Photosynthesis of soybean cultivars released in different decades after grafting onto record-yield cultivars as rootstocks

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Abstract

While photosynthesis of soybean has been enhanced by breeding, it remains to be clarified whether the improvement of root function could bring a further increase of photosynthetic capacity for the development of soybean cultivars. The objective of this grafting experiment was to determine the influence of record-yield soybean cultivars, Liaodou14 (L14) and Zhonghuang35 (Z35), as rootstocks on photosynthetic traits of cultivars released in different decades. Grafting of various soybean cultivars onto L14 or Z35 rootstocks showed a higher root physiological activity, which resulted in significant increases in some photosynthetic traits at the late grain-filling stage compared with the non-grafted and self-grafted plants. The genetic gain for some photosynthetic traits of cultivars released from 1966 to 2006 increased by using L14 and Z35 as rootstocks. It suggested that the photosynthetic traits of the recently released cultivars could increase more if their root functions are improved.

Additional key words: chlorophyll fluorescence; gas exchange; Rubisco.

Introduction

Grain yield of soybean (*Glycine max* L.) have been significantly improved during several decades by breeding. There was a positive correlation between the photosynthetic rate and yield in soybean cultivars (Wells *et al.* 1982, Boerma and Ashley 1988, Morrison *et al.* 1999), which suggested that improvement of photosynthetic capacity might be a promising target for further yield gains (Ainsworth *et al.* 2012). In Canada, the photosynthetic rate of short-season soybean cultivars was found to have increased by approximately 0.5% per year, which was synchronous with their higher yield (Morrison *et al.* 1999). In Northeast China, Jin *et al.* (2010) also reported that the photosynthetic rate of soybean cultivars increased by 0.59% per year. Thus, the genetic improvement for the yield and agronomic traits resulted in the increase of photosynthetic capacity (Wells *et al.* 1982, Morrison *et al.* 1999, Liu *et al.* 2012, Koester *et al.* 2014, Keep *et al.* 2016).

However, Koester *et al.* (2016) reported that more recently released cultivars have not changed their photosynthetic capacity compared with the older cultivars, except under adequate soil moisture conditions. This implied that the expression of photosynthetic capacity of modern cultivars showed a greater reliance on adequate soil moisture conditions than that of older cultivars. Because roots mediate uptake of water and ions, it seemed that their functions *per se* also may be a limiting factor of further increasing photosynthetic capacity. The growth and development of soybean result from an interaction of leaf photosynthesis and root absorption of water and nutrients (Somerville and Briscoe 2001). Cui *et al.* (2016) showed the positive correlation between the photosynthetic capacity and root growth vigor of soybean cultivars released in different years. As it is difficult to observe and measure roots *in situ*, breeders have seldom considered selection for root traits during the improvement

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Abbreviations: D/D – non-grafted and self-grafted cultivars released in different decades; D/L14 – the cultivars released in different decades grafted onto Liaodou14 rootstocks; D/R – the cultivars released in different decades grafted onto record-yield cultivars rootstocks; D/Z35 – the cultivars released in different decades grafted onto Zhonghuang35 rootstocks; *E* – transpiration rate; ETR – electron transport rate; EGFS – early grain-filling stage; FM – fresh mass; FS – flowering stage; *g_s* – stomatal conductance; LGFS – late grain-filling stage; *P_N* – net photosynthetic rate; *q_p* – photochemical quenching coefficient; R1 – beginning of flowering stage; R5 – beginning of seed stage; Φ_{PSII} – actual photochemical efficiency of PSII.

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of soybean. Thus, it remained to be clarified whether the genetic improvement for root functions could bring the further increase of photosynthetic capacity and yield.

For this study, it was critical to improve the root function while ensuring a consistent shoot genotype. Grafting, which combines scions and rootstocks of different phenotypes, could be helpful. Grafting has been widely used in plant research among many plant species including soybean (Cardwell and Poison 1972, Pantalone *et al.* 1999, Ookawa *et al.* 2001). Soybean cultivars Liaodou14 (L14) and Zhonghuang35 (Z35) had produced high yields of 4, 908 and 6, 089 kg ha⁻¹, respectively (Song *et al.* 2001, Jin and Wang 2014), and therefore they are known as the record-yield cultivars in China. Their roots appeared to be more physiologically active than those of

common cultivars at the grain-filling stage (Zhang *et al.* 2013b). In our grafting experiment, L14 and Z35 were used as the rootstocks in order to increase the root function.

Moreover, a large number of cultivars were used in most studies to evaluate the improvement of some agronomic traits, but the pedigree of the cultivars was not considered. In this study, a set of somewhat related cultivars, released in different decades from Liaoning, China, and Ohio, USA, and their common ancestors, were used to eliminate the interference of complex pedigrees among cultivars. The scions of these cultivars were grafted onto L14 and Z35 rootstocks in order to determine the effect of root function improvement on the photosynthetic traits of cultivars released in different decades and to provide the strategy for future breeding.

Materials and methods

Plant material and grafting procedure: A total of 11 cultivars, released in different decades from Liaoning (38.55°–42.32°N), China, and Ohio (38.45°–41.22°N), USA, and their common ancestors Amsoy and Williams (*i.e.* excellent ancestors in USA and China, Gizlice *et al.* 1994, Cui *et al.* 2002), were collected (Fig. 1S, *supplement available online*). These cultivars were classified into five groups according to years of their release and origins (*see* the text table below). Scions of the 11 cultivars released in different decades were grafted onto rootstocks of record-yield cultivar L14 (D/L14) or Z35 (D/Z35), which were

collectively represented as D/R. Non-grafted and self-grafted plants of the 11 cultivars released in different decades were used as controls, which were collectively represented as D/D. Selected soybean seeds were planted in plastic pots (12 cm × 12 cm × 12 cm) with soil on 7 May 2014 and 4 May 2015. Grafts were initiated 10 d after planting as described by Pantalone *et al.* (1999). Immediately after grafting, grafted and non-grafted plants were placed at a greenhouse under 23–28°C and approx. 90% of relative humidity with light intensity of approx. 360 μmol(photon) m⁻² s⁻¹ (~70% shade).

Cultivar name, year of release, and origin of soybean cultivars tested in this study. L57-0034 is a selection from Clark × Adams; L24 is a Williams backcross population with the *Rps1-k* gene for multi race *Pmg* resistance and is closely related to Williams 82; HS88-7363 is from Voris 311 × Resnik; HS88-4988 is from S1492 × Asgrow A3127; Xin3511 is a variant strain from Liaodou3; HS94-4533 is from the same F₂ plant as Kottman.

Group	Cultivar	Growth habit	Year of release	Pedigree	Origin
Common parents	Amsoy	Indeterminate	1966	Adams × Harosoy	Iowa State Univ., USA
	Williams	Indeterminate	1971	Wayne × L57-0034	USDA, Univ. of Illinois., USA
Liaoning middle	Liaodou3	Semi-determinate	1983	Tiefeng18 × Amsoy	Liaoning Academy of Agric.Sci., CN
	Liaodou10	Semi-determinate	1991	Liaodou3 × Liao82-5185	Liaoning Academy of Agric.Sci., CN
Ohio middle	Resnik	Indeterminate	1987	A3127 × L24	Ohio State Univ., USA
	Kottman	Indeterminate	1991	HS88-7363 × HS88-4988	Ohio State Univ., USA
Liaoning current	Liaodou11	Semi-determinate	1996	Liao84063 × Liaodou3	Liaoning Academy of Agric.Sci.,CN
	Liaodou12	Semi-determinate	2001	Liao85094 × Liaodou10	Liaoning Academy of Agric.Sci., CN
	Tiefeng31	Semi-determinate	2001	Xin3511 × Resnik	Tieling Academy of Agric.Sci., CN
Ohio current	Dilworth	Indeterminate	2002	Chapman × Probst	Ohio State Univ., USA
	Dennison	Indeterminate	2006	Athow × HS94-4533	Ohio State Univ., USA
Record-yield	Liaodou14	Semi-determinate	2003	Liaodou10 × Mecury	Liaoning Academy of Agric.Sci., CN
	Zhonghuang35	Determinate	2006	(PI486355 × Zheng8431) × Zheng6062	Chinese Academy of Agric.Sci., CN

The pot-culture experiments were carried out under open-field conditions in 2014 and 2015 at Shenyang Agricultural University (41°82'N, 123°57'E), Liaoning

Province, China. The surviving grafts (survival rate ≥ 90%) and non-grafts were transplanted into pots (25 cm × 30 cm × 25 cm, 12.5 kg of soil). Chemical characteristics

of soil were: 16.87 g(soil organic matter) kg⁻¹, 0.79 g(total nitrogen) kg⁻¹, 0.07 g(available nitrogen) kg⁻¹, 0.02 g(available phosphorus) kg⁻¹, 0.14 g(available potassium) kg⁻¹, and pH of 7.33. Using the drip irrigation, the moisture content in the soil was maintained at ~70% of the field water-holding capacity that could prevent the plants from drought stress. Each pot contained two plants of the same treatment and was considered as one experimental unit. A randomized complete block design with three replications per treatment was used.

Measurements of traits: As all cultivars used in the grafting experiments were of a maturity group III, both the grafted and non-grafted plants had a similar growth and development. The upper third leaf (the third fully unfolded leaf from the top of main stem) of each plant was used to measure the gas-exchange parameters and relative chlorophyll (Chl) content at the flowering (about 5 d after onset of flowering, R1), early grain-filling (about 5 d after onset of seed stage, R5) and the late grain-filling (about 25 d after R5) stages. The gas-exchange parameters were measured by the portable photosynthesis system (LI-6400, Li-Cor Inc., Lincoln NE, USA). The light intensity was set at 1,200 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$, which was the light-saturation point for soybean by the measurement of light-response curves. The leaf temperature was kept at 25–30°C, relative humidity was 60–65%, CO₂ concentration of 380 $\mu\text{mol}(\text{CO}_2) \text{mol}^{-1}$, and air flow of 500 $\mu\text{mol} \text{s}^{-1}$. The relative Chl content (leaf greenness) was measured by SPAD-502 leaf Chl meter (Minolta Camera Co., Osaka, Japan).

At the late grain-filling stage, the Chl fluorescence parameters were also measured by the fluorescence monitoring system (FMS-2, Hansatech, Kings Lynn, UK). Following 30-min-dark adaptation for leaves, the minimum fluorescence yield was measured, and the maximum fluorescence yield was obtained using a saturating pulse [3,000 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ during 0.7 s]. Then the actinic white light [1,200 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$] was switched on for 5 min, and a saturating pulse was applied to determine the actual photochemical efficiency of PSII (Φ_{PSII}) and electron transport rate (ETR). During a brief interruption (5 s) of actinic illumination in the presence of 6 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ of far-red (730 nm) light, the photochemical quenching coefficient (q_p) was determined. After the determination of these traits, the upper third leaves were collected into liquid nitrogen immediately and stored at -80°C until further use. All measurements and sampling were made in the morning (9:00–11:30 a.m.) on a sunny day. Gas exchange parameters and relative Chl content measured on six plants from three pots ($n = 6$), the other measurements were performed on four plants from two pots ($n = 4$).

Leaf samples of approx. 0.5 g fresh mass (FM) were extracted by vigorous shaking with 2 mL of enzyme

extraction buffer. The composition of the extraction buffer was 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 MgCl₂, 1 mM PMSF, and 0.5 mM DTT. The initial and total activity of Rubisco (EC 4.1.1.39) was determined with three replications per sample at 25°C by microplate reader (Varioskan Flash, Thermo Fisher Scientific, Inc., Waltham, MA, USA) using a rapid, nonradioactive microplate-based method (Sulpice *et al.* 2007). Enzyme-linked immune-sorbent assay (Plant Rubisco ELISA Kit, Shanghai BOYE Biology Science and Technology Co. Ltd., Shanghai, China) was used to measure the Rubisco content with three replications per sample.

The total RNA extraction kit (TaKaRa Bio Inc., Shiga, Japan) was used to isolate the total RNA and synthesize the first-strand cDNA. Expression levels of *GmRCA α* and *GmRCA β* , two genes encoding the Rubisco activase in soybean (Yin *et al.* 2010), were determined with two replications per sample using real-time PCR system (CFX96™, BIO-RAD, Munich, Germany). The primers of *GmRCA α* , *GmRCA β* and endogenous reference gene tubulin (GenBank accession number AY907703.1) were referred by Yin *et al.* (2010). Relative expression levels were calculated by 2^{- $\Delta\Delta\text{CT}$} (Livak and Schmittgen 2001).

To reveal the growth vigor of L14 and Z35 rootstocks, the root bleeding sap volume and root activity were also measured at the flowering, early grain-filling, and the late grain-filling stages in three pots. To collect the root bleeding sap samples, the shoots were cut just under the cotyledonous node of each plant from each pot with a very sharp cutter as described by Peoples *et al.* (1989). After the roots from a pot were washed thoroughly with water, the tips of roots were cut off to measure the root activity by triphenyl tetrazolium chloride (TTC) method (Wang *et al.* 2006).

Seeds of plants were sampled at the late grain-filling and maturity stage to determine the fraction of grain filling. The grain dry mass (DM) per plant was measured after oven drying at 85°C for 72 h.

Statistical analysis: The value of each plant was considered as a replicate for all photosynthetic traits in the statistical analysis by SPSS-17.0 (SPSS Inc., Chicago, USA). The data were subjected to an analysis of variance (ANOVA) in a general linear model (GLM) with the grafting treatment, genotype, and grafting treatment $\times$ genotype as fixed effects. Years, along with the grafting treatment $\times$ year, genotype $\times$ year, and grafting treatment $\times$ genotype $\times$ year were regarded as a random factor. Means were subjected to the least significant difference (LSD) test at the $P < 0.05$ level. Pearson's product moment correlations between parameters were determined. Traits were regressed over year of release to evaluate change over time for each of the different grafting treatments.

Results

Effect of grafting treatments: The difference of root vigor and photosynthetic traits between the grafting treatments was compared at the flowering, early grain-filling, and late grain-filling stage (Tables 1, 2, 3). All traits showed no significant difference between the non-grafted and self-grafted plants. Rootstocks of L14 or Z35 showed a significantly higher bleeding sap mass and root activity at the late grain-filling stage than those of other cultivars (Table 1). The cultivars released in different decades grafted onto the L14 or Z35 rootstocks showed no difference in gas-exchange parameters and leaf greenness at the flowering and early grain-filling stage, but averaged significantly the higher photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and SPAD at the late grain-filling stage than the means of their non-grafted and self-grafted plants (Table 2). At the late grain-filling

stage, on average, the cultivars released in different decades grafted onto the L14 or Z35 rootstocks also showed significantly higher Φ_{PSII} , ETR, Rubisco initial activities, Rubisco total activities, Rubisco contents, and *GmRCA* expression levels, as compared with their non-grafts and self-grafts (Table 3). At late grain-filling stage, the fraction of grain filling was about 66–79% of the final grain mass (Table 1S, *supplement available online*).

Genetic gain of cultivars after different grafting treatments: Most photosynthetic traits at the late grain-filling stage had significantly positive relationships with the year of cultivar release (Table 4). The linear regression equations of these traits on the year of release under different grafting treatments were shown in Figs. 1, 2, 3. From 1966 to 2006, the average annual increase rates of

Table 1. Root bleeding sap mass and root activity evaluated at different growth stages for four grafting treatments. FS – flowering stage, EGFS – early grain-filling stage, LGFS – late grain-filling stage. D/L14 or D/Z35 – the cultivars released in different decades grafted onto L14 or Z35 rootstocks, respectively. *, **, *** – significant at the 0.05, 0.01, and 0.001 probability level, respectively. NS – not significant.

Treatment	Bleeding sap mass			Root activity		
	FS	EGFS	LGFS	FS	EGFS	LGFS
	[mg h ⁻¹ plant ⁻¹]			[μg(TTF) g ⁻¹ (FM) h ⁻¹]		
Non-graft	142	100	79	97	105	82
Self-graft	143	97	77	99	106	84
D/L14	141	122	112	103	116	103
D/Z35	143	118	102	103	114	96
<i>LSD</i> _{0.05}	8	7	5	5	12	9
<i>LSD</i> _{0.01}	14	13	9	9	22	17
<i>ANOVA</i>						
<i>F</i> values						
Treatment (df = 3)	0.3 ^{NS}	63.3 ^{**}	242.4 ^{***}	6.8 ^{NS}	4.3 ^{NS}	23.0 [*]
Genotype (df = 10)	8.1 ^{***}	51.7 ^{***}	20.1 ^{***}	14.9 ^{***}	95.1 ^{***}	44.3 ^{***}
T × G (df = 30)	0.3 ^{NS}	1.0 ^{***}	2.3 [*]	0.4 ^{NS}	1.1 [*]	4.9 ^{***}

Table 2. Gas-exchange parameters and leaf greenness evaluated at different growth stages for four grafting treatments. FS – flowering stage, EGFS – early grain-filling stage, LGFS – late grain-filling stage. D/L14 or D/Z35 – the cultivars released in different decades grafted onto L14 or Z35 rootstocks, respectively. *, **, *** – significant at the 0.05, 0.01, and 0.001 probability level, respectively. NS – not significant. E – transpiration rate; g_s – stomatal conductance; P_N – net photosynthetic rate.

Treatment	P_N [μmol(CO ₂) m ⁻² s ⁻¹]			g_s [mol(H ₂ O) m ⁻² s ⁻¹]			E [mmol(H ₂ O) m ⁻² s ⁻¹]			Leaf greenness [SPAD units]		
	FS	EGFS	LGFS	FS	EGFS	LGFS	FS	EGFS	LGFS	FS	EGFS	LGFS
Non-graft	24.25	17.45	13.69	1.21	0.62	0.19	10.17	7.84	3.94	40.16	41.46	35.47
Self-graft	24.29	17.46	13.79	1.21	0.62	0.19	10.23	7.93	3.93	40.20	41.33	34.63
D/L14	24.25	19.24	16.19	1.22	0.69	0.31	10.53	8.88	5.85	43.83	44.74	41.25
D/Z35	24.26	18.77	15.62	1.22	0.68	0.21	10.43	8.80	5.40	43.67	45.24	38.54
<i>LSD</i> _{0.05}	0.11	1.74	1.45	0.03	0.04	0.04	0.60	1.09	1.18	3.57	3.76	2.26
<i>LSD</i> _{0.01}	0.20	3.20	2.66	0.05	0.07	0.07	1.10	2.00	2.18	6.56	6.90	4.15
<i>ANOVA</i>												
<i>F</i> values												
Treatment (df = 3)	0.56 ^{NS}	5.62 ^{NS}	15.72 [*]	1.77 ^{NS}	22.81 [*]	42.46 ^{**}	1.30 ^{NS}	5.19 ^{NS}	14.44 [*]	6.76 ^{NS}	6.24 ^{NS}	36.41 ^{**}
Genotype (df = 10)	5.57 ^{**}	8.61 ^{***}	22.30 ^{***}	17.68 ^{***}	283.43 ^{***}	11.16 ^{***}	6.54 ^{**}	14.09 ^{***}	2.36 ^{NS}	10.42 ^{***}	14.06 ^{***}	53.29 ^{***}
T × G (df = 30)	1.71 ^{NS}	5.40 ^{NS}	6.21 ^{***}	0.35 ^{NS}	2.29 ^{NS}	1.59 ^{NS}	0.01 ^{NS}	0.10 ^{NS}	3.26 ^{***}	1.02 ^{NS}	1.58 ^{NS}	2.40 [*]

Table 3. Chlorophyll fluorescence parameters and Rubisco evaluated at late grain-filling stages for four grafting treatments. qp – photochemical quenching coefficient; Φ_{PSII} – actual photochemical efficiency of PSII; ETR – electron transport rate. The normalized expression level for each cultivar was calculated as $\Delta\Delta C_T = (C_{T, Target} - C_{T, tubulin})_{treatment} - (C_{T, Target} - C_{T, tubulin})_{calibrator}$, which the cDNA from the treatment of self-grafted was used as calibrator on each RT-PCR plate. ‘D/L14’ or ‘D/Z35’ – the cultivars released in different decades grafted onto L14 or Z35 rootstocks, respectively. *, **, *** – significant at the 0.05, 0.01, and 0.001 probability level, respectively. NS – not significant.

Treatment	qp	Φ_{PSII}	ETR	Rubisco activity		Rubisco content [unit mg ⁻¹ (FM)]	Expression level	
				Initial [nmol(3-PGA) g ⁻¹ (FM) min ⁻¹]	Total		<i>GmRCAα</i>	<i>GmRCAβ</i>
Non-graft	0.80	0.51	36.79	2,513	3,072	6.06	1.28	1.33
Self-graft	0.80	0.52	39.22	2,736	3,326	6.59	1.00	1.00
D/L14	0.83	0.59	47.86	3,941	4,670	7.31	5.89	6.29
D/Z35	0.70	0.53	45.43	3,570	4,432	8.97	5.66	6.73
<i>LS</i> D _{0.05}	0.19	0.04	5.85	639	636	1.18	3.35	3.22
<i>LS</i> D _{0.01}	0.36	0.07	10.73	1,173	1,167	2.16	6.16	5.92
<i>ANOVA</i>								
<i>F</i> values								
Treatment (df = 3)	1.62 ^{NS}	15.71*	15.90*	22.68*	31.59**	23.54*	12.95*	18.66*
Genotype (df = 10)	2.54 ^{NS}	34.38***	43.39***	3.66*	3.38*	2.36 ^{NS}	4.80**	11.10***
T × G (df = 30)	0.73 ^{NS}	6.81***	4.95***	0.74 ^{NS}	1.05 ^{NS}	1.28 ^{NS}	3.38***	6.09***

Table 4. *Pearson's* correlation coefficients between photosynthetic traits at the late grain-filling stage and year of cultivars release. qp – photochemical quenching coefficient; Φ_{PSII} – actual photochemical efficiency of PSII; ETR – electron transport rate. The normalized expression level for each cultivar was calculated as $\Delta\Delta C_T = (C_{T, Target} - C_{T, tubulin})_{genotype} - (C_{T, Target} - C_{T, tubulin})_{calibrator}$, which the cDNA from the common parents (Williams and Amsoy) was used as calibrator on each RT-PCR plate. *, **, *** – significant at the 0.05, 0.01, and 0.001 probability level, respectively. NS – not significant. *E* – transpiration rate; *g_s* – stomatal conductance; *P_N* – net photosynthetic rate.

Trait	Year of release	Bleeding sap mass	Root activity
<i>P_N</i>	0.61***	0.84***	0.81***
<i>g_s</i>	0.45**	0.78***	0.66***
<i>E</i>	0.33*	0.75***	0.65***
Leaf greenness	0.23 ^{NS}	0.31*	0.24 ^{NS}
qp	0.22 ^{NS}	0.14 ^{NS}	0.15 ^{NS}
Φ_{PSII}	0.48***	0.47***	0.47***
ETR	0.43**	0.61***	0.56***
Rubisco initial activity	0.41**	0.77***	0.71***
Rubisco total activity	0.36*	0.73***	0.68***
Rubisco content	-0.21 ^{NS}	0.21 ^{NS}	0.13 ^{NS}
<i>GmRCAα</i>	0.46**	0.62***	0.67***
<i>GmRCAβ</i>	0.43**	0.61***	0.67***

P_N, *E*, Φ_{PSII} , ETR, and Rubisco total activities increased by the grafting onto record-yield cultivar rootstocks. For instance, the cultivars grafted onto record-yield cultivar rootstocks (D/R) had a higher annual increase rate of *P_N* (1.16% per year vs. 0.44% per year, Fig. 1A), *E* (1.25% per year vs. 0.26% per year, Fig. 1C), Φ_{PSII} (1.47% vs. 0.43% per year, Fig. 2B), ETR (1.54% per year vs. 0.56% per year, Fig. 2C), and Rubisco total activities (2.02% per year vs. 0.59% per year, Fig. 3B) than that of non-grafted and self-grafted plants (D/D).

Differential response of cultivars to rootstocks of record-yield cultivars: The plants of cultivars released in different decades showed a differential response to record-

yield cultivar rootstocks (Table 5). The plants of common parents and middle cultivars showed a lower responsiveness, with plants grafted onto record-yield cultivar rootstocks (D/R) having 5–10% higher *P_N*, 25–41% higher *g_s*, and 26–34% higher *E* than those of non-grafted and self-grafted plants (D/D), respectively. Conversely, the plants of current cultivars exhibited relatively higher responsiveness to record-yield cultivar rootstocks, with an increase of 23–26% in *P_N*, an increase of 42–46% in *g_s*, and an increase of 55–65% in *E* as compared with those of non-grafted and self-grafted plants (D/D), respectively. Furthermore, the plants of Liaoning current cultivars grafted onto L14 or Z35 rootstocks showed a larger increase in Φ_{PSII} and ETR at the

late grain-filling stage than other cultivars. The plants of Ohio current cultivars grafted onto L14 or Z35 rootstocks showed a larger increase in Rubisco initial activity,

Discussion

There was a significantly positive relationship between root physiological activity and photosynthetic traits in soybean (Zhang *et al.* 2013a, Cui *et al.* 2016). In a previous study, rootstocks of L14 or Z35 displayed greater root bleeding sap mass, root activity, nodule mass, nitrogenase activity, and amount of nitrogen fixed than common cultivars during the grain-filling stage (Zhang *et al.* 2013b). The present study showed that the L14 or Z35 as rootstocks could maintain a stronger root growth vigor in grafts combined with scions of different cultivars. Grafting

Rubisco total activity, and *GmRCA* expression levels at the late grain-filling stage than other cultivars.

Fig. 1. Regression of photosynthetic rate (A), stomatal conductance (B), and transpiration rate (C) on year of cultivar release with D/D and D/R treatment at late grain-filling stage. D/D – non-grafted and self-grafted cultivars released in different decades; D/R – cultivars released in different decades grafted onto record-yield cultivars rootstocks. The error bar indicates the least significant difference ($P < 0.05$) between treatment $\times$ genotype.

Fig. 2. Regression of leaf greenness (A), Φ_{PSII} (B), and ETR (C) on year of cultivar release with D/D and D/R treatment at late grain-filling stage. qp – photochemical quenching coefficient, Φ_{PSII} – actual photochemical efficiency of PSII; ETR – electron transport rate. D/D – non-grafted and self-grafted cultivars released in different decades; D/R – cultivars released in different decades grafted onto record-yield cultivars rootstocks. The error bar indicates the least significant difference ($P < 0.05$) between treatment $\times$ genotype.

of cultivars released in different decades onto L14 or Z35 rootstocks resulted in the improvement of some photosynthetic traits at the late grain-filling stage. In general, the photosynthetic capacity gradually declined with the leaf senescence after the onset of grain-filling stage. Leaf senescence in soybean reproductive stages is influenced not only by shoots but also by the physiological activity of root system (Garrison *et al.* 1984). The rootstocks of greater physiological activity could delay the leaf senescence (Ookawa *et al.* 2001). The gene expression of leaves could be regulated by some signal molecules synthesized in roots (Takei *et al.* 2002). Root physiological

Fig. 3. Regression of Rubisco initial activity (A) and Rubisco total activity (B) on year of cultivar release with D/D and D/R treatment at late grain-filling stage. D/D – non-grafted and self-grafted cultivars released in different decades; D/R – cultivars released in different decades grafted onto record-yield cultivars rootstocks. The error bar indicates the least significant difference ($P < 0.05$) between treatment $\times$ genotype.

status could affect photosynthesis *via* long-distance signaling. Better root function could also acquire more

water and nutrients from soil (Pantalone *et al.* 1999), which is beneficial to maintain the active photosynthetic carbon metabolism in leaf. They suggested that the root function improvement could increase the photosynthetic capacity at the grain-filling stage.

After more than 70 years of breeding, the photosynthetic capacity of modern Liaoning and Ohio cultivars were increased significantly compared to the old cultivars (Xie *et al.* 2010). It suggested that by selecting for the higher yield, higher photosynthetic capacity have been also selected during the soybean breeding. Some studies showed that the genetic gain of soybean cultivars released in different decades was changed under different environments (Rincker *et al.* 2014) and cultivations, such as the supply of nitrogen (Wilson *et al.* 2014), plant populations (Suhre *et al.* 2014), or planting date (Rowntree *et al.* 2013, 2014). The present study showed that the genetic gains of photosynthetic traits of cultivars could be also increased if their root functions *per se* were improved. The older cultivars showed a slight enhancement in photosynthetic traits with grafting onto record-yield cultivars rootstocks, which indicated that the scion but not roots restricted the further improvement of these traits in the older cultivars. However, the current cultivars expressed a higher responsiveness in their photosynthetic traits to the rootstocks of record-yield cultivars. Our results indicated that although the photosynthetic potentials of current cultivars largely increased during the genetic improvement, the roots function *per se* restricted the expression of their photosynthetic potential.

Table 5. Percentage increase of different cultivars for photosynthetic traits at the late grain-filling stage after grafting onto the rootstocks of record-yield cultivars. Increase [%] = $(D/R - D/D)/D/D \times 100\%$, D/D – the means of non-grafted and self-grafted of cultivars released in different decades, D/R – the means of cultivars released in different decades grafted onto L14 and Z35 rootstocks. qp – photochemical quenching coefficient; Φ_{PSII} – actual photochemical efficiency of PSII; ETR – electron transport rate. The normalized expression level for each cultivar was calculated as $\Delta\Delta C_T = (C_{T, Target} - C_{T, tubulin})_{treatment} - (C_{T, Target} - C_{T, tubulin})_{calibrator}$, which the cDNA from the treatment of self-grafted was used as calibrator on each RT-PCR plate. *, **, *** – significant at the 0.05, 0.01, and 0.001 probability level, respectively. *E* – transpiration rate; *g_s* – stomatal conductance; *P_N* – net photosynthetic rate.

Trait	Increase [%]				
	Common parents	Liaoning middle	Ohio middle	Liaoning current	Ohio current
<i>P_N</i>	5	8	10*	23***	26***
<i>g_s</i>	31	41*	25	46**	42**
<i>E</i>	26	27	34*	55**	65***
Leaf greenness	9	10*	19**	18***	12*
qp	-8	-3	-9	7	-12
Φ_{PSII}	-3	3	-11	31***	1
ETR	6	11	25*	42***	13
Rubisco initial activity	42	28	34	41	63*
Rubisco total activity	22	20	35	41	80**
Rubisco content	-15	-1	20	68	99*
Increase [fold]					
<i>GmRCAa</i>	3*	6***	4**	4**	7***
<i>GmRCAb</i>	3	5*	3	8***	7**

Conclusion: The rootstocks from the record-yield cultivars were used in the grafting experiment in order to evaluate the importance of root function improvement in soybean breeding. The rootstocks of the record-yield cultivars exhibited higher physiological activity at the late grain-filling stage than common cultivars, which could increase the photosynthetic capacity of soybean cultivars, particularly, of the current ones. Breeders have seldom

considered selection for root function, generally focusing selection on the grain yield, seed quality, and resistance of plants. The root function became a limiting factor of further increasing photosynthetic capacity. Thus, with the genetic improvement of agronomic and yield traits, breeders should pay more attention to the root function improvement in order to express the photosynthesis potential sufficiently.

References

- Ainsworth E.A., Yendrek C.R., Skoneczka J.A. *et al.*: Accelerating yield potential in soybean: potential targets for biotechnological improvement. – *Plant Cell Environ.* **35**: 38-52, 2012.
- Boerma H.R., Ashley D.A.: Canopy photosynthesis and seed-fill duration in recently developed soybean cultivars and selected plant introductions. – *Crop Sci.* **28**: 137-140, 1988.
- Cardwell V.B., Poison D.E.: Response of 'Chippewa 64' soybean scions to roots of different genotypes. – *Crop Sci.* **12**: 217-219, 1972.
- Cui X., Dong Y., Gi P. *et al.*: Relationship between root vigour, photosynthesis and biomass in soybean cultivars during 87 years of genetic improvement in the northern China. – *Photosynthetica* **54**: 81-86, 2016.
- Cui Z.L., Carter T.E., Burton J.W.: Genetic base of 651 Chinese soybean cultivars released during 1923 to 1995. – *Crop Sci.* **40**: 1470-1481, 2002.
- Garrison F.R., Brinker A.M., Noodén L.D.: Relative activities of xylem-supplied cytokinins in retarding soybean leaf senescence and sustaining pod development. – *Plant Cell Physiol.* **25**: 213-224, 1984.
- Gizlice Z., Carter T.E., Burton J.W.: Genetic base for North American public soybean cultivars released between 1947 and 1988. – *Crop Sci.* **34**: 1143-1151, 1994.
- Jin J., Liu X.B., Wang G.H. *et al.*: Agronomic and physiological contributions to the yield improvement of soybean cultivars released from 1950 to 2006 in Northeast China. – *Field Crop. Res.* **115**: 116-123, 2010.
- Jin X., Wang L.: Case studies of super yield in the soybean variety Tszhun Khuan 35. – *Selekt. Nasinnitsvo* **106**: 148-154, 2014.
- Keep N.R., Schapaugh W.T., Prasad P.V.V. *et al.*: Changes in physiological traits in soybean with breeding advancements. – *Crop Sci.* **56**: 122-131, 2016.
- Koester R.P., Skoneczka J.A., Cary T.R. *et al.*: Historical gains in soybean (*Glycine max* Merr.) seed yield are driven by linear increases in light interception, energy conversion, and partitioning efficiencies. – *J. Exp. Bot.* **65**: 3311-3321, 2014.
- Koester R.P., Nohl B.M., Diers B.W. *et al.*: Has photosynthetic capacity increased with 80 years of soybean breeding? An examination of historical soybean cultivars. – *Plant Cell Environ.* **39**: 1058-1067, 2016.
- Liu G., Yang C., Xu K. *et al.*: Development of yield and some photosynthetic characteristics during 82 years of genetic improvement of soybean genotypes in northeast China. – *Aust. J. Crop Sci.* **6**: 1416-1422, 2012.
- Livak K.J., Schmittgen T.D.: Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. – *Methods* **25**: 402-408, 2001.
- Morrison M.J., Voldeng H.D., Cober E.R.: Physiological changes from 58 years of genetic improvement of short-season soybean cultivars in Canada. – *Agron. J.* **91**: 685-689, 1999.
- Ookawa T., Nishiyama M., Takahiro J. *et al.*: Analysis of the factors causing differences in the leaf-senescence pattern between two soybean cultivars, Enrei and Tachinagaha. – *Plant Prod. Sci.* **4**: 3-8, 2001.
- Pantalone V.R., Rebetzke G.J., Burton J.W. *et al.*: Soybean PI 416937 root system contributes to biomass accumulation in reciprocal grafts. – *Agron. J.* **91**: 840-844, 1999.
- Peoples M.B., Faizah A.W., Rerkasem B. *et al.*: Methods for Evaluating Nitrogen Fixation by Nodulated Legumes in the Field. Pp. 76. ACIAR, Canberra 1989.
- Rincker K., Nelson R., Specht J. *et al.*: Genetic improvement of US soybean in maturity groups II, III, and IV. – *Crop Sci.* **54**: 1419-1432, 2014.
- Rowntree S.C., Suhre J.J., Weidenbenner N.H. *et al.*: Genetic gain $\times$ management interactions in soybean: I. Planting date. – *Crop Sci.* **53**: 1128-1138, 2013.
- Rowntree S.C., Suhre J.J., Weidenbenner N.H. *et al.*: Physiological and phenological responses of historical soybean cultivar releases to earlier planting. – *Crop Sci.* **54**: 804-816, 2014.
- Somerville C., Briscoe J.: Genetic engineering and water. – *Science* **292**: 2217, 2001.
- Song S.H., Wang W.B., Lu G.L. *et al.*: [Research on technology for super high yielding in spring soybean.] – *Chin. J. Oil Crop Sci.* **23**: 48-50, 2001. [In Chinese]
- Suhre J.J., Weidenbenner N.H., Rowntree S.C. *et al.*: Soybean yield partitioning changes revealed by genetic gain and seeding rate interactions. – *Agron. J.* **106**: 1631-1642, 2014.
- Sulpice R., Tschoep H., von Korff M. *et al.*: Description and applications of a rapid and sensitive non-radioactive microplate-based assay for maximum and initial activity of D-ribulose-1, 5-bisphosphate carboxylase/oxygenase. – *Plant Cell Environ.* **30**: 1163-1175, 2007.
- Takei K., Takahashi T., Sugiyama T. *et al.*: Multiple routes communicating nitrogen availability from roots to shoots: a signal transduction pathway mediated by cytokinin. – *J. Exp. Bot.* **53**: 971-977, 2002.
- Wang X.K., Zhang W.H., Hao Z.B. *et al.*: [Principles and Techniques of Plant Physiological Biochemical Experiment.] Pp. 118-119. Higher Education Press, Beijing 2006. [In Chinese]
- Wells R., Schulze L.L., Ashley D.A. *et al.*: Cultivars differences in canopy apparent photosynthesis and their relationship to seed yield in soybeans. – *Crop Sci.* **22**: 886-890, 1982.
- Wilson E.W., Rowntree S.C., Suhre J.J. *et al.*: Genetic gain $\times$ management interactions in soybean: II. nitrogen utilization. – *Crop Sci.* **54**: 340-348, 2014.
- Xie F.T., Zhang H.J., Wang H.Y. *et al.*: Effect of preplant fertilizer on agronomic and physiological traits of soybean cultivars from different breeding programs. – *Agr. Sci. China* **9**: 1602-1611, 2010.

- Yin Z.T., Meng F.F., Song H.N. *et al.*: Expression quantitative trait loci analysis of two genes encoding Rubisco activase in soybean. – *Plant Physiol.* **152**: 1625-1637, 2010.
- Zhang X., Huang G., Bian X. *et al.*: Effects of root interaction and nitrogen fertilization on the chlorophyll content, root activity, photosynthetic characteristics of intercropped soybean and microbial quantity in the rhizosphere. – *Plant Soil Environ.* **59**: 80-88, 2013a.
- Zhang X.X., Zhang H.J., Song S.H. *et al.*: [Comparison on root activity and nodulation characteristics of super-high-yielding soybeans.] – *Soybean Sci.* **32**: 496-500, 2013b. [In Chinese]