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Growth, gas exchange, and water-use efficiency response of two young apple cultivars to drought stress in two scion-one rootstock grafting system

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Abstract

The combination of two scion-one rootstock was used for two apple cultivars, 'Pink Lady' and 'Qinguan', budded on the same, one-year-old *Malus hupehensis* (Pamp.) Rehd. to reduce the impact of root and pot size and in order to understand the growth, water-use efficiency (WUE), and chlorophyll fluorescence characteristics. The two-scion grafted trees were planted in plastic pots under two water regimes, *i.e.* 70% field capacity (FC) and 55% FC. Results indicated that different scions were affected differently by drought stress. 'Pink Lady' had higher net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (E) compared with 'Qinguan' under both water treatments. However, 'Qinguan' had lower minimal fluorescence (F_0), higher maximum fluorescence (F_m), and higher maximum photochemical efficiency of photosystem II (F_v/F_m) than 'Pink Lady' at 55% FC. Moreover, 'Qinguan' had larger shoot dry mass (ShDM) and higher intrinsic WUE_I than 'Pink Lady' under both water status. Gas-exchange and growth parameters, except for P_N and scion diameter, were significantly affected by the cultivar and water treatment. At 70% FC, ShDM was significantly correlated with WUE_I. Moreover, WUE_I was negatively linearly correlated with g_s at either 70 or 55% FC. These results might indicate that 'Pink Lady' was more sensitive to drought than 'Qinguan'. 'Qinguan' apple was able to improve WUE more than 'Pink Lady' under both well-watered and drought conditions. The growth parameters and photosynthetic capacity of two different scions showed that the combination of double scion-one rootstock might eliminate the influences of the rootstock and pot size.

Additional key words: chlorophyll fluorescence; field capacity; growth parameters; *Malus hupehensis*.

Introduction

Drought can adversely affect production of most crops worldwide (Jiang and Zhang 2002, Connor and Fereres 2005, Zhang *et al.* 2010). Most of soil water is transpired by stomata. When drought occurs, stomata close to prevent a water loss, at the expense of photosynthesis, while intrinsic water-use efficiency (WUE_I) may increase. If drought stress is prolonged, withering, defoliation, and death of plants may occur (Nar *et al.* 2009, Wang *et al.* 2012). The limitations imposed by drought on net photosynthesis (P_N) have been traditionally analyzed in terms of stomatal and non-stomatal limitations (Egea *et al.* 2011), the former resulting from the resistance imposed by stomatal conductance and the latter often assumed as a metabolic constraint (Fanaei *et al.* 2009).

Apple (*Malus domestica* Borkh.) is one of the most widespread fruit trees in the world, and a part of them is cultivated in arid and semi-arid areas (Liu *et al.* 2012b). Apple trees can respond and adapt to water stress by altering their physiological, biochemical, and biomolecular processes, which invoke various defense mechanisms (Bohnert and Jensen 1996, Massonnet *et al.* 2007). Nowadays, water-use efficiency is an important index that indicates how plants adapt to adverse condition (Li *et al.* 2009). Water-use efficiency can be described as intrinsic water-use efficiency (WUE_I), instantaneous water-use efficiency (WUE = P_N/E), and long-term water-use

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Abbreviations: Chl – chlorophyll; DMG – plant dry mass gain; E – leaf transpiration rate; FC –field capacity; F_m – maximum fluorescence; F_v/F_m – maximum photochemical efficiency of PSII; F_0 – minimum fluorescence; g_s – stomatal conductance; LDM – leaf dry mass; PDT – plant daily transpiration; P_N – net photosynthesis rate; PSII – photosystem II; RDM – rootstock dry mass; SD – scion diameter; SH – shoot height; ShDM – shoot dry mass; StDM – stem dry mass; S/R – shoot/root ratio; WUE – water-use efficiency;WUE_I – intrinsic water-use efficiency; WUE_L – long-term water-use efficiency.
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efficiency (WUE_L). WUE_I was defined as P_N/g_s by Comstock and Ehleringer (1992), and WUE_L as the ratio of dry matter production to water use (Anyia and Herzog 2004, Liu *et al*. 2012a).

A large number of experiments concerning the plant growth is often conducted in some kind of pots, where the pot size has significant effect on a root volume and the plant growth, which may affect the relative differences between treatments (Poorter *et al*. 2012). Considering the influence of the pot size, two scion-one rootstock grafting system was used in our experiment to compare the gas exchange and water-use efficiency performance of two cultivars. The objective of this experiment was to investigate the different responses of 'Qinguan' and 'Pink Lady' to drought stress in such grafting system.

Materials and methods

Plant material and experimental design: This experiment was carried out in the greenhouse of Northwest A & F University, Yangling, China (34°20'N, 108°24'E) in 2011. In late March, 'Pink Lady' and 'Qinguan' apple trees were grafted on the same rootstock of one-year-old *M. hupehensis* (Pamp.) Rehd. The combination of two scion-one rootstock was 'Y' shaped. The trees were planted in about 8 L plastic pots containing a soil/sand/cow dung mixture 1:1:1 (v/v/v). After careful nursery, 12 uniform and healthy plants were selected and treated in two watering regimes, *i.e.* drought stress was maintained at 55% of soil moisture content (field capacity, FC) and control, well-watered conditions were maintained at 70% FC, between July 7 and August 22. During the experiment, the evapotranspiration loss was supplemented gravimetrically by weighing the pots every other day. FC was calculated as follows:

$$FC = (SM - DM)/DM$$

where SM was the mass of saturated soil and DM was the mass of dry soil. The pot masses were calculated for 70% and 55% FC according to the above equation. SM was determined after the pots were saturated for 24 h (the gravimetric water was allowed to drain). Then the soil was dried and DM was determined.

Gas-exchange and Chl fluorescence measurements: Leaf gas-exchange parameters were measured on upper, fully expanded leaves with a combined open gas-exchange system (*LI-6400-40*, *Li-Cor*, Lincoln, NE, USA) on sunny day in the greenhouse. The measured parameters included P_N, g_s, and E. Photosynthetic photon flux density was maintained at 1,000 µmol m^{-2} s^{-1} by using internal red/blue LED light source, CO_2 concentration was set to 400 µmol mol^{-1} by mixing external air with CO_2 from a source attached to the unit. Leaf temperature was 33–35°C and ambient humidity was 33–46%. The measurements were carried out between 10:00–11:00 h on 4 samples per treatment. Chl fluorescence parameters were measured between 20:00–21:00 h, by using the fluorescence chamber of a *LI-6400-40*, leaves were dark adapted in advance for 1 h. When F_0 was measured, a strong saturating flash (duration of 0.8 s, intensity of about 1,800 µmol m^{-2} s^{-1}) was used to measure F_m. Maximum photochemical efficiency of PSII (F_v/F_m) was calculated as follows:

$$F_v/F_m = (F_m - F_0)/F_m$$

Growth parameters: Shoot height was recorded from the grafting point to the top bud of the main stem. A scion and rootstock diameters were measured with a digital micrometer 2 cm from the top and the bottom of the grafting point, respectively. Finally, the plants were harvested, divided into leaf, stem, and rootstock and oven-dried for at least 48 h at 80°C before weighing. Shoot dry mass (ShDM) and shoot/root ratio (S/R) were calculated as follows:

$$ShDM = LDM + StDM$$
$$S/R = ShDM/RDM$$

where LDM was the leaf dry mass; StDM was the stem dry mass; and RDM was the rootstock dry mass.

WUE: WUE_I was calculated as P_N/g_s. WUE_L was calculated using this formula:

$$WUE_L = DMG/TIW$$

where DMG was plant dry mass gain and TIW was total amount of irrigation water.

Plant daily transpiration (PDT) was defined as:

$$PDT = MPM - EPM$$

where MPM and EPM were the pot mass in the morning and in the evening, respectively.

ShDM of cultivars could replace WUE_L because of sharing the same rootstock and total amount of irrigation water.

Statistical analysis: Values were expressed as mean ± SD of at least 4 replications. One- or two-way *ANOVA* comparisons between means were made by *Origin 8.0* following the *Fisher*'s LSD test at $P<0.05$. Significant inter-variable correlations were determined with a *Pearson*'s coefficient test at $P<0.05$ or $P<0.01$.

Results

Gas exchange and Chl fluorescence: Drought induced decrease of P_N, E, g_s, and PDT in both scions (Table 1). Values of P_N in 'Pink Lady' and 'Qinguan' were reduced by 33.7 and 35.8%, respectively. Values of g_s were reduced by 59.4 and 69.2%, respectively. The reduction of E was 49.3 and 59.4%, respectively. Values of P_N, E, and, g_s in 'Pink Lady' were higher than in 'Qinguan' for both water treatments. PDT was 0.36 kg d^{-1} in two scion-one rootstock under 55% FC, and it was significantly lower than that at 70% FC (0.82 kg d^{-1}). E and g_s were significantly affected by varieties (Table 2). All gas-exchange parameters were also significantly affected by irrigation, but the interaction of varieties and water treatments showed no significance (Table 2). At 70% FC, correlations between P_N, g_s, and E were significantly linear and they were enhanced by drought stress (Table 3).

Drought stress increased F_0 and decreased F_m and F_v/F_m in the dark adapted leaves. The cultivar 'Qinguan' showed higher F_v/F_m (0.82) under drought than 'Pink Lady' (0.80), while both cultivars had the same value at 70% FC (Table 1).

Table 1. Two scion-one rootstock trees preformed on net photosynthetic rate (P_N), stomatal conductance (g_s), leaf transpiration rate (E), intrinsic water-use efficiency (WUE_I), minimum fluorescence (F_0), maximum fluorescence (F_m), maximum photochemical efficiency of PSII (F_v/F_m), shoot dry mass (ShDM), and plant daily transpiration (PDT) at 70% and 55% soil field capacity (FC). Data are expressed as a mean ± SD (n = 4). *Different letters* in a column mean significant difference at P<0.05 by *Fisher*'s least significant (LSD) test. Last column stands for parameters at 55% FC divided by parameters at 70% FC.

Parameter	Cultivar	70% FC	55% FC	[%]
P_N [μmol(CO_2) m^{-2} s^{-1}]	'Pink Lady'	21.66 ± 1.54^a	14.36 ± 3.23^b	66.4
	'Qinguan'	20.39 ± 2.45^a	13.10 ± 1.62^b	64.2
g_s [mol(H_2O) m^{-2} s^{-1}]	'Pink Lady'	0.32 ± 0.04^a	0.13 ± 0.04^b	40.6
	'Qinguan'	0.26 ± 0.03^a	0.08 ± 0.03^b	30.8
E [mmol(H_2O) m^{-2} s^{-1}]	'Pink Lady'	9.36 ± 0.54^a	4.75 ± 1.25^b	50.7
	'Qinguan'	7.96 ± 0.70^a	3.23 ± 1.02^b	40.6
WUE_I [μmol mol^{-1}]	'Pink Lady'	67.8 ± 4.8^b	112.6 ± 9.7^a	166.1
	'Qinguan'	79.4 ± 10.2^b	162.1 ± 31.5^a	204.2
F_0	'Pink Lady'	165.9 ± 32.1^a	195.4 ± 10.1^a	-
	'Qinguan'	179.8 ± 1.6^a	187.2 ± 11.6^a	-
F_m	'Pink Lady'	951.7 ± 15.5^a	948.7 ± 10.1^a	-
	'Qinguan'	$1,052.1 \pm 81^a$	$1,027.5 \pm 56.4^a$	-
F_v/F_m	'Pink Lady'	0.83 ± 0.02^a	0.80 ± 0.01^a	-
	'Qinguan'	0.83 ± 0.01^a	0.82 ± 0.01^a	-
ShDM [g]	'Pink Lady'	20.00 ± 2.39^a	9.63 ± 2.06^b	48.0
	'Qinguan'	30.86 ± 1.95^a	10.49 ± 1.05^b	34.0
PDT [kg d^{-1}]	-	0.82 ± 0.14^a	0.36 ± 0.02^b	43.9

Table 2. Two-way *ANOVA* (F value) for effect of cultivar and watering regime on some morphological and gas exchange parameters in two scion-one rootstock graft system. E – leaf transpiration rate; P_N – net photosynthetic rate; g_s – stomatal conductance; WUE_I – intrinsic water-use efficiency; ShDM – shoot dry mass; SD – scion diameter; S/R – shoot/root ratio; StDM – stem dry mass; LDM – leaf dry mass; C × W – interaction of cultivar and water treatment. Level of significance: $^*P$<0.05, $^{**}P$<0.01. n = 8.

	E	P_N	g_s	WUE_I	ShDM	SH	SD	S/R	StDM	LDM
Cultivar (C)	7.56^*	0.89	6.97^*	9.22^*	38.77^{**}	8.92^{**}	0.06	15.76^{**}	14.03^{**}	5.16^*
Watering (W)	77.23^{**}	29.73^{**}	79.66^{**}	40.22^{**}	264.1^{**}	49.49^{**}	38.84^{**}	31.95^{**}	51.20^{**}	62.90^{**}
C × W	0.01	9.91E–5	0.21	3.54	28.28^{**}	5.88^*	1.98	7.15^*	8.06^*	4.91^*

Growth parameters: SD and SH of 'Pink Lady' were reduced at 55% FC by 23.1 and 37.2%, respectively (Figs. 1,2). Decrease in SD and SH of 'Qinguan' was 33.2 and 53.5%, respectively. The rootstock diameter was also reduced by 24.4% at 55% FC. All dry mass parts of two scion-one rootstock combination were significantly affected by irrigation (P<0.05). The drought induced percentage reductions of ShDM, LDM, and StDM in 'Qinguan' were larger than the reductions in 'Pink Lady'. But all parts of 'Qinguan' had higher dry mass than those of 'Pink Lady' even at 55% FC (Fig. 3). Moreover, S/R of 'Pink Lady' was smaller than that of 'Qinguan' even under drought stress. Drought reduced S/R of 'Qinguan' more severely than that of 'Pink Lady'. SH, S/R, and

Table 3. *Pearson*'s coefficients of correlations (*r* values) among net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), shoot/root ratio (S/R), intrinsic water-use efficiency (WUE_I), and shoot dry mass (ShDM) under 70% and 55% soil field capacity (FC), n = 8. Level of significance: $^*P<0.05$.

Treatment	Parameter	g_s	E	S/R	WUE_I
70% FC	P_N	0.69^*	0.54^*		
	g_s		0.96^*		
	WUE_I	-0.81^*			
	ShDM			0.86^*	0.68^*
55% FC	P_N	0.90^*	0.90^*		
	g_s		0.99^*		
	WUE_I	-0.88^*			
	ShDM			0.47	–0.05

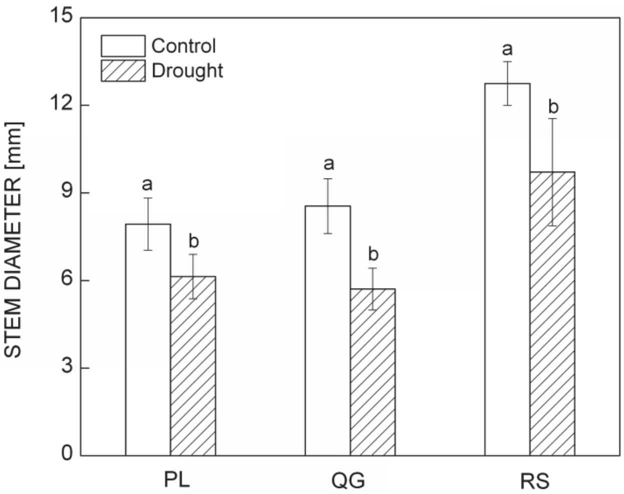

Fig. 1. Stem diameter of two scion-one rootstock trees at 70% (control) or 55% (drought) soil field capacity. PL – Pink Lady; QG – Qingguan; RS – rootstock. *Different letters* indicate significant differences between treatments (P<0.05). Data are the mean ± SD, n = 4.

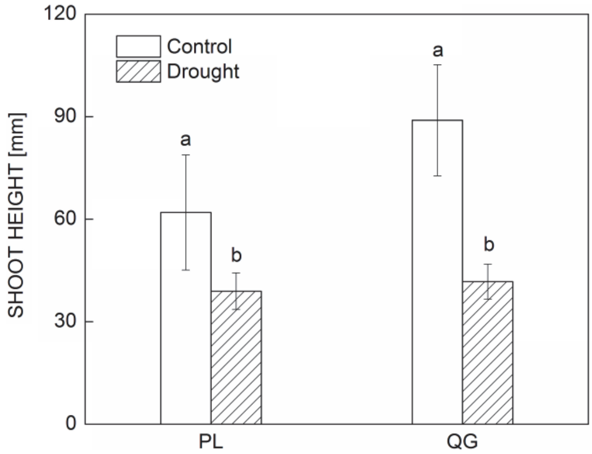

Fig. 2. Shoot height of two scion-one rootstock trees at 70% (control) or 55% (drought) soil field capacity. PL – Pink Lady; QG – Qingguan. *Different letters* indicate significant differences between treatments (P<0.05). Data are the mean ± SD, n = 4.

LDM were significantly affected by varieties, water treatments, and their interaction. However, SD was significantly affected only by water treatment (Table 2).

WUE: WUE_I of both 'Pink Lady' and 'Qinguan' was significantly larger at 55% FC than at 70% FC (Table 1). Cultivar 'Qinguan' had higher WUE_I than 'Pink Lady' under both drought and control conditions. WUE_I was significantly affected by cultivar and water treatment (Table 2). WUE_I exhibited significant negative correlation with g_s at both 70 and 55% FC (Table 3). At 55 or 70% FC, ShDM of 'Qinguan' was larger than that of 'Pink Lady'. Furthermore, drought reduced ShDM of 'Qinguan' and 'Pink Lady' by 66.6 and 52.7%, respectively (Table 1). ShDM was significantly affected by cultivar, water treatment, and their interaction. A strong positive correlation was found between S/R and ShDM at 70% FC, but not at 55% FC. In addition, ShDM was significantly correlated to WUE_I at 70% FC, but not at 55% FC (Table 3).

Discussion

Growth parameters of both 'Pink Lady' and 'Qinguan' were restricted by water deficit (Figs. 1,2,3). The rootstock and scion diameters, S/R, SH, LDM, and StDM were significantly reduced in comparison with those under well-watered conditions, similarly as it was observed in other plant species (Liu and Stützel 2004, Degu *et al.* 2008, Fernandes-Silva *et al.* 2010).With regard to these growth parameters, the difference between two cultivars was remarkable, except for the scion diameter (Table 2). Moreover, plant growth parameters except for SD were significantly affected by the interaction of cultivar and water treatment. Many reports also declared that cultivar difference led to different performance in growth, even under drought stress (Schultz 2003, Netto *et al*. 2010). In our experiment, 'Qinguan' grew better than 'Pink Lady'. From the DMG point of view, 'Qinguan' was more adaptive to water deficit. However, an index of tolerance to water deficit an index of tolerance to water deficit ($TOL_{wd\text{-}index}$) was reported in many papers (Monclus *et al.* 2006, 2009, Bauerle *et al.* 2011); it is calculated as plant dry or fresh mass of trees under drought treatment divided by plant dry (fresh) mass under well watered conditions. In our study, percentage of ShDM at 55% FC divided by that at 70% FC (Table 1) was similar to $TOL_{wd\text{-}index}$. In this perspective, 'Pink Lady' seemed to be more tolerant to drought than 'Qinguan'.

Values of P_N, g_s, and E declined, when apple trees were exposed to drought (Table 1). However, the degree of reduction differed between the cultivars. Larger

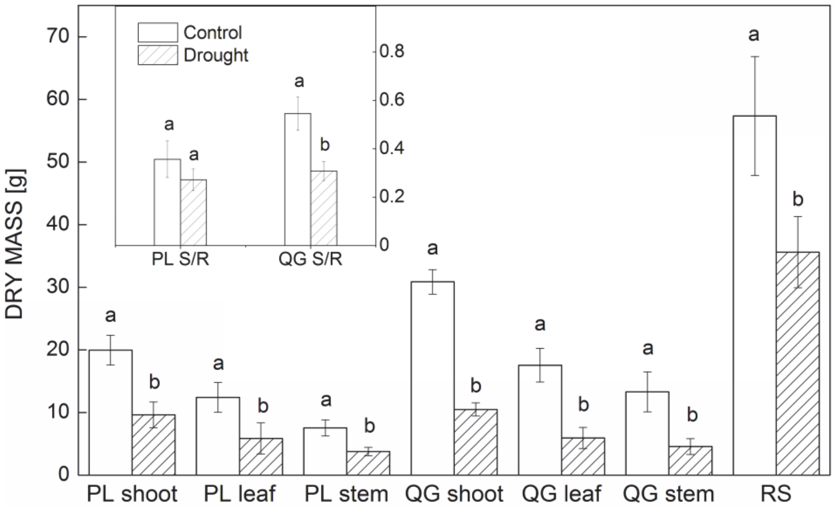

Fig. 3. Dry mass of scions shoot, leaf, stem, rootstock (RS) and shoot/root ratio (S/R) of double scion-one rootstock trees at 70% (control) or 55% (drought) soil field capacity. PL – 'Pink Lady' scion; QG – 'Qinguan' scion. *Different letters* indicate significant differences between treatments ($P<0.05$). Data are the mean ± SD, $n = 4$.

reductions of leaf P_N, g_s, and E under drought may indicate higher sensitivity to drought (Jabbari *et al.* 2009). But tolerant poplar clones showed lower g_s than sensitive ones (Silim *et al.* 2009), and drought-tolerant strawberry subjected to drought stress had lower P_N, g_s, and E than sensitive one (Ghaderi and Siosemardeh 2011). From this point of view, 'Qinguan' was more tolerant to drought, because 'Qinguan' had lower P_N, g_s, and E than 'Pink Lady'. The cultivar 'Qinguan' had lower g_s and larger ShDM than 'Pink Lady' under both water treatments, which seems to be contradictory. In fact, the plant gas exchange and growth can be determined within and between species. Generally, higher g_s permits faster growth (Atkinson *et al.* 2000, Li *et al.* 2002). The cultivar 'Qinguan' had lower P_N and g_s under well watered conditions, but it had larger total dry mass (Liu *et al.* 2012a). Plant growth is a complex process and it could be affected not only by gas-exchange parameters, but also by nutrition and hormone content (Yordanov *et al.* 2000).

On the other hand, 'Qinguan' had higher F_m and F_v/F_m, and lower F_0 than 'Pink Lady' under drought condition. Those results also indicated that 'Pink Lady' was more sensitive to water stress than 'Qinguan'. An increase in F_0 implies PSII inactivation, whereas a decline in F_m may indicate nonphotochemical quenching or close to the reaction center (Li *et al.* 2006, Elsheery and Cao 2008). It is well known that F_v/F_m is the maximum quantum yield of primary photochemical reactions in the dark-adapted leaves. When plants are exposed to stress, values of F_v/F_m are reduced, in particular, indicating the phenomenon of photoinhibition or the degree of damage to PSII complex (Liu *et al.* 2012c).

Different cultivars may use different water-use strategies to fight against water deficit. Plants under drought stress can invoke basically two water-use strategies: drought tolerance and water consumption (Chirino *et al.* 2011). The cultivar 'Qinguan' was more conservative in water use than 'Pink Lady', because the reduction of g_s limits P_N, E, and improves WUE_I. This result is similar to the study of Massonnet *et al.* (2007), in which 'Braeburm' was more conservative in water use than 'Fuji', due to stomatal limitation of P_N, higher WUE_I, and lower leaf carbon isotope discrimination. In our study, two cultivars shared one rootstock and they were irrigated in the same way to maintain 70% or 55% FC. Therefore, the difference of ShDM could indicate the difference of WUE_L. The cultivar 'Qinguan' had higher WUE_I and WUE_L. Our results agreed with Liu *et al.* (2012a), who reported 'Qinguan' had higher biomass production and WUE_L under drought stress than other 29 apple cultivars including 'Pink Lady'. However, WUE_I and WUE_L showed no linear correlation at 55% FC (Table 3). This result conformed to Tomás *et al.* (2012), who found out that none of the leaf-level estimates of water-use efficiency showed any significant or consistent correlation with whole-plant biomass-based WUE_L.

One scion-one rootstock combination was the main method in studying different treatments of scion-rootstock combination (Gonçalves *et al.* 2005, Rodríguez-Gamir *et al.* 2010, Zhen *et al.* 2010). Few studies have used combinations of one scion-double roots or double scion-one root (Dodd 2007, Dodd *et al.* 2008, Notaguchi *et al.* 2009, Tandonnet *et al.* 2010, Li *et al.* 2012). When we evaluated different WUE_L in different, potted cultivars, we had to evaluate RDM. The longer experiment time means the greater pot size effect. Dodd *et al.* (2008) advised to avoid the ratio of plant biomass to pot volume larger than 2 g L^{-1}. The lowest RDM in our experiment was 35.59 g (Fig. 2), which needed a 17.8 L pot to avoid the pot size effect. Therefore we used the two scion-one rootstock grafting system, which could eliminate the effect of the pot size.

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