



Full Length Article

Carbohydrate Metabolism in *Paeonia suffruticosa* Leaves Grown under Root Restriction

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Abstract

The goal of this study was to analyze the effect of root restriction (RR) on carbohydrate metabolism in 'Luoyanghong' tree peony (*Paeonia suffruticosa* Andr.) leaves. The results revealed that root restriction significantly reduced the net photosynthetic rate (Pn), sucrose phosphate synthase (SPS) activity and acid invertase (AI) activity of tree peony leaves. Before anthesis, sucrose and starch levels were significantly lower in root restriction (RR) tree peony leaves than in control leaves (CK). However, at 0 and 7 days after anthesis (DAA) sucrose and starch accumulated in RR tree peony leaves mainly because of the low sink strength (SPS and AI activity), which limited the driving force to transport sucrose to sink organs. At anthesis (0 DAA), there was a rapid increase in the hexose content in CK. Interestingly, this increase was not seen in RR. Based on correlation analyses, it can be seen that photo assimilates produced in RR tree peony leaves were used primarily for their own growth, while lesser were transported to sink organs and at a slower rate compared to CK. © 2020 Friends Science Publishers

Key word: Carbohydrate metabolism; Leaf; Root restriction; Sink strength; Tree peony

Introduction

Root restriction (RR) has become increasingly important in forestry, agriculture and horticulture (Yong *et al.* 2010; Graham and Wheeler 2016). Root cells undergo many complex biochemical reactions to sense and recognize stress signals, which were transported to shoot through xylem, thus regulating the growth and metabolism of the whole plant (Galvan-Ampudia and Testerink 2011). Plants undergo many physiological and morphological changes in response to RR. Plants grew spirally when growing in containers (Dominguez-Leren *et al.* 2006). It is commonly recognized that RR disturbs the nutrition balance and communication between the roots and shoots of a plant, with concomitant changes in gene expression and physiological processes (Yeh and Chiang 2001; Lu *et al.* 2011; Khan *et al.* 2014). Generally, RR reduces plant growth vigor, restrains shoot growth, reduces leaf area, slows down growth, decreases branch elongation and coarseness, reduces plant dry and fresh weight, and reduces relative growth rate (Ronchi *et al.* 2006; Shi *et al.* 2008a; Zaharah and Razi 2009; Mugnai and Al-Debei 2011). Research on RR has focused mainly on

photosynthesis, water and nutrition, gas conduction and oxygen absorption and endogenous phytohormones (Slewsinski and Braun 2010). Many studies have found that RR reduces photosynthetic rate of plants. However, Carmi (1986; 1995) found that RR increases the photosynthetic rate of cotton and bean. Peterson *et al.* (1991) found that RR had no effect on photosynthetic rate. Researchers explained the effect of RR on photosynthetic rate in different ways, but the mechanism is still unclear.

Some studies indicated that the decrease in photosynthetic rate caused by RR is related to the decrease of stomatal conductance in leaves (Rieger 1994; Yong *et al.* 2010; Huang *et al.* 2018). However, Thomas and Strain (1991) have found that the decrease of photosynthesis caused by RR has no relation to the decrease of stomatal conductance. Other scholars have explained this phenomenon from the feedback regulation mechanism of carbon metabolism. They believed that RR caused carbohydrate accumulation, which resulted in the feedback regulation mechanism of photosynthesis and the decrease of photosynthetic rate (Pezeshki and Santos 1998; Kharkina and Ottosen 1999, Yong *et al.* 2010).

Carbohydrate metabolism is a dynamic process, as metabolic fluxes and sugar concentrations alter dramatically both during development and in response to environmental signals (Coleman *et al.* 2010). Carbohydrates play a key role in plant metabolism, growth, defense, and senescence. It has been shown that carbohydrate metabolism determines the source-sink relationship and controls the allocation of carbon to different plant organs (Lazare *et al.* 2016). Under RR, the movement and distribution of nutrient elements in plants were changed, while carbohydrates were mostly studied. When field grown *Euonymus alatus* plants were potted, 46% of the assimilates were allocated to the main stem, while only 21% of the CK, and there was no difference in the distribution of the assimilates in the roots (Dubik *et al.* 1992). The differences in plants flowering depend largely on sink strength, activities of carbohydrate metabolism enzymes, and efficiency of energy conversion (sink activity).

For many plant species, the activities of source photosynthetic production and sink growth appear to be closely coordinated, such that a balance is maintained between source supply and sink demand (McCormick *et al.* 2006). The regulation of sink-to-source supply capacity is considered to be caused by the accumulation of carbohydrates in source tissues (Valantin-Morison *et al.* 2006).

Tree peony (*Paeonia suffruticosa* Andr.) is a perennial shrub native to China that possesses great landscaping value. It is Chinese traditional flower and has a long cultivation time. Tree peony grows well in the field. However, when grown in containers (RR), growth of tree peony tends to be slow, with sparse, small or abortive buds, which was inadequate for commercial use. At present, there is little information on the growth of tree peony in RR condition. To find solutions for poor growth and flowering of potted tree peony, we chose to study the 'Luoyanghong' tree peony variety, which grows well in Henan Province (China). The objective of this paper was to determine the effect of RR on carbohydrate metabolism in tree peony leaves. Net photosynthetic rate (Pn), carbohydrate pools (sucrose, glucose, fructose and starch) and the activities of key enzymes (acid invertase [AI, EC 3.2.1.2), neutral invertase (NI, EC 3.2.1.26), sucrose-phosphate synthase (SPS, EC 2.4.1.14) and sucrose synthase (SS, EC 2.4.1.13)] associated with sucrose metabolism were assessed.

Materials and Methods

Experimental design and sample preparation

Eighty uniform 7-year-old tree peony seedlings were selected and grown under ambient outdoor conditions (a moderate land climate) in the Science Park of Henan Agricultural University (Zhengzhou, China). Forty seedlings were grown in the field as control (CK). The other forty seedlings were grown in pots as root restriction (RR) treatment. Potting medium was the same soil found in the

field. The pot was 40 cm high and 35 cm in diameter. The CK and RR treatment plants were arranged alternately over a total of 10 rows and 8 columns. Space between the plants was 80 cm × 100 cm. One third of each pot was placed below ground to prevent lodging. Tree peony prefers dry and well-drained soil, so they were watered only when they showed signs of drought. The plants were fertilized three times every year (before anthesis, flower bud differentiation and before going into winter). The annual flowering time of 'Luoyanghong' tree peony in the Zhengzhou area is April 18. Sampling data were -28, -21, -14, -7, 0, 7 and 14 days after anthesis (DAA). Leaves were sampled from the third or fourth pair of completely expanded leaves from the apex of tree peony. All samples were washed immediately, treated with liquid nitrogen, then stored at -70°C until further analysis. For each sampling, 5 plants were taken from potted plants and 5 from field peony.

Measurement of net photosynthetic rate

The net photosynthetic rate (Pn) of the sec fully expanded leaf was measured under sunny conditions from 0900 to 1300 using the PP Systems-CIRAS-2 Portable Photosynthesis System (PP Systems, MA, USA). Moreover, the measurement using the intensity of sunlight and the concentration of CO₂ in the atmosphere was also made. Therefore, the CO₂ concentration in 'Cuvette environment' was set to 0. 'Par' was set to 0 and temperature was set to track ambient.

Carbohydrate pool assay and sucrose-metabolism key enzyme activity assay

The determination of sucrose content, glucose content, fructose content and starch content were carried out according to Zhang and Qu (2006) method. The determination of sucrose metabolism key enzymes (AI, NI, SPS, SS) were carried out according to Mi *et al.* (2011) method.

Data processing

Every physiological data are mean ± SE (n = 3~10). Field test and laboratory test were repeated at least three times. Excel and SPSS statistical analysis software were used for data processing. The differences among the data are analyzed by ANOVA and t-test. Pearson correlation analysis was done to analyze the correlation between the indicators.

Results

Effect of RR on the net photosynthetic rate of tree peony leaves

The leaf-expansion period of tree peony began at -28 DAA,

and they entered the fast-growth period at -14 DAA. The Pn of RR and CK increased with the growth of leaves and reached the maximum level at 0 DAA. At -28, -14, -7, 0, 7 and 14 DAA, the Pn of RR tree peony were significantly lower than that in the CK, accounting for only 53.73, 59.54, 61.81, 48.83, 43.87 and 32.64% of the Pn of CK, respectively (Fig. 1).

Effect of RR on the carbohydrate pools of tree peony leaves

From -28 DAA to 7 DAA, sucrose content in RR tree peony leaves increased with time and reached its maximum level at 7 DAA. In contrast, sucrose content in the CK tree peony reached the maximum level at -7 DAA, then declined at 0 DAA. The increase in sucrose content in RR tree peony was slower than that in the CK group. At -28, -21, -14, and -7 DAA, sucrose content in RR leaves was significantly lower, accounting for just 39.99, 47.35, 58.79 and 79.87% of CK, respectively, of that in CK leaves. At 0 and 7 DAA however, sucrose content was significantly higher in RR leaves than that in CK leaves (Fig. 2).

Sucrose can be transported to sink organs and utilized only by hydrolyzing into hexose (glucose and fructose). In the absence of hydrolysis, sucrose accumulates in those sink organs. At -21, -14, -7, 0, 7 and 14 DAA, hexose content in RR leaves was significantly lower, accounting for 85.28, 75.11, 85.39, 71.88, 77.82 and 77.15%, respectively, of the CK peony. Plants need large amount of energy for flowering, which can accelerate the hydrolysis of sucrose, causing a rapid increase in hexose levels. This phenomenon was seen in the CK tree peony, but not in the RR tree peony. From -7 DAA to 0 DAA, the hexose content increased 53.74% in CK leaves and 29.43% RR in leaves. These results indicated that sucrose was used less and more slowly in the RR tree peony than that in the CK at anthesis (Fig. 3-5).

The starch content of RR leaves increased at a slow rate until 7 DAA, while the starch content of CK tree peony increased rapidly and reached the maximum level at -7 DAA. At -28, -21, -14 and -7 DAA, the starch content of RR tree peony leaves was significantly lower, accounting for 71.55, 61.02, 56.23 and 49.83%, respectively, of that in the CK leaves (Fig. 2). At 7 DAA, starch accumulated in RR tree peony and starch content column was higher than that in CK, but the difference was not significant (Fig. 6).

Effect of RR on the sucrose metabolism enzymes of tree peony leaves

Maximum activity of AI in RR tree peony was observed at -7 DAA, indicating that this was the fastest-growing period. The maximum activity of AI in CK tree peony was observed at 0 DAA when buds need more energy for flowering. So the sink strength of CK tree peony was generally high, accelerating the hydrolysis of sucrose to transport to sink tissues (flower buds). At -28, -21, -14, 0, 7 and 14 DAA, AI

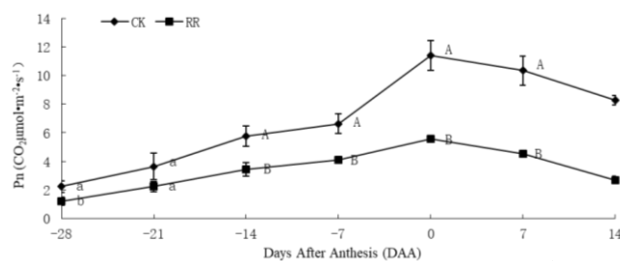


Fig. 1: Effects of root restriction on the Pn of tree peony leaves (aa, ab and AB above the standard error bars indicate that there is no significant difference, the difference is significant at $P < 0.05$ level, and the difference is significant at $P < 0.01$ level. The same as below)

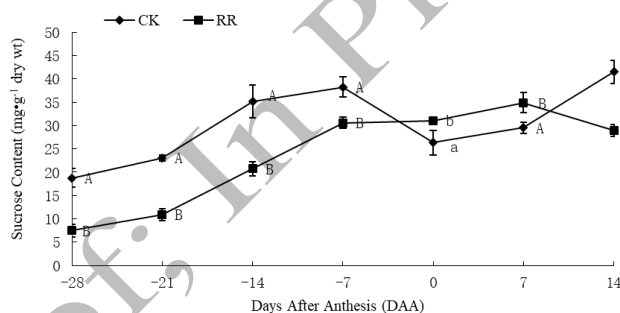


Fig. 2: Effects of root restriction on the sucrose content of tree peony leaves

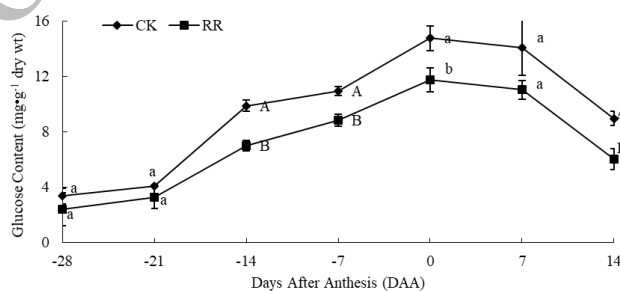


Fig. 3: Effects of root restriction on the glucose content of tree peony leaves

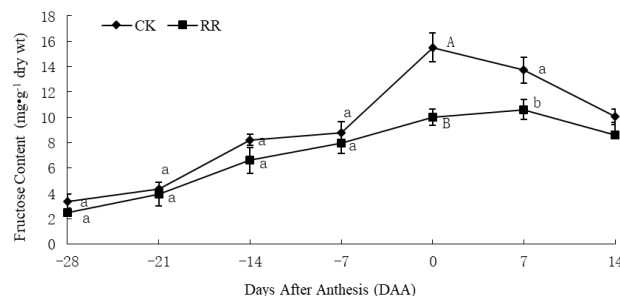


Fig. 4: Effects of root restriction on the fructose content of tree peony leaves

activity in RR tree peony leaves was significantly lower, accounting for 56.17, 65.67, 69.67, 61.49, 78.68 and

68.76%, respectively of that in CK leaves (Fig. 7). From Fig.8 it can be seen that the activity of NI was much lower than that of AI in both RR and CK tree peonies, showing that AI was mainly involved in the hydrolysis of sucrose in tree peony leaves.

The activities of SPS were significantly lower in RR tree peonies than that in CK tree peonies at all sampling dates. No obvious change of SPS activity was observed before or after anthesis in RR peonies. However, SPS activity in CK tree peony reached its maximum at 0 DAA, then declined, which seems due to large requirement of carbohydrates for flowering. Before anthesis (0 DAA), SS (synthesis direction) activity in RR tree peonies was significantly lower than that in CK tree peonies. However, compared to SPS, SS activity was much lower in both groups, which indicated that SPS plays a much more important role in sucrose synthesis in tree peony leaves (Fig. 9–10).

Discussion

Reduction in leaf Pn has been reported in plants grown under RR conditions in several earlier studies (Shi *et al.* 2008b; Yong *et al.* 2010; Wang *et al.* 2013). In the present study, we found that RR significantly reduced the Pn of tree peony (Fig.1). The activities of photosynthetic production at the source and growth at the sink are closely coordinated in many plant species (Quereix *et al.* 2001). Root growth is a major metabolic sink for photosynthetically fixed carbon. RR lowers sink strength and changes the ratio of root to shoot in plants. The nutrition imbalance between root and shoot results in the reduction of shoot growth (Arp 2010) and has a negative effect on photosynthesis (Paul and Pellny 2003). Before entering the fast-growing period (-14 DAA), tree peony leaves act as sink organs, so the low Pn of RR tree peony is correlated with the poor growth of the leaves. Flowers are main sink organs at anthesis (0 DAA); however, the sparse, small or abortion flower organs in RR tree peony limited the sink capacity, which had a pronounced negative effect on the rate of net carbon assimilation.

In most plants, sucrose is the primary product of photosynthesis and also the form of assimilated carbon that is transported (Chen *et al.* 2019). While some of the sucrose was used directly for leaf growth and maintenance, the major amount was transported to the growing plant organs. Starch is the main storage form of carbohydrate. When demand at the sink increases, starch was hydrolyzed to provide energy for plant growth and development. A decrease in sucrose and starch content in CK tree peony leaves we observed at anthesis (0 DAA) showing that carbohydrate was transported from source leaves to flowers (the major sink organ). However, sucrose and starch content did not decrease, but accumulated instead in RR peony leaves. This showed that less amount of sucrose was transported to sink organs at a slower rate in RR tree peony compared to that in CK tree peony. Induction of flowering

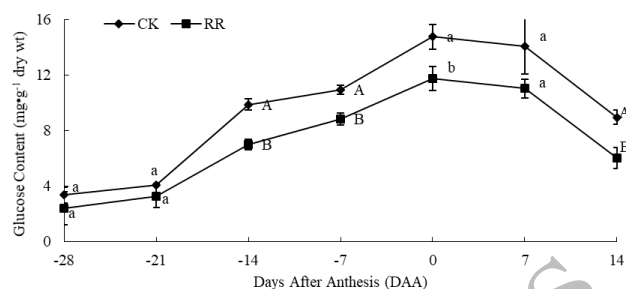


Fig. 5: Effects of root restriction on the glucose content of tree peony leaves

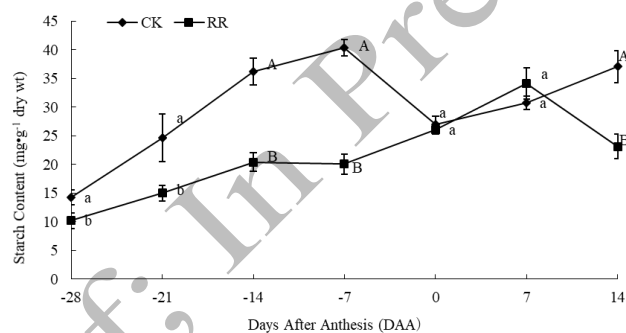


Fig. 6: Effects of root restriction on the starch content of tree peony leaves

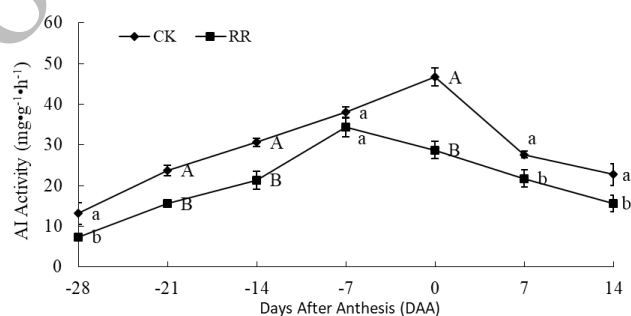


Fig. 7: Effects of root restriction on the AI activity of tree peony leaves

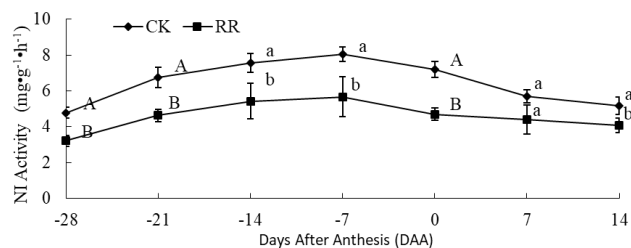


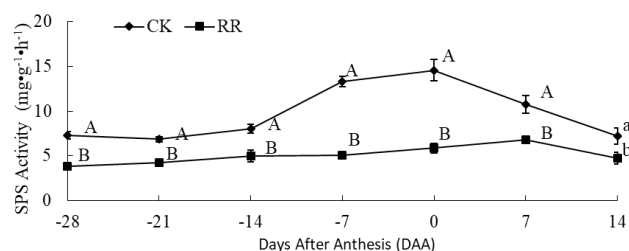
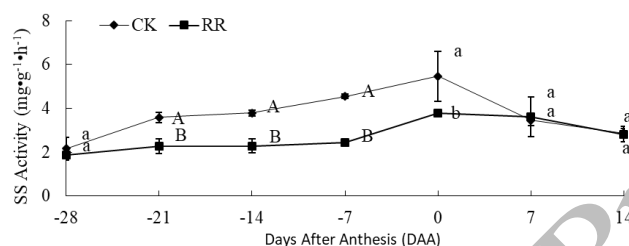
Fig. 8: Effects of root restriction on the NI activity of tree peony leaves

requires large amount of energy and a transient increase in leaf hexose content occurs during that period (Schmitz *et al.* 2014). Consistent with this, we observed a decline in starch and sucrose content with an increase in hexose content at

Table 1: Correlations of main carbohydrate metabolism physiological indicators in tree peony leaves

Related Index	Correlation coefficient					
	Sucrose	Starch	Hexose	Sucrose/starch	Hexose/sucrose	
Pn	CK	0.386	0.441	0.976*	-0.415	0.881**
	RR	0.843*	0.792*	0.948*	0.607	0.130
AI	CK	0.296	0.479	0.764	-0.604	0.709
	RR	0.724	0.490	0.744	0.810*	-0.107
SPS	CK	0.131	0.283	0.761	-0.319	0.764*
	RR	0.787*	0.778*	0.674	0.511	-0.570

*,**Correlation is significant at the 0.05 level and 0.01 level (2-tailed), respectively

**Fig. 9:** Effects of root restriction on the SPS activity of tree peony leaves**Fig. 10:** Effects of root restriction on the SS activity of tree peony leaves

anthesis (0 DAA) in CK tree peony. Strikingly, this phenomenon was not observed in RR tree peony (Fig. 2–10).

Both Pn and SPS were positively correlated with sucrose and starch content in RR tree peony but not in CK tree peony. This suggested that the carbohydrates produced by RR tree peony leaves are used mainly for its own growth. Sucrose and hexoses (mainly glucose and fructose) are recognized as the main sugar-sensing molecules and elicit sugar responses in both source and sink organs (Rosa *et al.* 2009; Zhang *et al.* 2017). Both Pn and SPS were found to be positively correlated to hexose-to-sucrose ratio in CK tree peony, but not in RR tree peony (Table 1). This implied that the ratio of hexose-to-sucrose ratio was disturbed in plants grown under RR.

The SPS catalyzes the regulatory step in sucrose synthesis during photosynthesis. AI hydrolyzes of sucrose to maintain sucrose gradient between the source and the sink (Wan *et al.* 2017). SPS and AI thus play important roles in carbohydrate metabolism. SPS and AI are coordinated in controlling long-distance transport of sucrose and sucrose metabolism in sink organs (Park *et al.* 2008). SPS and AI are also involved in the sugar-sensing system of plants by

adjusting the sink's capacity to regulate photosynthesis (Estornell *et al.* 2013). Under RR, SPS and AI activities of tree peony leaves were significantly reduced. So it can be seen that both sink and source activities were limited growing under RR condition.

Conclusion

When grown under RR conditions, the balance between the roots and shoots of tree peony was found to be disturbed. Such an imbalance can decrease the effectiveness of absorption and usage of nutrients. The levels of intermediate products and enzymes involved in carbohydrate metabolism and sugar signaling system were also found to be affected. Consequently, slow growth and low carbon assimilation rate were observed in addition to poor development of flower organs in tree peony grown under RR conditions.

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