
Evaluating and improving rootstocks for apple cultivation

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1 Introduction

The foundations of a productive and healthy orchard are the rootstocks that provide anchorage, water and nutrients essential to the above-ground portions of the trees. The utilization of composite trees has increased the efficiency of breeding productive apple trees by dividing the selection of scion traits and rootstock traits into two genetically (and functionally) different specimens, which are then brought together through grafting. The art and science of grafting scions onto rootstocks spans several millennia; it is thought that it was used initially to aid in the clonal propagation of desirable scion varieties for fruit and nut production (Janick, 2005). In these millennia, it is likely that very little attention was dedicated to the selection of a particular rootstock chosen for its properties (ease of propagation) and the properties it imparted to the scion (Tukey, 1964; Rom and Carlson, 1987; Webster, 2003; Webster and Wertheim, 2003). Clonal selection and the beginning of the science of rootstocks seems to have originated in the latter half of the last millennia, where at least for apple, certain rootstock clonal selections were identified to impart unique productivity and architectural properties (early bearing and dwarfing) onto the grafted scion variety (Monceau, 1768). It is very likely that these properties existed or were selected directly on own-rooted trees first as these trees were early bearing, inherently dwarfed and production of fruit from these curious apple plants was early and abundant compared to seedling trees (Loudon, 1822). The combination of small architecture and productivity is adapted to cultivation in fruiting gardens typical of monasteries, aristocratic and wealthy middle-class dwellings (Rivers, 1866). Someone with very practical acuity made the connection that perhaps, by grafting other apple varieties on these early bearing, dwarfed plants the scion would behave in much the same way. Specifically with

the 'Paradise' apple, 'Jaune de Metz' (Lindley, 1828) otherwise known as Malling 9 (and relatives) the supposition that grafting could make the scion dwarfed was correct and must have led to a revolution of some sort in apple culture because fruit fanciers and nurseries in the eighteenth and nineteenth centuries in central Europe were propagating and using these stocks to make composite dwarfed trees (Hatton, 1917). During this period of discovery there was another important element to these new rootstocks that must have been realized: the seeds from these rootstocks did not breed true, meaning that the early bearing and dwarfing properties were not wholly replicated in all seed progeny. The new rootstocks needed to be propagated clonally to maintain their properties unaltered, hence the possibility of confusion, due to uneven results if a nursery sold trees grafted on seedlings derived from these rootstocks, or had mixtures as their starting material (Hatton, 1920). Major credit needs to be given to the scientists at the East Malling Research Station in the United Kingdom, who in the midst of this confusion collected many clonal rootstocks from around Europe and painstakingly characterized each of them according to their architectural, leafing and flowering properties identified and eliminated duplicates and established what we would call today 'foundation material' of rootstocks that were named 'Malling 1-16' (Hatton, 1919). Some of these rootstocks ('Malling 9' and 'Malling 7') became very popular where 'Malling 9' (M.9) and its sport mutations became the primary rootstock that fuelled the green revolution of dwarfed apple orchards that occurred in the twentieth century in many apple production regions of the world.

In addition to clone characterization and selection, the East Malling Research Station undertook breeding activities aimed at resistance to woolly apple aphids (WAA), a major barrier for apple cultivation in the far reaches of the British Empire and other traits such as improved propagation and productivity. Following East Malling, several other countries (Russia, Germany, Poland, Sweden, Japan, Canada and the United States) hosted government- or university-supported apple rootstock breeding programmes in the latter half of the twentieth century with goals that ranged from improved disease resistance to increased propagability and productivity. All these breeding efforts utilized the initial Malling selections as the starting point for their improvement projects and crossed with parents that would complement the weaknesses of the Malling material (e.g. resistance to fire blight, cold hardiness). Narrow crosses among the Malling rootstocks resulted in two widely used rootstocks: Malling 26 (M.26) and Malling 27 (M.27) that have improved propagability, and different forms of the early bearing and dwarfing effects. Most of the dwarfing founding germplasm was interrelated and had a very narrow genetic base (Oraguzie et al., 2005; Gharghani et al., 2009). The best way to introduce new forms of disease resistance and improve on other horticultural characteristics was to make wide crosses with germplasm that exhibited the desired phenotypes (Aldwinckle et al., 1999; Momol et al., 1999; Forsline et al., 2002). The results of these wide crosses have produced a series of rootstocks that combine disease and insect resistance with productivity, and represent the second generation of rootstock technologies applied worldwide (Fischer, 1991; Wertheim, 1998; Fischer et al., 2000).

Tree fruit growers must look to alternative, economically and environmentally sustainable management schemes of production to remain competitive in the international fruit market (Robinson, 2008). They are doing this by establishing high-density plantings with much smaller trees using new cultivars. These high-density plantings may cost 10–20 times more to establish than low-density plantings, thus greatly enhancing the economic risk (Robinson et al., 2007). Potential returns of high-density plantings, however, far exceed those of low-density plantings, particularly during the first 10 years after planting, often

returning the growers' initial investment much sooner than the initially less-costly, low-density plantings. The central component of high-density systems is the rootstock, the part of the tree which provides size control to allow for high-density plantings (Barritt, 2000). As part of the tree, the rootstock influences many factors in addition to tree size, particularly productivity, fruit quality, pest resistance, stress tolerance and ultimately profitability (Barden and Marini, 2001).

As our understanding of physiology of apple trees, both at the whole tree level and at the cellular level, has increased, so has the understanding of how and what scion properties are modulated by rootstocks, thus increasing the target traits that may be selected to improve whole tree performance by improving rootstock performance (Fazio and Mazzola, 2004). It makes sense then that improving rootstock performance involves two sets of very different types of traits: the inherent apple rootstock traits (rooting for propagation, lack of spines and burr knots, resistance to root pathogens, cold hardiness, etc.) that deal with the interaction between rootstocks and the environment, and scion traits that are modulated by rootstocks (tree architecture, productivity, etc.) that represent the interaction between rootstocks and scions.

2 Apple breeding methods

Breeding apple rootstocks can be a very lengthy process (Johnson et al., 2001a); there are two ways to accelerate the process: the application of marker-assisted breeding (MAB) in the pipeline and/or the intensification of later stages of field testing (Fazio et al., 2015b). The first aims to eliminate substandard germplasm (non-precocious, non-dwarfing, susceptible to diseases, etc.) from the parental and progeny pools via the development and application of robust diagnostic markers. The second is to increase the number of clonal plants tested for each elite genotype and subject them to multiple phenotyping tests and environments that represent production regions. The theoretical benefits from the application of marker technologies to breeding have been reported in publications (Bus et al., 2000; Fazio et al., 2003; Antanaviciute et al., 2012; Bassett et al., 2015). In 2011 the USDA-ARS apple rootstock breeding programme located in Geneva, New York, conducted an internal analysis of the economic impact of applying molecular markers in the breeding programme by itemizing the cost per genotype for each stage of selection. A detailed description of the rootstock breeding programme stages can be found in Fazio et al. (2015b). Briefly, stages 1 and 2 deal with parent selection, crossing, culling seedlings with disease inoculations and propagation of survivors, stages 3 and 4 deal with the establishment of plants as rootstocks in field orchards and propagation beds (see Fig. 1), stage 5 includes the evaluation of propagation beds and replicated tests on biotic and abiotic resistance, stages 6 and 7 are secondary highly replicated tests and stages 8–10 deal with pre-commercial testing with multiple varieties and multiple locations. The Geneva breeding programme elected to conduct the first round of MAB before stage 3, which involves the initial propagation of plants surviving *Phytophthora* root rot and fire blight screens. The cost of genotyping with two markers including DNA extraction and labour was about \$10 per seedling. The cost to phenotype each seedling for dwarfing and precocity during stage 3 (9 years of evaluation) in 2010 dollars was \$15.40/year for 9 years = \$138. The cost savings by culling non-dwarfing individuals was significant, and in 2012 we were able to plant 2 orchard rows of well-replicated, high-density first-test orchard instead of the 12 previously planted.

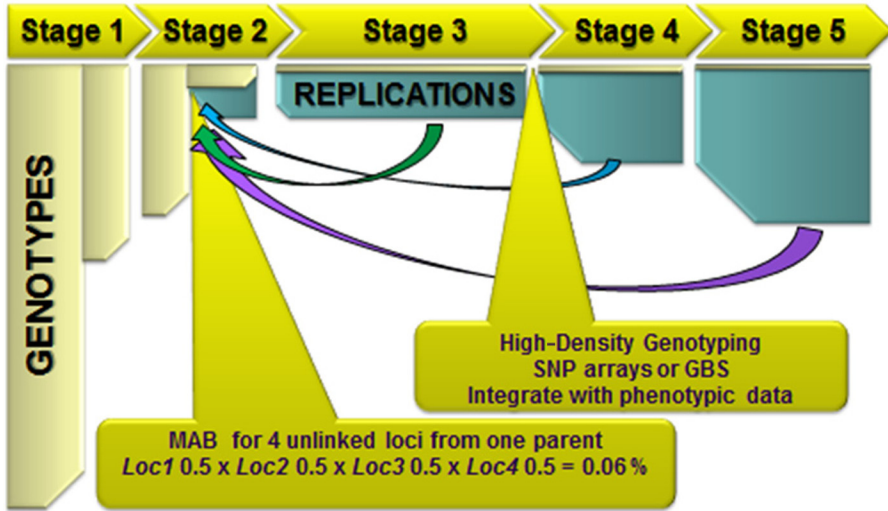


Figure 1 Integration of markers in the US national apple rootstock breeding programme has the impact of increasing highly replicated evaluation of breeding lines at earlier stages of the breeding programme allowing for time and throughput efficiency. Conventionally, stages 2–5 of the breeding programme include planting in fields as propagation sources (maintained as long as the rootstock trials last) and as rootstock trials (7–12 years). Any plant that can be culled (indicated by the arrows) at stage 2 saves the programme significant resources that would go into maintaining orchards and collecting data on useless material. In addition, maintaining the same evaluation capacity (e.g. number of plants tested in a specific stage) in stages 2–5, the programme is able to increase the number of replications and tests done in those stages, thereby increasing the confidence of selection and decreasing the time to release to industry. This cost saving allows for more in-depth genotyping applications with single-nucleotide polymorphisms (SNP) chips (Chagne et al., 2012) or genotyping by sequencing (GBS) methods (Migicovsky et al., 2016) to be applied to surviving individuals.

3 Scion traits affected by rootstocks

Until recently, the number of traits that were recognized to be modulated by apple rootstocks was pretty small: tree vigour, early bearing and water use. This list has been expanded to new architecture components such as canopy shape and bud break (sytleptic branching), and effects on fruit size and quality, on disease resistance and on nutrient availability in the scion. Perhaps the biggest breakthrough in our understanding of rootstock effects on scions is the study that monitored gene expression changes in scion tissue by different apple rootstocks (Jensen et al., 2003, 2010, 2011, 2012). At the cellular level, signals sent from the root system of different genotypes to the scion can change the expression levels of genes, which in turn change the composition of proteins and related metabolic processes and compounds in the scion. Although there are no experiments in apple that have described the opposite interaction, it is safe to assume that this dramatic change likely occurs as signalling from the scion affects the way roots behave and grow. The science behind understanding the issue of communication and affinity between scion

and rootstock seems to be in its infancy and has a lot of potential as the concept of 'designer rootstocks' gets more traction in the industry.

3.1 Tree vigour

The reduction in tree vigour is perhaps the most important trait imparted by apple rootstocks to the grafted scion (Tukey, 1964). It is imparted to the scion as an early termination of overall season growth (Seleznova et al., 2008). The benefits due to this trait in modern orchards are enormous and range from increased efficiency in picking and tree management operations, including mechanization, to the decrease of pesticide inputs, ladder accidents and other ergonomic issues (Groot, 1997a,b; Masseron and Roche, 1999; Robinson et al., 2007; James and Middleton, 2011). At the physiological level, the dwarfing trait has increased the effective light interception and partition to fruit production in the orchard and increased the production per unit area by at least 30% when compared to non-dwarfing rootstocks (Brown et al., 1985; Strong and Miller Azarenko, 1991; Atkinson et al., 1998). This means that for an industry worth \$3 billion like the US industry at least \$900 million are a result of the efficiency gained through dwarfing rootstocks. While the genetic components to this trait have been described to be the interaction of two main loci (Fazio et al., 2014b) and perhaps additional modifying loci (Harrison et al., 2016), it is important to mention that this is a complex trait that has fairly big interaction components and that the total effect of these components results in the overall vigour of the tree. Therefore for any scion 'S', the vigour 'V' is equal to the inherent growth dynamic genetics of the scion 'S_g', plus the dwarfing genetic components of the rootstock 'R_g', plus their interaction, plus the interaction of the whole composite tree with environmental effects 'E' such as fertility, water availability, diseases, soil type, soil pH, and soil type, or orchard management, weed competition and the like so that when scion vigour is measured, the genetic components of dwarfing rootstocks are only a part of the equation. This is exemplified by observing the effect of stunting caused by soil-borne replant disease, which has a similar effect to the dwarfing loci in apple and sometimes confuses the estimation of vigour potential of a rootstock.

Although several architectural dwarfs have been identified in domesticated and wild apple populations (Fazio et al., 2009a, 2014a), this material has not produced commercially viable rootstocks or has not been tested for similarity to the dwarfing characters offered by the alleles contained in M.9, M.8, M.13 and other Malling rootstocks belonging to the initial set selected in East Malling. The dwarfing trait has been shown to be highly heritable, modulated mainly by the combination of alleles of locus Dw1 found on chromosome 5 (Rusholme et al., 2004; Pilcher et al., 2008) and locus Dw2 found on chromosome 11 (Fazio et al., 2014b). Models that take into account some or all combinatorial allelic effects of these two loci have been able to explain upwards of 80% of the genotypic variation for dwarfing (Foster et al., 2015). The two loci interact with each other and do not necessarily seem to be additive, meaning that the lack of one dwarfing locus effect in the model negates the effect of the other. Several physiological models based on phenotypic observation have hypothesized the involvement of hormone signalling (Zhang et al., 2015; Tworowski and Fazio, 2016), graft union anatomy (Tworowski and Miller, 2007; Tworowski and Fazio, 2011), hydraulic conductivity (Atkinson et al., 2003; Cohen et al., 2007), dry matter partitioning to fruit production or a combination of these (van Hooijdonk et al., 2011), while the underlying causative genes are still largely unknown.

Although current science may have a good understanding of the genetics governing this trait and may have developed robust markers for culling out undesirable material in the breeding pipeline, nothing can substitute the required time in the field to accurately measure the potential of each rootstock to dwarf the scion. Evaluation of this trait for breeding still requires a lengthy period of 7–10 years for the first observation and perhaps another 10–12 years for multi-location trials with multiple scions. As mentioned in the beginning of this section, multiple field trials are needed to evaluate rootstock interactions with different scions and environmental factors.

3.2 Inducement of early bearing in scions

Apple seedlings planted on their own roots experience a juvenile period anywhere from 4–7 years before they reach sexual maturity and bloom and fruit (Visser, 1967). Some dwarfing rootstocks have the ability to induce early bearing or reduce the juvenile period to 2 years in extreme cases (Visser and Schaap, 1967; Visser, 1973). Early bearing is a major selection criterion for improved apple rootstocks because the intensive types of cultivation of apple require a quick return on investment (early production of apples) to offset the installation and infrastructure costs to build the orchard (Cummins et al., 1995; Robinson et al., 2007, 2011). The genetic loci underlying the rootstock-induced trait ‘early bearing’ were first described by Fazio et al. (2014), as two loci, Eb1 and Eb2, that roughly co-located with Dw1 and Dw2, perhaps indicating that the two traits may be physiologically and genetically interconnected. Several studies have described the rootstock-induced partitioning effect of photosynthate into sexual (fruit) and vegetative portions of the tree (Seleznyova et al., 2008), comparing the effects of different rootstocks (Marini et al., 2006a; Autio et al., 2011b,c), crop loads on tree growth (Marini et al., 2012), productivity and bienniality (Marini et al., 2013). However, there is paucity in the literature about the causative elements for these rootstock effects. Breeding for this trait requires field evaluation for four years for the first observation in a replicated experimental orchard and then an additional 5 years in multi-location, multi-scion trials. Visser (1967) showed that scions with reduced juvenility also seemed to be more productive when grafted on M.9 dwarfing rootstocks, indicating the possibility of an inherent scion effect on early bearing and the need to test this scion–rootstock interaction in replicated trials.

3.3 Induction of architectural changes

Early and abundant fruit production is related to the number of flowering buds produced in the nursery phase and early establishment of the tree in the orchard (Ferree and Rhodus, 1987; Robinson et al., 1991a,b; Theron et al., 2000). This number can be influenced not only by the early bearing effects of the rootstocks discussed previously, but also by the ability of the rootstocks to produce prolific sylleptic branching (feathers on a nursery tree) in the nursery and later in the orchard. Early yield has been associated with nursery tree calliper, tree height and number of feathers. Rootstocks with wider genetic diversity than M.9 and Budagovsky 9 (B.9) have been shown to influence the production of sylleptic branches and the formation of crotch angles, that produce trees with a more open (flatter branches) structure (Fazio and Robinson, 2008a,b). This characteristic is mostly observed in rootstocks developed by the Geneva, New York, breeding programme, especially with rootstocks G.935, G.213, G.41 and G.214 (Fig. 2 and 3). The strength of these effects vary with different scions and continue through the life of the tree in the orchard, as observed

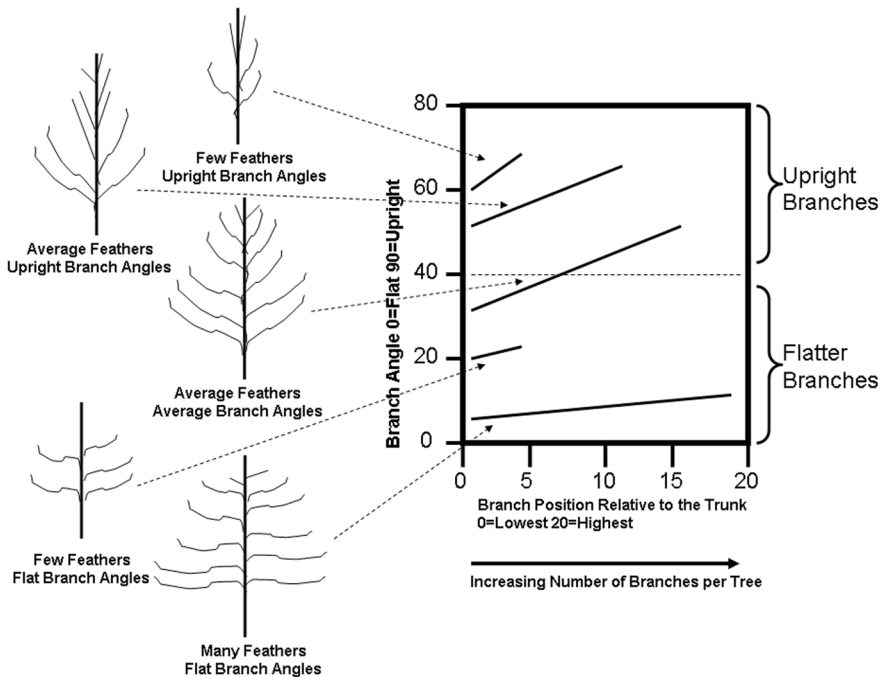


Figure 2 The number of feathers and average angle to the trunk can be captured in a simple graphic.



Figure 3 Comparison of nursery tree architecture featuring a flat branching rootstock, G.935 (right), versus an upright branching rootstock (JTE-B).

in the millions of trees planted on G.935 rootstock throughout the world. One additional characteristic that may be related to sylleptic branching is the ability of some rootstocks in the Geneva breeding programme to induce bud break and flowering in low chill

environments. This effect was observed in a replicated trial in Southern Brazil with 'Gala' grafted on three rootstocks G.213, M.9 and, Marubakaido with M.9 interstem (an interstem is a section of the trunk grafted in between the rootstock and the scion usually made from a third rootstock variety to bridge incompatibility or leverage the qualities of the interstem to increase precocity of the whole tree) with 10 trees per plot and 10 replicated plots per rootstock where it was observed that flowering and bud break were 35% higher with G.213 rootstocks, resulting in higher productivity of the trees (Francescato, pers. comm.). Breeding for these characteristics requires a lot of time and effort as the effects are confounded by the interaction with the dwarfing potential of the rootstocks and the difficulty of measuring crotch angle and branch length of thousands of replicated nursery trees. Our understanding of the genetic effects underlying these traits is in its infancy as the trait was first described in 2007. It is likely that research and breeding efforts aimed at uncovering the genetic factors for these traits will lead to more productive apple trees.

3.4 Propagation traits

Apple rootstocks can be clonally propagated by sterile *in vitro* methods, soft and hard wood cuttings (Bassuk and Howard, 1980), and by layer or stool cuttings (Adams, 2010). Whereas efforts to breed rootstocks amenable to *in vitro* culture are virtually impossible due to the complexity of media and growing conditions, efforts to improve rooting ability in layering beds and cuttings although difficult may result in superior rooting genotypes. Breeding for nursery performance can be quite complicated as many factors influence apple rootstock performance in the different nursery phases and at times may conflict with field performance. A prime example of this is the fast and easy adventitious rooting trait, highly desired in the propagation phase but correlated with the development of burr knots in the orchard – a harmful trait in certain orchard environments especially where dogwood borers and other insect borers may be present (Bergh and Leskey, 2003). Although it is possible to produce rootstocks with clean (no burr knots) shanks which also produce few or no suckers in the orchard, the selection of these orchard traits can result in difficult propagation properties with standard methods. These difficulties can be overcome with improved nursery management practices which include utilization of different propagation techniques like cuttings (Hansen, 1989; Deering, 1991) or micropropagation (Castillo et al., 2015; Geng et al., 2015), and the treatment with plant growth regulators such as prohexadione calcium in the nursery (Adams, 2010) to increase production of primary adventitious roots. The genetics of adventitious root formation have been investigated in the Geneva breeding programme revealing a complex trait with low heritability. Therefore, while it may be possible to breed for rooting traits, the importance of these traits is dwarfed by the importance of low suckering and lack of burr knots in the orchard.

Another characteristic affected by the rootstock is graft compatibility. Historically, most problems that were blamed on compatibility turned out to be virus related (Cummins and Aldwinckle, 1983; Lana et al., 1983), however, certain rootstock/scion combinations under unspecified grafting and nursery management conditions have shown a tendency for weak graft unions in very young trees (Robinson et al., 2003). Graft incompatibility can arise because of the disruption of normal healing between grafted tissues and can result in anatomical and physiological symptoms, biochemical and mechanical issues that lead to graft failure or tree death (Simons and Chu, 1983, 1985; Skene et al., 1983; Simons, 1985). Although it is likely that the method of grafting (chip budding, whip-and-tongue grafting, and machine V grafting) (Hartmann et al., 1997) has an effect on healing

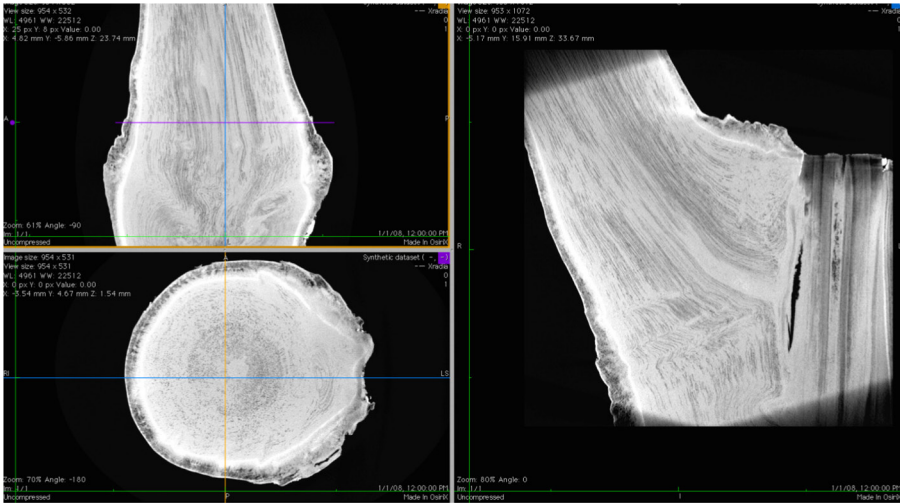


Figure 4 CAT scan of a graft union showing dead tissue where the graft was initially placed and unorganized tissue between scion and rootstock, a possible indication of weakness.

and union strength at various stages in the nursery cycle, there may be plant-growth-regulator-related and metabolic-compound-related signals that prevent the formation of a strong graft union. Efforts to understand the ability of the rootstock/scion combination to generate enough connective tissue where they meet is underway in the Geneva breeding programme through the use of X-ray tomography (CAT scans) shown in Fig. 4. Some nurseries report that large calliper stocks may not form as strong a graft union as small calliper stocks; therefore, a rootstock genotype that produces smaller calliper liners from the stool bed may be more suitable for nursery tree production. We have not been successful at evaluating finished nursery tree traits early in the breeding cycle because such traits are somewhat hard to evaluate and require high replication in order to detect rootstock effects on many different scions (Song et al. 2013).

Improvement of rootstocks sometimes means breeding interstems that can leverage dwarfing and precocity to improve the performance of a well-adapted vigorous clonal or seedling rootstock (Webster and Warrington, 1995; Luo et al., 2013). The length of the dwarfing interstem is inversely proportional to vigour induction and directly proportional to the induction of early bearing in apples (Carlson and Oh, 1975). Whereas this method of propagation is still used in some apple growing regions, most nurseries have moved away from this system because it adds extra costs (labour, materials, time, risks) to the development of a finished tree and it is not always reliable. It is possible, however, to select certain combinations of rootstocks and interstems that may leverage positive traits of root systems (disease resistance, nutrient uptake) (Ebel et al., 2000; Ma et al., 2013) and combine them with stem traits (dwarfing and early bearing) to generate even more productive trees, but no breeding programme to date has published research data on such combinations.

Apomixis, or the production of seedlings containing the same genetic information as the mother tree, has been observed in several wild apple accessions (Campbell and Dickinson,

1990). Breeding apomictic apple rootstocks has several attractive aspects in terms of nursery management as seed is more easily transported, free of viruses and provides opportunities for adjustment in production level to match demand. Although apomictic rootstock selections have been identified and evaluated, this material has not gained much traction because of inherent problems with other characteristics in this germplasm (Schmidt, 1972, 1982; Ferree, 1998) including the lack of early bearing induction so critical for modern production (Sax, 1949; Schmidt, 1970). Breeding for apomicts still continues in certain programmes in China (Sha et al., 2011; Ma et al., 2012), however, because of the difficulties related to hybridizing with apomictic species (Bisognin et al., 2008), most breeding programmes have abandoned this effort.

3.5 Drought tolerance

It is difficult to define drought tolerance without an objective reference or phenotype to measure and it is even more difficult to define in a rootstock independent of the scion-specific tolerance (Higgs and Jones, 1991; Virlet et al., 2015). The economic definition of drought tolerance (little or no loss of marketable fruit production) is different from some of the physiological definitions, which range from loss of photosynthetic activity (Massacci and Jones, 1990), to shoot and root growth under stress (Atkinson et al., 2000), to water use efficiency. Perhaps drought-tolerant rootstocks are of little value where irrigation water is available and relevant only in regions that utilize rainwater and may experience long stretches of drought. However, as climate changes and freshwater availability is threatened in traditional apple growing regions, the search for rootstocks that can thrive with less water is becoming more and more important (Ebel et al., 2001). It has long been recognized that there are differences in apple rootstock reaction to drought (Preston et al., 1972; Cummins and Aldwinckle, 1974; Ferree and Schmid, 1990), but those observations mostly dealt with spurious drought events and compared vigorous and non-vigorous types (Chandel and Chauhan, 1993; Fernandez et al., 1994). Decreased sensitivity to drought was attributed to 'Malling 9' rootstock when compared to 'Mark' (Fernandez et al., 1997) in a potted tree study. A comparison of hormonal drought response between M.9 and MM.111 rootstocks indicated that both rootstocks provided drought resistance but by mechanisms which appear to differ – M.9 produces higher levels of abscisic acid (ABA) that may regulate stomatal opening while MM.111 possesses a more extensive root system (increased soil exploration index) (Tworowski et al., 2016). Water use efficiency, defined as the ratio of biomass produced to the rate of transpiration, and decreased sensitivity to drought (Xiang et al., 1995; Bassett et al., 2011) has been described in wild apple populations indicating the possibility of using this descriptor as a selection method. Breeding for such a complex trait may be possible only at latter stages of selection as discernment of field-meaningful data requires experiments with high replication, special equipment to control water delivery and use, and very-high-density morphological and physiological measurements. Perhaps gain can be made by selection of components of the trait such as improved root morphology, plant growth regulator signals and nutrient uptake once their effect is identified in breeding populations possessing all the other 'important' traits.

3.6 Cold tolerance

Several rootstocks seem to be tolerant to the different types of cold events that can cause injury of cambial and root tissues (Embree, 1988). Damaging cold events can be quite

different in their mode of action as mid-winter events can have very different modes of action than late fall or spring cold events (Cline et al., 2012). Therefore, the methods used to evaluate sensitivity to differing cold injuring events need to address the physiological conditions specific to each event (Quamme et al., 1997; Moran et al., 2011a,b). Fluctuating temperatures in late fall, early winter and early spring are associated with hardening and de-hardening of tissues. This hardening and de-hardening process may have a strong genetic component (Forsline and Cummins, 1978), where a group of *Malus* rootstocks seem to have improved ability to be insensitive to such temperature fluctuations and remain dormant and cold-acclimated. Harvesting rootstock liners during these periods and subjecting them to increasingly low temperatures to show cambial damage is perhaps the most meaningful way to select cold hardy apple rootstocks. Observation of blackheart damage can also aid in the discernment of rootstock/scion combinations that are susceptible to mid-winter injury (Warmund and Slater, 1988; Warmund et al., 1996). Genes associated with cold response have been described for 'Gala' scions (Wisniewski et al., 2008), and similar genes may be found in apple rootstocks. However, the understanding of segregating factors that influence the different types of cold stress adaptation is virtually non-existent, making genetic or genomic-informed breeding impossible, and therefore selection relies entirely on highly replicated phenotyping.

3.7 Root morphology and architecture

Phenotypic variation in the morphology of roots has been associated with increases in yield and tolerance to abiotic stresses in several crops (Sousa et al., 2012; Chimungu et al., 2014; Lynch et al., 2014; Burton et al., 2015; Zhan et al., 2015). It makes sense that harnessing genetic and phenotypic variation in root morphology traits in apple rootstocks may improve productivity, tree size, drought tolerance, nutrient uptake, anchorage and other related whole tree functions (Eissenstat et al., 2001). Ample phenotypic variation has been characterized in wild *Malus sieversii* populations and within the Geneva apple rootstock breeding programme where genetic factors for fine root formation (highly branched fine roots) have been mapped to chromosomes 4 and 11 of the apple genome (Fazio et al., 2009b). Other traits that may be important to characterize may be the volume explored by the roots, the angle of the roots, the longevity of the roots and so on, which are all traits that are difficult to phenotype and for which robust genetic markers may be extremely useful. In Geneva, New York, the apple rootstock breeding programme measured several scion and root morphology characteristics of nursery trees of related (half-sibs) *Malus sieversii* seedlings. They found correlation between canopy volume/tree size and number of thick roots ($0.38, P < 0.001$), and a less pronounced correlation between tree size and root mass ($0.25, P < 0.001$), indicating a feedback loop between scion and root growth: the ability of the canopy to support the growth and expansion of a larger primary root system increased the vigour of young trees by their ability to produce root systems with strong primary hierarchy (Fazio et al., 2014a). Apple root systems vary in seasonal growth patterns (Eissenstat et al., 2006), which may affect their ability to forage for nutrients and water, and even colonization with beneficial mycorrhizae (Resendes et al., 2008). Root turnover rates may also play a significant role in tree nutrition and productivity as well as disease resistance or evasion as demonstrated by experiments that utilized replant-tolerant rootstocks from the Geneva breeding programme (Atucha et al., 2013; Emmett et al., 2014). Although these root traits can be targeted for MAB, the understanding of genes, gene expression patterns and physiological attributes associated

with these traits in rootstocks is limited compared with our understating of scion traits; therefore, more research is needed to understand these traits before they become the subject of selection based on genetic markers.

3.8 Nutrient uptake

Another set of root-related traits deals with the genetic variation and inheritance of absorption and translocation of macro- and micronutrients by the rootstock to the scion (Tukey et al., 1962). Rootstocks have been shown to vary significantly with regard to their intrinsic ability to forage for nutrients as well as transfer them up to various sinks in the scion, including fruit, perhaps affecting organoleptic, post-harvest qualities of the fruit and disease resistance (Lockard, 1976; Westwood and Bjornstad, 1980; Om and Pathak, 1983; West and Young, 1988; Chandel and Chauhan, 1990; Rom et al., 1991; Sloan et al., 1996; Chun and Chun, 2004; Kim et al., 2004). Transgenic, cisgenic or conventional breeding approaches have been suggested to increase nutrient uptake of minerals such as zinc to improve productivity of the orchard (Swietlik et al., 2007). Most research on nutrient uptake by apple rootstocks has focused on developing the best management practices for nutrient application on a genetically restricted set of rootstocks, and it was not until a large set of genetically diverse rootstocks were observed in different soils and pH treatments that the physiological diversity of apple roots was revealed (Fazio et al., 2012). Changes in soil pH, for example, showed differences in the expected curves of absorption of metal ions such as manganese and iron, indicating that some rootstocks performed better at certain pH than others. Soil pH is one of the most important predictors of soil fertility and developing a set of rootstocks well adapted to specific pH profiles may improve orchard performance and open up more land to the cultivation of apples.

The analysis of scion nutrient concentration in leaves and fruit in several rootstock field trials in New York State have indicated the possibility that specific rootstocks may affect fruit quality of Honeycrisp apples showing that certain rootstocks are able to transfer higher calcium levels to the fruit and that the calcium-linked disorders typical of Honeycrisp are a result of scion-specific intrinsic challenges in the movement of calcium into the fruit (Fazio et al., 2015a).

Investigation of the inheritance of nutrient uptake and translocation in a full-sib population of apple rootstocks revealed quantitative trait loci (QTL) influencing scion leaf mineral concentrations of potassium (K), sodium (Na), phosphorus (P), calcium (Ca), zinc (Zn), magnesium (Mg) and molybdenum (Mo) with the most significant ones on chromosome 5 for potassium; chromosome 17 for sodium; and lower significance QTLs for calcium, copper, zinc and phosphorous (Fazio et al., 2013). Concentrations of some nutrients were highly correlated (K and P, S and P), indicating common nodes in the networked pathway that takes nutrients from the soil through the rootstocks to diverse sinks in the scion. Subsequently, leaves and fruit of Honeycrisp scion in a rootstock research plot in Summerland, Canada, featuring 31 diverse rootstocks were tested for mineral nutrient concentrations and found to have significant differences, identifying a negative correlation between phosphorous and calcium in the first season fruit (Nielsen and Havipson, 2014). The very different mechanisms (interaction with soil biota, active and passive transport, vessel composition and size, etc.) that impact absorption and transport and the fact that crop load and irrigation can also influence mineral concentrations (Nielsen et al., 2015) makes these traits difficult to improve without the aid of a robust understanding of

molecular genetic factors involved. Modelling those factors to achieve a particular balance of nutrients in selected scions is therefore very complicated.

4 Disease and pest resistance

Commercial application of improved disease and insect resistance can be observed in the Geneva, New York, breeding programme. Since its inception, the programme focused on developing apple rootstocks resistant to fire blight, a North American disease caused by *Erwinia amylovora*, while maintaining the resistance to crown and root rot caused by *Phytophthora cactorum* (Aldwinckle et al., 1972; Gardner, 1977, 1980). This effort over three decades produced rootstocks that are not only resistant to fire blight and crown rot, but that are tolerant to the replant disease complex, and are also resistant to WAA (*Eriosoma lanigerum*).

4.1 Resistance to fire blight

Fire blight is a devastating disease caused by the anaerobic, gram-negative bacterium *E. amylovora*, which causes visible symptoms in blossoms, green tissues, fruit and some woody tissues of apple scions and rootstocks. Although this disease seems to have originated in the Eastern part of North America, it has now spread to most of the apple growing regions of the world. Rootstock blight on susceptible rootstocks (M.9, M.27 and M.26) can be devastating as the infection results in girdling and death of the rootstock shank eventually killing the whole tree – entire orchards and millions of trees have been destroyed because of rootstock blight. While spraying antibiotics like streptomycin can alleviate the onset of rootstock blight, genetic resistance of the rootstock is the best preventive treatment. Rootstock resistance to *E. amylovora* is found in several wild apple species and these have been utilized to breed a new series of fire blight-resistant rootstocks. There seem to be two main types of resistance in apple rootstock: a multi-genic type similar to that found in *Malus robusta* 'Robusta 5' where green tissues and flowers are not affected by the bacterium (Aldwinckle et al., 1974b; Cummins and Aldwinckle, 1974) and an ontogenic type of resistance found in Budagovsky 9 (B.9) rootstock where the green tissues are severely affected, but two-year-old and older wood seems not to react to the bacteria (Russo et al., 2008). Genetic inheritance of the 'Robusta 5' type of resistance has been described as having a strain-specific component on chromosome 3 identified as a gene belonging to the NBS-LRR class of resistance genes (Fahrentrapp et al., 2013; Broggini et al., 2014; Kost et al., 2015) and other minor QTLs on linkage groups 5, 7, 11 and 14, which do not seem to be strain-specific detected in a non-rootstock population 'Idared' × 'Robusta 5' (Wohner et al., 2014). Another locus that is non-strain specific was discovered on linkage group 7 in a rootstock population derived from a cross between 'Ottawa 3' and 'Robusta 5' (Gardiner et al., 2012). *Cis*-genic approaches with the LG03 gene proved only partially successful, suggesting a more complex pathway of resistance than just one gene recognition of the pathogen (Kost et al., 2015). The inheritance of the B.9 type of resistance is currently not known.

Screening and evaluation of resistance to fire blight is performed in two stages: the initial stage where a culture of a mix of *E. amylovora* strains are applied to actively growing shoots resulting in the elimination of any plant showing a visible necrotic lesion (Fig. 5), and



Figure 5 Side-by-side comparison of a susceptible (M.26 left) and resistant (G.41 right) rootstock inoculated with E2002a strain of *E. amylovora*. These types of inoculations are repeated several times during the process of breeding, utilizing multiple strains either independently or mixed.



Figure 6 Fire blight ooze from a blossom-inoculated tree: a sure sign that this rootstock will die killing the tree.

the second stage is where *E. amylovora* solution is sprayed during bloom onto the scion of two four-year-old finished trees in a replicated field trial (5–10 replicates) to simulate the same type of infection that would occur in a typical orchard. The second stage inoculation takes longer to appear with the initial symptoms being a small ooze point on the rootstock shank in the summer (Fig. 6), but the effects are quite dramatic in the beginning of fall when the leaves on the affected trees turn purple, indicating girdling and death of the tree (Russo et al., 2007). Combined these methods of inoculation can select for both types of resistance and have resulted in the release of several commercial rootstocks (G.65, G.11, G.16, G.30, G.202, G.41, G.935, G.214, G.814, G.213, G.969, G.890, G.222 and G.210) that show various levels of resistance to fire blight. MAB for this disease is possible but not necessary if greenhouse and field inoculation methods are available.

4.2 Replant disease complex

The specific apple replant disease complex is a syndrome observed as stunting and poor growth of young apple trees planted in soil that was previously planted with an apple or pear orchard. This complex disease causes major production losses throughout the life of the orchard. The main causative agents implicated in this syndrome are *Cylindrocarpon destructans*, *Phytophthora cactorum*, *Pythium* spp., *Rhizoctonia solani* and various pathogenic nematodes (Mazzola, 1998). The occurrence of one or more of these agents will affect the severity of the syndrome and may explain some of the site-to-site variation observed in replant land. This is one of the major problems faced by orchardists as virgin land becomes more rare, major infrastructure investments (hail nets, irrigation, etc.) become more prevalent and require a 'replant-in-place' type of renewal of the orchard and as fumigation chemistries are restricted by environmental laws (Auvil et al., 2011). The removal of the old orchard leaves a major pathogen load in the soil, which overwhelms the young root system of nursery trees. Fumigation treatments (methyl bromide, chloropicrin and nematicides) seem to be effective for less than a year as the pathogens implicated in this disease quickly recolonize the sterile soil, and fallow treatments (undesirable because they leave the land in an unproductive state) have shown mixed results, with replant symptoms sometimes appearing even after 4 years of fallow (Leinfelder and Merwin, 2006). Alternative treatments like seed meal amendments, fertilizers, compost teas and solarization have been proposed and are in various phases of research and development (Utkhede, 1999; Utkhede and Smith, 2000; Mazzola and Mullinix, 2005; Mazzola and Manici, 2012). In addition to the combination of pathogens involved in each orchard, factors like soil type, climate and other edaphic conditions seem to affect the severity of the complex, making it difficult to diagnose (Fazio et al., 2012). The effects of the disease complex are usually measured by comparing the growth of the same rootstock in sterile soil (pasteurization or chemical treatment) to a biologically active soil collected from the rhizosphere of the old orchard (Leinfelder et al., 2004; Rumberger et al., 2004; Yao et al., 2006a). A comprehensive study of multiple rootstock accessions and *Malus* species indicated that there was sufficient phenotypic diversity to enable growth in non-pasteurized soil (Isutsa and Merwin, 2000); however, the only reported commercially applicable genetic tolerance to the replant disease complex seems to be derived from progeny of 'Robusta 5' and other wild apple species (Auvil et al., 2011). Certain root genotypes have been reported to promote unique types of microbial communities, indicating a specificity or perhaps a pseudo-symbiotic effect of specific root systems that defeat the presence of pathogenic microbes (Yao et al., 2006b; Rumberger et al., 2007;



Figure 7 Seedling survivors of inoculation with *Phytophthora*. The tray was packed with live seedlings before inoculation.

St. Laurent et al., 2010). Breeding and selection for *Phytophthora* resistance performed by inoculating young seedlings (Fig. 7) (Aldwinckle et al., 1974a) and planting test orchards in replant soils might be the reason why several Geneva rootstocks have exhibited strong tolerance to apple replant disease components. New studies leveraging next-generation sequencing of *Pythium* challenged rootstock seedlings show upregulation of disease resistance-related pathways in resistant plant material indicating the possibility to select for specific resistance to *Pythium* components of replant disease (Shin et al., 2016). The placement of several apple rootstocks and breeding populations in sterile culture (micropropagation) has enabled identification of separate genetic effects of resistance to the individual replant components, as these rootstocks were inoculated with cultures of *Rhizoctonia* species and *Pythium* species independently. While this set of experiments is still ongoing (Zhu, personal communication), preliminary reports indicate segregation of QTLs affecting this trait and the possibility of developing molecular markers to select superior genotypes.

4.3 Resistance to WAA

The development of apple rootstocks resistant to WAA was one of the first breeding objectives developed in the Malling–Merton (MM) apple rootstock improvement programme as the disease pressure of these aphids made the cultivation of apples very difficult in the southern hemisphere. The donor parent of resistance to WAA was ‘Northern Spy’, which when crossed with several Malling selections resulted in the WAA-resistant vigorous rootstocks MM.106 (Northern Spy × M.1) and MM.111 (Northern Spy × Merton 793) (Wertheim, 1998). The ‘Northern Spy’ type of resistance seems to be monogenic (the Er1 locus) and has been mapped to chromosome 8 of apple. Monogenic resistance

to WAA derived from 'Robusta 5' has been mapped to chromosome 17 (Er2 locus) and has been utilized extensively in the Geneva, New York, and New Zealand breeding programmes (Bus et al., 2008). Another resistance locus (Er3) from Aotea rootstock has also been mapped on chromosome 8, although it is not as effective as Er1 and Er2 (Sandanayaka et al., 2003, 2005; Sandanayaka and Backus, 2008). Phenotypic evaluation of this trait consists of rearing insects on susceptible germplasm and then transferring a specific number of insects on actively growing shoots of seedlings or replicated clones in a confined space (usually a netted greenhouse), then observing feeding and proliferation of WAA during a 2 month period after transfer (Beers et al., 2006). The monogenic nature of this type of resistance makes it amenable to utilization of *cis*-haplotype-specific markers to select parents and cull progeny that do not possess the resistance locus (Bassett et al., 2015). Other sources of WAA resistance are known in the *Malus* germplasm but very little is known about the genetic inheritance of these sources.

4.4 Tolerance of phytoplasmas and viruses

Apple viruses and phytoplasmas can cause productivity losses by interdicting basic plant functions, deforming branches and roots, and by making fruit unmarketable. To date, these pathogens are known to be spread by grafting, where infected clonal rootstocks or scions are the media for transmission (Wood, 1996; James et al., 1997; Silva et al., 2008). While the goal of apple industries throughout the world should be to work only with material that has been certified tested for viruses, phytoplasmas and other graft-transmissible agents, the eradication of these agents has been elusive due to propagation practices of some growers and homeowners that use infected sources of budwood. It is recommended that apple rootstock improvement programmes pay some attention to phenotyping apple rootstocks for susceptibility to some or all of the possible graft-transmissible viruses or phytoplasmas (Lankes and Baab, 2011). Efforts have been made in Germany and Italy to produce rootstocks resistant to the proliferation phytoplasmas (*Candidatus Phytoplasma mali*) found in certain accessions of *M. sieboldii* (Seemuller et al., 2007, 2008) and *M. sargentii* (Bisognin et al., 2008, 2009; Jarausch et al., 2008). These efforts were hampered by the polyploid and apomictic nature of the donor parents. Susceptibility to apple stem grooving virus has been observed in 'Ottawa 3' rootstocks and some of its derivatives (G.16 and G.814) which exhibited stunting or death upon being grafted with an infected scion. Some nurseries and inspection agencies have suggested maintaining certified virus-free budwood on susceptible rootstocks to reduce the possibility of introducing tainted budwood into the system. The slow decline caused by graft union necrosis among certain rootstock/scion combinations in the presence of tomato ring spot virus (ToRSV) (Tuttle and Gotlieb, 1985a,b) observed in MM.106 rootstock grafted with 'Delicious' scion is also of concern when breeding apple rootstocks. There is no conclusive data on the sensitivity of more recent apple rootstocks to ToRSV-induced graft union necrosis, but a large trial is underway in New York State to evaluate 50 genotypes for this sensitivity (Robinson, personal communication). Furthermore, there is paucity of genetic studies that describe the inheritance of susceptibility of *Malus* germplasm to viruses and phytoplasmas, making genetically informed breeding impossible. In the Geneva breeding programme, virus-sensitive parents like G.16 are being utilized for crosses, and efforts to map susceptibility loci are underway as a prerequisite to marker development to be utilized for culling susceptible seedlings before resources are wasted on growing them in larger field trials.

5 Future trends and conclusions

The varied environments where apples are grown suggest that no one rootstock will be well adapted to all environments and that coordinated, independent evaluation of new material from breeding programme be performed by local pomologists. There are some organizations in certain apple growing regions in the world that aim to independently test rootstocks in a regimented way covering multiple environments and scions (Elfving and McKibbin, 1990; Schechter et al., 1991; Usa, 1991; Kviklys, 2011). A considerable programme of tree fruit rootstock evaluation in the United States, Canada and Mexico is conducted by a group of 35+ researchers, extension specialists and industry collaborators within the CREES (cooperative research and extension services of the USDA) multi-regional project NC-140 (www.nc140.org) and in Europe through EUFRIN (www.eufrin.org). As a group the NC-140 researchers have made significant contributions to tree fruit rootstock research over the last three decades and have conducted highly coordinated impactful research for the tree fruit industry (Rom and Rom, 1991; Fernandez et al., 1995; Perry, 1996; Autio et al., 1997, 2011a,b; Barritt et al., 1997; Marini et al., 2002, 2006b). The evolution from low- to high-density plantings has raised the bar for rootstock performance and has increased the complexity of the research problems that need to be addressed in order to satisfy the needs of the industry. The tree fruit industry supply chain is complex with rootstock propagation nurseries growing and selling rootstocks to finished tree nurseries that graft the scion variety, grow finished trees and then finally sell to the growers. This complexity makes the adoption of new rootstock technologies rather viscous. This viscosity is increased by issues of local adaptability and survivability of rootstocks and also by issues regarding intellectual property. As new apple rootstocks are developed worldwide, they cannot be recommended unless there is sustained research investigating soil and climatic adaptability, root anchorage, size control, precocity, productivity, disease and pest resistance, and propagation (Autio et al., 2011c). In addition, there needs to be awareness of research into these new technologies not just by growers, but by the nurseries that will provide such material to growers. In general, field testing of rootstocks in an orchard setting requires a minimum of eight years to accurately assess the potential for improved profitability, enhancement of production efficiency and reduction of external farm inputs. It also requires testing propagation qualities in a nursery or micropropagation facility setting. With year-to-year variation in weather, this time span is necessary to obtain a true indication of rootstock performance across multiple environments typical of worldwide apple fruit production (Johnson et al., 2001a,b).

Ongoing research and results from localized evaluation experiments are reported in several settings internationally, the main ones being the recurring (every four years) ISHS symposium on 'Integrating Canopy, Rootstock and Environmental Physiology in Orchard Systems' and the symposium on 'Eucarpia Fruit Breeding and Genetics'. Apple industry associations also play a very important role in dissemination of knowledge about apple rootstocks, the premiere on which was established to promulgate the beneficial effects of adopting dwarfing apple rootstocks is the 'International Dwarf Fruit Tree Association' recently renamed 'International Fruit Tree Association' (www.ifruittree.org) because the task of conversion to dwarfing rootstocks had been largely accomplished by the constituents. Other organizations featuring coordinated international research on apple are RosBREED (www.rosbreed.org) (Iezzoni et al., 2010), FruitBreedomics (www.fruitbreedomics.com) and the Genome Database for the Rosaceae (www.rosaceae.org) are advancing the

development of new knowledge about physiology, phenomics, genetics, and genomics of Rosaceous crops and providing useful infrastructure to the development and evaluation of new apple rootstocks (Evans et al., 2012; Evans, 2013a,b; Peace et al., 2014; Chagne et al., 2015; Guan et al., 2015; Liverani et al., 2015; Mauroux et al., 2015; Fresnedo-Ramirez et al., 2016). The ultimate goal for all these organizations is to make apple growing more efficient, more environmentally friendly, more profitable for those that grow apples and more nutritious for the customers that eat apples, and the development of new apple rootstocks is an important cog in this intricate effort.

6 Where to find further information

Further information can be found online in the fruit growing oriented magazine *New York Fruit Quarterly*. <http://www.nyshs.org/fq.php> and in the web pages of the Cornell Fruit Site <http://www.fruit.cornell.edu/>.

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