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Growth, gas exchange, water-use efficiency, and carbon isotope composition of 'Gale Gala' apple trees grafted onto 9 wild Chinese rootstocks in response to drought stress

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Abstract

To determine the effects of rootstock choice on the scion response to drought stress, we compared the vegetative growth, biomass accumulation, gas exchange, and water-use efficiency (WUE) of 'Gale Gala' apple (*Malus domestica* Borkh.) trees grafted onto nine wild Chinese *Malus* rootstocks. Compared with the well-watered control, drought treatment limited growth, as manifested by smaller increments in plant height (PH), trunk diameter (TD), total fresh biomass (TB), total dry biomass (TDB), total leaf area (LA), and relative growth rate (RGR). The extent of this effect differed among rootstocks. Stress conditions led to increases in the root/shoot ratio (RSR), leaf thickness (LT), water-holding capacity (WHC), carbon isotope composition (δ^{13}C), and WUE. Decreases were noted in stomatal density (SD), leaf relative water content (RWC), chlorophyll content (Chl), net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s), again varying by rootstock. Those that are generally considered more drought-tolerant, *e.g.*, *M. sieversii*, *M. prunifolia*, and *M. toringoides*, had smaller declines in PH, TD, TB, TDB, LA, RGR, SD, RWC, Chl, P_N, E, and g_s and proportionally greater increases in RSR, LT, WHC, δ^{13}C, and WUE compared with the drought-sensitive *M. hupehensis* and *M. sieboldii*. These results suggest that moisture stress has a significant dwarfing effect in the latter two species. Based on WUE calculations, trees on drought-tolerant rootstocks showed higher tolerance when stressed, whereas those on drought-sensitive rootstocks were less tolerant, as indicated by their lower WUE values.

Additional key words: carbon isotope composition; gas exchange; growth; *Malus domestica* Borkh.; water-use efficiency.

Introduction

Apple (*Malus domestica* Borkh.) is one of the most economically important fruits worldwide. It is mainly cultivated in arid and semiarid regions such as the Northwest Loess Plateau in China. However, an increasingly serious water deficit in those areas is a major limitation to apple cultivation. Grafting superior, commercial cultivars onto rootstocks capable of alleviating the effects of drought is a promising tool for reducing water losses in production and improving WUE under stress conditions (Schwarz *et al.* 2010). Valuable knowledge about the evolutionary and adaptive senses of intraspecific variability in WUE has already been gained from some important forest species, *e.g.*, *Nothofagus* spp. (Read and Farquhar 1991), *Pinus pinaster* (Brendel *et al.* 2002, Nguyen-Queyrens *et al.* 1998), *Pseudotsuga menziesii*, and *Larix occidentalis* (Marshall and Zhang 1993), and *Castanea sativa* (Lauteri *et al.* 2004). However, little research has been reported concerning the selection and breeding of both rootstocks and scions for fruit trees.

Rootstocks function in water and nutrient uptake, resistance to soil-borne pathogens, and tolerance to environmental stresses (Layne 1987). The selection of rootstock is usually based on its ability to promote strong vegetative growth from the scion and persistence of the root system in a given soil type (Cohen and Naor 2002). Various hypotheses (*see* reviews by Aloni *et al.* 2010,

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Abbreviations: Chl – chlorophyll; g_s – stomatal conductance; LA – total leaf area; LAR – leaf area ratio; LT – leaf thickness; P_N – net photosynthetic rate; PH – plant height; RGR – relative growth rate; RSR – root/shoot ratio; RWC – relative water content; SD – stomatal density; SLA – specific leaf area; E – transpiration rate; TB – total fresh biomass; TD – trunk diameter; TDB – total dry biomass; WHC – water-holding capacity; WUE – water-use efficiency; δ^{13}C – carbon isotope composition.

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Martínez-Ballesta *et al*. 2010, and Schwarz *et al*. 2010) have proposed that the rootstock influences scion vegetative growth mainly by affecting tree water status, mineral nutrition, and hormones.

A good rootstock should be compatible with the scion cultivar, resistant and/or tolerant to pests and diseases, and adaptable to a wide range of soil types and climatic conditions (Hernández *et al*. 2010). Many rootstock types are used for apple cultivation in China, each having particular advantages and limitations when matched to different geographical regions. For example, M.9, M.26, and M.27 are often used as an interstock grafted onto vigorous rootstock to reduce growth in high-density orchards (Di Vaio *et al*. 2009, Smolka *et al*. 2010, Tworkoski and Miller 2007). Bai *et al*. (2010) have reported that *M. hupehensis* is more hypoxia-tolerant than *M. toringoides* and can be used in areas prone to waterlogging. Ma *et al*. (2010) compared the drought tolerance and WUE of 10 *Malus* rootstocks and observed that *M. prunifolia* and *M. sieversii* are the best in those aspects. Previous studies of performance by commercial apple cultivars focused mainly on clonal rootstocks (Cohen *et al*. 2007, Di Vaio *et al*. 2009, Larsen *et al*. 1992, Samad *et al*. 1999, Zeller *et al*. 1991a,b). Little researches were reported on the influence of wild Chinese rootstocks on the growth and WUE of apple trees under a variety of environmental stresses.

Here, we compared growth parameters for *M. domestica* cv. 'Gale Gala' trees grafted onto nine wild rootstocks originating from China. We assessed the influence of rootstock on scion vegetative growth, biomass accumulation, gas exchange, and WUE. Our goal was to provide useful information about the selection of stock–scion combinations that are most appropriate for arid and semiarid regions.

Materials and methods

Plants and experimental design: One-year-old *Malus domestica* cv. 'Gale Gala' trees were grafted onto nine rootstocks. Their seeds had been collected from plants growing in their native regions within China (Table 1). For bud-grafting, we used one-year-old seedlings produced from those seeds at Northwest A&F University, Yangling (34°20′N, 108°24′E), Shaanxi, China. All plants were greenhouse-grown in plastic pots (38 cm × 23 cm, volume adequate for root growth) filled with a local topsoil:sand:grass peat mix (5:1:1, v:v:v) (pH = 8.31; bulk density, 0.84 g cm^{-3}; water field capacity, 18.3%; gravel content, 14.3%; and content of organic matter and available N, P, and K were 0.95 g kg^{-1}, 50.3 mg kg^{-1}, 31.0 mg kg^{-1}, and 50.7 mg kg^{-1}, respectively). Plants were grown without supplemental illumination at 20/25°C (day/night) and a relative humidity of 65 to 80%. Prior to the start of our experiments, all trees were irrigated daily and supplied weekly with 100% Hoagland's solution (pH = 6.5). After two months of growth under well-watered conditions, we initiated treatments on 19 May 2009. Two watering regimes were implemented over a 60-d period: (*1*) well-watered control, in which 15 trees grafted onto each rootstock were irrigated every other day to 80% field capacity; and (*2*) drought stress, with 15 trees grafted onto each rootstock being maintained at 50% field capacity by irrigating every other day. All treatments were applied at 18:00 h. Soil field capacities were determined with a digital moisture recorder (*ZTS-II*, Zhejiang, China) and the volume of water added to each pot was recorded for WUE calculations. To avoid edge effects, all pots were rotated weekly. These experiments were terminated on 19 July 2009.

Surface evaporation was minimized by covering the potting media surface with a 3-cm layer of sieved (2 mm) sand. As a reference for comparison, six pots per treatment that did not contain plants were used to determine evaporative water loss from the soil surface throughout the experimental period. Transpiration water loss was evaluated gravimetrically by weighing all pots and calculating the changes in mass that occurred between watering events. The amount of water lost *via* transpiration was then added back to each pot during irrigation.

Table 1. Origins in China for nine *Malus* rootstocks.

Rootstock	Origin	Elevation [m]	Mean annual precipitation [mm]	Mean annual temperature [ºC]
M. hupehensis (Pamp.) Rehd.	Pingyi, Shandong	154–1,156	849	14.1
M. mandshurica (Maxim.) Komarov	Xingcheng, Liaoning	330–702	600	8.7
M. micromalus Hesmel	Changli, Hebei	50–695	713	11.0
M. prunifolia (Willd.) Borkh.	Fuping, Shaanxi	380–1,420	533	13.1
M. robusta Rehd.	Huailai, Hebei	394–1,978	413	10.5
M. sieboldii (Regel.) Rehd.	Lushi, Henan	475–2,058	467	12.6
M. sieversii (Ledeb.) Roem	Yili, Xinjiang	600–770	352	8.3
M. spectabilis (Ait.) Borkh.	Huailai, Hebei	394–1,978	413	10.5
M. toringoides (Rehd.) Hughes	Maerkang, Sichuan	2,180–5,301	753	11.1

Growth: At the end of the experiment, plant height (PH) and trunk diameter (TD) were recorded. Afterward, six plants were harvested from each treatment and divided into leaf, stem, and root portions to determine aboveground (shoot) biomass, root biomass, total fresh biomass (TB), and the root/shoot ratio (RSR, root fresh biomass divided by shoot fresh biomass). Total leaf area (LA) was obtained with an *AM-100 Area Meter* (*Analytical Development Company*, Hertsfordshire, UK). Materials were oven-dried at 70°C to a constant mass before calculating the total dry biomass (TDB) for each tissue type. Specific leaf area (SLA) and the leaf area ratio (LAR) were computed as LA/leaf dry mass and LA/TDB, respectively. The relative growth rate (RGR) was calculated by the standard formula of $RGR = (\ln W_2 - \ln W_1)/(t_2 - t_1)$, where W_1 and W_2 were the initial and final dry biomasses at the beginning and the end of the experiment, respectively, and $(t_2 - t_1)$ was the time interval.

Leaf relative water content (RWC), leaf water-holding capacity (WHC), and content of leaf chlorophyll (Chl) were measured on days 10, 20, 30, 40, 50, and 60 of the experiment. Six fully expanded leaves were collected at midday from the mid-canopy position of treated plants, placed in dishes containing wet filter paper, and weighed immediately to record their fresh mass (FM). Turgid mass (TM) was determined from leaves floated for 24 h in distilled water in a closed container at 4°C under darkness. Dry mass (DM) was determined for those same leaves after oven-drying for 48 h at 70°C. RWC and WHC were calculated as: $RWC = [(FM - DM)/(TM - DM)] \times 100$ and $WHC = (TM - DM)/DM \times 100$, respectively. Chl was extracted from 0.5 g of fresh leaf material for 72 h in the dark, using acetone (80%). Absorbances at 647 and 664 nm were determined with a *Shimadzu UV-/Vis Spectrophotometer* (*UV2401PC*, *Shimadzu*, Columbia, MD, USA) and used to calculate Chl according to the method of García-Sánchez *et al.* (2002). Mean values for RWC, WHC, and Chl recorded at the six time points were used to demonstrate the effect of rootstock choice and soil water status over the experimental period.

Leaf thickness (LT) and stomatal density (SD): Eight healthy, undamaged leaves per treatment were selected at mid-day to record LT and SD with an electron microscope (*H-7650, Hitachi Co.*, Japan) according to the method of Khazaei *et al.* (2009). LT was measured on either side of the mid-vein and averaged. The numbers of stomata were counted under a microscope at 400 × from four different fields of vision for lamina impressions, obtained from four leaves per species and treatment (*i.e.*, 16 different fields of vision). To estimate SD, the number of stomata per field of view was converted to the number of stomata per mm^2 of leaf, using a standard scale.

WUE was defined as the ratio of dry biomass produced to total water transpired during the experimental period. It was calculated as: $WUE = (W_F - W_I)/W_W$, where W_F was the final DM, W_I was the initial DM, and W_W was the total amount of water transpired during the period (Liu *et al.* 2012).

Gas-exchange parameters were measured every 10 d on the 8th leaf from the shoot apex, using a *Li-Cor 6400* portable photosynthesis system (*Li-Cor Inc.*, Lincoln, NE, USA). The net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) were obtained from six plants per treatment. Measurements were made on sunny days (09:00 to 11:00 h) at 1,500 μmol m^{-2} s^{-1} PPFD (light-saturation point of 'Gale Gala' is 1,453 μmol m^{-2} s^{-1}), as provided by a Q-Beam (blue and red diode) light source. Leaf temperature, ambient water vapor pressure, and CO_2 concentration were maintained at 28.7 ± 1.0°C, 1.30 ± 0.15 kPa, and 380 μmol m^{-2} s^{-1}, respectively.

Carbon isotope composition ($\delta^{13}C$): Five apple trees per rootstock and treatment were selected at the end of the experiment. The ninth leaf from the top was taken from each plant, and all five from the same treatment combination were mixed as one sample. These were first oven-dried at 105°C for 0.5 h, then at 70°C to a constant mass after about 72 h. After being ground and sieved, the samples were used to determine their stable $\delta^{13}C$ in a Continuous Flow Isotope Ratio Mass Spectrometer (*Finnegan MAT-251*, *Delta-E*, Bremen, Germany). Stable-isotope ratios were expressed as deviations from the Pee Dee Belemnite (PDB) standard, *i.e.*, $\delta^{13}C$ (‰) = $[(R_P/R_S) - 1] \times 1{,}000$, where R_P and R_S were the $^{13}C/^{12}C$ values for the plant sample and PDB standard, respectively. An atmospheric correction was not necessary in this case because all plants in the greenhouse were exposed to approximately the same conditions (Liu *et al.* 2012).

Statistical analysis: The experiments comprised a nine (rootstock) × two (watering regime) factorial, completely randomized design. Each treatment was replicated six times, with each sample measured four times. Average values for each parameter were the means of six replicates. Data were analyzed with a statistical software package (*SPSS 13.0 for Windows*; *SPSS Inc.*, USA). Treatment means were separated by least significant difference (LSD) tests ($p \leq 0.05$).

Results

Growth: We found significant differences ($p \leq 0.05$) in PH, TD, TB, TDB, LA, RGR, SD, and LT among 'Gale Gala' trees grafted onto nine different rootstocks grown under two watering regimes (Table 2). Under either regime, trees with *M. sieversii* and *M. prunifolia* as their rootstock showed significantly higher values for these parameters than those on *M. hupehensis*. Compared with the well-watered plants, 60 d of drought stress was associated with smaller increments in PH, TD, TB, TDB, and LA. The extent of this effect differed among rootstocks. The smallest gains in height and diameter were noted for *M. hupehensis* (39.69% less than well-watered plants) and *M. toringoides* (27.63%), respectively, compared with *M. sieversii* (21.61%) and *M. spectabilis* (16.32%), respectively. Those on *M. prunifolia* rootstocks showed the widest gap in values for TB (34.49%), TDB (30.98%), and LA (61.01%) when compared with well-watered trees; *M. sieversii* had the smallest decrease in TB (14.92%) and TDB (7.57%), and *M. micromalus* had the smallest decrease in LA (17.08%). Drought reduced the RGR for all trees except those on *M. sieboldii*, with reductions of 40.0% for the *M. hupehensis* rootstock but only 16.8% for *M. sieversii*. Drought was also associated with fewer stomata (decreased SD) – ranging from a decline of 7.94% for *M. sieversii* to 43.35% for *M. hupehensis*. By contrast, leaf thicknesses were increased by drought exposure, with LT rising by 4.00% for the *M. toringoides* rootstock and by 22.96% for trees on *M. prunifolia*.

Rootstock selection had a significant impact ($p \leq 0.05$) on values for RSR, SLA, LAR, RWC, and WHC under both watering regimes (Table 3). Trees grafted onto *M. prunifolia* had significantly higher LAR, RWC, and WHC than those on the other eight rootstocks. Under well-watered conditions, trees on *M. hupehensis* had significantly higher values for RSR and SLA. That rootstock under drought stress induced significantly higher SLA but lower RSR compared with the others. The extent to which trees were affected by reduced irrigation depended upon rootstock choice when RSR, SLA, LAR, RWC, and WHC were considered (Table 3). Stress treatment increased the RSR for all trees except those grafted onto *M. hupehensis*; the greatest increase in that ratio was found with *M. spectabilis*. Drought decreased values for SLA, LAR, and RWC, especially for *M. spectabilis* (12.27%), *M. spectabilis* (11.73%), and *M. sieboldii* (12.46%), respectively. This impact was lowest for trees on *M. toringoides* (0.48% for SLA), *M. sieboldii* (0.56% for LAR), and *M. mandshurica* (4.01% for RWC). Water-holding capacity was increased by drought conditions for all rootstocks except *M. hupehensis*, with the greatest improvement found for trees on *M. micromalus* (6.09%).

Gas exchange and Chl content: Under either watering regime, values for Chl, P_N, E, and g_s were significantly different ($p \leq 0.05$) among trees on all nine rootstocks (Table 4). Plants on *M. sieversii* showed the largest rises in Chl, P_N, and g_s while those on *M. hupehensis* had the smallest increases. Furthermore, values for E were lowest for trees on *M. sieversii* under both well-watered (5.99 mmol m^{-2} s^{-1}) and drought (4.98 mmol m^{-2} s^{-1}) conditions. The highest transpiration rates were 7.40 mmol m^{-2} s^{-1} for well-watered tree on *M. mandshurica* and 7.02 mmol m^{-2} s^{-1} for drought-stressed tree on *M. toringoides*.

The extent to which drought diminished Chl, P_N, E, and g_s differed among rootstocks, with *M. hupehensis* showing the largest declines in Chl (58.12%), P_N (36.46%), and g_s (63.64%), and trees on *M. mandshurica* having the greatest decrease in E (25.95%). By contrast, values were least influenced by drought in trees grafted onto *M. prunifolia* (10.45% for Chl), *M. sieversii* (16.17% for P_N; 21.05% for g_s), and *M. toringoides* (15.95% for E).

WUE and δ^{13}C were significantly different ($p \leq 0.05$) among trees grafted onto nine rootstocks under either watering regime, with those values being significantly elevated for *M. prunifolia* and *M. sieversii*. The extent to which drought influence these responses depended upon rootstock choice (Table 5). Trees on *M. sieversii* and *M. spectabilis* showed much greater increases in WUE, which respectively rose from 27.34 to 45.17 g L^{-1} and from 22.41 to 45.13 g L^{-1}. Rootstock *M. toringoides* induced the smallest increase in WUE, only from 19.26 to 23.00 g L^{-1}. δ^{13}C increased the most for trees on *M. mandshurica* and *M. micromalus*, which were from –26.75 to –25.08‰ and from –26.83 to –25.12‰, respectively. By contrast, the carbon isotope composition was least increased (from –25.22 to –25.00%) in trees on *M. prunifolia*.

Discussion

Growth: Superior rootstocks are widely used by horticulturalists to enhance performance when the growing environment is influenced by suboptimal and/or supraoptimal factors (Castle *et al.* 2010a,b; 2011). In the present study, *M. hupehensis* and *M. sieboldii* induced lower PH, RGR, and LA of 'Gale Gala' trees under both water conditions (Table 2), which suggested their dwarfing effects. The extent of the drought-induced decrease in growth parameters was significantly different among different rootstocks (Table 2). Since scions were the same, the

Table 2. Influence of rootstock on plant height (PH), trunk diameter (TD), total fresh biomass (TB), total dry biomass (TDB), total leaf area (LA), relative growth rate (RGR), stomatal density (SD), and leaf thickness (LT) of ‘Gale Gala’ trees in response to drought stress. ‘Gale Gala’ trees were bud-grafted onto 9 wild Chinese *Malus* rootstocks. Two watering regimes: Control, 80% soil field capacity; drought, 50% soil field capacity. Data are means of 6 replicates. Within a row, values not followed by *the same letter* are significantly different at $p \leq 0.05$.

Parameter		*M. hupehensis*	*M. mandshurica*	*M. micromalus*	*M. prunifolia*	*M. robusta*	*M. sieboldii*	*M. sieversii*	*M. spectabilis*	*M. toringoides*
PH [cm]	Control	96.22^{d}	111.86^{bc}	112.48^{bc}	129.26^{a}	109.35^{c}	100.36^{cd}	124.65^{a}	118.39^{b}	116.72^{b}
	Drought	58.03^{e}	77.67^{c}	79.05^{bc}	81.17^{bc}	71.85^{cd}	73.82^{cd}	97.71^{a}	82.27^{bc}	72.33^{cd}
Variation of drought to control [%]		–39.69	–30.56	–29.72	–37.20	–34.29	–26.44	–21.61	–30.51	–38.03
TD [mm]	Control	9.37^{d}	10.61^{b}	10.84^{ab}	10.73^{ab}	9.98^{cd}	9.33^{d}	11.06^{a}	10.72^{ab}	10.46^{bc}
	Drought	7.15^{e}	7.80^{cd}	7.86^{cd}	8.42^{b}	7.47^{de}	7.79^{cd}	8.98^{a}	8.97^{a}	7.57^{d}
Variation of drought to control [%]		–23.69	–26.48	–27.49	–21.53	–25.15	–16.51	–18.81	–16.32	–27.63
TB [g]	Control	175.35^{e}	260.72^{c}	259.68^{c}	324.18^{a}	236.42^{cd}	224.16^{d}	285.42^{bc}	280.06^{bc}	256.27^{c}
	Drought	145.18^{e}	203.44^{c}	204.32^{c}	212.36^{bc}	168.00^{de}	175.30^{de}	242.83^{a}	236.17^{ab}	169.01^{de}
Variation of drought to control [%]		–17.21	–21.97	–21.32	–34.49	–28.94	–21.80	–14.92	–15.67	–34.05
TDB [g]	Control	74.29^{d}	100.16^{bc}	99. 89^{bc}	124.05^{a}	92.41^{c}	86.04^{cd}	110.86^{b}	107.09^{b}	97.42^{bc}
	Drought	65.60^{e}	83.42^{bc}	84.42^{bc}	85.62^{bc}	75.48^{cde}	76.52^{cd}	102.47^{a}	97.53^{ab}	76.28^{cd}
Variation of drought to control [%]		–11.70	–16.71	–15.49	–30.98	–18.32	–11.06	–7.57	–8.93	–21.70
LA [m^{2}]	Control	1.32^{f}	2.29^{d}	2.40^{cd}	3.36^{a}	2.03^{e}	2.03^{e}	3.25^{ab}	3.39^{a}	2.25^{de}
	Drought	1.00^{e}	1.71^{b}	1.99^{a}	1.31^{d}	1.48^{cd}	1.48^{cd}	1.76^{b}	1.46^{cd}	1.29^{d}
Variation of drought to control [%]		–24.54	–25.33	–17.08	–61.01	–27.09	–27.09	–45.85	–56.93	–42.67
RGR [mg day^{-1}]	Control	5.30^{g}	10.22^{cd}	10.16^{cd}	13.84^{a}	8.89^{e}	7.63^{f}	12.56^{b}	12.33^{b}	10.07^{cd}
	Drought	3.18^{e}	7.71^{cd}	7.67^{cd}	9.90^{ab}	6.24^{d}	7.72^{cd}	10.45^{a}	9.89^{ab}	6.93^{d}
Variation of drought to control [%]		–40.00	–24.56	–24.51	–28.47	–29.81	1.18	–16.80	–19.79	–31.18
SD [no. mm^{-2}]	Control	247.58^{e}	279.09^{d}	285.10^{d}	320.58^{bc}	300.06^{c}	266.10^{de}	370.58^{a}	283.28^{d}	291.25^{cd}
	Drought	140.25^{f}	211.32^{de}	222.06^{d}	282.10^{bc}	239.18^{cd}	168.39^{ef}	341.16^{a}	219.83^{d}	233.00^{cd}
Variation of drought to control [%]		–43.35	–24.28	–22.11	–12.00	–20.29	–36.72	–7.94	–22.40	–20.00
LT [µm]	Control	218.26^{f}	240.28^{cd}	238.25^{cd}	265.42^{a}	237.20^{d}	222.06^{ef}	255.57^{b}	239.26^{cd}	242.08^{cd}
	Drought	230.15^{e}	261.17^{d}	261.55^{c}	326.37^{a}	259.71^{d}	239.40^{e}	312. 40^{b}	270.12^{cd}	251.77^{de}
Variation of drought to control [%]		5.45	8.69	9.78	22.96	9.49	7.81	22.24	12.90	4.00

Table 3. Influence of rootstock on root/shoot ratio (RSR), specific leaf area (SLA), leaf area ratio (LAR), relative water content (RWC), water-holding capacity (WHC), stomatal density (SD), and leaf thickness (LT) of 'Gale Gala' trees in response to drought stress. 'Gale Gala' trees were bud-grafted onto 9 wild Chinese *Malus* rootstocks. Two watering regimes: Control, 80% soil field capacity; drought, 50% soil field capacity. Data are means of 6 replicates. Within a row, values not followed by *the same letter* are significantly different at $p \leq 0.05$.

Parameter		*M. hupehensis*	*M. mandshurica*	*M. micromalus*	*M. prunifolia*	*M. robusta*	*M. sieboldii*	*M. sieversii*	*M. spectabilis*	*M. toringoides*
RSR	Control	1.51^{a}	0.94^{d}	1.02^{c}	1.11^{bc}	0.98^{d}	0.92^{de}	1.34^{b}	0.97^{d}	0.81^{e}
	Drought	1.02^{e}	1.42^{d}	1.80^{c}	2.12^{b}	1.73^{cd}	1.76^{cd}	2.39^{a}	2.37^{a}	1.74^{cd}
Variation of drought to control [%]		–32.45	51.06	76.47	90.99	76.53	91.30	78.36	144.33	114.81
SLA [cm^2 g^{-1}(DM)]	Control	123.05^{a}	98.21^{c}	99.08^{c}	89.32^{d}	106.24^{bc}	112.06^{b}	94.41^{cd}	96.33^{cd}	99.20^{c}
	Drought	116.75^{a}	91.04^{c}	88.71^{c}	88.41^{c}	98.13^{bc}	97.53^{bc}	82.84^{d}	84.51^{cd}	98.72^{bc}
Variation of drought to control [%]		–5.12	–7.30	–10.47	–1.02	–7.63	–12.97	–12.26	–12.27	–0.48
LAR [cm^2 g^{-1}(DM)]	Control	19.72^{e}	22.57^{cd}	23.67^{c}	26.85^{a}	22.58^{cd}	19.72^{e}	25.10^{ab}	24.64^{bc}	22.08^{cd}
	Drought	19.04^{c}	20.26^{bc}	21.24^{b}	24.64^{a}	20.88^{bc}	19.61^{c}	23.57^{a}	21.75^{b}	20.04^{bc}
Variation of drought to control [%]		–3.45	–10.23	–10.27	–8.23	–7.53	–0.56	–6.10	–11.73	–9.24
RWC [%]	Control	79.26^{d}	85.22^{cd}	86.42^{c}	94.59^{a}	84.92^{cd}	73.70^{e}	91.50^{b}	91.02^{b}	84.18^{cd}
	Drought	74.53^{d}	81.80^{c}	81.83^{c}	90.75^{a}	76.30^{d}	70.24^{c}	80.14^{c}	85.43^{b}	79.69^{c}
Variation of drought to control [%]		–5.97	–4.01	–5.31	–4.06	–10.15	–4.93	–12.42	–6.14	–5.33
WHC [%]	Control	200.28^{d}	210.17^{cd}	216.18^{c}	237.11^{a}	207.26^{cd}	203.28^{d}	226.19^{b}	226.41^{b}	209.15^{cd}
	Drought	197.05^{e}	217.58^{c}	229.35^{b}	241.03^{a}	210.17^{d}	214.03^{cd}	231.30^{b}	234.00^{ab}	213.06^{cd}
Variation of drought to control [%]		–1.61	3.53	6.09	1.65	1.40	5.29	2.26	3.35	1.87

Table 4. Influence of rootstock on content of leaf chlorophyll (Chl), net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) of 'Gale Gala' trees in response to drought stress. 'Gale Gala' trees were bud-grafted onto 9 wild Chinese *Malus* rootstocks. Two watering regimes: Control, 80% soil field capacity; drought, 50% soil field capacity. Data are means of 6 replicates. Within a row, values not followed by *the same letter* are significantly different at $p \leq 0.05$.

Parameter		*M. hupehensis*	*M. mandshurica*	*M. micromalus*	*M. prunifolia*	*M. robusta*	*M. sieboldii*	*M. sieversii*	*M. spectabilis*	*M. toringoides*
Chl	Control	1.91^{e}	2.30^{cd}	2.74^{bc}	3.35^{a}	2.54^{c}	2.03^{d}	3.34^{a}	3.31^{a}	2.22^{cd}
[mg g^{-1} FM]	Drought	0.80^{g}	1.26^{e}	1.84^{d}	3.00^{a}	1.70^{de}	0.99^{ef}	2.62^{b}	2.28^{c}	1.31^{e}
Variation of drought to control [%]		–58.12	–45.22	–32.85	–10.45	–33.07	–51.23	–21.56	–31.12	–40.99
P_N	Control	14.48^{e}	16.28^{c}	15.41^{cd}	17.15^{b}	16.16^{c}	15.84^{cd}	18.49^{a}	16.88^{bc}	17.25^{b}
[µmol m^{-2} s^{-1}]	Drought	9.20^{e}	11.82^{d}	11.18^{de}	13.72^{b}	12.15^{bc}	11.83^{d}	15.50^{a}	12.18^{bc}	13.39^{b}
Variation of drought to control [%]		–36.46	–27.4	–27.45	–20.00	–24.81	–25.32	–16.17	–27.84	–22.38
E	Control	7.09^{b}	7.40^{a}	6.83^{bc}	6.42^{cd}	6.22^{d}	7.09^{b}	5.99^{e}	6.52^{cd}	7.02^{b}
[mmol m^{-2} s^{-1}]	Drought	5.47^{bc}	5.48^{bc}	5.25^{c}	5.20^{c}	5.02^{d}	5.45^{bc}	4.98^{d}	5.32^{bc}	5.90^{a}
Variation of drought to control [%]		–22.85	–25.95	–23.13	–19.00	–19.29	–23.13	–16.86	–18.40	–15.95
g_s	Control	0.33^{cd}	0.35^{bc}	0.34^{c}	0.36^{b}	0.32^{d}	0.33^{cd}	0.38^{a}	0.37^{ab}	0.35^{bc}
[mol m^{-2} s^{-1}]	Drought	0.12^{d}	0.18^{cd}	0.23^{bc}	0.26^{b}	0.21^{c}	0.18^{cd}	0.30^{a}	0.23^{bc}	0.22^{c}
Variation of drought to control [%]		–63.64	–48.57	–32.35	–27.78	–34.38	–45.45	–21.05	–37.84	–37.14

Table 5. Influence of rootstock on water-use efficiency (WUE) and carbon isotope compositions ($\delta^{13}C$) of 'Gale Gala' trees in response to drought stress. 'Gale Gala' trees were bud-grafted onto 9 wild Chinese *Malus* rootstocks. Two watering regimes: Control, 80% soil field capacity; drought, 50% soil field capacity. Data are means of 6 replicates. Within a row, values not followed by *the same letter* are significantly different at $p \leq 0.05$.

Parameter		*M. hupehensis*	*M. mandshurica*	*M. micromalus*	*M. prunifolia*	*M. robusta*	*M. sieboldii*	*M. sieversii*	*M. spectabilis*	*M. toringoides*
WUE [g L^{-1}]	Control	8.59^{e}	19.62^{c}	19.57^{c}	29.81^{a}	16.40^{cd}	13.64^{d}	24.28^{b}	22.41^{bc}	19.22^{c}
	Drought	11.92^{f}	30.44^{d}	32.17^{d}	42.72^{bc}	22.38^{e}	23.20^{e}	50.23^{a}	45.13^{b}	23.16^{e}
Variation of drought to control [%]		38.77	55.15	64.38	43.31	36.46	70.09	106.88	101.38	20.50
$\delta^{13}C$ [‰]	Control	-27.82^{e}	-26.75^{c}	-26.82^{c}	-25.18^{a}	-27.43^{cd}	-27.67^{de}	-25.60^{a}	-26.08^{b}	-27.00^{cd}
	Drought	-27.33^{d}	-25.08^{b}	-25.15^{b}	-25.01^{b}	-26.56^{c}	-26.24^{c}	-24.16^{a}	-24.61^{a}	-26.33^{c}
Variation of drought to control [%]		1.76	6.24	6.23	0.68	3.17	5.17	5.63	5.64	2.48

differences in their growth were mainly ascribed to the effects of rootstock on scion morphological and physiological responses to drought (reviewed by Martínez-Ballesta *et al.* 2010, Schwarz *et al.* 2010). Trees grafted onto *M. prunifolia* and *M. sieversii* had higher vegetative growth, as manifested by the higher value in PH, TB, LA, and RGR. These outcomes were probably associated with their much larger and more vigorous root systems (Yan *et al.* 2008, Zhou 1999) when compared with the low biomass accumulations and shallow-rooting *M. hupehensis* (Zhou 1999). Our findings are consistent with results reported from studies with other species on the interactive effect of stock–scion combination, where it has been suggested that a balance is maintained between canopy growth and the size of the root system (Koundouras *et al.* 2008, Weibel *et al.* 2003).

Under drought condition, RSR, RWC, WHC, LT, and SD increased regardless of rootstock type. This may be considered a strategy for drought avoidance and a morphological adaptation to minimize water loss (Koundouras *et al.* 2008, Gómez del Campo *et al.* 2003, Toumi *et al.* 2007). The mechanism by which leaves become thicker enables a plant to reduce transpiration by retaining more water in the mesophyll (Lo Gullo and Salleo 1988). Measures of SLA and LAR are widely used to describe leaf structural modifications; here, both were significantly decreased by drought, primarily because the surface area of individual leaves from stressed plants was significantly smaller (Gómez del Campo *et al.* 2003). Trees grafted onto *M. prunifolia* or *M. sieversii* showed less sensitivity to reduced irrigation, as manifested by larger increases in LT and SD and smaller decreases in RWC, WHC, SLA, and LAR. Their responses are probably associated with greater drought tolerance, as previously reported with those same genotypes (Ma *et al.* 2010, Yan *et al.* 2008, Zhou 1999). By contrast, plants on the sensitive *M. hupehensis* rootstock showed smaller increases in LT and SD, and larger decreases in RWC, WHC, SLA, and LAR, suggesting less tolerance to drought. This noticeable influence on the scion response has also been described with additional *Malus* rootstocks (Cohen *et al.* 2007, Tworkoski and Miller 2007), and other species such as grape (Koundouras *et al.* 2008) and pistachio (Gijón *et al.* 2010).

Gas exchange: Drought stress led to reduced gas exchange and chlorophyll contents, with this effect varying significantly among rootstocks. Leaves from trees on *M. prunifolia* and *M. sieversii* had the highest P_N under both well-watered and drought conditions, which could primarily be attributed to those plants having the highest values for LA, g_s, and Chl. Whereas *M. hupehensis* induced much larger decrease in values for $P_{N,}$ g_s, and Chl under drought condition (Table 4). It suggested that grafting in fact served to minimize those induced decreases in LA, g_s, and Chl, thereby improving P_N overall in those plants. Similar results have been reported by Etehadnia *et al.* (2008) and He *et al.* (2009). Rootstocks have been widely reported to affect gas exchange (Soar *et al.* 2006, Solari *et al.* 2006) but reasons for the effect remain obscure due to the possibly complex interactions among morphological factors, nutrient status, water absorption, and source/sink relations (Paranychianakis *et al.* 2004).

δ^{13}C and WUE: Evaluating the carbon isotope composition is an ideal indirect tool for analyzing plant responses to water deficits. It is widely used to assess long-term WUE because of the positive and significant correlations between those two components (Zhang *et al.* 1993). Ma *et al.* (2010) have previously compared the δ^{13}C and WUE for the nine rootstocks used in this study and observed much higher values for *M. prunifolia* and *M. sieversii* than for *M. hupehensis*. In the current examination, both *M. prunifolia* and *M. sieversii* had higher δ^{13}C and WUE under drought stress, while trees on *M. hupehensis* had the lowest values in both δ^{13}C and WUE (Table 5). The δ^{13}C and WUE of 'Gale Gala' trees was mainly ascribed to the difference in growth, biomass accumulation, stomatal conductance, and photosynthetic capacity, likely, caused by rootstocks as discussed above. In fact, a high δ^{13}C associated with *M. sieversii* and *M. prunifolia* implies a low ratio of intercellular to atmospheric CO_2 concentrations. Given the relatively high g_s values found here for some rootstocks, the decrease in intercellular concentration must have been caused by enhanced photosynthetic capacity.

Conclusion: Performance by 'Gale Gala' trees is a function of the rootstock genotype, which affects RSR, LA, LT, SD, RWC, WHC, Chl, and g_s, thereby influencing P_N, TB, TDB, and WUE. Both *M. hupehensis* and *M. sieboldii* showed a significant dwarfing effect, a desirable trait when using them in orchards planted at close spacings. Under drought conditions, trees on *M. prunifolia* and *M. sieversii* had better performance, meaning that they are more suitable than other rootstocks for use in arid and semiarid regions. We demonstrated here that scion vegetative growth and WUE can be improved by grafting onto the most appropriate rootstocks, thereby exploiting their drought-induced differences in RWC, WHC, stomatal regulation, and photosynthetic capacity.

Young, potted apple trees were used here to determine the influence of wild Chinese *Malus* rootstocks on scion vegetative growth, biomass accumulation, gas exchange, and WUE in response to drought. For future work, we will utilize older trees to assess the effect of rootstock choice on blossom initiation, fruit and flower set, and fruit size and quality. Further research should also focus on the effects of scion genotype on root growth. All of these studies will provide a useful foundation when tailoring the selection of effective stock–scion combinations to fit specific environmental conditions.

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