basis for the maintenance of normal photosynthesis under stress conditions. However, a number of studies have shown that stress can reduce the content of chlorophyll in the leaves (Al-aghabary et al., 2005). In the present study, the chlorophyll content in the tobacco leaves was significantly reduced under Cd stress, and chlorophyll a decreased to a greater extent as Cd concentration increased, compared to chlorophyll b, which was not significantly reduced until Cd concentration reached 75 μmol·L^{-1}. As chlorophyll a was more abundant in the tobacco leaves than chlorophyll b, the change in total chlorophyll

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content was similar to the trend of chlorophyll a. As the change in the content of chlorophyll a was more prominent than that of chlorophyll b, the ratio of chlorophyll a/b in the tobacco leaves declined as Cd concentration increased. Thus, these results indicate that chlorophyll a is more sensitive to Cd stress. Chlorophyll a not only captures light energy but is also an energy conversion. Hence, reduction in the amount of chlorophyll a directly compromises the absorption and utilization of light energy in the leaves of flue-cured tobacco.

It was previously reported that Cd can inhibit the absorption, transmission, and transformation of light energy in the leaf, and it also reduces the photochemical efficiency of PSII, leading to the decline of photosynthetic capacity (Dhir et al., 2008). Wang and colleagues (2013) previously found that 100 and 200 μmol·L^{-1} Cd markedly decreased the oxygen evolving activity and inhibited the function of PSII in *Chlorella pyrenoidosa*. In addition, Li and colleagues (2011) demonstrated that Cd stress can reduce the activity of the carboxylation system in corn leaves, thus blocking the transformation of solar energy to chemical energy and resulting in the accumulation of electrons and decrease in net photosynthetic rate. The fast chlorophyll florescence kinetic technique is a probe for the function of PSII reaction centers in plants, and it is a useful tool to study the mechanism underlying PSII damage under stress conditions. In this study, all concentrations Cd investigated altered the shape of the OJIP curve. The JIP-test analysis of the OJIP curves showed that F_v/F_m of the tobacco leaves was minimally changed under various concentrations of Cd, and it decreased by 7.29% under 100 μmol·L^{-1} Cd compared with CK. These results suggest that the PSII reaction centers in the leaves of tobacco exhibit a relatively high Cd tolerance, and the results are consistent with Lugon-Moulin and colleagues' findings that tobacco grew well in the culture solution containing 100 μmol·L^{-1} $CdCl_2$ (Lugon-Moulin et al., 2004). However, performance index on absorption basis (PI_{ABS}) decreased significantly as the Cd concentration increased, suggesting that Cd stress reduced the activity of PSII reaction centers in a concentration-dependent manner in the leaves of flue-cured tobacco. Moreover, PI_{ABS} in the tobacco leaves decreased by 61.36% under 100 μmol·L^{-1} Cd compared with CK. These results suggest that PI_{ABS} is more sensitive than F_v/F_m regarding the function of PSII reaction centers in the leaves under stress. Compared to F_v/F_m, PI_{ABS} not only indicates the state of photon capture in the PSII reaction centers, but also reflects the capacity of electron transmission between photo systems during photosynthesis (Wen et al., 2005; Li et al., 2009). This is one of the advantages of applying the fast chlorophyll florescence kinetics technique in the study of PSII function in plants, as compared with the conventional chlorophyll fluorescence parameters, and the above observation has been validated in other studies (Zhang et al., 2013).

Cadmium has been shown to be toxic to *Microcystis aeruginosa* by affecting the activities of the electron donor and acceptor sides of the PSII reaction center and OEC (Zhou et al., 2006). Our results are consistent with these findings. By standardizing the OJIP curves of the tobacco leaves with various treatments, we found that V_J in the tobacco leaves increased as Cd concentration increased V_J under 50 μmol·L^{-1} Cd was remarkably different from the V_J of the CK, whereas V_J under 25, 50, and 75 μmol^{-1} Cd was not significantly different compared to the CK. The relative viable fluorescence at point J(V_J) reflects the amount that the electron donor Q_A (i.e., the accumulated amount of Q_A^-) was reduced. An elevated V_J is considered a most significant indicator of the blockage of electron transmission from Q_A to Q_B at the electron acceptor side of the PSII reaction center, while the variance of fluorescence at point I indicates a

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heterogeneity in the PQ bank (Li et al., 2005). In our study, V_J in the leaves of tobacco increased to a greater extent than V_I under all concentrations of Cd, suggesting that a Cd-induced blockage of electron transmission at the electron acceptor side of PSII mainly occurred at Q_B. Cadmium stress reduced the capacity of Q_B in electron acceptance. When Cd reached 100 μmol·L^{-1}, reduction in the electron acceptance capacity of the PQ bank resulted in a rate limit and a reduced capacity in electron transmission on the electron acceptor side of PSII. Increases in V_K (the relative viable fluorescence at point K on the standardized O-J curve) and V_L (the relative viable fluorescence at L point on the standardized O-K curve) and specifically indicate OEC injury and dissociation of thylakoids, respectively (Strasser, 1997; Ye et al., 2013; Arena et al., 2017). In the present study, Cd stress led to an increase of V_K in the leaves of flue-cured tobacco, which reached a significant level when the concentration of Cd was 100 μmol·L^{-1}. These results indicate a relatively high tolerance of the OEC, which locates at the electron donor side of PSII, to Cd stress, and the activity of OEC in the tobacco leaves was significantly inhibited only under 100 μmol·L^{-1} Cd. The 33 kDa OEC might be damaged under 100 μmol·L^{-1} Cd, leading to a disruption of the functional link between OEC and PSII (Jiang et al., 2006). Inhibition of the activity of OEC can cause abnormal water-splitting, and incomplete water-splitting may produce H_2O_2 (Henmi et al., 2004). Moreover, blockage of electron transmission at the acceptor side of PSII may lead to the production of superoxide (Zhang et al., 2013). These reactive oxygen species (ROS) can damage thylakoid membranes and lead to thylakoid dissociation (Wei et al., 2012). In our study, Cd of different concentrations did not cause a significant increase of V_L in the leaves of flue-cured tobacco, indicating that Cd stress did not damage the thylakoid structure in the leaves. These results suggest that thylakoids in the tobacco leaves exhibit a relatively high tolerance to Cd stress. The relationship between Cd-induced ROS production and thylakoid structure or PSII function requires further elucidation in future studies.

φ*E* was significantly reduced in the tobacco leaves under Cd stress, while φD_o was markedly increased, suggesting that the proportion of the absorbed luminous energy used for photochemical reactions was reduced under Cd stress, and the excessive energy was dissipated as inefficient thermal energy. This reflects Cd-induced damage to the PSII reaction centers in tobacco leaves, and it is also an adaptive strategy of the tobacco leaf to Cd stress. The tobacco leaves dissipate excessive heat to reduce the Cd-induced energy burden to the PSII reaction centers. *ABS*/*RC* in the tobacco leaves did not change significantly under Cd stress below 75 μmol·L^{-1}, but it increased in response to 100 μmol·L^{-1} Cd, implying that 100 μmol·L^{-1} Cd led to an increased number of inactive PSII reaction centers, resulting in enhanced light absorption in the remaining active reaction centers. The inactive reaction centers may act as dissipating sites of non-radioactive energy; thus inactivation of reaction centers is also a photo-protective mechanism in the plant in response to stress (Lee et al., 2001). Although *ABS*/*RC* was increased under Cd stress, ET_o/*RC* did not significantly change while DI_o/*RC* markedly increased. These results suggest that dissipation of thermal energy from the inactive reaction center is an important self-protective mechanism of the PSII reaction center for reducing the pressure of excitation energy in the reaction centers under Cd stress.

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Conclusion

Cadmium stress can reduce the content of chlorophyll in the leaves of flue-cured tobacco, and chlorophyll a is more sensitive to Cd stress than chlorophyll b. In addition, Cd can inhibit the activity of PSII reaction centers in the tobacco leaves. Compared with F_v/F_m, PI_{ABS} is a more representative indicator of Cd toxicity to PSII reaction centers in tobacco leaves. In the 50 $\mu mol \cdot L^{-1}$ Cd treatment, electron transmission from Q_A to Q_B at the electron acceptor side of PSII was blocked in the leaves of flue-cured tobacco, whereas the activity of OEC at the electron donor side of PSII was significantly inhibited when Cd concentration reached 100 $\mu mol \cdot L^{-1}$. Cadmium stress of various concentrations exerts litter effect on the state of thylakoids in tobacco leaves, suggesting the function of PSII is quite tolerant to Cd. When Cd stress is below 100 $\mu mol \cdot L^{-1}$, tobacco leaves adapt to Cd stress by decreasing the electron transmission rate in PSII, reducing the activity of the reaction centers and increasing thermal dissipation. When Cd stress rises to 100 $\mu mol \cdot L^{-1}$, tobacco leaves adapt to the high concentration of Cd via thermal dissipation and inactivation of PSII in synergy. Our future study aims to reveal the toxicological effect of Cd on the mechanisms underlying the anti-Cd response in tobacco.

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REFERENCES

[1] Al-aghabary, K., Zhu, Z. J., Shi, Q. H. (2005): Influence of silicon supply on chlorophyll content, chlorophyll fluorescence, and antioxidative enzyme activities in tomato plants under salt stress. – Journal of Plant Nutrition 27: 2101-2115.

[2] Arena, C., Figlioli, F., Sorrentino, M. C., Izzo, L. G., Giordano, S., Spagnuolo, V. (2017): Ultrastructural, protein and photosynthetic alterations induced by Pb and Cd in, *Cynara cardunculus* L. and its potential for phytoremediation. – Ecotoxicology and Environmental Safety 145: 83-89.

[3] Bah, M. A., Sun, H., Fei, C., Dai, H., Zhang, G., Wu, F. (2010): Comparative proteomic analysis of *Typha angustifolia* leaf under chromium, cadmium, and lead stress. – Journal of Hazardous Materials 184: 191-203.

[4] Bayçu, G., Gevrek-Kürüm, N., Moustaka, J., Castari, I., Rognes, S. E., Moustakas, M. (2017): Cadmium-zinc accumulation and photosystem II responses of *Noccaea caerulescens* to Cd and Zn exposure. – Environmental Science and Pollution Research 24: 2840-2850.

[5] Cai, S., Yue, L., Jin, T., Nordberg, T. (1998): Renal dysfunction from cadmium contamination of irrigation water: Dose-response analysis in a Chinese population. – Bulletin of the World Health Organization 76: 153-159.

[6] Cao, C. L., Ma, Y. B., Li, J. M., Wei, D. P., Yi, S. (2015): Tobacco cadmium health risk assessment and reduction techniques: A review. – Chinese Journal of Applied Ecology 26: 1279-1288.

[7] Deng, G., Li, M., Li, H., Yin, L., Li, W. (2014): Exposure to cadmium causes declines in growth and photosynthesisin the endangered aquatic fern (*Ceratopteris pteridoides*). – Aquatic Botany 112: 23-32.

[8] Dewez, D., Geoffroy, L., Vernet, G., Popovic, R. (2005): Determination of photosynthetic and enzymatic biomarkers sensitivity used to evaluate toxic effects of copper and fludioxonil in alga *Scenedesmus obliquus*. – Aquatic Toxicology 74: 150-159.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2103_19171929

[9] Dhir, B., Sharmila, P., Saradhi, P. P. (2008): Photosynthetic performance of *Salvinia natans* exposed to chromium and zinc rich wastewater. – Brazilian Journal of Plant Physiology 20: 61-70.
[10] Dong, J., Wu, F. B., Zhang, G. P. (2006): Influence of cadmium on antioxidant capacity and four microelement concentrations in tomato seedlings (*Lycopersicon esculentum*). – Chemosphere 64: 1659-1666.
[11] Ekmekçi, Y., Tanyolaç, D., Ayhan, B. (2008): Effects of cadmium on antioxidant enzyme and photosynthetic activities in leaves of two maize cultivars. – Journal of Plant Physiology 165(6): 600-611.
[12] Hall, J. L. (2002): Cellular mechanisms for heavy metal detoxification and tolerance. – Journal of Experimental Botany 53: 1-11.
[13] Henmi, T., Miyao, M., Yamamoto, Y. (2004): Release and reactive-oxygen-mediated damage of the oxygen-evolving complex subunits of PSII during photoinhibition. – Plant and Cell Physiology 45: 243-250.
[14] Hossain, Z., Hajika, M., Komatsu, S. (2012): Comparative proteome analysis of high and low cadmium accumulating soybeans under cadmium stress. – Amino Acids 43: 2393-416.
[15] Jarup, L., Kesson, A. (2009): Current status of cadmium as an environmental health problem. – Toxicology and Applied Pharmacology 238: 201-208.
[16] Jiang, C. D., Jiang, G. M., Wang, X. Z., Li, L. H., Biswas, D. K., Li, Y. G. (2006): Increased photosynthetic activities and thermostability of photosystem II with leaf development of elm seedlings (*Ulmus pumila*) probed by the fast fluorescence rise OJIP. – Environmental and Experimental Botany 58: 261-268.
[17] Jiang, C. D., Shi, L., Gao, H. Y., Schanker, G., Toth, S. Z., Strasser, R. J. (2006): Development of photosystems 2 and 1 during leaf growth in grapevine seedlings probed by chlorophyll a fluorescence transient and 820 nm transmission in *vivo*. – Photosynthetica 44: 454-463.
[18] Kieffer, P., Dommes, J., Hoffmann, L., Hausman, J. F., Renaut, J. (2008): Quantitative changes in protein expression of cadmium-exposed poplar plants. – Proteomics 8: 2514-2530.
[19] Kola, H., Wilkinson, K. J. (2005): Cadmium uptake by a green alga can be predicted by equilibrium modeling. – Environmental Science & Technology 39: 3040-3047.
[20] Krantev, A., Yordanova, R., Janda, T., Szalai, G., Popova, L. (2008): Treatment with salicilyc acid decreases the effect of cadmium on photosynthesis in maize plants. – Journal of Plant Physiology 165: 920-31.
[21] Lee, H. Y., Hong, Y. N., Chow, W. S. (2001): Photoinactivation of photosystem II complexes and hotoprotection by nonfunctional neighbours in *Capsicum annuum* L. leaves. – Planta 212: 332-342.
[22] Li, P. M., Gao, H. Y., Strasser, R. J. (2005): Application of the fast chlorophyll fluorescence induction dynamics analysis in photosynthesis study. – Journal of Plant Physiology and Molecular Biology 31: 559-566.
[23] Li, G., Zhang, S. P., Liu, P., Gao, H. Y., Wang, J. F., Liu, C. X., Dong, S. T., Zhang, J. W. (2011): Effect of cadmium on photosystem activities of maize (*Zea mays* L.) leaves. – Scientia Agricultura Sinica 44: 3118-3126.
[24] López-Millán, A.-F., Sagardoy, R., Solanas, M., Abadia, A., Abadia, J. (2009): Cadmium toxicity in tomato (*Lycopersicon esculentum*) plants grown in Hydroponics. – Environmental and Experimental Botany 65(2-3): 376-385.
[25] Lugon-Moulin, N., Zhang, M., Gadani, F., Rossi, L., Wagner, G. J. (2004): Critical review of the science and options for reducing cadmium in tobacco (*Nicotiana Tabacum* L.) and other plants. – Advances in Agronomy 83: 111-180.
[26] Marmiroli, M., Imperiale, D., Maestri, E., Marmiroli, N. (2013): The response of *Populus* spp. tocadmium stress: chemical, morphological and proteomics study. – Chemosphere 93(7): 1333-1344.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2103_19171929

[27] Masood, A., Iqbal, N., Khan, N. A. (2012): Role of ethylene in alleviation of cadmium-induced photosynthetic capacity inhibition by Sulphur in mustard. – Plant Cell & Environment 35: 524-533.
[28] Qian, H. F., Li, J. J., Sun, L. W., Chen, W., Sheng, G. D., Liu, W., Fu, Z. (2009): Combined effect of copper and cadmium on *Chlorella vulgaris* growth and photosynthesis-related gene transcription. – Aquatic Toxicology 94: 56-61.
[29] Rodrıguez-Serrano, M., Romero-Puertas, M. C., Zabalza, A., Corpas, F. J., Sandalio, L. M. (2006): Cadmium effect on oxidative metabolism of pea (*Pisum sativum* L.) roots. Imaging of reactive oxygen species and nitric oxide accumulation in vivo. – Plant Cell & Environment 29: 1532-1544.
[30] Sebastian, A., Prasad, M. N. V. (2014): Vertisol prevent cadmium accumulation in rice: Analysis by ecophysiological toxicity markers. – Chemosphere 108: 85-92.
[31] Sheetal, K. R., Singh, S. D., Anand, A., Prasad, S. (2016): Heavy metal accumulation and effects on growth, biomass, and physiological processes in mustard. – Indian Journal of Plant Physiology 21(2): 219-223.
[32] Singh, O. V., Labana, S., Pandey, G., Budhiraja, R., Jain, R. K. (2003): Phytoremediation: an overview of metallic ion decontamination from soil. – Applied Microbiology and Biotechnology 61: 405-412.
[33] Strasser, R. J. (1997): Donor side capacity of photosystem II probed by chlorophyll a fluorescence transient. – Photosynthesis Research 52: 147-155.
[34] Strasser, R. J., Srivastava, A., Govindjee (1995): Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria. – Photochemistry and Photobiology 61: 32-42.
[35] Tukaj, Z., Bascik-Remisiewicz, A., Skowronski, T., Tukaj, C. (2007): Cadmium effect on the growth, photosynthesis, ultrastructure and phytochelatin content of green microalga *Scenedesmus armatus*: a study at low and elevated CO_2 concentration. – Environmental & Experimental Botany 60: 291-299.
[36] Wagner, G. J. (1993): Accumulation of cadmium in crop plants and its consequences to human health. – Advances in Agronomy 51: 173-212.
[37] Wang, S. Z., Zhang, D. Y., Pan, X. L. (2013): Effects of cadmium on the activities of photosystems of *Chlorella pyrenoidosa* and the protective role of cyclic electron flow. – Chemosphere 93: 230-237.
[38] Wei, X. D., Chen, G. X., Shi, D. W., Liu, D., Tang, J. (2012): Effects of drought on fluorescence characteristics of photosystem II in leaves of *Ginkgo biloba*. – Acta Ecologica Sinica 32: 7492-7500.
[39] Wen, X., Qiu, N., Lu, Q. T., Lu, C. M. (2005): Enhanced thermotolerance of photosystem II in salt-adapted plants of the halophyte *Artemisia anethifolia*. – Planta 220: 486-97.
[40] Wu, K., Wu, Z. H., Tai, F. J., Xie, Y., Xie, B. N., Yuan, Z. L., (2011): Effects of cadmium on the contents of phytohormones，photosynthetic performance and fluorescent characteristics in tobacco leaves. – Acta Ecologica Sinica 31: 4517-4524.
[41] Ye, H. L., Shen, W. J., Zheng, B. G. (2013): Changes of photosynthetic membrane function and protein complexes in flag leaves of liangyoupeijiu during leaf senescence. – Acta Agronomica Sinica 39: 2030-2038.
[42] Zhang, X. F., Wang, D. Y., Chu, K. F. (2006): Changes of SOD activity and MDA content in rice exposed to Cd stress as affected by genotype. – Chinese Journal of Rice Science 20: 194-198.
[43] Zhang, F. Q., Wang, Y. S., Luo, Z. P., Dong J. D. (2007): Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). – Chemosphere 67: 44-50.
[44] Zhang, H. H., Tian, Q., Liu, G. J., Hu, Y. B., Wu, X. Y., Tian, Y., Li, X., Sun, G. Y. (2013): Responses of antioxidant enzyme and PSII electron transport in leaf of transgenic tobacco carrying *2-Cys Prx* to salt and light stresses. – Acta Agronomica Sinica 39: 2023-2029.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2103_19171929

[45] Zhang, H. H., Zhang, X. L., Wang, J., Tian, Y., Hu, J. W., Li, X., Sun, G. Y. (2013): A comprehensive evaluation of salt resistance in three clove varieties by the fast fluorescence transient parameters. – Journal of Nanjing Forestry University (Natural Sciences Edition) 37: 13-19.
[46] Zhang, F., Wan, X. Q., Zhai, J. (2014): Effects of nitrogen supplement on chlorophyll synthesis and chloroplast ultrastructure of poplar plants under cadmium stress. – Journal of Nuclear Agricultural Sciences 28: 485-491.
[47] Zhang, H. H., Li, X., Xu, Z. S., Wang, Y., Teng, Z. Y., An, M. J., Zhang, Y. H., Zhu, W. X., Xu, N., Sun, G. Y. (2020): Toxic effects of heavy metals Pb and Cd on mulberry (*Morus alba* L.) seedling leaves: Photosynthetic function and reactive oxygen species (ROS) metabolism responses. – Ecotoxicology and Environmental Safety 195: 110469.
[48] Zhou, W., Juneau, P., Qiu, B. (2006): Growth and photosynthetic responses of the bloom-forming cyanobacterium *Microcystis aeruginosa* to elevated levels of cadmium. – Chemosphere 65: 1738-1746.
[49] Zou, Q. (2000): Plant physiological and biochemical experiments. – Beijing: China Agriculture Press, pp. 168-170.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2103_19171929