Breeding Apple Rootstocks in the Twenty-First Century – What Can We Expect Them to Do to Increase Productivity in the Orchard?

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Abstract

Rootstocks are the foundation of a healthy and productive orchard. As such, the choice of a rootstock can influence the productivity and profitability of an orchard in a very significant way. Rootstock performance is highly correlated with the genetic potential of such rootstock to provide anchorage, explore the soil profile, absorb and transfer nutrients to the scion, adapt to pedo-climatic conditions, tolerate extreme weather events, resist or cope with pathogens, propagate efficiently and impart positive architectural properties to the scion – like vigor control and precocity. The inheritance and control of all these desirable characters is complex, making breeding (the action of combining high performance traits in same rootstock) quite challenging. Recent advances in genomic technologies are allowing more efficient, and informed ways of selecting new rootstocks during the breeding process. Furthermore, breaking down complex traits like tree vigor into component traits (hormonal transport, nutrient uptake and transport, root architecture, water use efficiency) and further characterization of the inheritance of these component traits can simplify the understanding of complex traits and improve the breeding process and outcome. In the Geneva® breeding program we have been studying root architecture, nutrient uptake and translocation, and inheritance of gene expression to better characterize breeding populations and select parents and seedlings for the next generation of apple rootstocks. We present data relating to these traits and how they are associated with good performance of released and elite stage apple rootstocks.

A discussion about the progress that we may accomplish in the next century with regards to rootstock breeding would not be complete without some reflections on the accomplishments that have occurred in the past century. One hundred years ago, apple orchards looked very different from today – almost all the trees were grafted onto seedling rootstocks, with some seedlings from same mother tree type, but most from apple seed available from cider mills. Clonal rootstocks were in use in Europe, but not very common. A little over 100 years to the date of this symposium (November 1912) R. Wellington and R.G. Hatton in East Malling (England) began to collect and classify the many types of clonal rootstocks that had been in use throughout Europe in earlier centuries – they named these Malling 1-16. The germplasm that was collected and characterized then, contained some very important properties: dwarfing, precocity, phytophthora root rot tolerance which are governed by a handful of genes. That handful of rootstocks/genes has caused a slow but dramatic transformation in our orchards from tall, wide, wood-producing plantings to dwarf, slender, fruit-producing walls. This transformation has increased per acre productivity of high quality fruit and gradually decreased labor, fertilizer, and spray inputs. Several breeding programs then sprouted in several countries around the world and incorporated the initial Malling germplasm to develop new rootstock types better adapted to their specific pedo-climatic conditions. Among the traits targeted by these breeding programs were: cold tolerance, resistance to insects, bacterial and fungal pathogens. Today, orchardists enjoy the availability of several rootstocks that represent improvements on the initial Malling selections. Consequently, dwarfing, precocious rootstocks possessing cold tolerance, wooly apple aphid resistance, fire blight

resistance, and tolerance to replant disease are finding commercial application increasing, food security and productivity in apple orchards around the world. While much has been achieved in the past century, apple growers are still facing several challenges that can be addressed by breeding new rootstocks, so there is still much that can be accomplished in the next century.

Breeding apple rootstocks has to account for many of the interactions that occur between the rootstock genotype and the nursery/orchard. These interactions are numerous and complex, one of the foremost interaction being with the soil (pH, soil type/status, nutrient levels, cation exchange capacity, biology and other forms of fertility), then interactions with climatological conditions (precipitation, temperature extremes), followed by interactions with diseases and insects (root rots, borers, aphids), the interaction with scion varieties and ultimately the interaction between the rootstock genotype and orchard management (ground cover, sprays, fertilizers, fumigation, support systems, pruning, etc.). While the orchardist/nursery can manage/mitigate several of these interactions, they still find themselves in a predicament for things that they cannot change: soil type, climate, disease pressure, etc. While it might be desirable to find one rootstock that will handle variation/stress in all those interactions, such a feat may be nearly impossible, the opposite scenario – to develop a myriad of rootstocks that are adapted optimally to each micro-ecosystem that apples are grown in – is equally impractical. The best approach is to identify the few variables that impact rootstock performance the most and develop a number of rootstocks that can deal with most environments specifically in the most efficient way – in essence "designer" rootstocks. Nurseries are essential partners in this endeavor and may need to adopt novel, more malleable systems of propagation in order to provide the orchardist with the most up to date portfolio of rootstocks adapted to localized needs.

The tools available to breeding programs for understanding how apple rootstocks can modify tree architecture, interface with the soil at the physical (root penetration/ anchorage), chemical (pH, CEC, nutrient extraction) and biological level, resist infections and deal with climate stress are increasing. What is also increasing is the genetic base (source of new germplasm) that is available to discover more stable sources of a particular trait or new traits altogether. In recent years in the Geneva® breeding program we have learned that rootstocks modify the scion at the most fundamental level: gene expression. A series of experiments performed by grafting the same scion onto several, genetically diverse apple rootstocks and then monitoring global gene expression by means of a gene chip revealed that rootstocks have a tremendous influence on activation/ inactivation, up-regulation, and down-regulation of entire gene networks that involve basic tree physiological and cellular functions like sugar metabolism, lignin formation, photosynthesis, disease response, nutrient transport, cell cycle, etc (Jensen et al., 2010, 2003). All of this is accomplished by whatever two-way communication is happening between the scion and the rootstock. Up until the advent of the technologies that enabled high throughput screening of gene expression, we could only theorize that such changes were happening, now and in the future we will be able to take a look at gene networks, identify which ones go with the positive traits that we desire in a tree and select those rootstocks that increase the likelihood of achieving the most in that trait. Among the technologies that will influence new rootstock development are: 1) Marker assisted breeding and quantitative trait locus mapping (Antanaviciute et al., 2012; Celton et al., 2009; Gardiner et al., 2012); 2) Gene transfer either from unrelated source or (more desirable) from the same species (Borejsza-Wysocka et al., 2009; Malnoy et al., 2010); 3) High throughput DNA/RNA sequencing (Chagne et al., 2012; Velasco et al., 2010); 4) Exploitation of knowledge about long range root to shoot (and vice versa) communication through plant hormones, small and micro-RNAs; 5) High throughput affordable phenotyping under different treatments, understood to be the ability to monitor the physiological status of tree tissues on hundreds of plants at the same time and the ability to monitor hormone, nutrient concentration, transport, protein activity in tissues of hundreds of plants (Luby et al., 2010). All these tools will enable the breeder to discover

and harness subtle changes in the DNA sequence of apple trees to develop improved rootstocks.

The base for any progress in breeding is genetic and phenotypic diversity found in germplasm collections and in the wild (Fazio et al., 2009; Volk et al., 2008). Several apple collections representing a wealth of diversity exist in the world, but these collections have almost exclusively been characterized for traits that do not involve rootstocks (fruit, leaves). While there is abundant genetic diversity available in the germplasm, most of the variation in terms of root characteristics is still hidden to the breeder. A concerted effort in phenotyping rootstock traits germplasm in these collections will have positive repercussions on breeding new apple rootstocks.

Reliable and reproducible phenotyping is the key to the development of marker assisted breeding and discovery of meaningful QTLs. In Geneva the breeding team has developed several genetic maps (Fazio et al., 2011) to dissect the inheritance of important traits into haplo-contigs (unique segments of DNA that can be ascertained to a specific parent). So far we have collected phenotypic data on more than 80 traits that deal with root and scion architecture, fruit productivity, precocity, disease resistance and nutrition. The discovery of gene sequences that are associated with important phenotypes in apple rootstocks will impact the long term efficiency of the breeding program by reducing the number of plants that need to be evaluated in the field and increasing the predictability of germplasm in the breeding pipeline. In the Geneva breeding program in collaboration with scientists at Penn State University we measured gene expression of 26,000 unique gene sequences on a full sib population to discover genes that were associated with important apple rootstock traits (Jensen et al., 2012). We utilized the expression profiles for these genes to map expression QTLs (e-QTLs) (Holloway and Li, 2010) for all 26,000 unigenes and recorded the location in the apple genome of the DNA control regions that modulate the production of RNA for these genes. This process was followed by a compilation of genes/eQTLs that were in cis configuration with the traits of interest (correlated with the trait in the rootstock progeny) and contained DNA polymorphisms that also segregated with the trait. One of the traits of interest that we studied with this method is the induction of flat branch angles (IFB) in the scion by the rootstock (Fig. 1) which was characterized in the Geneva germplasm (Fazio and Robinson, 2008a, 2008b). Using a genetic map and a breeding population segregating for this trait we discovered a QTLs for IFB on the top of chromosome 3 of apple and proceeded to ascertain which eQTLs were associated with the phenotype. While several eQTLs were also discovered in the same region, only two had strong correlation with branch angle modification (APPLE-0F000019515 whose sequence is similar to a Mitogen Activated Protein Kinase and APPLE0F00019310 with similarities to a MYB Transcription Factor) (Fig. 2). High throughput next-gen sequencing of parents in the breeding population and further sequence analysis of the DNA regions in which these genes reside, then become the source for discovering nucleotide polymorphisms that can be harnessed to generate cheap, reliable, haplotype specific primers as outlined in Figure 3. These markers can then be used to select other desirable progenies in the breeding pipeline. While the genes identified by correlation analysis may or may not be responsible for the phenotype – the important aspect from a marker assisted breeding standpoint is that they provide a window to the discovery of meaningful, expression-haplotype specific DNA polymorphisms. This process can be performed with virtually any phenotype that is of interest in the breeding program.

Novel and more precise methods of phenotyping plants may include multi-spectral image analysis, or the use of microsensors to monitor growth, water status, nutrient concentration, or photosynthetic activity of a tree. Several years back, our breeding program began investigating the hypothesis that different rootstocks within the breeding program may possess unique capabilities with regards to nutrient uptake and transport (Fallahi et al., 1985; Joubert et al., 2011). It seemed logical because different apple rootstocks respond in a unique way to a range of soil conditions such as pH, water availability, structure, and composition. It also seemed important to investigate nutrition because several

fruit storage disorders can be traced back to nutrient imbalances or deficiencies as in bitter pit which is associated with high N/Ca ratios. The investigation of plant nutrients seemed important because rootstocks that are better at absorbing certain nutrients might need less fertilizer inputs generating efficiency savings for the farmer and the environment. We designed several pot experiments that were meant to monitor growth parameter of young grafted trees onto more than 30 rootstocks with different pH (4.5, 5.5, 6.5, 7.5, 8.5), soil types (Sandy Loam, Clay Loam) and replant disease treatments (Fazio et al., 2012b). What we found was that different rootstocks feature unique interactions with the soil variables that were tested resulting in very different concentrations of nutrients in the same scion variety. The germplasm tested showed positive correlations between nutrient concentration and growth of the scion (Fig. 4) suggesting that selection of optimal nutrient uptake genes of any combination of nutrients could result in better growth of the scion. A parallel experiment that utilized a rootstock mapping population also showed that the genetic factors modulating nutrient uptake and translocation can be identified (Fazio et al., 2012a). This experiment has led to the discovery of major effect QTLs associated with rootstock uptake of potassium, sodium, iron, phosphorous, zinc, calcium, copper, molybdenum and magnesium. The ability to monitor the allelic constitution of the rootstocks in the latter experiment has enabled understanding of how nutrient concentration gradients affect each other and the phenotype of grafted scions, for example a very strong relationship was discovered between days to bud break and potassium/sodium concentrations.

How will monitoring nutrient uptake help with breeding? Understanding how the landscape of nutrient concentration is affected by several inter-related QTLs will guide the prediction of tree size, productivity, fruit set, perhaps the prevention of blind wood, or the ability to tolerate a number of abiotic stresses. Perhaps nutrient uptake efficiency may have an effect on disease resistance in the scion or maybe rootstock architecture like the fine root system shown in Figure 5 which may have a global effect on nutrient uptake. It is pretty clear though that different rootstocks can be selected that optimize the storability of fruit and prevent some storage disorders like bitterpit. It is the opinion of the author that we have only scratched the surface of the potential for rootstock technologies to impact world production positively. What can we expect rootstocks to do to increase productivity in the orchard? If there is a rootstock trait that is correlated with production of high quality fruit and we can measure it (and select for it) then we can expect it to appear in rootstocks that support the orchards of the future – it may just take a bit of time and resources!

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Figures

Fig. 1. Induction of flat branches by rootstocks evidenced by the comparison of the nursery tree architecture of the same scion (Fuji) grafted onto a non inducing rootstock (JTE-B) and an inducing rootstock (G.935). This phenotype is expressed throughout the lifetime of the tree and is detectable in the orchard.

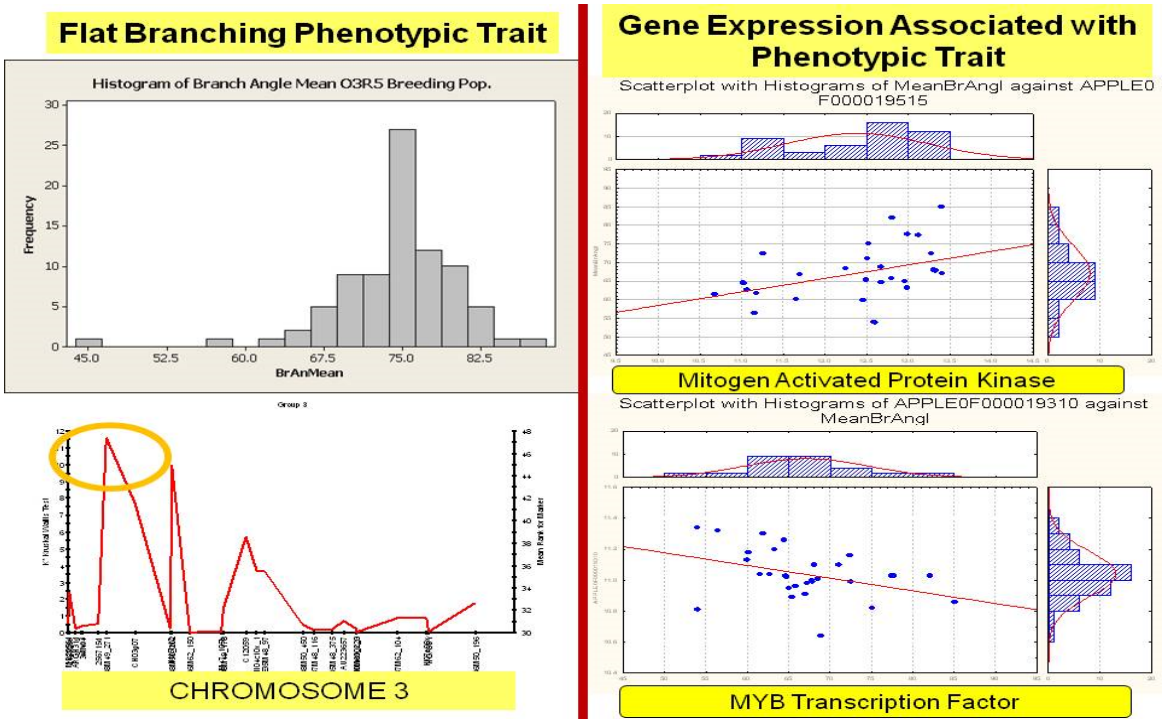

Fig. 2. Trait measurements on a breeding population and discovery of QTLs and eQTLs associated with the induction of flat branches by rootstocks.

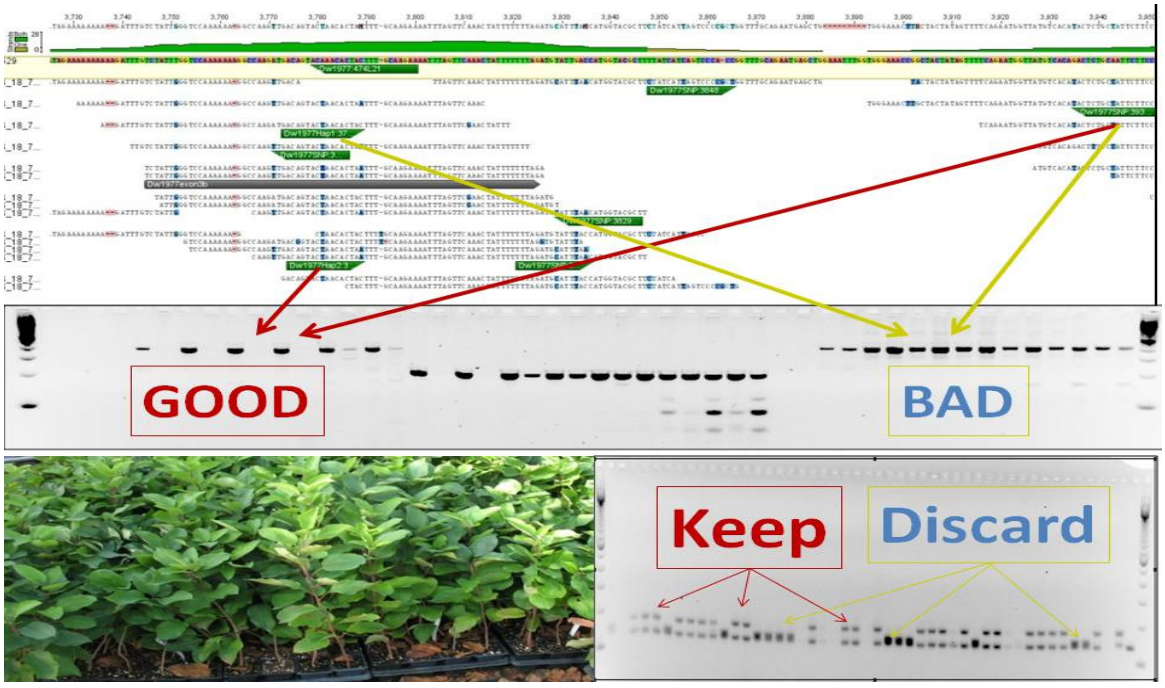

Fig. 3. Exploitation of DNA polymorphisms in associated eQTL gene sequences for marker development.

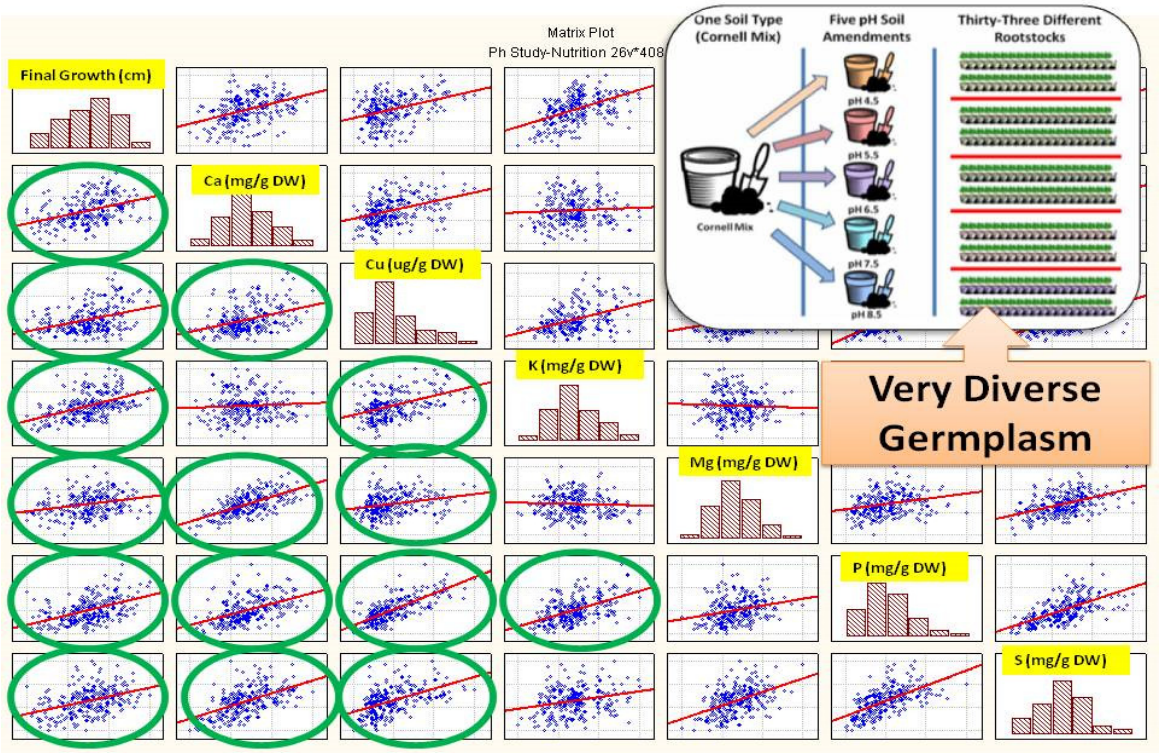

Fig. 4. Correlation of tree growth (final growth) with plant nutrient concentrations in the leaves.

Fig. 5. An example of apple root architecture features that may influence anchorage, soil exploration, water use and nutrient absorption.