Apple rootstocks' dwarfing loci relationships with mineral nutrient concentration in scion leaves and fruit

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Abstract

Apple rootstock mediated tree dwarfing is mainly controlled by genetic factors *Dw1* and *Dw2* located, respectively, on Chromosome 5 and 11 of apple. While the effect of these factors have been described with regards to tree architecture and productivity, very little is known about their effect on mineral nutrient status of the scion. We utilized an apple rootstock replicated field trial with 'Gala' scion grafted onto a population of 186 rootstocks (progeny of dwarfing rootstock 'Ottawa 3'), which is segregating for both dwarfing loci, providing dwarfing class combinations Dw1+/Dw2+ (fully dwarfing), Dw1-/Dw2+ or Dw1+/Dw2- (semi-dwarfing) and Dw1-/Dw2- (Vigorous) for comparisons. Scion fruit and leaf tissues were used to monitor the concentration (dry wt. basis) of nitrogen (N), potassium (K), phosphorous (P), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), copper (Cu) manganese (Mn) and aluminum (Al) as influenced by rootstocks. Molecular markers linked to the dwarfing loci were used to ascertain their effect on mineral nutrient concentrations by comparing means of contrasting alleles that were inherited by each rootstock in the population. Tree size was negatively correlated with fruit concentration of S, N, Cu, Mg in order of decreasing effect and significance. Tree size was positively correlated with leaf concentration of K, P and Ca in order of decreasing effect and significance. Markers associated with Dw1 showed that plants heterozygous for the dwarfing allele had significantly less leaf S than homozygous non-dwarfing plants, whereas Dw2 alleles had no significant effect. This is in contrast with fruit S which displayed significantly higher concentration in dwarfing plants where either Dw1 or Dw2 dwarfing alleles had displayed higher concentrations. Fruit N concentration was higher in fruit grown on rootstocks that possessed either of the dwarfing alleles following a similar pattern with S, the correlation between fruit S and N concentration was 0.79 (P<0.0001). More significant effects of Dw1 and Dw2 were observed on fruit concentration of Ca, P, K, Mg and Zn, and on leaf concentration of P, K, Ca, S and B. While Dw1 and Dw2 do not seem to be related directly in mode of action to mineral nutrition, segregation of these loci shows that they can have a significant effect on the nutrient status of leaves and fruit.

Keywords: *Malus* × *domestica*, dwarfing genes, fruit quality

INTRODUCTION

The ability of certain apple rootstocks to dwarf grafted apple scions has been known for centuries, however, this ability was not understood genetically until recently. A series of experiments utilizing structured bi-parental segregating populations identified two main loci responsible for this rootstock mediated dwarfing effect (Fazio et al., 2014). The effect of these loci seemed to be independent from other factors that may cause changes in tree growth parameters including water availability, temperature, soil health, disease pressure on roots, soil type, soil pH and soil nutrient availability. Among the vital functions performed by roots and rootstocks, foraging for nutrients has been observed to vary among cultivated varieties of the same crop species including wheat (Valle et al., 2011), rice (Onaga et al., 2013), maize (Mundim et al., 2013), cucumber (Waters and Troupe, 2012), and peach

(Reighard et al., 2013). Apple rootstocks have been shown to affect scion mineral nutrition (Kennedy et al., 1980; Lord, 1983; Simons and Swiader, 1985; West and Young, 1988; Rom et al., 1990, 1991; Fallahi et al., 2002; Fan and Yang, 2011). Monitoring the ability of apple rootstocks to increase nutrient concentrations in grafted scions has ramifications in the general health and productivity of the tree, the human nutritional value and organoleptic quality of apples (Autio, 1994; Fallahi, 2012) and the occurrence of storage disorders like bitter pit (Val et al., 2000; Kim et al., 2004; Volz et al., 2006). Nutrient uptake and transport to the scion by apple rootstocks is mediated by a complex array of mechanisms and pathways that can be divided in two types: absorbance by roots and conductance by transport tissues. Some factors influencing absorbance include co-absorption, production of root exudates, type of root structure, frequency of root turnover, length of root effective half-life, root exploration index, and root symbiotic and pseudo-symbiotic associations. Some factors that influence conductance which applies to any substance transported in vascular tissues are channel proteins, chaperones, active vs. passive processes, vessel size, shape, root pressure, solute gradient, and co-transport of molecules or ions. Such complexity is characteristic of calcium uptake, where there is both passive and active transport, competition among ions for exchange sites and competition among sinks (Shear and Faust, 1970; Saure, 2005). Genetic mechanisms underlying nutrition related traits in apple rootstocks may be influencing a number of 'component' traits such as vessel size, hydraulic conductivity (Atkinson et al., 2003; Tworkoski and Fazio, 2011), root morphology, root longevity (Eissenstat et al., 2000; Eissenstat et al., 2006), active and passive transport channels (Demidchik et al., 2002; Alemán et al., 2011), interaction with mycorrhizal fungi (Resendes et al., 2008), and production of exudates (Dong et al., 1997). Several of these component traits may be bundled in a variable that describes the specific conductivity of a tissue for a particular element. Differences among genotypes in specific conductivities have been detected in apple by testing the effect of an interstock on nutrient concentration in the scion (Lockard, 1976; Zhu et al., 1983; West and Young, 1988). This research aimed to understand the effects of the dwarfing loci on tree nutrient status that perhaps can be applied to other rootstock breeding populations.

MATERIALS AND METHODS

The genetic mapping and breeding population used in this study was the same used for previous apple rootstock nutrition studies (Fazio et al., 2013) and for the discovery of dwarfing loci (Fazio et al., 2014). Briefly the population hereafter referred to as O3R5 was comprised of 186 progeny from the interspecific cross between two apple rootstocks 'Ottawa 3' (Spangelo et al., 1974) and *Malus robusta* 'Robusta 5'. This population segregated for many important rootstock traits including resistance to fire blight (Gardiner et al., 2012), wooly apple aphid (*Eriosoma lanigerum*), powdery mildew, root architecture (Fazio et al., 2009), dwarfing, induction of early bearing, yield efficiency, root suckering, and formation of burr knots and spines.

Seedlings from this population underwent evaluation spanning 5 to 12 years designed to determine the degree of dwarfing and induction of early bearing by the rootstocks (Cummins et al., 1983). Briefly, 20 to 30 cm tall seedlings were planted in a rootstock nursery at the New York State Agricultural Experiment Station (NYSAES) in Geneva, NY in 2003. The plants were allowed to grow for one season to strengthen the mother plant and the top was removed in 2004 to stimulate sucker growth in preparation for propagation by stooling (Quamme and Brownlee, 1990). New shoots were mounded with sawdust during the second growing season to promote adventitious rooting on the shoots under the sawdust, and rooted shoots (rootstock liners) were harvested from mother plants in the late fall of 2005. Five to seven rootstock liners per genotype were planted in a tree nursery at the NYSAES in 2006 and were chip budded with 'Gala' scion at the end of 2006. In spring of 2007 the budded liners were topped to stimulate graft growth and budlings were trained to one shoot to produce an unbranched tree (whip). Finished trees were dug from the nursery in the fall of 2007. Root systems were graded and tree height and diameter measurements made on finished trees. Finished trees were planted in a completely randomized design at a

1.5×4-m spacing in a field at NYSAES, Geneva, NY. Diameter and trunk cross-sectional area measurements at 15 cm above the graft union, and number (or estimate) of flower clusters were collected on each tree during the Spring of 2008-2014. Trees were managed to a slender spindle training system, with typical fertilizer applications for apple orchards in Geneva, NY.

Number of fruit and weight of fruit per tree were measured during the fall of years 2008-2014. Fruit harvested in September of 2013 from each tree within a 32-h period was stored at 3°C until a sample of 10 fruit tree^{-1} was analyzed for sugar content (°Brix) with an handheld refractometer and fruit firmness with a penetrometer (EPT-1, Lake City Technical Products Inc., Kelowna, B.C. Canada, adjusted for apples). Material for mineral nutrient analysis was collected from all live trees in the orchard: ten fully expanded mid position leaves on new extension growth interspersed in the canopy were harvested 80-85 days after bloom and oven dried at 65°C. Concurrently five immature fruit from each tree were collected, cored, and four vertical quarter sections including the peel from 1 to 2 cm wide from opposite sides of the fruit were oven dried at 65°C. Oven dry leaves and fruit slices were sent to A&L Great Lakes Laboratories, Inc. (Fort Wayne, Indiana 46808) where concentration (dry weight basis) of nitrogen (N), potassium (K), phosphorous (P), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), copper (Cu) manganese (Mn) and aluminum (Al) was measured on samples. Total nitrogen was measured by the Dumas Method (nitrogen by combustion or nitrogen by thermal conductance.) AOAC 990.03. The mineral digestion was performed by the open vessel microwave method SW846-3051A (AOAC 991-10D(e)) and the mineral analysis was performed with an inductively coupled argon plasma (ICAP) AOAC 985.01.

Molecular marker data for Dw1 and Dw2 were as described in Fazio et al. (2014), rootstocks were divided into four classes depending on their dwarfing allele constitution. Phenotypic data means, contrasts, regressions, analyses of variance (ANOVA) and graphs were generated with a number of statistical packages including Minitab 16 (Minitab, State College, PA), Statistica 10 (Statsoft Inc. Tulsa, OK) and JMP 11 Pro (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

The results from the fruit and leaf mineral analyses indicated a significant effect ($p<0.05$) of rootstocks on the concentration in scion leaves and fruit for nitrogen (N), potassium (K), phosphorous (P), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), copper (Cu) manganese (Mn) and aluminum (Al). Histograms for genotypic means (Figure 1) in the population tested show for the most part normal distributions for leaf (Lf) and fruit (Fr) concentration of mineral nutrients with the exception for fruit P, leaf S (FrP, LfS, bimodal), and for fruit Mn and Zn (FrMN, FrZN, skewed left). Several nutrient concentrations in fruit were strongly correlated (FrP and FrK, 0.7, $p<0.001$; FrN and FrS, 0.5, $p<0.001$; FrMg and FrP, 0.47, $p<0.001$; FrB and FrP, -0.45, $p<0.001$) suggesting similar, parallel, or competitive mechanisms for absorption and translocation of nutrients to the scion (Table 1).

The negative correlation between FrB and leaf/fruit P and K is somewhat worrisome because it indicates that in this population of rootstocks, selection for higher P and K efficient rootstocks might result in boron deficiencies. While LfCa had a positive correlation with LfP and LfK this correlation did not translate to FrCa which did not show any significant positive correlations with any other nutrient, but did show negative correlations with FrCu, LfCu, LfAl and LfS suggesting perhaps competition for the same active transport slots for these ions. This relationship may have implications with regards to copper treatments that are applied routinely to apple orchards.

The relationship between dwarfing loci and leaf/fruit nutrient concentration values is shown in Table 2, where the effects were calculated by contrasting the means for groups of individual rootstocks possessing either of the dwarfing loci or both. The effect of Dw1 was negative on leaf P, K, Ca and S, and positive on fruit N, Ca, Mg, S, and Zn. The effect of Dw2 was negative for leaf P and Ca, and positive for fruit S and Cu. Together Dw1 and Dw2 had a small positive effect on leaf B, and a positive effect on fruit Mg and S. The relationship of tree

size (trunk diameter) showed that larger trees produced apples with smaller concentrations of nitrogen and sulfur than dwarfed trees, this observation seems to validate the concept that competition from other sinks (apical and radial meristems) more active in larger trees might be associated with lower concentration of N and S in fruit. The loose negative relationship between fruit firmness and K concentration was in accordance with other observations (Fallahi et al., 2010). Tree size was also negatively correlated with fruit sugars (°Brix) and fruit firmness (Figure 2), where larger trees on vigorous rootstocks tended to produce softer apples with less sugar compared to firmer apples with more sugars found on small trees on dwarfing rootstocks. While several studies have suggested an influence on fruit quality by apple rootstocks (Fallahi et al., 2011; Fallahi, 2012; Gjamovski et al., 2013), it is difficult to parse the effects of dwarfing and the dwarfing loci on nutrient concentration values connected to fruit quality, as these may be an indirect effect of other factors like tree architecture, fruit exposure to sunlight, crop load, water availability, which are all influenced by dwarfing.

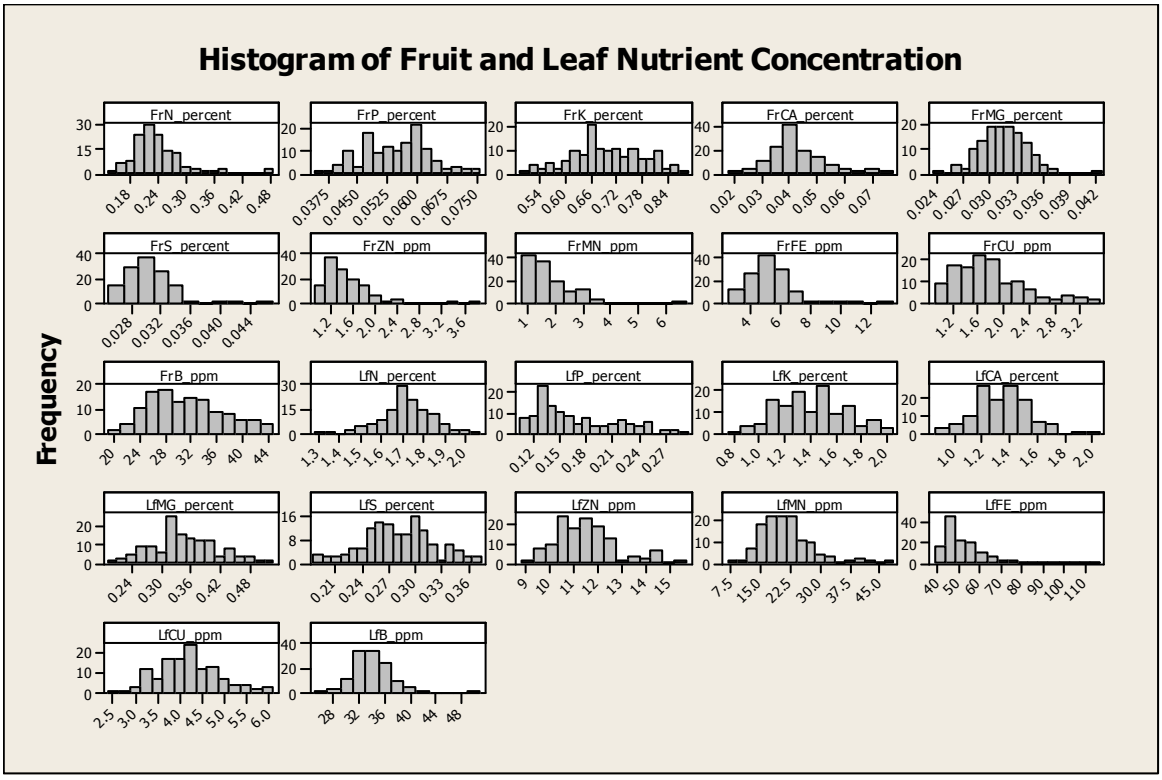

Figure 1. Histograms for fruit and leaf mineral nutrient concentration values in 'Gala' scion grafted onto a full sib population of apple rootstocks derived from the cross between 'Ottawa 3' and 'Robusta 5'.

Table 1. Pearson correlation coefficient and associated p-value between mineral nutrient concentration values in 'Gala' fruit from trees grafted on the O3R5 population of apple rootstocks.

	FrN %	FrP %	FrK %	FrCa %	FrMg %	FrS %	FrZn ppm	FrMn ppm	FrFe ppm	FrCu ppm	FrB ppm	FrAl ppm	LfN %	LfP %	LfK %	LfCa %	LfMg %	LfS %	LfZn ppm	LfMn ppm	LfFe ppm	LfCu ppm	LfB ppm
FrP %	0.29																						
p-value	0.00																						
FrK %	0.09	0.78																					
p-value	0.07	0.00																					
FrCa %	0.00	-0.01	-0.03																				
p-value	0.95	0.84	0.58																				
FrMg %	0.38	0.48	0.29	0.09																			
p-value	0.00	0.00	0.00	0.08																			
FrS %	0.50	0.30	0.15	-0.13	0.57																		
p-value	0.00	0.00	0.00	0.01	0.00																		
FrZn ppm	0.08	-0.02	-0.02	0.06	0.16	0.12																	
p-value	0.19	0.78	0.72	0.31	0.01	0.05																	
FrMn ppm	0.05	-0.03	-0.01	0.04	0.06	0.02	-0.03																
p-value	0.49	0.68	0.90	0.61	0.43	0.79	0.76																
FrFe ppm	-0.08	0.10	0.10	0.05	0.10	0.02	0.10	0.16															
p-value	0.12	0.07	0.07	0.39	0.06	0.65	0.11	0.04															
FrCu ppm	0.23	0.03	0.00	-0.13	0.19	0.36	0.07	0.35	0.02														
p-value	0.00	0.61	0.98	0.02	0.00	0.00	0.27	0.00	0.79														
FrB ppm	0.08	-0.45	-0.35	-0.05	-0.12	0.14	0.12	0.10	-0.14	0.24													
p-value	0.14	0.00	0.00	0.38	0.02	0.01	0.05	0.17	0.01	0.00													
FrAl ppm	0.03	-0.12	-0.07	0.01	0.02	0.22	-0.09	0.19	0.07	0.38	0.19												
p-value	0.69	0.15	0.37	0.95	0.77	0.01	0.30	0.08	0.39	0.00	0.02												
LfN %	0.08	-0.05	-0.01	-0.02	0.21	0.18	0.03	0.16	0.15	0.15	0.09	-0.03											
p-value	0.11	0.38	0.82	0.75	0.00	0.00	0.67	0.03	0.01	0.01	0.09	0.72											
LfP %	-0.07	0.60	0.69	0.10	0.11	-0.15	-0.14	-0.03	0.11	-0.22	-0.46	-0.19	0.07										
p-value	0.20	0.00	0.00	0.06	0.05	0.01	0.02	0.67	0.04	0.00	0.00	0.02	0.21										
LfK %	-0.09	0.54	0.79	0.08	0.07	-0.09	-0.08	-0.05	0.10	-0.13	-0.35	-0.09	0.01	0.78									
p-value	0.09	0.00	0.00	0.14	0.16	0.08	0.16	0.52	0.06	0.02	0.00	0.27	0.90	0.00									
LfCa %	-0.02	0.20	0.29	0.14	0.00	-0.05	-0.16	-0.11	0.04	-0.13	-0.16	-0.13	0.16	0.51	0.44								
p-value	0.76	0.00	0.00	0.01	0.99	0.37	0.01	0.16	0.47	0.02	0.00	0.12	0.00	0.00	0.00								
LfMg %	-0.09	-0.61	-0.62	0.05	-0.06	-0.02	-0.04	0.01	0.00	0.06	0.33	0.09	0.26	-0.39	-0.53	0.14							
p-value	0.10	0.00	0.00	0.32	0.22	0.68	0.52	0.89	0.99	0.33	0.00	0.27	0.00	0.00	0.00	0.01							
LfS %	-0.20	-0.23	0.07	-0.01	-0.25	0.02	-0.02	-0.01	-0.07	0.06	0.25	0.12	-0.20	0.04	0.18	0.05	0.05						
p-value	0.00	0.00	0.20	0.81	0.00	0.75	0.73	0.88	0.17	0.26	0.00	0.14	0.00	0.49	0.00	0.39	0.39						

Table 1. Continued.

	FrN %	FrP %	FrK %	FrCa %	FrMg %	FrS %	FrZn ppm	FrMn ppm	FrFe ppm	FrCu ppm	FrB ppm	FrAl ppm	LfN %	LfP %	LfK %	LfCa %	LfMg %	LfS %	LfZn ppm	LfMn ppm	LfFe ppm	LfCu ppm	LfB ppm
LfZn ppm	0.18	0.10	0.09	0.08	0.01	0.18	-0.09	-0.12	-0.05	0.09	0.05	0.08	0.03	0.13	0.12	0.29	0.06	0.24					
p-value	0.00	0.07	0.08	0.16	0.81	0.00	0.16	0.11	0.38	0.12	0.31	0.32	0.60	0.02	0.02	0.00	0.25	0.00					
LfMn ppm	0.02	0.24	0.34	-0.01	0.02	0.05	-0.16	0.21	0.14	0.09	-0.20	0.18	-0.01	0.24	0.37	0.16	-0.14	0.10	0.04				
p-value	0.77	0.00	0.00	0.90	0.68	0.31	0.01	0.01	0.01	0.11	0.00	0.03	0.88	0.00	0.00	0.00	0.01	0.05	0.50				
LfFe ppm	0.09	-0.04	-0.04	-0.03	-0.12	-0.16	-0.05	-0.15	0.10	-0.09	-0.01	-0.15	0.09	0.04	0.02	0.15	0.09	-0.12	0.10	0.00			
p-value	0.09	0.44	0.41	0.53	0.03	0.00	0.45	0.05	0.06	0.13	0.86	0.06	0.10	0.48	0.75	0.00	0.09	0.02	0.06	0.98			
LfCu ppm	0.21	-0.03	-0.02	-0.17	0.06	0.23	0.05	0.03	-0.06	0.33	0.22	0.15	0.35	-0.12	-0.01	-0.02	0.11	0.06	0.21	0.11	0.23		
p-value	0.00	0.55	0.70	0.00	0.28	0.00	0.45	0.67	0.22	0.00	0.00	0.06	0.00	0.02	0.83	0.75	0.05	0.28	0.00	0.04	0.00		
LfB ppm	0.18	0.01	-0.04	0.10	0.19	0.19	0.13	-0.03	0.04	0.14	0.44	0.14	0.22	0.05	0.04	0.06	0.12	0.01	0.15	-0.13	-0.04	0.12	
p-value	0.00	0.79	0.48	0.06	0.00	0.00	0.03	0.68	0.46	0.02	0.00	0.08	0.00	0.36	0.40	0.23	0.02	0.78	0.01	0.02	0.45	0.03	
LfAl ppm	-0.05	-0.16	-0.11	-0.12	-0.30	-0.02	0.07	-0.10	-0.16	0.05	0.14	-0.05	-0.22	-0.19	-0.17	-0.08	-0.12	0.18	0.08	-0.12	0.29	0.21	-0.18
p-value	0.33	0.00	0.05	0.02	0.00	0.68	0.27	0.18	0.00	0.41	0.01	0.51	0.00	0.00	0.00	0.12	0.03	0.00	0.15	0.03	0.00	0.00	0.00

Table 2. Influence of dwarfing loci Dw1 and Dw2 on nutrient concentration values in leaf and fruit of 'Gala' scions grafted on a segregating population of apple rootstocks. Only direction of the effect (not values) is listed for significant effects in individuals possessing either Dw1, Dw2 or both.

Dwarfing gene	N	P	K	Ca	Mg	S	Zn	Cu	Fe	Mn	B
Leaf-Dw1	NS	-	-	-	NS	-	NS	NS	NS	NS	NS
Leaf-Dw2	NS	-	NS	-	NS	NS	NS	NS	NS	NS	NS
Leaf Dw1*Dw2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	+
Fruit-Dw1	+	NS	NS	+	+	+	+	NS	NS	NS	NS
Fruit-Dw2	NS	NS	NS	NS	NS	+	NS	+	NS	NS	NS
Fruit Dw1*Dw2	NS	NS	NS	NS	+	+	NS	NS	NS	NS	NS

NS = Not significant ($p>0.05$); + = Significant positive effect ($p<0.05$); - = Significant negative effect ($p<0.05$).

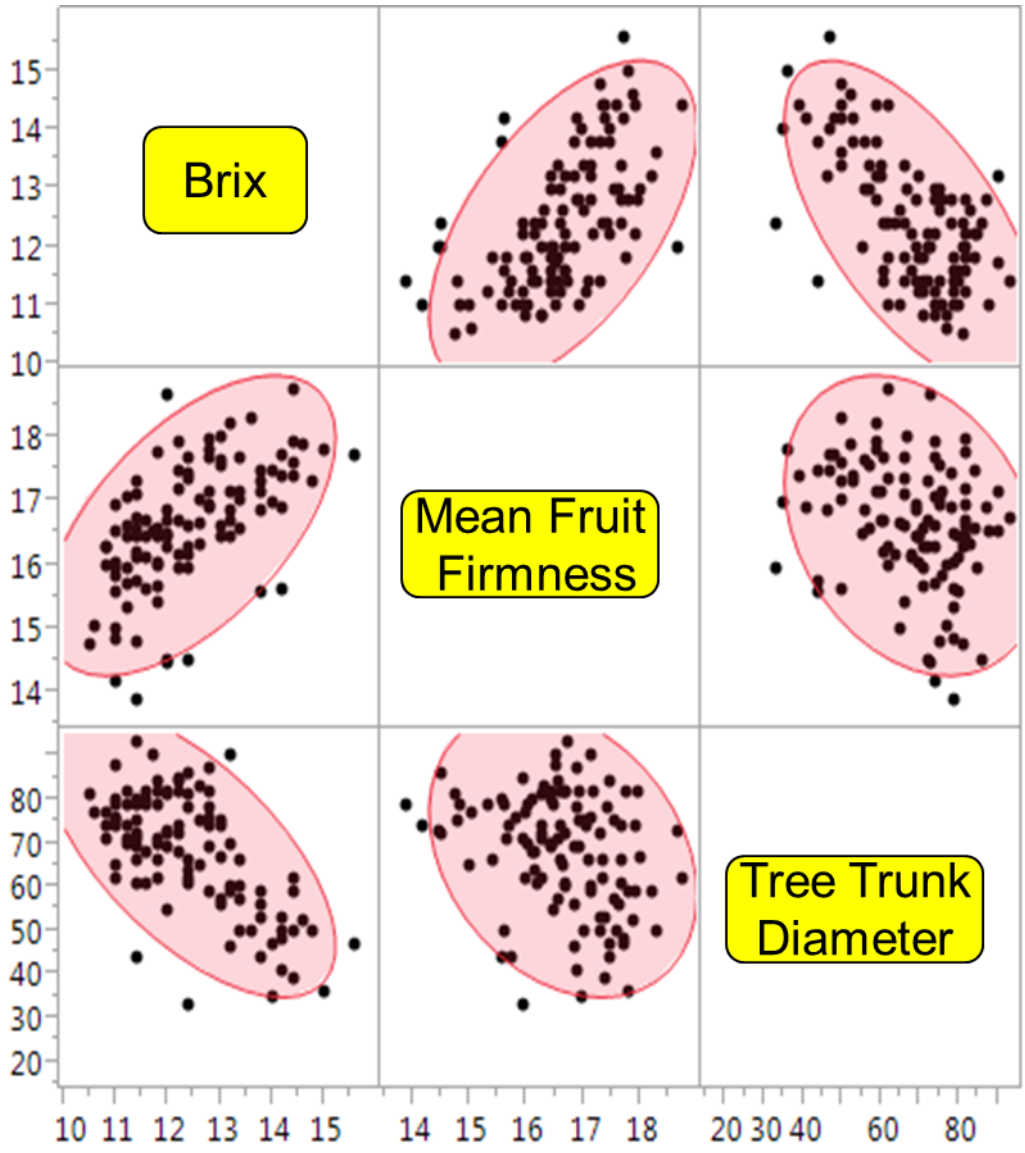

Figure 2. Relationship between trunk diameter, fruit firmness and fruit brix of 'Gala' apple trees on the O3R5 population of rootstocks. Trunk diameter was determined in large part by segregation of dwarfing loci Dw1 and Dw2 in the full sib population of rootstocks tested in this experiment.

CONCLUSIONS

We have determined that segregation of the dwarfing loci shows significant relationships with the mineral nutrient status of leaves and fruit in trees grafted with a 'Gala' scion. These relationships might be due to indirect effects related to tree canopy shape and size, source/sink relationships and other physiological characters affected by dwarfing and semi-dwarfing rootstocks.

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