Photosynthesis in reciprocal grafts of drought-tolerant and drought-sensitive cultivars of soybean under water stress

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Abstract

Maintaining greater photosynthetic performance is important to improve drought resistance in soybean. However, photosynthetic response mechanisms to drought *via* the interaction between root and shoot remain to be clarified. Here, we investigated the roles of roots and shoots in photosynthetic responses to water stress. Grafted plants of drought-tolerant cultivar L14 and drought-sensitive cultivar L21 were grown in the pot-culture experiments. Some photosynthetic and physiological traits were measured after water-stress treatments during a podding stage. The L14 as rootstock showed a stronger growth vigor under water stress, leading to increases in some photosynthetic traits. Although the photosynthesis of L21 scion was improved by grafting onto L14 rootstock, it was still lower than that of L14 scion. This indicated that not only the greater water absorption capacity *via* roots but also the physiological property of leaves conferred a higher photosynthetic capacity and drought resistance in soybean.

Additional key words: carboxylation efficiency; chlorophyll fluorescence; gas exchange; water status.

Introduction

Climatic changes with persistent water deficiency occur around numerous parts of the world, which is considered to be a major limiting factor in crop production worldwide (Shao *et al.* 2009). In soybean (*Glycine max*), a reduction of grain yield is maximal when water deficiency happens during flowering and podding stage, which causes a decrease in a pod number per plant (Kokubun *et al.* 2001). After anthesis, drought-induced lower photosynthetic capacity increased the rate of pod abortion in soybean, as a result of carbohydrate deprivation (Liu *et al.* 2004). Therefore, maintaining the greater capacity of photosynthesis and assimilate supply might be important to improve drought resistance in soybean.

Physiological responses in leaves to water stress included conservation of soil water by the decline of water loss, radiation absorption, and relative water content (Xoconostle-Cazares *et al.* 2010). Drought-induced lower leaf water potential led to decline in the photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (E) of soybean (Ohashi *et al.* 2006, Zhang *et al.* 2008). In response of leaves to water deficit, stomatal closure declines intercellular CO_2 concentration (C_i) which restrains CO_2 assimilation and results in an imbalance between electron requirement and photochemical activity at PSII (He *et al.* 1995, Flagella *et al.* 1998). Consequently, absorbed energy may exceed that required to generate NADPH and ATP for carbon fixation, which may result in photodamage (Kao and Tsai 1998).

The cultivars were empirically categorized as tolerant and sensitive to water stress on the basis of their capacity to maintain relative water content and sustained growth at decreased soil moisture conditions. Previous studies have found critical differences in photosynthetic responses to water stress between drought-sensitive and drought-tolerant genotypes (Fenta *et al.* 2012, Ries *et al.* 2012). The stability of photosynthesis under water deficit is also considered to be a crucial aspect of drought tolerance in soybean (Gilbert *et al.* 2011). In addition, the P_N of leaves is also closely related to the status of other tissues, such as root system. Developed roots, having a higher total surface area, enhance moisture and nutrient extraction in order to maintain P_N under drought conditions (Blum 2011, Comas *et al.* 2013). Root growth also affected the water absorption capacity of plants. The xylem sap is resulting

from root pressure and might mediate exchanges between shoots and roots (Li *et al.* 2009). The root bleeding sap mass (RBSM) was used as an indicator of the root growth vigor in previous study and had positive relationship with P_N in soybean (Cui *et al.* 2016). However, it remained to be clarified whether a relatively high photosynthesis of drought-resistant soybean under water stress is typical only for leaves or for the root system or for the whole plant.

The soybean cultivars Liaodou 14 (L14) and Liaodou 21 (L21) were categorized as drought-tolerant and drought-sensitive, respectively, based on their ability to maintain biomass and grain yield under drought conditions (Yan *et al.* 2018). In the practice, we found that the leaves of L14 had higher P_N than those of L21, especially under water stress conditions. We hypothesized that the greater P_N in the drought-tolerant soybean cultivar L14 was not only attributed to the photosynthetic advantage of leaves, but also due to the effect of stronger root growth vigor under water deficit. Grafting in order to connect a scion and rootstock of differing soybean genotypes has been used in our previous studies (Li *et al.* 2017a,b; 2019), which could be helpful to test this hypothesis. In the present study, L14 and L21 were used in the reciprocal grafting experiment to determine the shoot and root effects on leaf photosynthesis under well-watered and water-stress conditions. The results would provide the breeding strategies *via* root-shoot interaction to improve the drought-resistance in soybean.

Materials and methods

Plant material and grafting procedure: Two soybean (*Glycine max*) cultivars, L14 and L21, were released from Liaoning Academy of Agricultural Sciences and exhibited differing tolerance to drought stress for grain yield (Fig. 1S). The drought-tolerant cultivar L14 and drought-sensitive cultivar L21 were used as scion and rootstock in the reciprocal grafts. The above cultivars tested were of the maturity group III. Selected soybean seeds were planted in plastic pots (12 ×12 × 12 cm) with soil. Grafting was initiated at the cotyledon stage (VC), and the procedure was described in Pantalone *et al.* (1999). Six grafting treatments included rooted (non-grafted) L14, rooted (non-grafted) L21, and grafted scion/rootstock combinations: L14/L14, L21/L21, L14/L21, and L21/L14. Immediately after grafting, the rooted and grafted plants were placed in a glasshouse under the seedling management described in the previous study (Li *et al.* 2017a).

The pot-culture experiments were carried out under open field conditions in 2017 and 2018 at Liaoning Academy of Agricultural Sciences (41°83'N, 123°56'E), Liaoning Province, China. After seedling recovery, the surviving grafts (survival rate ≥ 90%) and non-grafts were transplanted into the pots (30 × 30 × 25 cm, 15.0 kg of soil). The soil was brown loam developed in loess parent material, which was derived from the 0–20 cm surface layer of field and sieved out. The particle size distribution was: clay grain (< 0.002 mm) of 23.3%, silt grain (0.002 –0.02 mm) of 36.3%, and sand grain (> 0.02 mm) of 40.5%. The soil bulk density was 1.12 g cm⁻³. The field

water-holding capacity was 26.5%, which was measured from above layer by gravimetric method. Chemical characteristics of soil were: soil organic matter of 22.30 g kg⁻¹, total nitrogen of 1.91 g kg⁻¹, available nitrogen of 0.09 g kg⁻¹, available phosphorus of 0.01 g kg⁻¹, available potassium of 0.11 g kg⁻¹, and pH of 6.8 in distilled water (1:5, v/v). The water-stress treatments began about 80 d after germination (podding stage) and were maintained during 10–12 d. The well-watered treatment maintained the soil moisture content at 80% of the field water-holding capacity, whereas water stress corresponded to 50%. The water content of soil was measured by a weighing method every day and replenished by water to the set value. There was a randomized complete block design with six replications (pots), and each pot contained two plants.

Gas-exchange parameters: After water-stress treatment, the upper third leaf was used to measure the gas-exchange parameters by the *LI-6400* portable photosynthesis system (*LI-6400*, *Li-Cor*, Lincoln, NE). The light intensity was set at a PPFD of 1,200 μmol m⁻² s⁻¹. The leaf temperature was kept at 25–30°C, relative humidity was 60–65%, CO_2 concentration of 380 μmol(CO_2) mol⁻¹, and air flow of 500 μmol s⁻¹. Six plants from six pots (*n* = 6) were measured in the morning on a sunny day.

P_N/C_i-response curves were also measured on six plants from six pots (*n* = 6). Cuvette conditions were maintained at a PPFD of 1,200 μmol m⁻² s⁻¹, relative humidity of 60–65%, and a leaf temperature of 25–30°C. A CO_2 mixer was used to control the ambient CO_2 concentration in the cuvette across the series of 50, 100, 200, 400, 500, 600, 700, and 800 μmol(CO_2) mol⁻¹. The P_N and C_i were recorded after equilibration to a steady state. Nonlinear regression, based on the equations of Cai and Xu (2000), was used to estimate the CE (initial carboxylation efficiency), P_{max} (photosynthetic capacity), and R_p (rate of respiration in the presence of light) for each P_N/C_i curve. The equations were represented as $P_N = CE \times P_{max} \times C_i/(CE \times C_i + P_{max}) - R_p$.

Chlorophyll (Chl) fluorescence parameters were measured on six plants from six pots (*n* = 6) by *PAM-2500* chlorophyll fluorometer (*Heinz Walz GmbH*, Germany). Following 30-min-dark adaptation for upper third leaves, the minimum fluorescence value was determined, and the maximum fluorescence was recorded using a 0.8-s saturation pulse at 3,000 μmol(photon) m⁻² s⁻¹. Then the actinic white light [1,000 μmol(photon) m⁻² s⁻¹ PAR] was switched on for 5 min. During the process, the light-adapted maximal fluorescence yield and minimal fluorescence at the light-adapted state was recorded. Then the actual photochemical efficiency of PSII [$Y_{(II)}$], electron transport rate (ETR), photochemical quenching (q_P), and nonphotochemical quenching (NPQ) were calculated by the *PamWin V3.12g* (control and data aquisition system).

Rubisco activity assay: The upper third leaves were collected into liquid nitrogen immediately and stored at –80°C for Rubisco activity assay. Leaf samples of approx. 0.5 g fresh mass (FM) were extracted with 2 mL of

enzyme extraction buffer [20% (v/v) glycerol, 1% (v/v) *Triton-X100* (*Sigma*, St. Louis, MO, USA), 0.25% (w/v) bovine serum albumin, 50 mM HEPES/KOH pH 7.5, 1 mM EDTA, 10 mM $MgCl_2$, 1 mM PMSF, and 0.5 mM DTT]. The initial and total activity of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase, EC 4.1.1.39) was determined with three replications per sample by microplate reader (*Varioskan Flash*, *Thermo Fisher Scientific*, *Inc.*, Waltham, MA, USA) using a rapid, non-radioactive microplate-based method (Sulpice *et al.* 2007). The initial activity was divided by the total activity to determine the Rubisco activation state.

Root bleeding sap: To reveal the growth vigor of L14 and L21 rootstocks, the RBSM was measured in six pots (*n* = 6) under well-watered and water stress conditions. To collect the root bleeding sap samples, the shoots were cut just under the cotyledons node of two plants from each pot with a very sharp cutter as described by Peoples *et al.* (1989).

Leaf water status: All leaves of two plants from each pot were collected to measure the leaf fresh mass (FM). Then the leaves were immersed in water for 4 h at room temperature (approximately 28°C) to measure the turgid mass (TM). Finally, the leaves were oven dried at 105°C for 30 min and then at 85°C until constant mass to measure the dry mass (DM). The leaf relative water content (LRWC) was determined according to the following equation: LRWC = (FM – DM)/(TM – DM).

Statistical analysis: Data analysis was conducted with an analysis of variance (*ANOVA*) in general linear model by *SPSS 19.0* (*SPSS*, *Inc.*, Chicago. USA). Genotype (grafting treatments), water-stress treatment, and genotype × water-stress treatment were as fixed effects. Years, along with the genotype × year, water-stress treatment × year, and genotype × water-stress treatment × year were regarded as a random factor. Means were subjected to the least significant difference (*LSD*) test at the *P*<0.05 level.

Results

The grafting procedure *per se* had no effect on photosynthetic traits. Non-grafted L14 *vs.* self-grafted L14 and non-grafted L21 *vs.* self-grafted L21 had insignificant differences in most photosynthetic traits in the *ANOVA*. A comparison of reciprocal grafts was undertaken to determine the effect of roots and shoots on photosynthesis under well-watered and water-stress conditions.

Water status: Under the water stress imposed, the RBSM decreased significantly (Fig. 1*A*). The reduction of RBSM followed the order L14/L14 (36%) ≤ L21/L14 (38%) < L21/L21 (55%) ≤ L14/L21 (56%). With the same scion genotype, compared to L21 rootstock, the L14 as rootstock showed the increases of 12.8 and 60.8% in RBSM under well-watered and water-stress conditions, respectively. In contrast, with the same rootstock genotype, insignificant difference in RBSM was observed between scions under either well-watered or water-stress conditions.

The LRWC also decreased significantly by water stress (Fig. 1*B*). The reduction of LRWC followed the order L14/L14 (14%) ≤ L21/L14 (15%) < L21/L21 (24%) ≤ L14/L21 (25%). With the same scion genotype, compared to L21 rootstock, the L14 as rootstock showed insignificant change in LRWC under well-watered condition, but the average increase of 16% under water-stress conditions. In contrast, with the same rootstock genotype, insignificant difference in LRWC was observed between scions under either well-watered or water-stress conditions.

Gas-exchange parameters: Water stress caused the decrease in P_N of leaves (Fig. 2*A*). The reduction of P_N followed the order L14/L14 (13%) < L14/L21 (22%) < L21/L14 (22%) < L21/L21 (29%). With the same scion genotype, compared to L21 rootstock, the L14 as rootstock showed insignificant change in P_N under well-watered conditions, but average increase of 12% under water-stress conditions. However, the L21 scion had always lower P_N than that of the L14 scion under both well-watered and water-stress conditions, even when grafted onto the L14 rootstock. Compared to the L21 scion, the L14 as scion showed a significant increase of 10% in P_N under well-watered conditions and of 22% under water-stress conditions, while grafted onto the same rootstock genotype.

Under the water stress imposed, the reduction of g_s followed the order L21/L14 (30%) < L14/L14 (40%) < L21/L21 (45%) < L14/L21 (54%) (Fig. 2*B*). With the same
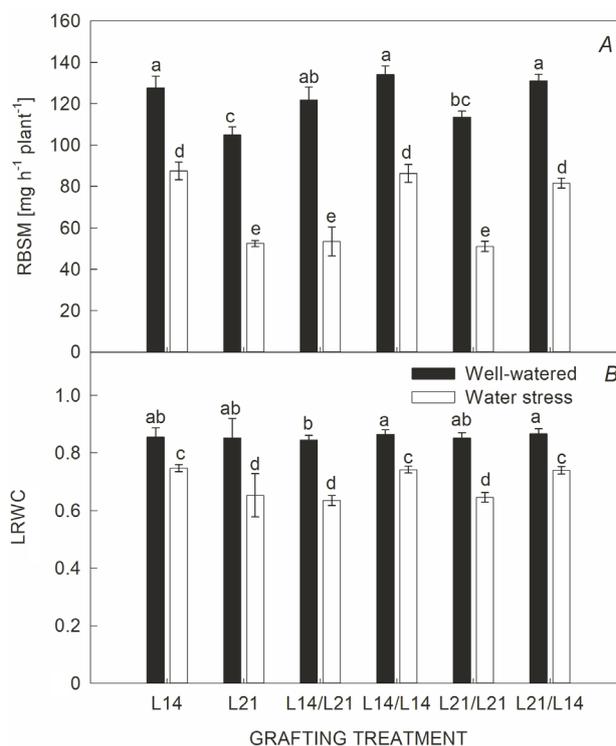

Fig. 1. Influence of water stress on root bleeding sap mass (RBSM) (*A*) and leaf relative water content (LRWC) (*B*) in different grafted soybean plants. Values are means ± SD (*n* = 12). *Different letters* following the mean values indicate significant differences between genotype × treatment at *P*<0.05 by *LSD* test.
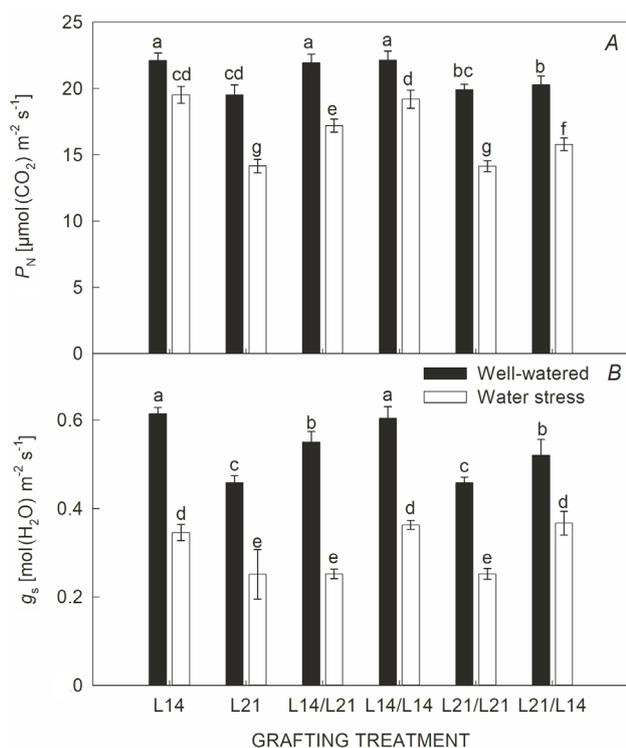
Fig. 2. Influence of water stress on the net photosynthetic rate (P_N) (*A*) and stomatal conductance (g_s) (*B*) in different grafted soybean plants. Values are mean ± SD (*n* = 12). *Different letters* following the mean values indicate significant differences between genotype × treatment at *P*<0.05 by *LSD* test.

scion genotype, the L14 as rootstock had a lower stomatal sensitivity than that of the L21 rootstock in response to water stress, as apparent from the lesser reduction in g_s. Compared to L21 rootstock, the L14 as rootstock exhibited the increase of 12 and 45% in g_s under well-watered and water-stress conditions, respectively. In contrast, with the same rootstock genotype, the L14 scion showed the higher stomatal sensitivity than that of the L21 scion in response to water stress, as evidenced by the larger reduction in g_s. Compared to L21 scion, the L14 as scion showed 18% higher g_s under well-watered condition, but the insignificant change under water-stress conditions.

Carboxylation efficiency: The P_N/C_i curves were fitted to calculate the CE under well-watered and water-stress conditions (Fig. 3, Table 1S). The P_N decreased by water stress, especially under high C_i conditions, resulting from a reduction in CE. The reduction of CE followed the order: L14/L14 (2%) < L14/L21 (10%) < L21/L14 (16%) < L21/L21 (22%). Rubisco enzyme activity assay showed the similar order: L14/L14 < L14/L21 < L21/L14 < L21/L21 for the reduction of initial Rubisco activity, total Rubisco activity, and Rubisco activation state by the water stress (Table 1). With the same scion genotype, compared to L21 rootstock, the L14 as rootstock exhibited the increase in initial Rubisco activity and Rubisco activation state by 11 and 10%, respectively, under well-watered conditions, while by 12 and 7%, respectively, under water-stress

conditions. In contrast, with the same rootstock genotype, compared to L21 scion, the L14 as scion increased its initial Rubisco activity, total Rubisco activity, and Rubisco activation state by 26, 7, and 18%, respectively, under well-watered conditions, while by 51, 8, and 40%, respectively, under water-stress conditions.

PSII of different grafts: Water stress increased the NPQ, but decreased the $Y_{(II)}$, q_P, and ETR (Table 2). The increase of NPQ followed the order: L21/L14 (4%) < L21/L21 (5%) < L14/L14 (12%) < L14/L21 (13%); the reduction of $Y_{(II)}$, q_P, and ETR followed the order: L14/L14 < L14/L21 < L21/L14 < L21/L21. With the same scion genotype, compared to L21 rootstock, the L14 as rootstock showed insignificant changes in $Y_{(II)}$, NPQ, q_P, and ETR under well-watered conditions, but increased by 20, 15, and 20%, respectively, under water-stress conditions. In contrast, with the same rootstock genotype, compared to L21 scion, the L14 as scion increased $Y_{(II)}$, NPQ, q_P, and ETR by 14, 15, 15, and 11%, respectively, under well-watered conditions, while by 38, 25, 32, and 37%, respectively, under water stress conditions.

Discussion

Grafting has appeared to be a useful tool to quickly improve the drought resistance for modern vegetable production (Schwarz *et al.* 2010, Sánchez-Rodríguez *et al.* 2012, 2016; Kumar *et al.* 2017). In the present study, the reciprocal grafts of soybean cultivars differing in their water stress tolerance were used to determine the role of roots and shoots in photosynthetic responses to water stress, in order to provide the theoretical basis for drought-resistance breeding in soybean.

Roots are known to play an important role in plant tolerance to water deficit by controlling and adjusting the water supply to shoot transpiration demand (Marguerit *et al.* 2012). Many studies found a larger root system with greater mass at depth, larger xylem diameters or larger lateral root systems with more root hairs in drought-tolerant cultivars, which were more advantageous than drought-sensitive cultivars under drought conditions (Serraj *et al.* 1997, Fenta *et al.* 2014, Tanaka *et al.* 2014, Vadez 2014). The root architecture was not considered in this study because of the limited substrate volume in the pot-culture conditions. Some grafting experiments reported that the difference of root system abilities to maintain a leaf water status was mainly attributed to root hydraulic conductance but was not the effect of root mass (Sanders and Markhart 1992). The RBSM was used as a root vigor index in previous study to reflect a greater physiological function in L14 rootstock (Li *et al.* 2017b). In present study, the L14 rootstock maintained its larger RBSM in the graft combination with L21 scion that normally confers lower RBSM, which resulted in higher LRWC under water stress. Interestingly, there was a lack of influence of the scion genotype on RBSM and LRWC, indicating that the roots rather than the shoots of L14 conferred the greater water absorption capacity and better water status under water stress.
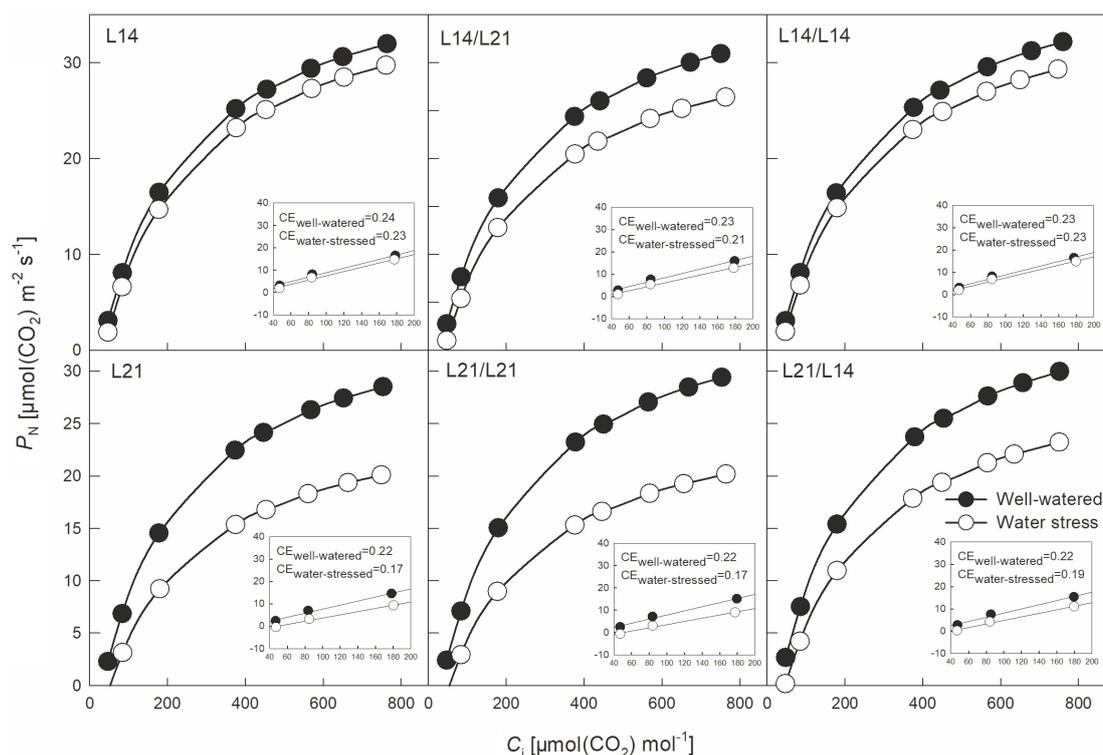
Fig. 3. Influence of water stress on P_N/C_i response curves in different grafted soybean plants ($n = 12$). C_i – intercellular CO_2 concentration; P_N – net photosynthetic rate.

Table 1. Influence of water stress on ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity in different grafted soybean plants. Values are means ± SD ($n = 12$). *Different letters* following the mean values indicate significant differences between genotype × treatment at $P<0.05$ by *LSD* test.

Genotype	Treatment	Rubisco activity [nmol(3-PGA) g⁻¹(FM) min⁻¹] Initial	Total	Rubisco activation state
L14	Well-watered	3,646.17 ± 155.6[ab]	4,325.11 ± 91.85[a]	0.84 ± 0.05[ab]
	Water stress	3,124.99 ± 137.41[c]	4,172.89 ± 87.20[b]	0.75 ± 0.04[c]
L21	Well-watered	2,725.65 ± 183.09[e]	3,934.59 ± 91.66[de]	0.69 ± 0.06[d]
	Water stress	1,847.76 ± 62.77[f]	3,451.38 ± 94.39[g]	0.54 ± 0.03[e]
L14/L21	Well-watered	3,554.34 ± 187.42[b]	4,239.40 ± 148.81[ab]	0.84 ± 0.06[ab]
	Water stress	2,777.46 ± 111.60[d]	3,801.19 ± 96.73[e]	0.73 ± 0.03[cd]
L14/L14	Well-watered	3,864.04 ± 145.73[a]	4,373.11 ± 123.09[a]	0.88 ± 0.05[a]
	Water stress	3,064.82 ± 149.79[c]	3,889.22 ± 108.68[de]	0.79 ± 0.05[bc]
L21/L21	Well-watered	2,754.12 ± 91.43[e]	3,966.73 ± 79.62[cd]	0.69 ± 0.03[d]
	Water stress	1,818.54 ± 91.58[f]	3,469.09 ± 113.10[g]	0.52 ± 0.04[e]
L21/L14	Well-watered	3,138.76 ± 138.95[c]	4,114.11 ± 91.55[bc]	0.76 ± 0.04[c]
	Water stress	2,048.78 ± 133.20[f]	3,658.29 ± 79.51[f]	0.56 ± 0.04[e]

The water status of plants under water deficit had a significant relationship with photosynthetic capacity (Mohsenzadeh *et al.* 2006, Rampino *et al.* 2006). Rootstock-induced increase in P_N under water stress was found in interspecific grafts (Nilsen *et al.* 2014, Liu *et al.* 2016). Grafting modern cultivars onto the L14 rootstock resulted in an increased P_N under well-watered conditions in our previous study (Li *et al.* 2017b). In present study, the P_N was increased by grafting onto the L14 rootstock under water stress, which was attributed to not only stomatal but also to nonstomatal factors. On the one hand, the P_N increased along with g_s, while the leaf water status under drought was improved by grafting on L14 rootstock. On the other hand, P_N/C_i curve and Rubisco enzyme activity assay in this study showed an increased CO_2 assimilation efficiency by grafting on the L14 rootstock under water stress.

Table 2. Influence of water stress on chlorophyll fluorescence parameters in different grafted soybean plants. Values are means ± SD (n = 12). *Different letters* following the mean values indicate significant differences between genotype × treatment at $P<0.05$ by *LSD* test. ETR – electron transport rate; NPQ – nonphotochemical quenching; q_P – photochemical quenching; $Y_{(II)}$ – the actual photochemical efficiency of PSII.

Genotype	Treatment	$Y_{(II)}$	NPQ	q_P	ETR
L14	Well-watered	0.25 ± 0.01^b	1.79 ± 0.10^d	0.43 ± 0.02^b	85.75 ± 3.69^b
	Water stress	0.19 ± 0.02^f	1.99 ± 0.10^{cd}	0.37 ± 0.03^e	68.06 ± 6.37^f
L21	Well-watered	0.21 ± 0.02^b	1.53 ± 0.05^{cd}	0.38 ± 0.05^b	78.15 ± 8.22^b
	Water stress	0.12 ± 0.02^e	1.60 ± 0.06^{cd}	0.24 ± 0.02^d	41.14 ± 3.20^e
L14/L21	Well-watered	0.25 ± 0.02^a	1.75 ± 0.07^b	0.42 ± 0.01^a	87.14 ± 6.87^a
	Water stress	0.17 ± 0.02^c	1.98 ± 0.08^a	0.32 ± 0.02^b	58.93 ± 6.34^c
L14/L14	Well-watered	0.25 ± 0.01^b	1.78 ± 0.09^{cd}	0.43 ± 0.02^b	88.08 ± 3.43^b
	Water stress	0.19 ± 0.01^f	1.99 ± 0.19^c	0.37 ± 0.02^e	67.98 ± 6.14^f
L21/L21	Well-watered	0.22 ± 0.02^a	1.52 ± 0.10^b	0.36 ± 0.04^a	77.00 ± 7.55^a
	Water stress	0.12 ± 0.02^d	1.59 ± 0.08^a	0.24 ± 0.01^c	41.23 ± 3.67^d
L21/L14	Well-watered	0.23 ± 0.01^a	1.54 ± 0.06^b	0.38 ± 0.05^a	80.43 ± 6.18^a
	Water stress	0.15 ± 0.03^c	1.59 ± 0.08^a	0.28 ± 0.01^b	51.73 ± 2.54^c

The L21 scion still had lower P_N than that of L14 scion, even if grafted onto the L14 rootstock. The greater P_N in the L14 scion was not due to the increased LRWC under well-watered or water-stress conditions, as evidenced by insignificant difference in LRWC between L14 scion and L21 scion grafted onto the consistent rootstock genotypes. In addition, there was the significant difference in g_s between L14 scion and L21 scion under well-watered rather than under water-stress conditions. The pathways of water loss and CO_2 absorption are diverse within the leaf, and their fluxes suffer from different restrictions, even though they are linked by the stomata (Egilla *et al.* 2005). The two processes could not be affected to the same degree by the stomatal closure. Soil drying induced the stomatal closure through root abscisic acid signal in order to reduce the water loss (Liu *et al.* 2003). Under water stress imposed, the leaves of L14 scion with higher stomatal sensitivity showed the larger reduction in g_s but minor reduction in P_N than those of L21 scion with their lower stomatal sensitivity. These suggested that the higher P_N in L14 scion seemed to be due to lower nonstomatal limitations.

There was a significant difference in nonstomatal factors between L14 scion and L21 scion under water stress, when they were grafted onto the same rootstock. The P_N/C_i curves showed higher CE and P_{max} in the L14 scion than that of L21 scion under water stress. Rubisco, a key enzyme of the Calvin Benson cycle, catalyzes the CO_2 assimilation by the carboxylation of ribulose-1,5-bisphosphate (Spreitzer and Salvucci 2002). The reduced initial Rubisco activity during water stress is not mediated by decreased relative water content, but somehow related to g_s (Flexas *et al.* 2006). Rootstock-induced increase of both CE and initial Rubisco activity was on account of the increased g_s under water stress. However, the L14 scion had higher CE, initial Rubisco activity, and Rubisco activation

state than those of the L21 scion under water stress, when they onto the consistent rootstock genotypes had a lack of difference in g_s. Previous study found the expression levels of *GmRCAβ*, encoding Rubisco activase in soybean, determined the initial Rubisco activity (Yin *et al.* 2010). The expression levels of *GmRCAβ* were regulated by the allelic variation in its promoter regions (Chao *et al.* 2014). Our results suggested that the differences in CO_2 assimilation efficiency between L14 scion and L21 scion were probably caused by the genotypic variation.

In addition, the Chl fluorescence measurements were also used to reveal the characteristic of PSII in differently grafted plants under water stress. The parameters of $Y_{(II)}$, q_P, and ETR are correlated with photochemical processes (Yang *et al.* 2014), while NPQ reflects the amount of redundant energy from photosynthetic electron transport that was dissipated innocuously as heat energy from PSII antennae (Veres *et al.* 2006). Previous studies found reductions of $Y_{(II)}$, q_P, and ETR, as well as an increase of NPQ in plants in response to water stress (Zlatev *et al.* 2004, Li *et al.* 2006, Brestič and Živčák 2013). Similar results were found in the study and showed that the effect of L14 rootstock or scion enhanced the photochemical efficiency and dissipation of unused light energy under water deficit, resulting in an active PSII and consequently in an increased photosynthetic capability.

Conclusions: The drought-tolerant cultivar as the rootstock could maintain greater water absorption capacity under water stress conditions, resulting in more favourable leaf water status and consequently in stronger photosynthetic capacity *via* both stomatal and nonstomatal factors. Although the photosynthetic capacity of the drought-sensitive scion cultivar was improved by grafting onto drought-tolerant cultivar rootstock, it was still lower than that of the drought-tolerant cultivar scion, which was

mainly due to the difference between scions in nonstomatal factors. The drought-tolerant cultivar as the scion conferred larger stomatal sensitivity, CO_2 assimilation efficiency, and PSII activity. These indicated that not only the greater water absorption capacity *via* roots, but also the physiological property of leaves conferred to higher photosynthetic capacity and drought resistance in soybean. We suggested that the coordinating improvement between roots and shoots could enhance the photosynthesis, which would be ideally considered for improving drought resistance in soybean.

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