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2 **Apple rootstock resistance to drought**

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7 **ABSTRACT**

8 Water for irrigation will likely be less available in apple-growing regions due to climate change
9 and competition with human needs other than agriculture. Apple cultivars and rootstocks may
10 differ in water use necessary for acceptable cropping. In two greenhouse experiments in 2014
11 and 2015 rootstocks (M.9 and MM.111) and scions ('Gala' and 'Fuji') with known differences in
12 size control and potential resistance to drought were compared under conditions of reduced water
13 availability. After 1 week without irrigation in 2014 the potting soil moisture and leaf water
14 potential (Ψ_L) were reduced more in trees on MM.111 than M.9 rootstocks and by 'Gala' than
15 'Fuji' scions. Absciscic acid (ABA) and associated metabolites **dihydrophaseic acid, absciscic acid**
16 **glucose ester, and phaseic acid** generally were greater for both scions on M.9 than MM.111.
17 Concentrations of ABA metabolites were greater in 'Gala' than 'Fuji' suggesting significant
18 metabolic rates in leaves. As noon Ψ_L decreased between -1.5 and -2.0 MPa leaf ABA levels
19 increased exponentially to concentrations above 500 ng / g dw and stomatal conductance (g_s)
20 decreased to less than 50 mmole H₂O m⁻² s⁻¹. At reduced g_s carbon assimilation (A) was low but
21 greater in trees on M.9 than on MM.111. Trees grown on M.9 partitioned less dry weight (dw)
22 to roots than MM.111. However the % dw partitioned to fine roots was greater in M.9 than

MM.111 which may have helped trees grow in a small environment such as a pot. In 2015 trees were grown in 45- and 75-L pots and irrigated over 3 weeks to maintain a steady Ψ_L between -1.5 and -2.5 MPa. This longer-term water stress in 2015 resulted in greater g_s and A in trees on MM.111 and in larger pots but WUE was still greater in trees grafted to M.9 in 3 of the 5 drought dates measured. Both rootstocks provided drought resistance, but by mechanisms which appear to differ and which may provide benefits to apple trees grown with reduced irrigation or with water stress. The dwarfing rootstock M.9 produces higher levels of ABA that may regulate stomatal opening and improve short-term drought resistance. The more invigorating rootstock MM.111 may be drought hardy in the longer-term due to development of a more extensive root system.

Keywords: Fruit tree, Hormone, Root system, Tree size, Water stress

1. Introduction

In recent years, there has been increased recognition that fruit is essential for a healthy and balanced diet. In the future, high demand for water for consumption, recreation, industry, and agriculture will almost certainly limit water availability for irrigation of fruit trees. New tools will be needed for sustainable fruit tree production, especially with concerns about climate change and the potential impact on precipitation. These tools may include trees with high water use efficiency (WUE, the ratio of net carbon assimilation to evapotranspiration) and drought resistant rootstocks, but mechanisms that impart water stress resistance in rootstocks are not clear (Jones, 2004). Elevated levels of the hormone *cis-abscisic acid* (ABA) and limited hydraulic

conductivity (ratio of water flow to pressure) of vascular systems have been found in some dwarfing rootstocks, and these factors appear to be part of the size-controlling process (Kamboj and Quinlan, 1998; Kamboj et al., 1999; Tworkoski and Fazio, 2011; Yadava and Dayton, 1972). ABA has been shown to be a root-produced message that regulates shoot dehydration resistance to drying soils (Davies et al., 2005). Root-drench applications of ABA have improved drought hardiness of whole apple trees (Tworkoski, et al., 2011). It is reasonable to postulate that under drought stress rootstocks that produce elevated ABA may lead to water conservation processes such as stomatal closure, and thus, some rootstocks used for size control of fruit tree scions may also be more drought-resistant. However, increased drought resistance may have a negative effect on yield. In grapes increased drought resistance often reduces yield while increasing WUE (Serra et al 2013). Research is needed to identify processes or mechanisms that may reduce this adverse linkage.

Rootstocks may provide drought resistance by altering processes other than stomatal closure. In apple rootstocks, drought resistance has been attributed to increased hydraulic conductance, recovery from embolisms in the xylem, and shift in assimilate partitioning to root growth (Atkinson et al., 1999; Bauerle et al., 2011; Cohen et al., 2007). Hydraulic conductance was 66% more in ‘Gala’ than ‘Fuji’ stems on any rootstock (Tworkoski and Fazio 2015a). Liu et al. (2012) found that ‘Gala’ had improved WUE when grafted to drought resistant apple rootstocks. In contrast to dwarfing rootstocks high vigor rootstocks displayed resistance to drought by increased assimilate partitioned to root development that increased the volume of soil accessible to roots for water uptake (Bauerle et al., 2011). Drought resistance may be further complicated by the effect that scion vigor can have on rootstock root growth and rootstock capacity for drought resistance (Tworkoski and Fazio 2015b). More work is needed to

understand the nature of root/shoot signals (e.g. ABA and related metabolites) and potential benefits of rootstock/high WUE scion combinations to develop technologies that are adapted to water stress. Improved understanding of root-to-shoot signals that may differ among rootstocks and affect drought hardiness will assist in the selection of rootstocks needed for efficient and sustainable production systems in apple.

The objective of our experiment was to determine whether two cultivars develop different water use strategies that may elevate resistance to water stress when they are grown on rootstocks with large vs. small root systems or rootstocks that have induced or constitutively high ABA. We hypothesized that: (1) Under well-watered conditions there are no differences in leaf water potential (Ψ_L) and carbon assimilation (A) of scions grafted to M.9 and MM.111 (~ 35 and 70% height of seedling, respectively). (2) Under drying conditions a vigorous rootstock (MM.111) maintains Ψ_L and A with its large root system that can access larger soil volumes. (3) Alternatively, under drying conditions M.9 rootstock physiologically maintains favorable water status by modulating stomatal conductance (g_s) with ABA production.

2. Materials and Methods

2.1. Trees

Trees were purchased from Stark Brothers Nursery (Louisiana, MO) in March 2014 and planted in 35 liter (L) pots with potting soil (Metromix 360/fine sand, 2.8/1, v/v) at the Appalachian Fruit Research Station. Scion-rootstock combinations included ‘Gala’ and ‘Fuji’ on M.9 and MM.111. Trees were approximately 1 m tall and 15.8 mm in diameter. During the

growing season, trees in the greenhouse were fertilized (1.75 g of 20N-8.8P-16.6K w/w/w per tree) on a weekly basis.

In March 2015 another group of trees from Stark Brothers Nursery with similar dimensions of the 2014 trees were planted in either 45 L or 75 L pots and water was withheld beginning in May 2015. In both 2014 and 2015 all pots were covered with aluminum foil and plastic sheet to reduce evaporation from the potting soil. It was assumed that water loss measured gravimetrically was due to evapotranspiration. In both years the trees were lightly pruned to a modified central leader.

2.2. Water

Three months after planting in June 2014 water was withheld from half the trees (drought) and the other half received water every day (controls). Water was withheld from 1 to 7 days and reapplied from 7 to 14 days. Plant moisture status was measured with three mature leaves from the top, middle, and lower canopy of each tree on 1, 4, 7, and 14 days. Predawn and noon Ψ_L were measured with a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, Calif.). Each leaf was covered and cut, and water potential was measured. Water was reapplied when wilt was evident or -2.5 MPa Ψ_L was reached, approximately 8 days from the time of withholding. The three leaves used for measuring water status were pooled, quickly frozen in liquid N₂, lyophilized and analyzed for ABA and related metabolites. Rate of leaf water potential decrease was used as an index of stress.

Moisture content (MC) of the potting soil at 1, 4, 7, 8, and 14 days was measured by collecting potting soil from the side of the pot with a probe half the distance from the top to the

bottom of the pot. Watering all trees resumed after 8 days. The soil was weighed immediately for fresh weight (fw) and after 7 days drying at 80 C for dry weight (dw).

$$MC (\%) = ((fw - dw) / dw) \times 100$$

In May 2015 trees in both 45 L and 75 L pots were well-watered for 4 weeks and then water was withheld for 14 days until Ψ_L at dawn was approximately -1.0 MPa and at noon was approximately -2.5 MPa. Significant plant stress occurs as water potential approaches -1.5 MPa (Fitter and Hay 1987). Daily water use was measured gravimetrically by measuring the change in mass of the tree and the pot and from soil cores. Control trees continued to receive full watering while drought trees received daily water that decreased over time. After 14 days without watering drought trees daily watering was then reduced every 3 days by 20, 40, and 60% of the daily water use, approximately 0.879, 0.659, and 0.439 L/day for the 45-L pots and 1.174, 0.880, and 0.587 L/day for 75-L pots, respectively.

2.3. Growth and physiological measurements

In 2014 trees were measured for early morning and midday stomatal conductance (g_s), evapotranspiration (E), and carbon assimilation (A) of three mature leaves per tree (CIRAS-3; PP Systems, Haverhill, Mass.) on 1, 4, 7, 8, and 14 days after drought was initiated. Water use efficiency (WUE) was calculated as A / E:

$$WUE = \text{Net CO}_2 \text{ assimilation rate } (\mu\text{mol m}^{-2} \text{ s}^{-1}) / \text{Evapotranspiration rate } (\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1})$$

After the final (14 days) gas exchange measurements, trees were harvested and dw was measured of scion leaves and stems, rootstock shank (stem portion of the rootstock), fine roots (< 1 mm diameter) and coarse roots (> 1 mm diameter). Leaf area (LA) was measured on a subset

of leaves from 5 trees of each scion/rootstock combination and a regression was developed to estimate LA based on leaf number (leaf area per tree = $(16.86 * \text{leaf number per tree}) + 1116.4$; $r^2 = 0.88$).

In 2015 trees were maintained in a drought state (-1.5 MPa or lower) for 3 wks.

Substantial plant stress occurs as water potential approaches -1.5 MPa (Fitter and Hay 1987).

Dawn and noon Ψ_L was measured daily. Assimilation, g_s , E at noon was measured and potting soil relative water content was measured twice per week.

In 2015 the root system adaptation to drought stress was calculated as the apparent root surface water potential (ARSWP, Jones, 1983):

$$\Psi_{s(D)} = \Psi_{l(D)} - \Psi_{l(C)} [g_{s(D)} / g_{s(C)}] [LA_{(D)} / LA_{(C)}]$$

Where:

$\Psi_{s(D)}$ = the apparent water potential at the root surface of the stressed treatment relative to the non-stressed treatment (MPa), $\Psi_{l(D)}$ = leaf water potential of the stressed treatment (MPa), $\Psi_{l(C)}$ = leaf water potential of the non-stressed (MPa) treatment, $g_{s(D)}$ = stomatal conductance of the stressed treatment (sm^{-1}), $g_{s(C)}$ = stomatal conductance of the non-stressed treatment (sm^{-1}), $LA_{(D)}$ = leaf area of the stressed treatment, and $LA_{(C)}$ = leaf area of the non-stressed treatment. The $A_{(D)}$ and $A_{(C)}$ are leaf area estimates based on regression equations between leaf number and leaf area. The ARSWP is an indirect measure of soil water potential at the root surface. ARSWP provides an estimate of soil water conditions when trees are transpiring during the day and but not pre-dawn (Jones 1983)

2.4. Hormone Analysis

In 2014 abscisic acid was measured in leaves of all scion-rootstock combinations at 1, 4, 7, 8, and 14 days after withholding water using methods described previously (Tworkoski and Fazio 2015b). ABA and metabolites dihydrophaseic acid (DPA), abscisic acid glucose ester (ABAGE), phaseic acid (PA) were measured (National Research Council, Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, Saskatchewan, S7N0W9). Briefly, samples were extracted in isopropanol:water:glacial acetic acid (80:19:1, v/v/v) that was spiked with stable isotopes of each hormone, dried, reconstituted in acidified methanol, partitioned with hexane, and the aqueous phase was then dried. Residue was reconstituted in acidified methanol, loaded on a C18 column, and the eluate was dried. Residue was reconstituted in acidified 40% methanol (v/v) and injected in an HPLC ESI-MS/MS (Chiwocha et al. 2003, Chiwocha et al. 2005). Individual hormones were quantified based upon the peak area of the native hormone and corrected for loss based on recovery of the internal standard (Ross et al. 2004). The limit of quantitation was established where the signal-to-noise ratio dropped below 8.

2.5. Experimental design

In both 2014 and 2015, the experimental designs were completely randomized. There were 5 and 3 whole-tree replications for greenhouse growth measurements during the drought experiments in 2014 and 2015, respectively. Main effects of scion, rootstock, and also pot size (2015 only) during the drought were analyzed by SAS Proc GLM followed by Duncan's New Multiple Range Test. Scion, rootstock, and their interaction were the main effects in the

analysis. Relationships among continuous variables were tested by Proc Corr and Proc Reg in SAS. All statistical tests were considered significant if the $P > f$ was at the 0.05 level or lower (SAS Institute, Inc. 2003. The SAS system for Windows. Release 9.1. SAS Inst. Inc., Cary, NC 27513).

3. Results

Interactive effects of scion and rootstock on potting soil moisture were found on days 1 and 4. Potting soil moisture was 25, 25, and 12 % lower in ‘Fuji’ trees grown on MM.111 than M.9 at 1, 4, and 7 days without irrigation (Fig. 1). Larger potting soil moisture reductions were also observed in ‘Gala’ on MM.111 compared with ‘Gala’ on M.9 on day 4. Scions and rootstocks differentially affected soil moisture under drought conditions. Soil moisture was depleted more by ‘Gala’ compared to ‘Fuji’ over both rootstocks by day 7.

In 2014 pre-dawn Ψ_L was lower in ‘Fuji’ on M.9 than on MM.111 on days 1, 4 and 7 (Fig. 2). At noon the trees without water for 4 and 7 days had Ψ_L ’s of -2 to -2.5 MPa and there were no treatment differences (Fig. 2). In 2014 dawn water content of potting soil above approximately 75% did not markedly affect Ψ_L (Fig. 3). Above 75% potting soil moisture content Ψ_L at dawn was less than -0.5 MPa. However, at noon Ψ_L of some trees at the same potting soil water content had a more negative Ψ_L . The Ψ_L changed quantitatively during drought but the Ψ_L among the different scion-rootstock combinations were relatively unchanged at the noon sampling (Fig. 2B).

Effects of drought on potting soil moisture content were also apparent on Ψ_L (Figs. 2 and 3). Scions and rootstocks that depleted potting soil moisture were also associated with lower Ψ_L .

For example ‘Fuji’ on M.9 had consistently more soil moisture and lower dawn Ψ_L than ‘Gala’ on MM.111 (Figs. 1 and 2). Noon Ψ_L tended to show a similar relationship between ‘Fuji’ on M.9 and ‘Gala’ on MM.111 but differences were not significant.

At dawn 4 days without water, Gala and Fuji had less negative Ψ_L on M.9 than on MM.111 (i.e. leaves on M.9 may have been less stressed) but by 7 days without water ‘Gala’ on M.9 Ψ_L was equivalent to scions on MM.111 and only ‘Fuji’ on M.9 had significantly less negative Ψ_L (Fig. 2). To more clearly determine the impact of M.9 on Ψ_L the drought would have to be extended for several weeks which was done in 2015.

In well-watered potting soil (control) leaf ABA was not consistently different between scions and rootstocks (data not shown). However, under drought conditions leaf ABA concentrations were affected by rootstock and time without water (Fig. 4). Under drought stress ABA levels in leaves were generally higher, although not always significantly, for either cultivar on a M.9 than a MM.111 rootstock (Fig. 4). ABA levels in cultivars were inconsistent at 7 and 8 days without water and differences were numerically but not statistically different: higher in ‘Fuji’ than ‘Gala’ on M.9 but the opposite on MM.111.

The conjugate (ABAGE) and degradation metabolites PA and DPA were affected by both scion and rootstock but differences were not statistically significant (Fig. 4). Within a scion ABAGE concentrations always were greater on M.9 than on MM.111 rootstocks under drought conditions. Unlike ABA, ABAGE tended to be greater under drought conditions in ‘Gala’ than ‘Fuji’.

G_s and E at dawn were quantitatively lower than at noon but treatment effects were relatively the same (data not presented). Consequentially, only noon results are presented (Table

1). During the time between 4 and 7 days without water drought-treated trees reduced g_s (Table 1). G_s measured at noon were significantly higher in MM.111 for days 7 and 14 without water. Reduced g_s coincided with drying soils, higher ABA concentrations, and lower Ψ_L (Figs. 1, 3, and 4). Evapotranspiration was not consistently affected by scion or rootstock. At day 1 E was higher in trees grown on M.9 than on MM.111. However, as drought progressed E was greater in MM.111 than M.9 (Table 1).

Assimilation at dawn and noon decreased with time without water (Fig. 5). Trees with M.9 rootstocks maintained numerically, if not statistically greater assimilation as drought increased from 1 to 7 days without water.

As Ψ_L at noon became more negative ABA concentrations increased and reduced stomatal conductance coincided with increased ABA concentrations (Fig. 6).

At 4 and 7 days without water and during the following period with daily watering (14 days) the water use efficiency (WUE) was significantly greater in scions grafted to M.9 than to MM.111 (Table 2). Assimilation was generally higher on trees grafted to M.9 rootstocks. Reduced evapotranspiration of trees on M.9 rootstock at 7 and 14 without water may also have contributed to greater WUE (Table 2).

Dry weight distribution was affected by scion and rootstock but not by the watering treatment (Tables 3 and 4). More dw partitioned to leaf in ‘Gala’ than ‘Fuji’ regardless of rootstock. ‘Fuji’ on MM.111 had significantly more dw partitioned to roots than to leaves as illustrated by the root-to-leaf ratio that was at least two-times greater than other scion-rootstock combinations (Table 4). Both cultivars tended to have less biomass partitioned to roots when grafted to M.9 but M.9 rootstocks tended to have more fine roots (Table 3). Dry weight

distribution might be altered with a more prolonged drought rather than the 1-week drought that was used in this study.

In the 2015 study the effects of scion, rootstock, and pot volume on physiological variables were determined during the controlled and persistent drought (Tables 5, 6, 7, and 8). Rootstock and pot volume affected g_s , and A with MM.111 and 75 L pot volume generally being greater (Tables 5 and 6). A significant scion-rootstock interaction affected g_s on days 14 and 24 (Table 5). On day 14 g_s was 162% higher for 'Fuji' on MM.111 than on M.9 compared with 'Gala' that was approximately 383% higher on MM.111 than on M.9. On day 24 'Fuji' had 61% the g_s on MM.111 than on M.9 and 'Gala' g_s on MM.111 was 109% higher than on M.9. On day 14 A was 117% higher for 'Fuji' on MM.111 than on M.9 compared with 'Gala' that was approximately 211% higher on MM.111 than on M.9. On day 35 'Fuji' on MM.111 had 42% the A than on M.9 and 'Gala' on MM.111 was 327% higher than on M.9. As drought proceeded it appears that 'Fuji' maintained higher g_s and A on M.9 than on MM.111. 'Gala' maintained higher g_s and A on MM.111 at all stages of drought.

WUE was greater in M.9 than MM.111 on days 14 and 28 and was affected by pot volume on days 17 and 35 (Table 7). Scion-rootstock and scion-pot volume were significant on days 17 and 28. On day 17 WUE was 87% for 'Fuji' on MM.111 than on M.9 compared with 'Gala' that was approximately 153% higher comparing the same rootstocks. On day 28 'Fuji' on MM.111 had 51% the WUE than on M.9 and 'Gala' on MM.111 was 128% higher than on M.9. The Ψ_L during the drought, as expected, was usually higher in trees grown 45 L than 75 L pot volumes (Table 8). Significant interactions of scion and rootstocks occurred on days 17 and 28; scion by pot volume interaction was significant on days 24 and 35. On day 24 Ψ_L of 'Fuji' on M.9

was 74% in a 75L pot compared to being in a 45L pot. Ψ_L of 'Gala' on M.9 was approximately 95% in a 75L pot compared to being in a 45L pot. On day 35 Ψ_L was 98% that for 'Fuji' on M.9 in a 75L pot compared to being in a 45L pot. 'Gala' Ψ_L was 117% comparing 75L to being in a 45L pot.

Scion also affected WUE and Ψ_L but only on days 17 and 24. In general, trees grown on M.9 or in 45 L soil volumes were most water stressed (more negative MPa) and had lower g_s and A. Although not always significant, trees on MM.111 more often maintained a less negative ARSWP than did trees on M.9 (Table 9).

Pot volume strongly affected physiological parameters in 2015 (Tables 5-8). However pot volume had few interactions with scion or rootstock. On day 24 Ψ_L was 74% that for 'Fuji' on M.9 in a 75L pot compared to being in a 45L pot. In 'Gala' on M.9 Ψ_L was approximately 95% in a 75L pot compared to being in a 45L pot. On day 35 Ψ_L was 98% that for 'Fuji' on M.9 in a 75L pot compared to being in a 45L pot. 'Gala' Ψ_L was 117% comparing 75L to being in a 45L pot.

4. Discussion

Water conservation is increasingly important to orchardists as the human population and associated activities demand more water from fixed or dwindling sources. New technologies and creative uses of existing technologies can help meet grower needs. Apple rootstocks have long been used to regulate size of scion and some rootstocks may avoid adverse dehydration by exploiting water sources within soil or by using water efficiently.

Water use was greater by ‘Gala’ than ‘Fuji’ trees and by trees on MM.111 than M.9 rootstocks (Fig. 1). Leaf water potential (Ψ_L) is an index of tree water status and Ψ_L changes reflected the depletion of potting soil water by trees (Fig. 2). Ψ_L declined (became more negative) more quickly in trees grafted to MM.111 than M.9. To determine the impact of the reduced water usage physiological processes such as carbon assimilation were measured.

Capacity for carbon assimilation (A) was greater in ‘Fuji’ trees and either cultivar grown on M.9 rootstocks when measured at dawn (Fig. 5). At noon these differences were much smaller but still apparent. It is likely that greater water use efficiency (WUE) of trees grown on M.9 rootstocks was due, at least in part, to higher A associated with M.9 under water stress conditions.

All scion-rootstock combinations responded to drought over time with lower (more negative) Ψ_L (Fig. 2). Over time, Ψ_L decreased more quickly at noon than dawn. At dawn Ψ_L ’s were similar between 0 and 4 days, reflecting little water stress. In contrast, noon Ψ_L decreased rapidly by day 4 and remained low between 4 and 7 days. It is possible that the trees were able to recover sufficient water overnight to reduce tree water deficits by dawn but the water was insufficient to overcome noon deficits. Tree mechanisms which enhance water stress resistance include reduced stomatal conductance of water vapor. Other resistance mechanisms may reduce water loss with efficient or extensive root systems that enhance water uptake.

Trees on MM.111 had lower Ψ_L even though more dw was partitioned to roots than trees on M.9 (Fig. 2 and Table 3). In the 2014 study large root systems may not have provided drought resistance. By growing all trees in the same-size (35 L) pot, trees grown on MM.111 rootstocks were larger than trees on M.9 and water use over time was greater. However, in the

field trees with larger root systems (i.e. MM.111) may exploit larger soil volumes to avoid dehydration stress. Indeed during the 2015 experiment trees grown in the 75 L soil volume had greater g_s and greater A than trees in the smaller, 45L volume (Tables 5 and 6).

Dry weight distribution within a rootstock (i.e. fine vs. coarse roots) may also affect drought resistance. The greater allocation of dw to fine roots in M.9 than MM.111 may have enabled exploitation of water from soil (Table 3). In ungrafted rootstocks grown with reduced irrigation, M.9 produced fewer coarse (> 3mm diameter) and more fine roots (< 3 mm diameter) under drought conditions (Atkinson et al. 1999). Neither coarse nor fine roots differed in MM.111 trees that were grown in well-watered or drought conditions.

Reduced stomatal conductance is a mechanism of water conservation that provides avoidance of adverse effects of drought. Under drying conditions a root-generated message, ABA, may translocate to leaves where it can stimulate stomatal closure (Davies 2005). As soil dried Ψ_L decreased and leaf ABA concentrations increased (Figs. 2 and 4). An inflection point for the rate of change of ABA leaf concentrations was observed (Fig. 6). As Ψ_L decreased below -1.5 MPa leaf ABA concentrations increased more rapidly. The rate of ABA concentration increase was more rapid for trees on M.9 than MM.111 rootstocks. However, g_s of trees on M.9 rootstocks continued, albeit at a reduced rate, at elevated leaf ABA concentrations (Fig. 6 – bottom). Scions on M.9 rootstock tended to have more negative ARSWP, suggesting the root was drawing more water to maintain Ψ_L which under drought had reduced g_s and A (Table 9). Continued g_s at reduced Ψ_L may allow assimilation (A) and evapotranspiration to continue at reduced levels which may explain greater WUE of scions grown on M.9 than MM.111 (Tables 2, 3, and Fig. 5). At 35 days with drought the ARSWP approached zero possibly due to reduced g_s of drought trees (Tables 5 and 9).

Concentrations of PA and DPA were nearly always higher in ‘Gala’ than ‘Fuji’ on both rootstocks (Fig. 4). PA and DPA were also higher in leaves of scion grafted to M.9 than to MM.111. Kondo et al. (2014) suggested that in apple reduced catabolism of ABA may improve drought resistance by preserving ABA-induced closure of stomates. In the current study, elevated concentrations of PA and DPA in ‘Gala’ and M.9 suggest that significant metabolic activity may be ongoing that controls even higher levels of ABA from developing (Fig. 4). Exogenous applications of ABA can result in leaf senescence (Tworkoski et al. 2011). It is possible that excessively high levels of endogenous ABA may contribute to leaf drop which may improve chances for tree survival at the cost of current-year growth and reproduction.

In some studies dwarfing rootstocks have been found to be less susceptible to dehydration than vigorous rootstocks. More vigorous rootstocks such as M.26 and MM.111 had higher g_s and lower Ψ_L than dwarfing rootstocks such as M.27 during severe drought (Atkinson et al. 2000). Reduced g_s is generally considered a short-term adaptation to avoid drought stress. Elevated sensitivity of MM.111 to drought may also be associated with retention of leaves (Atkinson et al. 1999). Based on weight loss of potted trees, ‘Imperial Gala’ on M.9 was less sensitive to drought than on MM.111 (Fernandez et al. 1997). In contrast other research found that the invigorating rootstock, MM.111, has been more resistant to dehydration. Anecdotally M.27, G.11, and G.30 are very susceptible, M.9 and M.26 are moderately susceptible, and M.7 and MM.111 have low susceptibility to drought stress (<https://web.extension.illinois.edu/mms/downloads/47281.pdf>). Our experiment in 2015 supports the idea that more vigorous rootstocks may be less susceptible to drought stress when trees are grown in large soil volumes with homogeneous water distribution. In the field intra- and inter-specific root competition and site conditions may also affect drought tolerance.

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353 Dwarfing rootstocks may be advantageous when trees are grown in drier or well-drained
354 soil. ‘Cox’s Orange Pippin’ on M.9 had higher yield / m² than more vigorous rootstocks on
355 sand; the opposite was found on loam (Preston 1959). Our results agree with Fernandez et al.
356 (1997) that ABA was higher in leaves of drought-stressed apple trees grown on M.9 EMLA than
357 on more vigorous rootstocks, possibly due to reduced evapotranspiration.

358 The provenance of apple germplasm can affect drought resistance. Species of *Malus* that
359 originated from dry environments responded to drought with higher levels of ABA and improved
360 WUE (Ma 2008). However, species originating from well-watered environments did not
361 respond with elevated ABA. Within a species differences in genetically-based ABA metabolism
362 can significantly alter and promote plant adaptation to drought (Mahajan and Tuteja, 2005).
363 Markers for elevated ABA or extensive root systems may assist in searching for and developing
364 rootstocks that could be targeted for use in specific growing environments, such as dry areas
365 where little irrigation may be available.

366 Age and stage of tree development may influence the capacity for drought resistance.
367 Massonnet et al. (2007) found ‘Braeburn’ to be more water-conserving than ‘Fuji’. They
368 suggested that stomatal conductance (i.e. functional components) and canopy architecture (i.e.
369 structural components) could be complementary when selecting drought-resistant apple trees.
370 However, structural components and functional components become more diverse as trees
371 mature. The complexity of apple tree development over time can produce challenges for
372 understanding and selecting apple rootstocks that are drought resistant (Nabi et al. 2000). We
373 worked with rootstocks that may affect both functional and structural components of scion.
374 Results support the following hypotheses: 1) Under moist conditions there are no differences

between M9 & MM111 in Ψ_L , A, and g_s . 2) Under drying conditions MM111 maintains its water potential and gas exchange with its large root system. 3) The M9 rootstock physiologically maintains water status by modulating stomatal conductance with ABA production.

A number of plant adjustments can occur in response to limited water availability. In this paper we have addressed only a few of these adaptations to drought and other mechanisms to improve drought resistance remain to be addressed. Short-term or rapid adjustments to prevent dehydration include reduced stomatal aperture, hydraulic conductivity, and leaf angle. Long-term adjustments include reduced leaf area or increased biomass distribution to the root. Dwarfing rootstocks such as M.9 may elevate leaf concentrations of proline and soluble sugars that modify osmotic potential and improve drought hardiness (Alizadeh et al. 2011).

Potential feedback loops between scion and rootstock may exist and affect drought resistance. Atkinson (1980) observed that scions may affect rootstock growth and function, e.g. by affecting available carbohydrates, stomatal density, and WUE – which may be affected by both scion and rootstock. The potential confounding effects that some scions may have when grafted on rootstock deserves scrutiny. Drought resistance may improve WUE and tree survival but it may also be associated with yield loss (Serra et al 2013). Soil water content necessary to avoid loss of yield or fruit quality differs among cultivars as well as rootstocks (Braun et al. 1989, Jones 2004).

5. Conclusion

In apple, long-term resistance to drought may be associated with vigorous root systems that can exploit soil whereas short-term adaptations may benefit from root signals that are sensitive to drying conditions. If these hypotheses prove to have merit then breeders may

identify the gene(s) associated with elevated ABA in rootstocks such as M.9 and include it into the genome of rootstocks with robust root systems such as MM.111 to attain multiple forms of drought protection.

Acknowledgements

The authors thank Tony Rugh, Chris Hott, and Kevin Webb for their technical assistance. Thanks also to Dr. M. Wisniewski and Dr. T. Artlip for reviews of this manuscript. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

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Table 1.

Main Effects of two scions ('Fuji' and 'Gala') and two rootstocks (M.9 and MM.111) during drought on stomatal conductance and transpiration measured in the greenhouse during 2014 at noon under drought conditions increasing with time.

Treatments	Days ¹			
	1	4	7	14
	Stomatal conductance (mmol H ₂ O m ⁻² s ⁻¹)			
Scion				
Fuji	484 a ²	458 a	51 a	726 b
Gala	213 a	344 a	50 a	1558 a
Rootstock				
M.9	nd	392 a	31 b	620 b
MM.111	nd	411 a	70 a	1664 a
<u>Main effects</u>			(P>f)	
Scion (S)	0.19	0.11	0.94	0.01
Rootstock (R)	nd ³	0.78	0.01	0.01
S x R	nd	0.10	0.67	0.01

Treatments	Transpiration rate (mmol H ₂ O m ⁻² s ⁻¹)			
Scion				
Fuji	2.2 a	4.0 a	1.2 a	4.9 a
Gala	3.5 a	3.3 b	1.1 a	5.3 a
Rootstock				
M.9	4.3 a	3.8 a	0.7 b	4.4 b
MM.111	1.4 b	3.6 a	1.6 a	5.7 a
<u>Main effects</u>			(P>f)	
Scion (S)	0.06	0.03	0.86	0.05
Rootstock (R)	0.01	0.52	0.01	0.01
S x R	0.05	0.07	0.99	0.25

¹ Water was withheld until day 8 and then trees were watered daily.

² Within each day and main effect, mean values followed by the same letter do not differ at the 0.05 level of significance.

³ nd designates no data.

Table 2.

Main Effects of two scions ('Fuji' and 'Gala') and two rootstocks (M.9 and MM.111) during drought on leaf water use efficiency (WUE)¹ and CO₂ assimilation at noon measured in the greenhouse during 2014.

Treatments	Days ²			
	1	4	7	14
	WUE ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) / ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)			
Scion				
Fuji	19.7 a ³	4.0 a	4.5 a	4.0 a
Gala	11.5 a	4.1 a	5.1 b	4.2 a
Rootstock				
M.9	14.0 a	4.2 a	6.7 a	4.9 a
MM.111	17.3 a	3.7 b	2.7 b	3.3 b
<u>Main effects</u>			(P>f)	
Scion (S)	0.16	0.42	0.75	0.52
Rootstock (R)	0.56	0.01	0.01	0.01
S x R	0.38	0.38	0.99	0.18

Treatments	CO ₂ assimilation rate ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)			
Scion				
Fuji	30.9 a	16.4 a	4.3 a	19.6 a
Gala	28.3 a	03.6 b	4.2 a	21.8 a
Rootstock				
M.9	36.3 a	16.1 a	5.0 a	21.9 a
MM.111	22.9 b	13.9 a	3.5 a	19.4 b
<u>Main effects</u>			(P>f)	
Scion (S)	0.13	0.05	0.75	0.06
Rootstock (R)	0.01	0.12	0.11	0.02
S x R	0.68	0.2	0.48	0.47

¹ WUE = CO₂ assimilation rate ($\mu\text{mol m}^{-2} \text{ s}^{-1}$) / Transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)

² Water was withheld until day 8 and then trees were watered daily.

³ Within each day and main effect, mean values followed by the same letter do not differ at the 0.05 level of significance.

Table 3.

Dry weight distribution in two scions ('Fuji' and 'Gala') and two rootstocks (M.9 and MM.111) measured in the greenhouse during 2014.

Scion	Rootstock	Total (g)	Leaf (%)	Branch (%)	Trunk (%)	Shank (%)	Root (%)	Root Fine (%)	Root Coarse (%)
Fuji	M9	347.6 a ¹	10.3 b	12.8 ab	30.9ab	40.0 a	6.0 b	54.3 a	45.7 a
Gala	M9	272.5 b	18.4 a	9.9 bc	27.2 b	38.1 a	6.5 b	56.3 a	43.7 a
Fuji	MM111	228.6 b	12.0 b	8.2 c	33.8 a	31.9 ab	14.1 a	48.8 a	51.2 a
Gala	MM111	260.0 b	17.1 a	15.8 a	31.2 ab	25.6 b	10.2 ab	47.7 a	52.3 a
<u>Main effects</u>					(P>f)				
Scion		0.27	0.01	0.53	0.01	0.10	0.42	0.50	0.42
Rootstock		0.01	0.10	0.08	0.04	0.01	0.09	0.09	0.18
S*R		0.02	0.72	0.01	0.01	0.19	0.72	0.69	0.85

¹ Within each response variable, means followed by the same letter do not differ at the 0.05 level of significance.

Table 4.

Whole tree leaf traits and root-to-shoot ratios that were based on different tree components measured in the greenhouse during 2014.

Scion	Rootstock	<u>Root-to-shoot ratios</u>			<u>Leaves per tree</u>	
		Total Weight ¹ (g)	Without Shank ² (g)	Root-to-leaf ³ (g)	Area (cm ²)	Number
Fuji	M9	0.85 a ⁴	0.11 b	0.57 b	8693 ab	449 ab
Gala	M9	0.80 ab	0.11 b	0.36 b	10566 a	560 a
Fuji	MM111	0.86 a	0.26 a	1.16 a	5757 b	275 b
Gala	MM111	0.56 b	0.15 b	0.60 b	7106 b	355 b

¹ Shank dw + Root dw / (Leaf dw + Branch dw + Trunk dw)

² Root dw / (Leaf dw + Branch dw + Trunk dw)

³ Root dw / Leaf dw

⁴ Within each response variable mean values followed by the same letter do not differ at the 0.05 level of significance.

Table 5.

Stomatal conductance of two scions ('Fuji' and 'Gala') grafted to two rootstocks (M.9 and MM.111) and grown in two pot volumes under drought conditions for 5 weeks in the greenhouse during 2015.

Treatments		Time with restricted irrigation (days) ¹				
		14	17	24	28	35
		(mmol H ₂ O m ⁻² s ⁻¹)				
Scion	Fuji	143	226	95	138	22
	Gala	167	214	101	156	28
Rootstock						
	M9	89 b ²	120 b	105	175	8 b
	MM111	215 a	314 a	91	116	40 a
Pot volume						
	45L	131 b	173 b	79 b	137	26
	75L	177 a	265 a	115 a	155	24
Control ³		221	398	247	452	196
<u>Main effects</u>				(P > f)		
Scion (Sn)		0.32	0.57	0.84	0.60	0.23
Rootstock (Rt)		0.01	0.01	0.48	0.07	0.01
Pot volume (Pv)		0.01	0.01	0.01	0.57	0.65
Sn x Rt		0.01	0.75	0.02	0.68	0.19
Sn x Pv		0.34	0.46	0.23	0.01	0.55
Rt x Pv		0.39	0.90	0.75	0.22	0.79

¹ Trees were grown with full water for 2 months and then water volumes were reduced to impose drought conditions. Data are presented for days 14 through 35 of restricted irrigation.

² Within each day and main effect, mean values followed by the same letter do not differ at the 0.05 level of significance.

³ Well-watered pots averaged across scion and rootstock.

Table 6.

Carbon assimilation of two scions ('Fuji' and 'Gala') grafted to two rootstocks (M.9 and MM.111) and grown in two pot volumes under drought conditions for 3 weeks in the greenhouse during 2015.

Treatments		Time with restricted irrigation (days) ¹				
		14	17	24	28	35
		(μmol CO ₂ m ⁻² s ⁻¹)				
Scion	Fuji	8.4	7.3	5.5	4.4	3.1
	Gala	8.5	6.1	5.1	4.1	2.7
Rootstock						
	M9	7.2 b ²	4.9 b	5.2	4.1	2.2 b
	MM111	9.6 a	8.4 a	5.4	4.4	3.4 a
Pot volume						
	45L	7.7 b	5.1 b	4.6 b	4.1	3.0
	75L	9.1 a	8.1 a	6.0 a	4.4	2.8
Control ³		11.6	12.1	10.1	11.1	11.5
<u>Main effects</u>				(P>f)		
Scion (Sn)		0.66	0.04	0.30	0.42	0.04
Rootstock (Rt)		0.01	0.01	0.64	0.39	0.01
Pot volume (Pv)		0.01	0.01	0.01	0.48	0.88
Sn x Rt		0.01	1.00	0.27	0.15	0.01
Sn x Pv		0.09	0.04	0.36	0.95	0.31
Rt x Pv		0.62	0.41	0.04	0.25	0.60

¹ Trees were grown with full water for 2 months and then water volumes were reduced to impose drought conditions. Data are presented for days 14 through 35 of restricted irrigation.

² Within each day and main effect, mean values followed by the same letter do not differ at the 0.05 level of significance.

³ Well-watered pots averaged across scion and rootstock.

Table 7.

Water use efficiency of two scions ('Fuji' and 'Gala') grafted to two rootstocks (M.9 and MM.111) and grown in two pot volumes under drought conditions for 3 weeks in the greenhouse during 2015.

Treatments		Time with restricted irrigation (days) ¹				
		14	17	24	28	35
		(μmol CO ₂ m ⁻² s ⁻¹) / (mmol H ₂ O m ⁻² s ⁻¹)				
Scion	Fuji	4.6	3.2 a ²	6.4 a	5.2	5.4
	Gala	4.3	2.3 b	4.4 b	4.0	8.8
Rootstock						
	M9	5.2 a	2.6	5.1	5.6 a	9.2 a
	MM111	3.7 b	2.8	5.8	3.7 b	5.1 b
Pot volume						
	45L	4.6	2.4 b	5.0	4.1	9.7 a
	75L	4.3	3.1 a	5.9	5.1	4.4 b
Control ³		4.6	3.8	5.1	5.2	5.8
<u>Main effects</u>				(P>f)		
Scion (Sn)		0.54	0.01	0.01	0.13	0.07
Rootstock (Rt)		0.01	0.16	0.29	0.05	0.04
Pot volume (Pv)		0.28	0.01	0.22	0.21	0.02
Sn x Rt		0.70	0.01	0.12	0.03	0.11
Sn x Pv		0.24	0.03	0.07	0.02	0.19
Rt x Pv		0.51	0.09	0.35	0.36	0.01

¹ Trees were grown with full water for 2 months and then water volumes were reduced to impose drought conditions. Data are presented for days 14 through 35 of restricted irrigation.

² Within each day and main effect, mean values followed by the same letter do not differ at the 0.05 level of significance.

³ Well-watered pots averaged across scion and rootstock.

Table 8.

Leaf water potential (-MPa) of two scions ('Fuji' and 'Gala') grafted to two rootstocks (M.9 and MM.111) and grown in two pot volumes under drought conditions for 3 weeks in the greenhouse during 2015.

Treatments		Time with restricted irrigation (days) ¹				
		14	17	24	28	35
		(-MPa)				
Scion	Fuji	1.7	1.8 b ²	2.0 b	1.8	2.3
	Gala	1.7	2.0 a	2.2 a	1.7	2.3
Rootstock						
	M9	1.8	2.0	2.0	2.0 a	2.3
	MM111	1.6	1.8	2.2	1.6 b	2.3
Pot volume						
	45L	1.9 a	2.2 a	2.3 a	1.9	2.2 b
	75L	1.5 b	1.6 b	1.9 b	1.7	2.4 a
Control ³		1.0	1.0	0.8	0.8	1.4
<u>Main effects</u>		(P>f)				
Scion (Sn)		0.97	0.01	0.05	0.49	0.25
Rootstock (Rt)		0.20	0.08	0.21	0.01	0.32
Pot volume (Pv)		0.01	0.01	0.01	0.08	0.02
Sn x Rt		0.59	0.03	0.21	0.01	0.50
Sn x Pv		0.97	0.83	0.03	0.29	0.01
Rt x Pv		0.43	0.11	0.53	0.05	0.06

¹ Trees were grown with full water for 2 months and then water volumes were reduced to impose drought conditions. Data are presented for days 14 through 35 of restricted irrigation.

² Within each day and main effect, mean values followed by the same letter do not differ at the 0.05 level of significance.

³ Well-watered pots averaged across scion and rootstock.

Table 9.

Apparent root surface water potential (ARSWP) of two scions ('Fuji' and 'Gala') grafted to two rootstocks (M.9 and MM.111) and grown in two pot volumes under drought conditions for 3 weeks in the greenhouse during 2015.

Treatments		Time with restricted irrigation (days)				
		14	17	24	28	35
		(-MPa)				
Scion	Fuji	0.208 b	0.125 b ¹	0.697	0.278	0.048 b
	Gala	0.398 a	0.431 a	0.523	0.251	0.131 a
Rootstock						
	M9	0.428 a	0.120 b	0.961 a	0.385 a	0.082
	MM111	0.181 b	0.409 a	0.296 b	0.156 b	0.092
Pot volume						
	45L	0.290	0.322 a	0.702	0.225	0.063
	75L	0.308	0.219 b	0.534	0.302	0.110
<u>Main effects</u>				(P>f)		
Scion (Sn)		0.01	0.01	0.47	0.73	0.02
Rootstock (Rt)		0.01	0.01	0.01	0.01	0.74
Pot volume (Pv)		0.98	0.03	0.48	0.27	0.16
Sn x Rt		0.81	0.01	0.06	0.18	0.13
Sn x Pv		0.85	0.90	0.05	0.02	0.07
Rt x Pv		0.28	0.20	0.26	0.22	0.12

¹ Within each day and main effect, mean values followed by the same letter do not differ at the 0.05 level of significance.

Fig. 1. Effect of drought and combinations of two scions ('Fuji' and 'Gala') and two rootstocks (M.9 and MM.111) on potting soil moisture measured in the greenhouse during 2014. Water was withheld from 1 to 7 days and reapplied from 7 to 14 days. Within each day bars with the same letter do not differ at the 0.05% level of significance.

Fig. 2. Effect of drought and combinations of two scions ('Fuji' and 'Gala') and two rootstocks (M.9 and MM.111) on leaf water potential at dawn (top) and noon (bottom) measured in the greenhouse during 2014. Water was withheld from 1 to 7 days and reapplied from 7 to 14 days. Within each day bars with the same letter do not differ at the 0.05% level of significance.

Fig. 3. Effect of water content of potting soil on leaf water potential at noon (top) and dawn (bottom) measured in the greenhouse during 2014.

Fig. 4. Effect of drought on leaf endogenous levels of ABA and ABA-metabolites ABAGE (abscisic acid glucose ester), PA (phaseic acid) and DPA (dihydrophaseic acid), in two scions ('Fuji' and 'Gala') and two rootstocks (M.9 and MM.111) measured in the greenhouse during 2014. Water was withheld from 1 to 7 days and reapplied from 8 to 14 days. Within each day bars with the same letter do not differ at the 0.05% level of significance.

Fig. 5. Effect of drought and combinations of two scions ('Fuji' and 'Gala') and two rootstocks (M.9 and MM.111) on leaf assimilation rate potential at dawn (top) and noon (bottom) measured in the greenhouse during 2014. Water was withheld from 1 to 7 days and reapplied from 7 to 14 days. Within each day bars with the same letter do not differ at the 0.05% level of significance.

Fig. 6. Relationship of leaf water potential and ABA (top) and ABA and stomatal conductance (bottom) measured at noon after 7 days without irrigation in the greenhouse during 2014.