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Relationship between root vigour, photosynthesis and biomass in soybean cultivars during 87 years of genetic improvement in the northern China

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Abstract

During the last century, the world soybean yield has been constantly enhancing at a remarkable rate. Factors limiting the soybean yield may be multiple. It is widely acknowledged that changes of root metabolism can influence aboveground characteristics, such as the seed yield and photosynthesis. In this study, we considered root bleeding sap mass (BSM) and root activity (RA) as indicators of the root growth vigour. We used 27 soybean cultivars, spanning from 1923 to 2009, to evaluate the contribution of root characteristic improvement to efficient photosynthesis and dry matter production. The BSM, RA, net photosynthetic rate (P_N), and organ biomass were measured at different growth stages, such as the fourth leaf node, flowering, podding, and seed-filling stage. Our results showed that the soybean cultivars increased their biomass and P_N thanks to genetic improvement. At the same time, BSM and RA also increased in dependence on a year of cultivar release. However, both P_N and biomass were positively correlated with root characteristics only at the podding stage. Our data revealed that the improved root characteristic may have contributed to the enhanced photosynthesis, biomass, and yield of soybean cultivars during last 87 years of genetic improvement. We suggest that BSM and RA could be used as important indexes for further practice in soybean production improvement.

Additional key words: breeding; gas exchange; *Glycine max*; root biomass.

Introduction

During the last century, the world soybean yield has been continuously enhancing at a remarkable rate. Many reports has focused on the effects of genetic improvement on agronomic and biological traits in soybean (Voldeng *et al.* 1997, Wang *et al.* 2006, Li *et al.* 2007, Tian *et al.* 2007, Zhao *et al.* 2008, Zheng *et al.* 2008, Liu *et al.* 2009). It is well-known that the genetic improvement of crops can elevate the photosynthetic rate and dry matter content under favorable growth conditions (Karmakar *et al.* 1996, Specht *et al.* 1999, Wilcox 2001). The factors limiting soybean yield increase in the world may be multiple. Considering the specific metabolism trait of soybean roots (*i.e.*, nodule nitrogen fixation), we may expect that the improvement of root characteristics contributes to the enhancement of the yield. Several researches documented that development of roots directly influenced the growth, biomass, and yield (Hilbert 1990, Lynch 1995, Smith *et al.* 2005, Wang *et al.* 2009), and that xylem sap might mediate exchanges between shoots and roots (Philips and Jones 1964, Shi *et al.* 2008, Li *et al.* 2009). Chihiro *et al.* (2001) and Dieleman *et al.* (1998) found that root bleeding intensity was correlated with plant height and root dry mass.

Lower photosynthetic photon flux density (PPFD) may result in reductions in the yield and quality (Challa and Schapendonk 1984, Cockshull *et al.* 1992). As stated by Austin *et al.* (1980), the high P_N is considered to be one of the most important breeding strategies. Most of experiments focused on the aboveground characteristics, photosynthetic rate and biomass of high-yielding genotypes, while the characteristics of roots have been largely ignored. However, there is a strong relationship between root growth and aboveground characteristics. For example, Yang *et al.* (2001) found that genetic improvement of soybean increased the root mass and root volume. Nodule numbers, nodule fresh mass of soybean cultivars also increased due to genetic improvement (Yao *et al.* 2009). Roots play critical roles in many physiological processes, such as initial uptake and subsequent transport of water and nutrients, production of stress response signals, the secretion of bioactive molecules, and the establishment of

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Abbreviations: BSM – bleeding sap mass; P_N – net photosynthetic rate; RA – root activity. R2 – flowering stage; R4 – podding stage; R6 – seed-filling stage; TTC – triphenyl tetrazolium chloride; V4 – fourth leaf node stage.
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microbiologic populations in rhizosphere. These physiological processes may directly influence the seed yield.

In this work, we evaluated if root BSM and RA could be considered as an indicator of a root growth vigour. Twenty-seven soybean cultivars spanning from 1923 to 2009 were used to evaluate the contribution of root characteristics to improve the photosynthetic efficiency and dry matter yield.

Materials and methods

Plant material: In total, 27 soybean cultivars bred in the Jilin Province of China from 1923 to 2009 (old cultivars from 1923 to 1984; modern cultivars from 1991 to 2009) were chosen. They were provided by Institute of Soybean Germplasm Resources, Jilin Academy of Agricultural Sciences, China. Yields were the means of 2010 and 2011.

Cultivar	Year of release	Yield [kg ha^{-1}]	Cultivar	Year of release	Yield [kg ha^{-1}]
Huangbaozhu	1923	1439	Jilin 36	1996	2409
Mancangjin	1929	1434	Jilin 38	1998	2535
Yuanbaojin	1929	1072	Jinong 7	1999	2233
Jinyuan 1	1941	1093	Jilin 45	2000	2201
Jiti 5	1956	1368	Jilin 58	2001	1933
Jilin 3	1963	2250	Changnong13	2002	2986
Jilin 1	1963	1992	Jinong 11	2002	2222
Jilin 5	1963	1714	Jiyu 66	2002	2252
Jilin 6	1963	1738	Changnong16	2003	3370
Jilin 8	1971	1840	Jinong 15	2004	2549
Jilin 16	1978	2008	Jinong 19	2006	3251
Jilin 20	1984	2170	Jiyu 95	2008	3013
Jilin 26	1991	1848	Oukedou 25	2009	2889
Jilin 35	1995	2445			

Field experiments were carried out in 2010 and 2011 at the experimental field of Jilin Agricultural University, Changchun, China (43.53°N, 125.10°E). Total soil organic matter was determined using potassium dichromate volumetric method (Lu 2000). Total soil nitrogen (N) was determined by the semi-micro Kjeldahl method, and alkaline hydrolysis of N was determined with the diffusion method (Lu 2000). Total soil phosphorus (P) was determined with colorimetric analysis after digestion with sulphuric acid and perchloric acid, and available P was extracted with sodium bicarbonate solution at pH 8.5 and determined by molybdenum-antimony colorimetry (Lu 2000). Soil available potassium (K) was extracted by ammonium acetate solution at pH 7.0 and determined by flame photometer (*FP 640, Shanghai Precision & Instrument Co., Ltd.*, China) (Lu 2000). Soil contained 26.9 g(total organic matter) kg^{-1}(soil), 1.65 g(total N) kg^{-1}, 0.86 g(total P) kg^{-1}, 0.12 g(alkali-hydrolysable N) kg^{-1}, 16.1 mg(available P) kg^{-1}, and 122.0 mg(available K) kg^{-1}. The soil was of a loamy texture and pH 6.8.

The plots were maintained weed-free with a post-emergent herbicide (*Chlorimuron-ethyl, Shandong Binnong Science Technology Co., Ltd.*, China) and hand weeding. Precipitation in the area was 724 mm from May to October in 2010, and 567 mm in 2011; accumulated temperature (≥ 10°C) was 2,860°C; annual average temperature was 4.6°C; frostless period was about 140 d in the Changchun region (data provided by the weather stations in the Jilin Province).

Each cultivar plot contained five rows, 0.65 cm apart and 5 m long. The seeds were sown on 1 May in both years. The density of plants was 200,000 plants ha^{-1}. The experiments were set-up in a complete randomized block design with three replication. Sampling was done at four individual dates throughout the growing season: at the fourth leaf node (V4), flowering (R2), podding (R4), and seed-filling stage (R6).

Measurement of root BSM: Cotton wool, plastic bag, and rubber belt were weighed, and the masses were recorded as M_1 (g). The plants were cut at the cotyledon node phloem, and cotton wool covered by a plastic bag was sealed immediately with rubber belt to prevent the evaporation of water and collect bleeding sap of xylem. Bleeding sap of five plants were pooled, and the mass per plant was considered as a replicate value of the BSW. Two hours later, the cotton wool was removed, and the masses were recorded as M_2 (g). Root bleeding sap was collected from 9:00 to 11:00 h. BSM rate [g h^{-1} per plant] was calculated as $(M_2 - M_1)/2$.

Measurement of total plant biomass: Plants from the field were separated into two parts: aboveground and roots. The separated plant materials were dried for over 72 h at 80°C. The dry matter was weighed. Five plants of each cultivar were pooled, and the mass per plant was considered as a replicate value of the biomass.

Net photosynthetic rate: Leaf P_N was measured from 9:00 to 11:00 h at V4, R2, R4, and R6 stages with a portable photosynthesis system (*LI-6400*, *LI-COR*, USA). The PPFD was set at 1,200 μmol m^{-2} s^{-1}, chamber temperature was 30–34°C, relative humidity was 53–65%, CO_2 concentration of 340–360 μmol(CO_2) mol^{-1}, and air flow of 500 μmol s^{-1}.

Measurement of root biomass and RA: The whole plant including roots was dug out carefully in a soil block, with a size of 30 × 30 × 30 cm, to keep the integrity of roots, and transferred back to the laboratory immediately. The plants were cut at the plant collar; the roots were washed carefully, dryied with filter paper, and used for determination of RA and root dry mass (nodules were removed before drying). RA was measured using triphenyl tetrazolium chloride (TTC) method (Zhou 2000). The fresh roots were incubated for 60 min at 37°C in the TTC solution (0.04% in phosphate buffer, pH 7.0). The red product in roots was extracted using ethyl acetate. The absorbances were determined by spectrophotometer (*UV-1240, Shimadzu Co., Ltd.*, Japan) at 485 nm. The activity of the root system was expressed as μg g^{-1} h^{-1}

Data analysis: The results of experiments were average data of two years with three replicates. The treatments were designed in a randomized complete block design with three replications. Data were analyzed by two-way analysis of variance (*ANOVA*) using a statistical program *SPSS 13.0* and LSD test was used for means separation when treatments were significant (*SPSS Inc*., Chicago, USA).

Results and discussion

Organ biomass, BSM and RA of 27 soybean cultivars: The BSM, RA, P_N, and biomass were measured at the V4, R2, R4, and R6 stages. We considered the cultivars from 1923 to 1984 as the old cultivars, and the cultivars from 1991 to 2009 as the modern cultivars. The averages of the old and modern cultivars were calculated. The averages of root BSM, RA, and biomass are shown in Fig. 1. All parameters showed significant differences between the old and modern cultivars. Also, two-way *ANOVA* indicated that all parameters significantly differed not only among cultivars but also between both years. Differences between the two years might be attributed to a factor of precipitation (Table 1). In addition, we observed that a significant interaction between the year and cultivar occurred in many parameters (Table 1). The significant interaction clearly revealed that meteorological conditions may influence the metabolic characteristics of the soybean cultivars, possibly affecting the seed yield. For example, P_N and RA showed

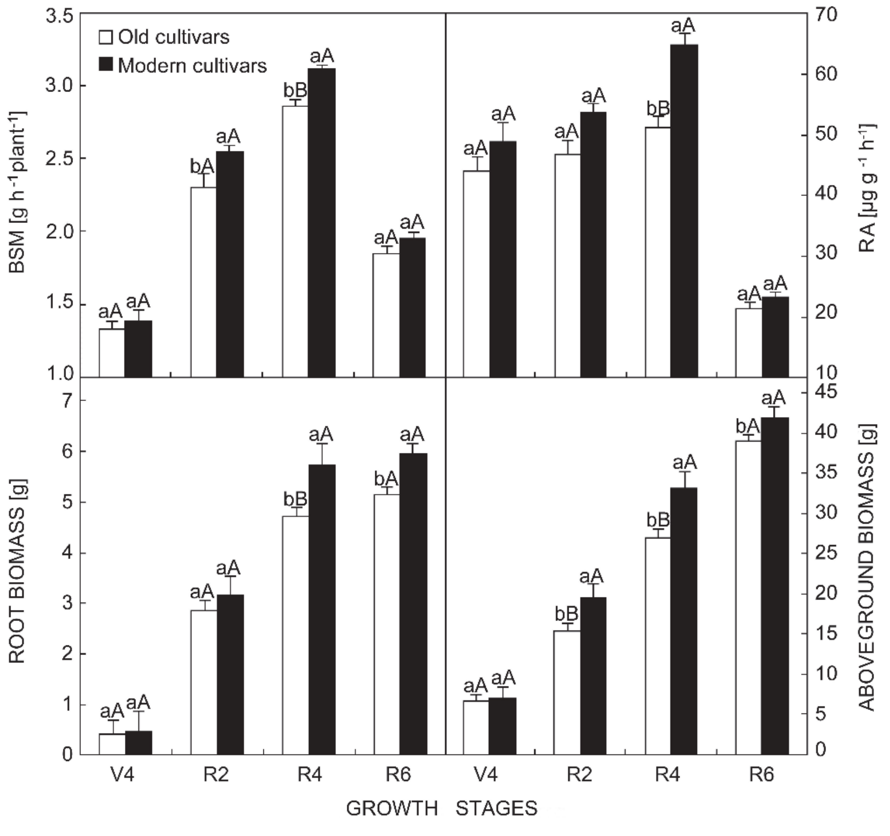

Fig. 1. Changes in BSM, RA, root biomass, and aboveground biomass between old and modern soybean cultivars. *The small letters* above bars indicate significant differences at the 0.05 level in means of the years 2010 and 2011. *The capital letters* indicate significant differences at the 0.01 level in means of the years 2010 and 2011. The values are means (± SE) of three replicates.

Table 1. Two-way variance analysis of different physiological indexes in four growth stages. The figures expressed *F* values of two-way variance analysis. * and ** – significant differences at the 0.05 and 0.01 level, respectively. P_N – net photosynthetic rate; RA – root activity; BSM – bleeding sap mass.

Index	Stages	Year	Variety	Interaction
P_N	V4	99.02**	14.98**	2.34*
	R2	14.91**	20.81**	0.91
	R4	144.09**	32.56**	1.71*
	R6	94.91**	27.40**	3.07*
RA	V4	25.81*	68.07**	5.23*
	R2	56.29**	81.50**	4.06
	R4	84.38**	112.14**	2.18*
	R6	41.36*	19.37**	4.63*
BSM	V4	141.71**	3.55*	0.78
	R2	79.17**	16.88**	7.61**
	R4	2.47	29.07**	2.97*
	R6	82.02**	32.91**	2.31*
Aboveground biomass	V4	156.91**	451.19**	0.19
	R2	123.33**	86.50**	1.48
	R4	100.98**	8.70*	1.22
	R6	48.28**	123.06**	4.37*
Root biomass	V4	121.92**	26.06**	1.54
	R2	128.24**	77.56**	1.29
	R4	213.02**	13.75*	0.67
	R6	35.10*	14.04*	4.88*

significant interaction between the year and cultivar, suggesting that more attention should be given to meteorological conditions in evaluations of photosynthesis and root traits during genetic improvement. The modern cultivars showed an increasing tendency in BSM, RA, and biomass compared to the old cultivars (Fig. 1), especially at the R4 stage. Regression analyses also demonstrated that BSM, RA, organ biomass, and P_N exhibited an increasing tendency with a year of release at all four stages, but it was statistically significant only at R4 (Figs. 2, 3). The P_N showed positive correlation with aboveground biomass, BSM, and RA at all the four stages, but it was significant only at R4 (Table 2). In addition, root biomass and total biomass were also positively correlated with RA and BSM only at R4 (Fig. 4).

Photosynthesis and biomass: Our results showed that the organ biomass increased due to genetic improvement of soybean cultivars (Figs. 2, 3). At the same time, BSM and RA also rose with the year of release. The P_N and biomass were positively correlated with root characteristics only at R4. Our data revealed that the improvements of the root characteristics may contribute to increase of photosynthesis, biomass, and the yield during last 87 years. The contribution of genetic improvement to the yield of soybean cultivars has been estimated to be around from 0.5 to 0.7% per year (Voldeng *et al.* 1999, Kumudini *et al.* 2001). Zhao *et al.* (2008) showed that specific leaf mass and chlorophyll content in leaves were elevated by genetic improvement of soybean cultivars. Zhuang *et al.* (2010) pointed that P_N of the modern cultivars was higher than that of the old cultivars, and maximum of P_N was observed at upper leaves. Xu *et al.* (2011) showed that genetic improvement of soybean enhanced plant biomass at upper and middle leaves. Efficient canopy photosynthesis may contribute to the increase of the yield and biomass in the modern cultivars (Hao *et al.* 1999, Gutiérrez-Rodríguez *et al.* 2000, Untiedt *et al.* 2004, Wang *et al.* 2008). In the last several decades, enormous progress has been made on the physiology of plant roots. Roots mediate uptakes of water and ions, and respond to external signals. Root BSM can reflect physiological activity of the roots; it can be used as a biological indicator of RA. Long-distant signaling derived from roots can regulate shoot gene expression following changes in nutrient supply to the root system (Takei *et al.* 2002). Some signal molecules synthesized in roots are transported into stems and leaves to regulate photosynthesis and growth. These signal molecules include hormones and nutrients that directly adjust the

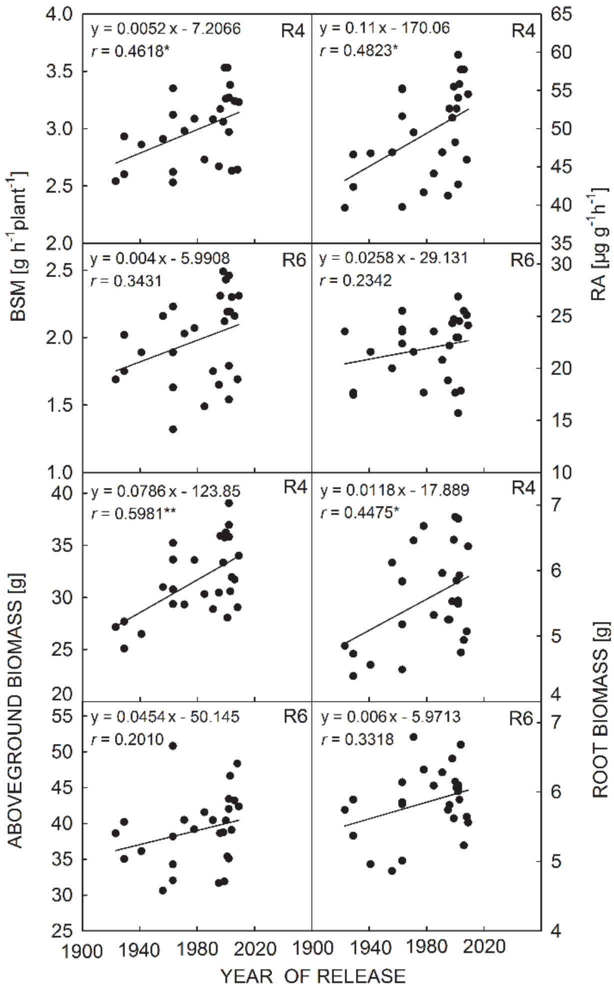

Fig. 2. Correlation between BSM, RA, root biomass, aboveground biomass, and the year of release among 27 soybean cultivars at the R4 and R6 stage. * and ** – significant differences at the 0.05 and 0.01 levels.

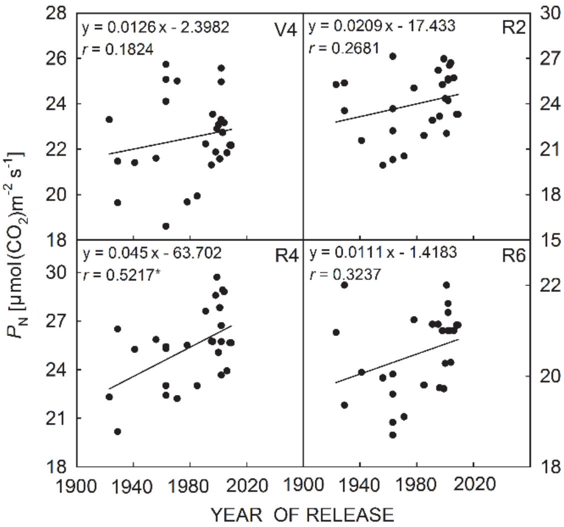

Fig. 3. Correlation between net photosynthetic rate (P_N) and the year of release among 27 soybean cultivars at different growth stages.

Table 2. Correlation between net photosynthetic rate and aboveground biomass, BSM, and RA among 27 soybean cultivars at different growth stages. * – significant differences at the 0.05 probability level in means of the years 2010 and 2011.

Growth stage	Correlation coefficient Aboveground biomass	BSM	RA
V4	0.1113	0.2648	0.1667
R2	0.1374	0.1728	0.0781
R4	0.4389*	0.4281*	0.4839*
R6	0.2173	0.2404	0.2649

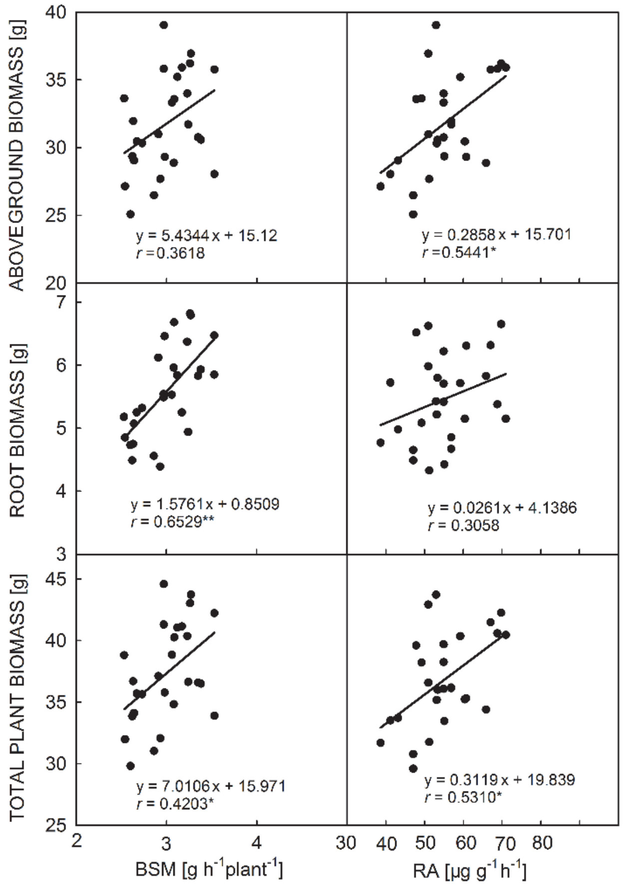

Fig. 4. Correlation between root characteristics (bleeding sap mass, BSM, and root activity, RA) and organ biomass among 27 soybean cultivars at the podding stage (R4).

gene expression and protein synthesis involved in photosynthesis and growth. As a result, photosynthesis and metabolism may be affected. For example, the genetic improvement of root growth contributes to more efficient P acquisition in maize (Zhang *et al.* 2013). Mu *et al.* (2015) found that genetic improvement of root growth increased maize yield *via* enhanced nitrogen uptake by roots at a post-silking stage. According to these data, we suggest that the improvement of root characteristics should be considered as an efficient approach to evaluate enhancing soybean productivity.

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