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Exogenous γ-glutamic acid (GABA) induces proline and glutathione synthesis in alleviating Cd-induced photosynthetic inhibition and oxidative damage in tobacco leaves

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ABSTRACT
The effects of exogenous γ-glutamic acid (GABA) on photosynthetic gas exchange capacity, PSII function, proline (Pro) synthesis and ROS metabolism of tobacco leaves under cadmium (Cd) stress were studied. The results showed that both stomatal and non-stomatal factors were the important factors to limit the photosynthetic capacity of tobacco leaves under Cd stress. Pro accumulation and glutathione (GSH) content increase are the important ways for tobacco leaves to adapt to Cd stress. Exogenous GABA significantly increased the stability of oxygen-evolving complex (OEC) and the electron transfer rate of PSII in tobacco leaves under Cd stress, and increased G_s and P_n in varying degrees. Exogenous GABA also increased the expression of *P5CS* and *P5CR* genes in tobacco leaves under Cd stress, thus promoting Pro accumulation. In conclusion, 0.5 mmol·L^{-1} exogenous GABA can not only improve the carbon assimilation ability by increasing the G_s, but also alleviate the oxidative damage by promoting the synthesis and accumulation of Pro and GSH, and improve the stability of photosynthetic function of tobacco leaves under Cd stress.

γ-glutamic acid (GABA) is a natural non-protein amino acid, which is mainly synthesized by Glutamic acid decarboxylase (GAD) catalyzed glutamic acid (Glu) (Chung et al. 1992; Zarei et al. 2017), it can also be oxidized by polyamine oxidase (DAO) to pyrroline, and then to GABA (Gemperlova and Eder 2005). GABA reacts with pyruvate in the presence of GABA transaminase (GABA-T), generating succinic acid semialdehyde and alanine. Succinic acid semialdehyde is oxidized to form succenic in the presence of succinic semialdehyde dehydrogenase (SSADH), then enters the tricarboxylic acid cycle and participates in carbon metabolism. Therefore, GABA is associated with two major metabolic pathways of carbon and nitrogen in plants (Albert et al. 2015). When the synthesis of glutamine (Gln) is blocked, the protein synthesis is reduced or the degradation is accelerated, the conversion of Glu to GABA will increase (Wang et al. 2002). Succinic acid and other metabolites produced by GABA synthesis pathway can provide the necessary substrate for the respiration cycle in plant cells (Bouche 2003). Exogenous GABA could induce the activities of glutamine synthetase (GS) and glutamic acid synthetase (GOGAT) in rice seedlings under high ammonium condition, thus reducing the accumulation of NH_4^+ and alleviating ammonium toxicity (Ma et al. 2016). GABA is also considered as a signaling molecule of plant growth and development (Lancien and Roberts 2006). The content of GABA in plants will increase rapidly under stress conditions (Hartzendorf and Rolletschek 2001; Bartyzel et al. 2003; Bown et al. 2006). GABA, as a kind of osmotic regulation substance with small molecules, can reduce the osmotic potential of cytoplasm under water stress, thus improving the water holding capacity of cells (Breitkreuz et al. 1999). The study of Lancien and Roberts (2006) on *Arabidopsis* showed that GABA synthesis was related to ABA and ethylene signaling pathways. GABA can also regulate intracellular Ca^{2+} concentration and stress response related to Ca^{2+} signaling through GAD and glutamate receptor family (Gut et al. 2009). GABA also enhances the adaptability of plants to stress by regulating reactive oxygen species (ROS) metabolism (Kinnersley A and Turano F 2000), such as activating antioxidant system and regulating H_2O_2 content (Shi et al. 2010; Yang et al. 2011). GABA can also induce the accumulation of proline (Pro) and polyamine (PAs) and improve the stress resistance ability of plants (Lei et al. 2016), for example, exogenous GABA inhibits ethylene synthesis under salt stress, which also promotes the accumulation of endogenous PAs (Turano et al. 2010).

At present, with the rapid development of industry and agriculture, a large number of excessive metal waste and agricultural sewage flow into the environment (Soffianian et al. 2014; Du et al. 2019). Heavy metals in nature are difficult to remove and degraded, which not only affects the safety of the ecological environment, but also poses a threat to food safety and human health (Alfven et al. 2000; Qian et al. 2009; Ogbomida et al. 2018). Although some heavy metals such as Cu, Zn and Mn are essential trace elements for plant growth and development (Seregin and Ivanov

2001), most of them are highly toxic to plants (Shahid et al. 2014). Among the heavy metal pollution, Cd is one of the most toxic and common heavy metal pollutants, and it is easy to enter the food chain (Chen et al. 2018). Cd can destroy the structure of plant chloroplast (Santos et al. 2018), inhibit the synthesis of chlorophyll and photosynthesis (Shukla et al. 2008; Hendrik et al. 2007; Zhang et al. 2015; Zhang et al. 2020a), reduce the activity of antioxidant enzymes (Wu et al. 2015; Khanna et al. 2019; Zhang et al. 2020b), lead to the outbreak of ROS (Zhang et al. 2007; Rizwan et al. 2019; Zhang et al. 2020c).

Tobacco exhibits a relatively high tolerance to Cd, 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). A large number of studies have shown that exogenous GABA can improve the stress resistance of plants (Shi et al. 2010; Yang et al. 2011; Rezaei-Chiyaneh et al. 2018), but there is no research on the mechanism of exogenous GABA regulating tobacco tolerance under Cd stress. Whether exogenous GABA can improve tobacco tolerance to Cd stress? If so, what is the main way to regulate it? Therefore, the effects of exogenous GABA on photosynthetic function of tobacco leaves under Cd stress were studied, and the effects of exogenous GABA on the metabolism of Pro and PAs under Cd stress were studied in order to reveal the mechanism of GABA in improving tobacco tolerance to Cd stress, and to provide a theoretical basis for tobacco cultivation with heavy metal tolerance.

1. Materials and methods

1.1 Experimental materials and treatment

The experimental tobacco variety, 'Longjiang 911,' used in this study is the major tobacco variety grown in Heilongjiang Province, China, and 'Longjiang 911' is one of the most sensitive varieties to Cd stress (Zhang et al. 2020a, p. 2020b). The seeds were provided by the Mudanjiang Tobacco Research Institute. The experiment was conducted at Northeast Agricultural University (Harbin, China) in 2019. The seeds were sown in peat and vermiculite (1:1 in volume) in early March, and the seedlings were cultivated indoors at $25 \pm 2°C$ under artificial light with an intensity of 200 μmol m^{-2} s^{-1} and a light/dark cycle of 12 h.

When the seedlings grow to four leaves, the seedlings were transplanted into a plastic pot with a diameter of 12 cm and a height of 15 cm, the culture medium was peat and vermiculite with the ratio of 1:1 (volume), and transplant one plant per pot. 30 days after transplanting, seedlings with the same growth characteristics were selected as the test subjects. A two-factors randomly design was used. According to the tolerance characteristics of tobacco to Cd in our preliminary experiment (Zhang et al. 2020a, p. 2020b), tobacco seedlings were treated with 0, 100 and 200 μmol·L^{-1} CdCl$_2$ respectively, each pot was irrigated with 200 mL CdCl$_2$ solution. The plants of different CdCl$_2$ treatments were divided into two groups, one group was sprayed with 50 mmol·L^{-1} GABA solution (GABA), the other group was sprayed with distilled water as control (CK). There are six treatments in total, ten pots for each treatment. A fine mist solution was sprayed both on the front and back of leaves until uniform, small liquid beads were about to drip from the leaf surface. On the 7th day, the differences of plants under different treatments were observed and this data was used to calculate the following indexes.

1.2 Measurements and methods

Determination of photosynthetic gas exchange parameters: From 9 am to 11 am, the second fully expanded functional leaf was used as the experimental material. A Li-6400 photosynthesis measurement system (Licor Corporation, USA) was employed to measure the net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r), and intercellular CO$_2$ concentration (C_i). The photon flux density (PFD) was measured using the default setting for the instrument, 1200 μmol m^{-2} s^{-1}, and the CO$_2$ concentration was constantly set to 400 μL L^{-1} using CO$_2$ cylinders. All measurements were repeated three times.

Determination of OJIP curves: fully expanded functional leaves on tobacco plants under different treatments were selected, and dark adaptation clips were used to treat leaves with 30 min of darkness. Using the multifunctional plant efficiency analyzer (M-PEA, Hansatech, UK), OJIP curves were obtained, and there were five replicates for each treatment. According to the formulae $V_{O-P}=(F_t-F_o)/(F_m-F_o)$, the OJIP curve was normalized, resulting in V_{O-P} curve, where F_m and F_o are the relative fluorescence intensities of P point (1000 ms) and O point (0.01 ms) on the OJIP curve, F_t denotes the relative fluorescence intensity at each time point on the OJIP curve. The relative variable fluorescence at the J point (2 ms) and K point (0.3 ms) on the V_{O-P} curve was represented as V_J and V_K. In addition, V_{O-P} a difference between treatments and CK were represented as ΔV_{O-P} a, and were used to analyze the range of variation of each feature point on the curve. A JIP-test analysis was conducted on the OJIP curve to obtain the maximum photochemical efficiency of PSII (F_v/F_m), performance index of PSII based on absorption (PI_{ABS}), JIP-test analysis of OJIP curves by the method of Strasser et al. (1995).

Determination of physiological indicators: Reduced glutathione (GSH) content were determined using the kits produced by Suzhou Comin Biotechnology Co., Ltd. H$_2$O$_2$ content was measured using the method of Alexieva et al. (2001); Proline (Pro) content was determined by 3% sulfosalicylic acid boiling water extraction and acid ninhydrin colorimetry; malondialdehyde (MDA) content was determined using the thiobarbituric acid chromogenic method (Wang et al. 2003). All the above indexes were measured three times of biological repetition.

Determination of the expression of Pro metabolism related gene: Total RNA was extracted from approximately 100 mg of plant tissue using OMEGA Plant RNA Kit (Biotek, Norcross, Georgia) according to the manufacturer's instructions. Extracted RNA was used for singlestrand cDNA synthesis with PrimeScript RT Reagent Kit (TaKaRa, Japan). Real-time PCR was carried out according to an SYBR Green fluorescence-based procedure using SYBR Premix Ex Taq (TaKaRa, Japan). The PCR cycling protocol consisted of an initial denaturation at 94°C for 10 min, followed by 40 cycles of 94°C for 20 s and 60°C for 20 s. After the final cycle, a melting curve analysis was performed over a temperature range of 60–95°C in increments of 1°C to verify the reaction specificity. Using the actin gene as a constitutive reference, relative expression was measured by the $2^{-\Delta\Delta Ct}$

method (Livak and Schmittgen 2001). Gene specific primers sequences (5′-3′) are as follows:

PsbA F: ATTCCAGGCTGAGCACAACA; R: GGTTGAAAGCCATAGTGCTG.

PsbO F: F: GTTGCTGGTTTTGCTCTGG; R: AGTTCCAGTTCCCTTTACTTCC.

P5CS: F: TCTGAAGTGGCTGAGACATTC; R: TCGAGCATGAATCCGACTTG

P5CR: F: AGGGTAATGCCTAACACAGC; R: CGTCAGCTTTCCATATTTTGCC

Reference genes: F: GGATGGGGAGGACATTCAAC; R: TGTATGTGAGACACCGTCGC.

1.3 Data analysis

Excel (2003) and SPSS (22.0) software were used for statistical analysis. Two-way ANOVA and least significant difference (LSD) were used for the comparison of the differences between different data sets.

2. Results

2.1 Photosynthetic gas exchange parameters

As show in Figure 1, with the increase of Cd concentration, P_n, G_s and T_r of tobacco leaves decreased, and C_i under Cd stress of 100 μmol L^{-1} decreased significantly compared with that no Cd stress, but when Cd concentration increased to 200 μmol L^{-1}, C_i increased slightly compared with that under Cd concentration of 100 μmol L^{-1}, but it did not reach the significant difference level. Exogenous GABA increased P_n, G_s and T_r of tobacco leaves slightly under different concentrations of Cd stress, but only at the

concentration of 200 μmol L^{-1} Cd was significantly different from CK.

2.2 PSII photochemical activity

It can be seen from Figure 2(a, b) that under Cd stress of 100 μmol L^{-1}, the relative fluorescence intensity (F_o) of O-point on the OJIP curve of tobacco leaves did not change significantly, while the relative fluorescence intensity (F_m) of P-point decreased significantly. Under Cd stress of 200 μmol L^{-1}, the F_m further decreased, and the F_o also increased significantly, F_o in tobacco leaves under exogenous GABA treatment was significantly lower than CK, but F_m was significantly higher than CK (Figure 3c, d). In Figure 2(e, f), with the increase of Cd concentration, F_v/F_m and PI_{ABS} of tobacco leaves show a decreasing trend, and the decreasing range of PI_{ABS} was significantly larger than that of F_v/F_m. Under Cd stress of F_v/F_m and 100 μmol L^{-1}, exogenous GABA slightly increases F_v/F_m and PI_{ABS} of tobacco leaves, but there is no significant difference with CK. Under Cd stress of 200 μmol L^{-1}, F_v/F_m and PI_{ABS} of tobacco leaves treated with exogenous GABA were higher than CK by 4.37% ($P < 0.05$) and 33.60% ($P < 0.05$), respectively.

2.3 PSII electron transfer

It can be seen from Figure 3(a, b) that F_o and F_m on the OJIP curve of tobacco leaves with different treatments are defined as 0 and 1 respectively. After standardizing the original OJIP curve according to $V_{O-P}=(F_t-F_o)/(F_P-F_o)$, with the increase of Cd concentration, the relative variable fluorescence of K point and J point on the V_{O-P} curve of tobacco leaves increases to different degrees compared with CK. In Figure
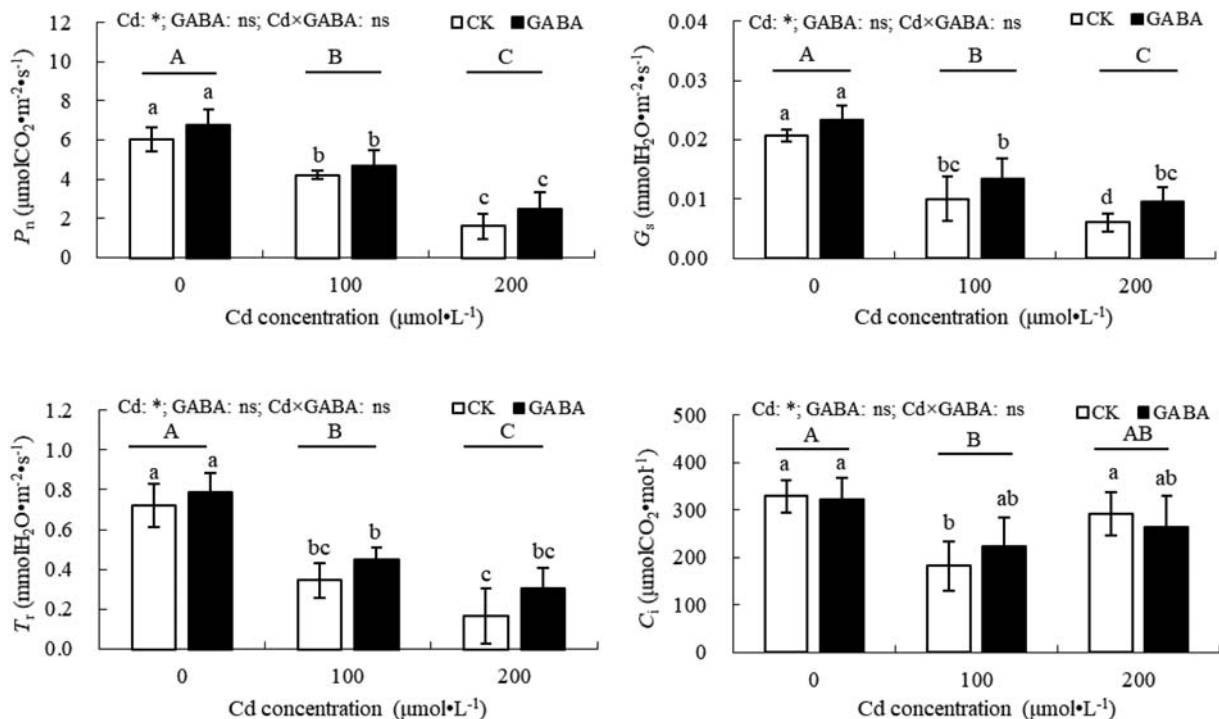

Figure 1 . Effects of exogenous GABA on photosynthetic gas exchange parameters of tobacco leaves under Cd stress. Note: Different capital letters indicated that there was a significant difference between different Cd treatment ($P < 0.05$), and different small letters indicated there was a significant difference among different treatments ($P < 0.05$). Cd, GABA, and Cd × GABA stand for Cd treatment, GABA treatment, and the mixed Cd and GABA, respectively. *: $P < 0.05$, ns: $P > 0.05$.

Figure 2 . Effects of exogenous GABA on the OJIP curve (a, b) and F_v/F_m (c) and PI_{ABS} (d) of tobacco leaves under Cd stress. Note: Different capital letters indicated that there was a significant difference between different Cd treatment ($P < 0.05$), and different small letters indicated there was a significant difference among different treatments ($P < 0.05$). Cd, GABA, and Cd × GABA stand for Cd treatment, GABA treatment, and the mixed Cd and GABA, respectively. *: $P < 0.05$, ns: $P > 0.05$.
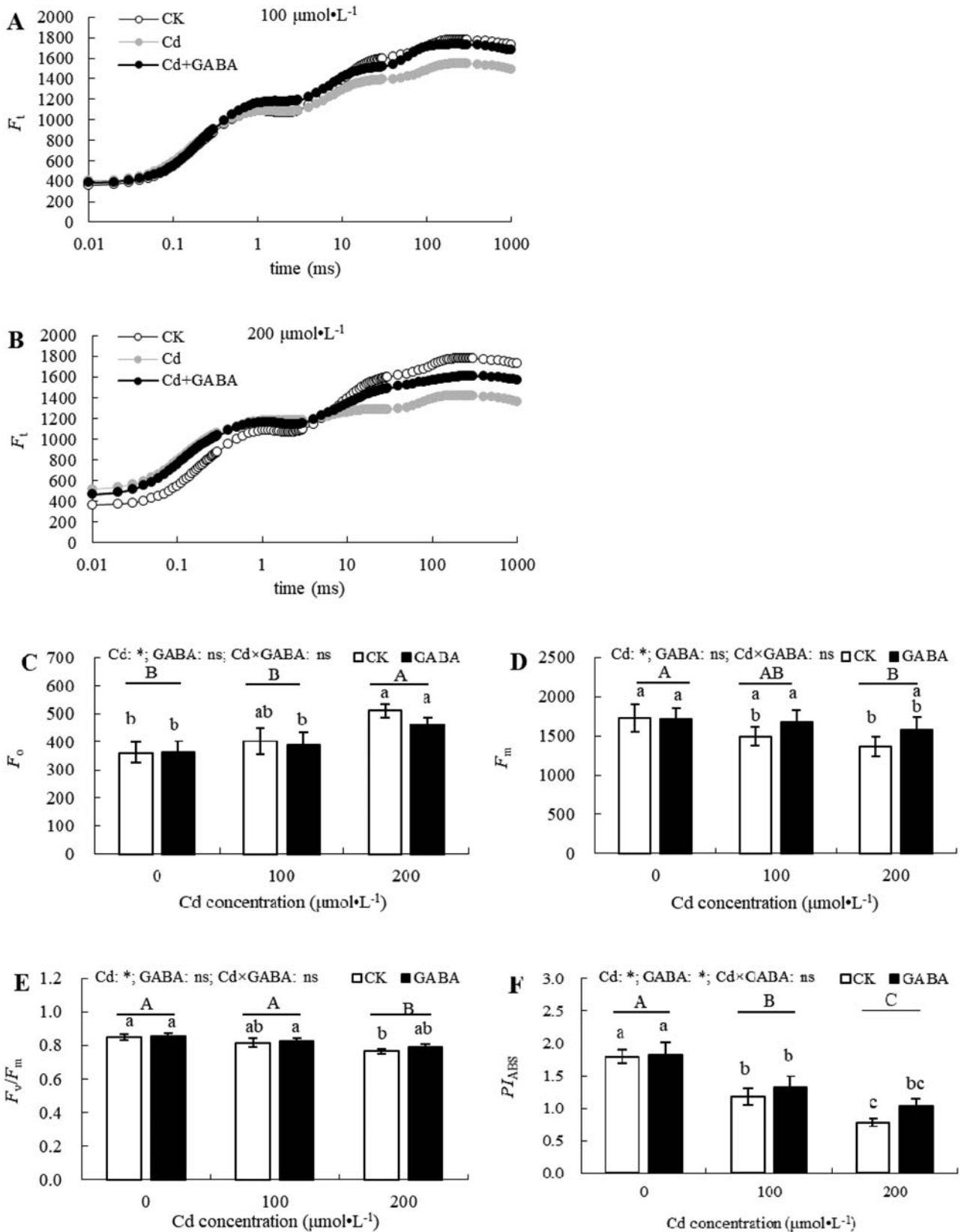
3(c, d), under 100 μmol L^{-1} Cd stress, exogenous GABA reduced the V_K by 12.98% ($P < 0.05$), but the V_J did not change significantly. Under 200 μmol L^{-1} Cd stress, exogenous GABA reduced the V_K and V_J of tobacco leaves by 19.59% ($P < 0.05$) and 20.85% ($P < 0.05$) respectively. Without Cd stress, the expression of *PsbO* and *psbA* genes in

tobacco leaves under exogenous GABA treatment were not significantly different from that of CK. However, under 100 and 200 μmol L^{-1} Cd stress, the expression levels of *PsbO* and *psbA* genes in tobacco leaves treated with exogenous GABA were significantly higher than those of CK (Figure 3(e, f)).
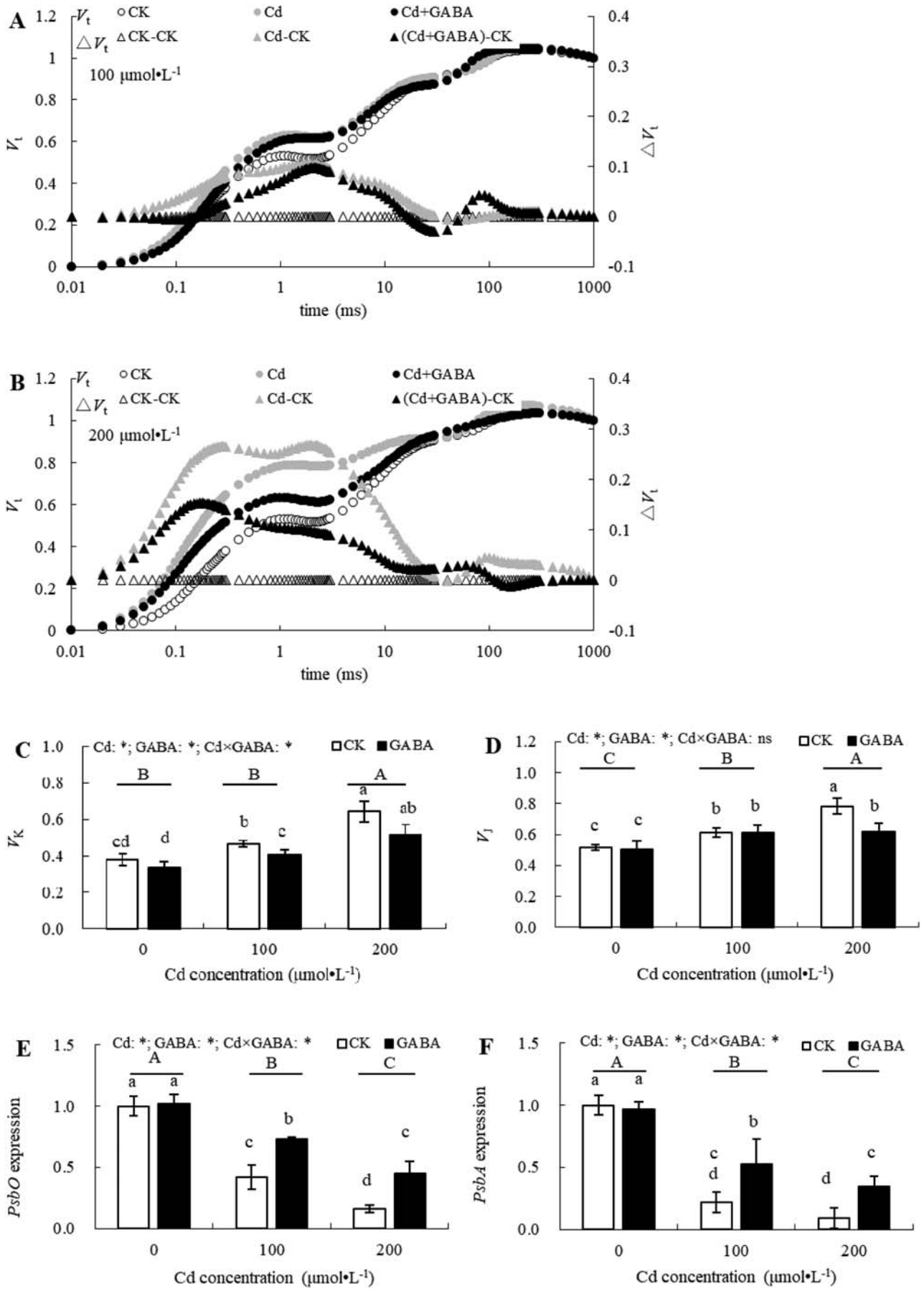
Figure 3 . Effects of exogenous GABA on the standardized OJIP curve (a, b), V_K (c), V_J (d) and related gene expression of *PsbO* (e) and *PsbA* (f) of tobacco leaves under Cd stress. Note: Different capital letters indicated that there was a significant difference between different Cd treatment ($P < 0.05$), and different small letters indicated there was a significant difference among different treatments ($P < 0.05$). Cd, GABA, and Cd × GABA stand for Cd treatment, GABA treatment, and the mixed Cd and GABA, respectively. *: $P < 0.05$, ns: $P > 0.05$.

2.4 GSH content and ROS metabolism

In Figure 4, under 100 μmol L^{-1} Cd stress, GSH content in tobacco leaves increased significantly, but when Cd concentration increased to 200 μmol L^{-1}, GSH content decreased compared with 100 μmol L^{-1} Cd stress. With the increase of Cd concentration, the H$_2$O$_2$ content, MDA content and electrolyte leakage rate of tobacco leaves increased significantly. Without Cd stress, exogenous GABA had no significant effect on the GSH and H$_2$O$_2$ content, but under different Cd stress, exogenous GABA significantly increased the GSH content of tobacco leaves, and significantly reduced the H$_2$O$_2$ content. Under 100 and 200 μmol L^{-1} Cd stress, exogenous GABA treatment reduced MDA content by 31.03% ($P < 0.05$) and 20.56% ($P < 0.05$), respectively.

2.5 Pro content related gene expression

It can be seen from Figure 5 that with the increase of Cd concentration, the Pro content of tobacco leaves increased. Without Cd stress, exogenous GABA had no significant effect on the Pro contents of tobacco leaves. Under Cd stress of 100 and 200 μmol L^{-1}, exogenous GABA increased the Pro contents of by 49.33% ($P < 0.05$) and 57.12% ($P < 0.05$), respectively. With the increase of Cd concentration, the expression of P5CS and P5CR genes in tobacco leaves showed an increasing trend. Under different Cd concentrations, exogenous GABA treatment significantly increased the expression of P5CS and P5CR genes.

3. Discussion

Photosynthesis is the basis for plants to obtain material and energy. More than 95% of dry matter in plants comes directly from photosynthetic products. Cd stress can significantly affect the photosynthetic capacity of plants, and the decrease of carbon assimilation capacity is mainly related to stomatal and non-stomatal factors (Deng et al. 2014). Among them, the non-stomatal factors are mainly related to the decrease of the activity of photosynthesis related enzymes (Krantev et al. 2008; Song et al. 2019), Cd^{2+} can combine with the sulfhydryl group in the active center of the enzyme to replace the essential elements in the metal protein, resulting in the change of the conformation of biological macromolecules and the loss of enzyme activity, photosynthesis related enzymes such as ribulose-1,5-bisphosphate (RuBP) carboxylase oxygenase (Mobin and Khan 2007), phosphoenolpyruvate carboxylase (Alexander et al. 2008) and so on. In the present study, the decrease of P_n was accompanied by the decrease of G_s, T_r and C_i in tobacco leaves under 100 μmol·L^{-1} Cd stress. Therefore, the decrease of photosynthetic capacity under 100 μmol L^{-1} Cd stress was mainly related to the decrease of stomatal conductance. However, with the decrease of P_n and G_s at 200 μmol L^{-1} Cd, C_i of tobacco leaves increased compared with that of 100 μmol L^{-1} Cd stress. According to Farquhar's theory (Farquhar and Sharkey 1982), it is suggested that Cd concentration of 200 μmol L^{-1} can reduce the photosynthetic capacity of tobacco leaves, and non-stomatal factors play an important role. The results showed that exogenous GABA could maintain the photosynthetic capacity of plants under stress by increasing chlorophyll synthesis (Nayyar et al. 2014; Luo et al. 2011), improving stomatal conductance and reducing stomatal limiting value (Wu et al. 2016), GABA is necessary and sufficient to reduce stomatal opening and transpirational water loss (Xu et al. 2021). In this experiment, exogenous
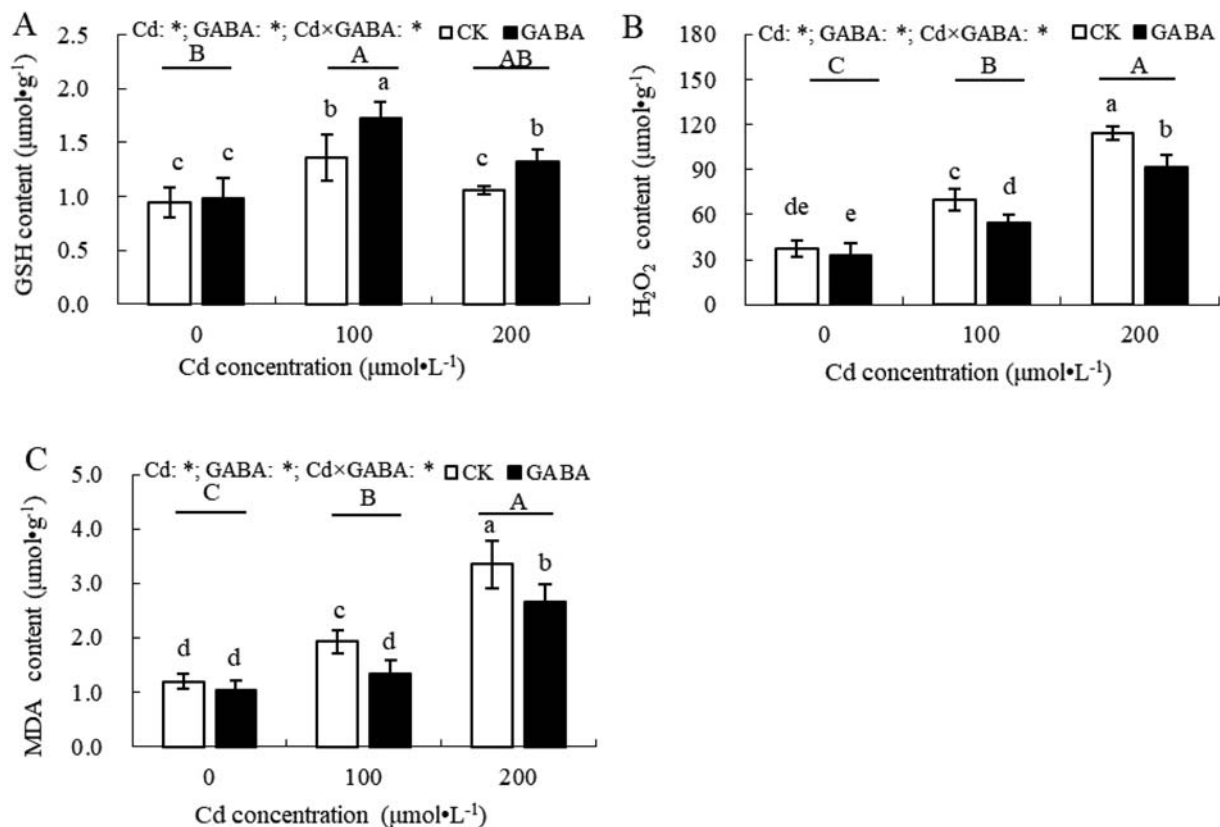

Figure 4 . Effects of exogenous GABA on GSH (a), H$_2$O$_2$ (b) and MDA content (c) of tobacco leaves under Cd stress. Note: Different capital letters indicated that there was a significant difference between different Cd treatment ($P < 0.05$), and different small letters indicated there was a significant difference among different treatments ($P < 0.05$). Cd, GABA, and Cd × GABA stand for Cd treatment, GABA treatment, and the mixed Cd and GABA, respectively. *: $P < 0.05$, ns: $P > 0.05$.
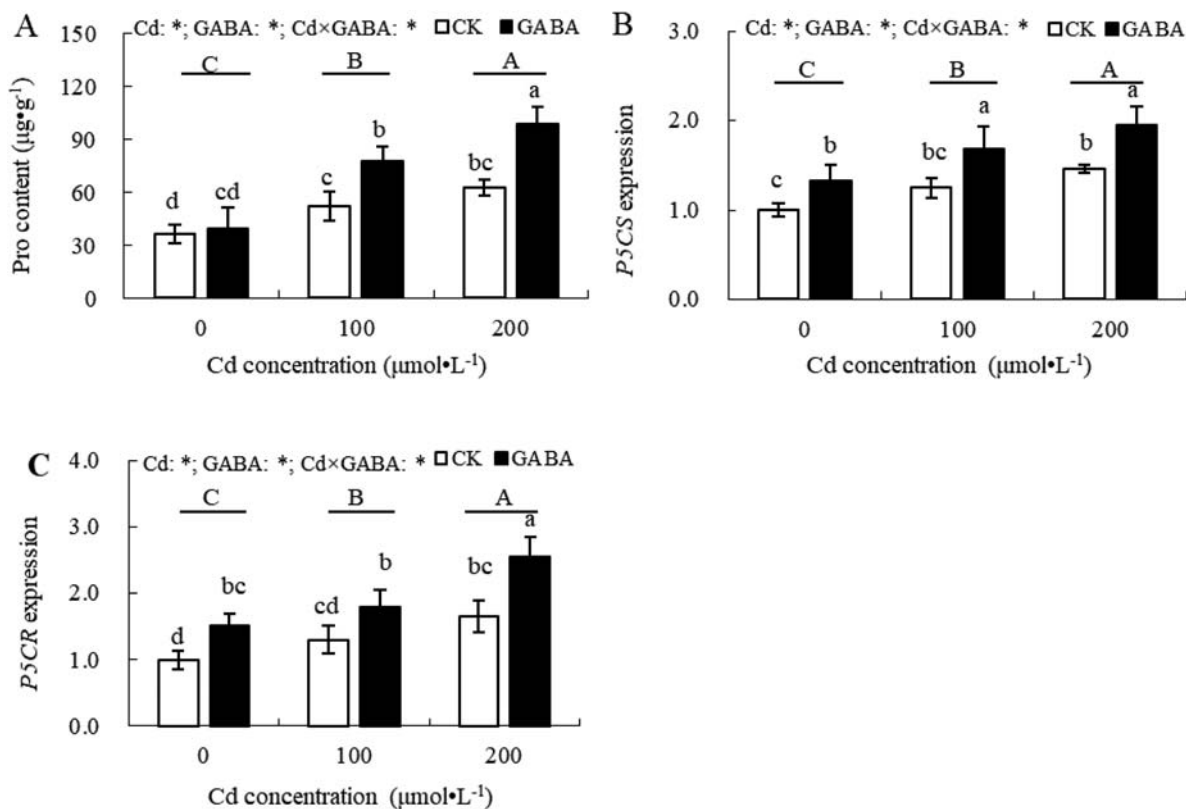
Figure 5 . Effects of exogenous GABA on Pro content (a), related gene expression of *P5CS* (b) and *P5CR* (c) in tobacco leaves under Cd stress. Note: Different capital letters indicated that there was a significant difference between different Cd treatment (*P* < 0.05), and different small letters indicated there was a significant difference among different treatments (*P* < 0.05). Cd, GABA, and Cd × GABA stand for Cd treatment, GABA treatment, and the mixed Cd and GABA, respectively. *: *P* < 0.05, ns: *P* > 0.05.

GABA promoted the increase of P_n, G_s and T_r in tobacco leaves under different Cd concentrations, exogenous GABA could improve the tolerance of tobacco to Cd stress to some extent by improving stomatal limitation of tobacco under Cd stress.

Cd stress inhibited the capture, transmission and transformation of light energy in plant leaves, and reduced the photochemical efficiency of PS II, PSII of photosynthesis is the primary target of Cd toxicity (Hendrik et al. 2007; Dhir et al. 2008). As a probe to study the function of PSII reaction center, chlorophyll fluorescence plays an important role in reflecting the intrinsic mechanism of PSII damage under stress. In this experiment, the results of JIP test on OJIP curve showed that F_v/F_m and PI_{ABS} of tobacco leaves decreased significantly with the increase of Cd concentration, indicating that Cd stress significantly reduced the activity of PSII reaction center in tobacco leaves. This is similar to the results of Wang et al. (2013), Vijayakumari and Puthur et al. (2015), and Zhang et al. (2018a), showed that exogenous GABA treatment could increase PSII activity in *Piper nigrum* Linn. leaves. Similar results were obtained in this study, the decrease of F_v/F_m and PI_{ABS} in tobacco leaves treated with exogenous GABA under different Cd concentrations were alleviated, which indicated that exogenous GABA could alleviate the photoinhibition of PSII in tobacco leaves under Cd stress. We further analyzed the effects of exogenous GABA on the electron transfer ability of PSII electron donor and acceptor side in tobacco leaves. Some studies have found that Cd stress can inhibit the activity of OEC (Mallicka 2003), the main reason is that the 33 kDa protein is destroyed under Cd stress, which results in the failure of functional link between OEC and PSII (Jiang et al. 2006).

The increase of relative variable fluorescence V_K at 0.3 ms on the normalized O-J curve was considered as a specific marker of damage to OEC activity of PSII (Zhang et al. 2018a). In this experiment, under exogenous GABA treatment, the V_K of tobacco leaves was significantly lower than that of CK, and the oxygen-evolving enhancer protein 1 gene (*PsbO*) expression was significantly higher than CK, indicating that exogenous GABA could promote the stability of OEC in PSII donor side of tobacco leaves under Cd stress. Some studies have shown that the stability of OEC depends on high concentration of Ca^{2+} in cystoid cavity, Ca^{2+} can prevent PSII photoinhibition by increasing the stability of OEC (Ettinger et al. 1999; Takahashi et al. 2009). Under Cd stress, Cd^{2+} can replace Ca^{2+} binding site in OEC and inhibit OEC activity (Faller et al. 2005). GABA can regulate intracellular Ca^{2+} concentration and stress response related to Ca signal (Gut et al. 2009), GABA treatment significantly increased the contents of calcium and calmodulin in barley (Gut et al. 2009), and induced the influx of Ca^{2+} in root tip cells of barley (Ma et al. 2019). Therefore, the increase of Ca^{2+} concentration in tobacco leaves induced by GABA may be one of the important reasons for the increase of OEC stability under Cd stress. The relative variable fluorescence V_J at 2 ms on the normalized OJIP curve indicated that the electron transfer from Q_A to Q_B in the photosynthetic electron transport chain was blocked (Zhang et al. 2019; Zhang et al. 2018b). Some studies found that Cd stress inhibited the electron transfer on the electron acceptor side of PSII reaction center of *Microcystis aeruginosa* (Zhou et al. 2006), Qian et al. (2009) showed that Cd stress could decrease the transcription of photosynthesis related genes such as *psbA*, *PsaB*, and inhibit the electron transfer from
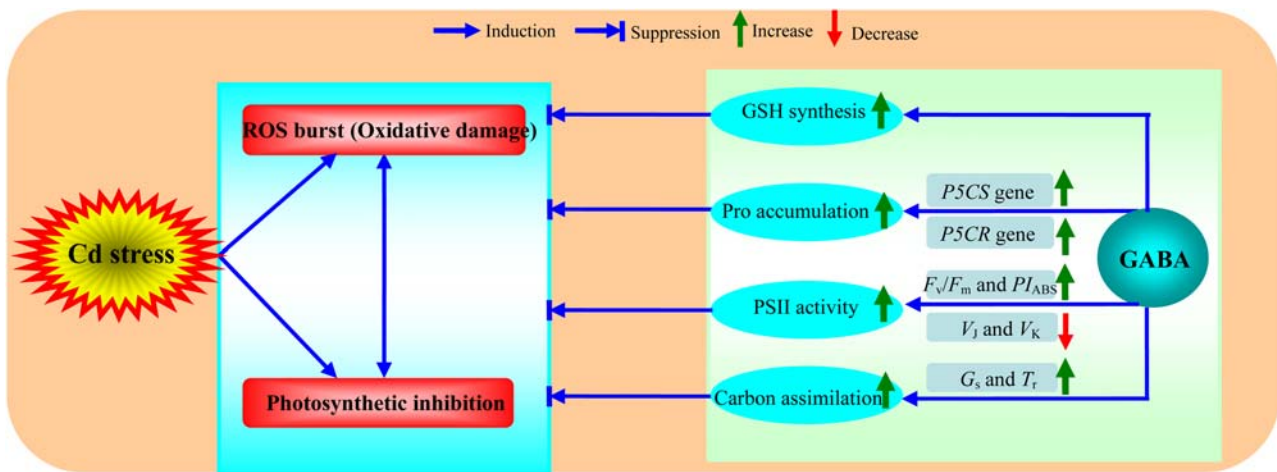
Figure 6 . The mechanism of GABA alleviated Cd-induced photosynthetic inhibition and oxidative damage in tobacco leaves.

Q_A to Q_B on PSII acceptor side. The present results showed that the V_J of tobacco leaves increased significantly with the increase of Cd concentration, which indicated that Cd stress inhibited the electron transport activity of PSII acceptor side. However, exogenous GABA alleviated the increase of V_J in tobacco leaves under 200 $\mu mol \cdot L^{-1}$ Cd stress, the down regulation of photosystem II protein D1 gene (PsbA) expression was alleviated, indicating that exogenous GABA could promote the electron transfer of PSII acceptor side in tobacco leaves under Cd stress. Xiang et al. (2016) also found that exogenous GABA could enhance the ability of PSII acceptor side electron transfer in Muskmelon seedlings leaves under saline alkali stress.

Stress often leads to excessive reduction of photosynthetic or respiratory electron transport chain in plants, excess electrons can induce a large number of ROS in cells (Ahmed et al. 2009). Excessive ROS breaks the redox balance in plants, causing membrane peroxidation, leading to membrane system damage and oxidative damage to cell components and structures (Gill et al., 2010; Kaya et al. 2019). The results showed that exogenous GABA could activate antioxidant system and inhibit ROS accumulation (Bouche et al. 2003; Shi et al. 2010; Wang et al. 2021). GSH is an important water-soluble antioxidant, which can directly reduce some ROS (Sorkheh et al. 2012). The increase of GSH content plays an important role in improving the tolerance of plants to Cd stress (Li et al. 2019). GABA-mediated shielding effect on membrane integrity by trolling lipid peroxidation was also observed by Song et al. (2010) in barley seedlings. As a non-protein amino acid, GABA participates in amino acid metabolism in plants (Brikis et al. 2018). In this experiment, under exogenous GABA treatment, the GSH content in tobacco leaves was significantly higher than that in CK treatment, while the contents of H_2O_2, MDA content were significantly decreased. Therefore, exogenous GABA could alleviate the oxidative damage induced by Cd by promoting GSH synthesis in tobacco leaves under Cd stress, which was one of the important reasons for the alleviation of photosynthetic inhibition.

The increase of Pro content can improve the water absorption capacity of plants under stress (Miransari 2010; Zhang et al. 2020d). In addition, Pro also plays a role of signal molecule in plant growth and development (Wang et al. 2014; Biancucci et al. 2015). In this study, with the increase of Cd concentration, tobacco leaves could adapt to Cd stress by increasing Pro content, which was consistent with the results of rice (Kumar and Khare 2016) and sesame (Koca et al. 2007) under stress through Pro accumulation. The synthesis of Pro in plants is mainly catalyzed by P5CS and P5CR (Kishor et al. 1995; Milosz et al. 2015). Under stress, the expression of P5CS andP5CR genes is increased, which promotes the synthesis of Pro (Chen et al. 2002). However, knockout of P5CS gene inhibited Pro synthesis in Arabidopsis and rice (Junghe et al. 2004; Székely et al. 2008). Exogenous GABA could promote Pro synthesis in leaves of white clover (Bin et al. 2017) and bread wheat (Farooq et al. 2017) under drought stress. Wang et al. (2021) also found that GABA also controls Al-induced proline biosynthesis. In this study, exogenous GABA significantly increased the expression of P5CS and P5CR genes in tobacco leaves under Cd stress, and promoted the accumulation of Pro. Pro not only has osmotic regulation function, but also has antioxidant activity, which can effectively remove ROS in cells, maintain redox balance in cells (Santiago 2016; Parvaiz et al. 2016), and improve the stability of plant cell membrane under stress (Mansour 2012). Therefore, exogenous GABA can promote the synthesis and accumulation of Pro under Cd stress, which is one of the important reasons for improving tobacco tolerance to Cd stress. The mechanism by which GABA alleviated Cd-induced PSII photoinhibition of and oxidative damage in tobacco leaves is summarized in Figure 6.

4. Conclusion

Cd stress significantly reduced the stomatal conductance of tobacco leaves, resulting in the limitation of photosynthetic gas exchange capacity. The ability of electron supply and transmission on the donor side and the acceptor side of PSII were significantly inhibited. However, tobacco leaves could improve their tolerance to Cd stress by accumulating Pro, GSH also played an important role in alleviating oxidative damage of tobacco leaves. Exogenous GABA (50 mmol L^{-1}) can significantly increase the expression of Pro synthesis related genes (P5CS and P5CR), thus promoting the accumulation of Pro. Exogenous GABA can also promote the synthesis of GSH, alleviate membrane peroxidation, and improve PSII activity and carbon assimilation ability of tobacco.

Disclosure statement

Funding

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