

Effects of Different Potassium Stress on Leaf Photosynthesis and Chlorophyll Fluorescence in Maize (*Zea Mays* L.) at Seedling Stage

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Abstract

Leaf early senescence caused by nutrition deficiency is one of the major limitation reasons in the world crop production. Potassium (K) is one of important nutrient elements in crop growth, which modifies dozens of enzyme activations and controls stomatal movement of photosynthesis. The yield and quality of maize (*Zea Mays* L.) have been limited due to K deficiency in plough layer soil. However, the mechanism of K deficiency tolerance is not fully understood in maize. In this study, two inbred lines, 099 (tolerance to potassium deficiency) and 835 (sensitive to potassium deficiency) were carried out to investigate the variations of chlorophyll content, photosynthetic and chlorophyll fluorescence parameters related with senescence under different K⁺ concentrations in maize at seedling stage. The results showed that the Chlorophyll *a*, *b* and (*a* + *b*) of 835 were significantly decreased under different K deficiency treatments, whereas those of 099 were remained normal. In addition, 099 showed a lower stomatal restriction and higher electronic transition capacity under different K deficiency treatments. The variations of F₀, F_v/F_m, Φ_{PSII}, qP and NPQ in 835 were largely higher than those in 099. These results indicated that the inbred line 099 tolerance to K deficiency could keep chlorophyll content to maintain photosynthesis and to alleviate the injury of PSII under K deficiency condition. This study should contribute to explaining the physiological mechanism tolerance nutrition deficiency and improving breeding program in maize.

Keywords

Potassium Deficiency, Maize, Leaf Senescence, Photosynthetic Parameters, Chlorophyll Fluorescence

1. Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops world-wide as well as an important source of feed, fibre, and biofuel. Potassium (K) as one of the essential nutrients for crop plays an important role in crop growth and development, metabolism and yield formation process [1] [2]. Because of long-term agricultural production of existing cultivated land, there is about 60% of massive arable land potassium deficiency, which inhibit crop yield and quality improvement [3] [4]. Meanwhile, the price of K fertilizers has been rapidly increased with rising energy prices, which lead to increased input in crop production. In Northeast China, an insufficient supply of K in soils often limits maize production to meet the demand for food from the growing population [4]-[6]. Therefore, it has been proposed that screening maize genotypes efficient use of potassium and clarifying the physiological mechanism of tolerance to potassium deficiency can reduce the use of expensive K fertilizers in agriculture [7]-[9].

The leaf senescence shortens the duration of the photosynthesis after heading, and reduces the photosynthetic production accumulation [10]-[12]. Potassium deficiency in plants usually induces brown scorching and curling of leaf tips as well as chlorosis (yellowing) between leaf veins, which accelerate leaf senescence. Leaf photosynthesis consists of the several physiological processes, including light harvesting, photosystem II (PSII) photochemistry and CO₂ assimilation [13]. Potassium deficiency disrupted the leaf photosynthetic organ, and led to declining in net photosynthetic rate, stomatal conductance, RuBPCase activity and obviously decreasing grain production in rice [14]. The study in soybean showed that a relatively high content of Chl *a*, *b* and (*a* + *b*), especially the increase of Chl *a* content in K efficiency variety, resulted in a high ability of photosynthesis. The effective transfer of photosynthetic electron was another factor in maintaining the ability, while the significant changes of those parameters led to decrease of photosynthesis ability for K inefficiency variety [15].

Chlorophyll fluorescence measurements have increasingly been used as a non-invasive tool in leaf ecophysiological studies. This method has been used extensively to investigate the response of plants to environmental stress, including the effects of low potassium on the photosynthetic apparatus of crops both in a controlled environment and in the field [16] [17]. In particular, it can assess PSII electron (e⁻) transport efficiency simultaneously, a number of photosynthesis parameters underlying physiological responses to environmental variables can be estimated [18] [19]. Increasing Nitrogen (N) nutrition could improve the PSII potential activity, increased maximum quantum yield in wheat, whereas decreased non-photochemical quenching and increased the net photosynthetic rate (P_n). However, nitrogen deficiency could decrease the quantum yield of PSII electron transport and the maximal efficiency of PSII (Fv/Fm) photochemistry rate. Nevertheless, little has been reported about the relations of leaf senescence, photosynthesis and chlorophyll fluorescence.

In present study, we designed an experiment to explore the different expression of photosynthesis and chlorophyll fluorescence using two typical maize inbred lines under four potassium treatment levels. Meanwhile, we want to verify the mechanization of resistance to senescence and the physiological mechanism of higher photosynthesis productivity in maize inbred lines tolerant to K deficiency.

2. Material and Methods

2.1. Plant Materials and Experimental Design

Two maize inbred line screened in our previous study, 099 (tolerance to K⁺ deficiency) and 835 (sensitive to K⁺ deficiency), were carried out to compare the different in hydroponic method at the experiment station of Shenyang Agricultural University. After disinfecting in 7% NaClO₃ solution, the selected full and uniform seed were germinated at 25°C in waterish sand on June 28, 2014. When two leaves were fully expanded, 12 seedlings per inbred line were washed with distilled water and carefully transplanted into an experimental lightproof plastic pot in the rainproof greenhouse under natural environment. The pot was 50 cm × 35 cm × 15 cm and contained 20 L nutrient solution. The row spacing was 7.5 cm and the plant spacing was 6.5 cm.

2.2. Treatment

The nutrient solution was modified with 1/2 Hoagland's nutrient solution and Arnon microelement, containing 2000 μmol Ca(NO₃)₂·4H₂O, 1000 μmol MgSO₄·7H₂O, 500 μmol NH₄H₂PO₄, 100 μmol FeSO₄·7H₂O, 100 μmol EDTA, 23 μmol·H₃BO₃, 6.3 μmol MnSO₄·H₂O, 0.16 μmol CuSO₄·5H₂O, 0.383 μmol ZnSO₄·7H₂O, 0.8092 μmol (NH₄)₆Mo₇O₂₄. Four levels of K⁺ treatment were adjusted at 0 mmol·L⁻¹, 0.625mmol·L⁻¹, 1.25mmol·L⁻¹ and 2.5 mmol·L⁻¹ (control) using KNO₃. A randomized complete block design was employed using a 2-way factorial

arrangement of treatments with three replications for each treatment. The oxygen was pumped to nutrient solution for 45 min every 15 min by automatic control electric pump. The pH value of solution was adjusted to 6.0 everyday by 0.1 mol/L NaOH or HCl and the solutions were replaced every week. After 9 days of treatment, the new expanded leaves of three representative seedlings per inbred line were selected to measure chlorophyll content, gas exchange parameters and chlorophyll fluorescence parameters in each treatment. All measurements were carried out between 9:30 and 11:30.

2.3. Measurement

2.3.1. Chlorophyll (Chl) Content

Chl *a*, *b* and Chl (*a* + *b*) were measured according to the methods of Li *et al.* (2011) [20]. Every Sample containing 100 mg of fresh leaf was extracted by 10 mL acetone and ethylalcohol (1:1, v/v) at dark place for 24 h until the sample changed to white [21]. Pigment concentrations were measured by using an UV-spectrophotometer (Shimadzu UV-1601, Japan). Then the absorbance was recorded at 645 nm and 663 nm.

2.3.2. Gas Exchange Parameters

Gas exchange was measured with a CIRAS-2 portable open-flow gas exchange system attached to internal LED with red/white light source (PP systems, Hansatech, UK). The chamber was set at airflow rate of 100 mL·min⁻¹, CO₂ concentration of 390 ± 5 μmol·mol⁻¹, photosynthetic photon flux density of 1200 μmol·m⁻²·s⁻¹. The net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (G_s) and intercellular CO₂ concentration (C_i) were measured on the upper fully expanded leaf.

2.3.3. Chlorophyll Fluorescence Parameters

Chlorophyll fluorescence parameters were measured with FMS-2 (Hansatech, UK) at the same leaves after keeping in the dark for 20 min, including minimal fluorescence (F_0), the maximum quantum efficiency of PSII photochemistry (F_v/F_m), actual photochemical efficiency of PSII (Φ_{PSII}), photochemical quenching (qP), non-photochemical quenching (NPQ).

2.4. Statistical Analysis

The mean values of three replications were calculated for each trait and used in data analysis using Excel 2013. Data among the different treatments and inbred lines were analyzed using TWO-way ANOVA ($p \leq 0.05$) method. The analysis was carried out using SPSS22.0 software for windows 8.

3. Results

3.1. Effects of K⁺ Deficiency on Chl Content

The variations of Chl *a*, Chl *b* and Chl (*a* + *b*) content were shown in **Table 1**. With the K⁺ concentration decreasing, the value of Chl *a*, Chl *b* and Chl (*a* + *b*) in 835 and 099 were largely reduced. The Chl *a*, Chl *b* and Chl (*a* + *b*) of 835 were significant decreased comparing with CK at 0.625 mmol/L. The Chl *a* and (*a* + *b*) of 835 were significantly decreased from 2.5 mmol/L to 0 mmol/L, while those of 099 were only slightly decreased. Under the same K⁺ concentration, the Chl *a*, Chl *b* and Chl (*a* + *b*) of 099 were significantly higher than those of 835.

3.2. Effects of K⁺ Deficiency on Photosynthetic Characteristics

3.2.1. Net Photosynthetic Rate (P_n)

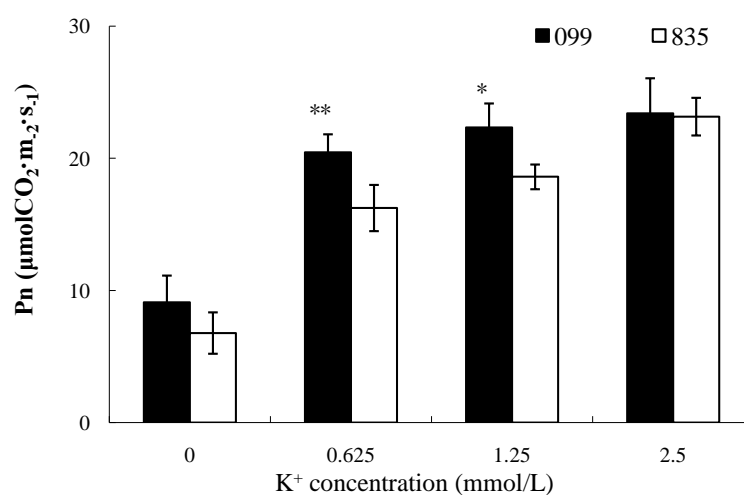
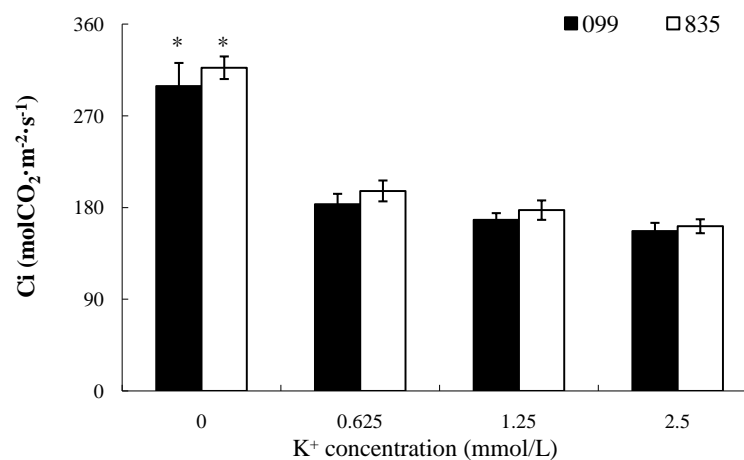
With the K⁺ concentration decreasing, the two inbred lines showed different declining (**Figure 1**). Comparing with the control, the P_n of 835 were significantly decreased at 0 mmol/L, 0.625 mmol/L and 1.25 mmol/L by 70.73%, 29.87%, 19.68%, respectively. However, the P_n of 099 were little decreased by 61.07%, 12.60% and 4.53% under K deficiency. Although there was no difference between 099 and 835 in control, the P_n of 099 were significantly higher than 835 at 0.625 mmol/L and 1.25 mmol/L, by 25.88% and 20.07%.

3.2.2. Intercellular CO₂ Concentration (C_i)

The C_i of two inbred lines was decreased with the K⁺ concentration increasing (**Figure 2**). There was no differ-

Table 1. Effects of potassium deficiency on chlorophyll content between two maize inbred lines.

Inbred line	K ⁺ concentration (mmol/L)	Chl <i>a</i> (mg/g)	Chl <i>b</i> (mg/g)	Chl (<i>a+b</i>) (mg/g)
099	0.000	1.129 ± 0.15bc	0.302 ± 0.03ab	1.431 ± 0.223bc
	0.625	1.77 ± 0.093a	0.475 ± 0.027a	2.244 ± 0.132a
	1.250	1.82 ± 0.129a	0.495 ± 0.029ab	2.315 ± 0.151ab
	2.500	1.853 ± 0.142a	0.523 ± 0.032a	2.376 ± 0.207a
835	0.000	0.641 ± 0.123e	0.225 ± 0.022c	0.866 ± 0.11e
	0.625	1.033 ± 0.092d	0.298 ± 0.022bc	1.331 ± 0.093de
	1.250	1.09 ± 0.131d	0.325 ± 0.03bc	1.415 ± 0.129de
	2.500	1.217 ± 0.127c	0.366 ± 0.027bc	1.583 ± 0.142cd

**Figure 1.** Effect of different K⁺ concentration on P_n in two inbred lines.**Figure 2.** Effect of different K⁺ concentration on C_i in two inbred lines.

ence in the control, whereas those of 835 were slightly higher than those of 099 at 0 mmol/L, 0.625 mmol/L and 1.25 mmol/L, by 6.03%, 7.09% and 5.60%. The C_i of 099 and 835 were significant increasing by 90.77% and 96.26% at 0 mmol/L, respectively.

3.2.3. Stomatal Conductance (G_s)

With the K^+ concentration decreasing, the G_s of 835 and 099 were significantly declined (Figure 3). Comparing with control, the G_s of 099 and 835 were decreased by 10.49%, 20.65%, 41.30% and 17.74%, 33.87%, 60.97% from 2.5 mmol/L to 0 mmol/L. Under K deficiency, those of 099 were significantly higher than those of 835 at 0 mmol/L and 0.625 mmol/L, whereas there was no significantly under other treatments.

3.2.4. Transpiration Rate (T_r)

The T_r of 099 were decreased from 4.65 mmol CO_2 $m^{-2}\cdot s^{-1}$ to 3.04 mmol CO_2 $m^{-2}\cdot s^{-1}$ with the K^+ concentration decreasing (Figure 4), while the T_r of 835 were little declined from 4.35 mmol CO_2 $m^{-2}\cdot s^{-1}$ to 2.66 mmol CO_2 $m^{-2}\cdot s^{-1}$. It was showed that the T_r of 835 were larger decreased than those of 099 under K deficiency. There was not significantly different between two inbred lines at 1.25 mmol/L and 2.5 mmol/L, whereas the T_r of 099 was significantly higher than that of 835 at 0.625 mmol/L.

3.3. Effects of K Deficiency on Chlorophyll Fluorescence Parameters

3.3.1. Minimal Fluorescence (F_0)

With the decreasing of K^+ concentration, the F_0 of two inbred lines were largely increased (Figure 5). The F_0 of 099 was decreased by 21.58% at 0 mmol/L comparing with control, whereas that of 835 was significantly decreased 45.06%. Under K deficiency, the F_0 of 835 were largely increased from 2.5 mmol/L to 0 mmol/L by

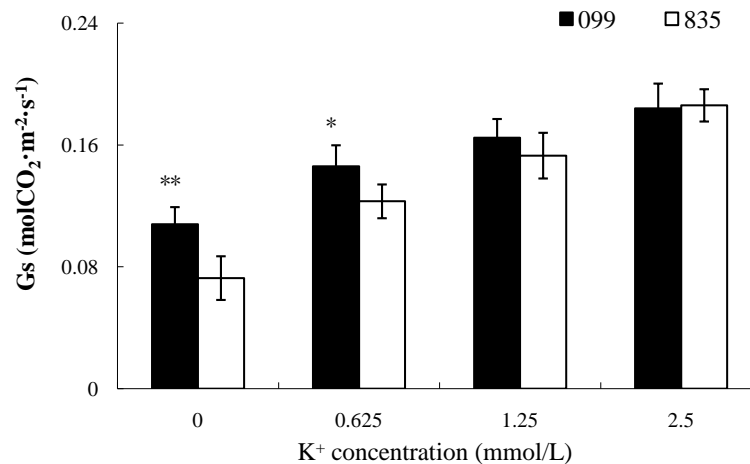


Figure 3. Effect of different K^+ concentration on G_s in two inbred lines.

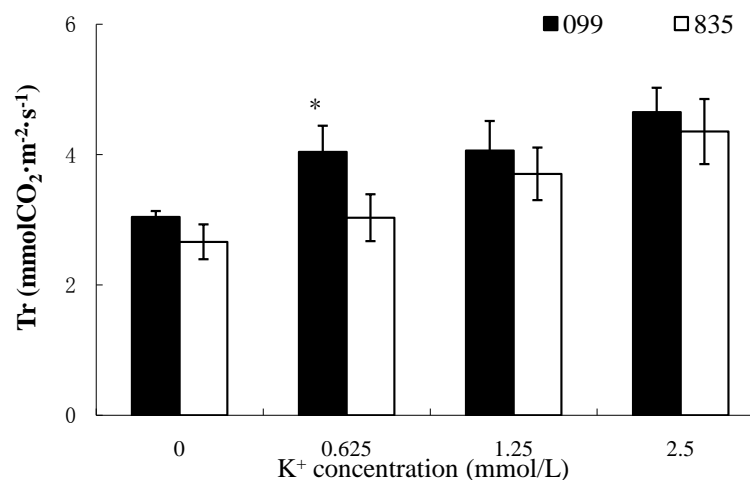


Figure 4. Effect of different K^+ concentration on T_r in two inbred lines.

13.92%, 10.89% and 6.91%, whereas that of 099 was slightly increased.

3.3.2. Maximum Quantum Efficiency of PSII Photochemistry (F_v/F_m)

The F_v/F_m of two inbred lines was decreased from 2.5 mmol/L to 0 mmol/L (Figure 6). The F_v/F_m of 099 was significantly higher than those of 835 at 0 mmol/L and 0.625 mmol/L, by 25.47%, 10.61% respectively. The F_v/F_m of 835 at 0.625 mmol/L was significantly lower than control, whereas there was no different for 099.

3.3.3. Actual Photochemical Efficiency of PSII (Φ_{PSII})

The Φ_{PSII} of two inbred lines were decreased with the decreasing of the K^+ concentration (Figure 7). The Φ_{PSII} of 099 were significantly higher than that of 835 from 0 mmol/L to 2.5 mmol/L by 88.66%, 92.35%, 42.80% and 30.33%, respectively. Comparing with the control, the Φ_{PSII} of 099 was not significantly lower except at 0 mmol/L, whereas that of 835 were largely decreased at 0 mmol/L and 0.625 mmol/L.

3.3.4. Photochemical Quenching (qP)

The qP is commonly used to reflect the degree of opening of the reaction center. With the decreasing of K^+ concentration, the qP of 835 were largely decreased from 2.5 mmol/L to 0 mmol/L (Figure 8). The qP of 099 were

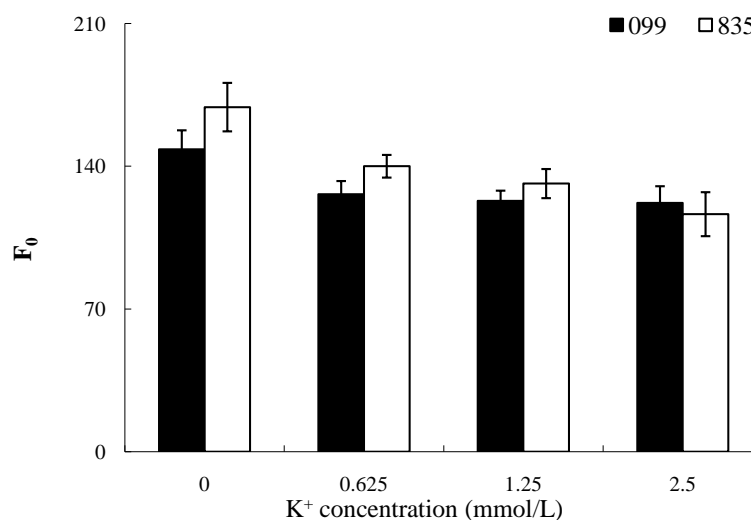


Figure 5. Effect of different K^+ concentration on F_0 in two inbred lines.

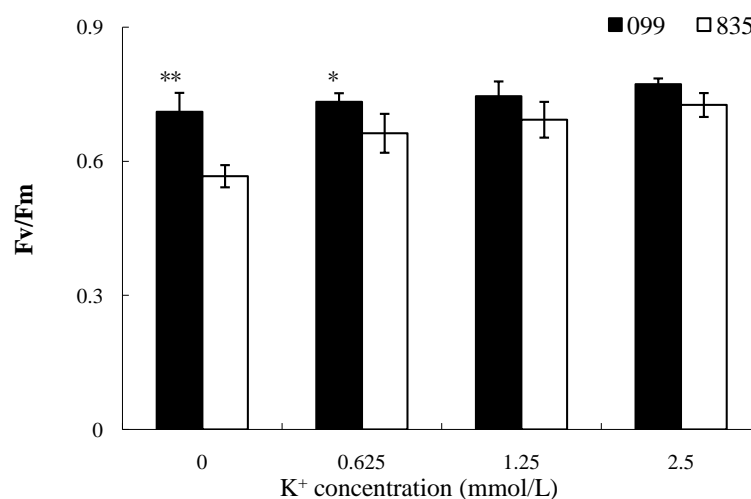


Figure 6. Effect of different K^+ concentration on F_v/F_m in two inbred lines.

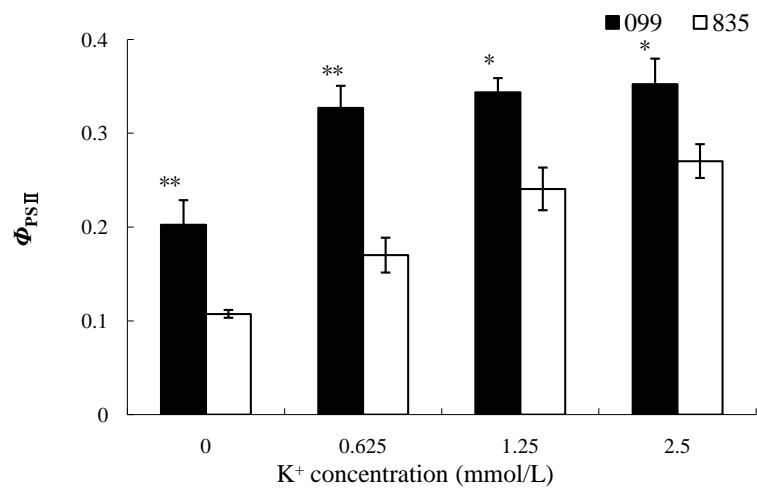


Figure 7. Effect of different K⁺ concentration on Φ_{PSII} in two inbred lines.

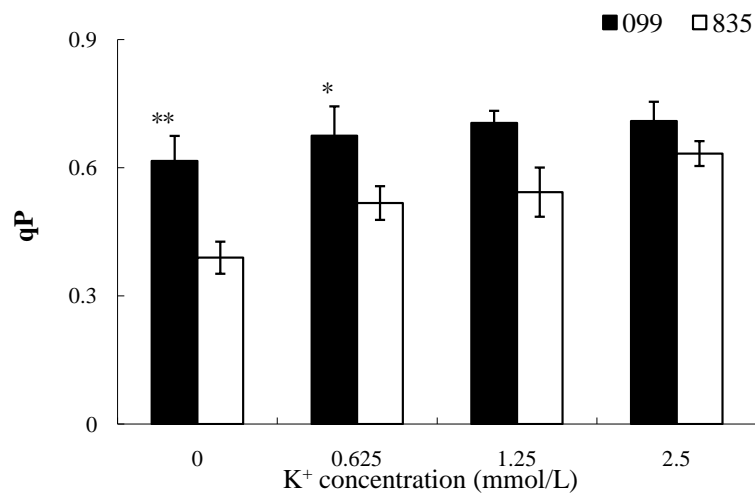


Figure 8. Effect of different K⁺ concentration on qP in two inbred lines.

significantly higher than those of 835 under K deficiency. Comparing with the control, the qP of 835 were significantly decreased by 38.49% and 18.29% at 0 mmol/L and 0.625 mmol/L.

3.3.5. Non-Photochemical Quenching (NPQ)

The NPQ reflects the change of the ability of plant heat dissipation. The NPQ of two inbred lines increased gradually from 2.50 mmol/L to 0mmol/L (Figure 9). The NPQ of 099 were largely higher than those of 835 at different K⁺ concentration. At 0mmol/L K⁺ concentration, the NPQ of 099 was significantly increased by 21.79%, while that of 835 was slightly increased.

4. Discussion

Leaf senescence comprises a series of biochemical and physiological events [11] [22]. The ability to maintain green leaf area is one of the important physiological traits that have an implication on yield potential related to increasing assimilate availability [23]-[25]. However, several reports has described that many abiotic stresses, including nutrient deficiency, could not result in uniform symptoms, but rather on visible patches or color variation on leaf surfaces and margin [26]. Chlorophyll degradation during senescence is an early and, for membrane polypeptides, essential event. If chlorophyll breakdown in senescence is prevented, the process of senescence could be delayed. In the present study, the Chl *a*, *b* and Chl (*a* + *b*) of 099 were not varied significantly at

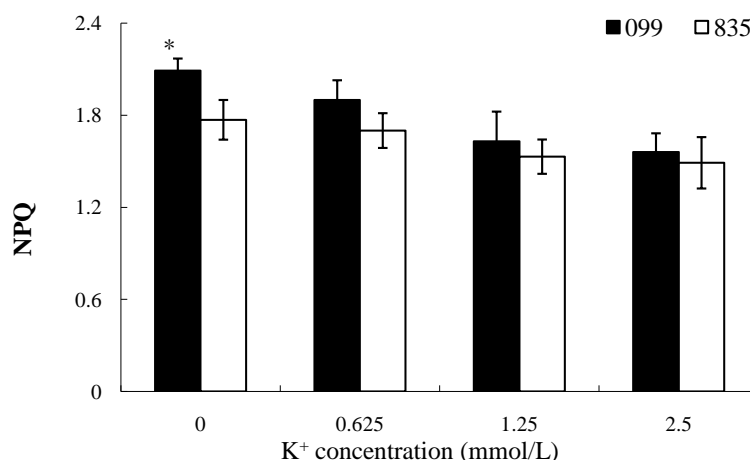


Figure 9. Effect of different K⁺ concentration on NPQ in two inbred lines.

0.625 mmol/L and 1.250 mmol/L, whereas those of 099 were significantly decreased at 0 mmol/L. To the contrary, those of 835 were significantly declined under K deficiency comparing with control. Under the same K⁺ concentration, the Chl *a*, *b* and Chl (*a* + *b*) of 099 were significantly higher than those of 835. Those results showed that the progress of leaf senescence was more slowly in 099 than in 835 under potassium deficiency stress.

Leaf photosynthesis consists of the several physiological processes, that is, light harvesting, PSII photochemistry, and CO₂ assimilation [13]. The photosynthetic functions, including PSII photochemistry, were inhibited as a result of nutrients deficiency, which directly influence the photosynthetic apparatus through biosynthesis and functioning of key photosynthetic components [27]. For example, nitrogen, sulphur and iron deficiencies could directly influence the synthesis of protein complexes in photosynthetic reactions [28] [29]. Potassium plays an important role in stomatal function by maintaining turgor pressure [30]. Under potassium deficiency stress, the leaf net photosynthetic rate, stomatal conductance, photosynthetic phosphorylation activity and electron transfer energy were decreased in rice, cotton [31] [32]. In addition, Xu *et al.* (2010) assumed that photosynthetic CO₂ conductance limit was divided into stomatal limitation and non-stomatal limitation, which the later is largely concerned with the transmission of CO₂ [33]. In the present study, the P_n, C_i, G_s and T_r of 099 and 835 were no significant different at 2.5 mmol/L. However, the P_n, G_s and T_r of 835 were significantly decreased under K deficiency and the C_i was largely increased. On the contrary, those of 099 were slightly affected under K deficiency. These results demonstrated that the photosynthetic system of 099 tolerance to K deficiency could remain natural function under K deficiency.

In recent years, the measurement of chlorophyll fluorescence parameters has provided a rapid non-destructive method to obtain precise information about the state of photosynthetic apparatus and especially of PSII [34] [35]. Osório *et al.* (2014) reported that Chlorophyll fluorescence parameters (F_v/F_m, Φ_{PSII}, NPQ, qP) showed a significant variation with greater values in midrib areas in strawberry on 42th day after Fe deficiency [26]. In addition, Kalaji *et al.* (2014) demonstrated that the electron donation by oxygen evolving complex (OEC) was significantly decreased in Mg and Ca deficient plants [27]. However, Sulphur deficiency resulted in a limitation of electron transport beyond PSI, probably on account of decreasing in the PSI content or activity of PSI electron acceptors. To the contrary, they considered that the PSII activity was affected much more than PSI in Ca deficiency plants. To further explore the internal causes of decline in photosynthesis due to potassium deficiency, we conducted chlorophyll fluorescence parameters measured at different K⁺ concentrations. It can be seen from the results that in the condition of potassium deficiency, Φ_{PSII} of 099 and 835 were declined, which was corresponding with the increasing of F₀. These results showed that the change and photoinhibition in 099 were lesser than in 835 under K potassium. The variable fluorescence and maximum fluorescence reflect the electron transport system smoothly or not in PSII. In the present study, the electron transferred in 099 was more smoothly than that in 835 under different K deficiency. These results can explain that PSII of 099 suffered damage to a lesser extent, maintaining relative high photochemical efficiency and strong photosynthetic capacity.

5. Conclusion

In the present study, two typical maize inbred lines were carried out to measure the different of photosynthesis and chlorophyll fluorescence at different K^+ concentration. The results indicated that the P_n , G_s , T_r , F_v/F_m , qP , Φ_{PSII} and ETR of 099, tolerance to K deficiency, were significantly higher than those of 835 under K^+ deficiency treatments, whereas the C_i and F_0 of 099 were lower. These results indicated that the 099 could prolong leaf senescence and have higher photosynthesis to maintain plant growth under K^+ deficiency condition.

Acknowledgements

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