DOI: 10.1007/s11099-011-0073-4 PHOTOSYNTHETICA 49 (4): 633-636, 2011

BRIEF COMMUNICATION

Comparison of gas exchange and chlorophyll fluorescence of low-potassium-tolerant and -sensitive soybean [*Glycine max* (L.) Merr.] cultivars under low-potassium condition

X.-T. LI, P. CAO, X.-G. WANG, M.-J. CAO+, and H.-Q. YU+

College of Agriculture, Shenyang Agricultural University, Shenyang 110866, P.R. China

Abstract

The effects of potassium (K) deficiency on chlorophyll (Chl) content, photosynthetic gas exchange, and photosystem II (PSII) photochemistry during the seedling stage were investigated in two soybean [*Glycine max* (L.) Merr.] cultivars, low-K sensitive Tiefeng31 and low-K tolerant Shennong6. The cultivars were grown hydroponically in K-sufficient (KS) and K-deficient (KD) solutions. Photosynthetic gas exchange and Chl content in Tiefeng31 were severely affected by the low K condition, but were almost unaffected in Shennong6. This difference is in accordance with the PSII photochemistry in the plants, indicating that the photosynthetic apparatus of Shennong6 is more tolerant to low-K stress than that of Tiefeng31.

Additional key words: chlorophyll fluorescence; low-K stress; mesophyll resistance; net photosynthetic rate; stomatal conductance.

Potassium (K), the most important cation in plants, plays an important role in plant growth, metabolism, and yield (Mäser *et al.* 2002, Meille and Pellerin 2004). Although K-deficient soils (KD) are common worldwide, their widespread occurrence in crop-growing regions of Northeast China has been recently observed. Moreover, increased K deficiency in soybean [*Glycine max* (L.) Merr.], the most important food commodity and oil crop in Northeast China, has been observed (Liu and Liu 1995, Tang *et al.* 2003).

The application of K fertilizer is an effective way to increase yield (Tiwari *et al.* 1985, Sale and Campbell 1987). The relatively low efficiency of fertilizer use is a present serious dilemma because low fertilizer utilization increases the potential hazards of environmental pollution (Römheld and Kirkby 2010). Therefore, improving nutrient efficiency in crops is an important issue both for reducing cost in agricultural production and for protecting the environment.

The genotype difference of soybean intraspecies in terms of appearance and yield under low-K stress may be used for screening tolerant cultivars (Sale and Campbell 1987), which change little in plant growth and yield development under low-K stress (Liu and Liu 1995). During the past decade, tolerant and sensitive cultivars have been screened in field experiments and pot trials according to yield, K^+ assimilation, and K^+ accumulation (Tang *et al.* 2003). Some cultivars were spread in Liaozhong county of Northeast China, where soil K^+ content is lower than 50 mg kg^{-1}. In addition, dozens of low-K-tolerant and -sensitive cultivars had been screened (Tang *et al.* 2003, Wang *et al.* 2005). Until recently, no adequate information was available concerning the influence of low-K stress on the photosynthetic physiology in leaves and on genotype differences under low-K tolerance.

The net photosynthetic rate, stomatal conductance,

Received 30 September 2010, *accepted* 10 September 2011.

+Corresponding author: fax: +86 24 88487135, e-mail: caominjian@163.com; qiuzikr@163.com

Abbreviations: Area – the space above the fluorescence curve between F_0 and F_m; Chl – chlorophyll; C_i – intercellular CO_2 concentration; E – transpiration rate; F_m – maximal Chl fluorescence yield of dark-adapted state; F_0 – minimal fluorescence yield of dark-adapted state; F_v – variable Chl fluorescence; F_v/F_m – maximal photochemical efficiency of PSII; g_s – stomatal conductance to water vapour; K – potassium; KD – K-deficient; KS – K-sufficient; P_N – net photosynthetic rate; Q_A– primary quinone acceptor of PSII; RC – reaction centre; V_j – relative variable fluorescence intensity at the J-step.

Acknowledgement: This work is supported by a grant from Shenyang Agricultural University for young teachers (2006109) and the Creative Team Project (CTP) from the education department of Liaoning province.

Table 1. Effects of low-K stress on chlorophyll (Chl) contents in the leaves of low-K-sensitive and low-K-tolerant cultivars. Significant differences between the respective values of the K-sufficient (KS) and K-deficient (KD) treatment are: n.s. = not significant; * $p<0.05$; ** $p<0.01$. Means ± SE ($n = 5$).

Parameters	Shennong6			Tiefeng31		
	KD	KS		KD	KS	
Chl *a* [mg g^{-1}]	1.50 ± 0.10	1.36 ± 0.14	n.s.	1.45 ± 0.12	1.18 ± 0.09	**
Chl *b* [mg g^{-1}]	0.43 ± 0.02	0.41 ± 0.03	n.s.	0.43 ± 0.06	0.39 ± 0.03	n.s.
Chl (*a*+*b*) [mg g^{-1}]	1.93 ± 0.11	1.77 ± 0.16	n.s.	1.87 ± 0.16	1.57 ± 0.11	*
Chl *a*/*b*	3.47 ± 0.26	3.32 ± 0.34	n.s.	3.44 ± 0.40	3.06 ± 0.27	n.s.

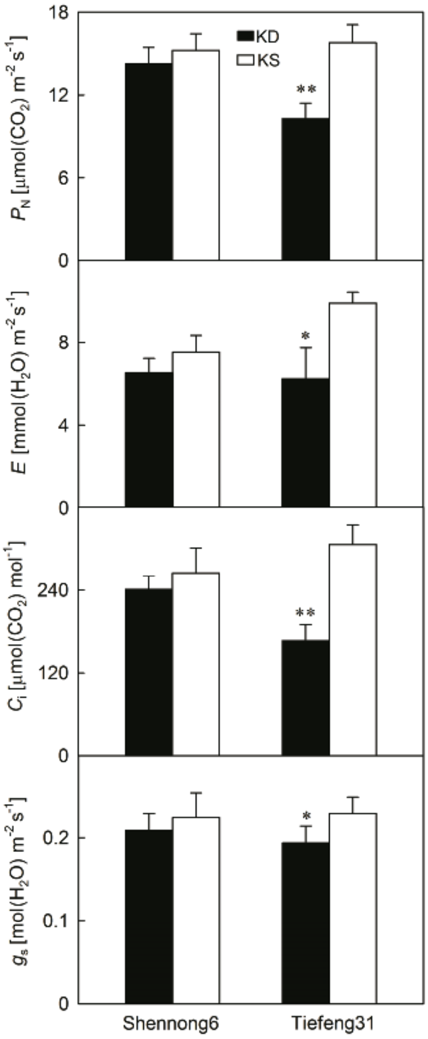

Fig. 1. Effect of low-K stress on P_N, E, C_i, and g_s in the leaves of low-K-sensitive and low-K-tolerant cultivars. Error bar means SD of five replicates in *Fisher*'s protected LSD test ($p<0.5$). Significant differences between the respective values of the K-sufficient (KS) and K-deficient (KD) treatment are: * $p<0.05$; ** $p<0.01$. Means ± SE ($n = 5$).

intracellular CO_2 concentration, Chl content, and fluorescence parameters of two soybean cultivars were investigated in this study. The adaptability and physiological metabolism of the low-K tolerant cultivar were discussed.

Two soybean cultivars were selected for this study, namely, low-K-sensitive Tiefeng31 and low-K-tolerant Shennong6. Seeds from both cultivars were germinated in washed sand, and the seedlings were transplanted into polyethylene buckets until they grew to 8 cm in early May 2009. Each bucket contained 5 L of nutrient solution, and three seedlings were transplanted per bucket. The water bath contained the nutrient solution according to Hoagland (Hoagland and Arnon 1938). It contained (in μM): 2,500 $NaNO_3$; 2,500 $Ca(NO_3)_2$; 1,000 $MgSO_4$; 5,000 NaH_2PO_4, 50 EDTA-Fe(III)Na, 115 H_3BO_3, 22.5 $MnCl_2$, 0.75 $CuSO_4$, 0.75 $ZnSO_4$, and 0.182 $(NH_4)_6Mo_7O_{24}$. Deionized water was used to make up the solution. The seedlings were grown in a glasshouse at 18 ± 0.5ºC at midnight and at 28 ± 0.4ºC at midday under natural sunlight. The K^+ treatment involved two levels with half-strength Hoagland's nutrient solution modified to supply K^+ at 0.5 mM ($K_{0.5}$) and 3.0 mM ($K_{3.0}$) as KNO_3, and 1.25 mM NH_4NO_3 was substituted for the lack of NO_3^- in $K_{0.5}$. $K_{0.5}$ nutrient solution was prepared according to Bednarz *et al.* (1998) and Zhao *et al.* (2001).

After four weeks, gas-exchange rate was measured with an open system using a temperature-controlled chamber under the following conditions: leaf temperature 25 ± 0.4ºC, 385 ± 5 μmol mol^{-1}(CO_2), 64 ± 2.8% relative humidity, and 1,000 μmol m^{-2} s^{-1} photosynthetic photon flux density. Leaf area was 5.9 cm^2, and the rate of airflow into the assimilation chamber was 706 μmol s^{-1}. CO_2 concentration and water-vapour pressure in the reference and sample air were monitored using an infrared gas analyzer (*Portable Photosynthesis System LI-6400, LI-COR Inc.*, Lincoln, NE, USA). All measurements were conducted from 10:00 to 11:00 h of the local time.

Chl *a* fluorescence parameters were measured using handy *PEA* fluorometer (*Hansatech*, Kings Lynn, UK). The leaves were placed under a dark-adaptation state (DAS) for 20 min using light exclusion clips before measurement. All reaction centres (RCs) and electron carriers of the PSII were reoxidized during DAS, which was essential for rapid fluorescence induction kinetics and for recording Chl *a* fluorescence parameters. Clips were randomly sampled at the centre of the leaves except for the nervure. Measurements were made with saturation irradiance up to 3,000 μmol m^{-2} s^{-1}.

After measuring all the parameters, fully expanded, nonsenescent, and undamaged leaves were collected.

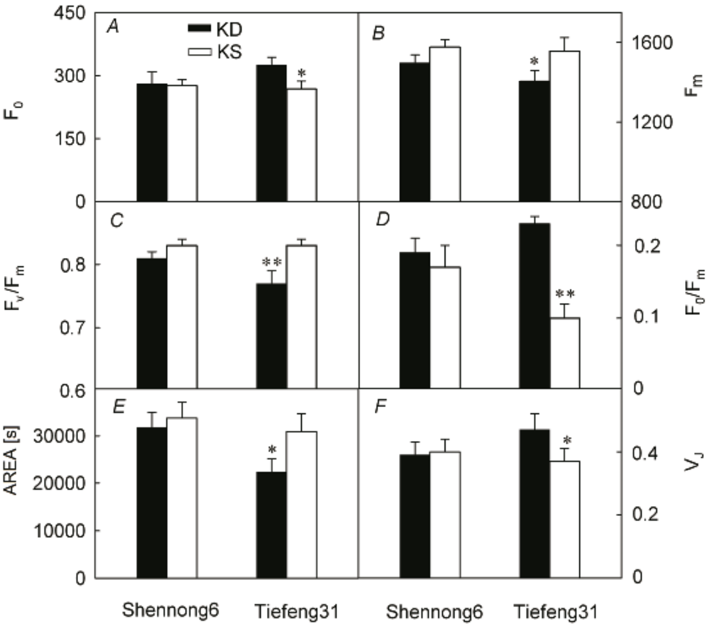

Fig. 2. Effects of low-K stress on F_0 (*A*), F_m (*B*), F_v/F_m (*C*) F_0/F_m (*D*), area (*E*), and V_J (*F*) in the leaves of low-K-sensitive and low-K-tolerant cultivars. Significant differences between the respective values of the K-sufficient (KS) and K-deficient (KD) treatments are: * $p<0.05$; ** $p<0.01$. Means ± SE ($n = 5$).

Then, 0.5 g of each sample was extracted using 80% acetone and placed in the freezer at –4°C for 48 h until they were blanched. Pigments were determined according to the method of Lichtenthaler (1987) using a spectrophotometer (*Uvikon 930, Kontron Instruments*, Watford, UK). Amounts of Chl *a* and Chl *b* [g kg^{-1}(FM)] were calculated according to the formula by Wellburn (1994).

Under greenhouse conditions, the degree of KD symptoms varied between the cultivars. KD symptoms were evident in the KD solution for 2 weeks, especially for the low-K-sensitive cultivar. Necrosis and brown spots on the old leaves occurred when the plants continued growing under KD, while Tiefeng31 showed more severe effects. Under hydroponic conditions, Chl content in the plants grown in the KD solution increased. In Tiefeng31, Chl (*a*+*b*) and Chl *a* significantly increased during the seedling stage (Table 1). In Shennong6, no significant difference in Chl content was observed between both treatments, suggesting that the increase in Chl *a* content primarily enhanced the total Chl content of Tiefeng31 under low-K stress.

P_N, *E*, C_i, and g_s of the soybean leaves decreased when the plants were grown under KD condition, but the decrease in values between Tiefeng31 and Shennong6 varied. Although P_N, *E*, C_i, and g_s in Shennong6 were reduced under KD condition, no significant difference among these parameters was observed in Shennong6 under both treatments. However, significant differences among these parameters in Tiefeng31 were observed under both treatments (Fig. 1). Zhao *et al.* (2001) argued that under severe K deficiency, g_s might not have primarily caused the decrease in P_N. Moreover, they observed that other factors that limit P_N, such as the mesophyll resistance as well as the quantity and structure of the photosynthetic RC, were dominant. Bednarz and Oosterhuis (1998) pointed out that during mild K deficiency, increased stomatal resistance was the primary factor for the decrease in P_N in leaves; biochemical factors also contributed as deficiency progressed. In this study, Tiefeng31 had significantly lower P_N, C_i, *E*, and g_s in its leaves under KD condition, signifying that stomatal resistance plays an important role in decreasing P_N of Tiefeng31 under low-K stress. Shennong6 had lower leaf P_N, g_s, C_i, and *E*, but the reduced range of their values were not significant under KD condition, indicating that the decreased stomatal resistance does not significantly affect the P_N of the Shennong6 leaf.

Minor alterations occurred in the fluorescence parameters of Shennong6 under both treatments but no significant changes were observed. F_0, F_m, F_v/F_m F_0/F_m, area, and V_J values of the fluorescence parameters of Tiefeng31 under KD treatment were significantly different from those obtained under the KS treatment (Fig. 2).

The level of F_0 increased under KD condition in both cultivars, but the extent of its increase in Tiefeng31 was higher than that in Shennong6 (Fig. 2*A*). An increase in F_0 is one of the most direct signs of photoinhibition (Aro *et al.* 1993); it is due to the damage of the acceptor side of PSII (Styring *et al.* 1990). Therefore, Tiefeng31 suffered from more photoinhibition effects than Shennong6 under low-K stress.

The maximal Chl *a* fluorescence yield F_m refers to complete reduction of PSII acceptor Q_A, the quencher of fluorescence. A decrease in F_m demonstrates the reducing ability of PSII for the primary acceptor Q_A (Joliot and Joliot 2002). Similarly to other abiotic stresses, low-K stress also affects the photosynthetic apparatus. In Tiefeng31, F_m decreased significantly compared with that in Shennong6, indicating that PSII in Shennong6 is more tolerant to low-K stress (Fig. 2*B*).

Kitajima and Butler (1975) have proposed that F_v/F_m is a useful parameter proportional to the quantum yield of PSII photochemistry, and exhibits a high degree of correlation with the quantum yield of net photosynthesis. Alteration in F_v/F_m implies changes in photochemical conversion efficiency of PSII and, therefore, possible photoinhibition of photosynthesis. Under nonstressed conditions, F_v/F_m is almost constant (from 0.80 to 0.86) (Björkman and Demming 1987). In the present study, the F_v/F_m ratio of KD treatment Tiefeng31 decreased to 0.77 ± 0.02, and it was significantly lower than that under KS treatment (Fig. 2*C*). However, the F_v/F_m ratios of Shennong6 leaves under both treatments were very similar. This reduction of Tiefeng31 under KD treatment provides clear proof for thermal dissipation processes (Scarascia Mugnozza *et al.* 1996). The principal cause of increase in F_0/F_m of Tiefeng31 (induced by KD treatment) can be attributed to a loss of excitation energy during its transfer from the pigment bed to the RCs and to an increase of energy loss through nonphotochemical quenching processes (Roháček 2002). These values show that photosynthesis was inhibited due to the low-K stress, but the photoinhibition was slighter in Shennong6.

The changes observed in the area of Shennong6 (Fig. 2*E*), which represents the electron acceptor pool size of PSII that includes Q_A, Q_B, and PQ (Joliot and Joliot 2002), were very similar in both treatments. A considerable decrease in area in Tiefeng31 was observed, signifying that the pool size of the acceptor in Tiefeng31 decreased significantly compared with that in Shennong6 under low-K stress.

Our results show F_0/F_m, and V_j values were higher under KD treatment than under KS treatment and higher increase in both ratios of Tiefeng31 than those of Shennong6 under KD condition (Fig. 2*D,F*). These ratios give rise to closed reaction centres (Dudeja and Chaudhary 2005). The results clearly indicate that there were more inactive reaction centres in Tiefeng31 in comparison to Shennong6 under KD treatment.

References

Aro, E.M., Virgin, I., Andersson, B.: Photoinhibition of photosystem II. Inactivation, protein damage and turnover. – Biochem. Biophys. Acta **1143**: 113-134, 1993.

Bednarz, C.W., Oosterhuis D.M., Evans R.D.: Leaf photosynthesis and carbon isotope discrimination of cotton in response to potassium deficiency. – Environ. Exp. Bot. **39**: 13-139, 1998.

Bednarz, C.W., Oosterhuis, D.M.: Development of a protocol to study the effects of potassium deficiency in cotton under controlled environmental conditions. – J. Plant Nutr. **21**: 329-339, 1998.

Björkman, O., Demmig, B.: Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. − Planta **170**: 489-504, 1987.

Dudeja, S.S. and Chaudhary, P.: Fast chlorophyll fluorescence transient and nitrogen fixing ability of chickpea nodulation variants. − Photosynthetica **43**: 253-259, 2005.

Hoagland, D.R., Arnon, D.I.: The water-culture method for growing plants without soil. – Agr. Exp. Stat. Circ. **347**: 1938.

Joliot, P., Joliot, A.: Cyclic electron transport in plant leaf. – Proc. Nat. Acad. Sci. USA **99**: 10209-10214, 2002.

Kitajima, M., Butler, W.L.: Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. – Biochim. Biophys. Acta **376**: 105-115, 1975.

Lichtenthaler, H.K.: Chlorophyll and carotenoids: pigments of photosynthetic biomembranes. – Method Enzymol. **148**: 350-382, 1987.

Liu, G.D., Liu, G.L.: [A new strategy alleviation shortage of potassium resource in China.] – Sci. Agr. Sin. **28**: 25-32, 1995. [In Chin.]

Mäser, P., Gierth, M., Schroeder, J.I.: Molecular mechanisms of potassium and sodium uptake in plants. – Plant Soil **247**: 43-54, 2002.

Meille, L.J., Pellerin, S.: Leaf area establishment of a maize (*Zea Mays L.*) field crop under potassium deficiency. – Plant Soil **265**: 75-92, 2004.

Roháček, K.: Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. − Photosynthetica **40**: 13-29, 2002.

Römheld, V., Kirkby E.A.: Research on potassium in agriculture: need sand prospects. – Plant Soil **335**: 155-180, 2010.

Sale, P.W.G., Campbell, L.C.: Difference responses to K deficiency among soybean cultivars. – Plant Soil **104**: 183-190, 1987.

Styring, S., Virgin, I., Ehrenerg, A., Andersson, B.: Strong light photoinhibition of electron transport in Photosystem II. Impairment of the function of the first quinone acceptor, Q_A. – Biochim. Biophys. Acta **1015**: 269-278, 1990.

Tang, J. C., Cao, M. J., Liu, X.: [Resistance mechanism and screening of soybean genotype resistance to low potassium.] – Soybean Sci. **22**: 18-21, 2003. [In Chin.]

Tiwari, K.N., Nigam, V., Patha,k A.N.: Studies on the potassium requirements of different crops. – Nutr. Cycl. Agroecosyst. **8**: 91-96, 1985.

Wang, W., Cao, M. J., Zhou, C. X., Li, Z., Zhang, H.: [Screening of tolerance low potassium soybean varieties.] – Soybean Bull. **4**: 7-8, 2005. [In Chin.]

Wellburn, A.R.: The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. – J. Plant Physiol. **144**: 307-313, 1994.

Zhao, D.L., Oosterhuis, B., Bednarz C.W.: Influence of potassium deficiency on photosynthesis, chlorophyll content, and chloroplast ultrastructure of cotton plants. – Photosynthetica **39**: 103-109, 2001.