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Improved drought resistance by intergeneric grafting in Salicaceae plants under water deficit

Qingquan Han [1, 2], Jianxun Luo [3], Zhijun Li [4], Helena Korpelainen [5] and Chunyang Li [1, *]

[1] College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 310036, China

[2] Key Laboratory of Mountain Surface Processes and Ecological Regulation, Institute of Mountain Hazards and Environment, Chinese Academy of Sciences, Chengdu 610041, China

[3] Sichuan Academy of Forestry, Chengdu 610081, China

[4] Tarim University, College of Plant Science, Alar 843300, China

[5] Department of Agricultural Sciences, Viikki Plant Science Centre, P.O. Box 27, FI-00014 University of Helsinki, Finland

[*] Corresponding author: Chunyang Li, E-mail: licy@hznu.edu.cn

Head title: Improved drought resistance by intergeneric grafting

Highlights

- Intergeneric grafting was conducted between *P. cathayana* and *S. rehderiana*.
- *P. cathayana* scion grafting combinations have a better biomass accumulation.
- *S. rehderiana* -rooted grafting combinations are more resistant to drought stress.
- Grafting *Populus* scions onto *Salix* rootstocks is an effective way to provide drought resistance.

Abstract In this study, intergeneric grafting was employed between *Populus cathayana* and *Salix rehderiana* to investigate the grafting compatibility of the two Salicaceae plants and to reveal whether grafting can improve the drought resistance. Under different grafting combinations, the survival and growth, biomass accumulation and allocation, photosynthetic traits, carbon isotope composition ($\delta^{13}C$), relative water content (RWC) and non-structural carbohydrates (NSCs) were measured. The results showed that the grafting compatibility between *P. cathayana* (P) and *S. rehderiana* (S) is good, as the survival rates of P/P, P/S, S/S and S/P were 100%, 92%, 90% and 76%, respectively. Compared with the controls, drought significantly decreased growth and biomass accumulation, photosynthetic pigment contents, net photosynthesis rates (P_n) and RWC, and increased $\delta^{13}C$ of all grafting combinations. Under well-watered conditions, growth and biomass accumulation, photosynthetic pigment contents, P_n, and NSC concentrations of P/P and P/S were higher than those of S/S and S/P. On the other hand, under drought stress, growth and biomass accumulation, photosynthetic pigment contents and P_n of P/P and P/S were higher than those of S/S and S/P. Moreover, P_n, $\delta^{13}C$, RWC and NSCs of P/S were the highest. Taken together, our results suggested that the individuals produced by grafting *P. cathayana* scion onto *S. rehderiana* rootstock had the best survival rate, growth performance and drought resistance among the studied grafting combinations.

Key words: Intergeneric grafting; drought resistance; photosynthesis; water use efficiency; non-structural carbohydrates

Introduction

Salicaceae species have a wide natural distribution all over the word. They are mainly distributed in temperate and subtropical zones of the northern hemisphere, and their area of occurrence exceeds 80 Mha globally (Ball et al., 2005). Salicaceae plants, such as *Populus* and *Salix*, are important multipurpose afforestation species. They are fast-growing, well-adapted and easy to cultivate, which makes them suitable for wood production, ecological restoration, bioenergy and land reclamation, etc. Among the environmental limitations for plant growth, water is one of the most critical factors and it impacts plant growth, production and survival (Niu et al., 2014; Pierik and Testerink, 2014; Mutava et al., 2015; Doffo et al., 2017). With global climate change, the earlier snowmelt, higher temperatures and higher variability in precipitation will promote more frequent droughts (Ryan, 2011); consequently, the effects of drought stress on plants are becoming more and more serious.

To cope with drought stress, plants may develop a range of strategies. Previous studies have reported that plants could increase their water use efficiency (WUE), carbon isotope composition ($\delta^{13}C$), and malondialdehyde (MDA), abscisic acid (ABA), proline and antioxidant enzyme (such as SOD, GPX, APX, and GR) contents, etc. when exposed to drought stress, which would enhance their ability to resist the water-deficit pressure (Lei et al., 2006; Xu et al., 2008; Dong et al., 2016). On the other hand, drought resistance can vary between different species or sexes of the same species (Ma et al., 2010; Zhang et al., 2012; Chen et al., 2014). Since drought causes serious economic losses, researchers have used conventional or molecular approaches to develop new varieties with drought tolerance or resistance (Kasuga et al., 1999; Zhou et al., 2013; Kumar et al., 2014). As one of the most ancient horticultural techniques, grafting continues being applied to many plant production questions, including improved drought resistance or water use efficiency of plants (Cantero-Navarro et al., 2016).

Grafting is a complex biochemical and structural process beginning with the adhesion of the grafted partners, followed by callus formation and the establishment of a functional vascular system, eventually resulting in a single functional plant (Pina and Errea, 2005; Cookson et al., 2013). Grafting is widely used in agriculture and horticulture, and it serves a spectrum of purposes (e.g. to modify plant growth and size, to strengthen biotic and abiotic stress resistance, to control the vigor of a shoot, to reduce disease incidence and to modify a cultivar) (Rivero et al., 2003a; Mudge et al., 2009; Lee et al., 2010; Temperini et al., 2013). Although grafting is rarely applied in forestry, there are some relevant studies about grafting between *Populus* or *Salix.* Han et al. (2013) used reciprocal grafting between *P. cathayana* males and females, and found that grafting a female scion onto a male rootstock was an effective method to improve drought resistance of females. So far, little is known about the physiological responses of intergeneric grafted seedlings of *Populus* and *Salix* to drought stress.

In this study, *Populus cathayana* and *Salix rehderiana* were employed for intergeneric grafting. *S. rehderiana* is the dominant woody plant during the primary stage of succession in the Gongga Mountain, which is located on the southeastern fringe of the Tibetan Plateau. During early stages of succession, the soil nutrient resources and water availability are relatively scarce (Song et al., 2017). In such stressful conditions, *S. rehderiana* has a strong resistance compared to *P. cathayana*. The present study aimed to examine the growth, photosynthesis, photosynthetic pigments, fluorescence, water use efficiency and non-structural carbohydrates (NSCs) of different grafting combinations under well-watered and drought stress conditions. The physiological characteristics and stress resistance of grafted plants are mainly influenced by the rootstock, as proved in many previous studies (Cantero-Navarro et al., 2016; Penella et al., 2016; Warschefsky et al., 2016; Huang et al., 2016). Therefore, we hypothesized that: (i) under well-watered conditions, *P. cathayana* scion grafting combinations have a better growth, and under drought stress conditions, *S. rehderiana*-rooted grafting combinations have a stronger resistance to drought stress; (ii) grafting can improve the drought resistance of Salicaceae plants, and grafting a *P. cathayana* scion onto a *S. rehderiana* rootstock provides the best survival under drought stress.

Materials and methods

Plant materials and experimental design

P. cathayana and *S. rehderiana* cuttings (~ 60 cm) were both collected from 60 different trees in 5 populations (12 cuttings per population, the sex ratio of males and females 1:1). *P. cathayana* cuttings were collected from the Xiaowutai mountain (elevation: 1,466 m a.s.l; 39°55' N, 114°59' E) and the *S. rehderiana* cuttings were collected from the Gongga mountain (elevation: 3,000 m a.s.l; 29°34' N, 101°59' E). Cuttings with approximately the same size were used for grafting. Four grafting combinations were created, including two intraspecific combinations, i.e., *P. cathayana* scion with *P. cathayana* rootstock (P/P) and *S. rehderiana* scion with *S. rehderiana* rootstock (S/S), and two intergeneric combinations, i.e., *P. cathayana* scion with *S. rehderiana* rootstock (P/S) and *S. rehderiana* scion with *P. cathayana* rootstock (S/P). The grafted plants were grown in 10-L plastic pots filled with 8 kg of homogenized soil without soil media. After two months of growth, the grafted plants were subjected to well-watered (100% field capacity) or drought stress (30% field capacity) conditions for three months. The experimental design was completely randomized. Each watering regime included 60 individuals (15 individuals per grafting combination). During the experiment, the pots were weighted and then re-watered to the designated soil water content. The experiment lasted from 17 April 2016 to 17 September 2016. At the end of the experiment, five cuttings from each treatment were randomly selected, and the 4^{th} and 5^{th} fully expanded leaves (counted from the top of the plant) were collected for physiological analyses.

Growth measurements

At the end of the experiment (17 September 2016), five seedlings from each treatment were randomly selected and measured for the height growth (HG) and basal diameter (BD). After that, all seedlings were harvested and separated into roots, stems and leaves. The biomass of all samples was weighted after oven-drying (80 °C for 48 h) to a constant mass, and the root dry weight (RDW), stem dry weight (SDW), leaf dry weight (LDW), total dry weight (TDW) and root/aboveground ratio (R/A) were calculated.

Gas exchange measurements

The 4th fully expanded and intact leaf of each cutting was used for gas exchange measurements. The parameters were measured between 08:00 and 11:30 a.m. using the LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA). The optimal parameters were as follows: leaf temperature 25 °C, relative air humidity 60%, CO_2 concentration 400 ± 5 μmol mol^{-1}, leaf-to-air vapor pressure deficit 1.5 ± 0.5 kPa and photosynthetic photon flux density (DP) 1500 μmol m^{-2} s^{-1}. After enclosure, the leaves were equilibrated under measurement conditions to achieve the full photosynthetic induction. A standard LI-COR leaf chamber (2 × 3 cm^2) was used. Once the steady-state gas exchange rates were observed under these conditions, the net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (E) and intercellular CO_2 concentration (C_i) were recorded.

Chlorophyll fluorescence measurements

The leaves that were used for the above gas exchange measurements were also utilized for chlorophyll fluorescence measurements using a PAM chlorophyll fluorometer (PAM 2500, Walz, Effeltrich, Germany). Before measurements, the leaf samples were dark-adapted for at least 30 min by a dark-adaptation blade clip (DLC-8), and then the minimum fluorescence yield (F_o) and the maximum fluorescence yield (F_m) were measured. The leaves were illuminated with actinic light at an intensity of 250 μmol m^{-2} s^{-1}, which was consistent with the light intensity inside the greenhouse at the time of measurements. The actinic light was then switched off, and each leaf was illuminated for 3 s with far-red light to determine the minimal fluorescence yield (F_o'). Then, a saturating white light pulse of 8000 μmol m^{-2} s^{-1} was applied for 0.8 s to measure the maximum fluorescence yield (F_m'). Chlorophyll fluorescence kinetics parameters (F_v/F_m, maximum efficiency of PSII; Yield, maximum effective quantum yield of PSII; *q*P, photochemical quenching coefficient; *q*N, nonphotochemical quenching coefficient) were measured and calculated, as described by van Kooten and Snel (1990).

Chlorophyll pigment measurements

The leaves used for chlorophyll fluorescence measurements were also used to determine leaf pigment contents. Leaf disks of 0.8 cm diameter were extracted in 80% chilled acetone (v/v) in darkness until the leaf turned white. The absorbance of the extract was measured using a spectrophotometer (Unicam UV-330; Unicam, Cambridge, UK) at 470, 646 and 663 nm. The contents of chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids (Caro) were calculated using the following formulae: *Chl* a= $12.21OD_{663}$-$2.81OD_{646}$; *Chl* b= $20.13OD_{646}$-$5.03OD_{663}$; Caro=

$(1000OD_{470}$-3.27*Chl* a-104*Chl* b) /229 (Porra et al., 1989). The total chlorophyll content (*Tchl*) was the sum of *Chl a* and *Chl b*.

Carbon isotope composition and relative water content measurements

Leaves used in photosynthesis measurements were also used for the measurements of the carbon isotope composition ($\delta^{13}C$). The leaf samples were dried at 80 °C for 48 h, dried leaves were homogenized and $^{13}C/^{12}C$ ratios were determined by an Isotope Ratio Mass Spectrometer (DELTA V Advantage: Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA). The carbon isotope composition was expressed as $\delta^{13}C$ values. The overall precision of $\delta^{13}C$ was better than 0.1‰, as determined from five repeated samples. The analysis was performed in the Stable Isotope Laboratory for Ecological and Environment Research, CAS.

Five cuttings from each treatment were randomly selected, and the 4^{th} fully expanded leaf of each cutting was used for the measurements of the relative water content (RWC)s. RWC was measured according to Zhang et al. (2012) by calculating it from fresh mass (FM), turgid mass (TM) and dry mass (DM) measurements on 10 leaf discs (1 cm in diameter) obtained from the central portion of each leaf: RWC = 100(FM - DM)/(TM - DM).

Nonstructural carbohydrate measurements

Starch, fructose, sucrose and total soluble sugars (TSS) concentrations of five replicates from each treatment were extracted from dried samples. About 50 mg fine powder was placed in a 10-ml centrifuge tube, 4 ml 80% (v/v) ethanol was added and the tube was placed into a water bath at 80 °C for 30 min, then centrifuged at 5000 g for 5 min, after which the supernatant was transferred to a new 10-ml centrifuge tube. After that, 2 ml 80% (v/v) ethanol was added to the sediment, followed by centrifugation at 5000 g for 5 min, after which the supernatant was transferred to a new 10-ml centrifuge tube. This procedure was repeated again, the supernatants were combined, and 80% (v/v) ethanol was added to make the final volume to 10 ml. Sugars were estimated in ethanol extracts, total soluble sugars were detected colorimetrically at 625 nm following the anthrone-sulfuric acid method (Yemm and Willis, 1954), and fructose and sucrose were detected colorimetrically at 480 nm following the modified resorcinol method (Murata et al., 1968). For the starch extraction, 2 ml distilled water was added to the residues, which were left in the centrifuge tubes after sugar extraction, and the tubes were placed into a boiling water bath for 25 min. After cooling, 1 ml 9.2 mol^{-1} $HClO_4$ was added and the tubes were shaken for 15 min. Another 1 ml distilled water was added, then the tubes were centrifuged at 5000 g for 5 min, and the supernatants were transferred to new 10-ml centrifuge tubes. Residues were re-extracted with 2 ml 4.6 mol^{-1} $HClO_4$, the procedure was repeated, the supernatants were combined, and distilled water was added to make the final volume to 10 ml. Starch was determined colorimetrically at 620 nm by the anthrone - sulphuric acid method using glucose as the standard (Dubois et al., 1956).

Statistical analyses

All data were analyzed using the software Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) version 17.0. Two-way ANOVAs were used to assess water treatment × grafting type interaction effects. Prior to analyses, the data were checked for the normality and homogeneity of variances. Individual differences among means were determined using the Tukey's test of one-way ANOVA at a significance level of $P < 0.05$. Mean values and standard errors were determined for each variable. A principal component analysis (PCA) was conducted for the eco-physiological traits to identify the most discriminatory effects of grafting types and drought stress. PCA analyses were performed using SIMCA-P14.1 (Umetrica AB, Umea, Sweden).

Results

Survival rates, morphology, and biomass accumulation and allocation

The survival rate of S/P was lower than that of the other three grafting combinations; the survival rates of P/P, P/S, S/S and S/P were 100%, 92%, 90% and 76%, respectively. As shown in Table 1, the interactions of watering and grafting significantly influenced growth and biomass indicators, except for basal diameter (BD). Under well-watered conditions, except for height and stem dry weight (SDW), other indicators of P/P and P/S were all significantly higher than those of S/S and S/P. Drought stress retarded the growth of all four grafting combinations, as the accumulation of biomass in roots, stems and leaves, and in the total biomass reduced significantly. Under drought stress conditions, the height and basal diameter of P/P and P/S were higher than those of S/S and S/P. Moreover, the biomass of roots, stems and leaves and the total biomass of P/P and P/S were significantly higher than those of S/S and S/P. It is noteworthy that under well-watered conditions, the root/aboveground ratios (R/A) of P/P and P/S were significantly higher than those of S/S and S/P, whereas the R/A ratios of P/S and S/S were significantly higher than those of P/P and S/P when exposed to drought stress.

Gas exchange, chlorophyll fluorescence and pigments

Under well-watered conditions, P_n and g_s of P/P and P/S were significantly higher than those of S/S and S/P, and there was no significant difference in C_i among different grafting combinations. The E

value was highest in P/S while lowest in S/P. Significant decreases in P_n, g_s, C_i and E were observed under drought stress conditions in all four grafting combinations. Under drought stress, P_n and E of P/P and P/S were significantly higher than those of S/S and S/P, whereas there were no significant differences in g_s and C_i among different grafting combinations (Table 2).

As shown in Table 3, F_v/F_m and Yield were significantly affected by watering, grafting and the interaction between watering and grafting, but qP and qN were significantly affected by only watering and grafting. In well-watered conditions, there were no significant differences in F_v/F_m, Yield, qP and qN among grafting combinations. Drought stress resulted in a significant decrease in F_v/F_m and qP in all grafting combinations, and Yield of S/S and S/P decreased significantly, but others showed no significant change. Under drought stress conditions, F_v/F_m of P/P and P/S was significantly higher than that of S/S and S/P, and Yield of P/S was significantly higher than that of S/S and S/P. There were no significant differences in qP and qN among different grafting combinations.

Drought stress significantly decreased *Caro*, *Chl a*, *Chl b* and *Tchl* contents in all four grafting combinations, and the content of photosynthetic pigments decreased most in S/P and was also lowest among the four grafting combinations (Fig. 1 a,b,c,d). There was no significant difference in the content of *Chl b* among the four grafting combinations under either control or drought stress conditions (Fig. 1 c). Under well-watered conditions, the *Caro*, *Chl a* and *Tchl* contents of P/P and P/S were significantly higher than those of S/S and S/P (Fig. 1 a,b,d).

Carbon isotope composition ($\delta^{13}C$) and relative water content (RWC)

It is clear from Fig. 2 that there were no significant differences in δ^{13}C and RWC among different grafting combinations under well-watered conditions. Drought significantly increased δ^{13}C and significantly decreased RWC in all four grafting combinations. Under drought stress, δ^{13}C and RWC of P/S were the highest but those of S/P were the lowest. Moreover, δ^{13}C and RWC of P/S were significantly higher than those of S/P, but there were no significant differences between P/P and S/S.

Non-structural carbohydrate concentrations (NSCs)

As shown in Fig. 3, watering, grafting and the interaction of watering × grafting significantly affected NSC (starch, fructose, sucrose and TSS) concentrations in roots, stems and leaves in all grafting combinations, except for leaf starch concentrations in P/P and root sucrose concentrations in S/P (Fig. 3 a,b,c,d). Under well-watered conditions, starch concentrations in roots, stems and leaves of P/P and P/S were higher than those of S/S and S/P. Under drought stress conditions, starch concentrations in roots, stems and leaves of P/P and stems of S/P significantly decreased, while starch concentrations in stems and leaves of P/S and leaves of S/S significantly increased. In addition, starch concentrations in roots, stems and leaves of P/S were significantly higher than those of other three grafting combinations, and the starch concentrations in roots, stems and leaves of S/P were the lowest (Fig. 3 a). Under well-watered conditions, the fructose concentrations in roots of P/S and S/S were significantly higher than those of P/P and S/P. The fructose concentrations in the roots of P/P and stems of S/S, and in the plant organs of other grafting combinations were all significantly reduced when exposed to drought stress; the fructose concentrations in roots, stems and leaves of S/P were the lowest (Fig. 3 b). Under well-watered conditions, the sucrose concentrations in roots, stems and leaves of P/P and P/S were higher than those of S/S and S/P, and the sucrose concentrations in roots and leaves of P/S were significantly higher than those of P/P. Except for roots of P/P, stems and leaves of S/S and stems of S/P, the plants showed significantly reduced sucrose concentrations under drought stress. Under drought stress conditions, the sucrose concentrations in roots, stems and leaves of P/S were significantly higher than those of S/S and S/P, but there was no significant difference when compared to P/P (Fig. 3 c). TSS concentrations in roots, stems and leaves of P/P and P/S were higher than those of S/S and S/P under well-watered conditions. Drought significantly decreased the TSS concentrations of stems and leaves in P/P, leaves in P/S and roots in S/P, while others showed no significant differences. Consistent with the starch concentration trend, the TSS concentrations of roots, stems and leaves of P/S were the highest, while the TSS concentrations of S/P were the lowest (Fig. 3 d).

Principal component analysis (PCA)

The principal component analysis (PCA) showed a clear delineation based on trait combinations in the four grafting combinations under different watering regimes (Fig. 4). Under both well-watered and drought stress conditions, different grafting combinations were well separated from each other, especially the distances from *P. cathayana* scion grafting combinations (P/P and P/S) to *S. rehderiana* scion grafting combinations (S/S and S/P) were relatively great. However, the distances between S/S and S/P under well-watered conditions and P/P and P/S under drought stress conditions were small (Fig. 4). However, compared with the controls, the distances between P/P and S/P were greater than those of P/S and S/S. Thus, this finding demonstrated that P/P and S/P are more sensitive to drought stress than P/S and S/S (Fig. 4). In all, principal components 1 (PC1) and 2 (PC2) accounted for 67% and 12.7% of the observed variance, respectively (Fig. 4). As shown in Table 4, P_n, *Caro*, *Chla*, *Tchl* and root sucrose were key contributors to PC1, and PC2 was strongly influenced by R/A ratio, C_i, $\delta^{13}C$, leaf starch and stem TSS.

Discussion

Many factors influence grafting success, such as inherent cellular incompatibility, the formation of plasmodesmata, vascular tissue connections, and the presence of growth regulators and peroxidases (Pina and Errea, 2005; Pina et al., 2009; Zarrouk et al., 2010; Melnyk and Meyerowitz, 2015). Incompatibility between rootstock and scion has been more often observed in interspecific than in intraspecifc grafts, possibly resulting from a graft dieback (Darikova et al., 2011). Our study indicated that the survival rate of S/P (76%) was clearly lower than that of P/P and S/S (≥ 90%). Yet, the results showed that there was a good interspecific grafting affinity between *P. cathayna* and *S. rehderiana* (P/S), the survival rate being 92%. Considering the consistency of the grafting technique, environmental conditions and experiment management, we speculated that the lower survival rate of S/P might be related to water and energy supply. Both hydraulic failure and carbon starvation have been implicated as likely mechanisms contributing to tree mortality (McDowell et al. 2008). Our results found that *P. cathayna* used as rootstocks developed roots slowly, and *S. rehderiana* used as scions had lower photosynthetic rates, which restricted the absorption of water and the production of photosynthetic energy. Furthermore, the carbon transport can be constrained through osmotic or hydraulic mechanisms (McDowell et al. 2013; Sevanto et al. 2013). These above might be the reasons leading to the death of the grafted S/P individuals.

Plant height, basal diameter and biomass are important indicators of plant growth. Previous studies have shown that drought stress has a great effect on plant height and biomass partitioning and production (Fazeli et al., 2007; Wu et al., 2008; Xu et al., 2008). Our results were in accordance with these previous studies.. Moreover, the growth and biomass accumulation of S/P was lowest under both well-watered and drought stress conditions, which indicated that it is not appropriate to graft a *S. rehderiana* scion onto a *P. cathayana* rootstock. The ratio of the root to the aboveground parts (R/A) is also one of the indicators when evaluating plant resistance to abiotic stresses. Under drought or nutrient deficiency conditions, plants enhance their resistance by increasing the investment in underground parts (Ma et al., 2010; Kano et al., 2011). Our results showed that the R/A ratio of P/S and S/S was significantly higher than that of P/P and S/P when exposed to drought stress, which indicated that *S. rehderiana*-rooted combinations (P/S and S/S) could resist drought better. Most studies have indicated that the grafted plants have a higher degree of resistance against stresses, if the rootstock is more resistant to stresses (Rivero et al., 2003b; Ruiz et al., 2005; Han et al., 2013; Kunwar et al., 2015; Penella et al., 2016). Thus, we could come to the conclusion that the anti-drought ability of *S. rehderiana* is better than that of *P. cathayna*, which also supports our hypothesis.

The growth and biomass accumulation of plants are closely related to their P_n. Different studies have found that drought stress significantly reduces P_n of plants, and plants with a better resistance to drought also have a smaller decrease in P_n when compared to drought sensitive plants (Xu et al., 2008; Zhang et al., 2012; Li et al., 2015). Our results showed that the P_n values of the four grafting combinations were all significantly reduced under drought stress, and P_n of P/P and S/P reduced more drastically than that of P/S and S/S. In addition, under drought stress conditions, P_n of P/P and P/S was significantly higher than that of S/S and S/P, but there were no significant differences in g_s

and C_i among the four grafting combinations, which showed that the differences in P_n were not caused by a limiting entry of external CO_2 by g_s. Since photosynthetic pigments participate in the light absorption, energy conversion, electron transfer, CO_2 fixation etc., significantly decreased pigment contents under drought stress could affect P_n of plants (Liu et al., 2011). The results of this study indicated that the contents of *Chl a* and *Tchl* of P/P and P/S were significantly higher than those of S/S and S/P. This result was consistent with the change of P_n under drought stress conditions. It is notable that the decrease in P_n might be caused by reduced photosynthetic pigments. Moreover, the PCA analysis indicated that P_n, *Caro*, *Chla* and *Tchl* were also the key contributors to PC1, and there were positive correlations among them. Ahmed et al. (2009) have found that drought-induced reductions in leaf pigments are considered to be typical oxidative stress indicators, which might be attributed to pigment photo-oxidation, chlorophyll degradation and / or chlorophyll synthesis deficiency. Damaged chloroplasts, reduced thylakoids and deteriorated thylakoid membranes have been found under drought stress (Zhang et al., 2012; Han et al., 2013; Chen et al., 2014), which may also be linked to the lower photosynthetic pigment contents under drought stress. Additionally, under drought stress, the decreased pigment contents detected in all grafting combinations also caused a decrease in the maximum quantum yield of PSII (F_v/F_m), which indicated that the pigment breakdown was accompanied by a decrease in the maximum photochemical efficiency. F_v/F_m is a reliable diagnostic indicator of photosynthetic activity. In particular, chlorophyll fluorescence can give insights into the ability of a plant to tolerate environmental stresses and into the extent to which those stresses have damaged the photosynthetic apparatus (Maxwell and Johnson, 2000; Roháček, 2002). Our results revealed that F_v/F_m of all grafting combinations decreased significantly under drought stress, and F_v/F_m of P/P and P/S was also significantly higher than that of S/S and S/P, just like P_n. Furthermore, the higher F_v/F_m observed in P/P and P/S suggests less disorder in the electron transport chain of PSII under drought (Zhang et al., 2012), which helps to ensure a higher P_n.

In this study, we used the carbon isotope composition (δ^{13}C) and leaf relative water content (RWC) to assess water use efficiency (WUE) of grafted plants. As a long-term indicator of WUE, δ^{13}C significantly increases under drought stress (Chen et al., 2014; Kenney et al., 2014; Dong et al., 2016), and RWC significantly decreases with increasing drought stress (Cocozza et al., 2010; Zhang et al., 2012). Our results were in a good agreement with previous studies, as δ^{13}C significantly increased and RWC significantly decreased in all grafting combinations. Furthermore, δ^{13}C and RWC of P/S and S/S were higher than those of P/P and S/P when exposed to drought stress conditions, which also was consistent with previous studies showing that rootstock traits contribute to plant resistance to drought (Han et al., 2013; Rolli et al., 2015). The higher δ^{13}C and RWC values of P/S and S/S are good for maintaining normal physiological functions and improving water use efficiency. Moreover, the increased water use efficiency could also mitigate the reduction of P_n caused by drought stress (Ma et al., 2010).

Non-structural carbohydrates (NSCs) are a major part of photosynthesis and play an important role in plant growth, physiological processes and resistance to stresses (Adams et al., 2013; Guo et al., 2016). As one kind of important osmotic adjustment substances in plants, NSCs are mainly used to lower the osmotic potential and maintain the normal turgor of the cells in order to reduce harmful effects on plants (Muller et al., 2011; Blum, 2017). Based on our results, we anticipate that *S. rehderiana*-rooted combinations (P/S and S/S) might survive longer than *P. cathayana*-rooted combinations under intensifying drought stress. The average leaf starch content is apparently correlated with the survival time under drought (Dickman et al., 2015). Comparably in this study, we found that under drought stress, the leaf starch concentration of P/S and S/S significantly increased while that of P/P and S/P decreased, which indicated that P/S and S/S have stronger resistance to drought stress. Another important factor is that the storage of NSCs, such as starch and soluble sugars, are thought to be critical for survival under stress and disturbance (Regier et al., 2009; Palacio et al., 2014). Our results showed that when compared to well-watered conditions, the reductions of fructose, sucrose and TSS concentrations were smaller in P/S and S/S than in P/P and S/P, which also indicated that *S. rehderiana* has a stronger resistance to drought stress.

The successful grafting between *Populus* and *Salix* conducted in our study will have important ecological applications. *Populus* and *Salix* are often used as urban street trees, and both of them are dioecious. We can change female trees to males by grafting to solve the problem of flocculation, which is caused by mature female seeds. Additionally, most studies have found that some *Salix* species could be used for phytoextraction of heavy metals (such as Cd, Cr, Cu, Ni, Pb and Zn) from the rhizosphere (Baum et al., 2006; Meers et al., 2007; Regvar et al., 2010; Vaculík et al., 2012). Moreover, *Salix* shows a higher heavy metal tolerance and a greater accumulation ability when compared to *Populus* species (Utmazian et al., 2007; Zacchini et al., 2011). Therefore, we can graft *Populus* onto *Salix* to improve the ability of absorption and transport of soil heavy metals, which would play an important role in the process of land reclamation.

In conclusion, the present study confirmed our hypothesis that *S. rehderiana* has a stronger drought resistance than does *P. cathayana*, and we also demonstrated that grafting *P. cathayana* scions onto *S. rehderiana* rootstocks is an effective approach to improve growth and resistance under drought stress conditions. We propose that P/S might combine the advantages of *P. cathayana* and *S. rehderiana*, although the molecular mechanisms need further research. Recent studies have shown that there is genetic information exchange between grafted plants, involving mitochondria, small RNAs and even entire nuclear genomes (Fuentes et al., 2014; Lewsey et al., 2016; Gurdon et al., 2016). Our future research will focus on the mechanisms of grafting on the genome level in woody plants, and we will explore the molecular mechanisms associated with plant resistance.

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Conflict of interest The authors declare that they have no conflict of interest.

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Table 1. The height growth (HG), basal diameter (BD), root dry weight (RDW), stem dry weight (SDW), leaf dry weight (LDW), total dry weight (TDW) and root/aboveground ratio (R/A ratio) of four grafting combinations under well-watered and drought stress conditions (mean ± SE).

Field capacity (%)	Scion/Rootstock	HG (cm)	BD (mm)	RDW (g)	SDW (g)	LDW (g)	TDW (g)	R/A ratio
100	P/P	86.40 ± 2.46 e	7.72 ± 0.21 e	4.94 ± 0.16 f	5.17 ± 0.15 e	6.36 ± 0.39 f	16.48 ± 0.60 e	0.43 ± 0.01 c
	P/S	57.80 ± 2.42 bcd	6.51 ± 0.29 d	3.12 ± 0.32 e	4.21 ± 0.32 d	5.15 ± 0.28 e	12.47 ± 0.89 d	0.33 ± 0.02 b
	S/S	58.80 ± 2.50 cd	5.30 ± 0.12 bc	1.51 ± 0.12 bc	3.22 ± 0.18 d	3.87 ± 0.25 d	8.59 ± 0.53 c	0.21 ± 0.01 a
	S/P	49.00 ± 1.22 bc	4.65 ± 0.20 ab	0.88 ± 0.04 ab	1.79 ± 0.12 b	2.07 ± 0.13 bc	4.75 ± 0.30 b	0.23 ± 0.01 a
30	P/P	60.60 ± 2.50 d	6.14 ± 0.15 cd	2.28 ± 0.13 d	4.19 ± 0.15 d	4.74 ± 0.22 de	11.21 ± 0.25 d	0.26 ± 0.01 a
	P/S	51.20 ± 2.94 bcd	5.50 ± 0.25 bc	1.78 ± 0.06 cd	2.60 ± 0.10 c	2.41 ± 0.14 c	6.84 ± 0.21 c	0.35 ± 0.01 b
	S/S	48.00 ± 1.64 ab	4.60 ± 0.21 ab	0.87 ± 0.03 ab	1.24 ± 0.12 ab	1.20 ± 0.06 ab	3.31 ± 0.19 ab	0.36 ± 0.01 b
	S/P	37.80 ± 2.27 a	3.75 ± 0.13 a	0.38 ± 0.03 a	0.73 ± 0.04 a	0.86 ± 0.06 a	1.97 ± 0.06 a	0.24 ± 0.02 a
	P: F_w	***	***	***	***	***	***	ns
	P: F_g	***	***	***	***	***	***	***
	P:$F_{w×g}$	***	ns	***	*	**	*	***

P, *Populus cathayana*; S, *Salix rehderiana*. Genotype notation is scion/rootstock. F_w, water treatment effect; F_g, grafting type effect; $F_{w×g}$, water treatment × grafting type effect. Values are means ± standard error (n = 5). Within a column, values followed by different letters are significantly different at the $P < 0.05$ level according to Tukey's test. ns, not significant; ** 0.001< $P \leq 0.01$; ***$P \leq 0.001$.

Table 2. Net photosynthesis rate (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and transpiration rate (E) of four grafting combinations under well-watered and drought stress conditions (mean ± SE).

Field capacity (%)	Scion/Rootstock	P_n (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	C_i (μmol mol^{-1})	E (mmol m^{-2} s^{-1})
100	P/P	15.06 ± 0.79 d	0.93 ± 0.04 c	355.68 ± 4.89 b	4.79 ± 0.22 cd
	P/S	14.53 ± 0.84 d	0.92 ± 0.05 c	351.93 ± 3.12 b	5.02 ± 0.19 d
	S/S	11.91 ± 0.58 c	0.75 ± 0.06 b	341.80 ± 5.23 b	4.37 ± 0.24 cd
	S/P	11.10 ± 0.81c	0.66 ± 0.05 b	349.26 ± 3.71 b	3.97 ± 0.22 c
30	P/P	5.96 ± 0.13 b	0.22 ± 0.02 a	309.94 ± 6.51 a	2.89 ± 0.14 b
	P/S	6.07 ± 0.28 b	0.24 ± 0.02 a	315.55 ± 4.83 a	2.57 ± 0.17 b
	S/S	3.61 ± 0.18 a	0.14 ± 0.02 a	308.28 ± 8.28 a	1.56 ± 0.07 a
	S/P	2.33 ± 0.15 a	0.12 ± 0.03 a	292.06 ± 5.42 a	1.35 ± 0.15 a
	P: F_w	***	***	***	***
	P: F_g	***	***	ns	***
	P:$F_{w×g}$	ns	ns	**	ns

P, *Populus cathayana*; S, *Salix rehderiana*. Genotype notation is scion/rootstock. F_w, water treatment effect; F_g, grafting type effect; $F_{w×g}$, water treatment × grafting type effect. Values are means ± standard error (n = 5). Within a column, values followed by different letters are significantly different at the $P < 0.05$ level according to Tukey's test. ns, not significant; *0.01 < P < 0.05; ** 0.001 < $P \leq 0.01$; ***$P \leq 0.001$.

Table 3. Maximum efficiency of PSII (F_v/F_m), maximum effective quantum yield of PSII (Yield), photochemical quenching coefficient (qP) and non-photochemical quenching coefficient (qN) of four grafting combinations under well-watered and drought stress conditions (mean ± SE).

Field capacity (%)	Scion/Rootstock	F_v/F_m	Yield	qP	qN
100	P/P	0.65 ± 0.02 d	0.80 ± 0.00 c	0.70 ± 0.04 c	0.65 ± 0.02 c
	P/S	0.64 ± 0.02 d	0.78 ± 0.01 c	0.75 ± 0.04 c	0.63 ± 0.01 bc
	S/S	0.63 ± 0.01 cd	0.75 ± 0.01 bc	0.74 ± 0.03 c	0.61 ± 0.02 bc
	S/P	0.61 ± 0.02 cd	0.75 ± 0.02 bc	0.68 ± 0.03 bc	0.59 ± 0.02 abc
30	P/P	0.55 ± 0.02 c	0.68 ± 0.02 ab	0.49 ± 0.01 a	0.56 ± 0.02 abc
	P/S	0.56 ± 0.01 c	0.74 ± 0.02 bc	0.57 ± 0.01 ab	0.57 ± 0.02 abc
	S/S	0.47 ± 0.01 b	0.62 ± 0.04 a	0.56 ± 0.02 ab	0.55 ± 0.02 ab
	S/P	0.38 ± 0.02 a	0.60 ± 0.03 a	0.47 ± 0.01 a	0.50 ± 0.03 a
	P: F_w	***	***	***	***
	P: F_g	***	**	**	*
	P:$F_{w\times g}$	***	**	ns	ns

P, *Populus cathayana*; S, *Salix rehderiana*. Genotype notation is scion/rootstock. F_w, water treatment effect; F_g, grafting type effect; $F_{w\times g}$, water treatment × grafting type effect. Values are means ± standard error (n = 5). Within a column, values followed by different letters are significantly different at the $P < 0.05$ level according to Tukey's test. ns, not significant; * $0.01 < P < 0.05$; ** $0.001 < P \leq 0.01$; *** $P \leq 0.001$.

Table 4. Contributions of all parameters to PC1 and PC2 in four grafting combinations. HG, height growth; BD, basal diameter; RDW, root dry weight; SDW, stem dry weight; LDW, leaf dry weight; TDW, total dry weight; R/A ratio, root/aboveground ratio; P_n, net photosynthesis rate; g_s, stomatal conductance; C_i, intercellular CO_2 concentration; E, transpiration rate; F_v/F_m, maximum efficiency of PSII; Yield, maximum effective quantum yield of PSII; qP, photochemical quenching coefficient; qN, non-photochemical quenching coefficient; $\delta^{13}C$, carbon isotope composition; RWC, relative water content; TSS, total soluble sugar.

	PC1	PC2
HG	0.806	0.189
BD	0.863	0.316
RDW	0.875	0.335
SDW	0.867	0.205
LDW	0.874	0.111
TDW	0.893	0.211
R/A ratio	0.452	0.658
P_n	0.914	-0.337
g_s	0.865	-0.407
C_i	0.701	-0.541
E	0.892	-0.347
F_v/F_m	0.876	-0.220
Yield	0.707	-0.329
qP	0.681	-0.493
qN	0.747	-0.147
$\delta^{13}C$	-0.687	0.500
RWC	0.804	-0.462
Carotenoids	0.965	-0.052
Chlorophyll a	0.953	-0.038
Chlorophyll b	0.859	-0.378
Total chlorophyll	0.963	-0.113
Root starch	0.833	0.439
Stem starch	0.745	0.464
Leaf starch	0.391	0.656
Root fructose	0.823	-0.142
Stem fructose	0.873	0.021
Leaf fructose	0.864	-0.272
Root sucrose	0.902	0.040
Stem sucrose	0.859	0.408
Leaf sucrose	0.897	0.083
Root TSS	0.740	0.334
Stem TSS	0.684	0.643
Leaf TSS	0.822	0.239

Figure legends

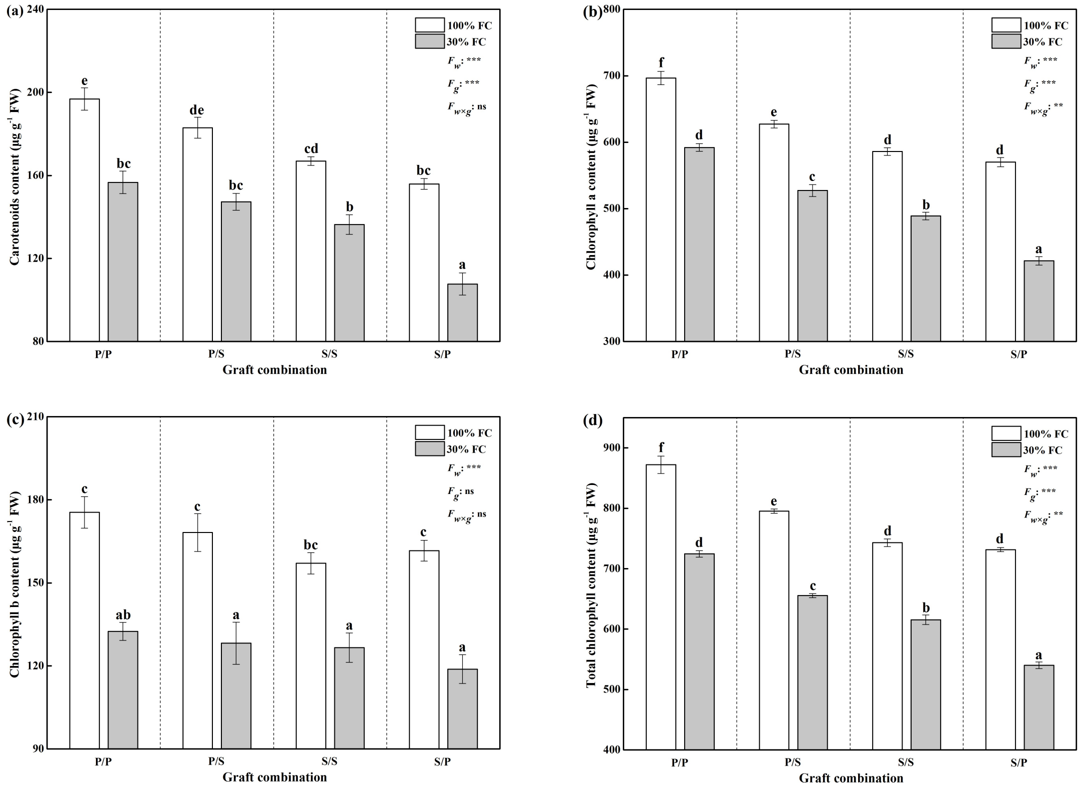
Figure 1. Carotenoid (a), chlorophyll a (b), chlorophyll b (c) and total chlorophyll (d) contents of four grafting combinations under well-watered and drought stress conditions. P, *Populus cathayana*; S, *Salix rehderiana*. F_w, water treatment effect; F_g, grafting type effect; $F_{w\times g}$, water treatment × grafting type effect. Values are the means ± standard error (n = 5). Different letters indicate significant differences at the $P < 0.05$ level according to Tukey's test.

Figure 2. $\delta^{13}C$ (a) and RWC (b) of four grafting combinations under well-watered and drought stress conditions. P, *Populus cathayana*; S, *Salix rehderiana*. $\delta^{13}C$, carbon isotope composition; RWC, relative water content. F_w, water treatment effect; F_g, grafting type effect; $F_{w\times g}$, water treatment × grafting type effect. Values are the means ± standard error (n = 5). Different letters indicate significant differences at the $P < 0.05$ level according to Tukey's test.

Figure 3. Non-structural carbohydrate concentrations of different organs in four grafting combinations under well-watered and drought stress conditions. P, *Populus cathayana*; S, *Salix rehderiana*. F_w, water treatment effect; F_g, grafting type effect; $F_{w\times g}$, water treatment × grafting type effect. Values are the means ± standard error (n = 5). Different letters indicate significant differences at the $P < 0.05$ level according to Tukey's test.

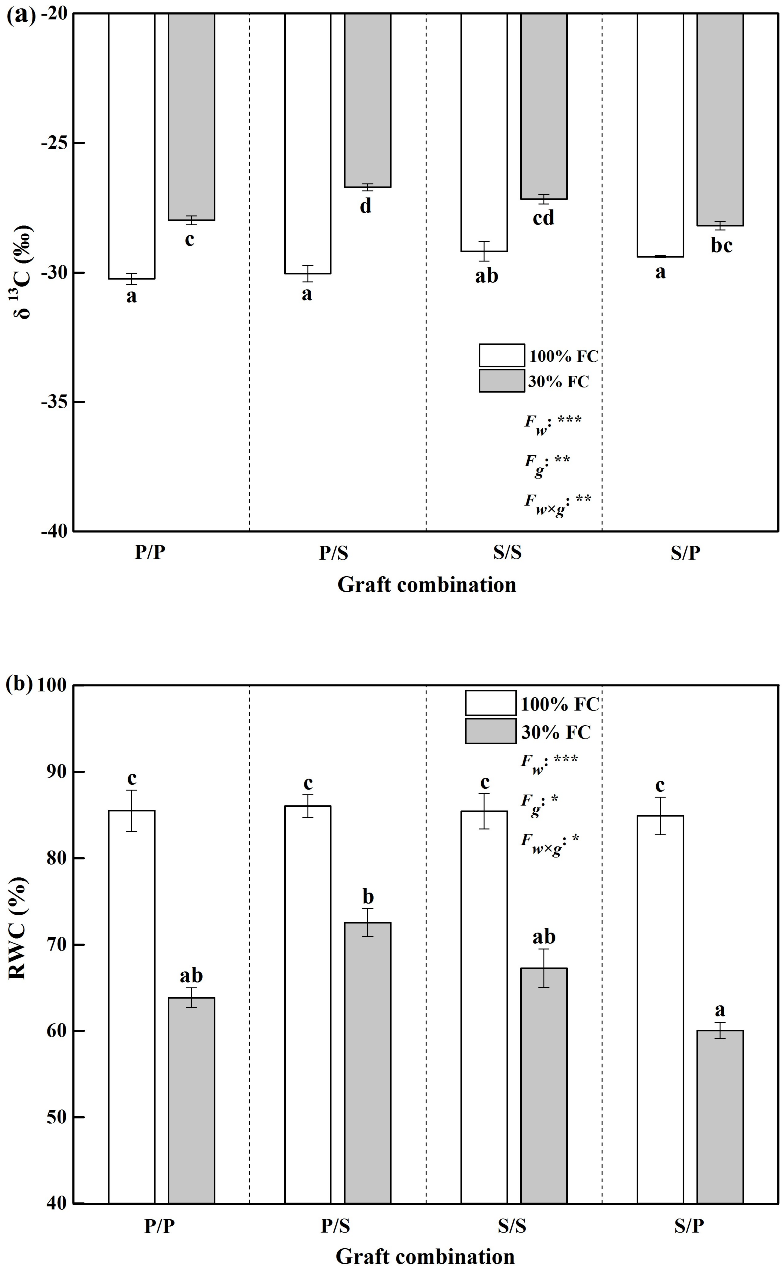
Figure 4. PCA plots of four grafting combinations under well-watered and drought stress conditions. P/P-C, P/P under well-watered conditions; P/S-C, P/S under well-watered conditions; S/S-C, S/S under well-watered conditions; S/P-C, S/P under well-watered conditions; P/P-D, P/P under drought stress conditions; P/S-D, P/S under drought stress conditions; S/S-D, S/S under drought stress conditions; S/P-D, S/P under drought stress conditions; PC1, principal component 1; PC2, principal component 2.

Figure 1



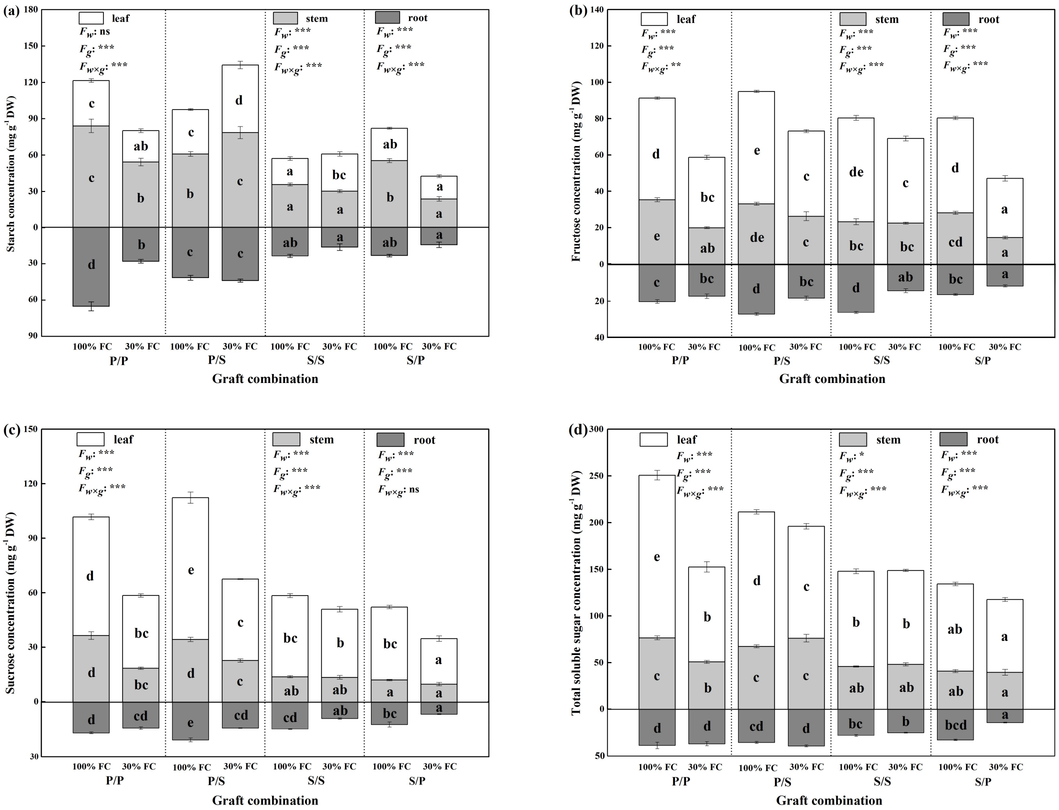
(a)
Carotenoids content (μg g^{-1} FW)
(b)
Chlorophyll a content (μg g^{-1} FW)
(c)
Chlorophyll b content (μg g^{-1} FW)
(d)
Total chlorophyll content (μg g^{-1} FW)
100% FC
30% FC
P/P
P/S
S/S
S/P
Graft combination

Figure 2



(a)
δ 13C (‰)
-20
-25
-30
-35
-40
c
d
cd
bc
a
a
ab
a
100% FC
30% FC
F_w: ***
F_g: **
$F_{w×g}$: **
P/P
P/S
S/S
S/P
Graft combination
(b)
RWC (%)
100
90
80
70
60
50
40
100% FC
30% FC
F_w: ***
F_g: *
$F_{w×g}$: *
c
c
c
c
ab
b
ab
a
P/P
P/S
S/S
S/P
Graft combination

Figure 3



(a)
(b)
(c)
(d)
leaf
stem
root
Starch concentration (mg g-1 DW)
Fructose concentration (mg g-1 DW)
Sucrose concentration (mg g-1 DW)
Total soluble sugar concentration (mg g-1 DW)
100% FC
30% FC
P/P
P/S
S/S
S/P
Graft combination

Figure 4