Grafting Tobacco onto Nutrient-efficient Rootstocks Improves Photosynthesis

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ABSTRACT. Potassium deficiency is a major problem limiting tobacco (*Nicotiana tabacum*) growth, and grafting has the potential to alleviate it. To compare the photosynthetic performance of grafted tobacco under different potassium levels, tobacco Yunyan 87 (main cultivar) and Wufeng No. 2 (potassium high-efficiency cultivar) were selected to conduct mutual grafting trials in the form of hydroculture with two potassium supply levels (5 mmol·L⁻¹ K and 0.5 mmol·L⁻¹ K). The plant growth, gas exchange parameters, chlorophyll *a* fluorescence, and the initial ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) activity were measured. The results showed that potassium deficiency could significantly decrease the net photosynthetic rate, stomatal conductance (*g_s*), and transpiration rate in the tobacco leaves, resulting in nonstomatal restriction. Grafting could effectively alleviate this problem. The actual quantum yield of photosystem II (PSII) photochemicals in ‘Yunyan 87’ increased 29.4% and 20.3% by grafting, respectively, under normal and low potassium levels. Compared with nongrafted ‘Yunyan 87’, grafting also effectively improved the electron transfer efficiency of PSII in the tobacco leaves under low potassium stress by reducing nonradiation energy dissipation and enhancing the initial activity of RuBisCO. From this study, it can be known that grafted tobacco plants can improve their photosynthesis by alleviating the nonstomata restriction of leaves under potassium stress and improving the electron transfer efficiency of PSII.

Grafting is one method of the propagation of plants. A branch or bud of one plant is grafted onto the stem or root of another so that the two parts joined together and function as a whole plant. Grafting will not only change the ability of tobacco (*Nicotiana tabacum*) to absorb nutrients, but a series of photoprotective mechanisms, including photorespiration and the malic reaction, may be influenced by rootstock induction, which is ultimately reflected in a change in the photosynthetic efficiency of the scion. This is a very important factor explaining why grafting is suitable for application under abiotic stress. Grafting experiments on different eggplant (*Solanum melongena*) rootstock materials showed that low-temperature tolerant eggplant rootstock could reduce the accumulation of reactive oxygen species (ROS) by enhancing the activity of respiratory burst oxidase. Meanwhile, the initial concentration of Ribulose 1,5 bisphosphate (RuBP) was increased to enhance the fixation efficiency of CO₂, so as to maintain the higher photosynthesis level of the scion (Gao et al., 2016). Previous research indicated that changes in environmental factors can alter the physiological processes of thylakoid electron

transport, carbon reduction, and CO₂ fixation (Allen and Ort, 2001). Under high-photon quantum flux density, the palisade tissue and sponge tissue arrangement of grafted pepper (*Capsicum annuum*) are closer than that in self-rooted seedlings (Jang et al., 2014). However, some studies have suggested that grafting does not have a significant effect on the photosynthesis of the scions (Huang et al., 2015; Rahmatian et al., 2014). In a grafting experiment of tomato (*Solanum lycopersicum*) and potato (*Solanum tuberosum*), it was found that there was no significant difference in photosynthetic CO₂ response between the grafted plants and the control plants when the tomato leaves were measured during the flowering period (Zhang and Guo, 2019).

Potassium cannot only promote the synthesis of chlorophyll in flue-cured tobacco, but also improve the structure of chloroplast, which has an important effect on the photosynthesis of tobacco leaves. At present, the main research on the effect of potassium on plant photosynthesis focuses on three aspects: *g_s* regulation, mesophyll tissue structure, photosynthetic phosphorylation processes and enzyme activity. For the first aspect of *g_s*, the regulation of K⁺ on stomatal opening and closing is realized by the change in concentration in the guard cells. The signal receptor of this process is H⁺-ATPase located on the guard cell membrane, which increases the level of K⁺ in the guard cells through the polarization of the K⁺ channel, thus generating cell turgor and promoting stomatal opening (Kim et al., 2010). The close relationship between potassium fertilizer application and *g_s* also has been verified (Jin et al., 2011; Wang et al., 2012). For the second aspect of mesophyll tissue structure, when

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potassium supply is sufficient, the leaf density decreases, the thickness increases, the ratio of leaf volume to leaf weight decreases, and the gap between the mesophyll tissues becomes larger, which is conducive to the passage of CO_2 (Pettigrew, 1999). At the same time, potassium can also increase the gas phase conductance of the mesophyll tissue (Battie-Laclau et al., 2014) and accelerate the decomposition of starch grains in the chloroplasts, which will reduce the transport resistance of CO_2 in the chloroplast (Zhao et al., 2001). For the last aspect of photosynthetic phosphorylation processes and enzyme activity, a lack of potassium will not only lead to decreased RuBP activity but also hinder the synthesis of chlorophyll (Weng et al., 2007). Water-soluble small molecules in cells will hinder the supply of inorganic phosphorus and the recycling of RuBP (Gerardeaux et al., 2010). An adequate supply of potassium can promote the decomposition of these small molecules and boost the activity of enzymes in the carbon metabolism system (Quentin et al., 2013).

According to our previous studies, grafting affects the absorption and utilization of K^+ in tobacco, especially that of a series of strategies for coping with low potassium stress, and also changes the circulation and redistribution pattern of K^+ in tobacco plants (Hu et al., 2019). This will have a significant impact on the photosynthesis of the tobacco leaves and will directly change the synthesis and transportation of assimilation products. Through the research of gas exchange parameters in grafted tobacco plants and the performance of photosystem II (PSII) electron transfer efficiency under different potassium levels, we hope to clarify the influence and mechanism of grafting on the photosynthesis of tobacco leaves.

Materials and Methods

PLANT MATERIALS AND GRAFTING TREATMENTS. The experiments were conducted in the greenhouse of the Chongqing Academy of Agricultural Sciences, Chongqing, China (lat. $29^{\circ}36'N$, long. $106^{\circ}29'E$). The main cultivar Yunyan 87 (Yunnan Tobacco Research Institute, Yuxi, China) and K-efficient genotype tobacco 'Wufeng No.2' (Yichang Tobacco Co., Yichang, China) were used in the present research. The industrial quality of the leaf of 'Yunyan 87' is excellent, and the potassium absorption capacity of the root of 'Wufeng No.2' is relatively strong. Using 'Yunyan 87' as the scion and 'Wufeng No.2' as the rootstock thus combines the advantages of the two. The experiment mainly examined the difference between this grafting combination and the nongrafted 'Yunyan 87'. To ensure the success rate of grafting, the seeds of the rootstock were planted 7 d before the scion. "Split grafting" was selected as the grafting method once six to eight true leaves had appeared on the seedlings of the rootstock (Ding et al., 2004). In the process of grafting, silicone catheter or a graft clip was used to fix the callus according to the situation of the combination of the rootstock and the scion (Fig. 1). When the new leaves had grown on the grafted plants, the plants were transplanted into a hydroponic box with 12 seedlings per container. The nutrient solution for the hydroponics was formulated as reported in a previous study (Hu et al., 2019). Air pumps were fixed to each container through a hose to supply oxygen to the tobacco seedlings during the experiment. The plants were grown at 22.5°C under a 16/8-h light/dark cycle using fluorescent lamps with an average photosynthetic photon flux density (PPFD) of $300\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the greenhouse. The relative humidity ranged from 60% to 95%.

Fig. 1. Silicone catheter or graft clip was used to fix tobacco callus. The left is the silicone catheter and the right is the transplant clip. Split grafting was selected as the grafting method once six to eight true leaves had appeared on the seedlings of the rootstock. In the process of grafting, a silicone catheter or a graft clip was used to fix the callus.

Two K levels were used for the experiments: +K, normal supply ($5\ \text{mmol}\cdot\text{L}^{-1}$) and -K, deficit ($0.5\ \text{mmol}\cdot\text{L}^{-1}$), using K_2SO_4 as the substance source to adjust the K levels. In addition, four graft treatments ['Wufeng No.2' (W), 'Yunyan 87' (Y), W grafted onto Y (W/Y), and Y grafted onto W (Y/W)] were used in the experiment. Eight treatments were replicated six times with 12 plants in each replicate. The solutions were replaced every 4 d. Tobacco samples were collected 20 d after the start of the treatment.

TOBACCO PLANT GROWTH. The roots, stems, and leaves of the plant samples were separately dried in an oven at 70°C for 24 h, and the dry weight was measured with an electronic balance. The penultimate true leaf on the stem of the tobacco seedling was selected as the object of leaf area measurement (LI-3000C; LI-COR Biosciences, Lincoln, NE). Plant height was measured with a leather tape. The root of the sample was carefully rinsed with deionized water, the excess water was absorbed with paper towels, and then the root surface area, root length, and root volume were measured using image analysis software (WinRhizo Pro 2.0; Regent Instruments, Ottawa, ON, Canada).

DETERMINATION OF THE INITIAL ACTIVITY OF RuBisCO. Fresh leaf samples of 0.2 g were weighed and enzymes were extracted using the method of (Weng et al., 2007). Initial RuBisCO activity was determined using a RuBisCOase enzyme-linked immunosorbent assay kit (CK-E91697P; Jinjin Chemical Technology Co., Shanghai, China). The determination method was according to the manufacturer's instructions.

DETERMINATION OF LEAF GAS EXCHANGE. Net photosynthetic rate (P_N), g_s , intercellular CO_2 concentration (C_i), and transpiration rate (E) were measured on fully expanded leaves between 8:30 and 10:30 AM using the portable open gas exchange system (LI-6400; LI-COR Biosciences) (Hu et al., 2018). Two blades were measured every day and finished the measurement in 6 d with little difference in climatic conditions. The environmental parameters during the measurement were as follows: light-emitting diode light source, PPFD of $800\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, flow rate $500\ \mu\text{mol}\cdot\text{s}^{-1}$, chamber temperature $30 \pm 2^{\circ}\text{C}$, and relative humidity $60\% \pm 1\%$.

DETERMINATION OF CHLOROPHYLL FLUORESCENCE. The chlorophyll *a* fluorescence parameters of each grafting treatment were determined on the same leaf used for the gas exchange parameter measurement using a leaf chamber fluorometer (6400-40; LI-COR Biosciences). The measurement indicators included

Table 1. Primers used in real-time fluorescence quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis. *CAOI* is the only gene in the chloroplast that regulates the relative content of chlorophyll *a* and *b*. *HemA1* dominates the rate of chlorophyll biosynthesis.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>CAOI</i>	TGGCCAGAACTAGTCAG TGGATG	CCTTCCATTAGCCT TTCCTCTCC
<i>HemA1</i>	AAGTGGGAATCCC GGTTTCGG	ACAAGAGAGTCAA GGCCTGC
Actin ^z	AACAGTTTGGTTGGA GTTCTGG	CATGAAGATTAAA GGCGGAGTG

^zReference gene for qRT-PCR analysis.

minimum fluorescence yield (F_0), photochemical quenching coefficient (q_p), PSII photochemical maximum quantum efficiency (F_v/F_m), chlorophyll fluorescence nonphotochemical quenching (NPQ), and actual quantum yield of PSII photochemistry (Φ_{PSII}). The specific measurement process and calculation method are the same as in our previous research (Hu et al., 2018).

TOTAL RNA EXTRACTION AND QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION. Total RNA was extracted from the leaves of each treatment with TRIzol reagent (Invitrogen, ThermoFisher, Waltham, MA) according to the manufacturer's instructions. Total RNA of 1 μ g was used to synthesize first-strand complementary DNA in combination with oligo (dT)-18 as a primer and M-MuLV reverse transcriptase (TaKaRa, Kusatsu, Japan). The quantitative real-time polymerase chain reaction (qRT-PCR) (ABI 7900HT; Applied Biosystems, Foster City, CA) was performed in a 20- μ L reaction system using a LightCycler480 SYBR Green I Master kit according to the protocols. Each sample analysis was repeated at least three times. Primer Premier 5.0 (Premier Biosoft, San Francisco, CA) software was used to design specific primers, and all primers were summarized in Table 1. Each primer possessed high specificity as determined by the melting curve analysis. The polymerase chain reaction (PCR) products were quantified by the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001).

STATISTICAL ANALYSIS. The data in the tables and figures are expressed as the means of all replicates $\pm$ SD. Data were

statistically analyzed by analysis of variance using statistical software (SAS version 9.2; SAS Institute, Cary, NC). Statistical significance was assessed using the least significant difference test ($P < 0.05$). Pearson's linear correlation analysis was performed on the gas exchange indexes.

Results

PLANT GROWTH. As shown in Table 2, in the case of the +K treatments, the grafting combination with the W genotype tobacco as the rootstock was significantly higher than Y in terms of the whole plant dry weight, leaf area, and plant height. In addition, there was no significant difference between the same rootstock group (e.g., W and Y/W). The growth parameters of each grafting combination were significantly reduced by potassium starvation, with the dry weight, leaf area, and plant height of the tobacco plants reduced on average by 37.8%, 12.5%, and 20.4%, respectively. However, different treatments led to different degrees of decline. Less reduction in dry weight was observed in W (31.1%) and Y/W (32.2%) in comparison with W/Y (44.5%) and Y (61.3%). The trends in plant height and leaf area were similar. It is worth noting that the growth of the flue-cured tobacco plants in W/Y under the condition of -K was significantly better than that in Y, which differed from that under the condition of +K.

INITIAL ACTIVITY OF RuBisCO. It can be seen in Fig. 2 that low potassium stress significantly reduced the initial activity of RuBisCO in each grafting combination. The RuBisCO activity in the grafting combinations with W as the rootstock decreased by 36.9%, whereas that with Y as the rootstock decreased by 47.1%, which indicates that the insufficient supply of potassium more greatly affected the initial activity of RuBisCO in the grafting combination of Y as the rootstock. For the different treatments, the initial activity of RuBisCO at normal and low potassium levels of the grafting combination with W as the rootstock was significantly higher than that of grafting with Y as the rootstock, whereas the difference between the same rootstock treatments was not significant.

EFFECTS OF GRAFTING AND POTASSIUM LEVEL ON GAS EXCHANGE IN TOBACCO LEAVES. Compared with the +K treatment, P_N , E , and g_s in each grafting combination decreased, respectively, by

Table 2. Effect of two K^+ level on growth of four tobacco graft combination.

Graft combination ^z	+K ^y					
	Whole-plant dry mass (g)	Leaf area (cm ²)	Plant ht (cm)	Root surface area (cm ²)	Root length (cm)	Root vol. (cm ³)
	mean $\pm$ SD ^x					
W	2.31 $\pm$ 0.11 a ^w	81.92 $\pm$ 4.09 a	8.72 $\pm$ 0.43 a	21.93 $\pm$ 1.09 a	271.09 $\pm$ 13.55 a	1.42 $\pm$ 0.07 a
Y/W	2.08 $\pm$ 0.10 a	74.83 $\pm$ 3.74 a	8.93 $\pm$ 0.44 a	19.82 $\pm$ 0.99 a	253.71 $\pm$ 12.65 a	1.34 $\pm$ 0.07 a
W/Y	1.48 $\pm$ 0.07 b	56.97 $\pm$ 2.84 b	7.44 $\pm$ 0.37 b	15.34 $\pm$ 0.76 b	195.57 $\pm$ 9.75 b	1.18 $\pm$ 0.06 b
Y	1.43 $\pm$ 0.07 b	52.9b $\pm$ 2.64 b	7.62 $\pm$ 0.38 b	14.19 $\pm$ 0.71 b	182.33 $\pm$ 9.11 b	1.07 $\pm$ 0.06 b
	-K					
W	1.63 $\pm$ 0.08 a	75.90 $\pm$ 3.79 a	6.72 $\pm$ 0.33 a	17.79 $\pm$ 0.88 a	243.72 $\pm$ 12.15 a	1.13 $\pm$ 0.06 a
Y/W	1.46 $\pm$ 0.07 a	69.86 $\pm$ 3.49 a	6.61 $\pm$ 0.33 a	16.75 $\pm$ 0.85 a	218.98 $\pm$ 10.92 a	1.06 $\pm$ 0.06 a
W/Y	0.86 $\pm$ 0.04 b	54.88 $\pm$ 2.74 b	6.03 $\pm$ 0.30 b	12.42 $\pm$ 0.62 b	170.19 $\pm$ 8.56 b	0.82 $\pm$ 0.04 b
Y	0.61 $\pm$ 0.03 c	41.11 $\pm$ 2.05 c	5.17 $\pm$ 0.25 c	9.28 $\pm$ 0.47 c	143.33 $\pm$ 7.16 c	0.63 $\pm$ 0.03 c

^zW = 'Wufeng No.2', Y = 'Yunyan 87', Y/W = Y grafted onto W, W/Y = W grafted onto Y.

^y+K, normal supply (5 mmol·L⁻¹) and -K, deficit (0.5 mmol·L⁻¹).

^xMean and SD of six independent replicates.

^wDifferent letters in same potassium supply level within the same column indicate a significant difference ($P < 0.05$).

Fig. 2. Effects of grafting on the initial activity of ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) in the flue-cured tobacco leaves. The tobacco graft combinations included the nongrafted tobacco ‘Wufeng No.2’ (W) and ‘Yunyan 87’ (Y) and the grafted tobacco Y grafted onto W (Y/W) and W grafted onto Y (W/Y). Different uppercase letters denote significant differences ($P < 0.05$) under normal K levels ($5 \text{ mmol}\cdot\text{L}^{-1}$), and lowercase letters indicate significant differences ($P < 0.05$) under K starvation ($0.5 \text{ mmol}\cdot\text{L}^{-1}$).

51.8%, 24.1%, and 19.0% with the $-K$ treatment. At the same time, the C_i increased by 45.1%. As indicated in Fig. 3, each gas exchange parameter of the W/Y and Y grafting combinations was significantly lower than that in W and Y/W, except for C_i ; however, there was no significant difference between the grafting combinations with the same rootstock (e.g., W and Y/W). As for potassium starvation, with the exception that P_N , E , and g_s of W/Y were significantly higher, and C_i was significantly lower, than Y, the trend among the grafting combinations was basically the same as that of the normal potassium supply. To determine the reasons for the decrease in photosynthesis under low potassium stress, Pearson’s linear correlation analysis was carried out on P_N , C_i , E , and g_s . The analysis results in Table 3

show that C_i was significantly negatively correlated with the other three indicators, suggesting that the decline in P_N rather than the stomatal closure was responsible; otherwise, C_i would have indicated a significantly positive correlation.

EFFECTS OF GRAFTING AND POTASSIUM LEVEL ON PHOTOCHEMICAL EFFICIENCY IN PSII. As indicated in Fig. 4, with the decrease in the potassium supply level, the average values of F_0 and NPQ in each grafting combination increased by 15.27% and 46.7%, respectively, whereas q_p and Φ_{PSII} decreased by 21.3% and 15.4%, respectively. The difference in F_v/F_m between each treatment was not significant. For the different grafting combinations, F_0 and NPQ of W and Y/W were significantly lower than Y and W/Y in the $+K$ treatment, whereas q_p and Φ_{PSII} in W and Y/W were significantly higher than Y and W/Y. The trends in the $-K$ treatments were similar. It is important to note that the differences between the treatments with the same rootstock were not significant in each chlorophyll fluorescence parameter.

EFFECTS OF GRAFTING AND POTASSIUM LEVEL ON THE EXPRESSION OF CHLOROPHYLL SYNTHASE CODING GENES. Compared with normal potassium supply, the expression level of *CAO1* and *HemA1* in each graft combination was downregulated under potassium deficit. As shown in Fig. 5, regardless of the potassium level, the expression levels of *CAO1* and *HemA1* were significantly higher in the treatment with W as the rootstock than in Y. However, there was no difference in the expression levels of *CAO1* and *HemA1* between the treatments with the same rootstock. In other words, grafting significantly alleviated the inhibition of the chlorophyll synthesis of the Y genotype flue-cured tobacco under low potassium stress.

Discussion

GRAFTING ALLEVIATED THE INHIBITION OF TOBACCO GROWTH UNDER LOW POTASSIUM STRESS. In recent years, grafting has been successfully applied to mitigate biotic and abiotic and improve mineral element uptake in many plant species (Dong et al.,

Fig. 3. Effects of grafting and potassium level on gas exchange in tobacco leaves. Different lowercase letters denote significant differences ($P < 0.05$) (C_i = intercellular CO_2 concentration, E = transpiration rate, P_N = net photosynthetic rate, g_s = stomatal conductance). The tobacco graft combinations included the nongrafted tobacco ‘Wufeng No.2’ (W) and ‘Yunyan 87’ (Y) and the grafted tobacco Y grafted onto W (Y/W) and W grafted onto Y (W/Y). To facilitate the comparison of various indicators with large magnitudes of difference in a single figure, the values of each parameter in the W treatment were converted to 1, whereas those in the other treatments were proportionally converted based on the W. $+K$, normal supply ($5 \text{ mmol}\cdot\text{L}^{-1}$) and $-K$, deficit ($0.5 \text{ mmol}\cdot\text{L}^{-1}$).

Table 3. Pearson's linear correlation coefficient between net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), transpiration rate (E), and stomatal conductance (g_s) of tobacco.

	P_N	C_i	E	g_s
P_N	1			
C_i	-0.95*	1		
E	0.93*	-0.98**	1	
g_s	0.91*	-0.92*	0.91*	1

*, **Significant at $P < 0.05$ or 0.01 , respectively.

2008; Huang et al., 2010; Qin et al., 2014), including tomato, watermelon (*Citrullus lanatus*), cotton (*Gossypium hirsutum*), and cucumber (*Cucumis sativus*). Nevertheless, the effects and mechanisms by which grafting alleviates K stress in tobacco are largely unknown. The present study shows that tobacco plant growth was obviously weakened under K starvation but that K-efficient rootstock grafting could alleviate this (Hu et al., 2019). Compared with the W/Y and Y grafting combination, W and Y/W only slightly decreased the plant dry weight, and the trends in plant height and leaf area were similar. Regardless of the K levels, the whole-plant dry weight, height, and leaf area of the W genotype tobacco were significantly higher than the Y genotype. By grafting, the growth index of the Y genotype tobacco, which used W as the rootstock, reached the level of the W genotype tobacco and was significantly higher than the nongrafted original cultivar. Combined with our previous research (Hu et al., 2019), the utility of grafting technology in improving the growth of tobacco plants has been confirmed again in the present study. In addition, as a feedback adjustment from the scion to the rootstock, W also improved the plant growth performance of W/Y. This feedback regulation mechanism in grafting is usually dominated by long-distance signaling, and microRNA or hormonal signals may act as signaling substances (Ferguson and Matheisius, 2003; Lorteau et al., 2001; Wang et al., 2002).

Fig. 4. Effects of grafting and potassium level on the minimal fluorescence yield of the dark-adapted state (F_0), maximal quantum yield of photosystem II (PSII) photochemistry (F_v/F_m), photochemical quenching coefficient (q_p), effective quantum yield of PSII photochemistry (Φ_{PSII}), and nonphotochemical quenching (NPQ) in tobacco leaves. The tobacco graft combinations included the nongrafted tobacco 'Wufeng No.2' (W) and 'Yunyan 87' (Y) and the grafted tobacco Y grafted onto W (Y/W) and W grafted onto Y (W/Y). Different uppercase letters denote significant differences ($P < 0.05$) under normal K levels ($5 \text{ mmol}\cdot\text{L}^{-1}$), and lowercase letters indicate significant differences ($P < 0.05$) under K starvation ($0.5 \text{ mmol}\cdot\text{L}^{-1}$).

Fig. 5. Relative expression levels of *CAOI* and *HemA1* in tobacco with two K supply conditions. The tobacco graft combinations included the nongrafted tobacco 'Wufeng No.2' (W) and 'Yunyan 87' (Y) and the grafted tobacco Y grafted onto W (Y/W) and W grafted onto Y (W/Y). Different uppercase letters denote significant differences ($P < 0.05$) under normal K levels ($5 \text{ mmol}\cdot\text{L}^{-1}$), and lowercase letters indicate significant differences ($P < 0.05$) under K starvation ($0.5 \text{ mmol}\cdot\text{L}^{-1}$).

GRAFTING ENHANCES THE INITIAL ACTIVITY OF RuBisCO IN THE TOBACCO LEAVES. RuBisCO activity includes initial activity and total activity, among which the initial activity represents the activity of ribulose 1 – 5 biphosphate carboxylase, involved in the Calvin cycle. The activity of RuBisCO in the leaves is closely related to the photosynthetic rate. At the same time, it catalyzes the initial reaction of the Calvin cycle and has an important influence on the entire cycle process (Flexas et al., 2016; Niyogi, 2017; Rogers and Humphries, 2000). As the affinity of RuBisCO to CO_2 is very low (Jensen, 2000) and the concentration of CO_2 in the chloroplasts is insufficient to meet the needs of the assimilation of RuBisCO (Li et al., 2009), the activity of RuBisCO is thus very low under normal conditions. In addition, as a bifunctional enzyme, RuBisCO not only catalyzes RuBP to combine with CO_2 for carboxylation reactions but also catalyzes RuBP to combine with O_2 for oxidation reactions, making CO_2 and O_2 compete for the reaction site of RuBisCO (Salvucci and Crafts-Brandner, 2004). The oxidation of RuBisCO is the first step in photorespiration and requires the consumption of electrons. The rate of electron transfer by PSII and the proportion of electron distribution in carboxylation and oxidation directly affect the net photosynthetic rate of the leaves. Studies on different crops have found that potassium application can improve the initial activity of RuBisCO in the leaves (Hu et al., 2016; Peoples and Koch, 1979). In this study, it was found that low potassium stress could significantly reduce the initial activity of RuBisCO in various grafted tobacco combinations. However, the initial activity of RuBisCO in the grafting combination with W as the rootstock was significantly higher than that with Y as the rootstock at any potassium level. This suggests that the initial RuBisCO activity of the Y genotype tobacco could be improved using W as the rootstock. Previous studies on rice corroborate our results that both differences in potassium supply and cultivar would cause changes in the initial activity of RuBisCO (Weng et al., 2007; Yang et al., 2004). A study on the effect of potassium starvation on the photosynthetic rate of pecan seedlings found that the regulation of potassium on the ratio of carboxylation and oxidation of RuBisCO may be the main reason that it can affect the initial activity of RuBisCO in the leaves; that is, potassium application

can increase the proportion of electrons allocated to carboxylation reactions (Jin et al., 2011). Grafting simply meets this requirement from the perspective of improving the potassium absorption efficiency of the tobacco roots (Hu et al., 2020).

REGULATION OF POTASSIUM LEVEL ON PHOTOSYNTHETIC EFFICIENCY. The most important effect of potassium on the photosynthetic characteristics of plants relates to two aspects. First, potassium regulates chloroplast morphology and promotes the transport of CO₂ from the stomatal cavity to the carboxylation site (Lu et al., 2016). Second, potassium promotes the content and activity of RuBisCO to promote CO₂ fixation (Lu et al., 2017). Some studies suggest that the photosynthetic rate of plant leaves is reduced in the absence of potassium (Chen et al., 2018; Du et al., 2019; Hou et al., 2019; Jin et al., 2011; Kanai et al., 2011), whereas a few reports have pointed out that potassium supply does not significantly improve the leaf photosynthetic rate (Sengupta et al., 1989). For different crops, there must be a threshold for the decrease in photosynthesis caused by potassium deficiency in a specific environment. Therefore, there is currently a different understanding of the effect of potassium on photosynthetic rate (Zorb et al., 2014). Experimental data show that potassium deficiency causes a significant decrease in P_N , E , and g_s in flue-cured tobacco leaves, whereas C_i increases significantly. According to Pearson's linear correlation analysis results, the reduction in photosynthetic efficiency caused by potassium deficit in this experiment was dominated by nonstomatal factors. This is consistent with our previous study on the leaf gas exchange status of tobacco leaves exposed to drought stress (Hu et al., 2018). Nonstomatal limitation is usually caused by obstacles in carbon assimilation (Weng et al., 2007; Yang et al., 2004) and reducing force (Pinheiro and Chaves, 2011) and also includes the obstruction of the mesophyll layer on CO₂ transport (Flexas et al., 2007). The environmental factors that cause nonstomatal limitation generally include low temperature, low light, high CO₂ adaptation, and reduction of RuBP content and activity. When the RuBP content and activity were decreased, the ability of CO₂ fixation through photosynthesis is significantly weakened. When the stomata are kept open, CO₂ accumulates in the mesophyll tissues in large quantities, resulting in increased intercellular CO₂ concentration. In this experiment, the lack of potassium caused the synthesis of RuBP to be affected and the Calvin cycle to be blocked, which is also the reason for the nonstomatal limitation. Grafting can effectively mitigate this problem, resulting in the net photosynthetic rate of the Y/W treatment being affected much less than that of the nongrafted treatments.

RELATIONSHIP BETWEEN POTASSIUM LEVEL AND PHOTOCHEMICAL EFFICIENCY IN PSII. The results of this experiment show that an increase in F_0 results in a decrease in nonradiative energy consumption, which causes a decrease in the efficiency of the total reaction center of PSII. F_v/F_m of each grafting combination did not change when potassium was scarce, indicating no photo-inhibition (Elgersma et al., 2015). At the same time, according to q_p and NPQ, the proportion of open reaction centers in the PSII system was reduced and the heat dissipation was increased when the potassium level was decreased (Lu and Vonshak, 2002). Based on the preceding three points, we can conclude that the decrease in the efficiency of the reaction center of PSII in this experiment was reversible inactivation rather than irreversible damage (Hu et al., 2007), which was inseparable from the reduction of potassium levels. An analysis of the chlorophyll

fluorescence of cyanobacteria confirms this conclusion (Campbell et al., 1998). It is worth noting that in a study on the relationship between potassium application and the photosynthetic physiology of cotton, the decrease in F_v/F_m and Φ_{PSII} is the factor that leads to the decline in P_N (Hu et al., 2016). These results corroborate our research. In this experiment, Φ_{PSII} decreased significantly in the absence of potassium, indicating that the reducing force was inhibited, which significantly affected P_N of the leaf. Moreover, an adequate supply of potassium could significantly improve the electron transfer efficiency, which is conducive to the formation of ATP and NADPH, thus improving the reduction force. At the same time, it is evident from the data of this experiment that grafting could effectively improve the electron transfer efficiency of PSII in the flue-cured tobacco leaves caused by the decrease in potassium supply level, which partly explains why the plant growth performance of the W/Y treatment was better than the Y treatment.

ANALYSIS OF *CAOI* AND *HEMAI* EXPRESSION LEVEL IN THE LEAVES. Chlorophyll *b* is formed by the oxidation of chlorophyllide *a* under the action of chlorophyll *a* oxygenase (CAO) to form chlorophyllide *b*, which is then formed by esterification (Rudiger, 2002). The gene that encodes the CAO enzyme is *CAOI*, which is the only gene in the chloroplast that regulates the relative content of chlorophyll *a* and *b* (Biswal et al., 2012; Espineda et al., 1999). Glutamyl-transfer RNA reductase (GluTR) is a rate-limiting enzyme for the synthesis of 5-amino-levalulinic acid (ALA) and is encoded by the *HemA* gene family (Goslings et al., 2004). The trial results of transferring the antisense *HemA1* gene into arabidopsis (*Arabidopsis thaliana*) indicate that *HemA1* is the most critical gene regulating GluTR in the *HemA* family (McCormac et al., 2001). ALA synthesis is the rate-limiting step of the chlorophyll biosynthesis pathway, which means that *HemA1* dominates the rate of chlorophyll biosynthesis (Zhao et al., 2014). As a cofactor for various enzymes, potassium affects the expression levels of *CAOI* and *HemA1* by participating in multiple enzymatic reactions throughout the photosynthetic pigment synthesis process. Potassium deficiency is closely related to decreased chlorophyll content (Zhao et al., 2001), and our results confirm the previous research conclusion from different perspectives. The expression level of *CAOI* and *HemA1* in each graft combination was downregulated under potassium deficit, but grafting significantly improved the inhibition of chlorophyll synthesis of the Y genotype flue-cured tobacco under low potassium stress. In this experiment, the difference in expression levels of *CAOI* and *HemA1* in each treatment originated from two aspects: potassium levels and different flue-cured genotypes. The difference from the grafting combinations with the same scion was significant (e.g., W and W/Y), suggesting that the dominant factor in the difference in the expression levels between *CAOI* and *HemA1* is potassium level rather than genotype; that is, grafting improved the synthesis of photosynthetic pigments by improving the potassium absorption capacity of Y-type flue-cured tobacco. At the same time, studies have noted that potassium deficiency also can lead to an increase in the chlorophyll *a* to chlorophyll *b* ratio, which is consistent with our experimental results (Jin et al., 2011).

Conclusions

The main effect of potassium on plant photosynthesis was achieved by changing the efficiency of CO₂ assimilation in the

leaves. In this experiment, the efficiency of photosynthesis in the tobacco leaves and the growth of plants under low potassium stress could be improved by grafting. The reasons can be summarized as follows: 1) In terms of gas exchange of leaves, grafting can alleviate the nonstomatal restriction due to potassium deficiency; and 2) grafting can effectively improve the electron transfer efficiency of PSII in the tobacco leaves under low potassium stress, reduce nonradiation energy dissipation, and enhance the initial activity of RuBisCO.

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