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Full Length Article

Effects of Different Forms of Potassium on Plant Growth and Photosystem II Function in Leaves of Tobacco

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Abstract

In order to understand the effects of different forms of potassium on tobacco plant growth and photosynthesis, tobacco seedlings were treated with KCl or K_2SO_4. Plants were then assayed for growth, chlorophyll content and chlorophyll fluorescence characteristics using the fast chlorophyll fluorescence kinetics. When applications of K_2SO_4 were less than 150 mg/kg plant growth was significantly stimulated but at K_2SO_4 treatments of 200 mg/kg or greater, growth was inhibited. Additionally all KCl applications significantly inhibited plant growth. Tobacco leaf chlorophyll content was highest for 150 mg/kg or lower applications of K_2SO_4. The synthesis of chlorophylls, especially chlorophyll b, was inhibited when KCl was applied. Under all K_2SO_4 treatments, PI_{ABS} and PI_{total} were all higher than in the control (no potash fertilizer, CK). However, KCl showed little effect on PI_{ABS} and PI_{total} in tobacco leaves indicating that a certain amount of K_2SO_4 can improve the activity of the photosystem II (PSII) reaction center. As the amount of KCl increased tobacco leaf V_J increased, while V_I did not change. When K_2SO_4 was applied the V_J and V_I values were both significantly lower than those of CK plants indicating that the main inhibition effect of KCl on the activity of the PSII reaction center lies in lowering the electron accepting ability of Q_B. In contrast, K_2SO_4 promotes electron transfer on the acceptor side of the PSII reaction center. As KCl applications increased tobacco leaf V_K increased while V_L did not significantly change. This indicates that KCl inhibits the function of the oxygen-evolving complexes at the donor side of the PSII reaction center but does not degrade tobacco leaf thylakoid [1]membranes. V_K and V_L in tobacco leaves showed significant decreases under a range of K_2SO_4 applications. A reasonable application of potassium in the form of K_2SO_4 can improve the photosynthetic capability of tobacco and promote plant growth but potassium in the form of KCl should not be applied for soil chloride contents of around 100 mg/kg. © 2018 Friends Science Publishers

Keywords: Chlorine; Chlorophyll fluorescence; Potassium fertilizer; PSII; Tobacco

Introduction

Potassium is a macronutrient necessary for plant growth and it affects the yield and quality of crops significantly (Liu *et al*., 2016). An appropriate potassium content is an important factor affecting tobacco leaf quality and high-quality tobacco leaves typically have a potassium content exceeding 2% (Leymonie and Etourneaud, 1996). Increasing potassium content in tobacco leaves not only facilitates tobacco sugar accumulation and aroma formation (Liu *et al*., 2004) but also improves leaf colour and flammability (Hu, 1996). Therefore, a reasonable application of potassium fertilizer can both promote the growth and metabolism of tobacco plants and improve the quality of tobacco leaves (Jie *et al*., 2005; Dai *et al*., 2012). At present, the commonly used potassium fertilizers in agriculture mainly include KCl and K_2SO_4 and the effects of different forms of potassium on plant yield and quality significantly differ (Guo *et al*., 2007). KCl is still the main fertilizer on the market today because it is inexpensive. Chloride is an essential nutrient for normal tobacco growth (Liu, 2003) and excessively low chloride content can decrease tobacco leaf quality. That is a moderate chloride content is able to not only improve the yield and quality of flue-cured tobacco but also enhance the drought and pest resistance of tobacco plants. However, tobacco is a chloride-intolerant crop; excessive chloride absorption can have negative effects on tobacco plant growth and thereby severely affect its yield and quality especially in terms of moisture absorption, combustion, aroma and taste of tobacco leaves (Li and Luo, 1995). Therefore, it is of great importance to study how to control the application of potassium and chloride-containing fertilizers for the purpose of ensuring the yield and quality of tobacco leaves.

Photosynthesis is the main source of materials and energy for plants carrying out normal life activities and fertilization application is an important means for enhancing photosynthesis (Zhang *et al*., 2013). Potassium plays an

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important role in promoting chloroplast synthesis, regulating stomata opening, improving photosynthetic capacity and maintaining normal cell turgor (Kang *et al*., 2014). Chloride also plays an important role in plant photosynthesis. As a catalyzer of the photosystem II (PSII) chloride has an important function in maintaining PSII activity in plant leaves and the chloride in chloroplasts has a protective effect on the stability of chlorophyll molecules (Zhang *et al*., 2015). Liu (Liu *et al*., 2005) claimed that chloride can affect chlorophyll synthesis and chlorophyll content in tobacco leaves at different growth stages. However, few studies have examined the effect of chloride on the photosynthetic function of tobacco leaves, especially on the function of the PSII reaction center. Chlorophyll fluorescence techniques are an important tool for exploring PSII function in plant photosynthesis and in particular, fast chlorophyll fluorescence induction kinetics technology can be used to quantitatively assay changes in the primary photochemical reaction of PSII and the structure and state of the photosynthetic apparatus (Zhang *et al*., 2011; Zhang *et al*., 2012). Strasser *et al*. (2000) established JIP test technique to study the fast chlorophyll fluorescence induction kinetic curves based on biofilm energy flow, which has become a powerful tool for studying the effect of stress on photosynthetic mechanisms (Appenroth *et al*., 2001; Van *et al*., 2003). Currently, there are few studies on the effect of different potassium fertilizers on the PSII function of tobacco leaves. For this reason, this experiment studied the effects of two different potassium fertilizers, KCl and K_2SO_4, on tobacco plant growth and PSII function, mainly the PSII reaction center activity electron transfer capacities on the electron donor side the electron acceptor side of PSII and so forth. This study was conducted with the aim of providing some basic data on the high-quality cultivation of tobacco plants and determining appropriate applications of potassium fertilizers for tobacco cultivation.

Materials and Methods

Experimental Details and Treatments

The experiment was conducted using tobacco plants grown in pots at the College of Resources and Environment, Northeast Agricultural University in Harbin City, Heilongjiang Province, China. The tobacco variety used in the experiment was 'Long Jiang 911'. The tobacco seedlings were provided by Mudanjiang Tobacco Research Institute of Heilongjiang Province. The seedlings were transplanted when the seedlings had just a single shoot with five leaves in May 2016. They were planted in plastic 30 cm×30 cm pots (one plant and 6 kg soil per pot). The basic soil nutrient contents and chloride contents are shown in.

Two forms of potassium fertilizers were applied in measured amounts of potassium per kg dry weight and a total of five gradient levels of potassium (*i.e*., 0, 50, 100, 150 and 200 mg/kg) were set up, respectively. Each treatment was repeated six times. Potassium fertilizer was applied before the seedlings were transplanted. A small amount of distilled water was used to dissolve the KCl or K_2SO_4 and the solution was sprayed in the soil evenly and mixed well.

Index Determination Method

Growth index determination: Five tobacco plants from each treatment were used to determine plant height, number of leaves and dry weight on July 20th 2016. Plant height was measured from the base of the stem on the soil surface to the top of the stem using a meter stick the number of leaves was determined by counting the number of leaves exceeding 1 cm in length. To determine plant biomass, representative plants with uniformed growth, including their roots were removed from their pots. The soil on the roots and stems was washed off with running water and plants then were wiped with clean absorbent paper. Afterwards, plants were placed in an envelope in an oven at 105°C for 30 min. Then, they were cooled to 80°C, dried to a constant weight and weighed.

Determination of Chlorophyll Content

The concentrations of chlorophyll a (Chl *a*) and chlorophyll *b* (Chl *b*) were determined on fresh fully expanded leaves of tobacco and were expressed as mg/g. A fresh leaf sample without main vein was sliced and incubated in pigment extraction solution containing acetone, anhydrous ethanol and distilled water (4.5:4.5:1; V:V:V). Contents of Chl*a*, Chl *b* were calculated according to (Qi, 1995). Chl *a*+Chl *b* and Chl *a*/Chl *b* also were calculated.

Fast Chlorophyll Fluorescence Induction Kinetics Curves Determination

Sixty days after the transplantation the plants entered their vigorous growth period. The second to last fully expanded leaf from tobacco plants in each of the different treatment groups was used to measure the fast chlorophyll fluorescence induction kinetics curves (O-J-I-P fluorescence induction curve) using a Handy-PEA continuous excitation fluorescence spectrometer (Hansatech, King's Lynn, UK). The leaves were dark-adapted for 30 min before measurement. The four characteristic points on the OJIP curve, *i.e*., O, J, I and P correspond to time points of 0, 2, 30 and 1000 ms, respectively and relative fluorescence intensities represented by F_O, F_J, F_I and F_M, respectively. The points of time corresponding to 0.15 ms and 0.3 ms on the OJIP curves were defined as the L and K2 characteristic points and the corresponding relative fluorescence intensities were represented by F_L and F_K, respectively. The OJIP curves from different treatments were standardized by O–P, O–J and O–K, respectively. That is the curves were standardized by defining the relative fluorescence intensity

at O point as 0 and the relative fluorescence intensities at P, J and K as 1. The formulas used for these standardizations were $V_{O-P}=(F_t-F_o)/(F_p-F_o)$, $V_{O-J}=(F_t-F_o)/(F_J-F_o)$ and $V_{O-K}=(F_t-F_o)/(F_k-F_o)$, respectively. In these formulas, F_t represents the relative fluorescence intensity at different points of time. The relative variable fluorescence intensities of the four characteristic points L, K, J and I on the standardized curves were represented by V_L, V_K, V_J and V_I, respectively. The PSII maximum photochemical efficiency (F_v/F_m), the overall performance index (PI_{total}) and the photosynthetic performance index based on the absorption of light energy (PI_{ABS}) was obtained by a JIP-test analysis of the O-J-I-P curves.

Statistical Analysis

Statistical analyses were performed using Microsoft Excel and DPS software. The data are the means ± standard error (SE) of five replicates. The differences between groups were analyzed with a one-way ANOVA, followed by the least significant difference (LSD) method.

Results

Effects of Different Forms of Potassium on Plant Growth

All KCl treatments had a significant inhibitory effect on the growth of tobacco plants (Fig. 1). Compared to control plants, increased KCl applications were associated with reduced root and stem dry weights, while there were no significant changes in the total plant masses of the tobacco leaves and stems relative to control plants. However, as K_2SO_4 applications increased the biomass of different parts of the tobacco plants and the total biomass initially increased and then decreased. The dry weight of the roots and stems reached their maximum values at potassium application amounts of 150 mg/kg and the leaves and total plant mass reached their maximum values at a potassium application amount of 100 mg/kg. Under different potassium levels the plant dry masses were all significantly lower when the plants were treated with KCl compared to those treated with K_2SO_4. Plant heights and the numbers of leaves under different potassium levels decreased when KCl was applied. When K_2SO_4 was applied, plant heights were higher to a certain degree under different potassium levels compared to those in the CK group; however, there was little difference in the numbers of leaves under the treatment of K_2SO_4 compared to those in the CK group.

Effects of Different Forms of Potassium on the Chlorophyll Content of Tobacco Leaves

When the amount of KCl application increased the chlorophyll a, chlorophyll b and total chlorophyll contents

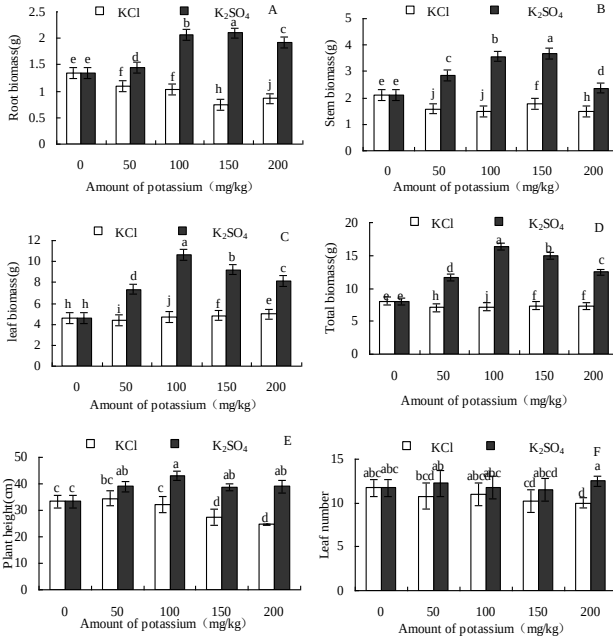

Fig. 1: Effects of different forms of potassium nutrition on root biomass (A), stem biomass (B), leaf biomass (C), total biomass (D), plant height (E) and leaf number (F) of tobacco plant. Bar graphs depict mean±SE, values followed by different small letters mean significant difference (p<0.05)

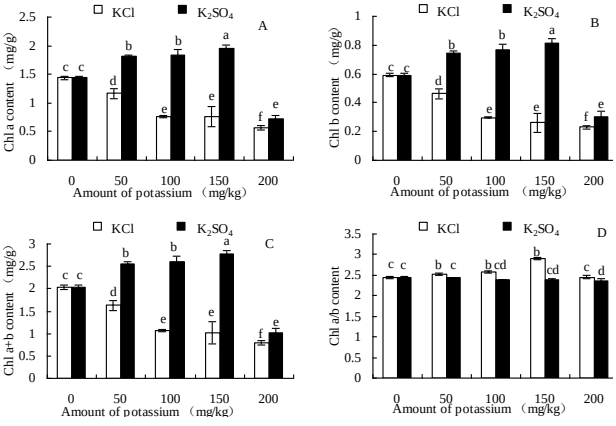

Fig. 2: Effects of different forms of potassium on Chl *a* content (A), Chl b content (B), Chl a+b content (C) and chla/b content (D) of tobacco leaves. Bar graphs depict mean±SE, values followed by different small letters mean significant difference (p<0.05)

all decreased significantly relative to plants in the CK group. When the application of KCl was lower than 150 mg/kg as the amount of KCl application increased the ratio of chlorophyll a to chlorophyll b in tobacco leaves increased; however, when the KCl application level reached 200 mg/kg, there was no difference in the chlorophyll a to

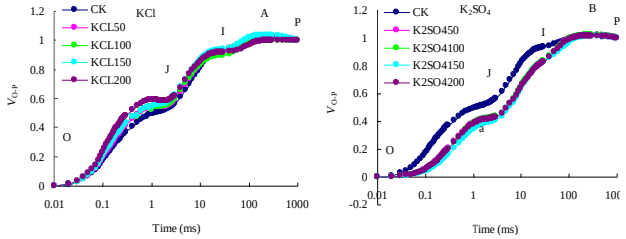

Fig. 3: Effects of different forms of potassium on OJIP curves of tobacco leaves. Bar graphs depict mean±SE, values followed by different small letters mean significant difference (p<0.05)

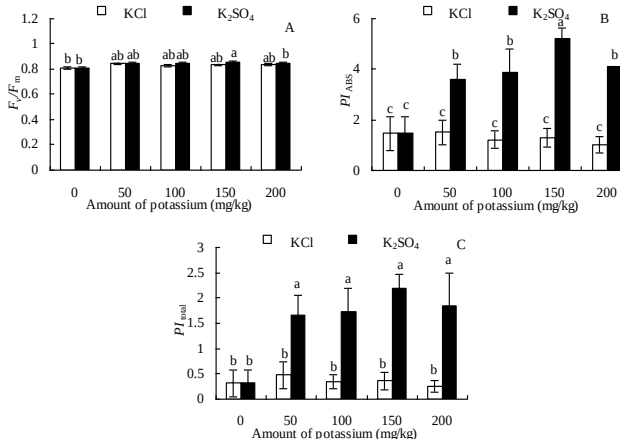

Fig. 4: Effects of different forms of potassium on F_v/F_m (A), PI_{ABS} (B), and PI_{total} (C) of tobacco leaves. Bar graphs depict mean±SE, values followed by different small letters mean significant difference (p<0.05)

chlorophyll b ratio compared to CK plants. When K_2SO_4 applications were lower than 150 mg/kg the contents of chlorophyll a, chlorophyll b and total chlorophyll in tobacco leaves were all significantly higher than those in the CK group and showed an obvious concentration effect; however, when K_2SO_4 application levels reached 200 mg/kg the contents of chlorophyll a and chlorophyll b in tobacco leaves were respectively 49.86% (P<0.05) and 48.36% (P<0.05) lower than those in CK. As K_2SO_4 applications increased, the chlorophyll a to chlorophyll b ratios in tobacco leaves showed a slight decrease.

Effect on the OJIP Curves

Significant changes occurred in the OJIP curves of tobacco leaves under different levels of KCl treatments compared with those in the control plant (Fig. 3). This is embodied in the decrease in the relative fluorescence intensities at points O, I and P consistent with an obvious concentration effect and in the relatively insignificant changes in the relative fluorescence intensity at point J. The relative fluorescence intensities at points O, J and I on the OJIP curve were significantly lower under different levels of K_2SO_4 treatments relative to KCl treatments, while the change in the relative fluorescence intensity at point P was relatively insignificant.

Effect on the PS II Photochemical Efficiency of Tobacco Leaves

Compared to the CK treatment, F_v/F_m values were increased in tobacco leaves under KCl and K_2SO_4 treatments and the degrees of increases under different levels of K_2SO_4 treatments were all higher than those under the KCl treatment at the same potassium level (Fig. 4). The changes in PI_{ABS} and PI_{total} were smaller under different amounts of KCl treatments than those in the CK group and they both decreased slightly. However, these differences were not statistically significant. PI_{ABS} and PI_{total} were both higher under K_2SO_4 treatments than those under KCl treatments at the same potassium level; PI_{ABS} and PI_{total} reached their maximum values when the potassium application was 150 mg/kg. When the application of K_2SO_4 reached 200 mg/kg, the PI_{ABS} and PI_{total} of the tobacco leaves slightly decreased but were still higher than those under the CK treatment.

Effect on Standardized OJIP Curves and Tobacco Leaf V_J and V_I Values

The OJIP curves of tobacco leaves under different treatments were standardized and the changes in V_J and V_I under the two forms of potassium treatments are shown in (Fig. 5). As KCl applications increased, V_J increased overall on the standardized OJIP curves; particularly, at potassium application levels of 200 mg/kg, there was an increase of 13.63% (P<0.05) compared to the CK treatment but V_I did not change significantly. Under the K_2SO_4 treatment, the values of V_J and V_I of the tobacco leaves both significantly decreased compared to those in the CK treatment, unlike under the KCl treatment. However, there were no significant differences among different levels of the potassium treatments.

Effect on Standardized O–J and O–K Curves and Tobacco Leaf V_K and V_L Values

The O–J, O–K and OJIP curves of tobacco leaves under the different treatments were standardized, respectively. Tobacco leaf V_K and V_L values on the standardized O–J and O–K curves showed significant differences between the two forms of potassium fertilizer treatments. As KCl applications increased, V_K showed an overall increasing trend, while V_L showed no significant change (Fig. 6). Under different K_2SO_4 application levels, both V_K and V_L showed significant decreases but there was no significant difference among different potassium concentrations.

Discussion

In this study, plant height, number of leaves and dry mass of the tobacco plants under different levels of K_2SO_4

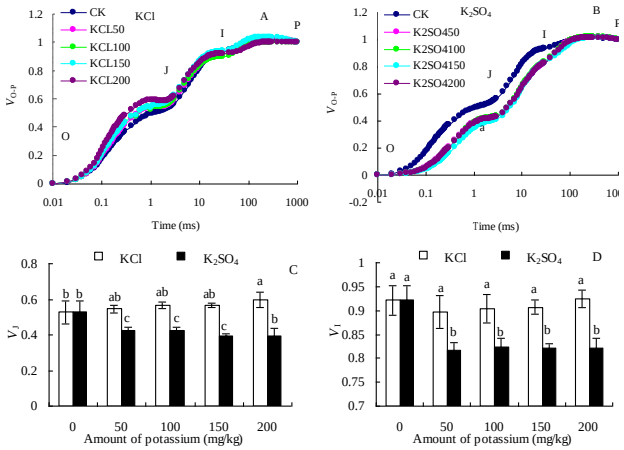

Fig. 5: Effects of different forms of potassium on the standardized OJIP curve (A and B) and tobacco leaf V_I (C) and V_J (D) Values. Bar graphs depict mean±SE, values followed by different small letters mean significant difference (p<0.05)

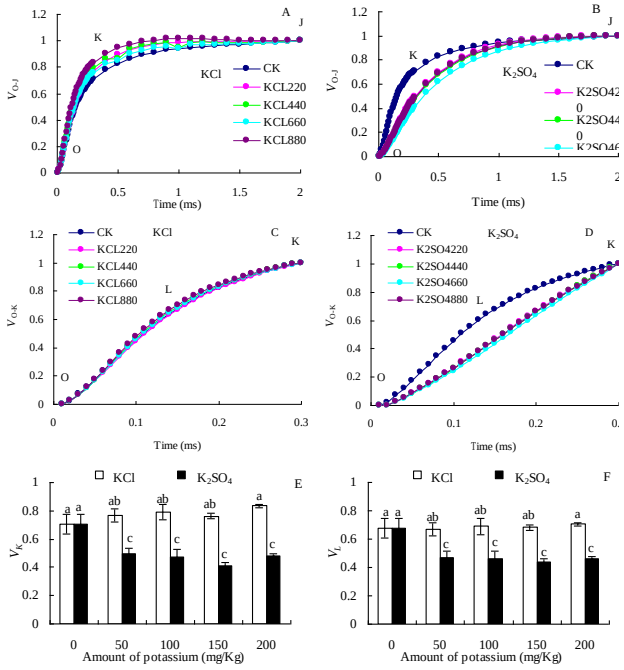

Fig. 6: Effects of different forms of potassium on standardized O–J (A and B) and O–K (C and D) curves and tobacco leaf V_K (E) and V_L (F) values. Bar graphs depict mean ± SE, values followed by different small letters mean significant difference (p<0.05)

treatments were all significantly higher than those under the CK treatment, suggesting that potassium fertilizer can promote tobacco plant growth and development, which is consistent with previous research (Qiu *et al*., 2015) However, under the KCl treatment, all plant growth indicators were significantly lower than those of the control and when potassium concentrations increased to 150 and 200 mg/kg tobacco plant heights decreased sharply and significantly. This indicates that the effects of different forms of potassium on tobacco plant growth differ significantly. The introduction of chloride in the form of KCl exerted significant stress effects on tobacco plants. This result agreed with the claim that tobacco is a chloride-intolerant plant (Wen *et al*., 2004).

Compared to the CK treatment, the chlorophyll a, chlorophyll b and total chlorophyll contents significantly increased when the tobacco plants were treated with less than 150 mg/kg K_2SO_4, indicating that applications of K_2SO_4 promote chlorophyll synthesis in tobacco leaves. When potassium applications reached 200 mg/kg, chlorophyll a, chlorophyll b and total chlorophyll contents were significantly decreased, possibly as a result of the osmotic stress caused by the excessive application of potassium fertilizer. As the KCl applications increased, the chlorophyll a, chlorophyll b and total chlorophyll contents of the tobacco leaves significantly decreased, exhibiting significant differences from the CK treatment and indicating that KCl inhibits chlorophyll synthesis in tobacco plant leaves. This result is inconsistent with the conclusion drawn by Li Mingde *et al*., who determined that an appropriate chloride application level can improve the chlorophyll content of tobacco leaves (Li *et al*., 2004). This discordant result may be related to the relatively high chloride content (113 mg/kg) of the soil used in this study.

Chlorophyll is the primary pigment for photosynthesis. Chlorophyll a is the main component of the light reaction center complex, while chlorophyll b is an important component of the light-harvesting pigment protein complex (Zhu *et al*., 1999). In this study, although under the KCl treatment the contents of chlorophyll a and chlorophyll b of tobacco leaves were significantly lower, under the treatments with less than 150 mg/kg of KCl the ratio of chlorophyll a to chlorophyll b increased. This indicated that the KCl application had a greater impact on chlorophyll b content. That is the toxic effect of chloride mainly affects the capture of light energy by tobacco leaves. As K_2SO_4 applications increased the overall chlorophyll contents increased while the chlorophyll a to chlorophyll b ratio slightly decreased. That is, K_2SO_4 application better enhances chlorophyll a synthesis in tobacco leaves, thus improving solar energy utilization capacity of tobacco plants.

Through fast chlorophyll fluorescence kinetics analysis, a series of reactions during the photosynthesis process, such as light energy absorption, distribution and dissipation, can be studied and the extent of toxic effects on plants can be studied as well (Ma *et al*., 2010). F_v/F_m, PI_{ABS} and PI_{total} are important indicators that reflect the activity of the PSII reaction center. In this study, when KCl and K_2SO_4 were applied as fertilizer for the tobacco plants, F_v/F_m

remained above 0.8, exceeding the F_v/F_m values under the CK treatment and indicating that different forms of potassium fertilizers can increase the PSII maximum photochemical efficiency of tobacco leaves. However, the trends in PI_{ABS} and PI_{total} were different from those of F_v/F_m when the tobacco plants were treated with KCl or K_2SO_4. Under different KCl treatment levels PI_{ABS} and PI_{total} showed no significant differences from the CK treatment but under K_2SO_4 treatments, PI_{ABS} and PI_{total} both substantially increased compared to those under the CK treatment, indicating that K_2SO_4 can substantially increase the activity of the PSII reaction center of tobacco leaves. Furthermore, the sensitivity of PI_{ABS} and PI_{total} in this study was significantly higher than that of F_v/F_m, indicating that PI_{ABS} and PI_{total} are better indicators of the response of the PSII reaction center to the application of potassium fertilizers, which is consistent with other studies (Zhang *et al*., 2013). However, when the amount of potassium applied reached 200 mg /kg, the application of K_2SO_4 decreased PI_{ABS} and PI_{total} slightly, indicating that too much fertilizer can also decrease the activity of the PSII reaction center, as is consistent with changes in chlorophyll content (Fig. 2).

The increases in V_J and V_I can reflect Q_A accumulation (Govindjee, 1995). Studies conducted (Schansher *et al.*, 2005; Li *et al*., 2009) found that increases in V_J could decrease the oxidation of plastoquinone Q_B, while increases in V_I could decrease the re-oxidation of PQH_2. That is, increases in V_J and V_I appear to be the results of both the blockage of electron transfer from the primary electron acceptor Q_A to the secondary electron acceptor Q_B and the blockage of electron transfer from Q_B to PQ of the PSII reaction center complex, respectively. In this experiment, as the amount of KCl fertilizer applied increased, tobacco leaf V_J showed an overall increasing trend, while V_I showed no significant change. This indicated that the application of KCl inhibits electron transfer from Q_A to Q_B on the acceptor side of the PSII reaction center but does not affect electron transfer from Q_B to PQ. That is the application of KCl mainly decreased the capacity of Q_B to accept electrons in the PSII reaction center of tobacco leaves but exerted no significant effect on the PQ pool. Studies have shown that D1 protein synthesis in plants can be easily inhibited under stress conditions. D1 proteins are rapidly synthesized and have high degradation turnover (Zhang *et al*., 2013) and Q_B is mainly linked to the D1 protein functionally in the electron transfer process. Therefore, the inhibition of electron transfer on the acceptor side of the PSII reaction center under KCl treatment in this study could be associated with the inhibition of D1 protein synthesis under high chloride ion stress. Both the V_J and V_I values decreased under the K_2SO_4 treatment compared to the CK treatment, indicating that K_2SO_4 can promote electron transfer on the acceptor side of the PSII reaction center in tobacco leaves.

The O–J and O–K curves were standardized for the quantitative analysis of the changes in V_K and V_L. As KCl applications increased, V_K showed an overall increasing trend on the standardized O–J curve, while no significant change in V_L was observed on the standardized O–K curves. The increase in V_K might have been influenced by factors such as the status of the oxygen-evolving complex (OEC) and the linkage status between PSII units (Jiang *et al*., 2006). That is, the increase in V_K is mainly associated with the inhibition of the activity on the electron donor side of PSII, especially OEC activity (Bertamini and Nedunchezhian, 2003). In contrast, the increase in V_L is mainly associated with damages to the chloroplast thylakoid membrane and the dissociation of the thylakoid. Therefore, KCl (Zhang *et al*., 2016) application not only inhibited electron transfer on the acceptor side of the PSII reaction center, KCl application also inhibited the OEC activity on the donor side of the PSII reaction center; however, it did not degrade the thylakoid membrane. Thylakoid membrane degradation mainly occurs through the production of reactive oxygen species during the metabolism process. In this study, although KCl application blocked electron transport in the tobacco leaves, which may lead to the leakage of electrons in the electron transport chain and create reactive oxygen species, it did not degrade thylakoid membranes. This might be associated with the enhanced function of the active oxygen scavenging system; however, this hypothesis requires confirmation by further studies. Under different K_2SO_4 treatment levels, the V_K and V_L values of the tobacco leaves were both significantly lower than those under the CK treatment; that is a reasonable application of K_2SO_4 can increase OEC function and increase the stability of the thylakoid membranes, which provides a solid foundation for the supply and normal transportation of photosynthetic electrons.

Conclusion

The effects of different forms of potassium fertilizers on plant growth and photosynthetic functions differed significantly. A reasonable application of K_2SO_4 can significantly improve the photosynthetic capabilities of tobacco leaves and promote overall plant growth; however, when soil chloride content is approximately 100 mg/kg KCl application can inhibit the growth of tobacco plants, mainly by suppressing chlorophyll synthesis in the tobacco leaves and decreasing the activity of the PSII reaction center during the photoreaction process in tobacco leaves. This result is consistent with both electrons transfer from Q_A to Q_B on the acceptor side of the PSII reaction center and OEC function being relatively sensitive to chloride.

Acknowledgments

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