Breeding apple rootstocks for modulation of mineral nutrients in scions

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Abstract

A major function of the root system of an apple tree is to gather mineral nutrients from soil and shuttle them into different sinks in the canopy including leaves and fruit. Significant phenotypic and associated genotypic diversity has been discovered in apple rootstocks with regards to modulation of mineral nutrient concentrations in grafted scion varieties. A series of replicated rootstock field experiments were conducted with 'Golden Delicious', 'Gala', 'Honeycrisp' and 'Fuji' scions aimed at unraveling the phenotypic and genotypic potential of apple rootstocks to modulate the concentration of potassium (K), sodium (Na), phosphorous (P), calcium (Ca), copper (Cu), sulfur (S), zinc (Zn), magnesium (Mg), nickel (Ni), and molybdenum (Mo) in fruit and leaves over multiple growing seasons. Several rootstocks in these experiments were genotyped with single nucleotide polymorphism (SNP) and microsatellite markers to discover quantitative trait loci (QTLs) associated with rootstock mineral nutrient traits and to follow the effect of these markers in the general breeding populations. Our work detected significant genotypic mean correlations between many of the measured mineral nutrients and significant mineral nutrient QTLs over multiple years. Where possible, we tested for the presence of strongly associated markers in diverse field experiments to learn if they could be used for marker aided breeding. Preliminary analyses indicate that gain from selection with markers is possible, however because of the multiple correlated mineral nutrient traits, it may not be possible to disentangle selection of a positive effect nutrient on productivity, with a correlated nutrient having negative effects on fruit quality. Breeding and selection for specific nutrient profiles is further complicated by the genetic and physiological characteristics of the grafted scion. While we may have been successful in identifying some rootstocks with preferable nutrient profiles with specific scions, more data are needed to build a genetic roadmap for breeding new rootstocks that improve productivity and fruit quality.

Keywords: nutrient uptake, rootstock, breeding, yield components, fruit quality.

INTRODUCTION

Apple rootstock breeding is a long-term process that has mostly focused on yields, disease resistance and efficiencies gained by tree architecture modification like dwarfism (Fazio et al., 2015b). Root systems have important roles in tree fruit production as they forage for mineral nutrients and water necessary for fruit development and canopy growth (Rom et al., 1990; Neilsen and Hampson, 2014). Traditionally, nutrient deficiencies found in soils of fruit orchards have been addressed with the addition of different formulations of fertilizers delivered by multiple means (Fallahi et al., 1984; George et al., 2002; Szewczuk et al., 2009; Milosevic and Milosevic, 2015). This was done with some knowledge of the inherent potential of a few traditional rootstocks to absorb more or less of a particular nutrient contained in the rhizosphere (Chun et al., 2002). However, most fertilizer recommendations were not tailored to a specific rootstock, creating the potential of making such applications less efficient (more or less than specifically needed by the rootstock-scion combination) and potentially wasteful. Rootstocks are embedded in a complex environment were interactions with pH, soil particles,

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fungi, bacteria, insects, soil water status, scion variety, cover crops (and their competing roots) all play into their performance as foragers of nutrients (Kang et al., 2011; Fazio et al., 2012). As an example, the scion variety's evapotranspiration potential can have a huge effect on the nutrients passively brought up to the leaves in the xylem (Nord and Lynch, 2009; Yasutake et al., 2012; Fallahi et al., 2013). Conversely the root's ability to exude citrates in the rhizosphere can influence the pH dependent availability of iron (Fe) and other micro-nutrients (Durrett et al., 2007; M'Sehli et al., 2008; Valentinuzzi et al., 2015). The ability of root systems to associate with specific bacterial, fungal and mycorrhizal colonies sometimes enhances the reach and intensity of absorbance of macro and micro-nutrients, allowing some plants to thrive in otherwise hostile environments (Heikham et al., 2012; Labidi et al., 2012; Chu et al., 2013). All these interactions have genetic components in the rootstock, meaning that there are specific genes and associated alleles that affect the outcome of such interactions to the point that their effect can be detected in genetic experiments with segregating populations (Fazio et al., 2013). Fruit size and quality have been shown to be influenced by nutrient status (Jivan and Sala, 2014) and subsequently by apple rootstocks (Andziak and Tomala, 2004), where a good portion of the variability may be explained by the rootstock potential to absorb and translocate nutrients to the scion which implies that selection of a particular rootstock may be used to match nutrient weaknesses or requirements of fruit (Rom et al., 1991; Fazio et al., 2015a). Recently, data obtained from a diverse set of rootstock field experiments featuring 35 or more genetically different apple rootstocks have indicated the possibility to select for particular genetically determined nutrient profiles (Reig, et al., 2017). In this manuscript, we discuss the prospects and challenges with the introduction of these new selection parameters in the Geneva® apple rootstock breeding program.

MATERIALS AND METHODS

A series of apple rootstock field trials established between 2003 and 2010 in the State of New York, USA were used as the source of leaf and fruit material, which was collected in 2013-2017. These trials featured 30 to 135 apple rootstocks (not listed) belonging to the apple rootstock breeding program and a core of control stocks from other breeding programs (Malling, Budagovsky, Pillnitz) such as M.9, M.7, M.26, and B.9 (Fazio et al., 2015a). Ten mid position leaves on new extension growth and ten fruit randomly distributed throughout the tree canopy were harvested 80-90 days after bloom on all tree replicates of each field trial. Fruit was cored and processed so that only a 0.5 cm thick horizontal doughnut shaped section obtained two cm from the calyx end of fruit. The ten sections obtained from ten fruit harvested from one tree were bulked into one sample per tree for analysis. Leaves and fruit were oven dried, ground into powder and shipped to the USDA-ARS Children's Nutrition Research Center in Houston, TX for mineral analysis of several macro- and micro-mineral nutrients via inductively coupled plasma optical emission spectrometry. Carbon and nitrogen concentration of the fruit and leaf samples from two field trials (Hudson 'Fuji' and Champlain 'Honeycrisp' experiments) were measured with a C/N analyzer at the Cornell University Drinkwater Horticulture Lab in Ithaca, NY. Soil samples were collected from 3-6 locations on the Champlain and Hudson research plots and were analyzed for nutrients after modified Morgan extraction at Agro-One, Ithaca, NY. Leaf and fruit nutrient concentration values were tabulated and analyzed with Minitab 10.0 and JMP 12.0 statistical software packages; the rootstock genotype was treated as the main effect in a randomized complete block analysis. Rootstock genotype means were used in a multivariate analysis to generate correlation matrices and two-way similarity cluster diagrams based on genotype and variable similarities.

RESULTS AND DISCUSSION

The implementation of new selection traits in a plant breeding program requires knowledge related to the complexity, heritability and reliability of the selection process for the new trait (Fazio and Mazzola, 2004). The complexity of a trait depends on the number of segregating factors and the importance (size) of their contribution. Specifically for apple rootstock nutrition traits, we found that in one breeding population the concentration of K was largely governed by one major factor (locus) on Chromosome 5 of apple, whereas in the same experiments the concentration of Mg was influenced by several factors (loci) residing on multiple chromosomes (Fazio et al., 2013). The implications of these results are that selection for simpler traits (fewer factors) will be easier to perform than more complex traits. Another feature of mineral concentration traits that complicates breeding and selection is that most of them are strongly or loosely correlated with each other. For example, in many of our experiments we observed that leaf concentration of P and K is strongly positively correlated while P and S are loosely positively correlated. Subsequently, direct selection for higher values of K will indirectly influence P and S. Predicting what the effects that selection for one nutrient will have on other nutrients requires modeling correlated nutrients as a system of connected linear equations. For example the empirical relationship between leaf concentration values of K, P and S for 2014-15 data is $K=2.97+3.21\times P+1.33\times S$. Once a selection target is chosen, one can solve for any element in the equation. Obviously, these predictive values will be subject to the statistical probabilities associated with the correlations. Physiologically, the correlated values mean that these nutrients share some genetically similar pathways in their journey from the rhizosphere to the destination tissues. Some correlations are not desirable (K and Ca are negatively correlated in many datasets). It remains to be seen if in such positively or negatively correlated traits those correlations can be broken by means of recombination between genetic factors or by selection of novel parents.

The stability of traits over time is very important. Specifically for mineral nutrient concentration traits, this can be measured by comparing the consistency of genotypic means year over year. Currently, the most repeated dataset for a particular trial has three years' worth of measurements. Year to year genotypic mean correlations for a 2010 Honeycrisp trial features values as high as 0.78 for leaf boron and phosphorous between 2014 and 2015 data (Table 1). The lowest year-to-year correlation was found with sulfur with 0.24. In essence, the year-to-year data reveal some reliable nutrient traits (K, P, and B) and some traits that may be influenced by the changes in year to year environmental conditions (Ca, S), the latter being more difficult to breed for. The reasons why some mineral concentration traits are so environmentally sensitive are subject to speculation. A thorough set of controlled experiments needs to be performed to investigate the effects of temperature, light, scion growth, crop load, etc. on the absorption and translocation of these mineral elements.

Table 1. Genotypic mean correlations for a set of leaf mineral nutrients measured on the same experiment over two years (2014 and 2015). Trait correlations range from low (Ca, S) to high (P, K) between years. Experiments like this allow discernment of stable mineral nutrient traits that can become simpler targets for breeding.

Corr. Coeff.	**B µg/g DW 2014**	**Ca mg/g DW 2014**	**K mg/g DW 2014**	**P mg/g DW 2014**	**S mg/g DW 2014**	**B µg/g DW 2015**	**Ca mg/g DW 2015**	**K mg/g DW 2015**	**P mg/g DW 2015**	**S mg/g DW 2015**
B µg/g DW 2014	1	-0.14	0.72	0.73	0.17	0.78	0.05	0.55	0.64	0.40
Ca mg/g DW 2014	-0.14	1	-0.25	-0.12	0.14	-0.15	0.38	-0.16	-0.07	-0.12
K mg/g DW 2014	0.72	-0.25	1	0.82	0.30	0.54	-0.13	0.70	0.69	0.44
P mg/g DW 2014	0.73	-0.12	0.82	1	0.28	0.59	-0.10	0.63	0.78	0.44

S mg/g DW 2014	0.17	0.14	0.30	0.28	1	0.25	-0.38	0.45	0.40	0.24
B µg/g DW 2015	0.78	-0.15	0.54	0.59	0.25	1	-0.22	0.75	0.80	0.50
Ca mg/g DW 2015	0.05	0.38	-0.13	-0.10	-0.38	-0.22	1	-0.46	-0.24	0.10
K mg/g DW 2015	0.55	-0.16	0.70	0.63	0.45	0.75	-0.46	1	0.88	0.51
P mg/g DW 2015	0.64	-0.07	0.69	0.78	0.40	0.80	-0.24	0.88	1	0.66
S mg/g DW 2015	0.40	-0.12	0.44	0.44	0.24	0.50	0.10	0.51	0.66	1

The stability of mineral concentration traits over different scion varieties can be measured by monitoring the same rootstocks grafted with different scion varieties. The rootstock genotypic means for mineral nutrient concentrations are compared to observe their rank. Figures 1 and 2 show the ranking of means in Fuji and Honeycrisp (lines connect same rootstocks) for K and Ca. Both nutrients show some rootstocks behaving similarly (parallel lines) or staying in 'rank' and others changing rank indicating a substantial scion-rootstock interaction. The scion clearly has its inherent tendencies with regards to controlling nutrients as observed by the differences in mean concentrations between varieties. This observation adds a complexity to breeding and selection for mineral nutrient concentrations because of the multiple scion varieties that a particular rootstock will accommodate during its commercial existence. While this task may be simplified by bundling similar scions based on growth habit, productivity, vigor etc. more research needs to be performed to study the rootstock-scion interaction for mineral nutrient traits.

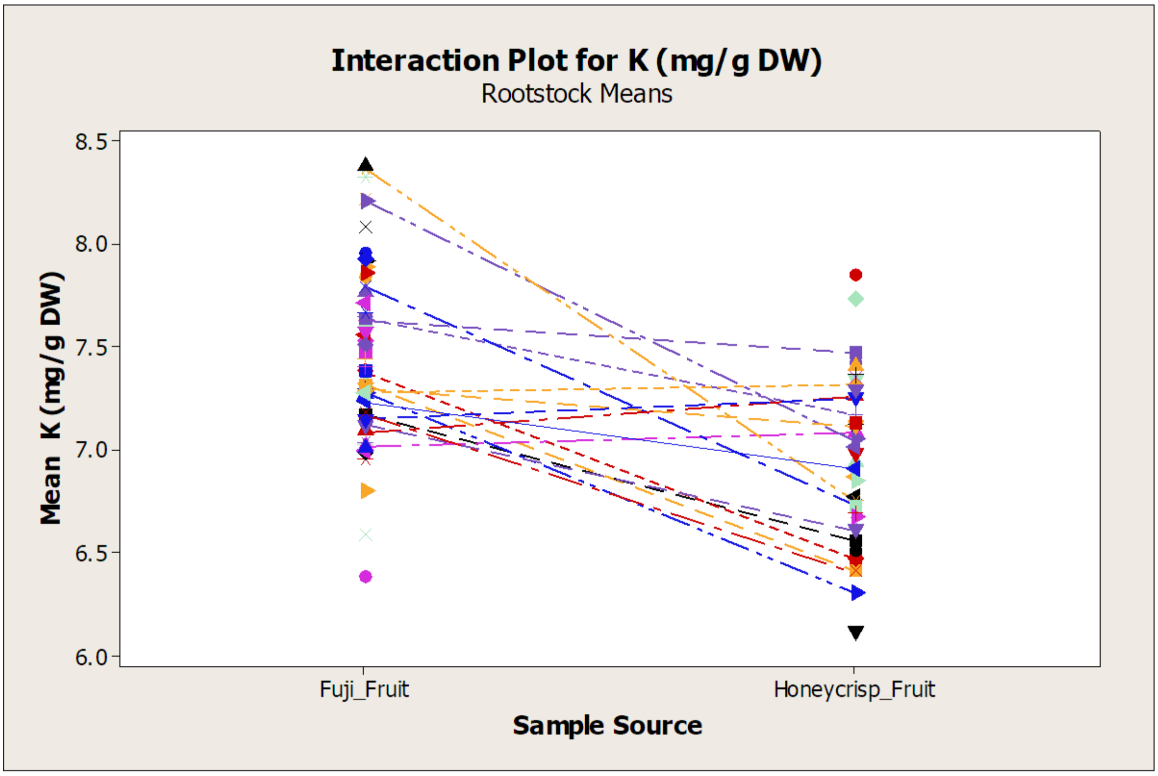


Figure 1. Interaction plot representing the genotypic means of fruit K concentrations in two experiments exhibiting the same rootstocks (connected by lines) grafted with two different scions: Honeycrisp and Fuji. Overall, Honeycrisp means are lower than Fuji indicating a physiological difference between the scions. Parallel lines indicate rootstocks that do not show an interaction with the scion. Crossover lines indicate rootstocks that show an interaction that changes the mean ranking in the experiments. This interaction might be leveraged to select rootstocks that match a specific nutrient requirement of the scion.

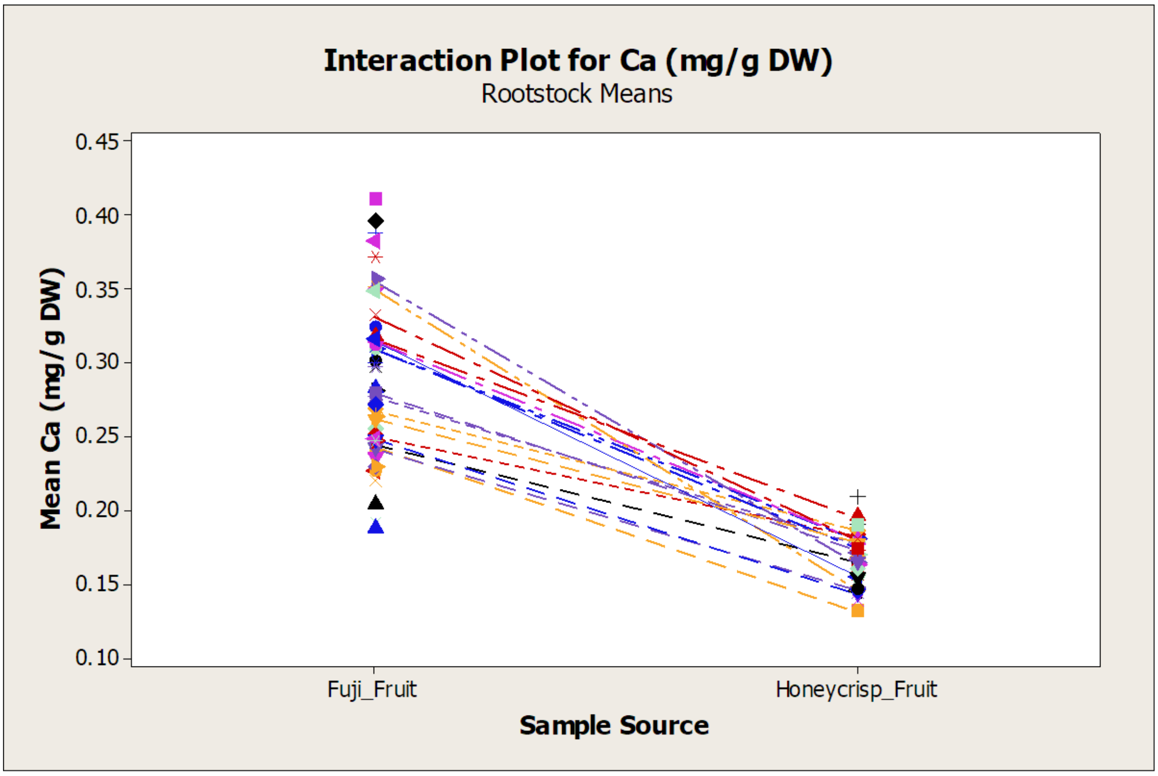
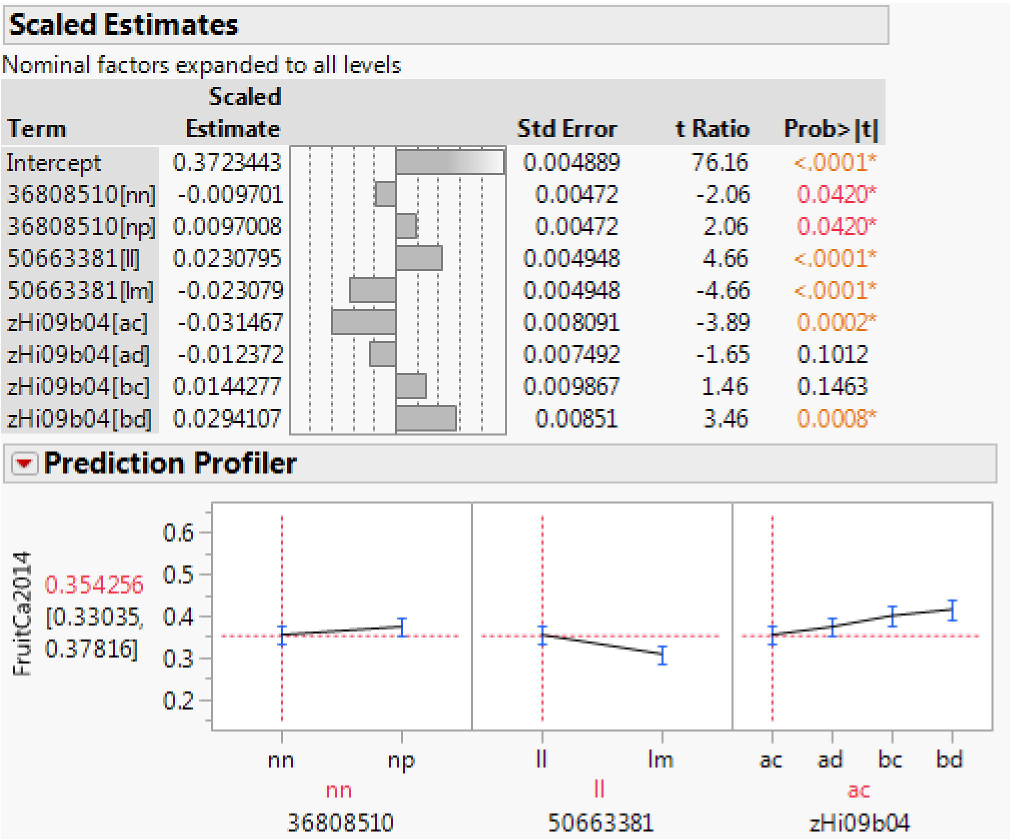


Figure 2. Interaction plot representing the genotypic means of fruit Ca concentrations in two experiments exhibiting the same rootstocks (connected by lines) grafted with two different scions: Honeycrisp and Fuji. Calcium concentration in Honeycrisp fruit is notoriously low, causing some fruit disorders like bitter pit. Parallel lines indicate rootstocks that do not show an interaction with the scion. Crossover lines indicate rootstocks that show an interaction that changes the mean ranking in the experiments. While the potential exists to leverage rootstocks to increase calcium levels in Honeycrisp, compared to Fuji, that potential is smaller.

Considerable effort has been placed within the Geneva® breeding program to learn about genetic factors (Quantitative Trait Loci or QTLs) associated with mineral nutrient traits (Fazio et al., 2013; Fazio et al., 2015a). The initial discovery of such factors was in experiments conducted in pots and we measured only leaf mineral concentrations. The same breeding populations were then used in orchard field experiments where the same traits were measured in leaves and fruit. Field experiments corroborated many of the QTLs found in the pot studies and added a few more because some fruit nutrient concentrations (Mg, Ca) behave differently than what we observed in leaves. In addition to fruit mineral nutrients, field experiments allowed monitoring of some fruit quality parameters like firmness, brix, and size. The QTLs for leaf K discovered on chromosome 5 were field confirmed in fruit and leaves in 2014 and 2015 data; this locus also seemed to affect fruit brix. The QTLs for Mg on chromosome 7 were field confirmed in fruit in 2014 data. The QTLs for P and S on chromosome 8 were field confirmed in 2014 data. However, several other minor QTLs detected for Cu, Zn, and Na were not reproducible in field studies, likely because a field soil environment is much more variable than the potting mix soil used for the initial pot experiments. The ultimate goal for monitoring genetic factors associated with mineral nutrient traits is to be able to use molecular markers to select for these traits since the phenotypic evaluations for mineral nutrient traits can be resource intensive (Fazio et al., 2011). In our marker assisted breeding scheme, once QTLs associated to a specific nutrient are discovered, they are broken down to their allelic effects and then modeled (Figures 3 and 4) to see what allelic combinations will deliver the desired concentrations and then develop a selection protocol that increases the frequency of those alleles in the breeding stocks. The Geneva® breeding program is in the process of developing such marker assisted breeding protocols for some of the simpler nutrient trait targets, and using molecular and phenotypic information to select for new parental combinations that should yield desired combinations.


Scaled Estimates

Nominal factors expanded to all levels

Term	Scaled Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.3723443	0.004889	76.16	<.0001*
36808510[nn]	-0.009701	0.00472	-2.06	0.0420*
36808510[np]	0.0097008	0.00472	2.06	0.0420*
50663381[ll]	0.0230795	0.004948	4.66	<.0001*
50663381[lm]	-0.023079	0.004948	-4.66	<.0001*
zHi09b04[ac]	-0.031467	0.008091	-3.89	0.0002*
zHi09b04[ad]	-0.012372	0.007492	-1.65	0.1012
zHi09b04[bc]	0.0144277	0.009867	1.46	0.1463
zHi09b04[bd]	0.0294107	0.00851	3.46	0.0008*

Figure 3. Estimates of the allelic effect of factors (QTLs) associated with calcium concentration in a breeding population of apple rootstocks. Estimating allelic effects (nn, np, ll, lm, ac, ad, bc, bd represent allele configurations) is an important step for the implementation of marker assisted breeding.

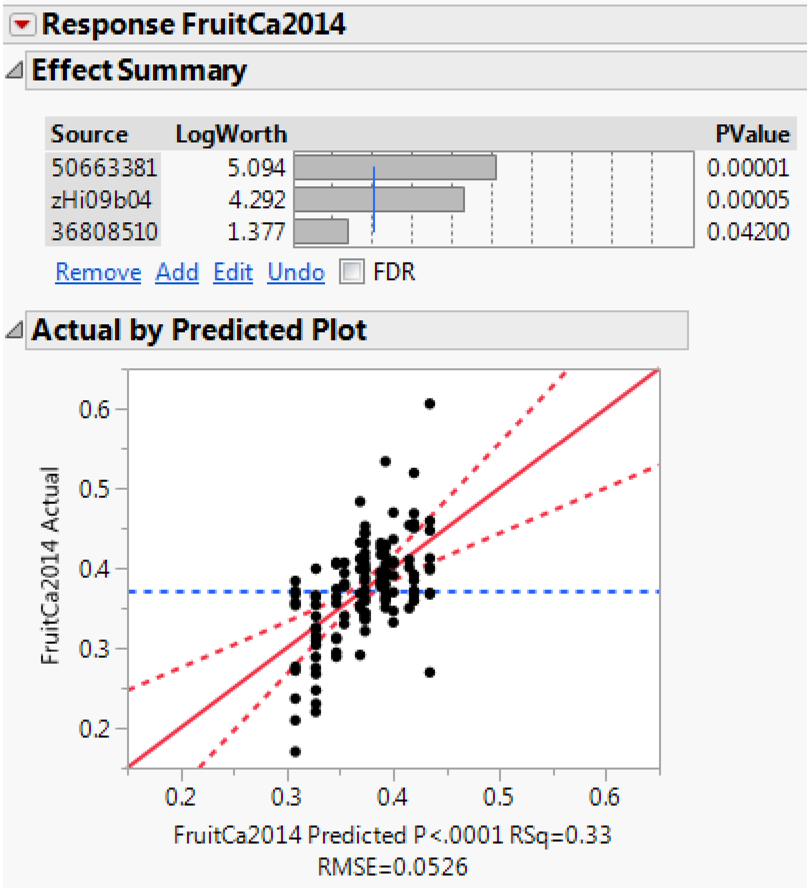


Figure 4. Modeling of the allelic effect of factors (QTLs) associated with calcium concentration in a breeding population of apple rootstocks. Modeling allelic combinations is an important step for the implementation of marker assisted breeding.

CONCLUSIONS

While we have made significant progress, we are still in the early stages of being able to breed apple rootstocks for mineral nutrient modulation in scions. Projects are underway to shed more light on apple rootstock functions related to mineral nutrient physiology and genetics. These projects leverage high throughput genotyping and more uniform growing conditions like aeroponics that allow better detection of minor effect QTLs and the painting of a more defined landscape for mineral nutrient traits.

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