

## Rootstock breeding in *Prunus* species: Ongoing efforts and new challenges

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The current global agricultural challenges imply the need to generate new technologies and farming systems. In this context, rootstocks are an essential component in modern agriculture. Most currently used are those clonally propagated and there are several ongoing efforts to develop this type of plant material. Despite this tendency, lesser number of rootstock breeding programs exists in comparison to the large number of breeding programs for scion cultivars. In the case of rootstocks, traits evaluated in new selection lines are quite different: From the agronomic standpoint vigor is a key issue in order to establish high-density orchards. Other important agronomic traits include compatibility with a wide spectrum of cultivars from different species, good tolerance to root hypoxia, water use efficiency, aptitude to extract or exclude certain soil nutrients, and tolerance to soil or water salinity. Biotic stresses are also important: Resistance/tolerance to pests and diseases, such as nematodes, soil-borne fungi, crown gall, bacterial canker, and several virus, viroids, and phytoplasmas. In this sense, the creation of new rootstocks at Centro de Estudios Avanzados en Fruticultura (CEAF) offers an alternative to stone fruit crop, particularly in Chile, where just a few alternatives are commercially available, and there are site-specific problems. The implementation of molecular markers in order to give support to the phenotypic evaluation of plant breeding has great potential assisting the selection of new genotypes of rootstocks. Marker-Assisted Selection (MAS) can shorten the time required to obtain new cultivars and can make the process more cost-effective than selection based exclusively on phenotype, but more basic research is needed to well understood the molecular and physiological mechanisms behind the studied trait.

**Key words:** Abiotic stress, biotic stress, breeding technique, clonal rootstocks, resistance, stone fruit crops, tolerance.

### INTRODUCTION

Rootstocks are an essential component in modern fruit production because of their capability of adapting a particular cultivar to diverse environmental conditions and cultural practices. Rootstocks can provide several or many traits that are absent in the scion, such as soil's pest and disease resistance, better anchorage, improved nutrient uptake, better tolerance to soils with high saline content or drought, as well as other limiting soil conditions. On the other hand, they can modify the performance of scion, like for example, by reducing tree vigor, and modify canopy structure that would allow the establishment of high density orchards. Rootstocks can

also reduce or extend the fruit maturation period; improve yield and fruit quality increasing profit returns. Therefore, each particular rootstock/scion combination can generate a plant with characteristic that neither component exhibits if grown separately.

Grafting was the key technological development that enabled the extensive use of rootstocks at present, which goes beyond fruit culture (Kubota et al., 2008).

The ways in which rootstock and scion interact to produce a variety of effects that modify the performance of the whole combination is well documented, has profound economic effects, and are generally referred to as rootstock/scion relationships. However, the mechanisms of these relationships are complex and partially understood.

There is a limit on the possibilities for making rootstock/scion combinations, since combinations beyond the species are generally not possible due to several incompatibility factors that prevent forming a solid and durable graft union. A degree of taxonomic relatedness should exist for the success of a particular rootstock/scion combination. Consequently, the degree of success goes as follows: intraclonal > interclonal > intraspecific > interspecific > intrageneric > intergeneric > intrafamilial (Andrews and Serrano, 1993).

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Received: 8 December 2014.

Accepted: 25 April 2015.

doi:10.4067/S0718-58392015000300002

Rootstocks currently available for fruit species are of two types: (1) Seedling rootstocks, which are those arising from a germinated seed of a particular cultivar. They are easy and almost inexpensive to propagate and are probably still the most widely used method. They have the additional advantage that they are free of those viruses that are not seed propagated (Mink, 1993). A lot of seedling rootstocks, however, have the disadvantage of presenting genetic variability, which leads to an uneven performance on grafted trees in the orchard. This variability can affect important agronomic characteristics such as vigor, productivity and other traits, including those for which the rootstock was selected. (2) Clonal rootstocks are those obtained from selected individuals that are vegetative propagated resulting in individuals with the exact genetic composition. They have the advantage of generating uniform orchards, easier to manage and more productive. Clonal rootstocks can be propagated by *in vitro* techniques, rooted cuttings or layering (Hartmann et al., 2002).

Seed propagated rootstocks were the most widely used alternative during the early development of modern fruit culture. Today however, clonally propagated cultivars are preferred for most species. According to Cummins and Aldwinckle (1995), the first commercial development of clonal rootstocks began in apples to control tree vigor and incorporate resistance to the woolly apple aphid (*Eriosoma lanigerum* Hausm.) This development took place 1922 at the John Innes Institute in cooperation with the East Malling Research Station, UK, which led to the release of the Merton Immune and the Malling-Merton series. Because of its great impact in apple culture, this initial development stimulated the initiation of similar programs for other fruit crops, like grapes (Whiting, 2004), cherries (Perry, 1987), and more recently *Prunus* (Rom and Carlson, 1987). Today there are several ongoing efforts to develop clonal rootstocks for many fruit crops. Despite this new tendency, lesser number of rootstock breeding programs exists in comparison to the large number of breeding programs for scion cultivars. These are probably due to the fact that rootstock programs are more expensive, long term; and require a large and well coordinated multidisciplinary effort. They also differ from scion program in the fact that released cultivars have a longer lifespan in comparison with scion cultivars, which become obsolete in a shorter period of time. Rootstock royalties collected by the breeder also tend to be much lower which in contrast, is not the case for scion cultivars. For these main reasons most rootstock programs are publicly funded.

In general, breeding programs for rootstocks use the similar methods than those implemented for obtaining scion cultivars. However, they differ in their strategy. Rootstock breeding programs make an extensive use of interspecific hybridization to broaden the genetic base, allowing the introgression of genes generally not present

in the breeding population. Since many interspecific hybrids obtained are not fertile, the F<sub>1</sub> population is the preferred population for testing, so the number of interspecific hybrids obtained in a given program is very important. Also, the traits that are evaluated are quite different: From the agronomic standpoint vigor is a key issue, in some species to enhance vigor, and in others, to control vigor allowing the establishment of high-density orchards.

Other important agronomic traits include the compatibility with a wide spectrum of cultivars from different species, a good aptitude for clonal propagation, modified chill requirements, tolerance to extreme temperatures and to root hypoxia, efficiency in the use of water, aptitude to extract or exclude certain soil nutrients, and tolerance to soil or water salinity. Biotic stresses are also important: Resistance/tolerance to pests, such as woolly aphids (pome fruits crops), *Phylloxera* (grapes), and nematodes which are problematic in most fruits species. In the case of *Prunus*, root-knot nematodes (*Meloidogyne* spp.) and lesion nematodes (*Pratylenchus* spp.) are the most damaging nematode pathogens. Also, resistance and/or tolerance is needed for diseases caused by a number of soil-borne fungi, crown gall (*Agrobacterium tumefaciens*), bacterial canker (*Pseudomonas syringae* pv. *syringae*), and several virus, viroids, and phytoplasms. The aim of this review was have an overall point of view about the most recent advances in each trait in which the CEAF rootstock breeding program is currently focused.

## BREEDING FOR SPECIFIC OBJECTIVES

### Vigor control

Rootstocks modify size and shape of the trees by shortening internodes size, altering the angle of the branches, dates and rates of active growth. A dwarfing rootstock is the one capable of suppressing growth of the grafted variety, as compared to growth of that variety on its own roots (Webster, 1995). In peach, rootstocks can induce or modify trunk cross-sectional area (TCSA), plant height, canopy volume, structure of branching, emission of suckers, fruit size, and production efficiency (Layne, 1994), as well as distribution of the dry matter and starch contents in old and new branches, both in winter dormancy and in active growth conditions (Caruso et al., 1997). The parameter TCSA is normally used to estimate tree vigor, but not always reflects well the true vigor of a tree, thus the need complement with other criteria such as tree height and canopy volume (Webster, 1995). The rootstock also modifies the concentration of nutrients in leaves and stems. For example, low-vigor cherry rootstocks are more sensitive under limiting soil conditions (Jiménez et al., 2007). Also, depending on the rootstock and the variety used, rootstocks with more TCSA have lower production efficiency (Zarrouk et al., 2005). This can be explained by

the fact that vigorous vegetative growth competes with fruit growth and production (Caruso et al., 1997). The different hydraulic properties of a rootstock can define vegetative growth rates of trees. The vigor is explained by a mechanical effect of the hydraulic conductance, where the most vigorous rootstock remains in a condition of high water status in trees as compared to low vigor rootstocks, leading to the more dwarfing rootstocks with lower daily growth of stems and reduction in photosynthesis, as observed in grafted peach varieties (Solari et al., 2006a). Among other properties, rootstocks alter transport rates of auxin and cytokinins within the plant, having an hormonal effect as observed in *Prunus* rootstocks (Sorice et al., 2002). Finally, we must consider that rootstocks inducing different vigor can modify the expression of genes in both parts of the plant, the graft zone and the aerial part, altering the time when meristematic activity stops in vegetative buds, and modifying the expression of genes related to metabolism of brassinosteroids, flavonoids and cell wall synthesis in cherry (Prassinis et al., 2009).

The implementation of pedestrian orchards has been used in recent decades to improve production efficiency and reduce labor costs. For this reason the new generation of low-vigor inducing rootstocks in *Prunus*, which will adapt to suit modern orchards, is regarded as a key objective in a breeding program.

### Scion-rootstock graft incompatibility

The limiting factor for the widespread use of some *Prunus* spp. for peach and cherry production is the lack of commercial rootstocks having a wide range of compatibility with various cultivars (Okie, 1987; Zarrouk et al., 2006). Scion/rootstock graft compatibility is a critical issue for orchard performance and longevity. It is perhaps more of a problem in cherry, almond, and especially apricot, than in peach or plum (Lang and Ophardt, 2000). In general, good compatibility occurs in cultivars and species closely related and some genera that have some degree of relatedness. In contrast, taxonomically distant species often manifest incompatibility (Rom and Carlson, 1987).

To remain physiologically healthy, rootstock and scion should be intimately unite, at least during the commercial life of the tree, providing a viable system for the uptake and translocation of minerals, water, assimilates, and hormones throughout the entire lifespan of the plant (Webster, 1995; Martínez-Ballesta et al., 2010). In this way, graft incompatibility leads to unhealthy trees, breakage at the graft union, premature dead or failure of the graft combination and incapacity to form a strong and lasting functional union (Zarrouk et al., 2006).

Previous studies described two types of incompatibility situations. The “translocated” graft incompatibility, which is usually expressed during the first year after grafting in the form of growth cessation, defoliation, and leaf discoloration (Herrero, 1951; Mosse, 1962). In peach/

plum combinations, this type of incompatibility has been associated with both biochemical and functional alterations at the graft interface, inducing a carbohydrate blockage in the scion above the graft union (Moing and Carde, 1988; Moing et al., 1990). Nevertheless, incompatibility symptoms may occur at later stage of development, known as “localized” incompatibility (Herrero, 1951; Mosse, 1962). In this case, the presence of some biochemical alterations across the graft union of *Prunus* may lead to a slight and delayed incompatibility as has been described in cherry and peach/plum combinations (Treutter and Feucht, 1991). This type of incompatibility is characterized by anatomical irregularities at the rootstock/scion union interface with breaks in vascular and cambial continuity patterns and poor vascular connections inducing mechanical weakness of the union (Errea et al., 2001), which may break after years of the orchard establishment, leading to the major economic losses (Herrero, 1951).

The physiological and metabolic mechanisms by which incompatibility is caused and expressed remain unclear (Zarrouk et al., 2006), and several hypotheses have been described. Improvements in the knowledge of metabolic compounds that control the incompatibility response and graft establishment in *Prunus* spp., could help the development of biochemical markers for their use in metabolite-directed rootstock breeding programs.

### Abiotic stresses

**Root asphyxia.** Proper relationship between oxygen and water at the root zone is necessary for an accurate stone fruit tree development. Soils could induce root asphyxia to different rootstocks. While hypoxia often occurs in specific productive areas, it is possible to induce this stress with excessive irrigation or soil compaction (Holzapfel et al., 2009). One of the main targets in rootstock breeding in *Prunus* species is to get asphyxia tolerance or resistance. The hypoxic condition occurs when partial oxygen pressure decreases to a point at which the ATP production is limited in the mitochondria, whereas anoxia occurs when available oxygen decreases until ATP production, by oxidative phosphorylation, is negligible compared to ATP generated by glycolysis and fermentation (Drew, 1997; Dat et al., 2004). The condition of flooded soils, where the diffusion of gases in soil (including oxygen) falls drastically, is one of the most important factors in the evolution of plants and yields achieved by crops worldwide.

The exact mechanism by which plants sense early oxygen deficiency is unknown. In nature it is common that plants under a flooding condition is first subjected to an hypoxic condition where oxygen decreases gradually to reach the condition of anoxia, which gives time to the plants to generate defense responses before the environmental condition becomes lethal (Drew, 1997). Response of sensitive rootstocks to water logging, such

as the peach-almond hybrids 'Felinem' and 'Garnem' exhibit increased activity of defense enzymes against oxidative damage as compared to tolerant Myrobalan plum rootstocks (Amador et al., 2012). They found that it was not possible to establish a direct relationship among the activity of the enzymes: peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT), and tolerance to water logging conditions. These enzymes have been associated with preventive protection against oxidative damage that could occur post-anoxia when roots are again under high oxygen conditions after water has drained from the soil (Drew, 1997; Igamberdiev et al., 2005).

Others responses in plants under flooding conditions have been linked to hormone-induced and ethylene signals (Dat et al., 2004). Apparently, there is a synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC), a precursor of ethylene in roots under hypoxia, which once reaching the aerial part is converted to ethylene in the presence of oxygen by the action of ACC oxidase, thus regulating shoot growth in the aerial part and triggering responses such as epinasty (Jackson, 2002) or leaf senescence (Arbona and Gómez-Cadenas, 2008). Also, in the submerged part of the plant, ethylene is involved in the generation of hypertrophied lenticels in the stems (Larson et al., 1992), and in the formation of aerenchyma (Drew, 1997).

In a study with apricot (*Prunus armeniaca* L. 'Búlida'), Nicolás et al. (2005) showed that after removing plants from water logging conditions, these were not able to decrease the hydraulic resistance to the flow of sap until the emission of new roots. The roots generated under hypoxia in some species have been observed to be coated with lignin and suberin to avoid radial oxygen losses (Sauter, 2013; Yamauchi et al., 2013). Herrera et al. (2008) postulated that there is some similarity in the symptoms displayed by plants under water deficit stress and hypoxia. In this context, acclimatization phenomenon has been studied in different species of *Prunus* (Ranney, 1994). This author observed in 11 taxa that increasing the time of flooding decreased net photosynthesis. Similarly, in a study using eight taxa of *Prunus*, with ornamental cherry, water logging affected net photosynthesis (Jacobs and Johnson, 1996). Other studies also revealed the high sensitivity of net photosynthesis and stomatal conductance as first symptoms of water logging in several *Prunus* hosts: the rootstock selection GxN-9 (*Prunus dulcis* (Mill.) D.A. Webb × *Prunus persica* [L.] Batsch) (Martinazzo et al., 2011), apricot (*P. armeniaca*) (Domingo et al., 2002; Nicolás et al., 2005), peach (Insausti and Gorjón, 2013), in peach-almond interspecific crosses and *Prunus cerasifera* Ehrh. (Xiloyannis et al., 2002), and the peach-almond hybrids 'Felinem' and 'Garnem' rootstocks (Amador et al., 2012).

Subsequently, in *Prunus*, by prolonging the time of flooding many physiological anomalies can be

observed or detected, the most common being lower chlorophyll content and less development (Amador et al., 2012; Insausti and Gorjón, 2013), increased defoliation (Ranney, 1994; Jacobs and Johnson, 1996), lower weight and root necrosis (Jacobs and Johnson, 1996), lower leaf water potential (Domingo et al., 2002; Nicolás et al., 2005; Insausti and Gorjón, 2013), less sap flow (Domingo et al., 2002; Nicolás et al., 2005), reduced turgor and leaf epinasty (Domingo et al., 2002), redness, and subsequent necrosis of leaf senescence and damage on vascular bundles (Iacona et al., 2013), all as symptoms of medium and long term appearance. In flooded peach (highly sensitive species) Insausti and Gorjón (2013) observed that fruit size was smaller and that harvested fruits produced ethylene earlier, advancing the climacteric ripening and softening of the fruit, seriously affecting fruit production. In most of these studies differences between sensitive and resistant plants of *Prunus* have been described in relation to the delay period to visualize first symptoms, degree of sensitivity, rate of recovery and survival of plants (Amador et al., 2012). Several of these indicators could be used in the selection of rootstocks for assessing tolerance to flooding conditions in *Prunus*.

The mechanisms of response to hypoxia and anoxia are still poorly investigated, especially in ligneous species such as *Prunus*. Most research in this species group are based on variables of growth, mainly morphological and physiological, but little has been done in reference to hormonal levels and even less at molecular level, focusing primarily on the activity of certain enzymes involved in the prevention of oxidative damage. So, it is necessary to validate the responses to hypoxia within *Prunus* species for their later use as indicators in breeding programs. This would allow implementing them as markers in early selection programs.

**Salinity.** Salinity problems often occur in arid and semi-arid areas. The main ions causing problems are  $\text{Cl}^{-1}$ ,  $\text{SO}_4^{-2}$ ,  $\text{HCO}_3^{-1}$ ,  $\text{Na}^{+1}$ ,  $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$ ,  $\text{NO}^{-3}$  and  $\text{K}^{+1}$ , although in some areas B is also involved (Bernstein, 1975). Approximately 6% of world cultivated land has salinity problems where NaCl is usually the most abundant and soluble salt (Hasegawa et al., 2000; Munns and Tester, 2008). It is unclear how plants can first detect increases in salinity, as well as the signal transduction transmitted to the rest of the plant (Hasegawa et al., 2000).

The mechanisms of perception to salt stress and the signals transduction within the plant are not fully elucidated, and yet, compounded by the limited research conducted in this subject matter for woody species. In general, stone fruit crops tend to manifest a moderate to high sensitivity to salinity. Several interesting studies have been conducted in *Prunus*. In a trial with 'GF-677' and 'Mr.S. 2/5' rootstocks, grafted with peach and subjected to different NaCl (0

to 120 mM) concentrations, an increased sensitivity in 'GF-677' in terms of growth and net assimilation of CO<sub>2</sub> was observed (Massai et al., 2004). These authors also observed a reduced growth of the roots and increased accumulation of sorbitol in leaves. In almond, Ranjbarfordoei et al. (2006) showed that the content of chlorophyll and fluorescence parameters in leaves are adversely affected when the electrical conductivity of the irrigation water exceeded 3 dS m<sup>-1</sup>. Also working with almond clones under different levels of salinity (0 to 75 mM NaCl), Najafian et al. (2008) found that with increased levels of stress, growth of stems, number of internodes, leaf area, fresh and dry weight of roots were reduced. Furthermore, *in vitro* cultured 'Gisela 6' cherry rootstock subjected to fluctuating saline concentrations evidenced an increase in the content of malondialdehyde and expression of SOD, ascorbate peroxidase, CAT, and glutathione reductase. El-Motaium et al. (1994) found that Myrobalan plum (*Prunus cerasifera* Ehrh.) is one of the most tolerant to salinity and B (less absorption of salts in the root), while 'Nemared' peach (*Prunus persica* (L.) Batsch) was one of the most sensitive (high salt content in the stem). By grafting these rootstocks with commercial varieties, the degree of tolerance of the rootstock is transferred to the variety.

In a 19-yr-old commercial Japanese plum (*Prunus salicina* Lindl. var. *salicina*) orchard grafted onto 'Marianna 2624' rootstock, different concentrations of a mixture of NaCl and CaCl<sub>2</sub> from 0 to 28 mM were applied (Ziska et al., 1991). These authors observed that much of Na<sup>+</sup> and Cl<sup>-</sup> was accumulated in woody tissues, while leaves mainly accumulated Cl<sup>-</sup>, causing lesions on leaves. They found that woody tissue is able to retain, apparently the movement of Na<sup>+</sup> to the leaves (which was not observed in young trees). In a second part of this study, these same authors observed that the most negative impact was caused by chlorides at leaf level, decreasing net photosynthesis, total carbohydrates, chlorophyll content, and leaf area (Ziska et al., 1990). In a 6-yr old plum trial, Catlin et al. (1993) observed that the recovery of trees following salt stress conditions may take several years, but ultimately achieved complete recovery. In this species, when salinity levels are higher (8 dS m<sup>-1</sup> on irrigation water), production can be reduced by half, both in fruit number per tree and in fruit weight, evidencing severe leaf damage (Hoffman et al., 1989).

In the case of *Prunus*, evaluation to salt tolerance should be careful with the choice of type material used for experimentation; e.g. the use *in vitro* plants vs. year old plants or trees in commercial orchards, if plants used in trials are grafted or un-grafted rootstocks, and type of salt they are exposed to. All these variables may influence the final answer. It is important to determine what type of test gives the best response in a commercial orchard to improve detection of better sources of tolerance in the rootstock selection process.

**Drought.** Among the environmental factors that push the evolution of plants, water availability is the most important factor (Zhu, 2002), and within abiotic stresses, drought has the most alarming impact on agriculture (Reddy et al., 2004). Water stress is generated not only by the shortage of water in soil, but also by a high vapor pressure deficit (VPD), which closes stomata even though the soil is well moisten due to an increase in transpiration rates. Both phenomena, shortage of water and high VPD, occur simultaneously in arid and semiarid climates (Reddy et al., 2004; Chaves et al., 2009).

It is not known how the lack of water is perceived at the molecular or biochemical level (Reddy et al., 2004). The information at the molecular level in woody species is more limited. Working under water stress conditions, rootstocks 'Cadaman', 'GF-677', 'ROOTPAC 20' (*P. besseyi* × *P. cerasifera*) and 'ROOTPAC R' (*P. cerasifera* × *P. dulcis*) grafted with 'Catherina' peach, increased the production of proline in roots and leaves, sorbitol in leaves and raffinose in roots, related to an increase in water use efficiency. A high expression of *P5SC* gene on 'GF-677' (tolerant) were associated with the high content of proline (Jiménez et al., 2013).

Several studies have observed the effects at stomatal level and photosynthesis in *Prunus* under drought. The values of midday water potential is related to stem growth, stomatal conductance, assimilation, and transpiration (Solari et al., 2006b). Interspecific *Prunus* hybrids under drought conditions decreases water potential, photosynthesis and transpiration, and significantly increase the activity of enzymes with antioxidant activity, which returns to normal values when plants are watered, while levels of ascorbate, glutathione and H<sub>2</sub>O<sub>2</sub> increase during water deficit (Sofa et al., 2005).

At the production level, moderate deficits in specific phenological stages that do not affect production have been evaluated. In a study conducted in a commercial peach orchard grafted onto 'GF-677', Gelly et al. (2004) found that performing a controlled water deficit in phase II of fruit growth (growth stops during the hardening of fruit pit) improves fruit quality increasing the amount of soluble solids and enhancing color. Carry water deficit at this stage of fruit growth allows saving water without affecting fruit size. In another study carried out with almond variety 'Nonpareil' grafted on 'Nemaguard' peach, Esparza et al. (2001) observed a decreased performance after 2 yr of water stress applied during floral initiation, mainly explained by a decrease in the renewal of fruitwood, without affecting the weight of seeds by the deficit of last season. Varying productive responses to water deficit is also related with the rootstock used, in terms of firmness of fruit, soluble solids content, and anthocyanin content. These variables can increase or decrease independently, improving or deteriorating various parameters of quality within a rootstock-scion combination, as has been observed in various combinations in peach (Besset et al., 2001).

A drought tolerant rootstock will not necessarily be successful if the scion is a sensitive variety. The current worldwide situation strongly advises to conduct a major effort into selecting drought tolerant rootstocks adapted to future climate change. Finally, the importance of evaluating grafted plants should be taken into account, where the interaction occurs between the two components, rootstock and scion, in order to determine the real level of tolerance.

**Iron chlorosis.** Iron chlorosis problems often occurs in calcareous soils (Abadía et al., 2011). In most soils there is Fe abundance but not available to plants, being highly insoluble in the presence of oxygen and much less soluble in the presence of carbonates (Schmidt, 2003). It is unclear the mechanism by which plants detect the lack of Fe. There are two main strategies to absorb Fe in the roots. Strategy I is mainly based on the activity of Fe-reductase enzyme belonging to the group ferric reductase oxidase (FRO) for the *Prunus* group, which reduces  $Fe^{+3}$  to  $Fe^{+2}$ , and then is absorbed by the specific transporter, while Strategy II is exclusive to grass (Abadía et al., 2011).

The use of tolerant rootstocks to iron chlorosis is the best way to overcome this problem (Cellini et al., 2011). Jiménez et al. (2008) working with several *Prunus* rootstocks reported that Fe-reductase activity was affected by Fe availability in an hydroponic solution in which rootstocks known as resistant genotypes such as 'Adesoto' (*P. insititia* L.), 'Felinem' (*P. dulcis* × *P. persica*) or 'GF 677' (*P. dulcis* × *P. persica*), had increased activity of this enzyme as compared to the sensitive rootstocks 'Barrier' (*P. persica* × *P. davidiana* (Carrière) N.E. Br.) and 'Cadaman' (*P. persica* × *P. davidiana*). They also found that rootstocks with higher SPAD values correlate well with increased tolerance to Fe chlorosis. Similarly, Cinelli et al. (1995) observed that resistant rootstocks are better suited to reduce Fe under stress, as compared to the sensitive 'Nemaguard' peach. Visible symptoms manifested mainly in new leaves and SPAD values fell faster in young leaves than older leaves. In another study with 'Nemaguard' established in a Fe deficient medium, Bohórquez et al. (2001) observed that Fe activity reduction of roots does not increase unless there is Fe or another metal such as Zn in the rhizosphere. In other study using potassium bicarbonate solutions to simulate the effects of a calcareous soil, with a pH increase of 8.5, almonds showed a tolerant response, while peaches exhibited a variable response according to genotype, being 'Nemaguard' sensitive (Shi and Byrne, 1995). The root level response in tolerant rootstocks, such as 'Adesoto' reached the highest contents of organic compounds and amino acids (Jiménez et al., 2011).

Comparing rootstocks with contrasting response to Fe chlorosis, such as 'GF 677', with 'Mr.S 2/5' and 'Ishtara' [(*P. cerasifera* × *P. salicina*) × (*P. cerasifera* × *P. persica*)], Cellini et al. (2011) observed that tolerant rootstock

has a higher content of glutathione and ascorbate, and additionally more glutathione reductase activity and NO content, with the highest antioxidant defense and less oxidative stress in the tolerant rootstock.

In commercial peach orchards growing on calcareous soils, Fe chlorosis deteriorates various physiological parameters, such as photosynthetic efficiency, assimilation, transpiration, and stomatal conductance (Eichert et al., 2010). Iron chlorosis decreased yield and fruit quality (Pestana et al., 2003). Visible symptoms of chlorosis rapidly appear followed by a decrease in performance with lesser fruit load and fruit size (Álvarez-Fernández et al., 2011).

Iron chlorosis is one of the most limiting factors in the production of *Prunus* and is determined by soil conditions that alter not only Fe availability, but also Fe uptake and transport within the plant. At the molecular and metabolic levels, several questions on perception and signal transduction, as well as its internal transport remain unanswered. The new generation of resistant rootstocks is one of the most effective ways to address this problem in stone fruit species and is, therefore, necessary to design tests that are effective in selecting tolerant rootstocks.

#### **Biotic stress**

**Root-knot nematode resistance.** One of the major pests in stone fruit orchards worldwide are plant parasitic nematodes (PPN) (Nyczepir and Esmenjaud, 2008). PPN are generally microscopic, colorless and un-segmented eel-shaped organisms. They are considered important crop pests but due to their small size and because they live in the soil, they are difficult to detect, identify and control (De Ley et al., 2005; Powers, 2004). Fruit tree crops like almond (*P. amygdalus* Batsch), cherry (*P. avium* (L.) L., and *P. cerasus* L.), peach (*P. persica*) and plum (*P. cerasifera*, *P. domestica* L., and *P. salicina*.), are susceptible to PPN attack worldwide (Nyczepir, 1991; Esmenjaud et al., 1996; 1997; Stalin et al., 1998; Pinochet, 2000; Rosso et al., 2004; Di Vito et al., 2005; Walters et al., 2008; Nyczepir and Thomas, 2009; Ye et al., 2009; Bosselut et al., 2011). The effects of PPN in crops are often underestimated but in general, it is accepted that on average, nematodes are annually reducing the global agricultural production by about 10% to 12% (Agrios, 2005).

The four major PPN associated with severe losses in stone fruits orchards worldwide are: root-knot (*Meloidogyne* spp.), ring (*Mesocriciconema* spp.), root-lesion (*Pratylenchus* spp.), and dagger (*Xiphinema* spp.) nematodes. Root-knot nematodes (RKN) are considered by far the most damaging nematodes in the world and reduce fruit production in several economically important *Prunus* species (Nyczepir and Esmenjaud, 2008). The host response of *Prunus* rootstocks to RKN species and populations has been studied for many decades in USA and Europe.

Traditionally, PPN control has been based mainly on chemical compounds (nematicides). However, the negative impact on environment and ineffectiveness after prolonged use have led to a total ban or restricted use of most chemical nematicides, especially soil fumigants, and an urgent need for safe and more effective alternatives (Esmenjaud et al., 1997; Perry et al., 2009; Radwan et al., 2012). The most economic and environmentally sound method for managing RKN in *Prunus* spp. crops is the use of resistant rootstocks (Fernández et al., 1994; Esmenjaud et al., 1997; Pinochet, 1997; Pinochet et al., 1999; Nyczepir and Esmenjaud, 2008; Perry et al., 2009; Verdejo-Lucas and Talavera, 2009; Khallouk et al., 2011; Pofu et al., 2012).

Resistance is used to describe the ability of plants to suppress nematode development and/or reproduction. It can range from low to moderate (partial or intermediate) resistance to high resistance. A completely or highly resistant plant allows no nematode reproduction or only very minor reproduction. Partially or moderately resistant plants allow a low reproduction. In contrast, susceptibility allows normal nematode development, enhancing the expression of any associated disease (Roberts, 2002). Studies focused in mechanisms of resistance to RKN have described diverse plant responses to nematode attacks, ranging from strong early hypersensitive-like reaction leading to rapid juvenile death to late inhibition in the formation of giant cells associated with an incomplete development of the nematode reproductive cycle (Marull et al., 1994; Khallouk et al., 2011).

Starr and Mercer (2009) summarize the key steps for identifying and evaluating crop resistance to RKN. First, it is necessary to identify RKN species present in the area of interest, and then evaluate the plant suitability (host-response) to RKN species and isolated populations. Therefore, the host response of *Prunus* rootstocks must be evaluated specially against populations with differences in virulence of a particular species or a mixture of virulent populations involving several RKN species, to ensure a broad spectrum of resistance (Esmenjaud et al., 1994; Pinochet et al., 1999; Pinochet, 2009). Thus, the detected source resistance can be identified and incorporated in a resistance breeding program. *Prunus* rootstocks with broad resistance to RKN have been found in the subgenus *Amygdalus* among wild peach (*P. davidiana*) or cultivated peaches (*P. persica*) or almond (*P. dulcis*) or peach-almond hybrid rootstocks (Esmenjaud et al., 1994; 1997; Pinochet, 1997; Nyczepir and Esmenjaud, 2008; Bosellut et al., 2011). Broad resistance has also been found in the subgenus *Prunophora* (*P. cerasifera*, *P. insititia*, *P. domestica*). In the plum group, high level of resistance to RKN has been found in 'Marianna' (*P. cerasifera* × *P. munsoniana* W. Wight & Hedrick) and Myrobalan (*P. cerasifera*) (Esmenjaud et al., 1994; 1997). The so called *Ma* genes of resistance in some Myrobalan clones suppress nematode reproduction and confer a complete spectrum,

high level and stable resistance to *Meloidogyne arenaria* (Neal, 1889), *M. incognita* (Kofoid & White, 1919), *M. javanica* (Treub, 1885), and *M. floridensis* (Nyczepir and Thomas, 2009).

Research conducted for developing nematode resistant crops worldwide have made major contributions to nematode management. Important economic profit has been achieved in the case of tomato, peach, grape, tobacco, and others major crops. The creation of nematode resistant crops leads to lower nematode population densities, with obvious long-term benefits in an agricultural system (Roberts, 2002; Starr and Mercer, 2009). New inputs through molecular biology, such as marker assisted selection (MAS) can aid plant breeders with new tools for accelerating the breeding process for incorporating nematode resistance (Esmenjaud et al., 1997).

**Disease resistance.** Breeding programs oriented towards developing new *Prunus* rootstocks require the use of germplasm resistant or tolerant to soil-borne fungal pathogens and orchard replant problems. Resistant rootstocks, and to a lesser degree tolerant rootstocks, provide an agronomic solution to *Prunus* fruit growers allowing an increase in productivity and improved efficiency via better tree survival in soils infested with pathogens like fungi, bacteria, virus, and virus-like diseases.

In poorly drained and dense clayish soils, *Prunus* rootstock are at risk of being infected with crown gall (*Agrobacterium tumefaciens*), crown rot (*Phytophthora* spp.), bacterial canker (*P. syringae* pv. *syringae*), oak root rot fungus, *Armillaria mellea* and *Armillaria tabescens*. Both fungi and bacteria are difficult to control or eradicate; therefore, incorporation of genetic resistance into rootstocks is a highly desirable option (Reighard and Loreti, 2008). However, this challenge has proven to be a difficult task for some pathogens since it first requires identifying sources of resistance, and second to transfer these sources into commercial material. For example, resistant genes against crown gall and oak root rot have been detected in a few wild sources but the trait has been difficult, if not impossible, to transfer into commercial or experimental *Prunus* (Pinochet et al., 2002). Furthermore, the difficulty in working with soil borne fungi and bacteria is the pathogenic diversity of these organisms.

Replant problems in stone fruit crop are of major concern and thus require a special mention. Replant disease is a syndrome expressed as a failure in tree establishment, suppressed growth, and shortened productive life. One of the most common practices in fruit tree cultivation worldwide is repeated cropping. Soil-borne pathogens (nematodes, fungi, and bacteria), are essential components associated with replant disease. Other abiotic stress factors are also involved. However, it is difficult to determine the primary causal agent or the predominating factor for each replant situation. In most cases, the existence of a combination of factors whose damaging effects over the

plant are accumulative is accepted. Thus, choosing a rootstock that would have multiple resistance or tolerance to several of these damage causing factors is a priority to assure success during the establishment of the tree, and afterwards, during its productive life (Calvet et al., 2000).

### Use of molecular markers

Molecular markers are based in the identification of inheritable DNA sequence differences (polymorphisms). This is a procedure that combines both traditional breeding strategies and molecular tools for selecting plant material with traits of interest, such as color, size, or biotic/abiotic stress resistance. A molecular marker may be a short DNA sequence, such as a single base-pair change (single nucleotide polymorphism, SNP), or a long one, like minisatellites, which are usually defined as the repetition in tandem of a short (6 to 100 bp) motif spanning 0.5 kb to several kilobases (Vergnaud and Denoeud, 2000). Other important kind of molecular markers are microsatellites or Simple Sequence Repeats (SSRs), which are sequences of 1 to 6 bp repeated in tandem that are frequently found in the genomes of prokaryotes and eukaryotes (Zane et al., 2002; Kalia et al., 2011). The increased genomic resources available from the last years, such as whole genome sequences and high-density genotyping platforms, are revealing important structural and regulatory genes, as well as molecular polymorphisms associated with important agronomic traits (Verde et al., 2012; Dirlwanger et al., 2012).

The identification of regions that contribute to the phenotypic variance of a character permits the selection of genotypes carrying these loci, which are not affected by interaction with the environment. Using the methodologies that identify genomic regions involved in the expression of other traits mainly developed for peach and cherry to a lesser extent, it would be possible to identify the genetic determinants involved in detecting tolerance for hypoxia in *Prunus* rootstocks. A review on this topic is presented in the article by Guajardo et al. included in this special issue.

### CONCLUSIONS

Current fruit production requires a rootstock incorporating traits like low vigor, compatibility with a wide number of varieties, and resistance or tolerance to biotic (diseases and nematodes) and abiotic (hypoxia, water stress, salinity and Fe chlorosis) stresses, which conforms a complex ideotype and a great challenge for the breeder. For this it is important to seek new sources of variation within the genus *Prunus* spp. by interspecific crosses. This requires have a broad collection of materials that will open new alternatives to the breeder to break the barriers of resistance and/or tolerance to pests and diseases. In this sense a quick way of access to new species is the implementation of a collection of cryopreserved pollen,

which offers the possibility to incorporate large number genotypes without the wait entry into the reproductive stage.

Moreover, the evaluation of progenies require understand physiological and molecular mechanisms behind the responses to biotic and abiotic stresses. Therefore it is important that rootstock breeding program has a multidisciplinary team of scientists supporting in the areas of genomics, physiology, and agronomy. The generation of basic knowledge of each area should be integrated to develop appropriate screening tests and early selection tools to shorten the evaluation time for each trait. At this point genomics can be helpful, both the possibility of using molecular markers and genetic characterization of the parent, which will guide the planning of crosses. This support will allow the agronomic evaluation of genotypes in the field in less time.

Finally the productive evaluation of genotypes requires several seasons of observation and different environments to obtain a variety adapted to different conditions of soil and climate. The implementation of a network of evaluation sites enables more information of performance of each material in every season, which added to the specific tests generates a detailed characterization of each genotype, facilitating the choice of the most appropriate rootstock for the conditions of each producer.

### ACKNOWLEDGEMENTS

We thank the Centro de Estudios Avanzados en Fruticultura (CEAF) and the CONICYT Regional project R08I1001 for financing this review.

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