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"Biochemical, metabolic and molecular characterization of pear-apple hybrids: PEARAPPLE-Omics"

in co-tutela con Fondazione Edmund Mach e Plant & Food Research

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Abstract

Apple and pear are economically important fruit crops well known for their unique textures, flavours and nutritional qualities. Both genera are characterised by a specific pattern of secondary metabolites, which directly affect not only the resistance or susceptibility towards certain diseases, but also have a significant impact on flavour and nutritional value of the fruits. The similar chromosome number, genome size, and their recent divergence date, together with DNA-markers have shown that apple and pear genomes are highly co-linear.

Since hybrids between apple and pear provide a unique germplasm resource for genomic, transcriptomic and metabolic profiling studies, the main task of this project was to understand whether putative apple-pear hybrids available from FEM, PFR and UniBo are true hybrids or not. This research work was in cooperation with the University of Udine.

This PhD project utilized comparative genomic approaches; high resolution melting, single nucleotide polymorphism (SNP) analysis, simple sequence repeats and SNP-chip analysis to identify genetic differences among the putative hybrids and its offspring. Furthermore, this work analysed the genus-specific metabolite pattern and the resistance or susceptibility to fire blight, apple scab and pear scab in these plant materials. Another study was to identify candidate genes, involved in the arbutin pathway.

The markers analysis and the biochemical analysis demonstrated that the Zwintzscher's Hybrid and the 'Abate' x 'Fuji' hybrid are true full hybrids. All PFR F1 progenies are partial hybrids. The hybridity of P26A19 4 and the F2 hybrid F2-FP12 1-1.2-OP were confirmed with the chemical analysis.

The UniBO accessions 'Decana' x 'Murray' 1 and 'Decana' x 'Murray' 2 are not hybrids, but only have pear genomes. Almost all the 'Fuji' progenies were susceptible and, in our work, were moderately susceptible or susceptible to fire blight. CO, P26A19, IP26, IP12, FP12 3 and three FP35 seems to be resistant to apple scab, but all the PFR F1 progenies were resistant to *V. pyrina*. CO population and P26A19 4 pear x apple hybrids exhibited low to no fire blight infection. The 'Imperial Gala' progeny, P26A19 3 and P26A17 are susceptible to fire blight.

Three candidate genes involved in the arbutin pathway, 4CL, HBS and CPL, were differentially expressed in pear and apple-pear, as compared to apple.

Future work can use the true and partially hybrids to introduce more traits of interest, such as, e.g., flavour or texture in apple or pear and to understand better the arbutin pathway.

CHAPTER I

General introduction

1 General introduction

The subfamily of *Pomoideae* (family Rosaceae) comprises a number of genera known as "pome fruits", which are valuable fruit crops for human nutrition and health (Espley and Martens, 2013). Among the most common pome fruits, apple (*Malus* x *domestica* Borkh.) is the major crop with respect to global consumption, followed by pear (*Pyrus communis* L.) and quince (*Cydonia oblonga* Mill.) (Cornille *et al.*, 2019).

Apple and pear are economically important fruit crops well known for their unique textures, flavours and nutritional qualities. Both genera are characterised by a specific pattern of secondary metabolites, which directly affect not only the resistance or susceptibility towards certain diseases, but also have a significant impact on flavour and nutritional value of the fruits. The identical chromosome number, similar genome size, their supposed recent divergence date, together with DNA-marker analysis has demonstrated that their genomes are highly co-linear (Dong *et al.*, 2020). Yamamoto *et al.* (2007), followed by Celton *et al.* (2009), were the first to show that the positions of SSR loci are well conserved between apple and pear. By that time, many SSR markers had been developed for apple (e.g. Liebhard *et al.*, 2002; Silfverberg-Dilworth *et al.*, 2006) and more than 100 apple SSR markers were positioned on pear genetic linkage maps (Pierantoni *et al.*, 2007).

Intergeneric F1 hybrids between apple and pear provide a unique germplasm resource not only for genomic, transcriptomic and metabolic profiling studies, but also for applying advanced breeding strategies. Using information derived from the recent apple and pear joint genome projects (Daccord *et al.*, 2017; Linsmith *et al.*, 2019) between Edmund Mach Foundation (FEM; Trento) and The New Zealand Institute for Plant & Food Research Limited (PFR), this PhD project will utilize comparative genomic approaches to identify genetic differences among the available putative hybrids and their offspring. Furthermore, the project will describe the genus-specific metabolite pattern found in these plants as well the use of genomics and other *-omics* technologies (metabolomics, transcriptomics) to provide insight into the genetic reorganization of the hybrids, including mapping the genomic segmentation between apple and pear in progeny.

The analyses were carried out on FEM and PFR plant materials available for this PhD.

Moreover, the University of Bologna (UniBo) had created additional putative hybrids between pear and apple that were available for this project too.

The findings will enhance and accelerate the breeding process for the development of superior crops for producers and consumers, by enabling the introduction of desired traits from the pear gene pool into apple and *vice versa*.

1.1 **Apple**

1.1.1 Taxonomy and origin of the genus *Malus*

The domesticated apple *M.* x *domestica* Borkh., and by extension the Maloidae, evolved from a chromosome doubling of *Gillenia* (Rosaceae, subfamily Spiraeoideae) (Velasco *et al.*, 2010). There are about 50 species of *Malus*, with five of them originating in the North American continent and all others in Eurasia (Pereira-Lorenzo *et al.*, 2009).

Linnaeus, in 1753, included the apple species in the genus *Pyrus*, with pear and quince classified as *Pyrus malus* and distinguishing the apple types as a botanical variety. This was in spite of the fact that Miller, in 1740, had divided apple from pear, considering them different genera because of graft incompatibility. This is the classification other botanists have used since (Walker, 1833).

The genus *Malus* belongs to the Rosaceae family and forms the subfamily *Maloideae* with its closely related fruit (*Pyrus* and *Cydonia*) and ornamental (*Amelanchier, Aronia, Chaenomeles, Cotoneaster, Crateagus, Pyracantha, Sorbus*) genera (Challice, 1974).

The domesticated apple is one of the most important fruit crop of the colder and temperate parts of the world and its domestication was driven by hybridization of different wild species and clonal propagation of genotypes with desirable traits (Sun *et al.*, 2020). Vavilov suggested that the wild apple of Turkestan (Kazakhstan, Kyrgystan, Uzbekistan, Turkmenistan and Tajikistan) and its close relatives were the progenitors of the domesticated apple, with the whole process of wild apple domestication being traced to the Almaty region (Kazakhstan) (Vavilov, 1997). Vavilov also reasoned that, because the wild apple bears similar fruits to the domesticated apple, it must have been the progenitor. Fieldwork in the region appeared to confirm the similarity between wild and cultivated apples (Harris *et al.*, 2002). Furthermore, Janick *et al.* (1996) suggested that 'this area (Central Asia and Tian Shan) is the area of greatest diversity and the centre of origin' of the domesticated apple. This is linked to Vavilov's 'oversimplified' idea that the centre of diversity is the place of origin. The Central Asian wild apple is a diverse species with a wide range of forms, colours and flavours, whilst its allozyme diversity is significantly greater than that found in five widely distributed North American wild apples (Harris et al., 2002). The wild species, *M. sieversii* and *M. sylvestris*, are proposed to be the major progenitors (Cornille *et al.*, 2012; Cornille *et al.*, 2014; Duan *et al.*, 2017). The chromosome number of apple is n = 17, which is evolved by autopolyploidization or by hybridization between two sister taxa with x = 9 (similar to extant *Gillenia*), followed by diploidization and aneuploidization to x = 17 (Velasco *et al.*, 2010).

Most *Malus* species are diploid (2n = 34), e.g., *M.* x *domestica*, *M. baccata* and *M. spectabilis*), while *M. hupehensis*, *M. sikkimensis* and *M. toringoides* are triploid (Pereira-Lorenzo et al., 2009), and *M. sargentii* is tetraploid. Some species show multiple ploidy levels, e.g., *M. coronaria* is triploid but can be tetraploid. At the same time, the polyploid species tend to be apomictic (develop an embryo without fertilization), e.g., *M. hupehensis*, *M. sikkimensis*, *M. sargentii*, *M. toringoides* and *M. coronaria* (Dickinson, 2018).

In the domesticated apple, most genotypes are diploid, with triploid (3n = 51) accessions being quite common, too (Arnal *et al.*, 2020), as they occur at a higher ratio than expected based on spontaneous polyploidisation (He *et al.*, 2018; Evans *et al.*, 2011; Pereira-Lorenzo *et al.*, 2018). Diploid individuals have regular meiosis and fully fertile seeds, whereas triploid cultivars tend to be the fusion between a normal gamete and an unreduced one (Larsen *et al.*, 2018). Triploid apple cultivars are more vigorous and the fruit size is larger than that of diploid cultivars (Larsen *et al.*, 2018; Luo *et al.*, 2020).

1.1.2 The apple tree

The 'concept of type' is central to classical morphology and expressions such as tree and branching habit or fruiting type and cropping habit, are used commonly in various species by breeders and geneticists (Lauri and Laurens, 2005), but also pomologists and physiologists (Sansavini and Musacchi, 1993).

Regulation of tree vegetative growth in intensive orchards is one of the most important tasks for fruit growers. To optimize the growth and yield of apple trees, different dwarfing rootstocks are usually chosen depending on the vigour of a particular apple cultivar (Kviklys *et al.*, 2020) and environmental and soil conditions (Zhang *et al.*, 2020). However, for example under Lithuanian climatic conditions, trees of medium- or strong-growing apple cultivars are too vigorous even on the currently available dwarfing rootstocks (Kviklys *et al.*, 2013).

From an architectural point of view, the apple tree is described by the following characteristics: branch orthotropic with rhythmic growth, monopodial branching at least during the first years following germination, then sympodial when flowering becomes terminal on long shoots. Flowering is terminal on short and possibly long shoots, and lateral on long shoots (Lauri and Laurens, 2005).

Apple tree architecture is thus intermediate between the architectural models of Rauh (monopodial branching with lateral flowering) and Scarrone (sympodial branching as a result of terminal flowering) (Crabbé, 1987; Lauri and Laurens, 2005). Although apple fundamentally conforms to

these two well-defined models, great differences exist between cultivars whether they belong to "spur" vs. "spreading" or "terminal bearing" growth habit (Lauri and Laurens, 2005).

1.1.3 Apple cultivation and economy

1.1.3.1 World apple production

Although apples are cultivated worldwide in temperate, subtropical and tropical environments, commercial production is limited to latitudes ranging from 25° to 52° (Palmer *et al.*, 2003). Over 63 countries produce apples with a great variation in growing conditions and utilizing a large number of cultivars (Musacchi and Serra, 2018). In 2019, apple production reached 87.23 million tons (MT) worldwide and the world apple area harvested was 4.7 million ha (FAOSTAT, 2019) (Figure 1.1).

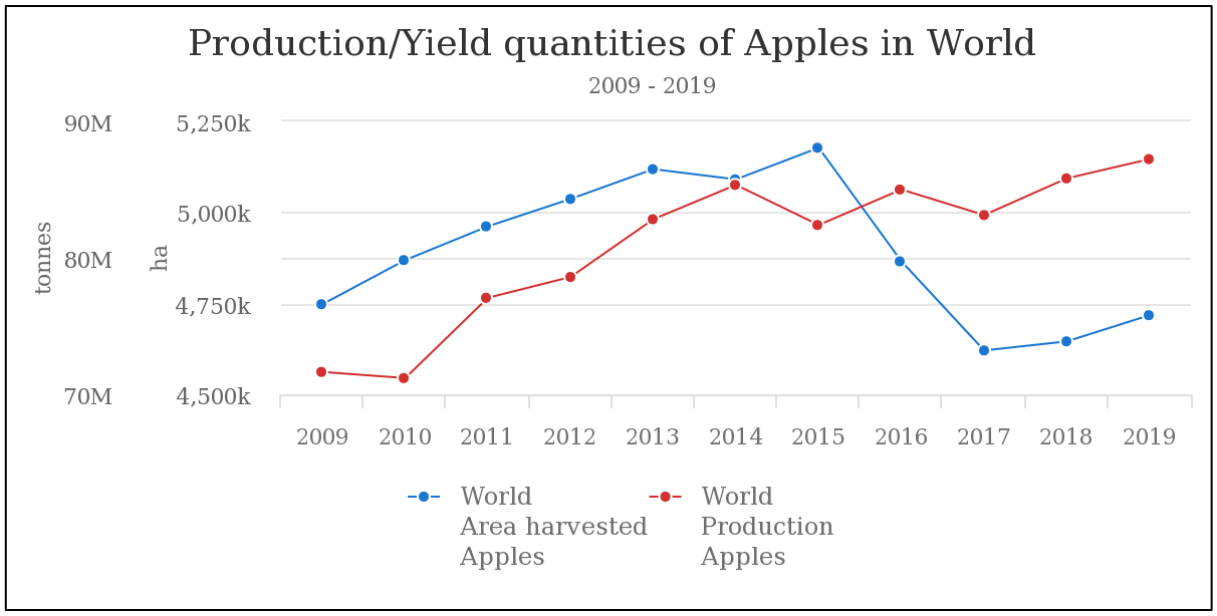

Figure 1.1: World production and area harvested area of apples in the period 2009 – 2019 (FAOSTAT, 2019).

The largest producer is China with 42.42 MT. The United States of America is the second largest producer with 4.9 MT, followed by 3.61 MT in Turkey, Poland 3.08 MT, Indian 2.31 MT and Italy with 2.30 MT (FAOSTAT, 2019) (Figure 1.2).

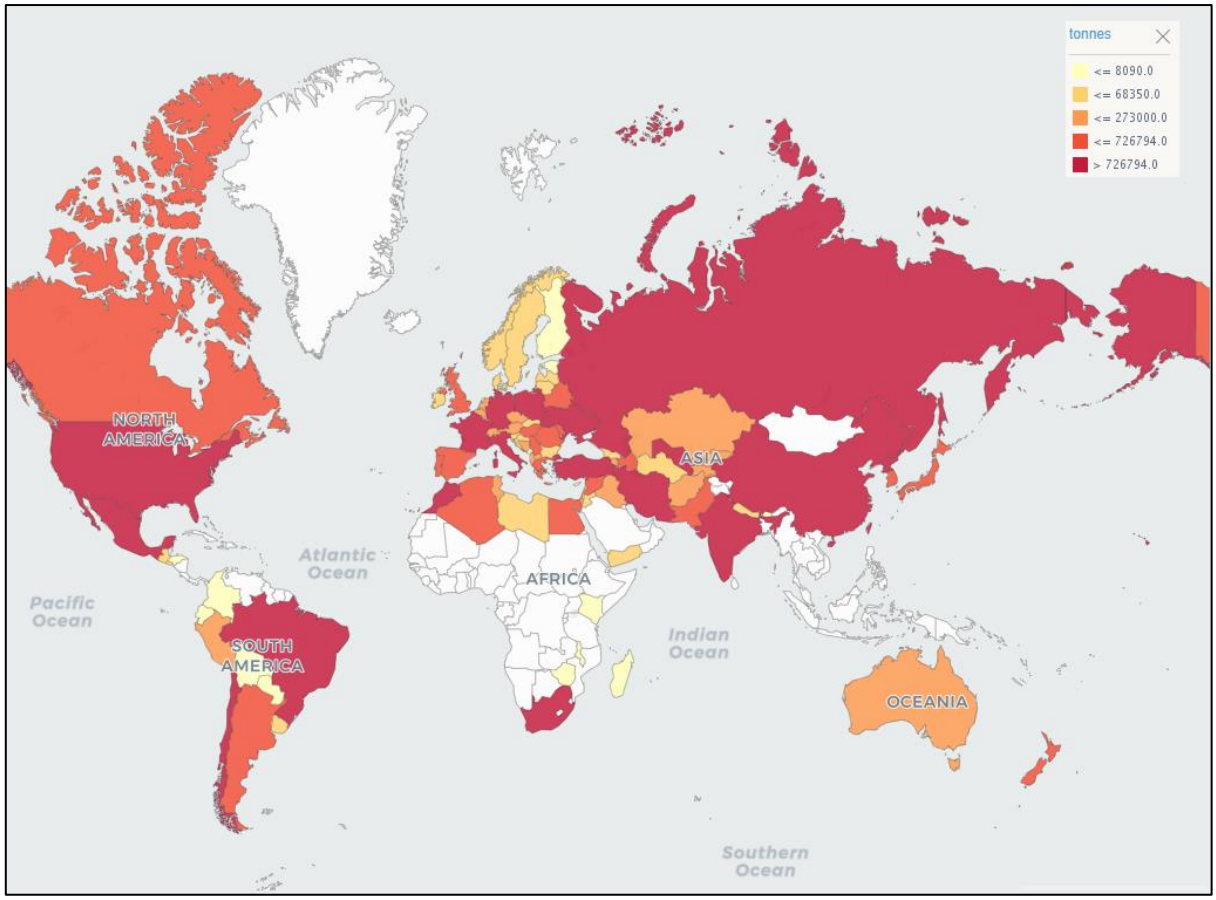

Figure 1.2: Production quantities of apples by country, 2019 (FAOSTAT, 2019).

1.1.3.2 Apple production in Italy

In 2019, apples represented the 6th most valuable commodity produced in Italy and the 5th crop produced after tomatoes, wheat, maize and tomatoes. The total Italian apple production was estimated to have decreased 0.9% between 2009 and 2019, while the area harvested decreased from 58,445 ha in 2009 to 55,000 ha in 2019 (FAOSTAT, 2019).

1.1.3.3 Apple production in New Zealand

In 2019 apples represented the 2nd largest crop in volume produced after kiwifruit in New Zealand and the 4th most valuable commodity produced. The total New Zealand production was estimated at 553,606 tons with an increase of 22% between 2009 and 2019, while the total area harvested increased 5% in that same period (FAOSTAT, 2019). This period masks a reduction in planted area, but it is on the rise again with the introduction of competitive new cultivars.

1.1.4 Apple genome and genotyping

The first whole genome sequence (WGS) for cultivated apples was reported by Velasco *et al.* (2010) as a high-quality draft sequence. This was followed by a high-quality *de novo* assembly of the doubled-haploid apple genome (Daccord *et al.*, 2017), a high-quality apple genome assembly (Zhang *et al.*, 2019), and a *de novo* diploid assembly of the apple cultivar 'Gala Galaxy' (Broggini *et al.*, 2020). Of the other species, only a draft genomes of the wild apple *M. baccata, M. sieversii* and *M. sylvestris* are available to date (Chen *et al.*, 2019, Sun *et al.*; 2020).

In 2012, next-generation sequencing (NGS) was used to detect single nucleotide polymorphisms (SNPs) covering the apple genome. This effort involved re-sequencing of a small set of cultivars, ancestors and founders, chosen to represent the pedigrees of apple breeding programs worldwide by RosBREED (www.rosbreed.org), a consortium established to enable marker-assisted breeding for rosaceous crops. They developed an initial Illumina 8K SNP array from whole-genome resequencing of 27 cultivars at low sequencing coverage (Chagné *et al.*, 2012), which contains 7,867 *Malus* SNP markers as well as 921 SNPs derived from *Pyrus* (Montanari *et al.*, 2013).

In 2014 an Illumina Infinium array targeting 20K SNPs was developed. The SNPs were predicted from re-sequencing data derived from the genomes of thirteen *M.* x *domestica* cultivars and one accession belonging to a crab apple species (*M. micromalus*) (Bianco *et al.*, 2014).

Bianco *et al.* (2016) described the development and validation of a 487K SNP Affymetrix Axiom® genotyping array for apple and discussed its potential applications. The array has been built from the high-depth resequencing of 63 different cultivars covering most of the genetic diversity in cultivated apples (Daccord *et al.*, 2017).

Sun *et al.* (2020) constructed an apple pan-genome uncovering thousands of new genes, with hundreds of them being selected from one of the progenitors and largely fixed in cultivated apple, revealing that introgression of new genes/alleles is a hallmark of apple domestication through hybridization.

In 2019, a review was written on the use of WGS to understand the species, geographical, and genomic origins of domesticated apples more precisely, as well as its relationship to wild relatives. This study included examples of basic and practical breakthroughs and challenges in using the apple WGS (roles and influences, intragenomic interactions, and germplasm distributions of variants of chromosomal modules—the genes, motifs, trait loci, haploblocks, base pairs and so on) (Peace *et al.*, 2019).

The causative variants for key plant breeding trait loci not only provide ideal genetic markers for efficient selection, but López-Girona *et al.* (2020) also demonstrated a new method to resolve single nucleotide and structural variants at the haplotype level in plant genomic regions. This method is

a combination of CRISPR-Cas9 target enrichment and Oxford Nanopore Technology sequencing for fine-mapping loci instead of genome-walking approaches.

1.2 **Pear**

1.2.1 Taxonomy and origin of the genus *Pyrus*

Several botanists proposed that the genus *Pyrus* differentiated in the Tertiary period in the mountainous territory that is occidental China today (Zheng *et al.*, 2014). From here, it would have dispersed towards both the East and the West, adapting to different climate and territorial conditions to differentiate into the 22 currently known species (Zheng *et al.*, 2014), with more than 5,000 accessions maintained worldwide. These accessions display wide morphological and physiological diversity, as well as broad adaptations to wide agro-ecological ranges (Wu *et al.*, 2018). Two primary points of origin were identified (Zukovskij, 1962; Vavilov, 1992):

a) China, where *P. pyrifolia*, *P. ussuriensis* and *P. calleryana* are cultivated; and
b) Middle Orient (Caucasus, Asia Minor), the primary point of origin of *P. communis.*

There is another secondary point of origin:

c) Central Asia (North-Occidental Asia, Afghanistan, Tajikistan, Uzbekistan and Tian-Shan), where *P. communis* has hybridized with *P. heterophylla* and *P.* x *bretschneideri.*

The genus *Pyrus* belongs, as *Malus*, to the Rosaceae family, subfamily *Pomoideae.* The chromosome number of pear is n = 17, same as apple (Velasco *et al.*, 2010), suggesting an allopolyploid origin since other Rosaceae have n = 7, 8 or 9. All the *Pyrus* species have a chromosome number 2n = 34 except for some *P. communis* cultivars that are polyploid (Wu *et al.*, 2013, 2018; Zheng *et al.*, 2014).

1.2.2 The pear tree

Most of the fruit trees, apple as well, are generated by grafting, which is an ancient, vegetative, asexual plant propagation technique. It is a combination of scion and rootstock of two different species, varieties or the same variety (Wertheim, 2000; Rouphael *et al.*, 2010; Goldschmidt, 2014). The formation of a successful graft is a complex biochemical and structural process that includes an immediate wound response, callus formation, establishment of new vascular tissue, and formation of a functional vascular system between the rootstock and scion (Hudina *et al.*, 2014). Quince has been used for many centuries as a dwarfing rootstock for pear (Fideghelli *et al.*, 2003). Some of the most commercially important European pear trees (*P. communis*), such as 'Williams Bon Chretien' and 'Abate Fetel', have shown graft incompatibility with quinces (Almeida *et al.*,

2020), and these combinations usually result in substantial tree mortality in the orchard, owing to disruptions in the graft union (Hudina *et al.*, 2014). Graft incompatibility is overcome by the use of a quince-compatible interstock, mostly 'Beurré Hardy'.

1.2.3 Pear cultivation and economy

1.2.3.1 World pear production

The world economic situation for pear cultivation is substantially different from that for apple. While there is a worldwide crisis in apple production and decrease of prices, the economics of pear production are much better and pears have become an interesting economic diversification (FAOSTAT, 2019).

In 2019, the world pear production was 23.91 MT with an agricultural area of 1.37 million ha (FAOSTAT, 2019) (Figure 1.3). The largest producer again is China with 17.00 MT. The United States of America is the second largest producer with 661,340 tons, followed by Argentina with 595,427 tons, 530,723 tons in Turkey and 429,290 tons in Italy (FAOSTAT, 2019) (Figure 1.4).

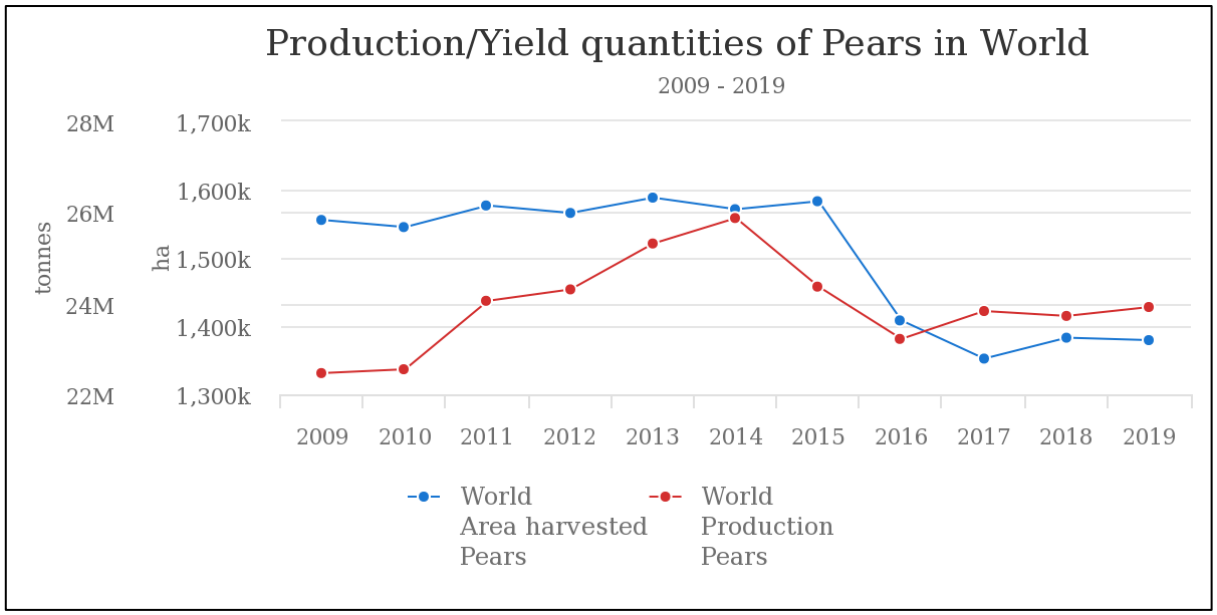

Figure 1.3: World production and area harvested area of pears in the period 2009 – 2019 (FAOSTAT, 2019)..

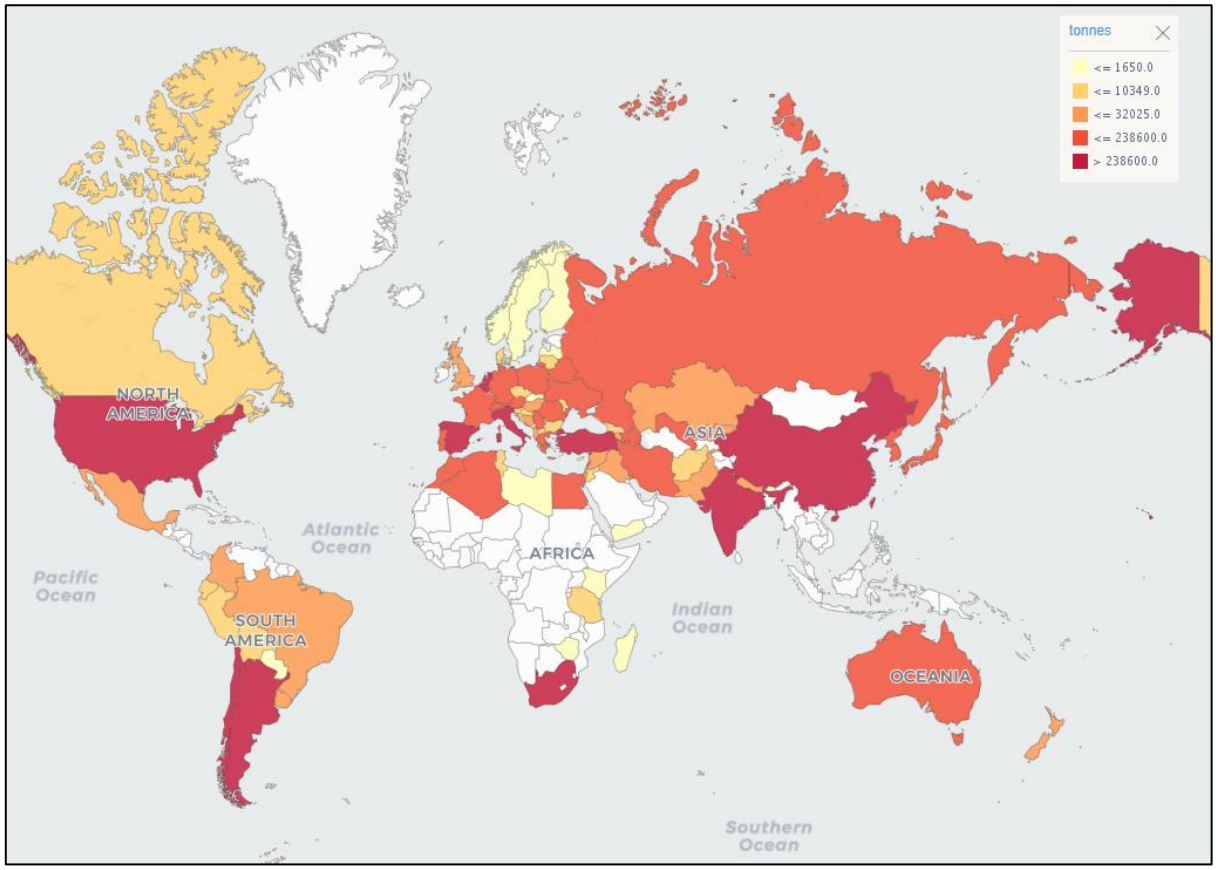

Figure 1.4: Production quantities of pears by country, 2019 (FAOSTAT, 2019).

1.2.3.2 Pear production in Italy

Italy was the second largest pear producer in Europe in 2019. The total Italian pear production was estimated to have decreased 50% in the 10 years from 2009 to 2019 from an area that decreased by 28% over the same period (from 40,190 ha in 2009 to 28,710 ha in 2019) (FAOSTAT, 2019).

1.2.3.3 Pear production in New Zealand

The total New Zealand production was estimated to be 17,563 tons in 2019. The total New Zealand area harvested decreased by 46% in the last 10 years (from 639 ha in 2009 to 340 ha in 2019) (FAOSTAT, 2019).

1.2.4 Pear genome and genotyping

In 2014 the first draft assembly of the genome of European pear (*P. communis*) 'Bartlett' was published, which was developed using Roche 454 sequencing technology and spans 577.3 Mb, containing 43,419 putative genes (Chagné *et al.*, 2014). Linsmith *et al.* (2019) developed a new pseudo-chromosome–length genome assembly of a double haploid 'Bartlett' pear. The 'Bartlett'

genome assembly is an invaluable tool for identifying the genetic control of key horticultural traits in pear and will enable the wide application of marker-assisted and genomic selection that will enhance the speed and efficiency of pear cultivar development (Linsmith *et al.*, 2019). In 2019 a *de novo* genome of a wild pear (*P. betulaefolia*) was assembled (Dong *et al.*, 2020).

Molecular markers are useful for the classification of cultivars and germplasm management because they are not affected by environmental conditions or plant phenology (Belaj *et al.*, 2002; Singh *et al.*, 2013). Kim *et al.* (2019) identified the genetic relationships and population structure of pears with a SNP approach. In the same year a study was conducted to find the candidate genomic regions for pear fruit traits, including sensory eating quality traits, and to evaluate the potential of genomic selection using a hybrid population derived from crosses between Asian and European pears (Kumar *et al.*, 2019). SNP markers involved in the pear red skin colour were identified (Kumar *et al.*, 2019).

1.3 Apple-pear hybrids

Interspecific hybridization is acknowledged as the most important source of genetic variation for breeding new varieties (Van Tuyl and De Jeu, 1997). Interspecific hybridization, with or without chromosome doubling, is also an important force in plant evolution that can lead to stabilized introgression and to homoploid (hybridization without a change in chromosome number), or allopolyploid (hybridization followed by chromosome doubling) speciation (Lowe and Abbott, 2000; Minder *et al.*,2007; Abbott *et al.*, 2009; Soltis and Soltis, 2009). Such hybrids can be used to create new morphological forms of plants and fruits, and to combine characteristics of two different plants into one.

Overcoming the intergeneric crossing barrier between the genera *Malus* and *Pyrus* to produce hybrids would allow the introduction of various chromosomal regions of the pear genome into *Malus* via subsequent backcrosses with *Malus*, or *vice versa* (Fischer *et al.*, 2014).

Crane and Marks (1952) first reported hybrids between pears and apples. An apple x pear hybrid, obtained in 1970 had intermediate morphological characteristics compared with its parents. The fruits were pear shaped, seeds were rarely found but appeared to be viable. Fruit yield was poor, the pollen was completely sterile and after a few years the hybrid died (Rudenko and Rotaru, 1970). Later, morphological features of such hybrid plants were described (Rudenko and Rotaru, 1989). Inoue *et al.* (2003) reported that lethality in hybrids between Japanese pear and apple was suppressed at high temperatures. Unfortunately, the intergeneric seedlings died within five months, which was presumed to have been caused by physical stress from the high temperatures. Gonai *et*

al. (2006) reported a study of hybrids between Japanese pear (*P. pyrifolia*) and apple, in which hybrid embryos were gamma-irradiated and cultured at normal temperature conditions to obtain viable intergeneric plants. In most cases, the embryos were aborted at an early developmental stage or seedlings died within six months. In 2014, an intergeneric hybrid Zwintzscher's Hybrid of *Malus* and *Pyrus* was confirmed (Fischer *et al.*, 2014). The german breeder Max Zwintzscher (now deceased) obtained this hybrid at the former Institut für gärtnerische Pflanzenzüchtung in Köln-Vogelsang, Germany, during the 1980s. This F1 hybrid was saved by grafting, further cultivation and leaf fertilization by Herrmann Schimmelpfeng (TU Munich Freising-Weihenstephan; Germany). It also has given rise to a fertile F2 generation, which forms part of this study.

1.4 **Secondary metabolites**

The plant secondary metabolites, which are a characteristic feature of all plants, are often referred to as compounds that have no fundamental role in the maintenance of life processes in the plants, but they are important for the plant to interact with its environment for adaptation and defence. In higher plants a wide variety of secondary metabolites are synthesized from primary metabolites (e.g., carbohydrates, lipids and amino acids) (Akula *et al.*, 2011).

Chemical protection plays a decisive role in the resistance of plants against pathogens and herbivores. Secondary metabolites are especially important and can protect plants against a wide variety of micro-organisms (viruses, bacteria, fungi) and herbivores (arthropods, vertebrates). As it is the case with all defence systems of plants and animals, a few specialized pathogens have evolved in certain plants by overcoming the physical and chemical defence barriers (Naveed *et al.*, 2020).

Plant secondary compounds are usually classified according to their biosynthetic pathways (Harborne, 1999). Three large families are generally considered: phenolics, terpenes and steroids, and alkaloids. A good example of a widespread metabolite family are the phenolics: these molecules include the well-known class of flavonoids, but also phenolic acid and other polyphenols and are, for example, involved in pigment and lignin synthesis and they are common to all higher plants. However, other compounds, such as alkaloids, are sparsely distributed in the plant kingdom and are much more specific to defined plant genera and species (Bourgaud *et al.*, 2001).

Apple fruits are a major source of diverse phenolic compounds including flavonols, anthocyanins, proanthocyanins and dihydroclacones (Lee *et al.*, 2003).

Arbutin (hydroquinone-*β*-D-glucopyranoside) is a natural phenolic glucoside found in various plant species of diverse families, such as Ericaceae (*Vaccinium* spp., *Arctostaphylos* spp.), Asteraceae (*Achillea millefolium*), Betulaceae (*Betula alba*) and Rosaceae (*P. communis*) (Petkou *et al.*, 2002).
Fischer *et al.* (2014) confirmed that Zwintzscher's Hybrid accumulates phloridzin, arbutin and their aglyca forms (phloretin and *p*-hydroquinone, respectively) in leaves, while only one or the other was present in the respective parents. Isorhamnetin 3-*O*-glucoside, described to be indicative of pear (Schieber *et al.*, 2001), was found in low amounts in apple, but was much higher in the pear and hybrid extracts.

1.4.1 Phloridzin

Phenolic secondary plant metabolites, including the class of flavonoids, contribute to both fruit colour and human health. They are widely believed to possess anti-oxidative, anti-microbial, anti-mutagenic and anti-carcinogenic properties (Awad *et al.*, 2000). The dihydrochalcone phloridzin (phloretin 2'-*O*-glucoside) is the major phenolic glucoside found in apple trees and contributes to the flavor, colour and health benefits of apple fruits and processed products (Li *et al.*, 2011). For a long time phloridzin was thought to occur only in *Malus* species, but recent research has shown that it is also present in a many other plant species, e.g., *Lithocarpus polystachyus* (Dong *et al.*, 2007), *Rosa canina* (Hvattum, 2002), *Fragaria* x *ananassa* (Hilt *et al.*, 2003), *Vaccinium macrocarpon* (Turner *et al.*, 2005) and in the leaves of Australian native sarsaparilla (*Smilax glyciphylla*) (Cox *et al.*, 2005). The presence of high amounts of phloridzin makes apple unique in the Rosaceae family, but also in the plant kingdom, as the other species accumulate significantly lower amounts, whilst closely related species like pear (*P. communis*) are not able to synthesize phloretin or phloridzin at all (Williams, 1964; Andreotti *et al.*, 2006). However, considering the large amounts present in apples, the physiological function of phloridzin *in planta* still remains a puzzle (Gosch *et al.*, 2009).
The dihydrochalcone phloridzin represents more than 90% of the soluble phenolic compounds in apple leaves (Gosch *et al.*, 2009) and is disproportionately distributed in apple tissues as it is more abundant in vegetative tissues, i.e. leaves and branches (66–90% of total phenolic content), and lower in mature fruit (2–6% of total phenolic content), where it concentrates primarily in the peel, along with other phenolics and nutraceuticals (Gosch *et al.*, 2010; Zhou *et al.*, 2017).
Gutierrez *et al.* (2018) observed that genetic factors and russeting were strong predictors of phloridzin content in the peel, but not in the fruit flesh or leaves. Conversely, other peel phenolics were negatively associated with russeting. Variable phloridzin content was related to russet incidence during fruit development in 'Golden Delicious' (low to medium russet) and its sports,

'Empress Spur' (low russet), 'Razor' (complete russet), and 'Sergeant Russet' (medium to high russet) (Gutierrez *et al.*, 2018).

1.4.2 Arbutin

The hydroquinone-β-D-glucopyranoside, also known as arbutin, is a widely distributed compound in various higher plants such as leaves of bearberry and pear (Arend *et al.*, 2000; Ahmadian *et al.*, 2019).

Arbutin exhibits numerous biological activities, including disinfectant, anti-microbial, anti-hyperglycaemic, anti-hyperlipidemic, anti-oxidant, free radical scavenging, alpha-amylase inhibitory and anti-tumor effects (Nawarak *et al.*, 2009; Shahaboddin *et al.*, 2011; Li *et al.*, 2011; Yousefi *et al.*, 2013; Wickramaratne *et al.*, 2016). In addition, arbutin is able to attenuate oxidative stress through reducing the production of reactive oxygen species (ROS) and superoxide (Lee and Eun, 2012). As a mild, safe and effective agent, it has been widely used in medical and cosmetic industries (Seo *et al.*, 2012).

In the past, the presence of arbutin in pear has been correlated with the biochemical processes that operate as defence mechanisms against bacterial invasion. It has been suggested that the oxidation pathway of arbutin degradation may be involved in fire blight resistance of some pear varieties via the formation of toxic substances (Petkou *et al.*, 2002). Furthermore, the high concentration of arbutin and several flavonols has been considered as a possible cause of graft incompatibility between pear and quince (Hudina *et al.*, 2014).

1.5 **Diseases**

1.5.1 Scab

During the growing season, many ornamental and fruiting apple (*Malus* spp.) and pear (*Pyrus* spp.) accessions are highly susceptible to the foliar fungal pathogens *Venturia inaequalis* and *V. pyrina* which cause apple and pear scab, respectively. As suppliers, vendors and growers of both fruits generally adopt a zero-tolerance policy towards scab, any scab infection reduces the quality and marketable fruit yield (Percival *et al.*, 2009).

The genus *Venturia* belongs to the phylum Ascomycota, class Dothideomycetes (Schoch *et al.*, 2009). Traditionally, this genus has been included in the family Venturiaceae, order Pleosporales, according to its "Pleospora-type centrum and bitunicate asci" (Sivanesan, 1977). However, recent molecular phylogenetic analyses of Dothideomycetes, using both nuclear and mitochondrial gene

regions, have indicated that the family Venturiaceae forms a well-supported monophyletic group separate from the Pleosporales (Kodsueb *et al.*, 2006; Kruy *et al.*, 2006; Zhang *et al.*, 2011). Thus, Zhang *et al.* (2011) recently reordered Venturiaceae into Venturiales *ord. nov.* (together with Sympoventuriaceae fam. nov.).

Venturia species are host-specific pathogens and each can only infect one plant genus or close relatives in each host family (Prokchorchik *et al.*, 2019). *V. inaequalis* infects apple (*Malus* spp.); while *V. pyrina* (or *V. pirina*) and *V. nashicola* infect European pear (*P. communis*) and Asian pear (*P. pyrifolia* var. culta and *P. ussuriensis*), respectively (González-Domínguez *et al.*, 2017).

Apple scab occurs in every country where apple is cultivated, with the exception of West Australia, where the disease was temporarily eradicated (McKirdy *et al.*, 2001), but unfortunately was found again later, particularly in the Perth Hills (Mathews, 2020). *V. inaequalis* probably emerged in Central Asia, the center of apple origin, and followed its host's expansion into Europe and, more recently, into other regions with the expansion of apple cultivation. *V. inaequalis* infections of apples in Europe and Central Asia consist of three distinct populations: (i) a large European population infecting the domesticated apple and the wild *M. sylvestris*; (ii) a large Central Asian population infecting the domesticated apple and populations of *M. sieversii*; and (iii) a more geographically restricted population associated with *M. sieversii* in areas where *M.* x *domestica* is absent (Gladieux *et al.*, 2010). Xu *et al.* (2008, 2013) found a higher variability in a population of *V. inaequalis* from the same orchard in the UK than in populations from different cultivars or regions in China. Overall, *V. inaequalis* appears to be a model invasive plant pathogen with a broad geographic distribution and well-established populations (González-Domínguez *et al.*, 2017).

Like *V. inaequalis*, *V. pyrina* has a worldwide distribution that is strongly associated with the distribution of its host, the European pear (Percival *et al.*, 2009) and is one of the most serious diseases affecting this species in its native district range (Pierantoni *et al.*, 2007). *V. pyrina* is classified in the same genus as *V. inaequalis*, with both species mutually exclusively infect pear, and apple, respectively, while within pear, Japanese and Chinese pears are generally resistant to *V. pyrina* and susceptible to *V. nashicola*, and *vice versa* for European pear (Percival *et al.*, 2009; Terakami *et al.*, 2006). Most scab resistances in *P. communis* are presumed to be polygenic, and recent genetic mapping in several partially resistant cultivars has confirmed this finding (Korban, 2019).

1.5.2 Fire Blight

Erwinia amylovora causes fire blight disease of apple, pear, quince, blackberry, raspberry and many wild and cultivated rosaceous ornamentals (Vanneste, 2000). The most economically important hosts are apple and pear. Generally, pear is more susceptible than apple. The pathogen is

distributed widely in temperate regions in which rosaceous plants thrive. It was described initially as *Micrococcus amylovorus*, and then *Bacillus amylovorus* (Burrill), under the erroneous assumption that it destroys starch. It is Gram negative, rod-shaped, and motile with peritrichous flagella, belonging to the family *Enterobacteriaceae*. It was renamed *E. amylovora* (Burrill) by Winslow *et al.* in the early 1900s and remains the type species of the genus. Closely related bacteria that elicit symptoms reminiscent of fire blight, particularly, but not exclusively, in pear, have been described as new species, e.g., *E. pyrifoliae* and *E. piriflorinigrans* (Mansfield *et al.*, 2012).

Fire blight was first reported in North America in 1900 (Aldwinckle and Zwet, 1979) and was later detected in New Zealand in 1920. Infection was first reported in Britain in 1957 and remains a notifiable disease there (Eastgate, 2000). In Italy, fire blight was first seen in 1990 (Puglia, southern Italy), and in 1997 a fire blight epidemic occurred in the Emilia Romagna region (northern Italy), causing severe damage to pear orchards. Since then, fire blight on pear has been endemic in the region (Calzolari *et al.*, 1998).

The initial symptom of fire blight is water soaking, followed by wilting and rapid necrosis leaving infected tissue with a scorched, blackened appearance. The severity of fire blight symptoms can vary depending on the host plant and climatic conditions (Eastgate, 2000). Climatic conditions, such as warm temperatures and rain or high humidity, predispose blossoming plants to infection. Most fire blight infections are localized to blossom bracts; however, in highly susceptible hosts bacteria can spread into mature tissue (Eden-Green and Billing, 1974).

Fire blight is a highly destructive disease and is of major economic concern to fruit growers worldwide. *E. amylovora* infection of blossoms can greatly reduce crop yield, hence economic returns. Systemic spread of *E. amylovora* may cause the loss of entire trees and orchards. The severity of fire blight outbreaks in California prevents the commercial production of pears in this region. A major difficulty encountered with fire blight is the lack of effective disease control (Eastgate, 2000). The disease develops sporadically, but, occasionally, it is highly destructive, especially to young fruit trees that may be killed outright by infections that girdle the trunk or the rootstock.

1.6 Technologies applied in this project

1.6.1 Metabolomics analysis

The aim of metabolomics analysis is to comprehensively characterize the present metabolites in given biological samples both qualitatively and quantitatively (Zheng *et al.*, 2020).

There are two major metabolomics methodologies: untargeted and targeted metabolomics (Dettmer *et al.*, 2007; Patti *et al.*, 2012; Gong *et al.*, 2019; Lee *et al.*, 2020). Untargeted metabolomics focuses on the comprehensive analysis of all the measurable metabolites in given biological samples, including the unknown chemicals. In contrast, targeted metabolomics aims to analyse a set of known and pre-selected metabolites (Cai and Zhu, 2019).

Multiple reaction monitoring (MRM) using triple-quadruple mass spectrometry, which monitors both the specific precursor and product ion of each metabolite, is the most frequently used technique in targeted methods, because it enables high sensitivity, high specificity and excellent quantification ability (Lu *et al.*, 2008; Wei *et al.*, 2010; Zheng *et al.*, 2020).

In this project the MRM method was used to detect the synthesis and accumulation of secondary compounds, including the genus-specific metabolites described above, in the putative hybrids.

1.6.2 Molecular Markers

1.6.2.1 Simple Sequence Repeat markers

Simple sequence repeat (SSR) markers were discovered and developed by Litt and Luty (1989) and by Edwards *et al.* (1991) in humans and were first applied to plants by Akkaya *et al.* (1992). SSRs or microsatellites are DNA stretches consisting of short, tandemly repeated di-, tri-, tetra-or penta-nucleotide motifs. SSRs have been found in all eukaryotic species that have been examined for them (Li *et al.*, 2004). They provide excellent species specificity and means of assessing genetic variation in samples. In general, the sequences mutate rapidly, so provide differentiation of even closely related samples (Foster *et al.*, 2011).

SSRs are co-dominant markers, so they can distinguish heterozygotes from homozygotes. Main advantages are their high level of polymorphism and their reliability. Other advantages are their high abundance, random distribution in the entire genome, high information content, and reproducibility (Hao *et al.*, 2015). Many studies have applied SSRs to various goals, e.g., determining germplasm diversity (Ben-Ari and Lavi, 2012; Gong *et al.*, 2019; Nag *et al.*, 2020; Patil *et al.*, 2020). Finally, thanks to the advent of new high-throughput technologies, these markers are a powerful genotyping instrument, which is used in different plant species, including pear and apple, to develop genetic maps as a basis for marker assisted selection (MAS) (Bus *et al.*, 2010; Emeriewen *et al.*, 2020; Fan *et al.*, 2020).

1.6.2.2 High Resolution Melting SNP analysis

High resolution melting (HRM) analysis is a relatively new, post-PCR analysis method used for identifying genetic variation in nucleic acid sequences, most often single nucleotide polymorphisms

(SNPs). In the past, other expensive and time-consuming techniques, such as fluorescence resonance energy transfer (FRET) probes and denaturating gradient gel electrophoresis (DGGE), were used for mutation scanning. Compared to these technologies, HRM offers a far easier, less time-consuming and more reproducible procedure (High Resolution Melting Analysis - an overview | ScienceDirect Topic, 2020).

The HRM method is based on analysis of PCR melting (dissociation) curves and is enabled by the recent availability of improved double-stranded DNA (dsDNA)–binding dyes along with next-generation real-time PCR instrumentation and analysis software. HRM analysis can discriminate DNA sequences based on their composition, length, GC content, or strand complementarity (Applied Biosystems, 2010).

The attractiveness of HRM lies in its simplicity: PCR fragments ranging between 50 and 120 bp are amplified using unlabelled primer pairs constructed to amplify a short sequence containing a SNP designed upstream and downstream of the SNP. Melting analysis is performed at the end of the PCR reaction in the presence of a high-fidelity dye that is an intercalant of double-stranded DNA (dsDNA).

Prior to the HRM analysis, the PCR products are denatured at 94-95°C as this quick melting underlies the subsequent HRM analysis. During that critical step, complementary strands for the unique allele of a homozygous sample re-anneal perfectly to form a complementary dsDNA product (homoduplex). However, in the case of heterozygous samples that have more than one allele present in the PCR product, half of the alleles re-anneal to the complementary strand of the same allele and the other half re-anneal to the complementary strand of the other allele (Figure 1.5).

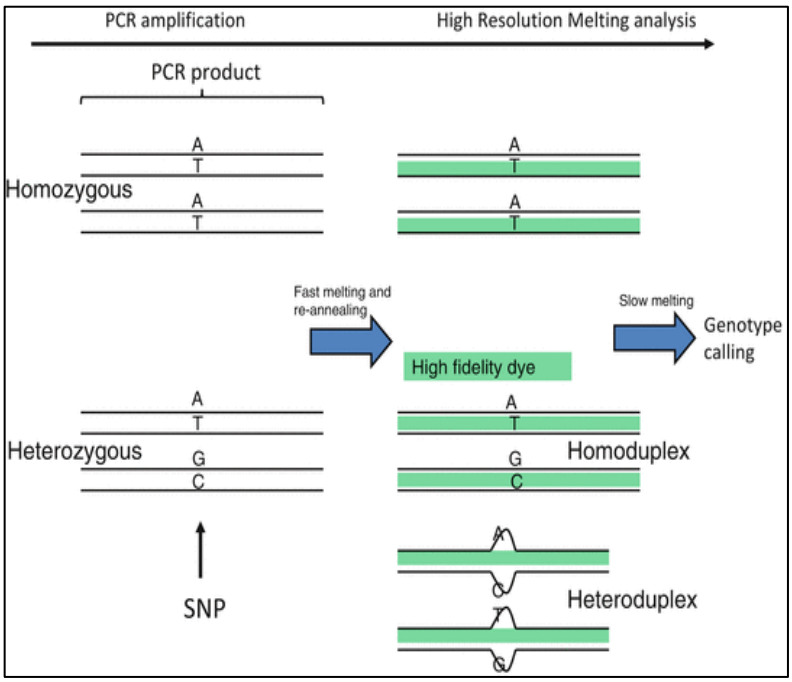

Figure 1.5: Principle of the High-Resolution Melting technique. Fast melting and reannealing promote the formation of heteroduplex PCR products for heterozygous individuals. Such heteroduplexes are less stable than homoduplexes and melt at a lower temperature (Chagné, 2015).

Such imperfectly annealed molecules are called hetero-duplexes and are less stable than homoduplexes. The last step of the HRM analysis involves a slow melting of PCR products from 65°C to 95°C, while a high frequency, high accuracy fluorescence capture (25 measurements per 1°C) is performed (Vossen *et al.*, 2009). Samples containing heteroduplexes (heterozygous alleles) melt at a lower temperature than homoduplex-containing samples (Chagné, 2015) (Figure 1.6). In this project HRM analysis was used to scan the genomes of the putative hybrids with specific 'apple/pear' primers, designed to detect the heterozygosity of putative hybrids compared with the respective parents for alleles in a homozygous state. The 'apple/pear' primers were designed at the beginning, the middle and end of each chromosome to identify parts of the 'hybrid' genomes that exhibited hybridity.

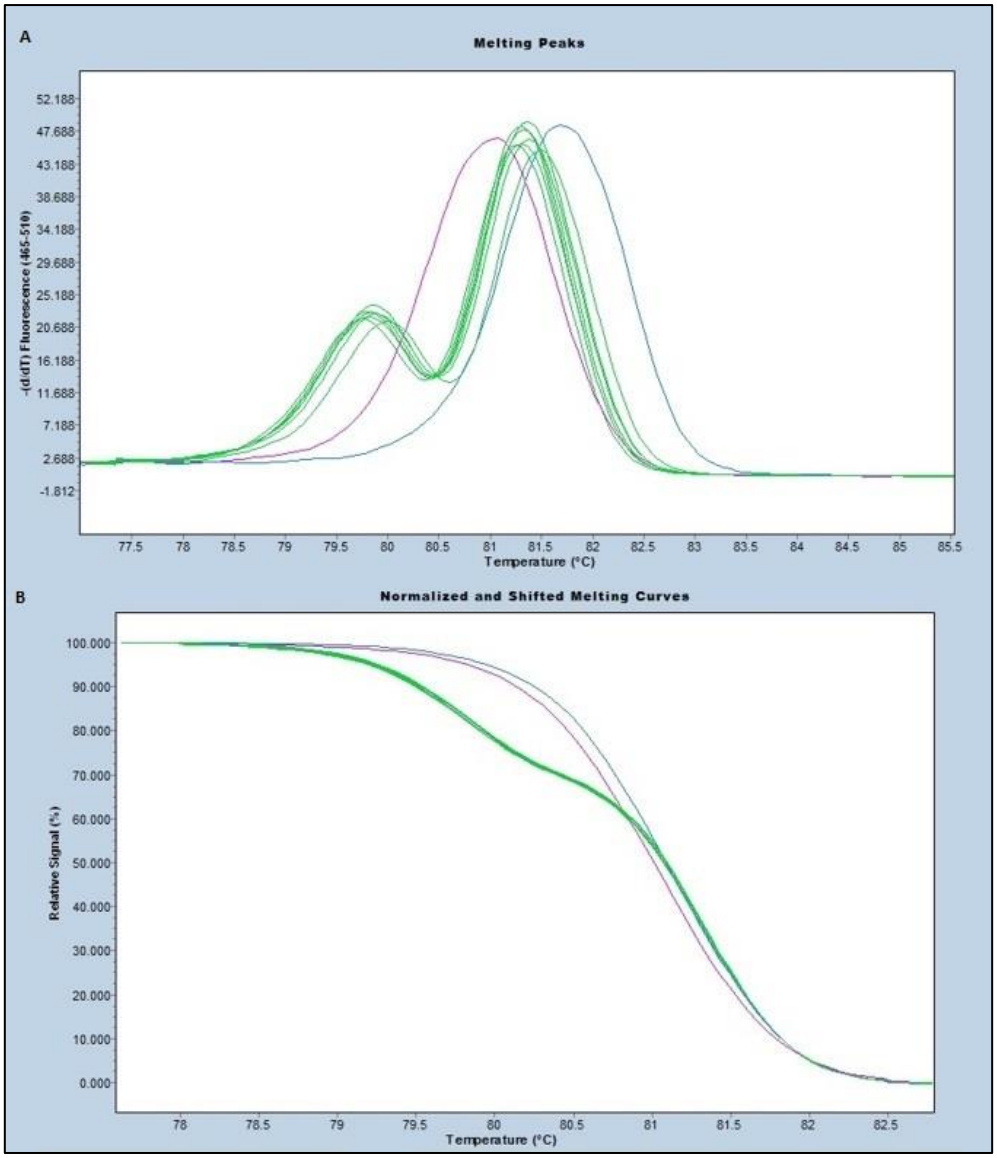

Figure 1.6: HRM result showing melting peaks (A) and normalized melting curves (B). In blue and pink are homozygous peaks for this marker; the green curves represent six heterozygous samples with double peaks in Figure 1.6A.

1.6.2.3 SNP-chip

The development of high-throughput SNP genotyping assays has radically changed the genetic dissection of complex traits in human, model organisms, and agricultural species (Groenen *et al.*, 2011).

SNPs are single-base variations in DNA sequences that are abundant in plant genomes and are highly suited to high-throughput assays, are useful for identifying differences within individuals or populations as well as identifying genetic loci associated with phenotypic variation (Montanari *et al.*, 2013). The low mutation rate of SNPs makes them valuable for understanding complex genetic traits and genome evolution (Dalton-Morgan *et al.*, 2014). High-throughput SNP arrays, such as

the Infinium® II assay (Illumina Inc.), are effective technologies for genotyping of large populations. The arrays contain probe sets to interrogate the two alleles for all the SNPs, conventionally referred to as allele A and allele B. The technology involves synthesizing 25mer oligonucleotide probes corresponding to a perfect match for the A and B allele sequences. In addition, a mismatch probe is synthesized for each allele to detect non-specific binding. This probe quartet is the basic unit for detecting different genotype groups: AA, AB or BB (Rabbee and Speed, 2006).

High-throughput SNP arrays have been developed for a range of fruit tree species. In 2014 an Illumina Infinium array targeting 20K SNPs was developed in *Malus*. The SNPs were predicted from re-sequencing data derived from the genomes of thirteen apple cultivars and one accession belonging to a crab apple species (*M. micromalus*) (Bianco *et al.*, 2014). In 2013 an apple and pear Infinium® II 9K SNP array for large-scale genotyping in pear across several species, using both pear and apple SNPs, was developed (Montanari *et al.*, 2013). The 1,096 pear SNPs were combined with the set of 7,692 apple SNPs on the International RosBREED SNP consortium (IRSC) apple Infinium® II 8K array, making this Infinium® II 9K SNP array the first cross-genera SNP array between *Malus* and *Pyrus*. It therefore enables, for the first time, the assessment of SNP marker transferability between these genera (Montanari *et al.*, 2013).

In this project high-throughput SNP array studies were performed to detect and identify differences within individuals or populations.

1.6.3 DNA content

The nuclear DNA content of a species, expressed as the C–value, is the total quantity of non-replicated nuclear DNA of a gamete, and it is constant and independent from the level of ploidy of the individuals (Martín–Martín *et al.*, 2020). Flow-cytometry determination of nuclear DNA content is a relatively simple, fast, and inexpensive method of verifying genome size stability (Miler *et al.*, 2020).

1.6.4 RNA sequencing

RNA sequencing (RNA-seq) uses the capabilities of high-throughput sequencing methods to provide insight into the transcriptome of a cell. Compared to previous Sanger sequencing- and microarray-based methods, RNA-seq provides far higher coverage and greater resolution of the dynamic nature of the transcriptome (Kukurba and Montgomery, 2015).

When RNA-seq is to be used for determining differential gene expression, the steps are: RNA extraction, followed by mRNA enrichment or ribosomal RNA depletion, cDNA synthesis and

preparation of an adaptor-ligated sequencing library. The library is then sequenced to a read depth of 10–30 million reads per sample on a high-throughput platform (usually Illumina). The final steps are aligning the sequencing reads to the reference transcriptome, quantifying reads that overlap transcripts, filtering and normalizing between samples, and statistical modelling of significant changes in the expression levels of individual genes and/or transcripts between sample groups (Stark *et al.*, 2019).

In this project RNA-seq analysis was performed to screen for putative genes involved in the arbutin biosynthesis.

1.6.4.1 Real-time quantitative PCR

Real-time quantitative PCR (RT-qPCR) is the reliable detection and measurement of products generated during each cycle of the PCR process, which are directly proportionate to the amount of template prior to the start of the PCR process (Ginzinger, 2002).

RT-qPCR has been the gold standard application with cDNA for gene expression analysis, applying relative quantification, and for absolute quantification using genomic DNA extracted from environmental, human and animal clinical samples, plant material, or food samples. Absolute quantification is the quantification of the absolute quantity of a target gene in a nucleic acid sample. This requires the application of a standard curve using a known concentration of starting sample. Relative quantification is the fold difference between a particular reference (control) sample relative to all other samples in the experiment. Typically, the calculation results in the control biological group giving a relative expression of 1 and the treatment groups are either fold increase or decrease compared with control (Taylor *et al.*, 2019).

The quantitative data generated can be used to relate variation in gene abundances and/or levels of gene expression (in terms of transcript numbers) in comparison with variation in abiotic or biotic factors and/or biological activities and process rates (Smith and Osborn, 2009).

In this project this analysis was used to quantify the candidate gene expression involved in the DHCs pathway, obtained from the RNA-seq analysis.

1.7 Aims of the study

In this study, different methods as described above were used to detect and characterise true apple-pear or *vice versa* hybrids raised from the intergeneric crosses made in Germany, New Zealand and Italy.

This thesis is divided in six chapters: General introduction, Chapter I; Plant materials, Chapter II; Three experimental chapters, the content of which is briefly summarised below; and General conclusion, Chapter VI.

CHAPTER III

This chapter covers the molecular characterization of putative apple-pear and pear-apple hybrids with different molecular markers (SSRs, SNPs using both HRM, and SNP-chips) and with DNA content measurements.

CHAPTER IV

This chapter focuses on disease evaluation of the materials from New Zealand for apple and pear scab, and fire blight.

CHAPTER V

This chapter focuses on detecting the presence/absence of arbutin and phloridzin in the hybrids using metabolomics analysis and to develop a first scheme of arbutin biosynthesis. These two secondary metabolites are genus-specific, so it is possible to detect true hybrids by the pattern of these two phenolic in samples. To date, the biosynthetic pathway branch branch leading to arbutin is not well known. In this work, putative genes involved in this pathway were identified by comparing the gene expression pattern in 'Kalco', 'André Desportes' and Zwintzscher's Hybrid.

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CHAPTER II

Plant materials

2 Plant materials

2.1 Plant materials

The plant material used in this thesis included putative apple/pear hybrids from three different sources: the first was the F1 Zwintzscher's Hybrid described in Figure 2.1.

Figure 2.1: Phenotype of Zwintzscher's Hybrid. A Flowers with intermediary habit, B vegetative habit, C leaves with small stipules, D shoot with rupturing bark, E fruit with intermediate form from which five putative F2 seeds were obtained and could be grown to fertile trees. Scale bars 2 cm (Fischer *et al.*, 2014).

This plant was also the open-pollinated parent of five putative F2 seedlings (Fischer *et al.*, 2014), which are being grown in the field at FEM. Ninety-five putative F1 hybrid trees grown in New

Zealand at the PFR sites at Hawke's Bay (HB) and Palmerston North (PN) resulted from crosses between apple and pear or *vice versa*. In addition, 29 trees, which were putative F2 hybrids from open pollination events from several of the putative New Zealand F1 trees, were included. Furthermore, six putative F1 pear-apple hybrids obtained and grown at the University of Bologna (UniBo, Italy) were available.

2.1.1 F1 Zwintzscher's Hybrid and F2 progeny

The progenitors of the Zwintzscher's Hybrid and putative F2 progeny are only partly known and only some of this plant material was available (Figure 2.2).

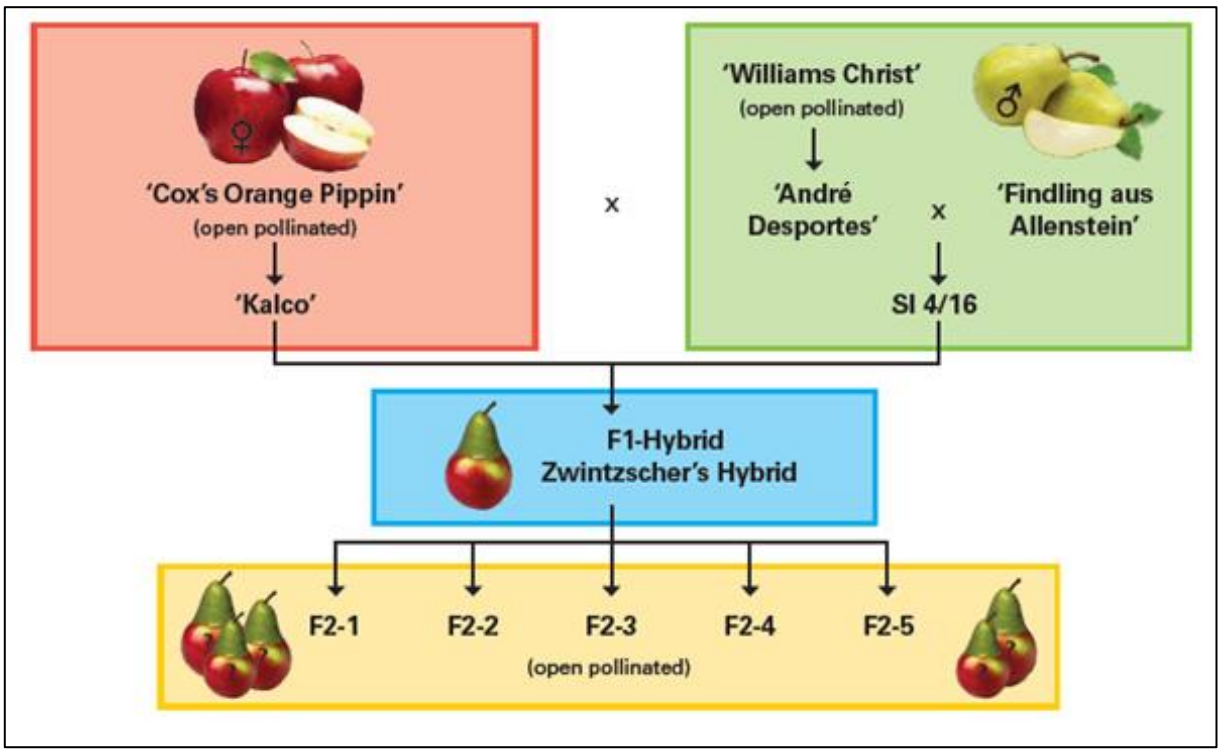

Figure 2.2: Modification of the Fischer *et al.* (2014) pedigree of Zwintzscher's Hybrid (blue) and F2 (yellow) from grandparental and parental apple (red) and pear (green) progenitors. All progenitors are known cultivars except SI 4/16, which was a breeding selection.

In the pedigree of the F1, open pollination occurred in the 1980s and therefore the cross is not known but is supposed to be *M.* x *domestica* 'Kalco' as the female parent and *P. communis* SI 4/16, a seedling of the former breeding programme at Cologne, as the male. Only one seedling survived and was grown to an adult stage. The female apple parent 'Kalco' is a selection from open pollination of 'Cox's Orange Pippin'. Within the male parental line, the grandparental cultivar 'André Desportes' arose as a selection from open-pollinated 'Williams Christ'. The male parent, breeding line SI 4/16 was selected from a cross between 'André Desportes' and 'Findling von Allenstein', like 'Williams Christ', a spontaneous seedling of unknown origin.

The characterised F1 hybrid, Zwintzscher's Hybrid, could only be cultivated by grafting it onto M9 rootstock and foliar fertilization with the main nutritious elements in critical vegetative periods in subsequent years. Three plants of Zwintzscher's Hybrid were grown in the experimental field at FEM (San Michele all'Adige, Italy) (Figure 2.3).

Figure 2.3: The three plants of Zwintzscher's Hybrid in the experimental field at FEM (San Michele all'Adige, Italy, 2020).

All trees flower regularly (Figure 2.1A), but only very rarely fruits are obtained by open pollination. Flowers and leaf shape of the F1 hybrid are intermediate between *Malus* and *Pyrus* (Figure 2.1A, B). Small stipules, which are common in pear, can be seen at leaf bases of the hybrid (Figure 2.1C). The bark of older shoots is ruptured (Figure 2.1D). The shape of the seldom occurring fruits is intermediate between that of apple and pear (Figure 2.1E). One of these fruits contained five viable seeds, which gave rise to fertile F2 plants (Fischer *et al.*, 2014).

During the years of the thesis, the three plants of Zwintzscher's Hybrid available for this study, surprisingly bore several fruits each season. The fruits were obtained spontaneously from open pollination (Figure 2.4). In 2019, only one mature seed was found, which was put into tissue culture. Unfortunately, the plantlet died during further *in vitro* culture. In 2020, one tree set six fruits, another zero and the third tree set eight fruits (Figure 2.5-2.6). In October 2020 these fruits were harvested. Of only the tree with six fruits, four contained mature seeds: one seed each in three fruits and one fruit with two seeds. These seeds were transferred into tissue culture and can be used for further characterisation in future studies.

Figure 2.4: The two Zwintzscher's Hybrid trees in the experimental field at FEM (San Michele all'Adige, Italy) bearing 6 (**A**) and 8 (**B**) fruits from open pollination.

Figure 2.5: Three fruits harvested in October 2020 from the open pollination of the Zwintzscher's Hybrid in the experimental field at FEM (San Michele all'Adige, Italy).

Figure 2.6: Four fruits harvested in October 2020 from the open pollination of the Zwintzscher's Hybrid in the experimental field at FEM (San Michele all'Adige, Italy)

The one seed harvested in 2019 and five seeds obtained in 2020 were sterilized in water with 10% bleach for 24 hours at room temperature on an orbital shaker (IKA® KS 501 Digital). After 24 hours the seed coats were removed and placed in a small jar (Karunairetnam, 2016) with a shoot propagation medium (Pessina *et al.*, 2016) (Figure 2.7). The plantlets were maintained in a growth chamber at 24 ± 1°C with a 16/8-h light/dark period (100 mmol/m2/s).

Figure 2.7: One of the five seedlings obtained from open pollination of the Zwintzscher's Hybrid.

2.1.2 New Zealand trees

The New Zealand samples were developed within the project "Development of apple/pear hybrids" - Discovery Science project PFR internal funding - Future Science Sector 1/10/2012 – 30/06/2015. The aim of this project was to provide a proof of concept of the possibility of generating novel apple-pear hybrids, which might be used either as rootstocks, or as parents in further crosses towards new variety development. The main outcomes were:

- True hybrids growing *in vitro* confirmed by SSR analyses;
- Hybrids showing linkage to the dwarfing ability of apple due to the presence of the *Dwarfing 1* (*Dw1*) allele;
- Development of further hybrids using a greater range of parents; it was noted that successful fertilization depends on the compatibility of the parents.

Sixty-one putative F1 trees grown at the PFR site in Hawke's Bay (HB) (Figure 2.8) and 34 F1 trees grown in Palmerston North (PN) resulted from crosses between apple and pear or *vice versa* (Table 2.1). In Table 2.2 the parentage of the parents of the putative F1 progeny are reported.

Figure 2.8: Sixty-one putative F1 trees grown in the experimental field at the PFR site at Hawke's Bay (New Zealand, April 2019).

There were also 29 trees of putative F2 hybrids from open pollination events from three of the New Zealand F1 trees located at the PFR site in HB (Figure 2.9). These were:

- Eight from FP18 3
- Eight from an IP26 1

- Thirteen from FP12 1

Seedlings (Table 2.3) were raised in tissue culture in 2017, 2018 and 2019 as described above after the fruits were stored in the fridge at 4°C for four months. This time, the seeds without seed coats were placed in petri dishes (Figure 2.10 B) and after ten days the resulting plantlets were transferred to plastic tubs. The petri dishes and the tubs contained apple medium (Table 2.4). When the plants were grown sufficiently with roots present, they were transplanted into pots with potting mix (Bark fibre 30%, CAN Fines 50% (www.daltons.co.nz) and pumice 7 mm 20%); we added fertiliser (per 600 L: Osmocote 8-9 month 3 kg, dolomite 1 kg, superphosphate 300 g, potassium sulphate 300 g, CAN 200 g (www.daltons.co.nz) and gypsum 400 g) and transferred them to the greenhouse (Figure 2.10 E), and later to the hardstand at PFR in HB.

For this study, we used only the 29 F2 plants from fruits harvested in 2018.

Table 2.1: Putative F1 hybrids from crosses between apple and pear or *vice versa* in New Zealand. The growth site is indicated as HB or PN for Hawke's Bay and Palmerston North, respectively

Hybrid	Female		Male	Location
CO 1	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 2	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 3	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 4	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 5	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 6	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 7	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 8	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 9	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 10	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 11	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 12	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 13	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 14	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 15	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 16	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 17	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 18	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 19	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 20	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 21	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 22	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 23	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 24	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 25	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 26	'Cox's Orange Pippin'	x	'Old Home'	HB

Hybrid	Female		Male	Location
IP12 1	'Imperial Gala'	x	P125R095T002	HB
IP12 2	'Imperial Gala'	x	P125R095T002	HB
FP18 1	'Fuji'	x	P186R125T002	HB
FP18 2	'Fuji'	x	P186R125T002	HB
FP18 3	'Fuji'	x	P186R125T002	HB
FP18 4 (PN)	'Fuji'	x	P186R125T002	PN
FP18 5 (PN)	'Fuji'	x	P186R125T002	PN
FP18 6 (PN)	'Fuji'	x	P186R125T002	PN
FP18 7 (PN)	'Fuji'	x	P186R125T002	PN
FP18 8 (PN)	'Fuji'	x	P186R125T002	PN
FP18 9 (PN)	'Fuji'	x	P186R125T002	PN
FP18 10 (PN)	'Fuji'	x	P186R125T002	PN
FP18 11 (PN)	'Fuji'	x	P186R125T002	PN
FP12 1	'Fuji'	x	P125R095T002	HB
FP12 2	'Fuji'	x	P125R095T002	HB
FP12 3	'Fuji'	x	P125R095T002	HB
FP12 4 (PN)	'Fuji'	x	P125R095T002	PN
FP12 5 (PN)	'Fuji'	x	P125R095T002	PN
FP12 6 (PN)	'Fuji'	x	P125R095T002	PN
FP12 7 (PN)	'Fuji'	x	P125R095T002	PN
FP26 1	'Fuji'	x	P266R231T015	HB
FP26 2	'Fuji'	x	P266R231T015	HB
FP26 3	'Fuji'	x	P266R231T015	HB
P26S	P265R232T018	x	'Scilate'	PN
FP35 1	'Fuji'	x	P354R200T138	HB
FP35 2	'Fuji'	x	P354R200T138	HB

CO 27	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 28	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 29	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 30	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 31	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 32	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 33	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 34	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 35	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 36	'Cox's Orange Pippin'	x	'Old Home'	HB
CO 37 (PN)	'Cox's Orange Pippin'	x	'Old Home'	PN
CO 38 (PN)	'Cox's Orange Pippin'	x	'Old Home'	PN
CO 39 (PN)	'Cox's Orange Pippin'	x	'Old Home'	PN
CO 40 (PN)	'Cox's Orange Pippin'	x	'Old Home'	PN
CO 41 (PN)	'Cox's Orange Pippin'	x	'Old Home'	PN
P26A17	P265R232T018	x	A174R01T204	HB
P26A19 1	P265R232T018	x	A199R45T055	HB
P26A19 2	P265R232T018	x	A199R45T055	HB
P26A19 3	P265R232T018	x	A199R45T055	HB
P26A19 4	P265R232T018	x	A199R45T055	HB
IP26 1	'Imperial Gala'	x	P266R231T015	HB
IP26 2	'Imperial Gala'	x	P266R231T015	HB

FP35 3	'Fuji'	x	P354R200T138	HB
FP35 4	'Fuji'	x	P354R200T138	HB
FP35 5	'Fuji'	x	P354R200T138	HB
FP35 6	'Fuji'	x	P354R200T138	HB
FP35 7	'Fuji'	x	P354R200T138	HB
FP35 8 (PN)	'Fuji'	x	P354R200T138	PN
FP35 9 (PN)	'Fuji'	x	P354R200T138	PN
FP35 10 (PN)	'Fuji'	x	P354R200T138	PN
FP35 11 (PN)	'Fuji'	x	P354R200T138	PN
FP35 12 (PN)	'Fuji'	x	P354R200T138	PN
FP35 13 (PN)	'Fuji'	x	P354R200T138	PN
FP35 14 (PN)	'Fuji'	x	P354R200T138	PN
FP35 15 (PN)	'Fuji'	x	P354R200T138	PN
FP35 16 (PN)	'Fuji'	x	P354R200T138	PN
FP35 17 (PN)	'Fuji'	x	P354R200T138	PN
FP35 18 (PN)	'Fuji'	x	P354R200T138	PN
FP35 19 (PN)	'Fuji'	x	P354R200T138	PN
FP35 20 (PN)	'Fuji'	x	P354R200T138	PN
FP35 21 (PN)	'Fuji'	x	P354R200T138	PN
FP35 22 (PN)	'Fuji'	x	P354R200T138	PN
FP35 23 (PN)	'Fuji'	x	P354R200T138	PN

Table 2.2: Pedigree of the parents of the putative F1 trees grown in New Zealand at the PFR sites at HB and at PN.

Accession	Female		Male
P265R232T018	P128R68T003	x	P202R136T118
P037R048T081	NJ 10	x	29-52
P266R231T015	P128R68T003	x	P202R136T118
P125R095T002	'Kosui'	x	'Torch Pear'
P186R125T002	'Pinguoli'	x	P124R074T045
P354R200T138	P202R136T118	x	OP
P128R68T003	'Snowflake'	x	'Max Red Bartlett'
P202R136T118	P125R95T002	x	P098R01T045
P124R074T045	'Nijisseiki'	x	'Torch Pear'
P098R01T045	'Nijisseiki'	x	'Max Red Bartlett'
A174R01T204	'Sciearly'	x	A047R08T037
A199R45T055	'Scired'	x	ABGF0348M018T068
A047R08T037	'Royal Gala'	x	'Fiesta'
'Royal Gala'	'Kidd's Orange Red'	x	'Golden Delicious'
'Kidd's Orange Red'	'Cox's Orange Pippin'	x	'Delicious'
'Fiesta'	'Cox's Orange Pippin'	x	'Idared'
'Idared'	'Jonathan'	x	'Wagener'
ABGF0348M018T068	'Sangrado'		open-pollinated

Figure 2.9: Two fruits from F1 trees grown in the experimental field at the PFR site at Hawke's Bay (New Zealand).

Table 2.3: F2 hybrids from open pollination of putative hybrids F1 from New Zealand. The growth site is Hawke's Bay (HB). Open pollination (OP).

Hybrid	Female		Male	Location
F2-IP26 1-1.3-OP	IP26 1	x	OP	HB
F2-IP26 1-1.4A-OP	IP26 1	x	OP	HB
F2-IP26 1-1.4B-OP	IP26 1	x	OP	HB
F2-IP26 1-1.5-OP	IP26 1	x	OP	HB
F2-IP26 1-2.1-OP	IP26 1	x	OP	HB
F2-IP26 1-2.3-OP	IP26 1	x	OP	HB
F2-IP26 1-1.1-OP	IP26 1	x	OP	HB
F2-IP26 1-1.2-OP	IP26 1	x	OP	HB
F2-IP26 2-1.2-OP	IP26 2	x	OP	HB
F2-IP26 2-1.4-OP	IP26 2	x	OP	HB
F2-IP26 2-2.5-OP	IP26 2	x	OP	HB
F2-IP26 2-3.2-OP	IP26 2	x	OP	HB
F2-IP26 2-3.4-OP	IP26 2	x	OP	HB
F2-IP26 2-3.7-OP	IP26 2	x	OP	HB
F2-IP26 2-1.1-OP	IP26 2	x	OP	HB
F2-IP26 2-3.6-OP	IP26 2	x	OP	HB
F2-FP12 1-1.2-OP	FP12 1	x	OP	HB
F2-FP12 1-1.3A-OP	FP12 1	x	OP	HB
F2-FP12 1-1.5A-OP	FP12 1	x	OP	HB
F2-FP12 1-1.6-OP	FP12 1	x	OP	HB
F2-FP12 1-1.7-OP	FP12 1	x	OP	HB
F2-FP12 1-1.8-OP	FP12 1	x	OP	HB
F2-FP12 1-3.3-OP	FP12 1	x	OP	HB
F2-FP12 1-3.4-OP	FP12 1	x	OP	HB
F2-FP12 1-3.7-OP	FP12 1	x	OP	HB
F2-FP12 1-3.8-OP	FP12 1	x	OP	HB
F2-FP12 1-3.5-OP	FP12 1	x	OP	HB
F2-FP12 1-1.11-OP	FP12 1	x	OP	HB
F2-FP12 1-3.1-OP	FP12 1	x	OP	HB

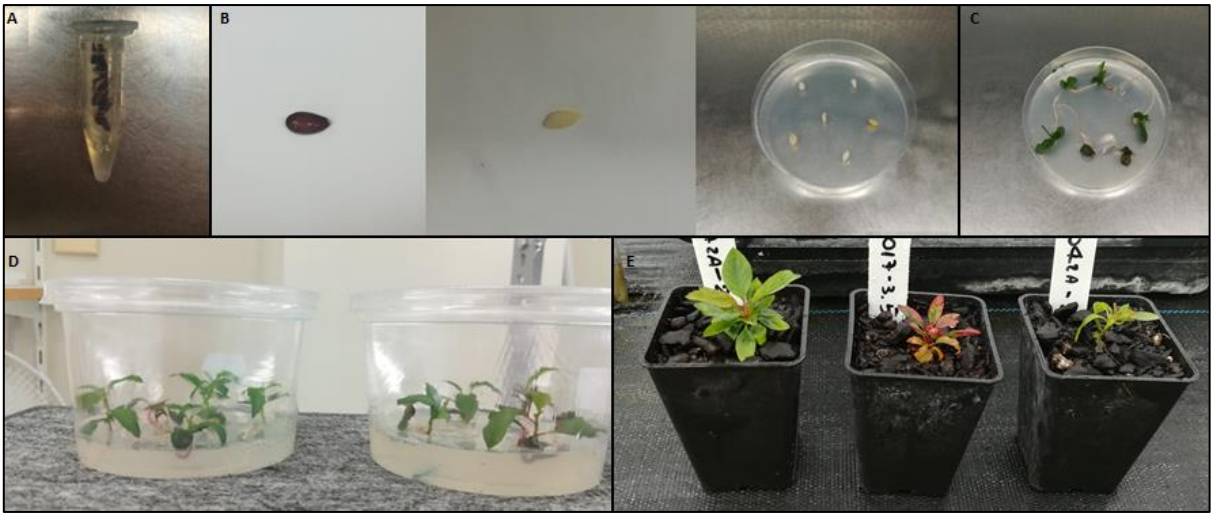

Figure 2.10: Steps of the plants *in vitro* at the PFR in HB. (A) Seeds sterilization, (B) seed before and after removal of seed coat, (C) after ten days, (D) after fifteen days the plants were transferred from petri dish to tubs, and (E) plants in the greenhouse.

Table 2.4: Ingredients for 1L of apple medium used for *in vitro* seedling raising at PFR, NZ

Ingredient	Litre Rate	Stock Concentration
MS Macro	25 mL	20x
MS Micro	5 mL	200x
Ferric Sodium EDTA	5 mL	200x
LS Vitamins	5 mL	200x
Sucrose	30 g	neat
Agar (Davis)	7.5 g	neat

2.1.3 Bologna samples

Three putative F1 hybrids were obtained from the controlled pollination of pear flowers with apple pollen at the UniBo. One hybrid was from the cross 'Abate' x 'Fuji' and two from the pollination of 'Decana' by 'Murray'. Several flowers were pollinated, but only three fruit with three fertile seeds in total were obtained.

These materials were propagated *in vitro*. Baby jars containing plantlets in a shoot propagation medium (Pessina *et al.*, 2016) were maintained in a growth chamber at 24 ± 1°C with a 16/8-h light/dark period (100 mmol/m^2/s). To promote rooting the *vitro* plants were transferred from the shoot propagation medium to a Murashige and Skoog medium supplemented with indole-3-butyric acid (Pessina *et al.*, 2016) and maintained in a growth chamber at 24 ± 1°C with a 16/8-h light/dark period (100 mmol/m^2/s) (Figure 2.11). Rooted plants were acclimatized in soil ('Terriccio Vegetal Radic' - TerComposti S.p.a., Brescia, Italy) by progressively reducing humidity for 3 weeks.

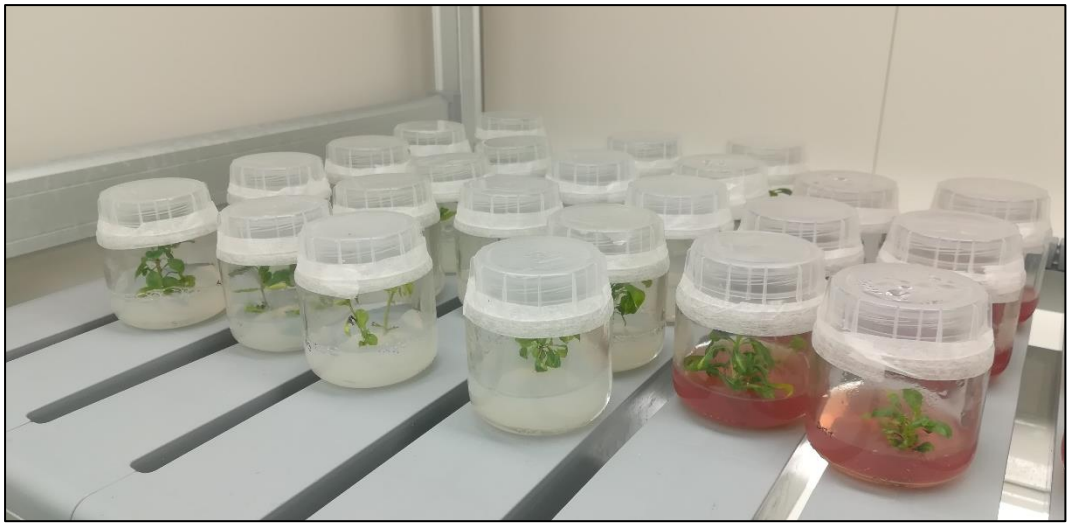

Figure 2.11: Baby jars containing pear-apple hybrids from UniBo in rooting (white) medium and propagation (red) media.

Well-acclimatized plants were maintained at greenhouse conditions (24 ± 1°C, 16/8-h light/dark period, relative humidity of 70% ± 5%) (Figure 2.12).

Figure 2.12: Replicated plants of an 'Abate' x 'Fuji' hybrid in the greenhouse.

2.2 References

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CHAPTER III

Molecular characterization of apple and pear hybrids

3 Molecular characterization of apple and pear hybrids

3.1 Abstract

The objective of this study was to genetically characterise putative hybrids between apple and pear. The plant material comprised three sources of apple-pear and pear-apple hybrid plants: Zwintscher's Hybrid (Fischer *et al.*, 2014) and its F2 progeny held at FEM; putative hybrids developed at PFR; and hybrids obtained at UniBo. Our objective was to determine which of the putative hybrids are true hybrids by characterising their genomes and determining their chromosomal structure. For the genome characterisation, we scanned the genomes with a set of SSRs, HRM markers, the 9K apple/pear and the 20K apple SNP arrays, and DNA content. In addition, the PFR hybrids were assessed for resistance to apple and pear diseases (see chapter IV). The findings showed that Zwintzscher's Hybrid and the 'Abate' x 'Fuji' hybrid developed at UniBO are true full hybrids with the parents having contributed equally to the hybrid genome. Furthermore, the results suggest that all the PFR F1 progeny are hybrids, too, but that the parents did not contribute equally to the hybrid genomes. The UniBO accessions 'Decana' x 'Murray' 1 and 'Decana' x 'Murray' 2 are not hybrids, but only have pear genomes. The hybrid material can be used for future crosses to introduce more traits of interest, such as eg flavour texture.

The study further confirmed that SSR and HRM markers, SNP arrays, and DNA content assessments can be used to identify apple/pear hybrids. Informative markers from this set can be used in breeding to develop novel crops by introducing desired traits from the pear gene pool into apple and *vice versa*.

Keywords: SSR, SNP, HRM, SNP-chip, DNA content

3.2 Introduction

In agriculture, one of the main objectives of plant breeding is to improve on existing cultivars by fixing their deficiencies in one or more traits and/or introducing novel traits by hybridizing them with lines that possess the desired traits.

Marker assisted selection (MAS) utilizing DNA markers associated with important agronomic traits is now often applied to improve existing cultivars of many crops, including apple (Bianco *et al.*, 2014; Chagné *et al.*, 2019; Heo *et al.*, 2019; Mansoor *et al.*, 2019; Peace *et al.*, 2019; Vanderzande *et al.*, 2019; Heo and Chung, 2020; Luo *et al.*, 2020; Muranty *et al.*, 2020; Cmejlova *et al.*, 2021) and pear (Verde *et al.*, 2012; Montanari *et al.*, 2013; Montanari *et al.*, 2020; Kumar *et al.*, 2017; Cao *et al.*, 2019; Han *et al.*, 2019; Kocsisné *et al.*, 2020; Ouni *et al.*, 2020; Weng, *et al.*, 2020; Zurn, *et al.*, 2020), rice (*Oryza sativa* L.) (Nogoy *et al.*, 2016), sweetpotato (*Ipomoea batatas* L.) (Monden and Tahara, 2017).

Genetic markers are broadly grouped into two categories: classical markers and DNA/molecular markers (Nadeem *et al.*, 2018). Morphological, cytological and biochemical markers are examples of classical markers. A molecular marker is a sequence of DNA, which is located within a known position on the chromosome (Kumar, 1999) adjacent to the controlling element of interest. Some examples of DNA markers are Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), SSRs, SNPs and Diversity Arrays Technology (DArT) markers (Jiang, 2013).

The main molecular marker techniques used currently include SNPs and SSRs, which are used in research projects involving genetic mapping and diversity analysis, as well as for MAS and cultivar identification in breeding programs. In any research project, the researcher is not limited to carrying out a single analysis of molecular markers, but is able to perform a combination of several of them (Kumar, 1999; Garrido-Cardenas *et al.*, 2018).

SSRs

SSRs are among the most widely used markers for cultivar identification and determination of hybridity because of their ease of use, high level of polymorphism, great discriminatory power, reproducibility and relatively low cost compared with some other molecular techniques (Larsen *et al.*, 2018). They utilize polymorphisms at DNA repeat sequence level, are stable and reproducible. SSRs are ubiquitous in the genome, not influenced by the environment and have Mendelian inheritance that is ideal for genetic studies. The number of microsatellite repetitions can vary from one individual to another and within the same individual in homologous chromosomes, revealing

multi-allelic bands with codominant inheritance. The repeats of tandem motifs can vary from 1 to 6, and these can be mono-nucleotides, di-nucleotides, tri-nucleotides or tetra-nucleotides (Silvanini *et al.*, 2011).

SSR markers also are very useful in plant breeding and genomic studies (Gianfranceschi *et al.*, 1998). They have been used to investigate genetic and evolutionary relationships within Rosaceae members, such as apple, pear and peach (Illa *et al.*, 2011), and in constructing consensus maps in plant species such as tea (*Camellia sinensis* L.) (Tan *et al.*, 2013), pea (*Pisum sativum* L.) (Guindon *et al.*, 2019) and pistachio (*Pistacia vera* L.) (Khodaeiaminjan *et al.*, 2018) as well as apple (Silfverberg-Dilworth *et al.*, 2006; N'diaye *et al.*, 2008). Using Bayesian analysis, it is possible to establish the membership of an anonymous sample of a certain variety by comparing the genetic profile of a sample variety/species with those known and already recorded, *e.g.* in a SSR database. These results were used in fingerprinting studies as a tool to characterize and conserve genetic collections (germplasm or core collections) that can be used in breeding programs (Testolin *et al.*, 2009; Zurn *et al.*, 2020). Finally, in a number of studies on individuals belonging to different animal and plant species, SSRs have been used to determine the population structure, without any prior information to assign individuals to different populations (Arnold and Schnitzler, 2020).

SNPs

SNPs have received increasing attention from researchers for their critical role in allele mining, genetic mapping and germplasm identification.

The HRM technique was developed for the detection of DNA sequence variants and it was applied first for genotyping (Wittwer *et al.*, 2003). Simplicity, low cost, ease of use, and a high sensitivity/specificity have been the most prominent features, making HRM single marker assays an attractive new tool for SNP genotyping and application in diagnostic laboratories (Vossen *et al.*, 2009). Over the last 20 years, SNP genotyping assays have generated large amounts of data that can be used to calculate the frequency of SNP alleles in different populations, to track the inheritance of those alleles across generations, and to associate SNP variation with phenotypic variation (Rafalski, 2002).

The hypothesis was that a more detailed SNP scan would be more effective in identifying smaller introgressions than the previous techniques. In 2013, an apple and pear Infinium® II 9K SNP array for large-scale genotyping, which could be used across several species, was developed (Montanari *et al.*, 2013). The 1,096 pear SNPs were combined with the set of 7,692 apple SNPs on the International RosBREED SNP consortium (IRSC) apple Infinium® II 8K array (Chagné *et al.*, 2012). The distinguishing feature of the apple and pear Infinium® II 9K SNP array is its combination of SNPs from both *Malus* and *Pyrus*, making it the first SNP array created across these

genera. It therefore enables, for the first time, the assessment of SNP marker transferability between genera (Montanari *et al.*, 2013).

In 2014 an Illumina Infinium® array targeting 20K apple SNPs was developed. The SNPs were predicted from re-sequencing data derived from the genomes of 13 *Malus* x *domestica* Borkh. cultivars and one accession belonging to a crab apple species (*M. micromalus*) (Bianco *et al.*, 2014).

Recently, an Applied Biosystems Axiom Pear 70K Genotyping Array was developed to reconstruct the largest pedigree of pear cultivars conserved in the USDA-ARS National *Pyrus* Collection (Montanari *et al.*, 2020).

In the present study, an initial bioinformatics analysis was performed for the detection of variants unique to the apple or pear genomes, before designing HRM primers positioned near both ends and the middle of all 17 chromosomes. In addition, in this study the 9K SNP array was used because it comprises both *Malus* and *Pyrus* SNPs, enabling the detection of intergeneric introgressions in apple x pear and reciprocal hybrids.

Nuclear DNA content and genome size

Nuclear DNA content is a specific feature and its assessment by flow cytometry can be helpful in differentiating taxa (Podwyszyńska, 2020). In many studies of crops, laser flow cytometry has demonstrated that nuclear DNA content is closely correlated with chromosome number (Bennett and Leitch, 2005; Zhang *et al.*, 2019). Apart from the utility of genome size data in ongoing molecular studies in the important Rosaceae plant family, blueberry (Sakhanokho *et al.*, 2018), maize (Santeramo *et al.*, 2020) and other plants, the amount and distribution of nuclear DNA content variation among related taxa may give insights into genomic evolution that underlies or parallels speciation (Dickson *et al.*, 1992).

Comparison of SSR, HRM and SNP analyses

SSR, HRM and SNP arrays have advantages and disadvantages when used to assess identity, pedigree and genetic diversity. Both SSRs and SNPs are abundant in the genome of most organisms and therefore potentially useful for detecting the population genetic structure and reconstructing the evolutionary history of species. SSRs are generally abundant and polymorphic in non-expressed genomic regions and consequently considered selectively neutral. The rapid mutation rates of SSRs may confound signals of population structure and divergence (Tsykun *et al.*, 2017; Montanari *et al.*, 2020; Zurn *et al.*, 2020).

In recent years, SNPs have started to replace SSRs in population genetic studies as well as in a wide range of other applications. SNP array screening of a large number of samples can be performed at a lower cost than SSR marker and HRM analysis. A limitation of SNP array analysis

is that they are very specific to the species, while the HRM analysis is cheaper than the SSR technology. SSR screening used in this study involved only 39 primer pairs, which is few compared with the SNP 20K and 9K arrays.

The objective of this part of the thesis was the genome screening of the putative hybrids with different combinations of molecular markers (SSRs and SNPs) using high-throughput technologies (HRM and SNP array) and nuclear DNA content analysis. The SSR analysis enabled the assessment of the genetic diversity among the crossing parents, the putative hybrid progeny and their offspring. The HRM-based 'apple/pear' primers at the middle and ends of each chromosome and the SNP-chip arrays enabled the identification of parts of their genomes that exhibited hybridity in the putative hybrids.

3.3 Materials and Methods

3.3.1 DNA extraction

For the SSR analysis of the FEM samples, DNA was extracted from leaves of the four available accessions in the pedigree of Zwintzscher's Hybrid (Chapter II), as well as Zwintzscher's Hybrid and the five putative F2 individuals using the NucleoSpin Plant II® Macherey Nagel kit. DNA quality and quantity were determined with a NanoDrop™ 8000 Spectrophotometer (Thermo Scientific™). For the PFR samples, DNA was extracted from milled freeze-dried leaves using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987) (C. Kirk, pers. comm.). The DNA quality and quantity were determined with a Qubit® 2.0 Fluorometer. For the UniBo samples, DNA was extracted from freeze-dried young leaf material using the DNeasy® Plant Mini Kit and the DNA quality and quantity were assessed with a NanoDrop™ 8000 Spectrophotometer (Thermo Scientific™).

For the HRM analysis of FEM and PFR leaf material, DNA was extracted from freeze-dried leaves samples using a modified CTAB method at PFR (C. Kirk, pers. comm.) as mentioned above. The leaves for each sample were placed in tubes with five beads (3 mm diameter) of stainless steel and kept in the freezer at -80°C for 24 h, after which the leaves were milled directly from frozen at 3.55 m/s for 20 s. Polyvinylpyrrolidone (PVP) and RNase A were added to an aliquot of 1.11x stock CTAB extraction buffer sufficient for the number of samples being processed. The buffer was warmed as required to aid the dissolving of PVP, then mixed well with a vortex mixer. An aliquot of 900 µL of buffer was added to each sample in a 2 mL o-ring tube and milled at 3.55 m/s

for 40 s. 100 µL 10% *n*-lauroyl sarcosine was added to each sample and proper mixing was achieved by inversion. The tubes were incubated at 65°C for 30 min and occasionally mixed during incubation. After this, 200 µL 24:1 chloroform: octanol was added to each tube and mixed, after which they were placed on ice for 5 min to cool. At the end of this incubation, the samples were centrifuged at 12,000 rpm for 8 min and the top layer (800-900 µL) was transferred to a new 2 mL tube. To each sample, 2/3 volume isopropanol (535-600 µL) was added to precipitate the gDNA. The samples were incubated for 5 min at room temperature, after which they were centrifuged at full speed for 10 min and the supernatant was discarded. The pellet was washed with 500 µL 76% ethanol with 10 mM ammonium acetate and centrifuged again at full speed for 1 min, after which the ethanol was decanted. The pellets were washed for a second time with 500 µL 70% ethanol, spun at full speed for 2 min, and the ethanol was decanted. The pelleted samples were dried in a Vacuum Concentrator (Labconco™ CentriVap DNA Vacuum Concentrator, Labconco Corporation, Kansas City, MO, USA) for 7 min and the pellets were resuspended in 100 µL TE buffer. The DNA quality and quantity were assessed with a Qubit® 2.0 Fluorometer (Invitrogen, Life Technologies Corporation). For the DNA extraction from leaves of the UniBo accessions the NucleoSpin Plant II® Macherey Nagel kit (Macherey-Nagel, Düren, Germany) was used. The DNA quality and quantity were determined with a NanoDrop™ 8000 Spectrophotometer (Thermo Scientific™).

The SNP array analysis used DNA extracted from freeze-dried young leaves using the DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany http://www.qiagen.com/). The DNA quality and quantity were assessed with a Qubit® 2.0 Fluorometer (Invitrogen, Life Technologies Corporation) for the New Zealand samples and with a NanoDrop™ 8000 Spectrophotometer (Thermo Scientific™) for the Italian samples.

3.3.2 SSR analysis

Forty published apple and pear SSR markers distributed over 15 of the 17 linkage groups (LGs) (Table 3.1) were selected to assess the degree of relationship among the PFR hybrids and their parents. PCR was performed in a 15.5 µL reaction volume comprising 2.5 µL of DNA, 8.85 µL of water, 1.5 µL of 10x reaction buffer, 0.45 µL of Mg^{2+} (50 mM), 1.5 µL of dNTPs (2 mM), 0.0195 µL of forward primer F (10 µM), 0.3 µL of reverse primer (10 µM), 0,3 µL of either FAM or Vic/Hex or NED or PET, and 0.1 µL of Taq DNA polymerase. PCR amplification was carried out using a modified version of the fluorescent M13 universal primer system and a touchdown PCR programme with annealing temperature 60–55°C (94°C/2 min 45 s; 10 cycles: 94°C/55 s, 60°C/55 s (−0.5°C per cycle); 72°C/1 min 30 s; 30 cycles: 94°C/55 s, 55°C/55 s, 72°C/1 min 30

s; 72°C/10 min). The fragments were separated using an ABI 3500 sequencer, and their size analysed with GeneMarker® v 2.2.0 software (© SoftGenetics, LLC.). Samples were multiplexed prior to analysis in a 124 µL reaction volume including 100 µL of water, 6 µL of PCR FAM amplification product, 6 µL of PCR Vic/Hex product, 6 µL of PCR NED product and 6 µL of PCR PET product held at 95ºC for 5 min. Amplification products were analyzed on an ABI 3500 Genetic Analyzer (Applied Biosystems) according to manufacturer's instructions and the raw data were processed using GeneMarker® v 2.2.0 software (© SoftGenetics, LLC.) to determine allele size in base pairs (bp).

Table 3.1: Details of the primers used for SSR analysis of the New Zealand samples. LG indicates linkage group and Tm the annealing temperature.

Marker	LG	Tm (°C)	Forward primer sequence	Reverse primer sequence	Genus	Reference
NH013a	1	57	GGTTTGAAGAGGAATGAGGAG	CATTGACTTTAGGGCACATTTC	*Pyrus*	(Yamamoto *et al.*, 2004)
CH02b10	2	57	CAAGGAAATCATCAAAGATTCAAG	CAAGTGGCTTCGGATAGTTG	*Malus*	(Liebhard *et al.*, 2002)
CH02c02a	2	59	CTTCAAGTTCAGCATCAAGACAA	TAGGGCACACTTGCTGGTC	*Malus*	(Liebhard *et al.*, 2002)
NH002b	2	58	GGAGTCAGCGGCAAAAAAAG	CCCACTCCCTCCTCTTATTGT	*Pyrus*	(Yamamoto *et al.*, 2002)
CH02b12	5	60	GGCAGGCTTTACGATTATGC	CCCACTAAAAGTTCACAGGC	*Malus*	(Liebhard *et al.*, 2002)
CH03a09	5	62	GCCAGGTGTGACTCCTTCTC	CTGCAGCTGCTGAAACTGG	*Malus*	(Liebhard *et al.*, 2002)
TsuENH086	5	61	CTCTGTTCTGCTTCGATTCTGCT	GTTTCTTGTCCACGTTCACCATTTTTCAGT	*Pyrus*	(Jean Marc Celton *et al.*, 2009)
CH04e05	7	58	AGGCTAACAGAAATGTGGTTTG	ATGGCTCCTATTGCCATCAT	*Malus*	(Liebhard *et al.*, 2002)
CH01h10	8	60	TGCAAAGATAGGTAGATATATGCCA	AGGAGGGATTGTTTGTGCAC	*Malus*	(Liebhard *et al.*, 2002)
NH029a	9	62	GAAGAAAACCAGAGCAGGGCA	CCTCCCGTCTCCCACCATATTAG	*Pyrus*	(Yamamoto *et al.*, 2002)
TsuENH008	9	63	CTGAGGTCTCATTCGGTGATTCT	GTTCTTCCTTCTCTGCTTTCTTCTTCACG	*Pyrus*	(Jean Marc Celton *et al.*, 2009; Emeriewen *et al.*, 2020)
NH045a	10	61	ATCGAGAGACGAGGGTAGCA	TCTCTTGGCGTCTTCCTCTC	*Pyrus*	(Jean Marc Celton *et al.*, 2009)
CH03d02	11	60	AAACTTTCACTTTCACCCACG	ACTACATTTTTAGATTTGTGCGTC	*Malus*	(Liebhard *et al.*, 2002)
NB105a	11	56	AAACAACCGACTGAGCAACATC	AAAATCTTAGCCCAAAATCTCC	*Pyrus*	(Jean Marc Celton *et al.*, 2009)
CH03c02	12	59	TCACTATTTACGGGATCAAGCA	GTGCAGAGTCTTTGACAAGGC	*Malus*	(Liebhard *et al.*, 2002)
CH05d04	12	59	ACTTGTGAGCCGTGAGAGGT	TCCGAAGGTATGCTTCGATT	*Malus*	(Liebhard *et al.*, 2002)
KA16	12	56	GCCAGCGAACTCAAATCT	AACGAGAACGACGAGCG	*Pyrus*	(Yamamoto *et al.*, 2002)
CH02g01	13	60	GATGACGTCGGCAGGTAAAG	CAACCAACAGCTCTGCAATC	*Malus*	(Liebhard *et al.*, 2002)

NH021a	13	58	ATCTCAATTTTCTCGGTAACCA	CTGATATCTCTCTGCACTCCCT	*Pyrus*	(Yamamoto *et al.*, 2002)
CH01g05	14	60	CATCAGTCTCTTGCACTGGAAA	GACAGAGTAAGCTAGGGCTAGGG	*Malus*	(Liebhard *et al.*, 2002)
CH03g06	14	54	ATCCCACAGCTTCTGTTTTTG	TCACAGAGAATCACAAGGTGGA	*Malus*	(Liebhard *et al.*, 2002)
NH004a	14	59	AGGATGGGACGAGTTTAGAG	CCACATCTCTCAACCTACCA	*Pyrus*	(Yamamoto *et al.*, 2002)
TsuENH058	14	61	AGAAGAAGGATAAGAAGAAGGATGG	GTTTCTTGTAACGAAAAGGAAACAGGACTTG	*Malus*	(J. M. Celton *et al.*, 2009)
CH02c09	15	60	TTATGTACCAACTTTGCTAACCTC	AGAAGCAGCAGAGGAGGATG	*Malus*	(Liebhard *et al.*, 2002)
CH02d11	15	60	AGCGTCCAGAGCAACAGC	AACAAAAGCAGATCCGTTGC	*Malus*	(Liebhard *et al.*, 2002)
NH027a	15	56	TAATGTGTTGGGGAGAGAGAG	GCTCTTGTTCCTTGCTCCTAA	*Pyrus*	(Yamamoto *et al.*, 2002)
CH05a04	16	57	GAAGCGAATTTTGCACGAAT	GCTTTTGTTTCATTGAATCCCC	*Malus*	(Liebhard *et al.*, 2002)
CH01b12	17	59	CGCATGCTGACATGTTGAAT	CGGTGAGCCCTCTTATGTGA	*Malus*	(Liebhard *et al.*, 2002)
CH04c10	17	56	GGGTTAGGTTGTCTTCTCTCCT	GCTTCTCGGGTGAGTTTTTC	*Malus*	(Liebhard *et al.*, 2002)
Ch-Vf1	1, 10	58	ATCACCACCAGCAGCAAAG	CATACAAATCAAAGCACAACCC	*Malus*	(Vinatzer *et al.*, 2004)
CH04c06	10, 17	60	GCTGCTGCTGCTTCTAGGTT	GCTTGGAAAAGGTCACTTGC	*Malus*	(Liebhard *et al.*, 2002)
TsuENH004	4, 12	59	CGCATTAAAGTCTGGCTTTCTTC	GAATTGGCAGAGAGATTGAGTGG	*Malus*	(J. M. Celton *et al.*, 2009)
CH05a02	8, 15	60	GTTGCAAGAGTTGCATGTTAGC	TTTTGACCCCATAAAACCCAC	*Malus*	(Liebhard *et al.*, 2002)
CH01h02	9, 17	62	AGAGCTTCGAGCTTCGTTTG	ATCTTTTGGTGCTCCCACAC	*Malus*	(Liebhard *et al.*, 2002)

To assess the genetic relationship among the five F2 progeny from open-pollinated Zwintzscher's Hybrid, this accession and its grandparents, the F1 progeny of 'Abate' x 'Fuji' and its parents, the two F1 hybrids of the cross 'Decana' x 'Murray' and their parents, 30 published apple SSR markers mapping to 16 of the 17 LGs (Table 3.2) were selected. PCR was performed in a 15 µL reaction volume including 30 ng of DNA, 0.5 units of GoldTaq DNA polymerase (Applied Biosystems, Foster City, CA, USA), 1.5 µL reaction buffer, 2 mM of $MgCl_2$ and supplemented with 0.2 mM dNTPs and 0.2 µM of forward and reverse primer mix. The thermal profile consisted of 4 min at 94ºC; ten cycles of 30 s at 94ºC, 45 s at 5ºC above the specific primers' annealing temperature, decreasing by 0.5ºC at each cycle and 60 s at 72ºC, followed by 25 cycles of 30 s at 94ºC, 45 s at their specific annealing temperature and 60 s at 72ºC, and a final extension of 7 min at 72ºC. Amplification products were analyzed on an ABI Prism 3130xl Genetic Analyzer sequencer (Applied Biosystems) according to manufacturer's instructions. The raw data were processed using GeneMapper 4.0 software (Applied Biosystems) to determine allele size in bp and a data matrix with allele sizes for each locus was created.

This matrix was used to calculate genetic diversity parameters to determine the molecular markers informative value and discrimination power for each population. The 'discrimination power at each locus for parent and progeny' (PD), which provides an estimate of the probability that two randomly sampled accessions of the study would be differentiated by their allelic profiles (Kloosterman *et al.*, 1993), was calculated as follows: PD=1-PI (probability of identity (PI)) was calculated using GenAlex v. 6.51b2 software (Peakall and Smouse, 2012). Finally, the SSR data were analysed using GeneMarker (GeneMarker® by SoftGenetics) and the GenAlex v. 6.51b2 (Peakall and Smouse, 2012) software to assess genetic relationships among the hybrids of a progeny.

A Neighbour joining clustering was performed for all populations using the Dice's and Euclidean's index. The Neighbour joining clustering is a method for hierarchical cluster analysis. These clusters were carried out using the PAST v. 4.03 software (Hammer *et al.*, 2001).

Table 3.2: Details of the primers used for apple SSR marker analysis of the FEM and Bologna populations. LG indicates the linkage group and Tm the annealing temperature.

Marker	LG	Tm °C	Forward primer sequence	Reverse primer sequence	Reference
Hi21g05	1	59	GACGAGCTCAAGAAGCGAAC	GTTTGCTCTTGCCATTTTCTTTCG	(Silfverberg-Dilworth *et al.*, 2006)
CH02f06	2	59	CCCTCTTCAGACCTGCATATG	ACTGTTTCCAAGCGATCAGG	(Liebhard *et al.*, 2002)
CH02c02a	2	57	CAAGGAAATCATCAAAGATTCAAG	CAAGTGGCTTCGGATAGTTG	(Liebhard *et al.*, 2002)
Ch03g07	3	57	AATAAGCATTCAAAGCAATCCG	TTTTTCCAAATCGAGTTTCGTT	(Liebhard *et al.*, 2002)
MS14h03	3	55	CGCTCACCTCGTAGACGT	ATGCAATGGCTAAGCATA	(Liebhard *et al.*, 2002)
CH02h11a	4	57	CGTGGCATGCTTATCATTTG	CTGTTTGAACCGCTTCCTTC	(Liebhard *et al.*, 2002)
NZ05g08	4	59	CGGCCATCGATTATCTTACTCTT	GGATCAATGCACTGAAATAAACG	(Guilford *et al.*, 1997)
CH02a08z	5	55	GAGGAGCTGAAGCAGCAGAG	ATGCCAACAAAAGCATAGCC	(Liebhard *et al.*, 2002)
Hi09B04	5	59	GCGATGACCAATCTCTGAAAC	TGGGCTTGAATTGGTGAATC	(Silfverberg-Dilworth *et al.*, 2006)
CH05a05	6	55	TGTATCAGTGGTTTGCATGAAC	GCAACTCCCAACTCTTCTTTCT	(Liebhard *et al.*, 2002)
CH01c06	8	59	AAACTTTCACTTTCACCCACG	ACTACATTTTTAGATTTGTGCGTC	(Liebhard *et al.*, 2002)
Hi04b12	8	59	CCCAAACTCCCAACAAAGC	GTTTGAGCAGAGGTTGCTGTTGC	(Silfverberg-Dilworth *et al.*, 2006)
CH01h02	9	57	AAACTTTCACTTTCACCCACG	ACTACATTTTTAGATTTGTGCGTC	(Liebhard *et al.*, 2002)
CH05c07	9	57	TGATGCATTAGGGCTTGTACTT	GGGATGCATTGCTAAATAGGAT	(Liebhard *et al.*, 2002)
Hi22f04	10	59	TCAATCCTCTGCTCTTCAAGG	GTTTAATCACCTGCTGCTGCTTG	(Silfverberg-Dilworth *et al.*, 2006)

MS06g03	10	59	CGGAGGGTGTGCTGCCGAAG	GCCCAGCCCATATCTGCT	(Liebhard *et al.*, 2002)
CH04h02	11	59	GGAAGCTGCATGATGAGACC	CTCAAGGATTTCATGCCCAC	(Liebhard *et al.*, 2002)
CH05d11	11	57	CACAACCTGATATCCGGGAC	GAGAAGGTCGTACATTCCTCAA	(Liebhard *et al.*, 2002)
CH03c02	12	55	TCACTATTTACGGGATCAAGCA	GTGCAGAGTCTTTGACAAGGC	(Liebhard *et al.*, 2002)
CH03a08	13	57	AAACTTTCACTTTCACCCACG	ACTACATTTTTAGATTTGTGCGTC	(Liebhard *et al.*, 2002)
CH03b10	13	57	CCCTCCAAAATATCTCCTCCTC	CGTTGTCCTGCTCATCATACTC	(Liebhard *et al.*, 2002)
AJ000761	14	55	CTGGGTGGATGCTTTGACTT	TCAATGACATTAATTCAACTTACAAAA	(Silfverberg-Dilworth *et al.*, 2006)
CH03d08	14	55	CATCAGTCTCTTGCACTGGAAA	TAGGGCTAGGGAGAGATGATGA	(Liebhard *et al.*, 2002)
CH04c07	14	59	GGCCTTCCATGTCTCAGAAG	CCTCATGCCCTCCACTAACA	(Liebhard *et al.*, 2002)
CH02d11	15	55	AGCGTCCAGAGCAACAGC	AACAAAAGCAGATCCGTTGC	(Liebhard *et al.*, 2002)
NZ02b1	15	59	AAGAGGGTGTTCCCAGATCC	TGTTCGATGTGACTTCAATGC	(Guilford *et al.*, 1997)
CH05a04	16	59	GAAGCGAATTTTGCACGAAT	GCTTTTGTTTCATTGAATCCCC	(Liebhard *et al.*, 2002)
CH05c06	16	57	ATTGGAACTCTCCGTATTGTGC	ATCAACAGTAGTGGTAGCCGGT	(Liebhard *et al.*, 2002)
CH01h01	17	55	GAAAGACTTGCAGTGGGAGC	GGAGTGGGTTTGAGAAGGTT	(Liebhard *et al.*, 2002)
CH05g03	17	59	GCTTTGAATGGATACAGGAACC	CCTGTCTCATGGCATTGTTG	(Liebhard *et al.*, 2002)

3.3.3 HRM analysis of SNPs

For the HRM analysis, re-sequenced reads of 34 apple accessions (including two apple parents, 'Cox's Orange Pippin' and 'Fuji', used in the crosses) were first aligned to the double haploid 'Bartlett' pear genome (Linsmith *et al.*, 2019) with Bowtie2 (Langmead and Salzberg, 2012). With the ".sam" output files being very large at several Gigabytes, they were converted to ".bam" file format using SAM tool software (https://jupyter.org/), which in turn were converted to variant calling format (VCF) (Danecek *et al.*, 2011) to search for the gene sequence variations with bcftools software (https://jupyter.org/). All data of the apple accessions were merged into one, so that it was possible to search for "apple" *vs* "pear". Primer 3 software (ver. 4.1.0, https://primer3.ut.ee/) was used to design the primers around identified SNPs (Table 3.3) to obtain amplicons of 50-120 bp for the HRM analysis of genetic variations in the amplicons at each point on the genome represented by an HRM marker. The pear genome was used to design these primers. The amplicons were as short as possible to reduce the possibility of including more than one SNP in the amplicon. The chromosome positions targeted for SNP primer development were at the beginning, middle and at the end of each chromosome. As the apple-pear hybrids are heterozygotes, their amplicons should have lower melting points than the apple or pear homozygotes.

To determine the efficiency of each designed primer set, a LightCycler® 480 System (Roche Life Science) was used to screen each pair in two columns in a 96-well plate over DNA samples from: 'Cox's Orange Pippin', 'Old Home', a mixture between of these two accessions, five putative hybrids, 'Kalco', 'Williams Christ', Zwintzscher's Hybrid and one of its F2s from open-pollination, and two mixtures of DNA from 'Cox's Orange Pippin' and 'Williams Christ'.

HRM analyses with the primers that passed this pre-screen were conducted in 96- or 384-well plates using a LightCycler® 480 System (Roche Life Science) for the New Zealand and FEM samples. The total volume of 10 µL contained 2.5 µL of 1 ng genomic DNA, 0.2 µL of each 10 µM primers, 1 µL of 25 mM $MgCl_2$, 5 µL of 2x HRM master mix and 1.1 µL of water. The conditions for the HRM reactions were as follows: initial denaturation at 98°C for 5 min, which was followed by 45 cycles of amplification (95°C for 10 s, 55°C for 30 s, and 72°C for 15 s) and then the melting curve (95°C for 1 min, 40°C for 1 min, and 65°C for 1 min heating from 65°C to 95°C at 0.02°C/sec and cooling to 40°C). Data evaluation was carried out using LightCycler® 480 Software release 1.5.0 SP4, version 1.5.0.39 (Roche Life Science). After normalization and temperature shift determination, the different melting curves of the several plots were generated.

For the Bologna samples, HRM analysis was done in a 96-well plate using a Bio-Rad CFX Real-Time PCR Thermocycler (Bio-Rad). The total volume of 10 µL contained 2 µL of genomic DNA,

0.4 µL of each primer, 5 µL of the reaction mixture with EvaGreen dye (SSoFast Supermix; Bio-Rad) and 2.2 µL of water. The conditions for the HRM reactions were as follows: initial denaturation at 98°C for 2 min, which was followed by 45 cycles of amplification (95°C for 5 s, 55°C for 10 s, and 72°C for 1 min) and at the end of the amplification, one additional cycle was performed, starting with 70°C for 1 s, with the temperature subsequently increased to 95°C in 0.2°C for 10 s increments (HRM analysis). Data evaluation was carried out using the Bio-Rad CFX Manager Software version 3.1 and Bio-Rad Precision Melt Analysis Software (Bio-Rad). After normalization and temperature shift determination, the different melting curves of the plots were generated. Three biological replicates were performed for each HRM assay.

A binary matrix was created considering 1-1 when the sample was homozygous like the mother, 2-2 when the sample was homozygous like the father, and 1-2 when it was heterozygous. This matrix was used for the subsequent statistical analysis.

As previously described for SSRs, for each locus present in each population the number of different alleles (Na), number of effective alleles (Ne), Shannon's information index (I), observed heterozygosity (Ho) and expected heterozygosity (He) were estimated using GenAlex v. 6.51b2 software (Peakall and Smouse, 2012). As above, PD was calculated.

A Neighbour-joining clustering using the Dice's and Euclidean's index was performed for all populations using the software PAST software v. 4.03 (Hammer *et al.*, 2001).

Table 3.3: Details of primers used for HRM analysis.

Name	Genome location	SNP	Forward primer sequence	Reverse primer sequence	Expected amplicon size (bp)	Melting temperature (ºC)
1	Chr1: 4650907-4651009	4650907	GTCAATGCAACCTATCGCCA	TTAGTCACTGTGGGAAACGC	103	55
2	Chr1: 38997511-38997627	38997511	CTCTAGCATGCAAAGGAACCC	TGGCACCATACACATACAATGA	96	55
3	Chr2: 306135-306226	306135	GTGTTACTCCCTACATTGCCA	GTTTGACTTCACACACGGGA	92	55
4	Chr2: 14077723-14077841	14077723	TCAGGTCTTGACAGGAAGGT	TCTTTGTCCCTTACCCTAGAGT	119	55
5	Chr2: 22240468-22240585	22240468	TTGTTTCTTCGGCCATGGTG	CCGTCGAATTTGAAGCCCTT	118	55
6	Chr3: 3468716-3468771	3468716	TGGTGTCAGCAATAACATACCG	TTCACAGCACATCCTTCTGC	56	55
7	Chr3: 6077512-6077630	6077512	TGGCCACGGCACCTTATG	GGATTCGGTTTCTTCTTCTCTCAC	119	55
8	Chr4: 10571099-10571175	10571099	GGGATGCAAAGTGACAATGG	TTCGTCAACACTTTGCATCTG	77	55
9	Chr4: 27271699-27271789	27271699	TCACCAATCCACTCCTGTCA	GGCAAGAAATGAGGCACACA	91	55
10	Chr5: 4426301-4426418	4426301	AAATCTCTGCCAGCAAAGAGG	GCTTGGCCAACTATCCCAAC	118	55
11	Chr5: 8856650-8856736	8856650	ACAGCTCGAATCGATGTTCTG	GAGATCGAGAGCGTTGAAGC	87	55
12	Chr5: 23625942-23625997	23625942	CGGAAGACTTCACACAAGCC	CATGCCTATGGATCCAAAAGC	56	55
13	Chr6: 1400544-1400660	1400544	CTGCTTCTCTGTACGTCCTTC	GGAAGACGTATATTCACTTGAAGC	117	55
14	Chr6: 17485718-17485793	17485718	CACTCTAGCCCAACGAACCT	TCTCGAGGTGTGGATGTAGC	76	55
15	Chr6:23002193-23002247	23002193	TTAGAAGGATCGCCAGCAAC	TGAGCTCAAGGTATCTCCACC	55	55
16	Chr7: 3410996-3411051	3410996	ACATTGTGATCAGGGTCTTCC	ACCACCAAGAACCTCAGCAT	56	55
17	Chr7: 20289452-20289564	20289452	CATTCTGCAGTCACACCAAGA	TGCGGTTGATACAGTTCATCA	113	55
18	Chr8: 344642-344754	344642	ACTCTCATTCCACTCCACCA	TGTGTCCCAAATGCATACGT	113	55
19	Chr8: 16391169-16391286	16391169	GTTGAGCCATGATCTGCCTG	TGGAAGGCCATGCTTGAATG	118	55

20	Chr9: 2196631-2196718	2196631	CAGCAGAATCGTCAAGAGCC	GATGTTCCAGGTTGAGCTGT	88	55
21	Chr9: 5441428-5441547	5441428	GGGCCTGTACCAAACAAATTG	TGGTTGATGCTTGAAGAATGTTC	120	55
22	Chr10: 2643959-2644012	2643959	TCTCTTGCTTGGTTCATGTCG	TTGACCAATGGTGACGAAGC	54	55
23	Chr10: 10168201-10168317	10168201	CATCAAGACCAAGCTTCTCGG	TCAGGCAGGCTACTAAAGAGG	117	55
24	Chr11: 463545-463660	463545	GTCAGCCGGCTCTTTATTCG	TGGTTCAAGCAGAGCAGC	116	55
25	Chr11: 23428118-23428212	23428118	GCCGACCTATCAACCCAGTT	AGCTCTCGTAATCAGTGGCT	95	55
26	Chr12: 925714-925782	925714	CACGACATTGACAAGGTGGA	AGGGGAACTATGAACAGCCA	69	55
27	Chr12: 6731573-6731673	6731573	TCCTGCATCGTAAACATAGGTTC	TGCCGGCTTTCTGTTGATTC	101	55
28	Chr12: 20068163-20068254	20068163	CCTTTACAAACGCTGACCTGT	AGCAGCAGAGTTCTCAGGTT	92	55
19	Chr13: 8624037-8624123	8624037	GATATATTTCACCACCTCATGCAG	TCCGTGGAAAGTAAATATTGTAGC	87	55
30	Chr13: 26326450-26326524	26326450	CATCCGTTAAATGGTGGGACA	ACAGAGACAACCCTTCCACA	75	55
31	Chr14: 2857003-2857097	2857003	TGGGCGTCAAACTTCAATGA	ACTATGCCATGGTTTTACTCGT	95	55
32	Chr14: 19912302-19912416	19912302	AGGCTAAACTCATAAACGTGTGG	CCACAAACCTCAGGCCATTC	115	55
33	Chr14:21391636-21391742	21391636	CATGAATATGCTCTTTCTCTGATGG	CAGTGAAACAGCTGAGGGTG	107	55
34	Chr15: 326327-326425	326327	AGCTGCAAATTGTCACAAGC	GAAACACTGAAGAGGCGAGG	99	55
35	Chr15: 773670-773764	773670	CCATACCTATGCTCACGCTC	ATGGCTCCATTTCTGTCCTG	95	55
36	Chr15:25892571-25892671	25892571	ACAATGGTGCTGACTGTGTT	TCCACAACTGCGAGACAATC	101	55
37	Chr16: 823477-823583	823477	TCGAACGAGGACTGGGATG	AGGCATGTCTATCAAGCGTG	107	55
38	Chr16: 17140174-17140230	17140174	TGAACTGTTGAACGAGGAGTC	AAAGCTGCATCTGTCGGAAG	57	55
39	Chr16: 29098000-29098108	29098000	ATCTGGAGTAGCCCACGAAC	GTAAGTTCATAGTGAGGGCTGAC	109	55

3.3.4 SNP array analysis

DNA extracted from plant samples (Table 3.4) as described above was amplified and hybridized to the apple 20K SNP array (for the Bologna materials) and the apple and pear Infinium® II 9K SNP array (for the New Zealand and FEM samples) following the Infinium® HD Assay Ultra protocol, and scanned with the Illumina HiScan (Illumina Inc., San Diego, USA). Some materials from New Zealand used in the SSR and HRM analyses described above were not available for this analysis.

Table 3.4: The accessions that were used for SNP array analysis.

Accession	Female		Male
CO 2	'Cox's Orange Pippin'	x	'Old Home'
CO 3	'Cox's Orange Pippin'	x	'Old Home'
CO 4	'Cox's Orange Pippin'	x	'Old Home'
CO 5	'Cox's Orange Pippin'	x	'Old Home'
CO 6	'Cox's Orange Pippin'	x	'Old Home'
CO 7	'Cox's Orange Pippin'	x	'Old Home'
CO 9	'Cox's Orange Pippin'	x	'Old Home'
CO 10	'Cox's Orange Pippin'	x	'Old Home'
CO 11	'Cox's Orange Pippin'	x	'Old Home'
CO 12	'Cox's Orange Pippin'	x	'Old Home'
CO 13	'Cox's Orange Pippin'	x	'Old Home'
CO 14	'Cox's Orange Pippin'	x	'Old Home'
CO 15	'Cox's Orange Pippin'	x	'Old Home'
CO 16	'Cox's Orange Pippin'	x	'Old Home'
CO 17	'Cox's Orange Pippin'	x	'Old Home'
CO 19	'Cox's Orange Pippin'	x	'Old Home'
CO 20	'Cox's Orange Pippin'	x	'Old Home'
CO 22	'Cox's Orange Pippin'	x	'Old Home'
CO 23	'Cox's Orange Pippin'	x	'Old Home'
CO 24	'Cox's Orange Pippin'	x	'Old Home'
CO 25	'Cox's Orange Pippin'	x	'Old Home'
CO 26	'Cox's Orange Pippin'	x	'Old Home'
CO 27	'Cox's Orange Pippin'	x	'Old Home'
CO 29	'Cox's Orange Pippin'	x	'Old Home'
CO 31	'Cox's Orange Pippin'	x	'Old Home'
CO 33	'Cox's Orange Pippin'	x	'Old Home'
CO 34	'Cox's Orange Pippin'	x	'Old Home'
CO 35	'Cox's Orange Pippin'	x	'Old Home'

Accession	Female		Male
FP18 6 (PN)	'Fuji'	x	P186R125T002
FP18 10 (PN)	'Fuji'	x	P186R125T002
FP18 11 (PN)	'Fuji'	x	P186R125T002
FP12 1	'Fuji'	x	P186R125T002
FP12 2	'Fuji'	x	P186R125T002
FP12 3	'Fuji'	x	P186R125T002
FP12 4 (PN)	'Fuji'	x	P186R125T002
FP12 6 (PN)	'Fuji'	x	P186R125T002
FP12 7 (PN)	'Fuji'	x	P186R125T002
FP26 1	'Fuji'	x	P266R231T015
FP26 2	'Fuji'	x	P266R231T015
FP26 3	'Fuji'	x	P266R231T015
P354R200T138			
FP35 1	'Fuji'	x	P354R200T138
FP35 2	'Fuji'	x	P354R200T138
FP35 3	'Fuji'	x	P354R200T138
FP35 4	'Fuji'	x	P354R200T138
FP35 5	'Fuji'	x	P354R200T138
FP35 6	'Fuji'	x	P354R200T138
FP35 7	'Fuji'	x	P354R200T138
FP35 8 (PN)	'Fuji'	x	P354R200T138
FP35 9 (PN)	'Fuji'	x	P354R200T138
FP35 10 (PN)	'Fuji'	x	P354R200T138
FP35 12 (PN)	'Fuji'	x	P354R200T138
FP35 13 (PN)	'Fuji'	x	P354R200T138
FP35 14 (PN)	'Fuji'	x	P354R200T138
FP35 15 (PN)	'Fuji'	x	P354R200T138
FP35 17 (PN)	'Fuji'	x	P354R200T138

CO 37 (PN)	'Cox's Orange Pippin'	x	'Old Home'
CO 40 (PN)	'Cox's Orange Pippin'	x	'Old Home'
'Cox's Orange Pippin'			
'Old Home'			
A199R45T055			
P26A19 3	P265R232T018	x	A199R45T055
P26A19 4	P265R232T019	x	A199R45T056
'Imperial Gala'			
P266R231T015			
IP26 1	'Imperial Gala'	x	P266R231T015
IP26 2	'Imperial Gala'	x	P266R231T015
P125R095T002			
IP12 1	'Imperial Gala'	x	P125R095T002
IP12 2	'Imperial Gala'	x	P125R095T002
'Fuji'			
P186R125T002			
FP18 1	'Fuji'	x	P186R125T002
FP18 2	'Fuji'	x	P186R125T002
FP18 3	'Fuji'	x	P186R125T002
FP18 4 (PN)	'Fuji'	x	P186R125T002

FP35 18 (PN)	'Fuji'	x	P354R200T138
FP35 19 (PN)	'Fuji'	x	P354R200T138
FP35 20 (PN)	'Fuji'	x	P354R200T138
FP35 22 (PN)	'Fuji'	x	P354R200T138
'Kalco'			
'Williams Christ'			
'André Desportes'			
Zwintzscher's Hybrid			
F2-1	Zwintzscher's Hybrid	x	OP
F2-2	Zwintzscher's Hybrid	x	OP
F2-3	Zwintzscher's Hybrid	x	OP
F2-4	Zwintzscher's Hybrid	x	OP
F2-5	Zwintzscher's Hybrid	x	OP
'Abata'			
'Decana'			
'Murray'			
AF	'Abata'	x	'Fuji'
DM1	'Decana'	x	'Murray'
DM1	'Decana'	x	'Murray'

Data were analysed using Illumina's GenomeStudio v 1.0 software Genotyping Module, setting a GenCall threshold of 0.15. The software automatically determines the cluster positions of the AA/AB/BB genotypes for each SNP and displays them in normalized graphs (Figure 3.1). A systematic method was used to evaluate and filter the SNP array data employing quality metrics from GenomeStudio (Illumina): GenTrain score $\geq$0.60, null alleles and null allele results for the parents.

The filtered SNPs from the 9K and 20K SNP arrays were allocated to LGs of apple and pear for each population. The mapping of data for each sample was performed using R Statistics Package version 3.5.1 (R. Core Team, 2019). The R script used to create the graphs is reported in supplementary text.

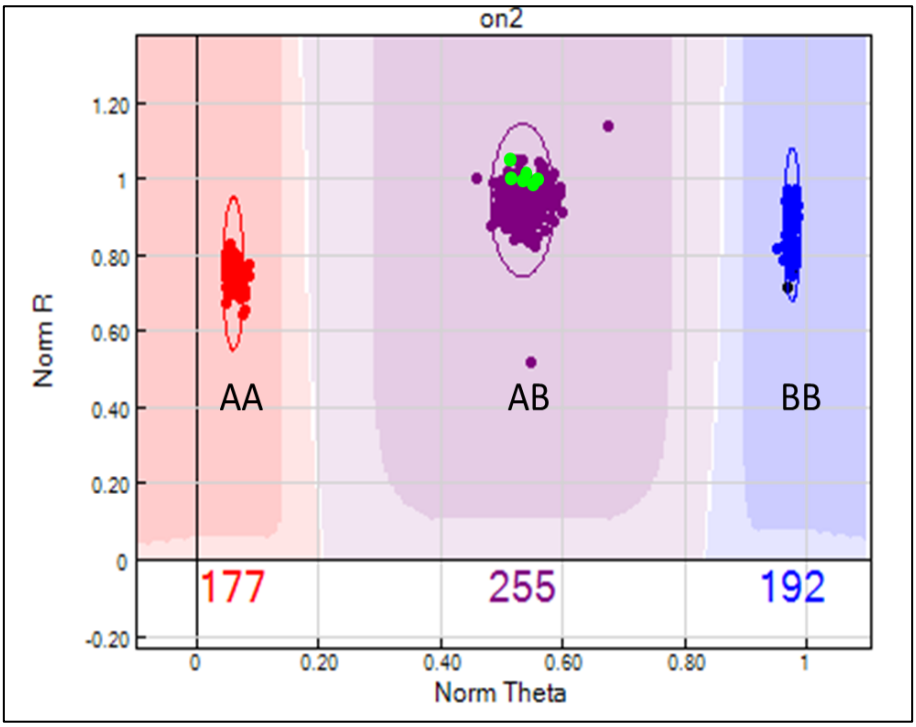

Figure 3.1: Example of normalized graphs of the cluster positions of the AA/AB/BB genotypes for each SNP generated with the GenomeStudio v 1.0 software. The cluster AA indicates the mother, BB the father, and AB the progeny (in green).

The SNP 20K array data were analysed by chromosome using the GenAlex Software (Peakall and Smouse, 2012) to assess genetic relationships among progeny and parents.

Before performing the mapping for the 20K SNP array data, we Blasted the SNP array sequences (35 nt upstream and downstream) using the double haploid apple genome (GDDH13) as a reference (Daccord *et al.*, 2017), then the best hit with identity >95% and query coverage (aligned part of the probe) >90% was selected for each SNP, and finally all SNPs with more than one best hit or no hit satisfying the parameters of step 2 were assigned position 0 on chromosome 0; all

other SNPs were uniquely positioned on the assembly. Chromosome 0 contains all the scaffolds that could not be placed on any of the chromosomes or organelles during assembly.
Where parental data were available for mapping populations, the colour green was allocated when the SNP results supported the hybridity of the F1, red when the results were the same as the mother, blue when the results were the same as the father, and black when the progeny were different from both mother and father (for example. AA result for the mother, AA result for the father and BB results for the progeny) (Figures 3.16-3.25).
The mapping was not performed for the FEM population and P266R232T018 x A199R45T055 accessions, since the plant material was not available for SNP array analysis.

3.3.5 DNA content analysis

The DNA content of the FEM and UniBo samples was measured by Plant Cytometry Services (Plant Cytometry Services, Schijndel, the Netherlands). Leaf material (a few cm^2, corresponding to 20–50 mg) together with a leaf sample of an internal standard with known DNA content was chopped with a razor blade in a plastic petri dish in 500 µL ice-cold extraction buffer. After 30–60 s of incubation, 2.0 mL of staining buffer was added (0.1% dithiothreitol, 1% PVP, 1% propidium iodide, 1% RNase A, pH 7.0). The sample, containing cell constituents and large tissue remnants of the leaf together with the internal standard (*Pachysandra terminalis* (DNA: 3.5 pg/2C)), was passed through a nylon filter of 50 mm mesh size. After incubation at room temperature for at least 30 min, the filtered solution with stained nuclei was passed through a flow cytometer with a 50 mW, 532 nm green laser beam (CyFlow ML Partec GmbH, Münster, Germany). The fluorescence of the stained nuclei was measured by a photomultiplier and converted into voltage pulses, which yielded integral and peak signals for processing by computer. The DNA content of each sample was determined using the software Flomax version 2.4 d (Partec, Germany).

3.4 **Results**

3.4.1 SSR results

Of the 34 SSR markers analysed for the NZ populations, 21 were informative for CO, 20 for P26A17, 15 for P26A19, 21 for IP26, 20 for IP12, 23 for FP18, 23 for FP12, 21 for FP26, 6 for P26S, and 23 for FP35 by showing good amplification profiles or polymorphisms useful for the genotyping analysis (Table 3.5). Similarly, out of 30 SSRs markers for the FEM and UniBo populations, only 25 for FEM, 17 for 'Abate' x 'Fuji', and 10 for 'Decana' x 'Murray' were informative (Table 3.6).

Table 3.5: SSR marker analysis of the New Zealand populations. x indicates the SSR marker informative for that population.

Marker	CO	P26A17	P26A19	IP26	IP12	FP18	FP12	FP26	P26S	FP35
NH013a	x		x	x	x	x	x	x	x	x
CH02b10			x		x					
CH02c02a	x	x		x	x	x	x	x		x
NH002b					x	x			x	x
CH02b12	x					x	x		x	
CH03a09	x									
TsuENH086									x	
CH04e05	x	x	x	x	x	x	x	x	x	x
CH01h10	x			x			x	x		
NH029a										x
TsuENH008	x	x	x	x	x	x	x	x	x	x
NH045a									x	
CH03d02		x	x	x			x		x	x
NB105a		x	x	x	x	x	x	x	x	x
CH03c02										x
CH05d04	x	x	x	x	x	x	x		x	x
KA16									x	
CH02g01	x	x	x	x	x	x	x	x	x	x
NH021a	x	x	x	x		x	x	x	x	x
CH01g05	x	x	x	x	x	x	x	x	x	x
CH03g06		x	x	x	x	x	x	x	x	x
NH004a										x
TsuENH058		x		x		x	x	x	x	
CH02c09	x	x		x	x	x	x	x	x	
CH02d11	x	x		x		x	x	x		
NH027a		x							x	
CH05a04	x	x			x	x		x	x	x
CH01b12	x	x	x	x	x	x	x	x	x	x
CH04c10	x	x	x	x	x	x	x	x	x	x
Ch-Vf1	x	x	x	x	x	x	x	x	x	x
CH04c06	x			x	x	x	x	x	x	x
TsuENH004	x	x	x	x	x	x	x	x	x	x
CH05a02	x	x		x	x	x	x	x	x	x
CH01h02	x	x			x	x	x	x	x	x

Table 3.6: Details of primers used for apple SSR marker analysis of Bologna (AF and DM) and FEM populations (FEM). x indicates the apple SSR marker infomative for that population.

Marker	AF	DM	FEM
Hi21g05		x	x
CH02f06	x		x
CH02c02a		x	x
Ch03g07	x		
MS14h03	x		x
CH02h11a			x
NZ05g08	x		x
CH02a08z			x
Hi09B04	x	x	x
CH05a05			x
CH01c06	x	x	x
Hi04b12	x		
CH01h02	x		x
CH05c07			x
Hi22f04		x	x
MS06g03			x
CH04h02		x	
CH05d11	x		x
CH03c02	x	x	x
CH03a08	x		x
CH03b10			x
AJ000761	x		
CH03d08	x		x
CH04c07	x	x	x
CH02d11	x		x
NZ02b1	x		x
CH05a04		x	
CH05c06		x	x
CH01h01	x		x
CH05g03			x

The discrimination power and the robustness of the SSR markers used was assessed by the genetic diversity analysis and are reported in Table 3.7. The extreme values for each population of all analysed parameters are provided. More details of these results for each population are shown in Supplementary paragraphs S 3.1 to S 3.13.

Table 3.7: Genetic diversity of SSR results of means of all the population. Na (number of different alleles), Ne (number of effective alleles, I (Shannon's information index), Ho (observed heterozygosity), He (expected heterozygosity) and PD (discrimination power at each locus for parent and progeny).

	Na	Ne	I	Ho	He	PD_Parent	PD_F1	
CO	1.5 - 4	1.3 - 3.06	0.28 - 1.13	0 - 0.99	0.19 - 0.64	0 - 0.89	0 - 0.82	
P26A17	1.5 - 3	1.5 - 3	0.35 - 1.04	0 - 1	0.25 - 0.63	0.54 - 0.89	0 - 0.63	
P26A19	1.5 - 3	1.4 - 2.83	0.32 - 1.07	0 - 0.58	0.22 - 0.65	0 - 0.51	0 - 0.54	
IP26	1.5 - 2.5	1.3 - 2.33	0.28 - 0.87	0 - 0.75	0.19 - 0.56	0 - 0	0 - 0.79	
IP12	1 - 3	1 - 2.67	0 - 1.04	0 - 0.5	0 - 0.63	0 - 0.79	0 - 0.79	
FP18	1.5 - 4	1.33 - 3.41	0.29 - 1.25	0 - 0.82	0.2 - 0.7	0 - 0.89	0.29 - 0.83	
FP12	1.5 - 3.5	1.16 - 3.48	0.21 - 1.24	0 - 0.86	0.12 - 0.71	0 - 0.89	0 - 0.87	
FP26	1 - 3	1 - 3	0 - 1.07	0 - 1	0 - 0.65	0 - 0.89	0 - 0.81	
P26S	1 - 2.5	1 - 2.33	0 - 0.87	0 - 0.75	0 - 0.56	0 - 0.79	0 - 0.63	
FP35	1.5 - 3.5	1.31 - 3.48	0.29 - 1.24	0 - 1	0.19 - 0.71	0 - 0.89	0.16 - 0.82	
AF	1.5 - 3	1.3 - 3	0.28 - 1.04	0.25 - 1	0.19 - 0.63	0.54 - 0.89	0 - 0.63	
DM	1.5 - 3	1.5 - 3	0.35 - 1.04	0 - 1	0.25 - 0.63	0.63 - 0.89	0 - 0.63	
	Na	**Ne**	**I**	**Ho**	**He**	**PD_Parent**	**PD_F1**	**PD_F2**
FEM	1.67 - 4.33	1.38 - 3.82	0.3 - 1.28	0.08 - 1	0.18 - 0.68	0.72 - 0.96	0 - 0.63	0 - 0.95

In the GenAlex population assignment of SSR data in the graphical analysis, a higher log-likelihood value on the X axis indicates population 1 as the most likely for pear as confirmed by the pear parents ('Williams Christ', 'André Desportes', 'Old Home', P265R232T018, P266R231T015, P125R095T002, P186R125T002, P354R200T138, 'Abate' and 'Decana'). A higher log-likelihood value on the Y axis indicates population 2 as the most likely for apple as demonstrated by the apple parents ('Cox's Orange Pippin', 'Kalco', A174R01T204, A199R45T055, 'Imperial Gala', 'Fuji', 'Scilate', Fuji' and 'Murray').

The 35 'Cox's Orange Pippin' x 'Old Home' (CO) progeny were confirmed to be true apple-pear hybrids with various levels of pear introgressions into apple as reflected by their assignment values, which ranged from 0.60 to 5.89 (Figure 3.2A). The same applied to 'Fuji' x P354R200T138 population FP35, but with a larger continuum of F1 progeny along the X-axis as the assignment values ranged between 8.99 and 27.03 (Figure 3.2B). Both the FP12 (Figure 3.2C) and FP18 (Figure 3.2D) showed two distinct progeny clusters: one closer to the 'Fuji' parent and another between the two parents with high log-likelihood values for both.

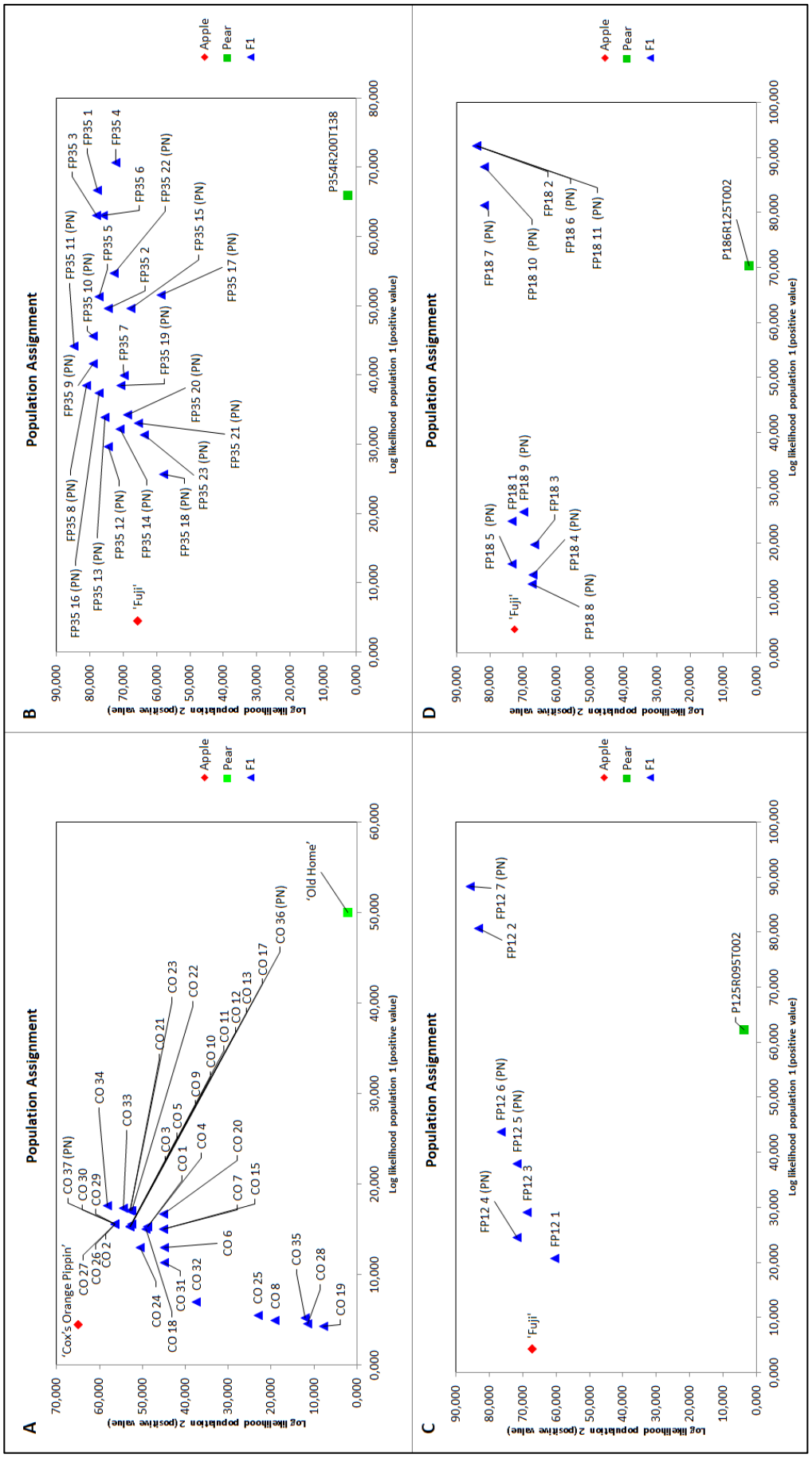

Figure 3.2: Population assignment of putative hybrids as deduced from the SSR marker analysis. The plots represent the positive log-likelihood of assignment of each sample by GenAlex. The lower log-likelihood value for apple parents ('Cox's Orange Pippin' and 'Fuji') on the X axes indicates population 1 as the most likely for apple; a lower log-likelihood value for pear parents ('Old Home', P186R125T002, P125R095T002, and P354R200T138) on Y axes indicates population 2 as the most likely for pear. The 35 putative apple-pear hybrids from 'Cox's Orange Pippin' x 'Old Home' (CO) (A), 'Fuji' x P354R200T138 population (B), the seven 'Fuji' x P125R095T002 (FP12) (C) and the 'Fuji' x P186R125T002 (FP18) (D).

With similar X- and Y-axis values, hybrids IP26 2 (Figure 3.3B) and the P26S (Figure 3.3C) are located between the two parents with assignment values of 49.1 and 1.81, respectively. Both P26A17 (Figure 3.3A) and IP26 1 (Figure 3.3B) are located high on the Y-axis for apple and halfway on the X-axis for pear (Figure 3.3B) with an assignment value for apple of 43.4, pear 70.3 and F1 cluster 49.2.

Figure 3.4 shows the population assignment for the putative apple-pear hybrids from 'Fuji' x P266R231T015 (A) and IP12 1 and IP12 2 (B) and P265R232T018 x the A199R45T055 (C). FP26 1 and FP26 2 (Figure 3.4A) were clustered between the two parents, but close to apple, while FP26 3 clustered near the apple mother 'Fuji'. Similarly, IP12 1 was located near the apple parent, while IP12 2 showed a high likelihood for both apple and pear, but clustered with a low assignment value in the progeny group (assignment value for apple 59.6, pear 53.4 and F1 46.2 (Figure 3.4B). The P265R232T018 x A199R45T055 (Figure 3.4C) progeny P26A19 2 clustered near pear, while P26A19 3 and P26A19 4 showed similar likelihoods in respect to their parents.

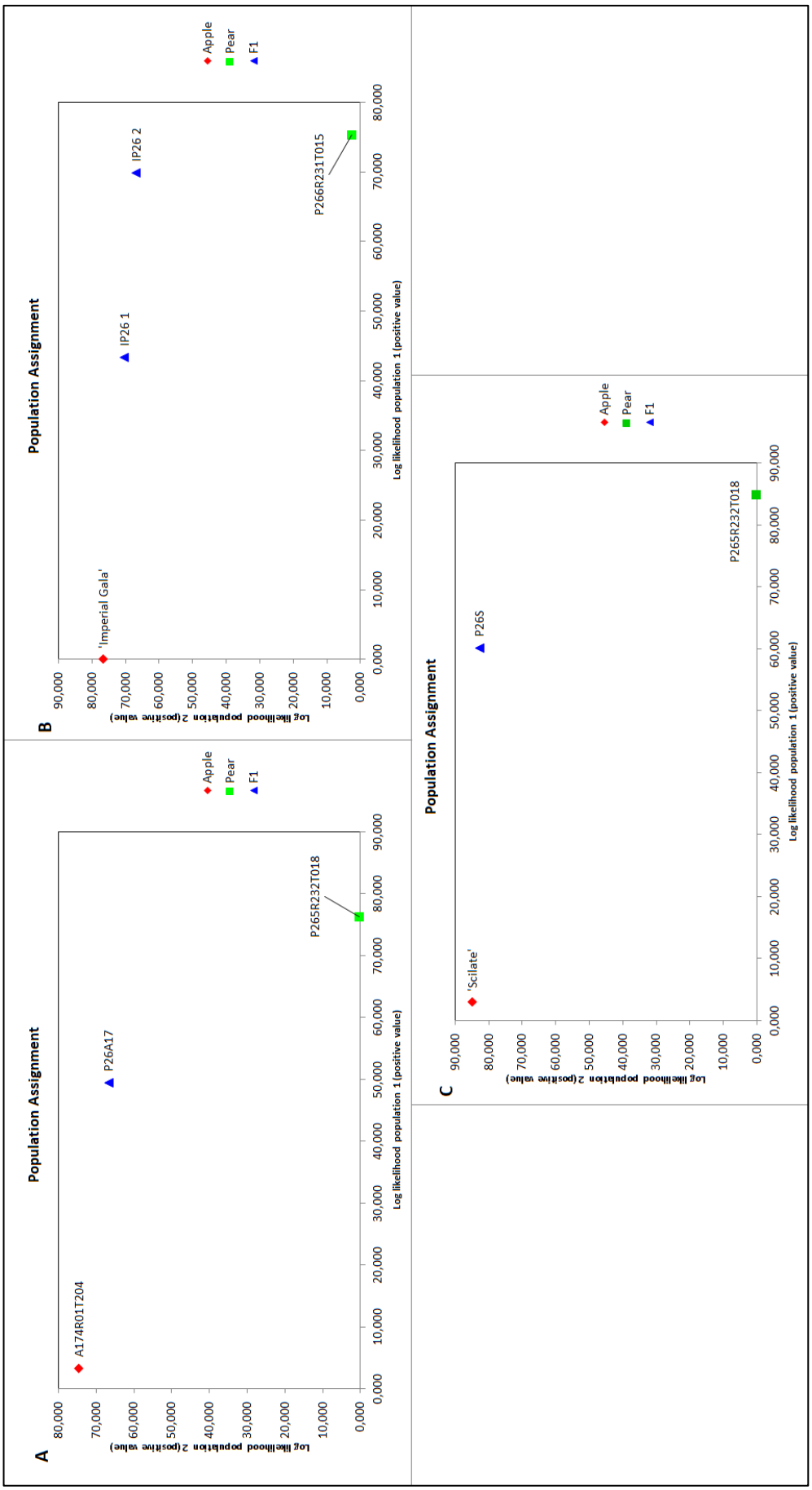

Figure 3.3: Population assignment of putative hybrids as deduced from the SSR marker analysis. The plots represent the positive log-likelihood of assignment of each sample by GenAlex. The lower log-likelihood value for apple parents (A199R45T055, 'Imperial Gala' and 'Scilate') on the X axes indicates population 1 as the most likely for apple; a lower log-likelihood value for pear parents (P265R232T018 and P266R231T015) on Y axes indicates population 2 as the most likely for pear. The P265R232T018 x A174R01T204 putative hybrid (P26A17) (A), the 'Imperial Gala' x P266R231T015 (IP26) (B) and the P26S (C).

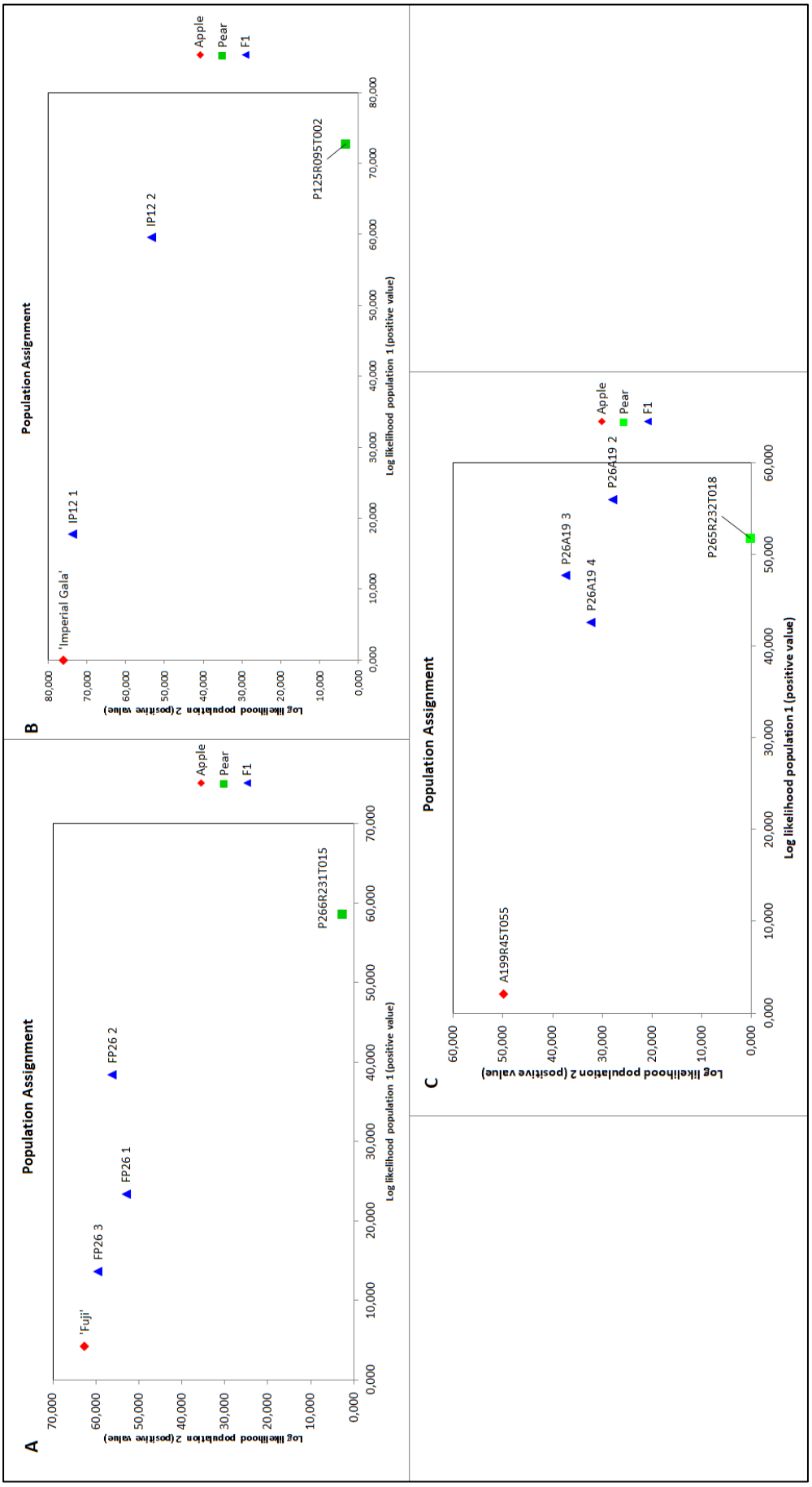

Figure 3.4: Population assignment of putative hybrids as deduced from the SSR marker analysis. The plots represent the positive log-likelihood of assignment of each sample by GenAlex. The lower log-likelihood value for apple parents ('Fuji', 'Imperial Gala' and A199R45T055) on the X axes indicates population 1 as the most likely for apple; a lower log-likelihood value for pear parents (P266R231T015 and P125R095T002) on Y axes indicates population 2 as the most likely for pear. The putative pear-apple hybrids from 'Fuji' x P266R231T015 (A) and IP12 1and IP12 2(B) and the P265R232T018 x A199R45T055 (C).

In the Italian samples, the means of three replicates of the putative hybrid from 'Abate' x 'Fuji' (AF) (Figure 3.5A) and Zwintzscher's Hybrid itself (Figure 3.5C) were located perfectly between the two parent groups. In contrast, the means of three replicates of two putative pear-apple hybrids from 'Decana' x 'Murray' (DM1, DM2) were located near pear (Figure 3.5B) and the five Zwintzscher's Hybrid F2 (F2) progeny were located somewhat closer to apple (Figure 3.5C).

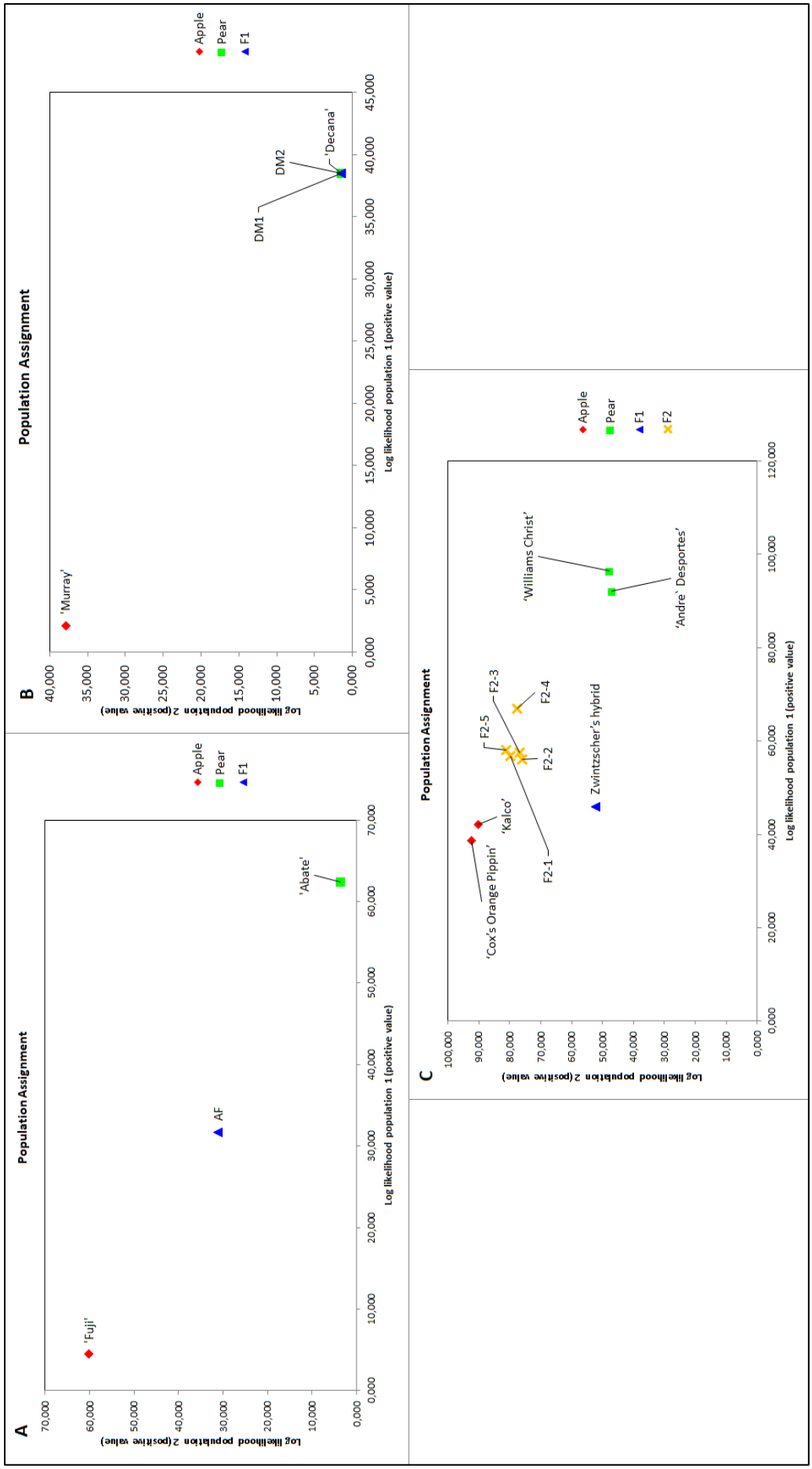

Figure 3.5: Population assignment of putative hybrids as deduced from the SSR marker analysis. The plots represent the positive log-likelihood of assignment of each sample by GenAlex. The lower log-likelihood value for apple parents ('Fuji', 'Murray', 'Cox's Orange Pippin', 'Kalco') on the X axes indicates population 1 as the most likely for apple; a lower log-likelihood value for pear parents ('Abate', 'Decana', 'Williams Christ' and 'André Desportes') on Y axes indicates population 2 as the most likely for pear. The three replicates of the putative hybrid from 'Abate' x 'Fuji' (AF) (A) and the Zwintzscher's Hybrid (C) were located between the two parent groups, the three replicates of two putative pear-apple hybrids from 'Decana' x 'Murray' (DM1, DM2) were located near pear (B) and the five Zwintzscher's Hybrid F2 (F2) progeny were located near apple (C).

The Neighbour-joining clustering using the Dice index for the PFR populations showed almost the same population clustering as the GenAlex analysis (Figure 3.6), supporting these results of the population assignment.

Figure 3.7 shows the results of Neighbour-joining clustering using the Euclidean's index for the FEM population. Zwintzscher's Hybrid is located perfectly between the two parental groups. The five F2 from open pollination of Zwintzscher's Hybrid were clustered in the same group, separate from the mother parent. This clustering supports the results of the GenAlex assignment for this population.

The Neighbour-joining clustering using the Euclidean's index for the UniBo population showed that AF is located perfectly between the two parents (Figure 3.8A), and that DM1 and DM2 were clustered in the same group as the pear mother ('Decana') (B). This clustering supports the results of the GenAlex population assignment for this population.

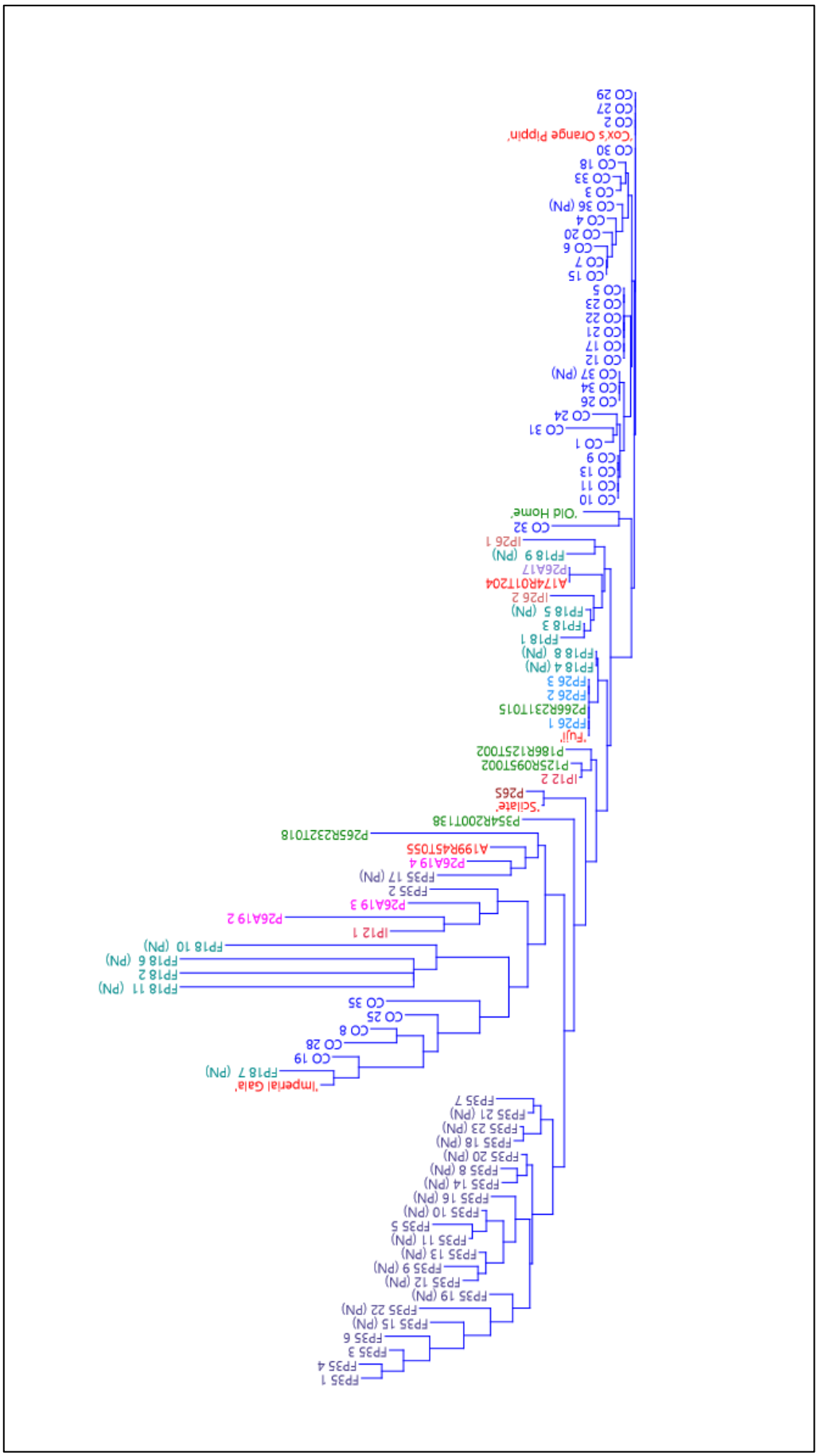

Figure 3.6: Neighbour joining clustering using Dice's index for SSR results of the PFR samples used for this analysis. In red' are all the apple parents ('Imperial Gala', A174R01T204, A199R45T055, 'Cox's Orange Pippin', 'Fuji', 'Imperial Gala' and 'Scilate'), in blue (all 'Cox' Orange Pippin' x 'Old Home' population), in aquamarine, ('Fuji' x P125R095T002 population), darkcyan, ('Fuji' x P186R125T002), dodgerblue, (FP261, FP26 2 and FP26 3), darkslateblue, ('Fuji' x P354R200T138), crimson, (IP12 1 and IP12 2), indianred, (IP26 1 and IP26 2), mediumpurple, (P26A17), fuchsia,(P26A19 1, P26A19 2, P26A19 3 and P26A19 4), darkred, (P26S), and in green are all the pear parents (P125R095T002, 'Old Home', P125R095T002, P186R125T002, P265R232T018, P266R231T015 and P354R200T138).

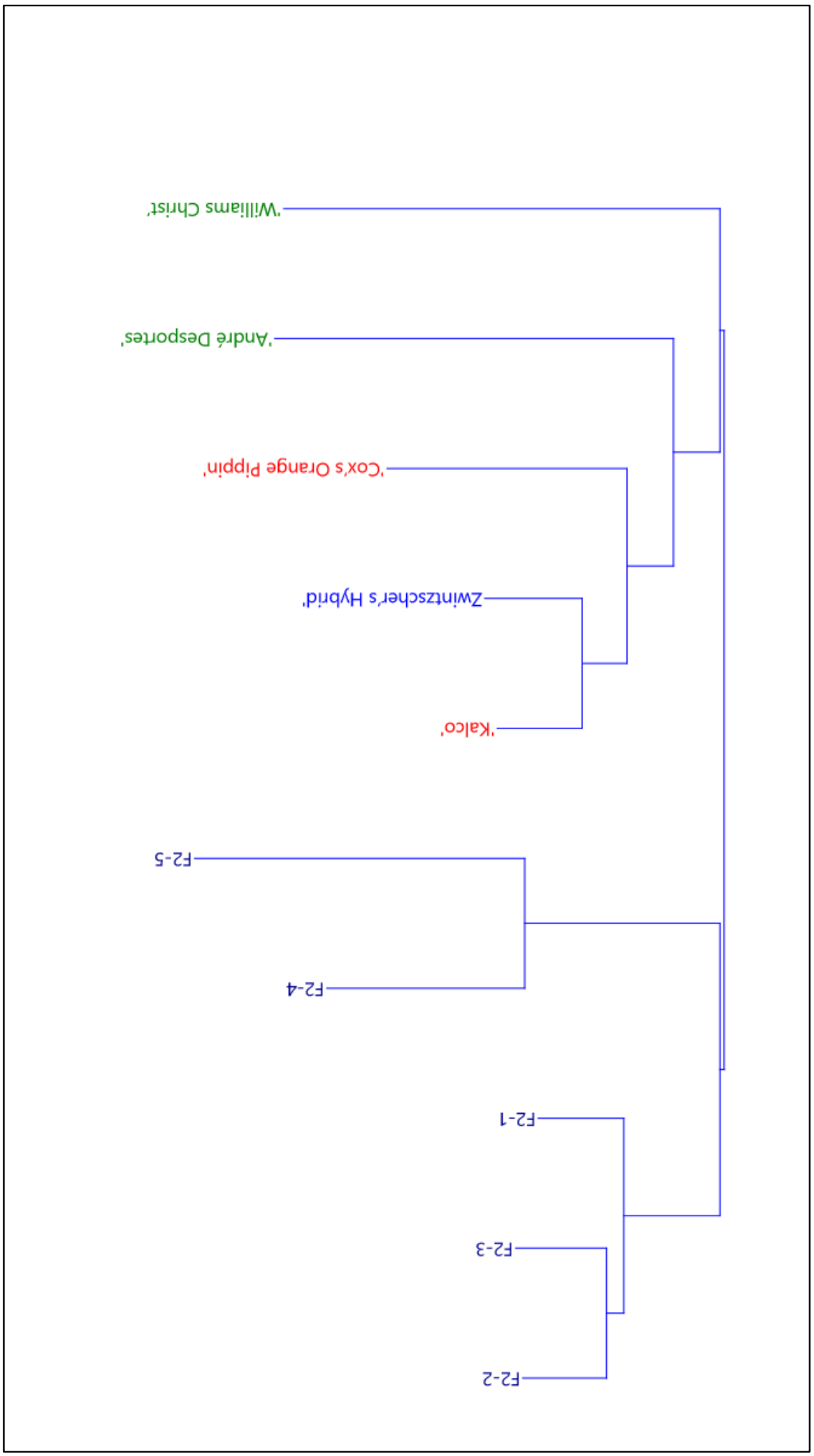

Figure 3.7: Neighbour joining clustering using Euclidean's index with SSR results of all the FEM samples used for this analysis. In red, all the apple parents ('Cox's Orange Pippin', and 'Kalco'), blue, (Zwintzscher's Hybrid), darkblue (F2-1, F2-2 F2-3, F2-4 and F2-5) and in green, the pear parents ('André Desportes' and 'Williams Christ').

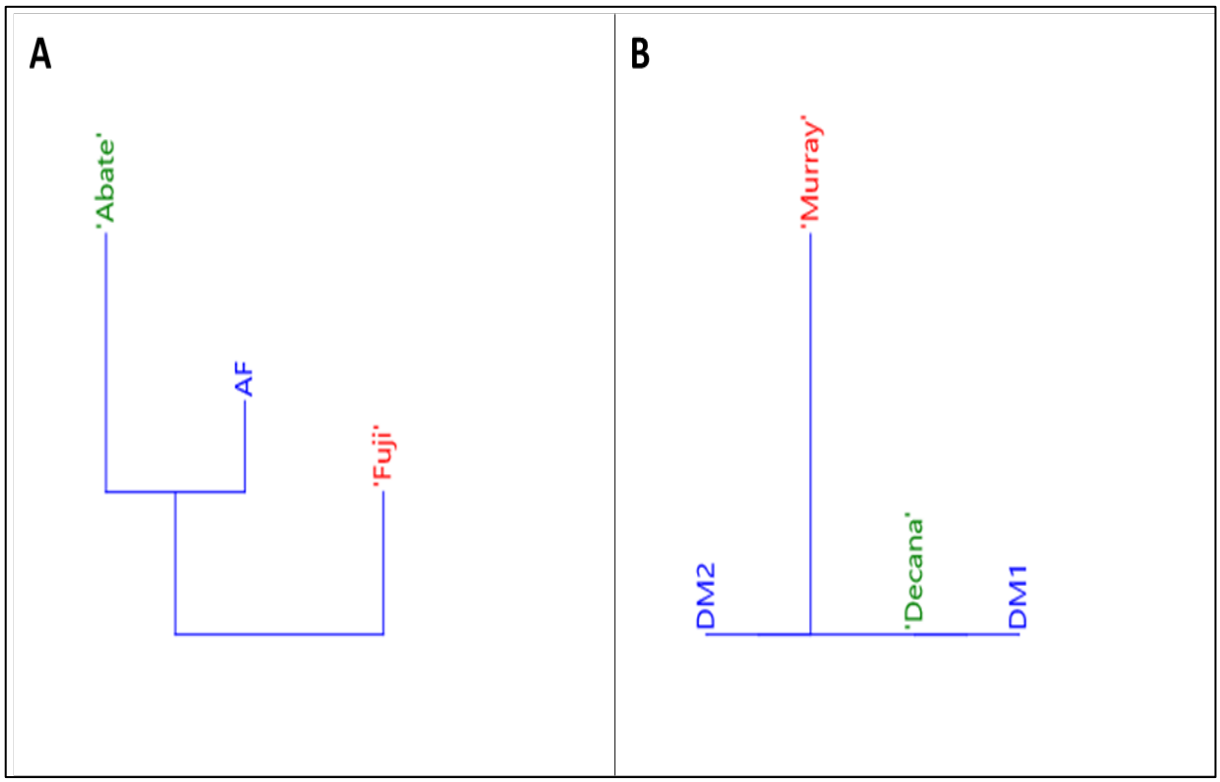

Figure 3.8: Neighbour joining clustering using Euclidean's index with SSR results of all the UniBo samples used for this analysis. A 'Abate' x 'Fuji' population and B 'Decana' x 'Murray' population. In red, the apple parents ('Fuji' and 'Murray'), in blue (AF, DM1 and DM2), and in green, the pear parents ('Abate' and 'Decana').

3.4.2 HRM results

The discrimination power and the robustness of the HRM markers used was assessed by the genetic diversity analysis. The extreme values for each population of all analysed parameters indicate that the genetic diversity of the NZ and FEM populations is very similar (Table 3.8). More details of these results for each population are shown in in Supplementary paragraphs S 3.14 to S 3.26.

Table 3.8: Genetic diversity of HRM results of means of all the population. Na (number of different alleles), Ne (number of effective alleles, I (Shannon's information index), Ho (observed heterozygosity), He (expected heterozygosity) and PD (discrimination power at each locus for parent and progeny).

	Na	Ne	I	Ho	He	PD_Parent	PD_F1	
CO	1.5 - 2.5	1.3 - 2.15	0.28 - 0.8	0 - 1	0.19 - 0.53	0.54 - 0.63	0 - 0.71	
P26A17	1.5 - 2	1.3 - 2	0.28 - 0.69	0 - 1	0.19 - 0.5	0.54 - 0.63	0 - 0.63	
P26A19	1.5 - 2.5	1.44 - 2.45	0.35 - 0.89	0 - 0.88	0.25 - 0.58	0.54 - 0.63	0 - 0.81	
IP26	1.5 - 2	1.3 - 2	0.28 - 0.69	0 - 1	0.19 - 0.5	0.54 - 0.63	0 - 0.63	
IP12	1.5 - 2	1.3 - 2	0.28 - 0.69	0 - 1	0.19 - 0.5	0.54 - 0.63	0 - 0.63	
FP18	1.5 - 2.5	1.3 - 2.41	0.28 - 0.88	0.25 - 1	0.19 - 0.57	0.54 - 0.63	0 - 0.81	
FP12	1.5 - 2.5	1.3 - 1.98	0.28 - 0.76	0.25 - 0.93	0.19 - 0.49	0.54 - 0.63	0 - 0.75	
FP26	1.5 - 2.5	1.3 - 2.5	0.28 - 0.9	0.25 - 1	0.19 - 0.58	0.54 - 0.63	0 - 0.81	
P26S	1.5 - 2	1.3 - 2	0.28 - 0.69	0 - 1	0.19 - 0.5	0.54 - 0.63	0 - 0.63	
FP35	1.5 - 2.5	1.3 - 2.17	0.28 - 0.81	0.25 - 1	0.19 - 0.54	0.54 - 0.63	0 - 0.77	
AF	2 - 2	2 - 2	0.69 - 0.69	0.5 - 0.5	0.5 - 0.5	0.63 - 0.63	0.63 - 0.63	
DM	1.5 - 1.5	1.5 - 1.5	0.35 - 0.35	0 - 0	0.25 - 0.25	0.63 - 0.63	0 - 0	
	Na	**Ne**	**I**	**Ho**	**He**	**PD_Parent**	**PD_F1**	**PD_F2**
FEM	1.67 - 2	1.53 - 2	0.42 - 0.69	0 - 0.68	0.29 - 0.5	0.54 - 0.63	0 - 0.63	0 - 0.63

HRM analysis with 36 markers confirmed that Zwintzscher's Hybrid is a full hybrid and also indicated that the 41 CO apple-pear progeny are partial hybrids, with 9-15 primers per genotype providing evidence for hybridity (Supplementary Table 3.27-3.28). One to six markers per genotype provided evidence for hybridity of the five Zwintzscher's Hybrid F2 progeny maintained at FEM. Twenty-nine markers per genotype provided evidence for hybridity of the pear-apple hybrid between P265R232T018 x A174R01T204. Thirty to eight markers per genotype provided evidence for hybridity of the four pear-apple hybrids between P265R232T018 x A199R45T055. Two putative apple-pear hybrids from 'Imperial Gala' x P266R231T015 were full hybrids, with five markers per genotype providing evidence for hybridity. Two putative apple-pear hybrids from 'Imperial Gala' x P125R095T002 were full hybrids, with three markers. Twenty-eight to two markers per genotype provided evidence for hybridity of the eleven 'Fuji' x P186R125T002 F1 progeny. Seven 'Fuji' x P125R095T002 F1 were full hybrids, with three to six markers per genotype

providing evidence for hybridity. Four to five per genotype provided evidence for hybridity of the four apple-pear putative hybrid between 'Fuji' x P266R231T015. One putative pear-apple hybrid from P265R232T018 x 'Scilate' was a full hybrid, with six markers per genotype providing evidence for hybridity. Two to seven markers per genotype provided evidence for hybridity of the twenty three 'Fuji' x P354R200T138 F1 apple-pear progeny. One 'Abate' x 'Fuji' F1 pear-apple progeny held at UniBO proved to be a full hybrid, with seven primers per genotype providing evidence for hybridity. No marker provided evidence for hybridity of two 'Decana' x 'Murrray' pear-apple putative hybrids obtained at UniBO.

As a result of population assignment of HRM data using GenAlex, the higher log-likelihood value for the apple parents ('Cox's Orange Pippin', 'Kalco', A174R01T204, A199R45T055, 'Imperial Gala', 'Fuji', 'Scilate', Fuji' and 'Murray') on the Y axis indicates population 1 as the most likely for apple; a higher log-likelihood value for pear parents ('Williams Christ', 'André Desportes', 'Old Home', P265R232T018, P266R231T015, P125R095T002, P186R125T002, P354R200T138, 'Abate' and 'Decana') on the X axis indicates population 2 as the most likely for pear. The 41 putative apple-pear hybrids from CO, the P265R232T018 x A174R01T204 putative hybrid (P26A17), the 4 putative P265R232T018 x A199R45T055 (P26A19) and the 2 'Imperial Gala' x P266R231T015 (IP26), were located between the two parent groups (Figure 3.9).

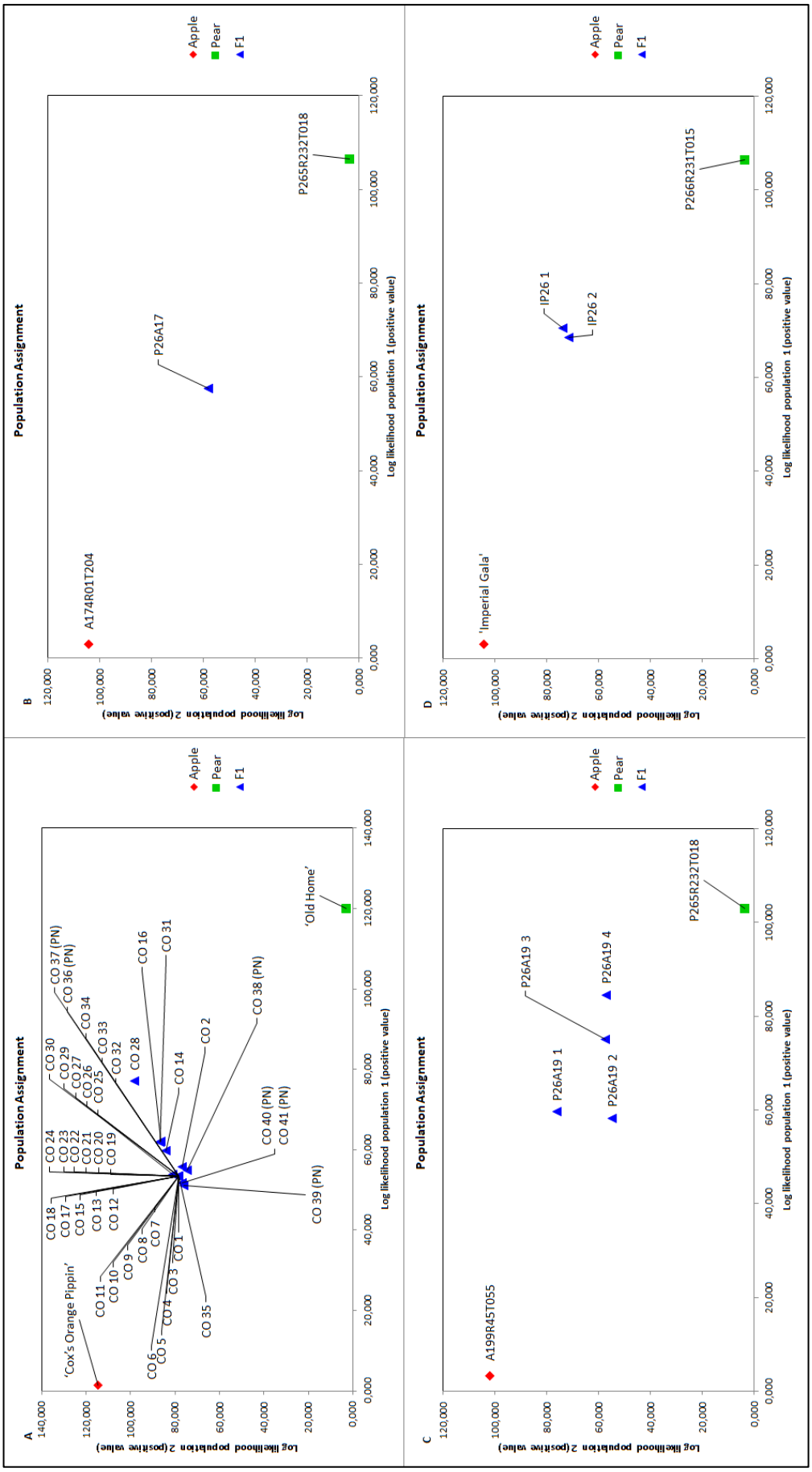

Figure 3.9: Population assignment of putative hybrids as deduced from the HRM marker analysis. The plots represent the positive log-likelihood of assignment of each sample by GenAlex. The lower log-likelihood value for apple parents ('Cox's Orange Pippin', A174R01T204, A199R45T055 and 'Imperial Gala') on the X axes indicates population 1 as the most likely for apple; a lower log-likelihood value for pear parents ('Old Home', P265R232T018 and P266R231T015) on Y axes indicates population 2 as the most likely for pear. The 41 putative apple-pear hybrids from 'Cox's Orange Pippin' x 'Old Home' (CO) (A) the P265R232T018 x A174R01T204 putative hybrid (P26A17) (B) the four putative P265R232T018 x A199R45T055 (P26A19) (C) and the two 'Imperial Gala' x P266R231T015 (IP26) (D) were located between the two parent groups

The two putative apple-pear hybrids from 'Imperial Gala' x P125R095T002 (IP12) (A) the ten putative hybrids 'Fuji' x P186R125T002 (FP18) (B), the seven 'Fuji' x P125R095T002 (FP12) (C), and the three 'Fuji' x P266R231T015 (FP26) (D) were located between the two parent groups and the FP1810 1 (PN) hybrid between 'Fuji' x P186R125T002 (B) was located near apple (Figure 3.10). The putative pear-apple hybrids from P265R232T018 x 'Scilate' (P26S) (A), the twenty-three putative hybrids 'Fuji' x P354R200T138 (FP35) (B), and the Zwintzscher's Hybrid and the five F2 (F2) (C) were located between the two parent groups (Figure 3.11). The putative pear-apple hybrids from 'Abate' x 'Fuji' (AF) (A) and 'Decana' x 'Murray' (DM1, DM2) were located near pear (B) (Figure 3.12).

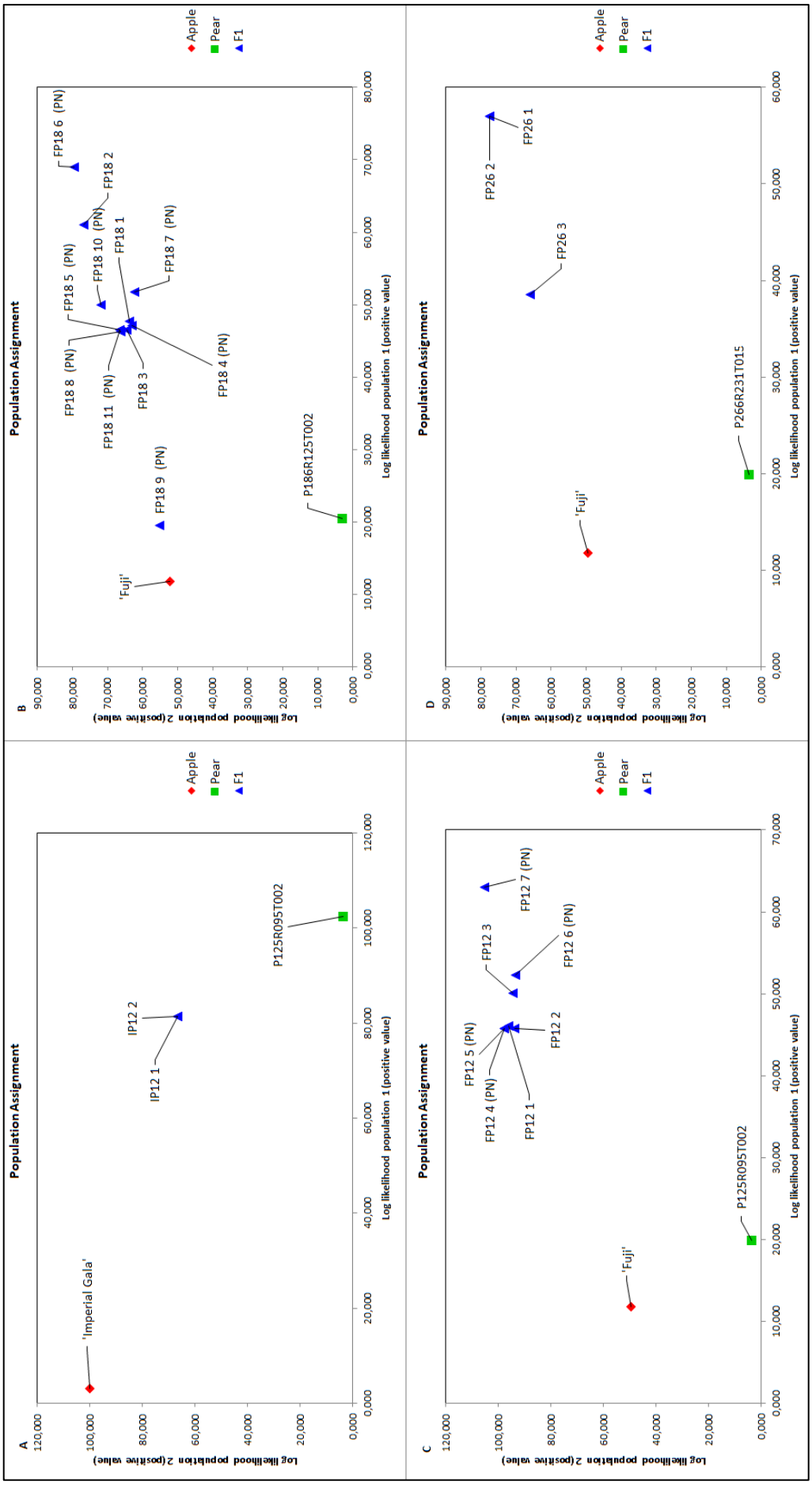

Figure 3.10: Population assignment of putative hybrids as deduced from the HRM marker analysis. The plots represent the positive log-likelihood of assignment of each sample by GenAlex. The lower log-likelihood value for apple parents ('Imperial Gala' and 'Fuji') X axes indicates population 1 as the most likely for apple; a lower log-likelihood value for pear parents (P125R095T002, P186R125T002 and P266R231T015) on Y axes indicates population 2 as the most likely for pear. The two putative apple-pear hybrids from 'Imperial Gala' x P125R095T002 (IP12) (A) the ten putative hybrids 'Fuji' x P186R125T002 (FP18) (B) the seventh 'Fuji' x P125R095T002 (FP12) (C), the three 'Fuji' x P266R231T015 (FP26) (D) were located between the two parent groups and the FP18 10 (1PN) hybrid between 'Fuji' x P186R125T002 (B) was located near apple.

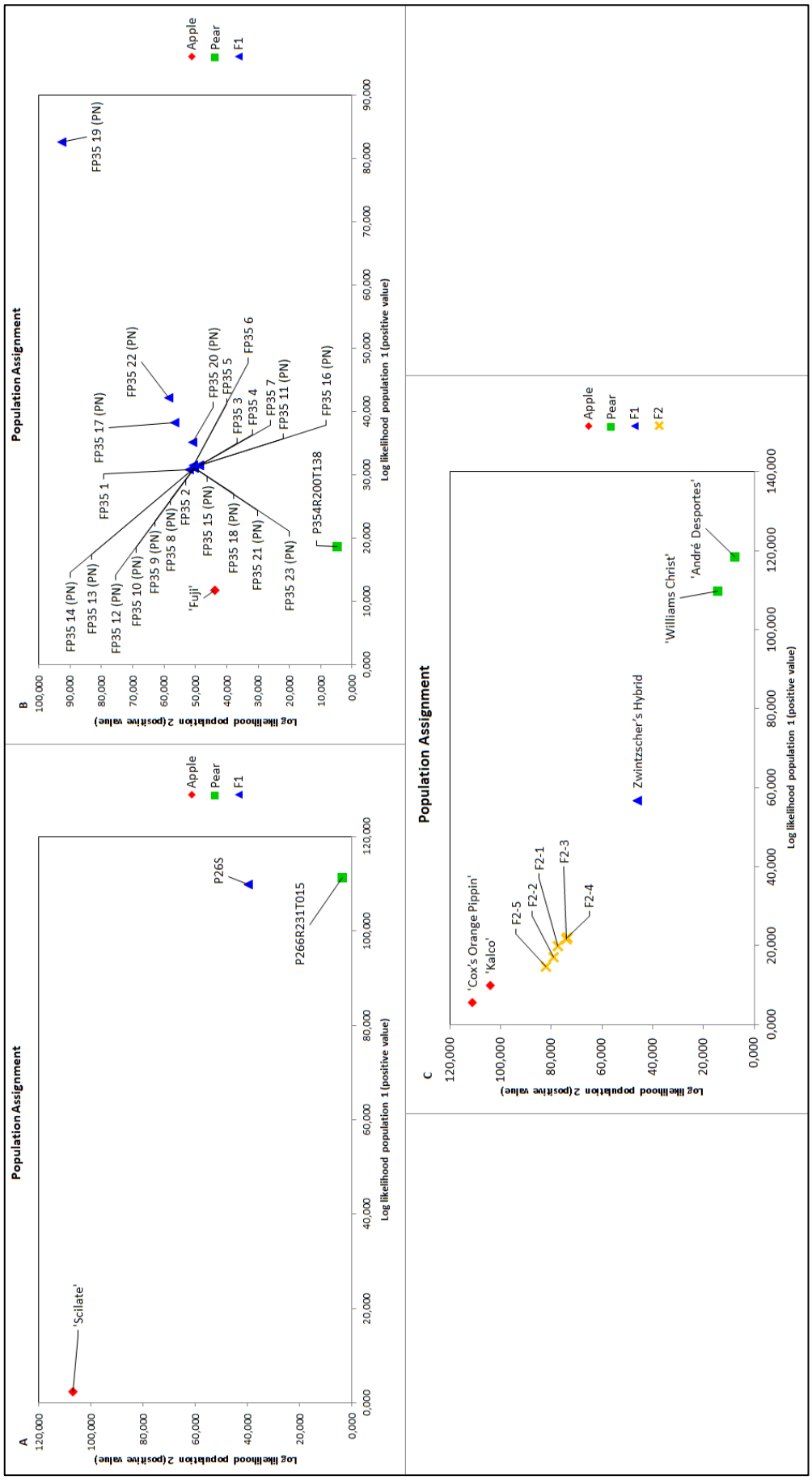

Figure 3.11: Population assignment of putative hybrids as deduced from the HRM marker analysis. The plots represent the positive log-likelihood of assignment of each sample by GenAlex. The lower log-likelihood value for apple parents ('Sciate', 'Fuji', 'Cox's Orange Pippin' and 'Kalco') on the X axes indicates population 1 as the most likely for apple; a lower log-likelihood value for pear parents (P266R231T015 P354R200T138, 'Williams Christ' and 'Andre` Desportes') on Y axes indicates population 2 as the most likely for pear. The putative pear-apple hybrids from P265R232T018 x 'Sciate' (P26S) (A), the twenty-three putative hybrids 'Fuji' x P354R200T138 (FP35) (B) and the 'Zwintzscher's Hybrid and the five F2 (F2) (C) were located between the two parent groups.

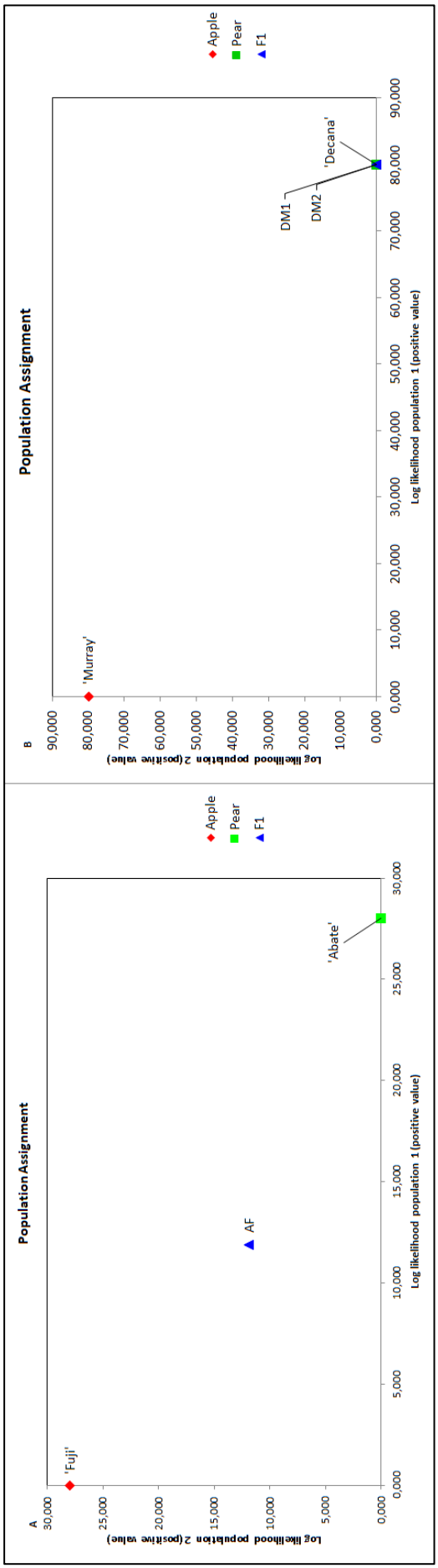

Figure 3.12: Population assignment of putative hybrids as deduced from the HRM marker analysis. The plots represent the positive log-likelihood of assignment of each sample by GenAlex. The lower log-likelihood value for apple parents ('Fuji' and 'Murray') on the X axes indicates population 1 as the most likely for apple; a lower log-likelihood value for pear parents ('Abate' and 'Decana') on Y axes indicates population 2 as the most likely for pear. The putative hybrid from 'Abate' x 'Fuji' (AF) (A) and the putative pear-apple hybrid from 'Decana' x 'Murray' (DM1, DM2) were located near pear (B).

In Figure 3.13 the results of the Neighbour-joining clustering using the Dice index are presented for the PFR populations. The population clustering was almost the same as the GenAlex analysis, supporting these results of the population assignment. Only the samples FP12 7 (PN), FP35 19 (PN), FP26 3 and FP18 9 PN clustered distantly from the other progeny.

The results of the Neighbour-joining clustering using the Euclidean's index for the FEM population shows that Zwintzscher's Hybrid is located perfectly between the two parental groups (Figure 3.14 in blue). The five F2 from open pollination of Zwintzscher's Hybrid (Figure 3.14 in dark blue) were clustered in the same group and more distant from the mother, Zwintzscher's Hybrid. This clustering supports the results of the GenAlex population assignment for this population.

The AF progeny positioned between the two parental group ('Abate' in green and 'Fuji' in red) in the Neighbour-joining clustering using the Euclidean's index (Figure 3.15A), agreeing with the GenAlex analysis, which indicated that AF is a hybrid.

It is obvious that DM1 and DM2 are not close to the apple cluster (Figure 3.15B in red for apple and in blue for F1). Their close mapping to 'Decana' again agrees with the GenAlex analysis.

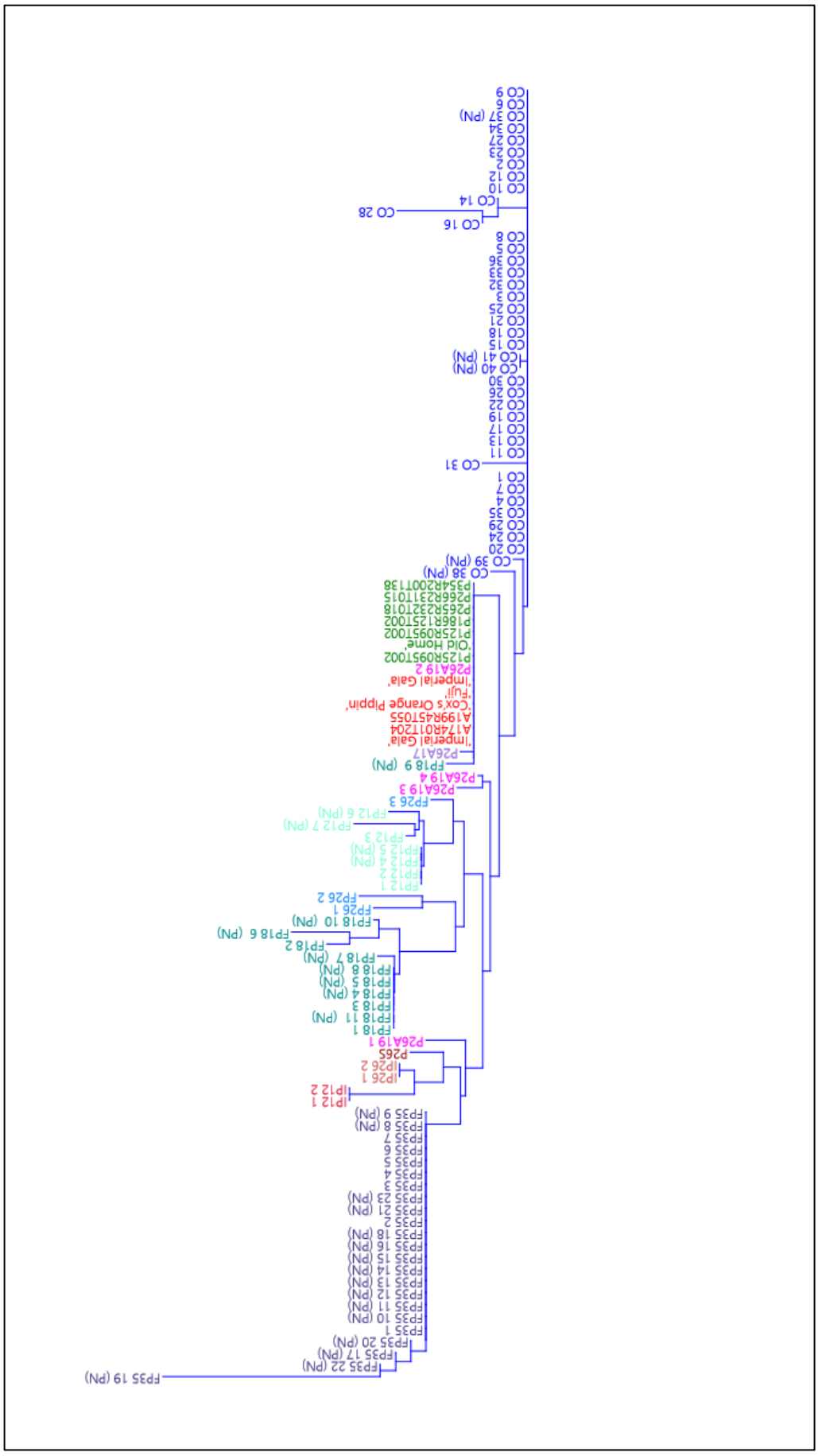

Figure 3.13: Neighbour joining clustering using Dice's index for HRM results of the PFR samples used for this analysis. In red' are all the apple parents ('Imperial Gala', A174R01T204, A199R45T055, 'Cox's Orange Pippin', 'Fuji', 'Imperial Gala' and 'Scilate'), in blue (all 'Cox's Orange Pippin' x 'Old Home' population), in aquamarine, ('Fuji' x P125R095T002 population), darkcyan, ('Fuji' x P186R125T002), dodgerblue, (FP261, FP26 2 and FP26 3), darkslateblue, ('Fuji' x P354R200T138), crimson, (IP12 1 and IP12 2), indianred, (IP26 1 and IP26 2), mediumpurple, (P26A17), fuchsia,(P26A19 1, P26A19 2, P26A19 3 and P26A19 4), darkred, (P26S), and in green are all the pear parents (P125R095T002, 'Old Home', P125R095T002, P186R125T002, P265R232T018, P266R231T015 and P354R200T138).

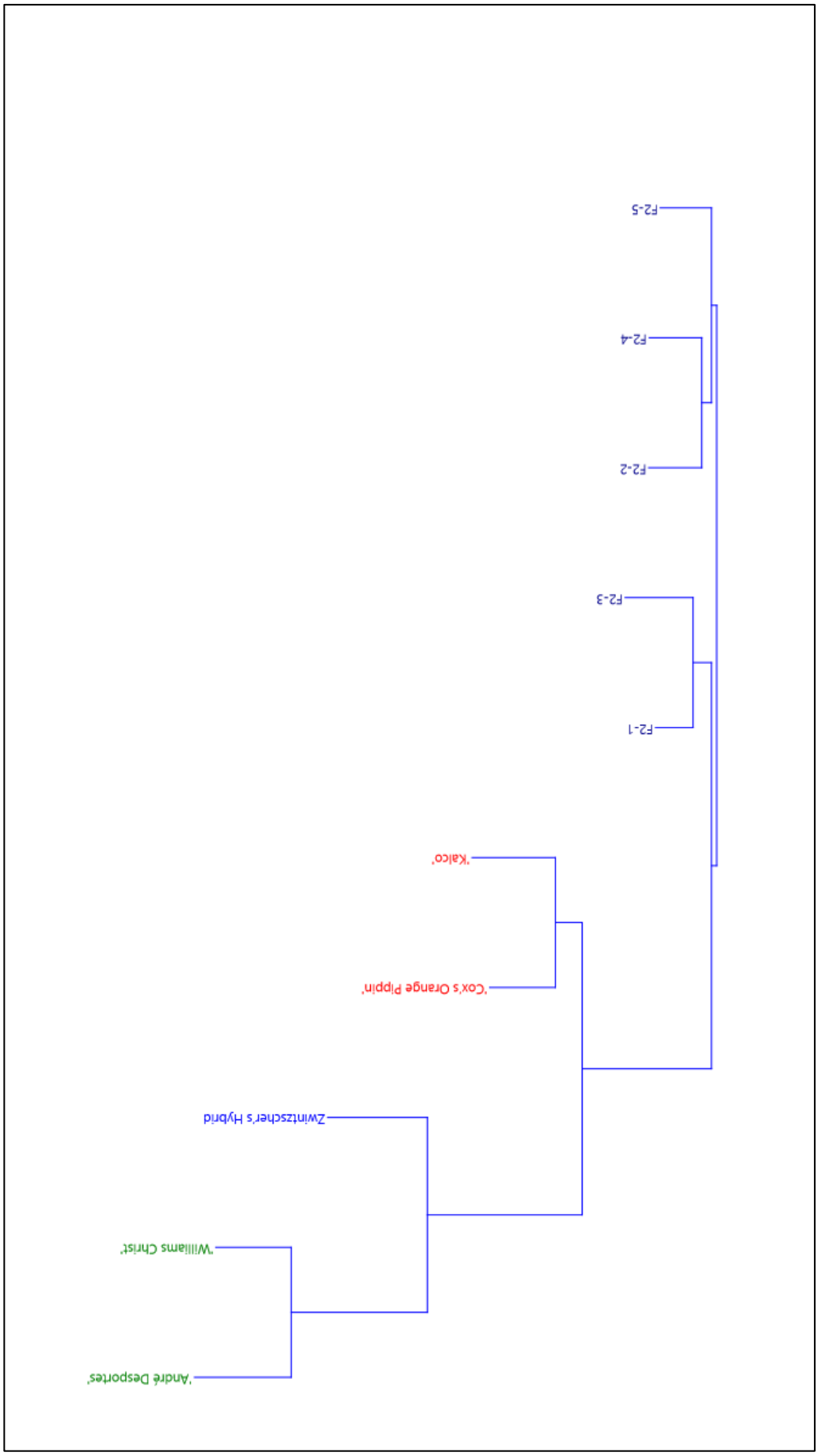

Figure 3.14: Neighbour joining clustering using Euclidean's index with HRM results of all the FEM samples used for this analysis. In red, all the apple parents ('Cox's Orange Pippin', and 'Kalco'), blue, (Zwintzscher's Hybrid), darkblue (F2-1, F2-2 F2-3, F2-4 and F2-5) and in green, the pear parents ('André Desportes' and 'Williams Christ').

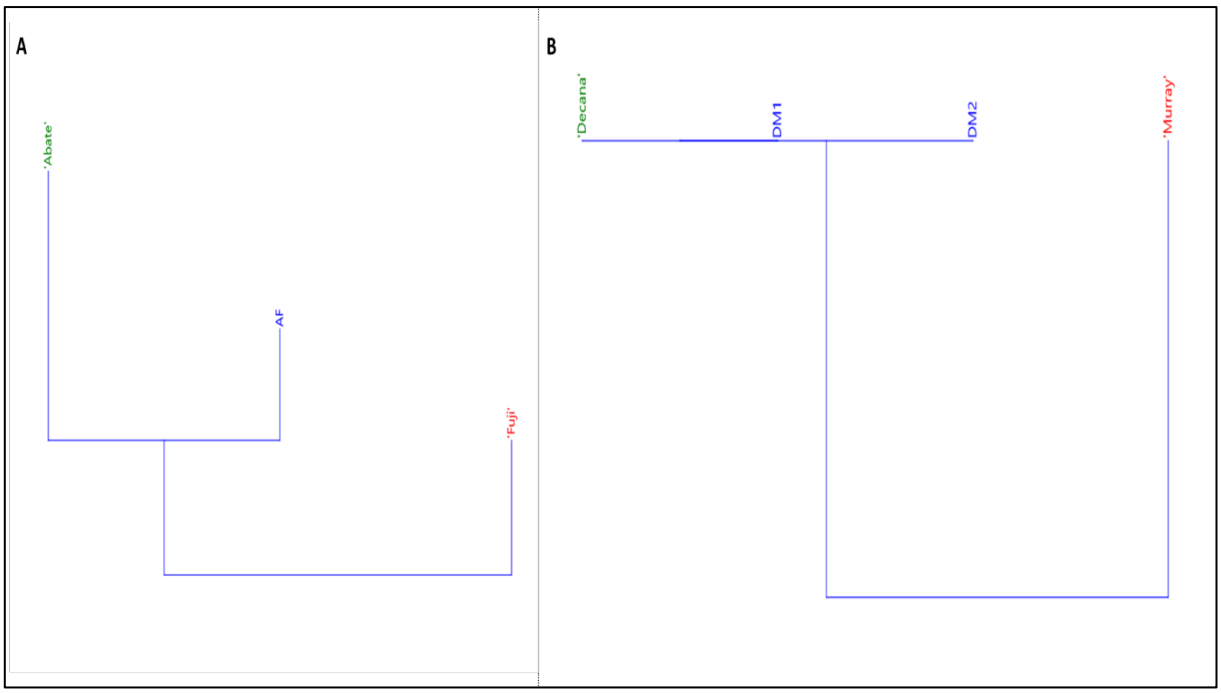

Figure 3.15: Neighbour joining clustering using Euclidean's index with HRM results of all the UniBo samples used for this analysis. A 'Abate' x 'Fuji' population and B 'Decana' x 'Murray' population. In red, the apple parents ('Fuji' and 'Murray'), in blue (AF, DM1 and DM2), and in green, the pear parents ('Abate' and 'Decana').

3.4.3 SNP-array results

In total, 1090 SNPs were obtained for the CO population after filtering of the apple and pear Infinium® II 9K SNP array data. The population 'Imperial Gala' x P266R231T015 yielded 1089 filtered SNPs; 'Imperial Gala' x P125R095T002 1092 SNPs; 'Fuji' x P186R125T002 1085 SNPs; 'Fuji'x P125R095T002 1088 SNPs; 'Fuji' x P266R231T015 1088 SNPs; 'Fuji' x P354R200T138 1086 SNPs; and the FEM population for the apple and pear Infinium® II 9K SNP array yielded 1061 SNPs (Table 3.9).

The number of filtered SNPs for the population 'Abate' x 'Fuji' were 8860; for the DM1 population 8793, and for the population DM2 8890 from the apple Infinium® II 20K SNP array (Table 3.10).

Supplementary Tables S3.29 to S3.38 show the number of SNPs that support hybridity by pear sample and by LG.

Table 3.9: Filtered SNPs for the NZ and FEM population for the apple and pear Infinium® II 9K SNP array.

		CO	IP26	IP12	FP18	FP15	FP26	FP35	FEM
Pear SNP	**LG0**	120	119	120	119	120	117	120	117
Apple SNP	**LG1**	43	43	43	43	43	42	43	41
	LG2	94	94	94	93	94	94	94	93
	LG3	60	61	61	61	61	61	61	56
	LG4	43	43	43	43	43	43	43	43
	LG5	81	81	81	81	81	81	80	79
	LG6	46	46	46	46	46	46	46	45
	LG7	37	37	37	36	37	37	37	36
	LG8	50	50	50	50	50	50	50	48
	LG9	69	69	69	68	68	69	68	67
	LG10	71	72	72	71	72	72	70	72
	LG11	49	49	49	49	49	49	49	47
	LG12	55	55	55	55	55	55	55	54
	LG13	46	46	46	46	46	46	46	43
	LG14	39	39	39	39	39	39	39	39
	LG15	92	92	92	91	91	91	91	89
	LG16	35	35	35	35	35	35	34	33
	LG17	60	58	60	59	58	58	60	59

Table 3.10: Filtered SNPs for the UniBo population for the apple Infinium® II 20K SNP array.

		AF	DM1	DM2
Apple SNP	**LG0**	628	876	883
	LG1	343	328	330
	LG2	567	535	548
	LG3	526	501	510
	LG4	417	419	410
	LG5	536	516	521
	LG6	425	392	408
	LG7	368	369	373
	LG8	430	432	436
	LG9	458	448	448
	LG10	556	544	549
	LG11	564	543	543
	LG12	545	508	517
	LG13	457	452	455
	LG14	440	420	422
	LG15	662	629	655
	LG16	448	417	414
	LG17	490	464	468

The preliminary results of population assignment are not presented here since some samples exhibit the same results, and are understood to be identical. These duplicated samples were removed in the final analysis. It is possible that duplicates were generated during the *in vitro* culture of plants prior to this study. For this reason, the mapping of genomic segments was performed only for progeny with unique results.

For the sample analysis of the 9K SNP array data, the markers were mapped by chromosome, i.e. 17 LGs, while all the 1K pear SNPs were placed into one group, Chr0.

Figure 3.16 represents the genomic segmentation maps for population CO: 26 F1 in Figure 3.16A; CO 7 and CO 26 in Figure 3.16B; CO 16 in Figure 3.16C; and CO 37 (PN) in Figure 3.16D.

Comparison of the four maps shows that the population in Figure 3.16A exhibited more SNPs supporting hybridity (824 SNPs) of these samples (in green), than the others in Figure 3.16 (B, C and D), where the hybridity is supported by respectively, 656 SNPs (B), 692 SNPs (C) and 682s SNP (D). 266 SNPs did not support hybridity of twenty-six CO (A), 434 SNPs (B), 398 SNPs (C) and 408 SNPs (D). The other 3 populations in Figure 3.16 (B, C and D) have similar results and more SNPs originated from the father than the population in Figure 3.16A. Only one (in black) of these 266 SNPs (A), 89 SNPs (B), 68 SNPs (C) and 74 SNPs (D) have a completely different result compared with the parent; 264 SNPs (A), 265 SNPs (B), 255 SNPs (C) and 253 SNPs (D) have the same results as the mother (in red); and only one SNP (A), 80 SNPs (B), 75 SNPs (C) and 81 SNPs (D) originating from the father (in blue).

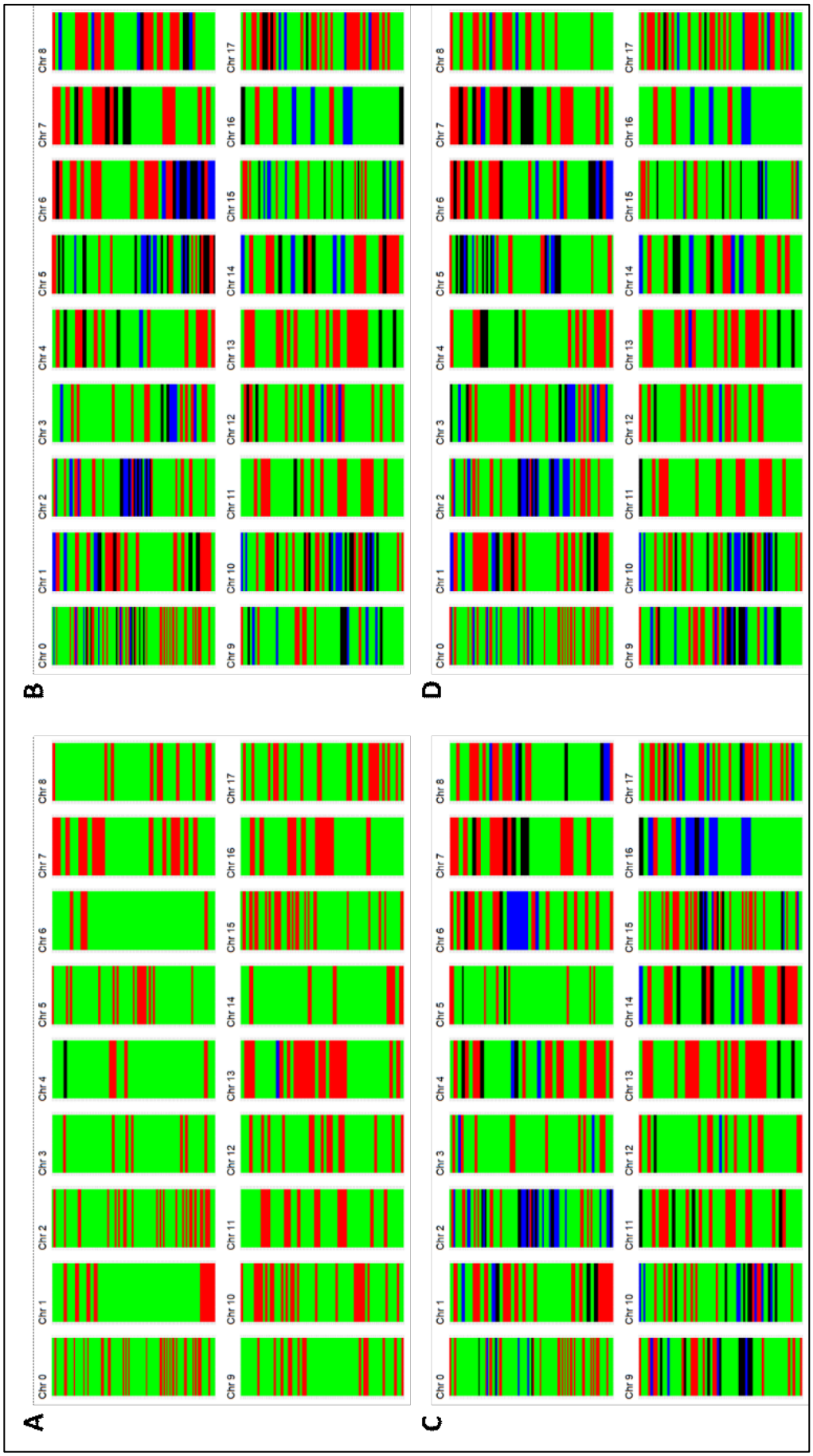

Figure 3.16: Mapping of genomes of the 'Cox's Orange Pippin' x 'Old Home' population with the 9K apple-pear SNP array by chromosome for the apple SNPs, while the pear SNPs were all put in one group, Chr0. In green when the results of the SNPs support the hybridity of the F1, red when the results were the same as the mother, blue when the results were the same as the father, and black when the progeny have different results to both mother and father. The mapping A represent the map of the CO 2, CO 3, CO 4, CO 5, CO 9, CO 10 CO 11, CO 12, CO 13, CO 14, CO 15, CO 17, CO 19, CO 20, CO 22, CO 23, CO 24, CO 25, CO 27, CO 29, CO 31, CO 33, CO 34, CO 35, CO 40 (PN); B represent the map of CO 7 and CO 26; C CO 16 and D for the CO 37 (PN).

The mapping of the 'Imperial Gala' x P266R231T015 population, IP26 1 (A) and IP26 2 (B), progeny is shown in Figure 3.17. These two progeny have similar results, with 779 (A) and 785 (B) SNPs (in green of Figure 3.17) supporting hybridity, and with 310 (A) and 294 (B) SNPs (in red of Figure 3.17) originating from the mother, 'Imperial Gala'.

Mapping of IP12 1 and IP12 2 is shown in Figure 3.18. These two samples have identical results, with 1051 SNPs (in green in Figure 3.18) supporting the hybridity of this progeny and with 41 SNPs (in red of the Figure 3.18) originating from the mother, 'Imperial Gala'.

The results of the mapping of the 'Fuji' x P186R125T002 population are shown in Figure 3.19. Figure 3.19A represents the map of the FP18 4 (PN); B FP18 1, C FP18 2 and FP18 3, D FP18 6 (PN), E FP18 11 (PN) and FP18 10 (PN). Comparing these, the map shown in Figure 3.19D is different from the other four (A, B, C, E).

In the map of the FP18 6 (PN) (D) samples there are 324 SNPs that do not support the hybridity of this progeny, but 761 SNPs do support the hybridity of this progeny. The other four maps have 348 SNPs (A) 348 SNPs (B), 344 SNPs (C) and 355 SNPs do not support the hybridity of this progeny, where 737 SNPs (A), 737 SNPs (B), 741 SNPs (C) and 750 SNPs (D) support the hybridity of this progeny. The difference between these samples is the number of SNPs with the same results of the P186R25T002, zero SNPs for the mapping shown in Figure 3.20A, B and E; 2 SNPs for the samples FP18 2 and FP18 3 (C) and 57 SNPs for the Figure 3.20D. Two SNPs (A and B); zero SNPs(E), one SNPs (C) and 41 SNPs for the Figure 3.20D do not support the hybridity of this progeny that exhibits completely different results from the parents 'Fuji' and P186R125T002. 364 SNPs (A and B), 335 SNPs (E), 341 SNPs (C) and 226 SNPs (D) originating from the mother ('Fuji').

The mapping of the 'Fuji' x P125R095T002 population is represented in Figure 3.20. It is clear that two of the maps have completely different appearances (A and B compared with C). Comparison of these three maps indicates that the map of the FP12 1, FP12 4 (PN), FP12 6 (PN), FP12 7 (PN) (A) and the FP12 2 map (B) have similar results with 1063 SNPs (A) and 1046 SNPs (B) supporting the hybridity of this progeny and only 25 SNPs (A) and 42 SNPs (B) originating from the mother.

These two maps have completely the same Chr in green, which supports the hybridity of these samples. The Figure 3.20A has the Chr2, Chr3, Chr4, Chr5, Chr7, Chr8, Chr11, Chr14, Chr16 and Chr17 to support this thesis. Figure 3.20B has Chr6, Chr7, Chr14, Chr15, Chr16 and Chr17 completely hybrid.

Zero SNPs exhibit a completely different result from the parents (A and B), only two SNPs, both on Chr13, originated from the father (A and B), and 23 (A) and 40 (B) SNPs originated from the mother.

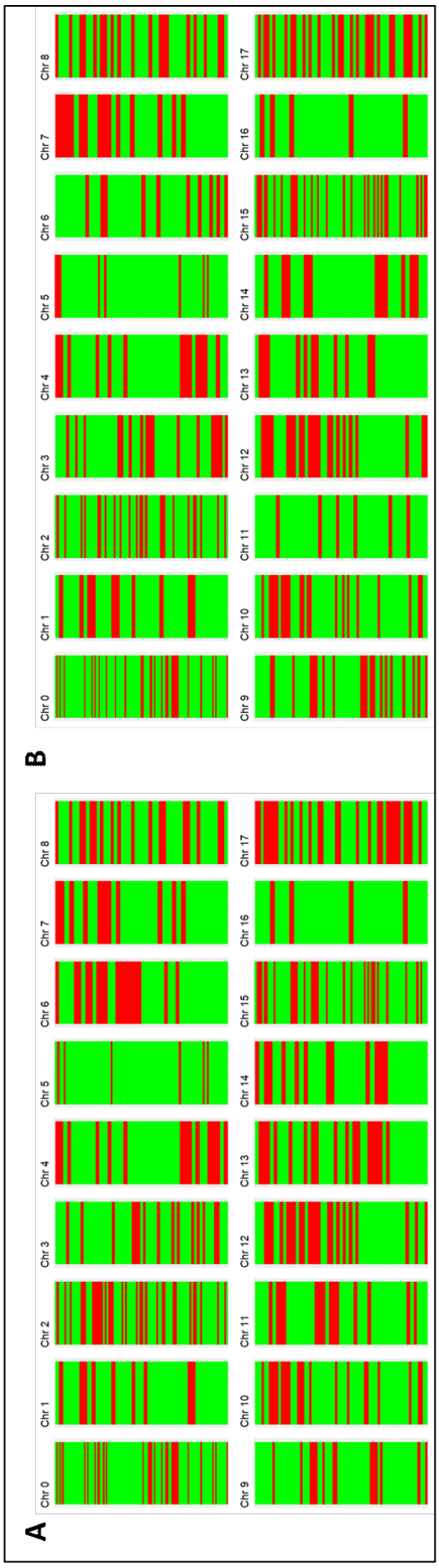

Figure 3.17: Mapping of genomes of the 'Imperial Gala' x P266R231T015 population with the 9K apple-pear SNP array by chromosome for the apple SNPs, while the pear SNPs were all put in one group, Chr0. In green when the results of the SNPs support the hybridity of the F1, red when the results were the same as the mother, blue when the results were the same as the father, and black when the progeny have different results to both mother and father. The mapping A represents the map of the IP26 1 and B IP26 2 progeny.

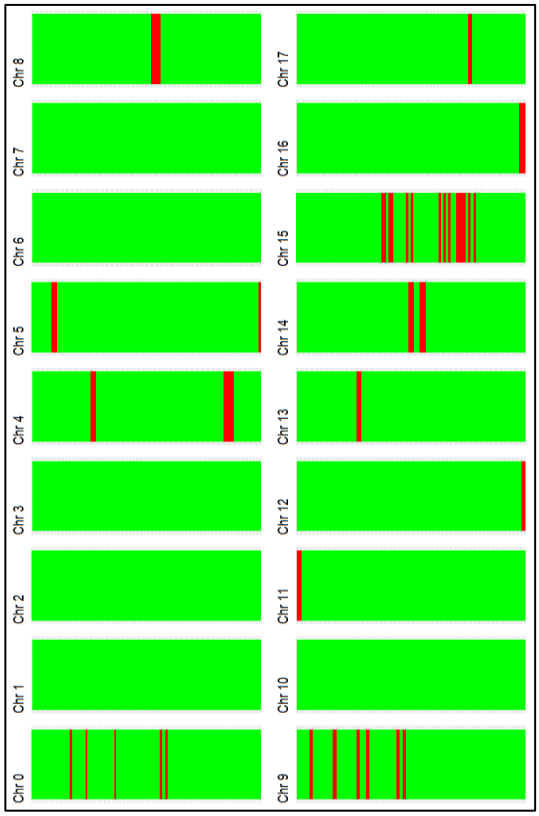

Figure 3.18: Mapping of genomes of the 'Imperial Gala' x P125R095T002 population with the 9K apple-pear SNP array by chromosome for the apple SNPs, while the pear SNPs were all put in one group, Chr0. In green when the results of the SNPs support the hybridity of the F1, red when the results were the same as the mother, blue when the results were the same as the father, and black when the progeny have different results to both mother and father. The mapping represent the map of the IP12 1 and IP12 2 progeny

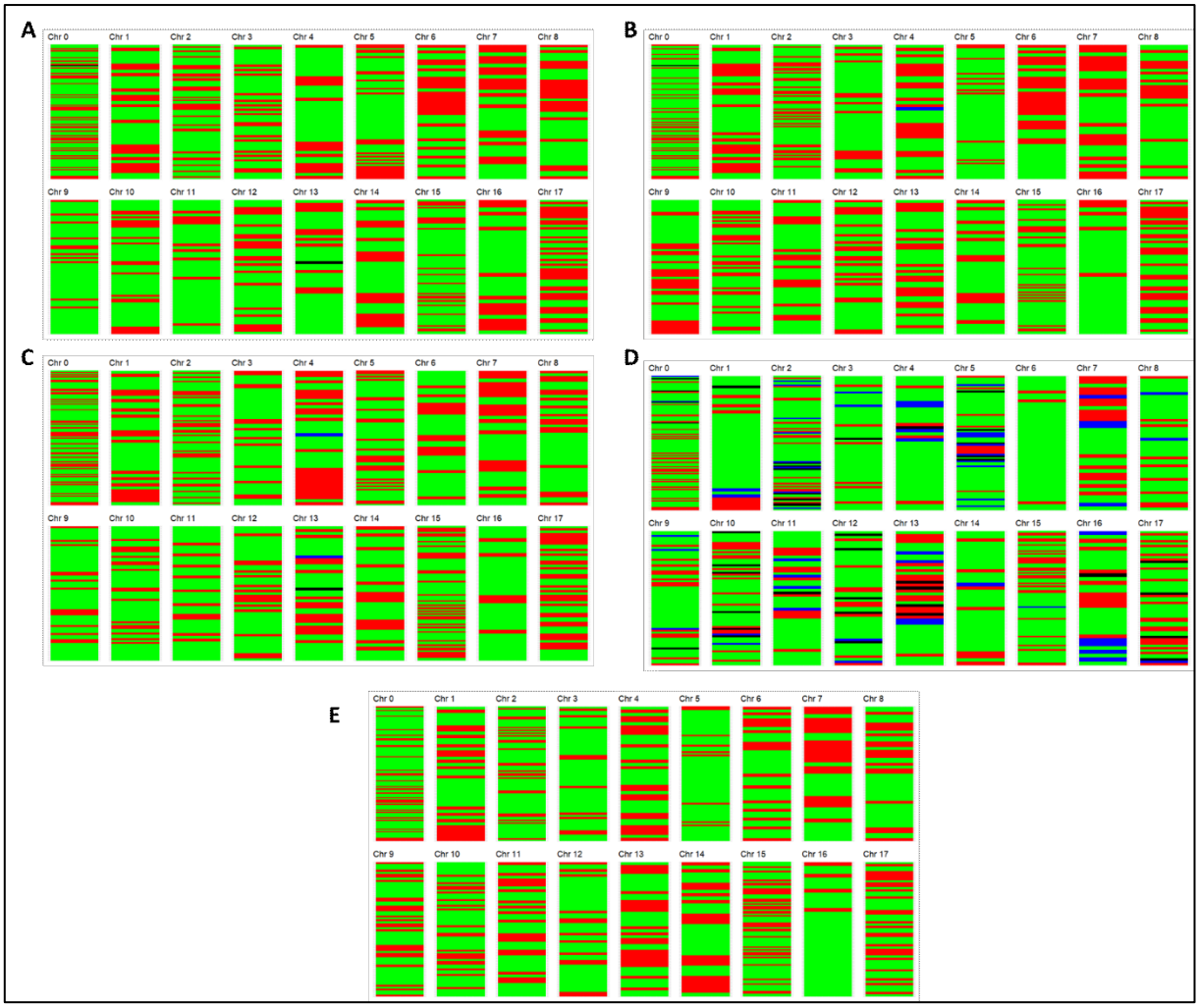

Figure 3.19: Mapping of genomes of the 'Fuji' x P186R125T002 population with the 9K apple-pear SNP array by chromosome for the apple SNPs, while the pear SNPs were all put in one group, Chr0. In green when the results of the SNPs support the hybridity of the F1, red when the results were the same as the mother, blue when the results were the same as the father, and black when the progeny have different results to both mother and father. The mapping A represents the map of the FP18 4 (PN); B the FP18 1, C FP18 2 and FP18 3, D FP18 6 (PN), E FP18 11 (PN) and FP18 10 (PN).

Figure 3.20C is completely different from A and B, with only 61 SNPs supporting the hybridity of progeny FP12 3, while 417 SNPs do not support it. Different from the previous samples, these samples have a large number, 204 SNPs with a different result from the parent, while 105 SNPs originating from the father and 108 SNPs from the mother.

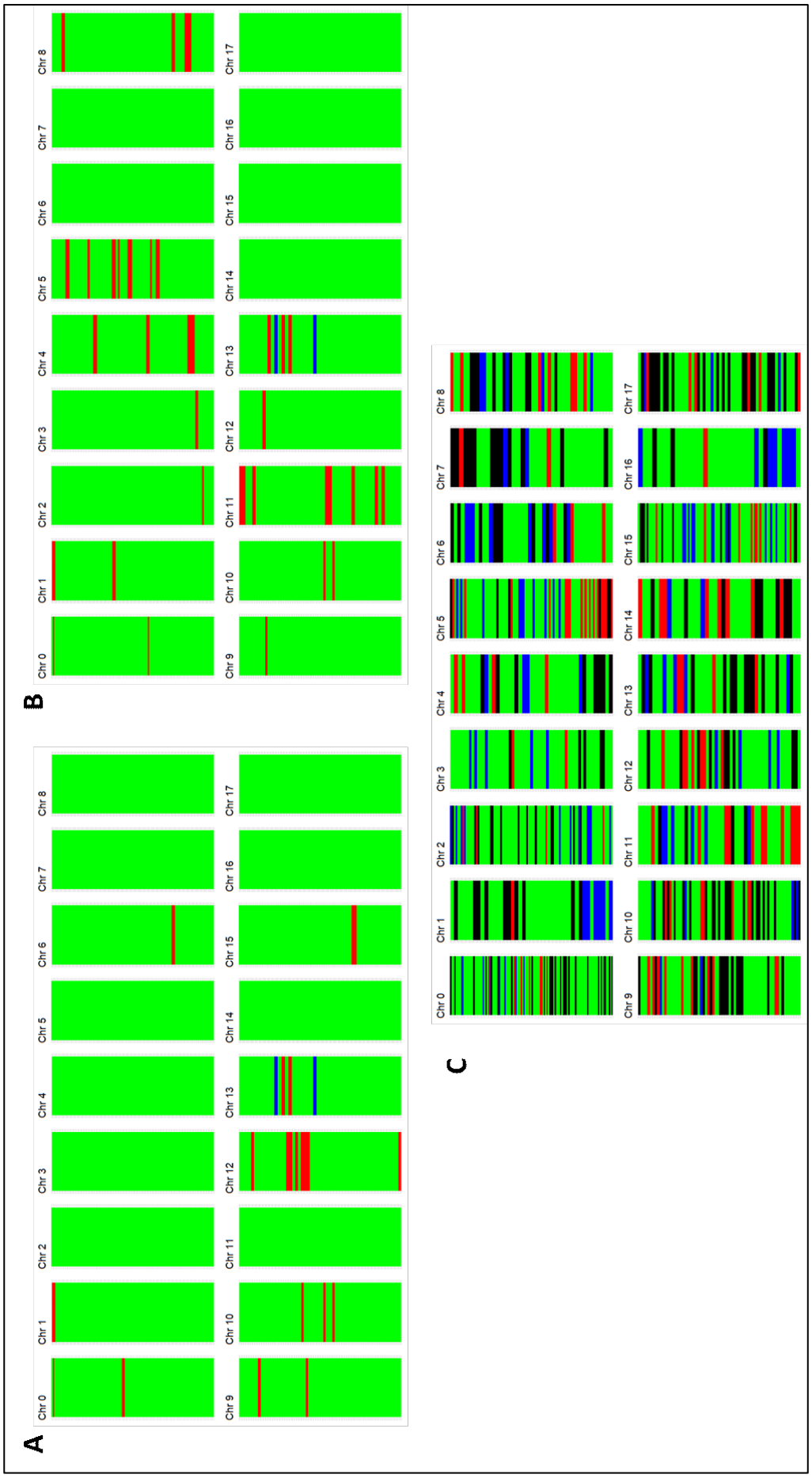

Figure 3.20 Mapping of genomes of the of the 'Fuji' x P125R095T002 population with the 9K apple-pear SNP array by chromosome for the apple SNPs, while the pear SNPs were all put in one group, Chr0. In green when the results of the SNPs support the hybridity of the F1, red when the results were the same as the mother, blue when the results were the same as the father, and black when the progeny have different results to both mother and father. The mapping A represents the map of the FP12 1, FP12 4 (PN), FP12 6 (PN), FP12 7 (PN); B FP12 2 and C FP12 3.

The genomic segmentation maps for the 'Fuji' x P266R231T015 population are reported in Figure 3.21. Figure 3.21A represents the map of FP26 1 and FP26 2 and the Figure 3.21B represents the map of FP263. Both maps are similar with one SNP (A) and zero SNPs (B) with completely different results from the parents; zero SNPs (A) and one SNPs (B) with results as the father, P266R231T015; 325 SNPs (A) and 331 SNPs (B) with results as the mother; 759 (A) and 753 (B) SNPs are supporting the hybridity of this progeny.

The results of the mapping of the 'Fuji' x P354R2200T138 population are shown in Figure 3.22 and Figure 3.23. Figure 3.22A represents the map of the FP35 2, in B FP35 3, in C FP35 4 and in D the samples FP35 5 and FP35 6. Figure 3.23A represent the map of FP35 7; FP35 8 (PN) FP35 12 (PN), FP35 9 (PN) and FP35 13 (PN) in Figure 3.23B, in C the FP35 20 (PN), FP35 14 (PN), FP35 15 (PN), FP35 17 (PN), FP35 18 (PN), FP35 19 (PN); D the samples FP35 22 (PN) and FP35 10 (PN); and in E FP35 1.The mapping of this population have similar result in the Figure 3.22 A, B and D and Figure 3.23 A, B; C and E than the Figure 3.22C and Figure 3.23D. This map has respectively zero SNPs for Figure 3.22 A and D, one SNPs (B), zero for Figure 3.23 C and E, one SNPs (A and B) with completely different results from the parent.

In Figure 3.22A, and Figure 3.23 A, B, C and E have zero SNPs with the same result as the father. 354 SNPs Figure 3.22A, 354 SNPs (B) and 301 SNPs (D) have the same results as the mother. 640 SNPs Figure 3.23A, 334 (B), 353 (C), and 329 (E) have same results as the mother.

63 SNPs (Figure 3.22C) and 41 SNPs (Figure 3.23D) do not support the hybridity of this progeny that exhibits completely different results from the parents. 57 SNPs (Figure 3.22 C) and 57 SNPs (Figure 3.23 D) support the hybridity of this progeny, with the same result as the father. 318 SNPs (Figure 3.22 C) and 227 SNPs (Figure 23 D) support the hybridity of this progeny, with the same results as the mother.

The mapping of this population has similar result for number of SNPs supporting the hybridity of this progeny. 732 SNPs (Figure 3.22 A), 730 SNPs (B), 632 SNPs (C) and 785 SNPs (D) support the hybridity of this progeny. 745 SNPs (Figure 3.23 A), 751 SNPs (B), 733 SNPs (C), 761 SNPs (D) and 757 SNPs (E) support the hybridity of this progeny.

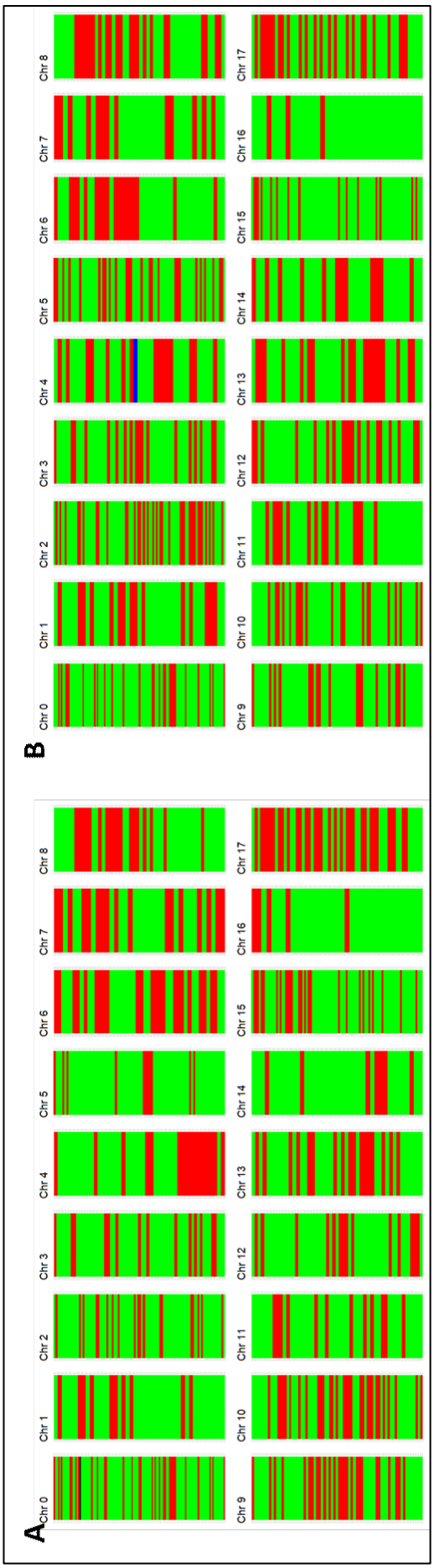

Figure 3.21: Mapping of genomes of the 'Fuji' x P266R231T015 population with the 9K apple-pear SNP array by chromosome for the apple SNPs, while the pear SNPs were all put in one group, Chr0. In green when the results of the SNPs support the hybridity of the F1, red when the results were the same as the mother, blue when the results were the same as the father, and black when the progeny have different results to both mother and father. The mapping A represents the map of the FP26 1 and FP26 2; B FP26 3.

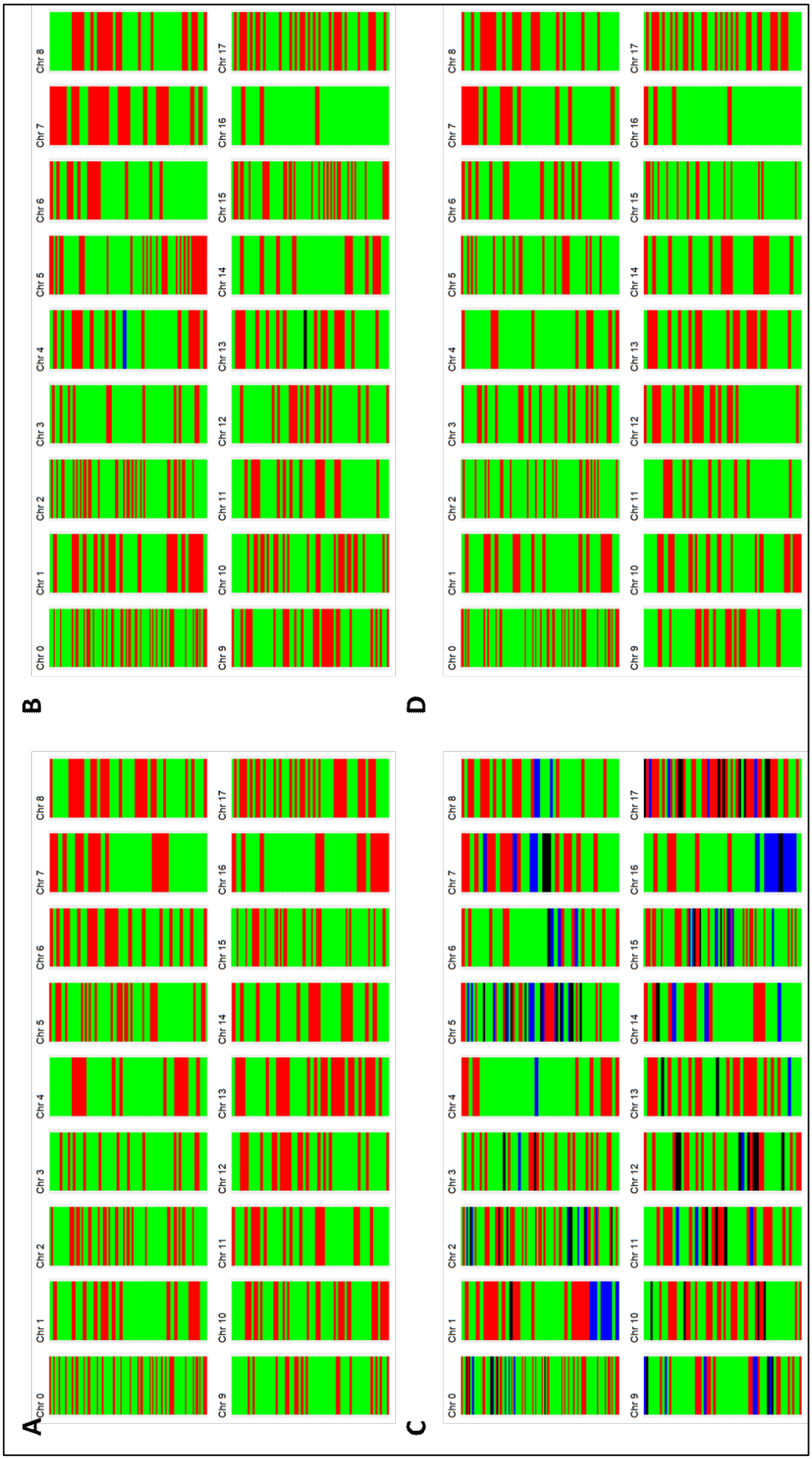

Figure 3.22: Mapping of genomes of the 'Fuji' x P354R200T138 population with the 9K apple-pear SNP array by chromosome for the apple SNPs, while the pear SNPs were all put in one group, Chr0. In green when the results of the SNPs support the hybridity of the F1, red when the results were the same as the mother, blue when the results were the same as the father, and black when the progeny have different results to both mother and father. The mapping A represents the map of the FP35 2, in B FP35 3, in C FP35 4 and in D the samples FP35 5 and FP35 6.

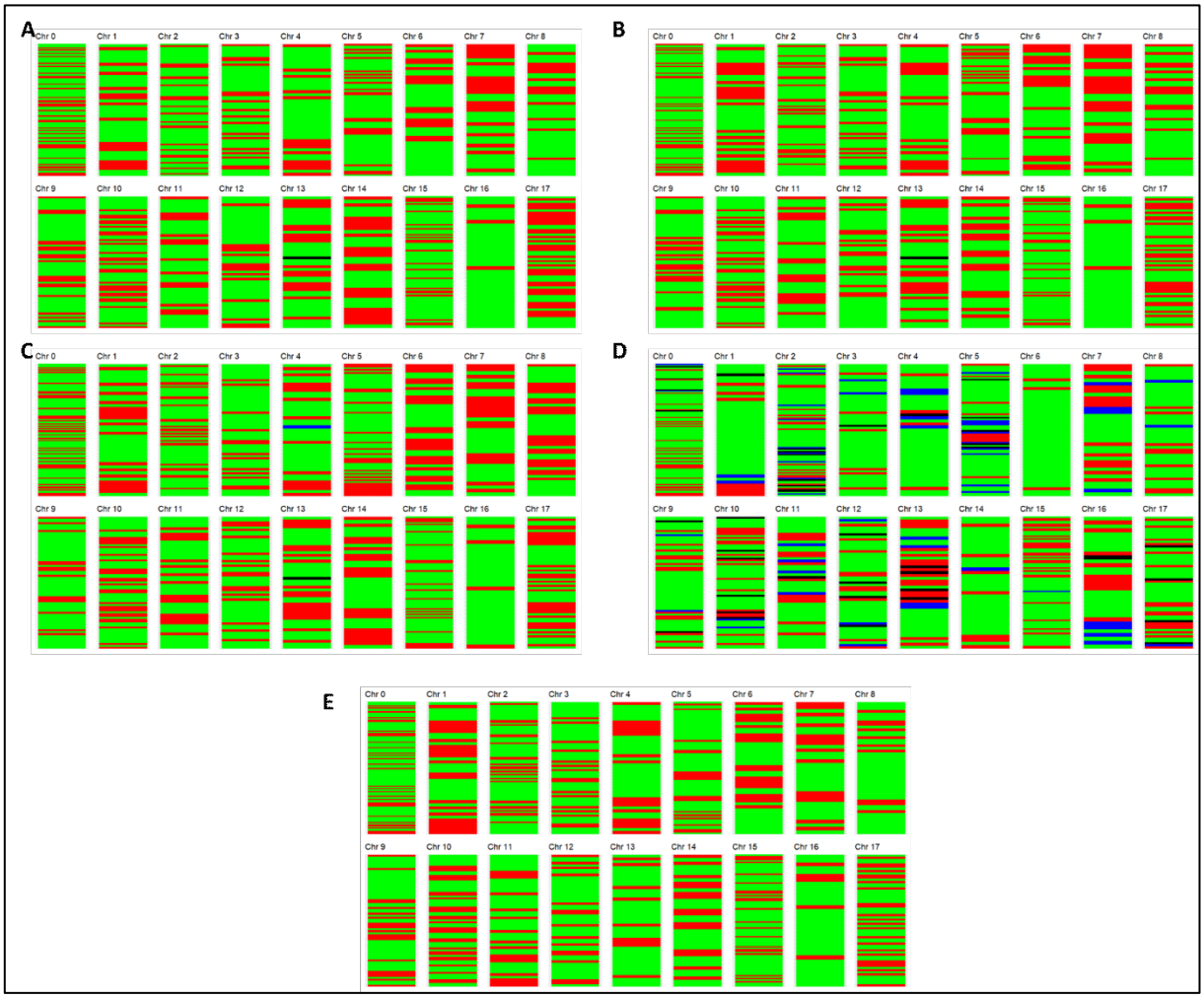

Figure 3.23: Mapping of genomes of the of the 'Fuji' x P354R200T138 population with the 9K apple-pear SNP array by chromosome for the apple SNPs, while the pear SNPs were all put in one group, Chr0. In green when the results of the SNPs support the hybridity of the F1, red when the results were the same as the mother, blue when the results were the same as the father, and black when the progeny have different results to both mother and father. The mapping A represents the map of the FP35 7; FP35 8 (PN) FP35 12 (PN), FP35 9 (PN) and FP35 13 (PN) in Figure 3.23 B, in C the FP35 20 (PN), FP35 14 (PN), FP35 15 (PN), FP35 17 (PN), FP35 18 (PN), FP35 19 (PN); D the samples FP35 22 (PN) and FP35 10 (PN); and in E FP35 1.

For segmentation mapping with the 20K SNP array, the mapping was divided among each of the 17 chromosomes.

Figure 3.24 represents the map for the population 'Abate' x 'Fuji', with 8605 SNPs supporting hybridity of AF, but 215 SNPs do not as they originate from the father and a further 40 originating from the mother.

Figure 3.25 represents the mapping for the population 'Decana' x 'Murray' with the samples DM1 in A and DM2 in B. These two samples have similar results. In Figure 3.25 7896 (A) and 7993 (B) SNPs support the hybridity DM1, 897 (A and B) SNPs do not support the hybridity. Of these 897 SNPs, 13 SNPs (A) and 10 SNPs (B) with completely different result from the parents, 22 SNPs (A) and 13 SNPs (B) with same results as the father and 862 SNPs (A) and 874 SNPs (B) with the same result as the mother.

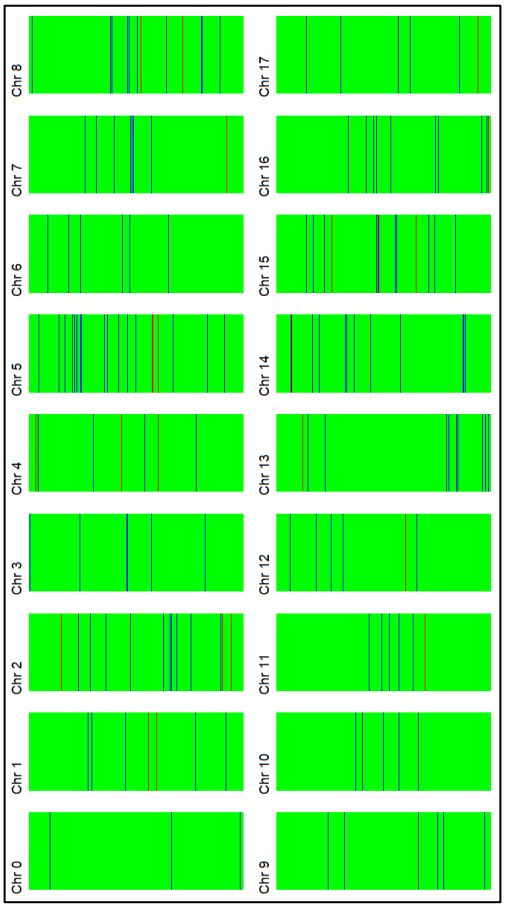

Figure 3.24: Mapping of the 20K SNP- chip array, the mapping was divided per each chromosome (Chr 0,1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17). In green when the results of SNP support the hybridity of the F1, red when the results were the same to the mother, blue when the results were the same to the father and black when the progeny have different results of mother and father. The mapping of the AF.

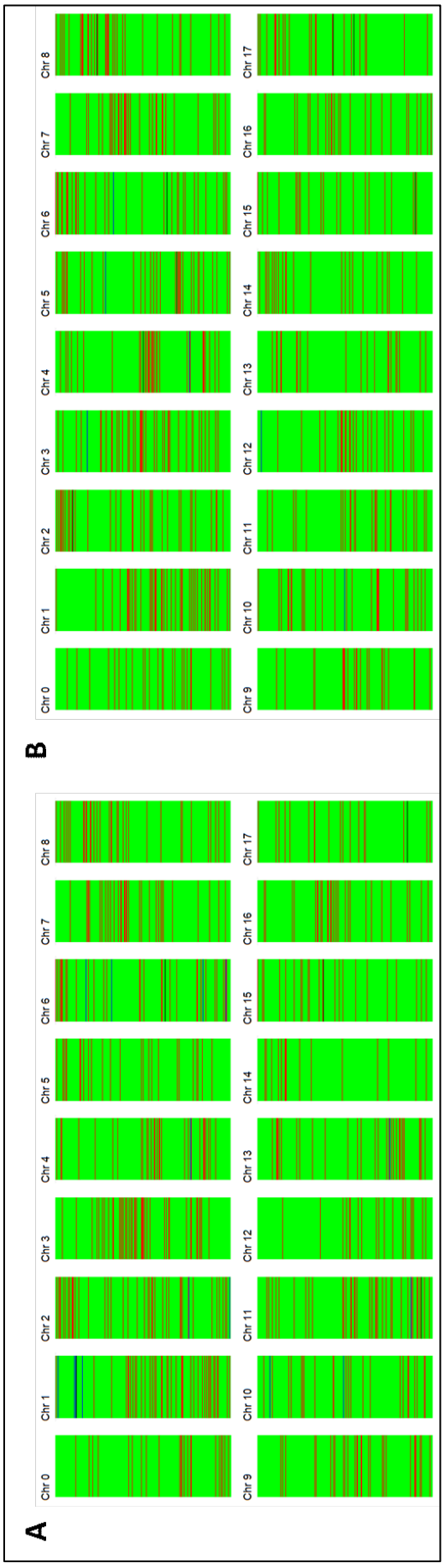

Figure 3.25: Mapping of the 20K SNP- chip array, the mapping was divided per each chromosome (Chr 0,1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17). In green when the results of SNP support the hybridity of the F1, red when the results were the same to the mother, blue when the results were the same to the father and black when the progeny have different results of mother and father. The mapping of the 'Decana' x 'Murray' population. In A the samples DM1 and in B the samples DM2.

3.4.4 DNA content results

Significant differences (p=0.0001) in absolute DNA content of the *Malus* and *Pyrus* genotypes, as well as for the putative hybrids were found by flow cytometry. The DNA content of apples 'Gala' and 'Murray' was on an average 1.51 pg/2C in comparison with the pear cultivar 'Andre´Desportes' of 1.12 pg/2C. The DNA content of Zwintzscher's Hybrid and the 'Abate' x 'Fuji' hybrid was 1.30 pg/2C, which is clearly intermediate between the DNA content of the *Malus* and *Pyrus* parents. The two other putative hybrids ('Decana' x 'Murray' 1, 'Decana' x 'Murray' 2) have a DNA content closer to pear (Table 3.11).

Table 3.11: Cellular DNA content of Zwintzscher's Hybrid, 'Murray' (Malus) 'Gala' (Malus) *in vitro* 'André Desport', 'Abate' x 'Fuji', 'Decana' x 'Murray' 1 and 'Decana' x 'Murray' 2.

Cultivar	Mean DNA content [pg] ± SD
Zwintzscher's Hybrid	1.30 ± 0.01
'Murray' (*Malus*)	1.54 ± 0.01
'Gala' (*Malus*) *in vitro*	1.49 ± 0.01
'André Desportes' (*Pyrus*)	1.12 ± 0.01
'Abate' x 'Fuji'	1.29 ± 0.01
'Decana' x 'Murray' 1	1.19 ± 0.01
'Decana' x 'Murray' 2	1.20 ± 0.01

3.5 Discussion

The SSR markers, chosen from previously published work (Guilford *et al.*, 1997; Liebhard *et al.*, 2002; Yamamoto *et al.*, 2002; Vinatzer *et al.*, 2004; Silfverberg-Dilworth *et al.*, 2006; J. M. Celton *et al.*, 2009; Emeriewen *et al.*, 2020) to have one or more markers on each chromosome for apple or pear or both, suggested that almost all the NZ individuals are hybrids, but closer to the mother than the male parent. This is in contrast to the findings for Zwintzscher's Hybrid and both AF samples which were full hybrids and are perfectly placed between the two parental groups. The SSR results for the five Zwintzscher's Hybrid F2 progeny suggest that these samples are hybrids too, but more similar to the apple than the Zwintzscher's Hybrid. The two offspring from 'Decana' x 'Murray' were not supported to be hybrids by the SSR results described above.

These results were again further confirmed by the Neighbour joining clustering of SSR results using the Dice's index for the NZ population and Euclidean's index for FEM and UniBo materials.

The most informative marker in this study was TsuENH008 with the highest number of alleles at 4.00, number of effective alleles 2.79, Shannon's information index 1.13, observed heterozygosity 0.68 and expected heterozygosity was 0.64. The high level of observed heterozygosity by SSR loci agrees with previous results in pear. Ghosh *et al.* (2006) found the observed heterozygosity of 0.63 by using nine SSR loci in discrimination of 60 pear genotypes (Ghosh *et al.*, 2006). Erfani *et al.* (2012) found the Ho of 0.68 by using 28 SSR loci (Erfani *et al.*, 2012).

The least informative marker used in this study was CH01b12 with the lowest number of alleles at 1.00, number of effective alleles, Shannon's information index, observed heterozygosity and expected heterozygosity were 0.

The high level of observed heterozygosity of the CH01c06 marker in the FEM populations agrees with the results of Liebhard *et al.* (2002). The highest number of alleles observed was 4.33, number of effective alleles 3.82, Shannon's information index 1.28, observed heterozygosity 0.70 and expected heterozygosity was 0.68 in this marker. The less informative marker was Hi21G05a.

In the UniBo population the results of genetic diversity analysis for the highest number of alleles and the number of effective alleles observed were 3.00, Shannon's information index was 1.04, observed heterozygosity 1.00 and expected heterozygosity was 0.63 for the CH05a04 marker. The high level of observed heterozygosity by SSR loci in all populations agrees with the results of Liebhard *et al.* (2002).

Shannon's information index is an important index for assessing the level of polymorphism (Erfani *et al.*, 2012). The SSR markers with the discrimination power low for the parent, higher for the progeny and with a low Shannon's information index were TsuENH008, CH02g01, Ch04e05 and NB105a. The value of PD and I agrees with previous results in pear (Erfani *et al.*, 2012). This demonstrate these markers are more informative than the others which exhibited a discrimination power higher for the parent (0.54), low for the progeny (0.00) and with a higher Shannon's information index (0.28).

The value of CH02g01 and Ch04e05 of PD and I in the results is in agreement with the previous result of Liebhard *et al.* (2002).

CH01b12 was the most informative SSR for the NZ population, and Hi21G05a was the most informative for the FEM population with a PD_Parent=0.72, PD_F1 and PD_F2=0.00 and I=0.30. The CH02c02a marker for 'Abate' x 'Fuji' population and the Hi21g05 and Hi09B04 markers for 'Decana' x 'Murray' population were found to be most informative for this material. The value of CH01b12 of PD and I agrees with previous results of Liebhard *et al.* (2002).

The most informative TsuENH008 marker in the PFR population was in the 'Fuji' x P186R125T002 population.

Compared the alleles observed, number of effective alleles, Shannon's information index, observed heterozygosity and expected heterozygosity in all the population the most significant marker was CH01c06 in the FEM populations. The makers used in the FEM, and UniBo population can give more information results than the PFR population probable because there are more markers with discrimination power low for the parent, higher for the progeny and with a low Shannon's information index than the markers used for PFR population.

For genetic diversity studies, a subset of loci that are highly informative to detect the hybridity or not and, representative of the genome (we used one or more SSRs for chromosome, robust, and well defined are useful) were selected.

The markers used in the HRM analysis seems to be more informative to the SSR markers because almost all the markers used have discrimination power low for the parent, higher for the progeny and with a low Shannon's information index. In the HRM analysis the primers were designed specifically for the parents used for crosses/populations different from the SSR markers used. In the SSR analysis, the markers were found from the bibliography; for this region the HRM analysis seems to be more informative to the SSR markers.

The results of the genetic diversity analysis of the markers used to perform the HRM analysis demonstrated that the highest Na observed was between 2.00 and 2.50, Ne 2.00-2.30, I 0.69-0.83, Ho 0.68-0.58 and He 0.50-0.52 in the marker number 17 (Chr7: 20289452-20289564) in the NZ and FEM populations. In the 'Abate' x 'Fuji' population the highest values of Na and Ne were 2.00, I 0.69, Ho and He 0.50 for the markers 5, 7, 11, 27, 28, 30 and 39 (Chr2: 22240468-22240585, Chr3: 6077512-6077630, Chr5: 8856650-8856736, Chr12: 6731573-6731673, Chr12: 20068163-20068254, Chr13: 26326450-26326524 and Chr16: 29098000-29098108, respectively). In the genetic diversity analysis of the markers used to perform the HRM analysis for the 'Decana' x 'Murray' population the highest Na and Ne obtained were 1.50, I 0.35, Ho 0.00 and He 0.63.

The HRM markers with the lowest PD (0.00) were 31 (Chr14: 2857003-2857097) and these markers were the most informative for NZ, FEM and UniBo population.

The HRM results suggest that the 41 progenies of CO, P26A17 are pear-apple hybrids, as well as the four P265R232T018 x A199R45T055, the two 'Imperial Gala' x P266R231T015, the two 'Imperial Gala' x P125R095T002, the 11 'Fuji' x P186R125T002, the 7 'Fuji' x P125R095T002, the P265R232T018 x 'Scilate', the 23 'Fuji' x P354R200T138 and the five Zwintzscher's Hybrid F2 progeny. It is noteworthy that the apple and pear parents did not appear to contribute equally to the genomes of their progenies, unlike the Zwintzscher's Hybrid and the pear-apple hybrids between 'Abate' x 'Fuji', where the parents did contribute equally to the hybrid genome. Maybe in these cases the parents were more compatible for Zwintzscher's Hybrid and AF showed more

chromosome recombination than the other hybrids. The HRM results for DM1, DM2 and the three 'Fuji' x P266R231T015 progeny suggest that these samples are not hybrids.

These results were confirmed by the Neighbour-joining clustering using the Dice's index for NZ population and Euclidean's index for FEM and UniBo materials.

The HRM and SSR results were further confirmed by the results of the 20K and 9K SNP array, respectively. Unexpectedly, though, the results for some SNPs in the progeny did not agree with those from the mother and father. This might be due to the fact that the two SNP-arrays were designed on different varieties, and/or because some mutations occurred in the progenies, or the SNP-arrays were designed with more apple SNPs than pear SNPs on the 9K chip, or apple SNPs only on the 20K chip. Nevertheless, with the results described here it can be concluded that the SNP-chip is a good approach for such genetic analysis as many samples can be analysed in parallel, with the 9K and 20K SNP arrays showing specific performances based on the germplasm used for their designs.

The hybridity of AF, Zwintzscher's Hybrid and DM progenies were cross-validated with the HRM, SSR and SNP results.

Both SSRs and SNPs are abundant in the genome of most organisms and therefore potentially useful for detecting the population genetic structure and reconstructing the evolutionary history of species (Tsykun e*t al.*, 2017; Montanari *et al.*, 2020; Zurn *et al.*, 2020).

In recent years, SNPs have started to replace SSRs in population genetic studies as well as in a wide range of other applications. SNP arrays can be used with a large number of samples at a lower cost than the SSR markers and HRM analysis, with the latter being not as costly as the SSRs. The issue of SNP-chip analysis is that they are very specific to the species used (Montanari *et al.*, 2020).

The methodology applied in this study used 39 primers, which detect different SNPs than those on the 9K and 20K arrays. In comparison, the number of filtered SNPs ranged between 1090 and 1061 for the analysis with the 9K array, and between 8860 and 8890 for the analysis with the 20K array.

In this study, good results were achieved with the HRM analysis, as the primers were designed and tested specifically for the apple and pear hybrids available in this study and can maybe be used in future work in combination with the 20K array (Bianco *et al.*, 2014) and 70K array (Montanari *et al.*, 2019) to provide improved results for the apple-pear hybrids. Main issue regarding the SNP-array results in this study was the balance of the apple and pear SNPs used, as for the sample analysis with the 9K SNP assay (Verde *et al.*, 2012) almost all pear SNPs were removed after the screen. SNP-chip results were limited compared with the HRM ones, as the SNPs used for the SNP-chip analysis were specific to the material used in the SNP array design. This problem has

also been shown for the SSR markers used for NZ population (Yamamoto *et al.*, 2002; Liebhard *et al.*, 2002; Saito *et al.*, 2003; Vinatzer *et al.*, 2004; Celton *et al.*, 2009). A repeated SSR analysis with the markers used for the FEM and UniBo populations gave better results, as different markers were used (Guilford *et al.*, 1997; Liebhard *et al.*, 2002; Silfverberg-Dilworth *et al.*, 2006). From this it was concluded that these markers are more suitable for the hybrids compared to the markers used for the NZ populations.

The estimation of nuclear DNA content is one of the important applications of flow cytometry and a reliable and efficient method for the characterization of plant nuclear DNA content. Nuclear DNA content represents an important biodiversity character with fundamental biological significance (Bennett *et al.*, 2000). Studies on nuclear DNA content within *Malus* and *Pyrus* in the Rosaceae family, and hybrids between *Malus* x *Pyrus* have been reported previously (Dickson *et al.*, YEAR; Tatum *et al.*, 2005; Höfer and Meister, 2010; Fischer *et al.*, 2014).

In this present study, nuclear DNA content of two apples ('Murray', 'Gala'), a pear ('André Desportes'), Zwintzscher's Hybrid, a seedling from 'Abate' x 'Fuji', and two seedlings from 'Decana' x 'Murray' was determined using flow cytometric analysis. The nuclear DNA content of the two apple cultivars analysed was almost equal and that of the pear cultivar analysed was 1.12 pg/2C. The nuclear DNA contents of Zwintzscher's Hybrid and 'Abate' x 'Fuji' were almost equal and intermediate between apple and pear, thus confirming the findings of the HRM and SSR results, which demonstrated the hybridity for these two samples. The nuclear DNA contents of the two putative pear-apple hybrids DM1 and DM2 were almost similar to the 'André Desportes' pear cultivar.

3.6 Conclusion

In conclusion, this research demonstrated that SSR and SNP markers as well as DNA content can be useful tools to assess the degree of hybridity of samples derived from crosses between apple and pear.

The SSR and HRM markers were more powerful than the SNP-chip probably due to the species specificity of these markers and the low number of samples analysed (Montanari *et al.*, 2020).

SSR analysis gave better results compared with the SNP-chip, but with the same problem of specificity of the SSR markers for apple or pear. In conclusion, when all the molecular markers used were compared here the best and most robust analysis was the HRM method, because for this analysis the primers were designed specifically for the parents used for crosses/populations ('Cox's Orange Pippin' and 'Fuji') in this study and were pre-screened on the parents and hybrids.

One drawback of the HRM analysis compared with the SNP array is the low number of pairs of primers, only 39, designed and used in this study.

The evaluated nuclear DNA contents levels are useful descriptors for identifying/demonstrating putative hybrids between pear and apple. The nuclear DNA content analysis demonstrated that AF is a true pear-apple hybrid, and the DM1 and DM2 are close to the pear, suggesting they are not intergeneric hybrids.

Our results suggest that the NZ F1 are all hybrids, but the parents did not contribute equally to the hybrid genome. Nevertheless, putative introgressions for fire blight and scab resistance are traits of interest for backcrossing into new cultivars, i.e. this method can be used to identify apple/ pear hybrids for use in breeding novel crops by introducing intergeneric traits from the pear gene pool into apple and *vice versa*.

3.7 References

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3.8 Supplementary

3.8.1 Data analysis

3.8.1.1 Structure of datebase

```
> str(AF)
Classes ‘tbl_df’, ‘tbl’ and 'data.frame': 8860 obs. of  4 variables:
 $ Name: chr  "SNP_FB_1055488" "SNP_FB_1115014"
"RosBREEDSNP_SNP_GT_16455193_1_00107_MAF30_475684_exon1"
"SNP_FB_0441428" ...
 $ Abate: chr  "BB" "BB" "AA" "BB" ...
 $ Fuji : chr  "AB" "AA" "AA" "BB" ...
 $ AF   : chr  "AB" "AB" "AA" "BB" ...

> str(newMap)
Classes ‘tbl_df’, ‘tbl’ and 'data.frame':   20017 obs. of  3 variables:
 $ Chr     : num  0 0 0 0 0 0 0 0 0 0 ...
 $ SNP     : chr  "GDsnp01263" "GDsnp01667" "GDsnp00544" "MdBFTa" ...
 $ Position: num  0 0 0 0 0 0 0 0 0 0 ...
```

3.8.1.2 R script

```
##### R package
require(ggplot2)
require(gridExtra)

chromo.plot(AF,newMap,3)
#position 1 = column, position 2 = database, posizione 3 = results of the column AF

###### input
chromo.plot<-function(foglio,database,colfiglio){

  matrice=as.data.frame(foglio)
  posizioni=as.data.frame(database)
  figlio=colfiglio
```

```
 ######## etichette colori
combo.res=NULL
for(i in 1:nrow(matrice)){
  combo=paste(matrice[i,2], matrice[i,3], matrice[i,figlio])
  combo.res=rbind(combo.res, combo)
}

combo.res2=combo.res
combo.res2=gsub("AA AA AA", "green", combo.res2)
combo.res2=gsub("AA AA AB", "green", combo.res2)
combo.res2=gsub("AA AA BB", "black", combo.res2)
combo.res2=gsub("AA AB AA", "green", combo.res2)
combo.res2=gsub("AA AB AB", "green", combo.res2)

combo.res2=gsub("AA AB BB", "blue", combo.res2)
combo.res2=gsub("AA BB AA", "red", combo.res2)
combo.res2=gsub("AA BB AB", "green", combo.res2)
combo.res2=gsub("AA BB BB", "blue", combo.res2)
combo.res2=gsub("AB AA AA", "green", combo.res2)

combo.res2=gsub("AB AA AB", "green", combo.res2)
combo.res2=gsub("AB AA BB", "red", combo.res2)
combo.res2=gsub("AB AB AB", "green", combo.res2)
combo.res2=gsub("AB AB AA", "green", combo.res2)#
combo.res2=gsub("AB AB BB", "green", combo.res2)#
combo.res2=gsub("AB BB AA", "red", combo.res2)
combo.res2=gsub("AB BB AB", "green", combo.res2)

combo.res2=gsub("AB BB BB", "green", combo.res2)
combo.res2=gsub("BB AA AA", "blue", combo.res2)
combo.res2=gsub("BB AA AB", "green", combo.res2)
combo.res2=gsub("BB AA BB", "red", combo.res2)
combo.res2=gsub("BB AB AA", "blue", combo.res2)
```

```
combo.res2=gsub("BB AB AB", "green", combo.res2)
combo.res2=gsub("BB AB BB", "green", combo.res2)
combo.res2=gsub("BB BB AA", "black", combo.res2)
combo.res2=gsub("BB BB AB", "green", combo.res2)
combo.res2=gsub("BB BB BB", "green", combo.res2)

combo.res2=gsub("AA AA NC", "out", combo.res2)
combo.res2=gsub("AA AB NC", "out", combo.res2)
combo.res2=gsub("AA BB NC", "out", combo.res2)
combo.res2=gsub("AB AA NC", "out", combo.res2)
combo.res2=gsub("AB AB NC", "out", combo.res2)
combo.res2=gsub("AB BB NC", "out", combo.res2)
combo.res2=gsub("BB AA NC", "out", combo.res2)
combo.res2=gsub("BB AB NC", "out", combo.res2)
combo.res2=gsub("BB BB NC", "out", combo.res2)

matrice2=cbind(matrice,combo.res2)

### ricerca nome
matrice3=NULL
for(n in 1:nrow(matrice2)){
 elem.riga1=matrice2[n,]
 elem.riga2=subset(posizioni, SNP==matrice2[n,1])
 if(length(elem.riga2)==0){elem.riga2[1,]<-c(NA,NA,NA)} else {elem.riga2[1,]=elem.riga2[1,]}
 riga.matrice2=cbind(elem.riga1, elem.riga2, row.names = NULL)
 matrice3=rbind(matrice3, riga.matrice2)
}

#### rimozione OUT
row.rem=matrice3$combo.res2=="out"
matrice4=matrice3[!row.rem,]

#### rimozione NA
```

```
  row.rem.na=is.na(matrice4$Chr)
  matrice5=matrice4[!row.rem.na,]

  ##### graphic
  all.p=list()
  for(i in sort(unique(matrice5$Chr))){
    matrice5s=subset(matrice5, Chr==i)
    matrice6=cbind(ORD=1:nrow(matrice5s), OCC=rep(1, nrow(matrice5s)),
matrice5s[order(matrice5s$Chr, matrice5s$Position),])
    matrice7=matrice6[,c(2,(ncol(matrice)+3),1)]

    colnames(matrice7)<-c("OCC","COL","LAB")
    matrice6$LAB<-as.character(matrice7$LAB)
    matrice6$COL<-as.character(matrice7$COL)

    colori=as.character(unique(matrice7$COL))
    matrice7=as.data.frame(matrice7)

    matrice8=matrice7[order(nrow(matrice7):1),]

    matrice8$LAB <- factor(matrice8$LAB, levels = matrice8$LAB)

    p=ggplot(data=matrice8, aes(x=LAB, y=OCC, fill=COL)) +
      geom_bar(stat="identity", width = 1)+
      scale_fill_manual(values = sort(colori))+
      coord_flip()+
      theme(axis.title.x=element_blank(),
            axis.text.x=element_blank(),
            axis.ticks.x=element_blank())+
      theme(axis.title.y=element_blank(),
            axis.text.y=element_blank(),
            axis.ticks.y=element_blank())+
      theme(legend.position="none")+
      ggtitle(paste("Chr", unique(matrice5s$Chr)))
```

```
    all.p[[i+1]]=p

  }

  grid.arrange(all.p[[1]],
               all.p[[2]],
               all.p[[3]],
               all.p[[4]],
               all.p[[5]],
               all.p[[6]],
               all.p[[7]],
               all.p[[8]],
               all.p[[9]],
               all.p[[10]],
               all.p[[11]],
               all.p[[12]],
               all.p[[13]],
               all.p[[14]],
               all.p[[15]],
               all.p[[16]],
               all.p[[17]],
               all.p[[18]],
               ncol = 9, nrow=2)
}
```

3.8.2 Genetic diversity SSR results

S 3.1: SSR Genetic diversity results of 'Cox's Orange Pippin' x 'Old Home' population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He), allele range and discrimination power at each locus for parent and F1 (PD).

	CH01b12	CH01g05	CH01h02	CH02b10	CH02b12	CH02c02a	CH2c09	CH02d11	CH02g01	CH03g06	CH04c06
Na	3.00	2.50	3.00	1.50	2.00	1.50	3.00	3.00	3.00	3.00	2.00
Ne	2.65	1.96	2.76	1.48	2.00	1.30	2.62	1.75	1.98	2.68	1.74
I	1.03	0.70	1.06	0.34	0.69	0.28	1.02	0.67	0.73	1.04	0.60
Ho	0.68	0.50	0.87	0.00	0.50	0.25	0.66	0.06	0.26	0.91	0.00
He	0.62	0.41	0.64	0.24	0.50	0.19	0.62	0.42	0.43	0.63	0.41
Allele Range	142 - 177	162 - 191	223 - 267	150 - 150	151 - 159	159 - 174	255 - 274	130 - 165	218 - 246	155 - 188	188 - 193
PD_Parent	0.79	0.79	0.79	0.00	0.63	0.54	0.79	0.63	0.79	0.79	0.63
PD_F1	0.78	0.34	0.80	0.62	0.63	0.00	0.77	0.53	0.38	0.78	0.49

	CH4c10	Ch04e05	CH05a02	CH05a04	CH05d04	ChVf1	NH013a	NH021a	TsuENH004	TsuENH008
Na	3.00	3.50	3.00	3.00	3.00	3.50	3.00	2.50	4.00	1.50
Ne	2.68	3.06	2.70	2.67	2.82	2.74	2.53	2.14	2.79	1.50
I	1.04	1.09	1.04	1.04	1.07	1.07	1.01	0.80	1.13	0.35
Ho	0.66	0.99	0.66	0.89	0.61	0.91	0.46	0.25	0.68	0.00
He	0.63	0.64	0.63	0.63	0.64	0.64	0.60	0.50	0.64	0.25
Allele Range	174 - 202	193 - 219	122 - 154	173 - 199	188 - 214	152 - 186	187 - 270	159 - 186	164 - 184	154 - 154
PD_Parent	0.79	0.89	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.63
PD_F1	0.78	0.66	0.79	0.78	0.81	0.80	0.76	0.55	0.82	0.00

S 3.2: SSR Genetic diversity results of P265R232T018 x A174R01T204 population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He), allele range and discrimination power at each locus for parent and F1 (PD).

	CH01g05	**CH01h02**	**CH02b12**	**CH02c02a**	**CH2c09**	**CH02d11**	**CH02g01**	**CH03d02**	**CH03g06**	**CH4c10**
Na	2.50	2.50	2.00	2.50	2.50	1.50	2.00	1.50	2.50	2.50
Ne	2.33	2.33	2.00	2.33	2.33	1.50	2.00	1.50	2.33	2.33
I	0.87	0.87	0.69	0.87	0.87	0.35	0.69	0.35	0.87	0.87
Ho	0.75	0.75	0.50	0.75	0.75	0.00	0.50	0.00	0.75	0.75
He	0.56	0.56	0.50	0.56	0.56	0.25	0.50	0.25	0.56	0.56
Allele Range	158 - 162	223 - 255	155 - 159	159 - 194	249 - 274	138 - 149	206 - 246	201 - 218	155 - 188	159 - 202
PD_Parent	0.79	0.79	0.63	0.79	0.79	0.63	0.63	0.63	0.79	0.79
PD_F1	0.63	0.63	0.63	0.63	0.63	0.00	0.63	0.00	0.63	0.63

	Ch04e05	**CH05a02**	**CH05a04**	**CH05d04**	**ChVf1**	**NH021a**	**TsuENH004**	**TsuENH008**	**TsuENH058**	**NB105a**
Na	2.00	1.50	3.00	2.50	2.00	1.50	2.00	1.50	2.00	2.00
Ne	1.83	1.50	3.00	2.33	2.00	1.50	1.80	1.50	2.00	1.83
I	0.52	0.35	1.04	0.87	0.69	0.35	0.63	0.35	0.69	0.52
Ho	0.25	0.00	1.00	0.75	0.50	0.00	0.75	0.00	0.50	0.25
He	0.31	0.25	0.63	0.56	0.50	0.25	0.44	0.25	0.50	0.31
Allele Range	193 - 219	154 - 154	109 - 206	188 - 208	152 - 193	159 - 171	174 - 184	154 - 183	311 - 369	169 - 199
PD_Parent	0.79	0.63	0.89	0.79	0.63	0.63	0.54	0.63	0.63	0.79
PD_F1	0.00	0.00	0.63	0.63	0.63	0.00	0.63	0.00	0.63	0.00

S 3.3: SSR Genetic diversity results of P265R232T018 x A199R45T055 population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He), allele range and discrimination power at each locus for parent and F1 (PD).

	CH01b12	**CH01g05**	**CH02b10**	**CH02g01**	**CH03d02**	**CH03g06**	**Ch04e05**	**CH05a04**
Na	1.50	2.50	2.50	2.00	2.50	2.50	3.00	2.50
Ne	1.40	2.23	2.23	1.83	2.50	2.23	2.83	2.23
I	0.32	0.84	0.84	0.52	0.90	0.84	1.07	0.84
Ho	0.00	0.25	0.25	0.25	0.00	0.25	0.58	0.25
He	0.22	0.53	0.53	0.31	0.58	0.53	0.65	0.53
Allele Range	142 - 142	158 - 175	150 - 159	206 - 246	199 - 235	175 - 188	193 - 221	109 - 183
PD_Parent	0.00	0.51	0.51	0.51	0.41	0.51	0.51	0.51
PD_F1	0.37	0.37	0.37	0.00	0.54	0.37	0.54	0.37

	CH05d04	**ChVf1**	**NH013a**	**NH021a**	**TsuENH004**	**TsuENH008**	**NB105a**
Na	2.50	2.50	2.00	2.00	2.00	1.50	2.50
Ne	2.23	2.23	1.90	1.90	1.50	1.50	2.50
I	0.84	0.84	0.66	0.66	0.43	0.35	0.90
Ho	0.25	0.25	0.00	0.00	0.17	0.00	0.00
He	0.53	0.53	0.47	0.47	0.25	0.25	0.58
Allele Range	188 - 208	161 - 193	187 - 232	159 - 186	178 - 184	154 - 183	169 - 193
PD_Parent	0.51	0.51	0.41	0.41	0.00	0.41	0.41
PD_F1	0.37	0.37	0.37	0.37	0.42	0.00	0.54

S 3.4: SSR Genetic diversity results of 'Imperial Gala' x P266R231T015 population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He), allele range and discrimination power at each locus for parent and F1 (PD).

	CH01b12	**CH01g05**	**CH02b10**	**CH02c02a**	**CH2c09**	**CH02d11**	**CH02g01**	**CH03d02**	**CH03g06**	**CH04c06**	**CH4c10**
Na	2.50	2.00	2.00	1.50	2.00	2.50	2.50	2.50	2.50	2.00	2.50
Ne	2.33	1.80	2.00	1.50	2.00	2.33	2.33	2.33	2.33	1.83	2.33
I	0.87	0.63	0.69	0.35	0.69	0.87	0.87	0.87	0.87	0.52	0.87
Ho	0.25	0.25	0.00	0.00	0.50	0.25	0.75	0.25	0.25	0.25	0.25
He	0.56	0.44	0.50	0.25	0.50	0.56	0.56	0.56	0.56	0.31	0.56
Allele Range	142 - 177	158 - 175	155 - 159	159 - 159	249 - 274	134 - 165	195 - 246	199 - 218	155 - 188	181 - 203	153 - 202
PD_Parent	0.63	0.63	0.63	0.00	0.63	0.63	0.79	0.79	0.63	0.79	0.79
PD_F1	0.79	0.54	0.63	0.63	0.63	0.79	0.63	0.63	0.79	0.00	0.63

	Ch04e05	**CH05a02**	**CH05d04**	**ChVf1**	**NH013a**	**NH021a**	**TsuENH004**	**TsuENH008**	**TsuENH058**	**NB105a**
Na	1.50	2.00	2.50	1.50	2.00	2.50	2.00	1.50	2.00	2.50
Ne	1.30	2.00	2.33	1.50	2.00	2.33	1.83	1.50	1.83	2.33
I	0.28	0.69	0.87	0.35	0.69	0.87	0.52	0.35	0.52	0.87
Ho	0.25	0.00	0.25	0.00	0.00	0.25	0.25	0.00	0.25	0.25
He	0.19	0.50	0.56	0.25	0.50	0.56	0.31	0.25	0.31	0.56
Allele Range	193 - 221	154 - 154	188 - 214	161 - 170	187 - 232	159 - 186	174 - 184	154 - 183	299 - 369	169 - 181
PD_Parent	0.54	0.63	0.63	0.63	0.63	0.79	0.79	0.63	0.79	0.79
PD_F1	0.00	0.63	0.79	0.00	0.63	0.63	0.00	0.00	0.00	0.63

S 3.5: SSR Genetic diversity results of 'Imperial Gala' x P125R095T002 population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He), allele range and discrimination power at each locus for parent and F1 (PD).

	CH01b12	**CH01g05**	**CH01h02**	**CH02b10**	**CH02c02a**	**CH2c09**	**CH02g01**	**CH03g06**	**CH04c06**	**CH4c10**
Na	2.00	2.50	2.50	2.00	1.00	3.00	2.00	3.00	2.50	3.00
Ne	2.00	2.33	2.33	2.00	1.00	2.67	1.83	2.67	2.33	2.67
I	0.69	0.87	0.87	0.69	0.00	1.04	0.52	1.04	0.87	1.04
Ho	0.00	0.25	0.25	0.50	0.00	0.50	0.25	0.50	0.25	0.50
He	0.50	0.56	0.56	0.50	0.00	0.63	0.31	0.63	0.56	0.63
Allele Range	142 - 142	158 - 175	223 - 267	154 - 159	159 - 159	249 - 261	195 - 246	155 - 182	181 - 203	153 - 202
PD_Parent	0.63	0.63	0.63	0.63	0.00	0.79	0.79	0.79	0.79	0.79
PD_F1	0.63	0.79	0.79	0.63	0.00	0.79	0.00	0.79	0.63	0.79

	Ch04e05	**CH05a02**	**CH05a04**	**CH05d04**	**ChVf1**	**NH002b**	**NH013a**	**TsuENH004**	**TsuENH008**	**NB105a**
Na	1.50	2.50	2.50	2.50	3.00	1.50	1.50	2.50	2.00	2.00
Ne	1.50	2.33	2.33	2.33	2.67	1.50	1.50	2.33	1.83	1.83
I	0.35	0.87	0.87	0.87	1.04	0.35	0.35	0.87	0.52	0.52
Ho	0.00	0.25	0.25	0.25	0.50	0.00	0.00	0.25	0.25	0.25
He	0.25	0.56	0.56	0.56	0.63	0.25	0.25	0.56	0.31	0.31
Allele Range	193 - 221	146 - 154	183 - 206	196 - 208	152 - 193	198 - 198	187 - 232	174 - 184	154 - 193	169 - 190
PD_Parent	0.63	0.63	0.79	0.79	0.79	0.63	0.63	0.79	0.79	0.79
PD_F1	0.00	0.79	0.63	0.63	0.79	0.00	0.00	0.63	0.00	0.00

S 3.6: SSR Genetic diversity results of 'Fuji' x P186R125T002 population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He), allele range and discrimination power at each locus for parent and F1 (PD).

	CH01b12	**CH01g05**	**CH01h02**	**CH02b10**	**CH02c02a**	**CH2c09**	**CH02d11**	**CH02g01**	**CH03g06**	**CH04c06**	**CH4c10**	**Ch04e05**
Na	1.50	2.50	3.50	3.00	3.00	3.50	3.00	2.50	3.50	3.00	3.50	3.00
Ne	1.33	2.29	2.72	2.83	2.83	2.83	2.30	2.33	3.41	2.74	2.83	2.81
I	0.29	0.83	1.09	1.07	1.07	1.11	0.89	0.86	1.23	1.05	1.11	1.06
Ho	0.00	0.57	0.43	0.57	0.82	0.52	0.14	0.25	0.77	0.52	0.52	0.52
He	0.20	0.52	0.63	0.65	0.65	0.65	0.56	0.56	0.70	0.63	0.65	0.64
Allele Range	142 - 142	158 - 162	223 - 263	150 - 159	159 - 194	249 - 261	134 - 165	195 - 246	162 - 188	177 - 188	140 - 205	193 - 221
PD_Parent	0.00	0.54	0.79	0.79	0.79	0.79	0.63	0.79	0.89	0.79	0.79	0.79
PD_F1	0.56	0.81	0.80	0.81	0.81	0.83	0.78	0.62	0.80	0.80	0.83	0.81

	CH05a02	**CH05a04**	**CH05d04**	**ChVf1**	**NH002b**	**NH013a**	**NH021a**	**TsuENH004**	**TsuENH008**	**TsuENH058**	**NB105a**
Na	2.00	2.00	2.50	3.50	2.00	2.50	2.50	2.00	4.00	2.00	2.50
Ne	1.79	1.93	2.27	3.41	1.60	2.05	2.33	1.99	3.30	1.99	2.33
I	0.63	0.67	0.84	1.23	0.50	0.78	0.86	0.69	1.25	0.69	0.86
Ho	0.25	0.00	0.14	0.77	0.00	0.48	0.25	0.50	0.73	0.00	0.25
He	0.44	0.48	0.55	0.70	0.33	0.49	0.56	0.50	0.68	0.50	0.56
Allele Range	146 - 154	109 - 206	188 - 199	152 - 186	154 - 198	187 - 232	159 - 171	174 - 184	160 - 193	299 - 369	169 - 181
PD_Parent	0.54	0.63	0.63	0.89	0.63	0.54	0.79	0.63	0.89	0.63	0.79
PD_F1	0.62	0.60	0.76	0.80	0.29	0.77	0.62	0.62	0.80	0.62	0.62

S 3.7: SSR Genetic diversity results of 'Fuji' x P125R095T002 population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He), allele range and discrimination power at each locus for parent and F1 (PD).

	CH01b12	CH01g05	CH01h02	CH02b10	CH02b12	CH02c02a	CH2c09	CH02d11	CH02g01	CH03d02	CH03g06	CH04c06
Na	1.50	2.50	3.00	2.00	2.00	2.50	3.00	3.00	2.50	2.50	3.50	3.50
Ne	1.16	1.97	2.42	1.98	1.84	2.09	2.50	2.81	2.18	1.67	3.48	3.48
I	0.21	0.76	0.96	0.69	0.65	0.78	1.00	1.01	0.82	0.60	1.24	1.24
Ho	0.00	0.39	0.32	0.00	0.00	0.68	0.64	0.36	0.25	0.07	0.86	0.86
He	0.12	0.47	0.58	0.49	0.45	0.49	0.60	0.61	0.52	0.38	0.71	0.71
Allele Range	142 - 142	158 - 162	223 - 263	150 - 153	155 - 159	159 - 194	249 - 261	138 - 165	195 - 246	199 - 235	169 - 188	177 - 203
PD_Parent	0.00	0.54	0.79	0.63	0.63	0.54	0.79	0.63	0.79	0.63	0.89	0.89
PD_F1	0.40	0.75	0.70	0.62	0.57	0.77	0.75	0.87	0.57	0.43	0.81	0.81

	CH4c10	Ch04e05	CH05a02	CH05d04	ChVf1	NH013a	NH021a	TsuENH004	TsuENH008	TsuENH058	NB105a
Na	2.50	3.00	2.50	3.50	3.50	2.50	2.50	2.50	2.50	2.50	2.50
Ne	2.18	2.77	2.20	2.53	3.48	2.28	2.31	2.14	2.50	2.31	2.18
I	0.82	1.06	0.81	1.05	1.24	0.83	0.86	0.80	0.69	0.86	0.82
Ho	0.50	0.54	0.46	0.64	0.86	0.61	0.25	0.57	0.50	0.25	0.25
He	0.52	0.64	0.51	0.60	0.71	0.52	0.56	0.53	0.38	0.56	0.52
Allele Range	153 - 202	193 - 221	146 - 154	196 - 208	152 - 186	187 - 232	159 - 186	174 - 184	160 - 193	311 - 369	169 - 190
PD_Parent	0.79	0.79	0.54	0.79	0.89	0.54	0.79	0.63	0.89	0.79	0.79
PD_F1	0.57	0.81	0.80	0.76	0.81	0.81	0.62	0.71	0.00	0.62	0.57

S 3.8: SSR Genetic diversity results of 'Fuji' x P266R231T015 population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He), allele range and discrimination power at each locus for parent and F1 (PD).

	CH01b12	**CH01g05**	**CH01h02**	**CH02b10**	**CH02c02a**	**CH2c09**	**CH02d11**	**CH02g01**	**CH03g06**	**CH04c06**	**CH4c10**
Na	1.00	1.50	3.00	3.00	2.00	2.50	2.00	2.00	3.00	3.00	2.50
Ne	1.00	1.30	2.62	2.83	1.70	2.33	1.90	1.83	2.62	3.00	2.33
I	0.00	0.28	1.03	1.07	0.60	0.87	0.66	0.52	1.03	1.04	0.87
Ho	0.00	0.25	0.75	0.58	0.58	0.75	0.33	0.25	0.75	1.00	1.00
He	0.00	0.19	0.62	0.65	0.41	0.56	0.47	0.31	0.62	0.63	0.56
Allele Range	142 - 142	158 - 162	223 - 263	150 - 159	159 - 194	249 - 274	134 - 149	195 - 246	155 - 188	177 - 203	153 - 202
PD_Parent	0.00	0.54	0.79	0.79	0.54	0.79	0.63	0.79	0.79	0.89	0.79
PD_F1	0.00	0.00	0.77	0.81	0.59	0.63	0.59	0.00	0.77	0.63	0.63

	Ch04e05	**CH05a02**	**CH05a04**	**ChVf1**	**NH013a**	**NH021a**	**TsuENH004**	**TsuENH008**	**TsuENH058**	**NB105a**
Na	3.00	2.00	2.50	2.50	2.50	2.50	2.50	3.00	2.50	2.00
Ne	2.33	1.70	2.23	2.33	2.30	2.23	2.33	2.33	2.23	1.83
I	0.95	0.60	0.84	0.87	0.83	0.84	0.87	0.95	0.84	0.52
Ho	0.67	0.58	0.25	0.75	0.58	0.25	1.00	0.42	0.25	0.25
He	0.56	0.41	0.53	0.56	0.52	0.53	0.56	0.56	0.53	0.31
Allele Range	193 - 221	146 - 154	183 - 206	161 - 186	187 - 232	159 - 186	174 - 184	160 - 183	299 - 369	169 - 181
PD_Parent	0.79	0.54	0.79	0.79	0.54	0.79	0.79	0.79	0.79	0.79
PD_F1	0.70	0.59	0.59	0.63	0.81	0.59	0.63	0.70	0.59	0.00

S 3.9: SSR Genetic diversity results of P265R232T018 x 'Scilate' population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He), allele range and discrimination power at each locus for parent and F1 (PD).

	CH01b12	**CH01g05**	**CH01h02**	**CH02b12**	**CH2c09**	**CH02g01**	**CH03d02**	**CH03g06**	**CH04c06**	**CH4c10**	**Ch04e05**	**CH05a02**	**CH05a04**
Na	1.50	1.50	2.00	1.50	2.00	2.50	1.50	2.50	2.00	2.00	2.00	1.50	2.50
Ne	1.30	1.50	1.83	1.50	1.83	2.33	1.50	2.33	2.00	1.83	1.83	1.50	2.33
I	0.28	0.35	0.52	0.35	0.52	0.87	0.35	0.87	0.69	0.52	0.52	0.35	0.87
Ho	0.25	0.00	0.25	0.00	0.25	0.75	0.00	0.75	0.50	0.25	0.25	0.00	0.75
He	0.19	0.25	0.31	0.25	0.31	0.56	0.25	0.56	0.50	0.31	0.31	0.25	0.56
Allele Range	142 - 177	158 - 158	223 - 255	155 - 159	249 - 274	195 - 246	199 - 218	155 - 188	181 - 203	153 - 202	193 - 221	154 - 154	183 - 206
PD_Parent	0.54	0.63	0.79	0.63	0.79	0.79	0.63	0.79	0.63	0.79	0.79	0.63	0.79
PD_F1	0.00	0.00	0.00	0.00	0.00	0.63	0.00	0.63	0.63	0.00	0.00	0.00	0.63

	CH05d04	**ChVf1**	**NH002b**	**NH013a**	**NH021a**	**NH027a**	**NH045a**	**TsuENH004**	**TsuENH008**	**TsuENH086**	**TsuENH058**	**NB105a**	**KA16**
Na	2.00	1.50	1.50	1.50	1.50	1.00	2.00	1.00	1.50	1.50	2.00	1.50	1.50
Ne	1.83	1.50	1.50	1.30	1.50	1.00	1.83	1.00	1.50	1.50	2.00	1.50	1.50
I	0.52	0.35	0.35	0.28	0.35	0.00	0.52	0.00	0.35	0.35	0.69	0.35	0.35
Ho	0.25	0.00	0.00	0.25	0.00	0.00	0.25	0.00	0.00	0.00	0.50	0.00	0.50
He	0.31	0.25	0.25	0.19	0.25	0.00	0.31	0.00	0.25	0.25	0.50	0.25	0.25
Allele Range	199 - 208	152 - 161	192 - 192	187 - 232	159 - 186	165 - 165	229 - 238	184 - 184	154 - 183	211 - 211	311 - 369	169 - 181	144 - 155
PD_Parent	0.79	0.63	0.63	0.54	0.63	0.00	0.79	0.00	0.63	0.63	0.63	0.63	0.00
PD_F1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.00	0.63

S 3.10: SSR Genetic diversity results of 'Fuji' x P354R200T138 population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He), allele range and discrimination power at each locus for parent and F1 (PD).

	CH01b12	**CH01g05**	**CH01h02**	**CH02c02a**	**CH02g01**	**CH03c02**	**CH03d02**	**CH03g06**	**CH04c06**	**CH4c10**	**Ch04e05**	**CH05a02**
Na	2.50	3.50	3.00	2.00	2.00	3.00	2.00	2.50	3.00	3.00	2.50	3.00
Ne	1.92	2.86	2.32	1.61	1.65	2.05	1.61	2.25	2.35	2.76	1.84	2.60
I	0.75	1.10	0.92	0.57	0.54	0.81	0.57	0.84	0.92	0.95	0.69	1.02
Ho	0.45	0.83	0.83	0.25	0.00	0.29	0.25	0.25	0.60	0.50	0.62	0.34
He	0.46	0.65	0.56	0.38	0.36	0.46	0.38	0.54	0.57	0.55	0.45	0.61
Allele Range	133 - 142	158 - 175	223 - 263	159 - 194	206 - 246	143 - 179	235 - 242	182 - 188	177 - 188	147 - 202	193 - 219	122 - 154
PD_Parent	0.54	0.79	0.79	0.54	0.63	0.79	0.54	0.79	0.79	0.89	0.54	0.79
PD_F1	0.74	0.82	0.66	0.55	0.38	0.49	0.55	0.60	0.67	0.51	0.67	0.77

	CH05a04	**CH05d04**	**ChVf1**	**NH002b**	**NH004a**	**NH013a**	**NH021a**	**NH029a**	**TsuENH004**	**TsuENH008**	**NB105a**
Na	3.00	3.50	3.00	1.50	3.00	3.00	2.00	2.00	2.50	3.00	2.00
Ne	2.12	3.48	2.77	1.31	2.36	2.54	1.65	1.55	2.28	3.00	1.87
I	0.86	1.24	1.06	0.29	0.96	1.01	0.54	0.44	0.83	1.04	0.65
Ho	0.36	0.80	0.49	0.00	0.40	0.70	0.00	0.00	0.47	1.00	0.00
He	0.49	0.71	0.64	0.19	0.57	0.61	0.36	0.29	0.52	0.63	0.46
Allele Range	183 - 206	188 - 214	152 - 186	154 - 154	109 - 158	187 - 270	159 - 186	107 - 112	174 - 184	160 - 177	169 - 173
PD_Parent	0.79	0.89	0.79	0.00	0.79	0.79	0.63	0.63	0.54	0.89	0.63
PD_F1	0.57	0.81	0.80	0.55	0.71	0.76	0.38	0.16	0.81	0.63	0.58

S 3.11: SSR Genetic diversity results of FEM population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He), allele range and discrimination power at each locus for parent, F1 and F2 (PD).

	Hi21G05a	CH02f06a	CH02c02aa	MS14h03a	CH02h11aa	NZ05g08a	HI09B04a	CH02a08za	CH05a05a	CH01c06	CH05c07a	CH01h02a	Hi22f04a
Na	1.67	3.33	3.33	2.33	3.67	3.67	3.33	3.33	3.67	4.33	3.00	3.33	3.33
Ne	1.38	2.72	2.62	1.96	3.38	3.39	3.07	2.92	3.12	3.82	2.49	2.98	2.88
I	0.30	1.03	1.00	0.69	1.19	1.03	1.09	1.09	1.14	1.28	0.97	1.07	1.07
Ho	0.08	0.85	0.78	0.57	0.83	0.37	0.70	0.63	0.85	0.70	0.77	1.00	0.70
He	0.18	0.60	0.57	0.44	0.67	0.52	0.63	0.64	0.64	0.68	0.58	0.62	0.62
Allele Range	160 - 174	152 - 177	127 - 187	112 - 137	100 - 124	112 - 238	223 - 280	126 - 150	122 - 229	136 - 240	112 - 144	203 - 246	126 - 138
PD_Parent	0.72	0.90	0.90	0.79	0.92	0.94	0.92	0.84	0.92	0.84	0.84	0.92	0.90
PD_F1	0.00	0.63	0.63	0.63	0.63	0.00	0.63	0.63	0.63	0.63	0.63	0.63	0.63
PD_F2	0.00	0.71	0.66	0.31	0.87	0.88	0.78	0.87	0.81	0.95	0.75	0.74	0.78

	MS06g03a	CH03c02a	CH05D11a	CH03b10a	CH03a08a	CH04c07a	CH03d08a	NZ02b1a	CH02d11a	CH05c06a	CH05g03a	CH01h01a
Na	3.67	2.00	3.00	2.67	3.67	4.00	3.67	4.00	4.00	3.00	3.67	3.67
Ne	3.30	1.89	2.67	2.10	2.88	3.59	3.17	3.19	3.37	2.83	3.12	3.28
I	1.18	0.58	0.96	0.82	1.09	1.18	1.15	1.20	1.20	1.03	1.14	1.18
Ho	0.83	0.50	0.70	0.62	0.68	0.73	0.93	0.70	0.93	0.92	0.93	0.85
He	0.66	0.38	0.58	0.52	0.62	0.64	0.65	0.66	0.65	0.63	0.64	0.66
Allele Range	166 - 187	112 - 123	167 - 186	83 - 117	148 - 244	106 - 161	127 - 160	139 - 265	112 - 137	86 - 112	114 - 192	100 - 130
PD_Parent	0.92	0.79	0.90	0.75	0.75	0.96	0.92	0.90	0.94	0.87	0.92	0.90
PD_F1	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63
PD_F2	0.86	0.00	0.63	0.66	0.91	0.74	0.82	0.88	0.81	0.81	0.81	0.88

S 3.12: SSR Genetic diversity results of 'Abate' x 'Fuji' population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He), allele range and discrimination power at each locus for parent, F1 and F2 (PD).

	CH02f06	**Hi09B04**	**Hi04b12**	**CH01c06**	**MS96g03**	**NZ02b1**	**CH05a04**	**CH02c02a**	**CH05c07**
Na	2.50	2.50	2.50	2.50	3.00	3.00	3.00	1.50	3.00
Ne	2.33	2.33	2.33	2.33	3.00	3.00	3.00	1.30	3.00
I	0.87	0.87	0.87	0.87	1.04	1.04	1.04	0.28	1.04
Ho	0.75	0.75	0.75	0.75	1.00	1.00	1.00	0.25	1.00
He	0.56	0.56	0.56	0.56	0.63	0.63	0.63	0.19	0.63
Allele Range	137 - 174	223 - 273	135 - 156	150 - 159	160 - 178	217 - 289	155 - 193	127 - 141	106 - 124
PD_Parent	0.79	0.79	0.79	0.79	0.89	0.89	0.89	0.54	0.89
PD_F1	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.00	0.63

	CH01h02	**CH05d11**	**CH03a08**	**MS14h03**	**CH03c02**	**AJ00076**	**CH02d11**	**CH03d08**
Na	3.00	3.00	2.00	3.00	3.00	3.00	3.00	3.00
Ne	3.00	3.00	2.00	3.00	3.00	3.00	3.00	3.00
I	1.04	1.04	0.69	1.04	1.04	1.04	1.04	1.04
Ho	1.00	1.00	0.50	1.00	1.00	1.00	1.00	1.00
He	0.63	0.63	0.50	0.63	0.63	0.63	0.63	0.63
Allele Range	199 - 240	171 - 191	150 - 184	126 - 162	111 - 133	245 - 261	102 - 129	125 - 133
PD_Parent	0.89	0.89	0.63	0.89	0.89	0.89	0.89	0.89
PD_F1	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63

S 3.13: SSR Genetic diversity results of 'Decana' x 'Murray' population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He), allele range and discrimination power at each locus for parent, F1 and F2 (PD).

	Hi21g05	**Hi09B04**	**CH01c06**	**Hi22f04**	**CH04b02**
Na	2.00	2.00	1.50	3.00	1.50
Ne	1.83	1.83	1.50	3.00	1.50
I	0.52	0.52	0.35	1.04	0.35
Ho	0.25	0.25	0.00	1.00	0.00
He	0.31	0.31	0.25	0.63	0.25
Allele Range	157 - 163	223 - 274	150 - 157	126 - 143.3	177 - 183
PD_Parent	0.79	0.79	0.63	0.89	0.63
PD_F1	0.00	0.00	0.00	0.63	0.00

	CH04c07	**CH05a04**	**CH02c02a**	**CH05c06**	**CH03c02**
Na	3.00	3.00	3.00	1.50	3.00
Ne	3.00	3.00	3.00	1.50	3.00
I	1.04	1.04	1.04	0.35	1.04
Ho	1.00	1.00	1.00	0.00	1.00
He	0.63	0.63	0.63	0.25	0.63
Allele Range	104 - 147	162 - 181	127 - 179	131 - 158	111 - 121
PD_Parent	0.89	0.89	0.89	0.63	0.89
PD_F1	0.63	0.63	0.63	0.00	0.63

3.8.3 Genetic diversity HRM results

S 3.14: HRM Genetic diversity results of 'Cox's Orange Pippin' x 'Old Home' population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He) and discrimination power at each locus for parent and F1 (PD).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Na	2.50	2.00	2.00	2.50	2.50	2.50	2.00	2.50	2.00	2.50	2.50	2.00	2.50	2.00	2.00	1.50	2.50	2.00	1.50	1.50
Ne	2.05	2.00	2.00	2.05	2.05	2.05	2.00	2.05	1.80	2.05	2.10	2.00	2.15	1.80	1.32	1.50	1.53	1.38	1.30	1.50
I	0.74	0.69	0.69	0.74	0.74	0.74	0.69	0.74	0.63	0.74	0.77	0.69	0.80	0.63	0.34	0.35	0.41	0.41	0.28	0.35
Ho	0.49	0.50	0.50	0.49	0.49	0.99	0.49	0.49	0.75	0.49	0.48	0.50	0.46	0.75	0.25	0.00	0.01	0.25	0.25	0.00
He	0.51	0.50	0.50	0.51	0.51	0.51	0.50	0.51	0.44	0.51	0.52	0.50	0.53	0.44	0.21	0.25	0.27	0.26	0.19	0.25
PD_Parent	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.54	0.63	0.63	0.63	0.63	0.54	0.54	0.63	0.63	0.54	0.54	0.63
PD_F1	0.66	0.63	0.63	0.66	0.66	0.66	0.62	0.66	0.63	0.66	0.69	0.63	0.71	0.63	0.09	0.00	0.09	0.24	0.00	0.00

	21	22	23	24	25	26	27	28	19	30	31	32	33	34	35	36	37	38	39
Na	2.00	1.50	2.00	1.50	2.00	1.50	2.50	1.50	2.00	2.00	2.00	1.50	2.00	2.50	2.50	2.00	2.00	2.00	2.50
Ne	1.52	1.50	1.52	1.50	2.00	1.30	1.82	1.50	1.80	2.00	2.00	1.50	1.80	1.53	2.05	2.00	1.80	2.00	1.85
I	0.40	0.35	0.40	0.35	0.69	0.28	0.66	0.35	0.63	0.69	0.69	0.35	0.63	0.41	0.74	0.69	0.63	0.69	0.68
Ho	0.02	0.00	0.00	0.00	0.49	0.25	0.74	0.00	0.75	1.00	0.50	0.00	0.75	0.01	0.50	0.50	0.75	0.50	0.74
He	0.27	0.25	0.27	0.25	0.50	0.19	0.44	0.25	0.44	0.50	0.50	0.25	0.44	0.27	0.51	0.50	0.44	0.50	0.45
PD_Parent	0.63	0.63	0.63	0.63	0.63	0.54	0.54	0.63	0.54	0.63	0.63	0.63	0.54	0.63	0.63	0.63	0.54	0.63	0.54
PD_F1	0.09	0.00	0.09	0.00	0.62	0.00	0.64	0.00	0.63	0.63	0.63	0.00	0.63	0.09	0.66	0.63	0.63	0.63	0.66

S 3.15: HRM Genetic diversity results of P265R232T018 x A174R01T204 population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He) and discrimination power at each locus for parent and F1 (PD).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Na	2.00	1.50	2.00	1.50	1.50	1.50	1.50	2.00	2.00	2.00	2.00	2.00	1.50	2.00	1.50	2.00	2.00	2.00	1.50	2.00
Ne	2.00	1.30	2.00	1.50	1.50	1.50	1.50	2.00	1.80	1.80	2.00	1.80	1.50	2.00	1.50	2.00	2.00	1.80	1.50	2.00
I	0.69	0.28	0.69	0.35	0.35	0.35	0.35	0.69	0.63	0.63	0.69	0.63	0.35	0.69	0.35	0.69	0.69	0.63	0.35	0.69
Ho	0.50	0.25	0.50	0.00	0.00	0.50	0.00	1.00	0.75	0.75	0.50	0.75	0.50	1.00	0.00	0.50	0.50	0.75	0.00	0.50
He	0.50	0.19	0.50	0.25	0.25	0.25	0.25	0.50	0.44	0.44	0.50	0.44	0.25	0.50	0.25	0.50	0.50	0.44	0.25	0.50
PD_Parent	0.63	0.54	0.63	0.63	0.63	0.63	0.63	0.63	0.54	0.54	0.63	0.54	0.63	0.63	0.63	0.63	0.63	0.54	0.63	0.63
PD_F1	0.63	0.00	0.63	0.00	0.00	0.00	0.00	0.63	0.63	0.63	0.63	0.63	0.00	0.63	0.00	0.63	0.63	0.63	0.00	0.63

	21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**19**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**
Na	2.00	1.50	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	1.50	2.00	1.50
Ne	2.00	1.50	2.00	2.00	1.80	1.80	2.00	2.00	1.80	2.00	2.00	2.00	2.00	2.00	1.80	2.00	1.30	2.00	1.50
I	0.69	0.35	0.69	0.69	0.63	0.63	0.69	0.69	0.63	0.69	0.69	0.69	0.69	0.69	0.63	0.69	0.28	0.69	0.35
Ho	0.50	0.00	0.50	0.50	0.75	0.75	0.50	0.50	0.75	1.00	0.50	0.50	0.50	0.50	0.75	0.50	0.25	0.50	0.50
He	0.50	0.25	0.50	0.50	0.44	0.44	0.50	0.50	0.44	0.50	0.50	0.50	0.50	0.50	0.44	0.50	0.19	0.50	0.25
PD_Parent	0.63	0.63	0.63	0.63	0.54	0.54	0.63	0.63	0.54	0.63	0.63	0.63	0.63	0.63	0.54	0.63	0.54	0.63	0.63
PD_F1	0.63	0.00	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.00	0.63	0.00

S 3.16: HRM Genetic diversity results of P265R232T018 x A199R45T055 population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He) and discrimination power at each locus for parent and F1 (PD).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Na	2.00	2.00	1.50	2.00	2.00	2.00	2.50	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.50	2.50	2.00	2.00	2.00
Ne	1.44	1.94	1.50	1.64	2.00	1.64	2.07	1.94	1.94	1.60	1.64	1.60	1.74	2.00	1.80	2.07	2.33	1.80	1.64	1.80
I	0.47	0.68	0.35	0.53	0.69	0.53	0.80	0.68	0.68	0.56	0.53	0.56	0.61	0.69	0.63	0.80	0.87	0.63	0.53	0.63
Ho	0.38	0.38	0.00	0.13	0.25	0.63	0.13	0.63	0.88	0.50	0.13	0.50	0.63	0.75	0.00	0.13	0.25	0.50	0.13	0.25
He	0.30	0.48	0.25	0.36	0.50	0.36	0.52	0.48	0.48	0.38	0.36	0.38	0.42	0.50	0.44	0.52	0.56	0.44	0.36	0.44
PD_Parent	0.54	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.54	0.63	0.54	0.54	0.63	0.63	0.63	0.63	0.54	0.63	0.63
PD_F1	0.37	0.61	0.00	0.37	0.63	0.37	0.72	0.61	0.61	0.54	0.37	0.54	0.61	0.63	0.54	0.72	0.79	0.63	0.37	0.54

	21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**19**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**
Na	2.00	2.00	2.00	2.50	2.00	2.00	2.50	2.50	2.00	2.00	2.50	2.00	2.00	2.50	2.00	2.00	2.00	2.00	2.00
Ne	2.00	1.80	1.64	1.84	1.74	1.74	2.45	2.33	1.60	1.94	1.84	1.64	1.44	2.33	1.74	1.64	1.74	1.64	1.94
I	0.69	0.63	0.53	0.71	0.61	0.61	0.89	0.87	0.56	0.68	0.71	0.53	0.47	0.87	0.61	0.53	0.61	0.53	0.68
Ho	0.00	0.00	0.13	0.13	0.63	0.38	0.38	0.25	0.25	0.88	0.13	0.13	0.38	0.25	0.38	0.13	0.63	0.13	0.63
He	0.50	0.44	0.36	0.45	0.42	0.42	0.58	0.56	0.38	0.48	0.45	0.36	0.30	0.56	0.42	0.36	0.42	0.36	0.48
PD_Parent	0.63	0.63	0.63	0.63	0.54	0.54	0.63	0.63	0.54	0.63	0.63	0.63	0.54	0.63	0.54	0.63	0.54	0.63	0.63
PD_F1	0.63	0.54	0.37	0.61	0.61	0.61	0.81	0.79	0.54	0.61	0.61	0.37	0.37	0.79	0.61	0.37	0.61	0.37	0.61

S 3.17: HRM Genetic diversity results of 'Imperial Gala' x P266R231T015 population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He) and discrimination power at each locus for parent and F1 (PD).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Na	1.50	1.50	1.50	1.50	1.50	1.50	2.00	1.50	2.00	1.50	1.50	1.50	2.00	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Ne	1.30	1.50	1.50	1.50	1.50	1.50	1.80	1.30	1.80	1.30	1.50	1.30	2.00	1.30	1.50	1.50	1.50	1.30	1.50	1.50
I	0.28	0.35	0.35	0.35	0.35	0.35	0.63	0.28	0.63	0.28	0.35	0.28	0.69	0.28	0.35	0.35	0.35	0.28	0.35	0.35
Ho	0.25	0.00	0.00	0.00	0.00	0.50	0.25	0.25	0.75	0.25	0.00	0.25	1.00	0.25	0.00	0.00	0.00	0.25	0.00	0.00
He	0.19	0.25	0.25	0.25	0.25	0.25	0.44	0.19	0.44	0.19	0.25	0.19	0.50	0.19	0.25	0.25	0.25	0.19	0.25	0.25
PD_Parent	0.54	0.63	0.63	0.63	0.63	0.63	0.63	0.54	0.63	0.54	0.63	0.54	0.63	0.54	0.63	0.63	0.63	0.54	0.63	0.63
PD_F1	0.00	0.00	0.00	0.00	0.00	0.00	0.54	0.00	0.54	0.00	0.00	0.00	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00

	21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**19**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**
Na	1.50	1.50	2.00	1.50	2.00	1.50	1.50	1.50	2.00	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	2.00	2.00
Ne	1.50	1.50	1.80	1.50	1.60	1.30	1.50	1.50	1.60	1.50	1.50	1.50	1.50	1.50	1.30	1.50	1.50	2.00	1.80
I	0.35	0.35	0.63	0.35	0.56	0.28	0.35	0.35	0.56	0.35	0.35	0.35	0.35	0.35	0.28	0.35	0.35	0.69	0.63
Ho	0.00	0.00	0.25	0.00	0.50	0.25	0.00	0.00	0.50	0.50	0.00	0.00	0.00	0.00	0.25	0.00	0.50	0.50	0.75
He	0.25	0.25	0.44	0.25	0.38	0.19	0.25	0.25	0.38	0.25	0.25	0.25	0.25	0.25	0.19	0.25	0.25	0.50	0.44
PD_Parent	0.63	0.63	0.63	0.63	0.54	0.54	0.63	0.63	0.54	0.63	0.63	0.63	0.63	0.63	0.54	0.63	0.63	0.63	0.63
PD_F1	0.00	0.00	0.54	0.00	0.54	0.00	0.00	0.00	0.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.54

S 3.18: HRM Genetic diversity results of 'Imperial Gala' x P125R095T002 population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He) and discrimination power at each locus for parent and F1 (PD).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Na	1.5	2.0	2.0	1.5	2.0	2.0	2.0	1.5	2.0	1.5	2.0	2.0	1.5	2.0	1.5	1.5	2.0	2.0	1.5	1.5
Ne	1.3	2.0	1.8	1.5	1.8	2.0	2.0	1.5	1.8	1.5	1.8	1.8	1.3	1.8	1.5	1.5	1.8	2.0	1.5	1.5
I	0.3	0.7	0.6	0.3	0.6	0.7	0.7	0.3	0.6	0.3	0.6	0.6	0.3	0.6	0.3	0.3	0.6	0.7	0.3	0.3
Ho	0.3	0.5	0.8	0.0	0.8	1.0	0.5	0.0	0.8	0.5	0.8	0.8	0.3	0.8	0.0	0.0	0.8	0.5	0.0	0.0
He	0.2	0.5	0.4	0.3	0.4	0.5	0.5	0.3	0.4	0.3	0.4	0.4	0.2	0.4	0.3	0.3	0.4	0.5	0.3	0.3
PD_Parent	0.5	0.6	0.5	0.6	0.5	0.6	0.6	0.6	0.5	0.6	0.5	0.5	0.5	0.5	0.6	0.6	0.5	0.6	0.6	0.6
PD_F1	0.0	0.6	0.6	0.0	0.6	0.6	0.6	0.0	0.6	0.0	0.6	0.6	0.0	0.6	0.0	0.0	0.6	0.6	0.0	0.0

	21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**19**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**
Na	1.5	2.0	2.0	1.5	2.0	2.0	1.5	1.5	2.0	2.0	1.5	2.0	1.5	1.5	2.0	1.5	2.0	2.0	2.0
Ne	1.5	2.0	2.0	1.5	1.8	1.8	1.5	1.5	1.8	2.0	1.5	2.0	1.5	1.5	1.8	1.5	1.8	2.0	2.0
I	0.3	0.7	0.7	0.3	0.6	0.6	0.3	0.3	0.6	0.7	0.3	0.7	0.3	0.3	0.6	0.3	0.6	0.7	0.7
Ho	0.0	0.5	0.5	0.0	0.8	0.8	0.0	0.0	0.8	1.0	0.0	0.5	0.0	0.0	0.8	0.0	0.8	0.5	1.0
He	0.3	0.5	0.5	0.3	0.4	0.4	0.3	0.3	0.4	0.5	0.3	0.5	0.3	0.3	0.4	0.3	0.4	0.5	0.5
PD_Parent	0.6	0.6	0.6	0.6	0.5	0.5	0.6	0.6	0.5	0.6	0.6	0.6	0.6	0.6	0.5	0.6	0.5	0.6	0.6
PD_F1	0.0	0.6	0.6	0.0	0.6	0.6	0.0	0.0	0.6	0.6	0.0	0.6	0.0	0.0	0.6	0.0	0.6	0.6	0.6

S 3.19: HRM Genetic diversity results of 'Fuji' x P186R125T002 population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He) and discrimination power at each locus for parent and F1 (PD).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Na	2.00	2.00	2.00	1.50	2.00	2.00	2.00	2.50	2.00	2.50	2.50	2.00	2.50	2.00	2.50	2.50	2.50	2.50	2.50	2.50
Ne	1.93	1.63	2.00	1.30	2.00	2.00	1.80	1.97	1.80	1.97	1.40	2.00	2.41	2.00	1.40	1.40	1.40	2.28	1.40	1.40
I	0.67	0.57	0.69	0.28	0.69	0.69	0.63	0.75	0.63	0.75	0.46	0.69	0.88	0.69	0.46	0.46	0.46	0.83	0.46	0.46
Ho	0.86	0.52	1.00	0.25	1.00	1.00	0.75	0.66	0.75	0.66	0.30	1.00	0.77	1.00	0.30	0.30	0.30	0.61	0.30	0.30
He	0.48	0.39	0.50	0.19	0.50	0.50	0.44	0.47	0.44	0.47	0.27	0.50	0.57	0.50	0.27	0.27	0.27	0.52	0.27	0.27
PD_Parent	0.63	0.54	0.63	0.54	0.63	0.63	0.54	0.54	0.54	0.54	0.54	0.63	0.63	0.63	0.54	0.54	0.54	0.54	0.54	0.54
PD_F1	0.60	0.56	0.63	0.00	0.63	0.63	0.63	0.73	0.63	0.73	0.30	0.63	0.80	0.63	0.30	0.30	0.30	0.81	0.30	0.30

	21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**19**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**
Na	2.00	2.00	2.50	2.50	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.50	2.50	2.00	2.00	2.50	2.50	2.00
Ne	1.79	1.79	2.03	1.53	1.51	1.79	1.80	1.35	2.00	2.00	1.80	1.45	1.83	1.40	1.80	1.80	2.03	1.97	1.51
I	0.63	0.63	0.78	0.58	0.52	0.63	0.63	0.37	0.69	0.69	0.63	0.48	0.71	0.46	0.63	0.63	0.78	0.75	0.52
Ho	0.70	0.70	0.43	0.34	0.43	0.70	0.75	0.30	1.00	1.00	0.75	0.39	0.52	0.30	0.75	0.75	0.77	0.66	0.43
He	0.44	0.44	0.49	0.34	0.34	0.44	0.44	0.23	0.50	0.50	0.44	0.31	0.44	0.27	0.44	0.44	0.51	0.47	0.34
PD_Parent	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.63	0.63	0.54	0.54	0.54	0.54	0.54	0.54	0.63	0.54	0.54
PD_F1	0.62	0.62	0.77	0.51	0.46	0.62	0.63	0.16	0.63	0.63	0.63	0.39	0.69	0.30	0.63	0.63	0.69	0.73	0.46

S 3.20: HRM Genetic diversity results of 'Fuji' x P125R095T002 population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He) and discrimination power at each locus for parent and F1 (PD).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Na	2.00	2.00	1.50	1.50	1.50	1.50	2.00	2.00	2.00	1.50	1.50	2.00	2.00	2.00	1.50	2.00	2.00	2.00	1.50	1.50
Ne	1.46	1.46	1.50	1.30	1.50	1.50	1.46	1.46	1.72	1.50	1.50	1.92	1.46	1.98	1.30	1.38	1.66	1.64	1.30	1.30
I	0.49	0.49	0.35	0.28	0.35	0.35	0.49	0.49	0.61	0.35	0.35	0.67	0.49	0.69	0.28	0.41	0.55	0.58	0.28	0.28
Ho	0.39	0.25	0.50	0.25	0.50	0.50	0.39	0.25	0.61	0.50	0.50	0.86	0.25	0.93	0.25	0.32	0.50	0.25	0.25	0.25
He	0.31	0.31	0.25	0.19	0.25	0.25	0.31	0.31	0.42	0.25	0.25	0.48	0.31	0.49	0.19	0.25	0.37	0.39	0.19	0.19
PD_Parent	0.54	0.54	0.63	0.54	0.63	0.63	0.54	0.54	0.54	0.63	0.63	0.63	0.54	0.63	0.54	0.54	0.63	0.54	0.54	0.54
PD_F1	0.40	0.40	0.00	0.00	0.00	0.00	0.40	0.40	0.60	0.00	0.00	0.60	0.40	0.62	0.00	0.24	0.40	0.57	0.00	0.00

	21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**19**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**
Na	1.50	1.50	2.50	1.50	2.00	1.50	1.50	1.50	2.00	1.50	1.50	1.50	2.50	1.50	1.50	1.50	1.50	1.50	2.00
Ne	1.30	1.30	1.97	1.30	1.78	1.30	1.30	1.30	1.66	1.50	1.30	1.30	1.80	1.30	1.50	1.30	1.30	1.30	1.92
I	0.28	0.28	0.76	0.28	0.62	0.28	0.28	0.28	0.55	0.35	0.28	0.28	0.70	0.28	0.35	0.28	0.28	0.28	0.67
Ho	0.25	0.25	0.25	0.25	0.68	0.25	0.25	0.25	0.50	0.50	0.25	0.25	0.32	0.25	0.50	0.25	0.25	0.25	0.71
He	0.19	0.19	0.47	0.19	0.43	0.19	0.19	0.19	0.37	0.25	0.19	0.19	0.44	0.19	0.25	0.19	0.19	0.19	0.48
PD_Parent	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.63	0.63	0.54	0.54	0.54	0.54	0.63	0.54	0.54	0.54	0.63
PD_F1	0.00	0.00	0.75	0.00	0.62	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.68	0.00	0.00	0.00	0.00	0.00	0.60

S 3.21: HRM Genetic diversity results of 'Fuji' x P266R231T015 population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He) and discrimination power at each locus for parent and F1 (PD).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Na	2.00	2.00	2.00	1.50	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.50	2.50	2.00	1.50	2.00	2.50	2.50	2.50	2.50
Ne	1.80	1.80	1.80	1.30	1.80	2.00	1.80	2.00	2.00	1.49	1.80	2.50	2.29	2.00	1.30	1.80	2.30	2.50	1.80	1.80
I	0.63	0.63	0.63	0.28	0.63	0.69	0.63	0.69	0.69	0.51	0.63	0.90	0.85	0.69	0.28	0.63	0.83	0.90	0.71	0.71
Ho	0.75	0.75	0.75	0.25	0.75	1.00	0.75	1.00	1.00	0.42	0.75	0.83	0.67	1.00	0.25	0.75	0.58	0.83	0.42	0.42
He	0.44	0.44	0.44	0.19	0.44	0.50	0.44	0.50	0.50	0.33	0.44	0.58	0.56	0.50	0.19	0.44	0.52	0.58	0.44	0.44
PD_Parent	0.54	0.54	0.54	0.54	0.54	0.63	0.54	0.63	0.63	0.54	0.54	0.63	0.63	0.63	0.54	0.54	0.54	0.63	0.54	0.54
PD_F1	0.63	0.63	0.63	0.00	0.63	0.63	0.63	0.63	0.63	0.44	0.63	0.81	0.77	0.63	0.00	0.63	0.81	0.81	0.70	0.70

	21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**19**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**
Na	2.00	2.00	2.50	2.50	2.50	2.00	2.00	1.50	1.50	2.00	1.50	2.50	2.50	1.50	2.00	2.00	2.00	2.00	2.00
Ne	1.80	1.80	2.30	1.80	1.80	1.80	1.80	1.30	1.50	2.00	1.30	1.80	2.09	1.30	2.00	1.80	1.69	1.80	2.00
I	0.63	0.63	0.83	0.71	0.71	0.63	0.63	0.28	0.35	0.69	0.28	0.71	0.79	0.28	0.69	0.63	0.57	0.63	0.69
Ho	0.75	0.75	0.58	0.42	0.42	0.75	0.75	0.25	0.50	1.00	0.25	0.42	0.42	0.25	1.00	0.75	0.67	0.75	1.00
He	0.44	0.44	0.52	0.44	0.44	0.44	0.44	0.19	0.25	0.50	0.19	0.44	0.49	0.19	0.50	0.44	0.39	0.44	0.50
PD_Parent	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.63	0.63	0.54	0.54	0.54	0.54	0.63	0.54	0.63	0.54	0.63
PD_F1	0.63	0.63	0.81	0.70	0.70	0.63	0.63	0.00	0.00	0.63	0.00	0.70	0.77	0.00	0.63	0.63	0.44	0.63	0.63

S 3.22: HRM Genetic diversity results of P265R232T018 x 'Scilate' population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He) and discrimination power at each locus for parent and F1 (PD).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Na	1.50	1.50	2.00	1.50	1.50	1.50	1.50	2.00	2.00	1.50	1.50	1.50	1.50	1.50	1.50	1.50	2.00	2.00	1.50	1.50
Ne	1.50	1.50	2.00	1.50	1.50	1.50	1.50	2.00	1.80	1.30	1.50	1.30	1.50	1.50	1.50	1.50	2.00	1.80	1.50	1.50
I	0.35	0.35	0.69	0.35	0.35	0.35	0.35	0.69	0.63	0.28	0.35	0.28	0.35	0.35	0.35	0.35	0.69	0.63	0.35	0.35
Ho	0.00	0.00	0.50	0.00	0.00	0.50	0.00	1.00	0.75	0.25	0.00	0.25	0.50	0.50	0.00	0.00	0.50	0.75	0.00	0.00
He	0.25	0.25	0.50	0.25	0.25	0.25	0.25	0.50	0.44	0.19	0.25	0.19	0.25	0.25	0.25	0.25	0.50	0.44	0.25	0.25
PD_Parent	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.54	0.54	0.63	0.54	0.63	0.63	0.63	0.63	0.63	0.54	0.63	0.63
PD_F1	0.00	0.00	0.63	0.00	0.00	0.00	0.00	0.63	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.63	0.00	0.00

	21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**19**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**
Na	1.50	1.50	1.50	1.50	2.00	1.50	1.50	1.50	2.00	1.50	1.50	1.50	2.00	1.50	1.50	1.50	1.50	1.50	1.50
Ne	1.50	1.50	1.50	1.50	1.80	1.30	1.50	1.50	1.80	1.50	1.50	1.50	2.00	1.50	1.30	1.50	1.30	1.50	1.30
I	0.35	0.35	0.35	0.35	0.63	0.28	0.35	0.35	0.63	0.35	0.35	0.35	0.69	0.35	0.28	0.35	0.28	0.35	0.28
Ho	0.00	0.00	0.00	0.00	0.75	0.25	0.00	0.00	0.75	0.50	0.00	0.00	0.50	0.00	0.25	0.00	0.25	0.00	0.25
He	0.25	0.25	0.25	0.25	0.44	0.19	0.25	0.25	0.44	0.25	0.25	0.25	0.50	0.25	0.19	0.25	0.19	0.25	0.19
PD_Parent	0.63	0.63	0.63	0.63	0.54	0.54	0.63	0.63	0.54	0.63	0.63	0.63	0.63	0.63	0.54	0.63	0.54	0.63	0.54
PD_F1	0.00	0.00	0.00	0.00	0.63	0.00	0.00	0.00	0.63	0.00	0.00	0.00	0.63	0.00	0.00	0.00	0.00	0.00	0.00

S 3.23: HRM Genetic diversity results of 'Fuji' x P354R200T138 population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He) and discrimination power at each locus for parent and F1 (PD).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Na	2.00	2.00	2.00	1.67	2.00	2.00	2.00	1.67	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Ne	2.00	2.00	2.00	1.67	1.96	2.00	2.00	1.57	1.96	1.96	2.00	1.87	2.00	1.96	1.87	2.00	2.00	1.96	2.00	2.00
I	0.69	0.69	0.69	0.46	0.68	0.69	0.69	0.43	0.68	0.68	0.69	0.65	0.69	0.68	0.65	0.69	0.69	0.68	0.69	0.69
Ho	0.67	0.67	0.67	0.33	0.75	1.00	0.67	0.20	0.75	0.75	0.67	0.83	0.67	0.92	0.83	0.67	0.67	0.75	0.67	0.67
He	0.50	0.50	0.50	0.33	0.49	0.50	0.50	0.31	0.49	0.49	0.50	0.46	0.50	0.49	0.46	0.50	0.50	0.49	0.50	0.50
PD_Parent	0.63	0.63	0.63	0.63	0.61	0.63	0.63	0.63	0.61	0.61	0.63	0.54	0.63	0.61	0.54	0.63	0.63	0.61	0.63	0.63
PD_F1	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.00	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63

	21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**19**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**
Na	2.00	2.00	2.50	1.50	2.50	2.00	2.00	2.00	2.00	2.00	1.50	1.50	2.50	1.50	2.00	1.50	2.50	2.00	2.50
Ne	1.80	1.80	2.07	1.30	1.89	2.00	2.00	2.00	2.00	2.00	1.30	1.30	1.62	1.30	2.00	1.30	2.17	1.80	2.09
I	0.63	0.63	0.78	0.28	0.70	0.69	0.69	0.69	0.69	0.69	0.28	0.28	0.64	0.28	0.69	0.28	0.81	0.63	0.77
Ho	0.75	0.75	0.55	0.25	0.73	1.00	1.00	1.00	1.00	1.00	0.25	0.25	0.36	0.25	1.00	0.25	0.91	0.75	0.98
He	0.44	0.44	0.49	0.19	0.46	0.50	0.50	0.50	0.50	0.50	0.19	0.19	0.38	0.19	0.50	0.19	0.54	0.44	0.52
PD_Parent	0.54	0.54	0.54	0.54	0.54	0.63	0.63	0.63	0.63	0.63	0.54	0.54	0.54	0.54	0.63	0.54	0.63	0.54	0.63
PD_F1	0.63	0.63	0.77	0.00	0.68	0.63	0.63	0.63	0.63	0.63	0.00	0.00	0.60	0.00	0.63	0.00	0.73	0.63	0.68

S 3.24: HRM Genetic diversity results of FEM population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He) and discrimination power at each locus for parent and F1 (PD).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Na	2.00	1.67	1.67	1.67	1.67	1.67	1.67	1.67	2.00	1.67	1.67	1.67	2.00	1.67	1.67	1.67	2.00	1.67	1.67	1.67
Ne	1.91	1.67	1.67	1.67	1.63	1.67	1.67	1.64	1.94	1.63	1.67	1.53	1.91	1.63	1.53	1.67	1.74	1.63	1.67	1.67
I	0.67	0.46	0.46	0.46	0.45	0.46	0.46	0.46	0.68	0.45	0.46	0.42	0.67	0.45	0.42	0.46	0.57	0.45	0.46	0.46
Ho	0.53	0.33	0.33	0.33	0.42	0.67	0.33	0.00	0.68	0.42	0.33	0.50	0.53	0.58	0.50	0.33	0.40	0.42	0.33	0.33
He	0.47	0.33	0.33	0.33	0.32	0.33	0.33	0.33	0.48	0.32	0.33	0.29	0.47	0.32	0.29	0.33	0.39	0.32	0.33	0.33
PD_Parent	0.63	0.63	0.63	0.63	0.61	0.63	0.63	0.63	0.61	0.61	0.63	0.54	0.63	0.61	0.54	0.63	0.63	0.61	0.63	0.63
PD_F1	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.00	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63
PD_F2	0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.61	0.61	0.00	0.00	0.00	0.58	0.00	0.00	0.00	0.31	0.00	0.00	0.00

	21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**19**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**
Na	1.67	1.67	2.00	1.67	1.67	2.00	1.67	1.67	2.00	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67
Ne	1.63	1.67	2.00	1.67	1.67	1.78	1.63	1.63	1.69	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.63	1.67
I	0.45	0.46	0.69	0.46	0.46	0.62	0.45	0.45	0.59	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.45	0.46
Ho	0.42	0.33	0.67	0.33	0.33	0.58	0.42	0.42	0.63	0.67	0.33	0.33	0.33	0.33	0.33	0.33	0.50	0.42	0.50
He	0.32	0.33	0.50	0.33	0.33	0.43	0.32	0.32	0.40	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.32	0.33
PD_Parent	0.61	0.63	0.63	0.63	0.63	0.61	0.61	0.61	0.54	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.61	0.63
PD_F1	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63
PD_F2	0.00	0.00	0.63	0.00	0.00	0.49	0.00	0.00	0.49	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

S 3.25: HRM Genetic diversity results of 'Abate' x 'Fuji' population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He) and discrimination power at each locus for parent and F1 (PD).

	5	**7**	**11**	**27**	**28**	**30**	**39**
Na	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Ne	2.00	2.00	2.00	2.00	2.00	2.00	2.00
I	0.69	0.69	0.69	0.69	0.69	0.69	0.69
Ho	0.50	0.50	0.50	0.50	0.50	0.50	0.50
He	0.50	0.50	0.50	0.50	0.50	0.50	0.50
PD_Parent	0.63	0.63	0.63	0.63	0.63	0.63	0.63
PD_F1	0.63	0.63	0.63	0.63	0.63	0.63	0.63

S 3.26: HRM Genetic diversity results of 'Decana' x 'Murray' population. Number of different alleles (Na), number of effective alleles = 1 / (Sum pi^2) (Ne), shannon's information index = -1* Sum (pi * Ln (pi)) (I), observed heterozygosity = No. of Hets / N (Ho), expected Heterozygosity = 1 - Sum pi^2 (He) and discrimination power at each locus for parent and F1 (PD).

	1	**2**	**3**	**4**	**7**	**8**	**10**	**16**	**18**	**19**
Na	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Ne	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
I	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Ho	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
He	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
PD_Parent	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63
PD_F1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

	21	**22**	**27**	**19**	**30**	**31**	**32**	**35**	**36**	**39**
Na	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Ne	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
I	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Ho	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
He	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
PD_Parent	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63
PD_F1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

S 3.27: HRM results for each primer (1-19) and per each samples. he (heterozygous), ho (homozygous) and n/c (no result).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
CO 1	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 2	he	he	ho	he	ho	ho	ho	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 3	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 4	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 5	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 6	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 7	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 8	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 9	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 10	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 11	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 12	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 13	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 14	he	he	ho	he	ho	ho	he	ho	ho	he	ho	he	n/c	he	ho	ho	ho	n/c	ho
CO 15	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 16	he	he	ho	he	ho	ho	he	he	ho	he	n/c	he	n/c	he	ho	ho	ho	n/c	ho
CO 17	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 18	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 19	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 20	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 21	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 22	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 23	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 24	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 25	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 26	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 27	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 28	he	he	ho	he	ho	ho	he	n/c	ho	n/c	n/c	he	n/c	he	n/c	ho	ho	n/c	ho
CO 29	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho

CO 30	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 31	n/c	he	ho	he	n/c	n/c	he	he	he	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 32	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 33	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 34	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 35	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 36 (PN)	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 37 (PN)	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 38 (PN)	he	he	he	ho	ho	ho	he	he	ho	he	ho	ho	he	he	ho	ho	he	ho	ho
CO 39 (PN)	he	ho	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 40 (PN)	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
CO 41 (PN)	he	he	ho	he	ho	ho	he	he	ho	he	ho	he	he	he	ho	ho	ho	ho	ho
P26A17	he	ho	he	ho	ho	ho	ho	he	he	he	he	he	ho	he	ho	he	he	he	ho
P26A19 1	ho	he	ho	ho	ho	ho	ho	he	he	ho	ho	he	he	ho	ho	ho	he	ho	ho
P26A19 2	he	he	ho	he	ho	ho	he	ho	he	ho	he	he	he	he	ho	he	he	he	he
P26A19 3	ho	ho	ho	ho	he	he	n/c	ho	he	he	ho	ho	he	he	ho	ho	ho	he	ho
P26A19 4	ho	he	ho	ho	he	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho
IP26 1	ho	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	he	ho	ho	ho	ho	ho	ho
IP26 2	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho
IP12 1	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
IP12 2	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP18 1	ho	ho	ho	ho	ho	ho	he	ho	he	ho	ho	ho	ho	he	ho	ho	ho	ho	ho
FP18 2	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	n/c	ho	ho	ho	ho	n/c	ho
FP18 3	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP18 4 (PN)	ho	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP18 5 (PN)	ho	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	he	ho	ho	ho	ho	ho
FP18 6 (PN)	ho	ho	ho	ho	ho	ho	ho	he	he	n/c	ho	ho	n/c	he	ho	ho	ho	n/c	ho

FP18 7 (PN)	ho	ho	ho	ho	ho	ho	ho	he	he	ho	ho	ho	ho	he	ho	ho	ho	n/c	ho
FP18 8 (PN)	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	he	he	ho	ho	ho	ho	ho	ho
FP18 9 (PN)	he	ho	he	ho	ho	he	ho	ho	he	ho	he	he	ho	he	he	he	he	he	he
FP18 10 (PN)	ho	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	n/c	he	ho	ho	ho	ho	ho
FP18 11 (PN)	ho	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	he	he	ho	ho	ho	ho	ho
FP12 1	he	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho
FP12 2	ho	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	he	ho	he	ho	ho	ho	ho	ho
FP12 3	he	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP12 4 (PN)	ho	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	he	ho	he	ho	ho	ho	ho	ho
FP12 5 (PN)	ho	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	he	ho	he	ho	ho	ho	ho	ho
FP12 6 (PN)	ho	ho	ho	ho	ho	ho	ho	n/c	he	ho	ho	he	ho	he	ho	he	ho	n/c	ho
FP12 7 (PN)	ho	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	he	n/c	he	ho	ho	n/c	n/c	ho
FP26 1	he	ho	ho	ho	ho	ho	he	ho	he	ho	ho	n/c	ho	he	ho	ho	n/c	ho	n/c
FP26 2	he	ho	ho	ho	ho	ho	he	ho	he	ho	ho	ho	n/c	he	ho	ho	ho	ho	n/c
FP26 3	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho
P26S	ho	ho	he	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	he	he	ho
FP35 1	ho	ho	ho	ho	ho	ho	ho	he	he	ho	ho	ho	ho	he	ho	ho	ho	ho	ho
FP35 2	ho	he	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	he	he	ho	ho	ho	ho	ho
FP35 3	ho	ho	ho	ho	ho	ho	ho	he	he	ho	ho	ho	ho	he	ho	ho	ho	ho	ho
FP35 4	he	ho	ho	ho	ho	ho	ho	he	he	he	ho	ho	he	ho	ho	ho	ho	ho	ho
FP35 5	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho
FP35 6	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho
FP35 7	he	ho	ho	ho	ho	ho	ho	he	he	he	ho	ho	he	ho	ho	ho	ho	ho	ho
FP35 8 (PN)	he	he	ho	ho	ho	ho	ho	he	he	ho	ho	ho	ho	he	ho	ho	ho	ho	ho
FP35 9 (PN)	he	he	ho	ho	ho	ho	ho	he	he	ho	ho	ho	he	he	ho	ho	ho	ho	ho

FP35 10 (PN)	he	he	ho	ho	ho	ho	ho	he	he	ho	ho	ho	he	he	ho	ho	ho	ho	ho
FP35 11 (PN)	he	he	ho	ho	ho	ho	ho	he	he	ho	ho	ho	ho	he	ho	ho	ho	ho	ho
FP35 12 (PN)	he	he	ho	ho	ho	ho	ho	he	he	ho	ho	ho	he	he	ho	ho	ho	ho	ho
FP35 13 (PN)	he	he	ho	ho	ho	ho	ho	he	he	ho	ho	ho	he	he	ho	ho	ho	ho	ho
FP35 14 (PN)	he	he	ho	ho	ho	ho	ho	he	he	ho	ho	ho	ho	he	ho	ho	ho	ho	ho
FP35 15 (PN)	he	he	ho	ho	ho	ho	ho	he	he	ho	ho	ho	ho	he	ho	ho	ho	ho	ho
FP35 16 (PN)	he	ho	ho	ho	ho	ho	ho	he	he	ho	ho	ho	ho	he	ho	ho	ho	ho	ho
FP35 17 (PN)	he	he	ho	ho	ho	ho	ho	he	he	ho	ho	ho	ho	he	ho	ho	ho	ho	ho
FP35 18 (PN)	he	he	ho	ho	ho	ho	ho	he	he	ho	ho	ho	ho	he	ho	ho	ho	ho	ho
FP35 19 (PN)	n/c	n/c	ho	ho	n/c	n/c	n/c	n/c	n/c	he	ho	ho	ho	n/c	n/c	ho	ho	ho	ho
FP35 20 (PN)	ho	he	ho	ho	he	ho	ho	ho	he	he	ho	ho	ho	he	ho	ho	ho	ho	ho
FP35 21 (PN)	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho
FP35 22 (PN)	ho	he	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	he	ho	ho	ho	ho	ho
FP35 23 (PN)	he	he	ho	ho	ho	ho	ho	he	he	ho	ho	ho	he	he	ho	ho	ho	ho	ho
Zwintzscher's Hybrid	he	he	he	he	he	he	he	ho	he	ho	he	he	he	ho	he	he	he	he	he
F2-1	he	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	he	ho	ho	ho	ho	ho	ho
F2-2	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
F2-3	he	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	he	ho	ho	ho	he	ho	ho
F2-4	he	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	he	ho	ho	ho	ho	ho	ho
F2-5	ho	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
AF	n/c	n/c	n/c	n/c	he	n/c	he	n/c	n/c	n/c	he	n/c	n/c	n/c	n/c	n/c	n/c	n/c	n/c
DM1	ho	ho	ho	ho	n/c	n/c	ho	ho	n/c	ho	n/c	n/c	n/c	n/c	n/c	ho	n/c	ho	ho
DM2	ho	ho	ho	ho	n/c	n/c	ho	ho	n/c	ho	n/c	n/c	n/c	n/c	n/c	ho	n/c	ho	ho

S 3.28: HRM results for each primer (20-39) and per each samples. he (heterozygous), ho (homozygous) and n/c (no result).

	20	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**
CO 1	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 2	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 3	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 4	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 5	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 6	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 7	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 8	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	he
CO 9	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 10	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 11	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 12	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 13	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 14	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 15	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 16	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 17	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 18	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 19	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 20	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 21	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 22	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 23	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 24	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 25	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 26	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 27	ho	ho	ho	ho	ho	he	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 28	ho	ho	ho	n/c	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	n/c
CO 29	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 30	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 31	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he

CO 32	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 33	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 34	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 35	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 36 (PN)	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 37 (PN)	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 38 (PN)	ho	he	ho	ho	ho	he	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	he	he	he
CO 39 (PN)	ho	he	ho	ho	ho	ho	ho	he	ho	he	ho	ho	ho	ho	he	ho	ho	ho	he	he
CO 40 (PN)	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
CO 41 (PN)	ho	ho	ho	ho	ho	he	ho	he	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
P26A17	he	he	he	he	he	he	he	he	he	he	he	he	he	he	he	he	he	he	ho	he	ho
P26A19 1	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	he	ho	he
P26A19 2	he	he	he	he	he	ho	he	he	he	ho	he	he	he	he	he	he	he	he	he	ho
P26A19 3	ho	ho	ho	ho	ho	he	ho	he	he	ho	he	ho	ho	ho	ho	ho	ho	he	ho	ho
P26A19 4	he	ho	ho	ho	ho	he	ho	he	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	he
IP26 1	ho	ho	ho	he	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	he	ho
IP26 2	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	he	he
IP12 1	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	he	he
IP12 2	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	he	he
FP18 1	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP18 2	ho	ho	ho	n/c	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP18 3	ho	ho	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP18 4 (PN)	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho
FP18 5 (PN)	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP18 6 (PN)	ho	ho	ho	n/c	ho	ho	ho	ho	ho	ho	ho	ho	ho	n/c	ho	ho	ho	n/c	ho	ho
FP18 7 (PN)	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP18 8 (PN)	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP18 9 (PN)	he	ho	he	he	he	he	ho	he	he	he	ho	he	he	he	he	he	he	he	he	ho

FP18 10 (PN)	ho	ho	ho	n/c	he	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho
FP18 11 (PN)	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP12 1	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP12 2	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
FP12 3	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP12 4 (PN)	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
FP12 5 (PN)	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
FP12 6 (PN)	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho
FP12 7 (PN)	n/c	ho	ho	n/c	ho	he	ho	ho	ho	ho	ho	ho	ho	n/c	ho	ho	ho	ho	ho	ho
FP26 1	n/c	ho	ho	ho	ho	n/c	ho	ho	ho	ho	ho	ho	n/c	ho	ho	ho	ho	ho	ho	ho
FP26 2	n/c	ho	ho	n/c	he	n/c	ho	ho	ho	ho	ho	ho	n/c	n/c	ho	ho	ho	ho	ho	ho
FP26 3	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho
P26S	ho	ho	ho	ho	ho	he	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP35 1	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP35 2	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
FP35 3	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP35 4	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
FP35 5	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
FP35 6	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
FP35 7	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
FP35 8 (PN)	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP35 9 (PN)	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP35 10 (PN)	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP35 11 (PN)	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
FP35 12 (PN)	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho

FP35 13 (PN)	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP35 14 (PN)	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	he
FP35 15 (PN)	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP35 16 (PN)	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP35 17 (PN)	ho	ho	ho	n/c	ho	he	ho	ho	ho	ho	ho	ho	ho	n/c	ho	ho	ho	ho	ho	ho
FP35 18 (PN)	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP35 19 (PN)	ho	ho	ho	n/c	ho	n/c	ho	ho	ho	ho	he	ho	ho	n/c	ho	ho	ho	n/c	ho	n/c
FP35 20 (PN)	ho	ho	ho	n/c	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP35 21 (PN)	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho
FP35 22 (PN)	ho	ho	ho	n/c	ho	he	ho	ho	ho	ho	ho	ho	ho	n/c	ho	ho	ho	n/c	ho	he
FP35 23 (PN)	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
Zwintzscher's Hybrid	he	he	he	he	he	he	he	he	he	he	he	he	he	he	he	he	he	he	he	he
F2-1	ho	ho	ho	he	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
F2-2	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
F2-3	ho	ho	ho	he	ho	ho	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
F2-4	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
F2-5	ho	ho	ho	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho	ho
AF	n/c	n/c	n/c	n/c	n/c	n/c	n/c	he	he	n/c	he	n/c	n/c	n/c	n/c	n/c	n/c	n/c	n/c	he
DM1	n/c	ho	ho	n/c	n/c	n/c	ho	n/c	n/c	ho	ho	ho	ho	n/c	n/c	ho	ho	n/c	n/c	ho
DM2	n/c	ho	ho	n/c	n/c	n/c	ho	n/c	n/c	ho	ho	ho	ho	n/c	n/c	ho	ho	n/c	n/c	ho

3.8.5 SNP chip results

S 3.29: Number of SNPs supporting the hybridity (Hybrid), have same results as the mother (Mother) and father (Father) or have a completely different result compared with the parent (N) for the 'Cox's Orange Pippin' x 'Old Home' population for the apple and pear Infinium® II 9K SNP array by chromosome for the apple SNPs, while the pear SNPs were all put in one group, Lg0. The Twenty-six CO F1 represent the results of the CO 2, CO 3, CO 4, CO 5, CO 9, CO 10 CO 11, CO 12, CO 13, CO 14, CO 15, CO 17, CO 19, CO 20, CO 22, CO 23, CO 24,CO 25, CO 27, CO 29, CO 31, CO 33, CO 34, CO 35, CO 40 (PN).

		Twenty-six CO F1				**CO 16**				**CO 7-CO 26**				**CO 37 (PN)**			
		Hybrid	**Mother**	**Father**	**N**	**Hybrid**	**Mother**	**Father**	**N**	**Hybrid**	**Mother**	**Father**	**N**	**Hybrid**	**Mother**	**Father**	**N**
Pear SNP	**Lg0**	90	30	0	0	87	26	4	3	76	31	6	7	84	29	6	1
Apple SNP	**Lg1**	35	8	0	0	23	15	2	3	23	13	3	4	20	16	3	4
	Lg2	70	24	0	0	51	13	20	10	58	16	12	8	54	16	17	7
	Lg3	53	7	0	0	48	10	2	0	42	11	5	2	37	12	7	4
	Lg4	38	4	0	1	23	15	2	3	28	11	1	3	29	11	0	3
	Lg5	66	15	0	0	71	8	0	2	46	11	9	15	54	10	6	11
	Lg6	42	4	0	0	23	14	7	2	16	17	6	7	23	12	5	6
	Lg7	23	14	0	0	20	12	0	5	19	13	0	5	16	14	1	6
	Lg8	40	10	0	0	30	13	4	3	24	19	4	3	34	14	2	0
	Lg9	55	14	0	0	41	15	4	9	51	8	5	5	41	11	8	9
	Lg10	48	23	0	0	45	12	7	7	37	16	8	10	39	15	8	9
	Lg11	36	13	0	0	31	14	0	4	33	15	0	1	33	15	0	1
	Lg12	41	14	0	0	41	12	1	1	36	15	2	2	39	14	1	1
	Lg13	25	20	1	0	26	18	0	2	27	17	0	2	27	16	1	2
	Lg14	33	6	0	0	21	11	3	4	20	11	4	4	23	9	4	3
	Lg15	65	27	0	0	55	26	5	6	65	15	7	5	71	12	4	5
	Lg16	25	10	0	0	22	2	9	2	26	3	4	2	28	3	4	0
	Lg17	39	21	0	0	34	19	5	2	29	23	4	4	30	24	4	2

S 3.30: Number of SNPs supporting the hybridity (Hybrid), have same results as the mother (Mother) and father (Father) or have a completely different result compared with the parent (N) for the 'Imperial Gala' x P125R095T002 population for the apple and pear Infinium® II 9K SNP array by chromosome for the apple SNPs, while the pear SNPs were all put in one group, Lg0.

		IP26 1				IP26 2			
		Hybrid	**Mother**	**Father**	**N**	**Hybrid**	**Mother**	**Father**	**N**
Pear SNP	**Lg0**	91	28	0	0	91	28	0	0
Apple SNP	**Lg1**	34	9	0	0	33	10	0	0
	Lg2	61	33	0	0	70	24	0	0
	Lg3	46	15	0	0	44	17	0	0
	Lg4	29	14	0	0	30	13	0	0
	Lg5	75	6	0	0	73	8	0	0
	Lg6	29	17	0	0	36	10	0	0
	Lg7	26	11	0	0	23	14	0	0
	Lg8	32	18	0	0	31	19	0	0
	Lg9	55	14	0	0	49	20	0	0
	Lg10	51	21	0	0	51	21	0	0
	Lg11	35	14	0	0	43	6	0	0
	Lg12	33	22	0	0	33	22	0	0
	Lg13	29	17	0	0	35	11	0	0
	Lg14	27	12	0	0	28	11	0	0
	Lg15	65	27	0	0	63	29	0	0
	Lg16	31	4	0	0	30	5	0	0
	Lg17	30	28	0	0	32	26	0	0

S 3.31: Number of SNPs supporting the hybridity (Hybrid), have same results as the mother (Mother) and father (Father) or have a completely different result compared with the parent (N) for the 'Imperial Gala' x P125R095T002 population for the apple and pear Infinium® II 9K SNP array by chromosome for the apple SNPs, while the pear SNPs were all put in one group, Lg0.

		IP12 1-IP12 2			
		Hybrid	**Mother**	**Father**	**N**
Pear SNP	**Lg0**	115	5	0	0
Apple SNP	**Lg1**	43	0	0	0
	Lg2	94	0	0	0
	Lg3	61	0	0	0
	Lg4	40	3	0	0
	Lg5	78	3	0	0
	Lg6	46	0	0	0
	Lg7	37	0	0	0
	Lg8	48	2	0	0
	Lg9	63	6	0	0
	Lg10	72	0	0	0
	Lg11	48	1	0	0
	Lg12	54	1	0	0
	Lg13	45	1	0	0
	Lg14	37	2	0	0
	Lg15	77	15	0	0
	Lg16	34	1	0	0
	Lg17	59	1	0	0

S 3.32: Number of SNPs supporting the hybridity (Hybrid), have same results as the mother (Mother) and father (Father) or have a completely different result compared with the parent (N) for the 'Fuji' x P186R125T002 population for the apple and pear Infinium® II 9K SNP array by chromosome for the apple SNPs, while the pear SNPs were all put in one group, Lg0.

		FP18 1				FP18 2-FP18 3				FP18 4 (PN)				FP18 6 (PN)				FP18 10 (PN)-FP18 11 (PN)			
		Hybrid	**Mother**	**Father**	**N**	**Hybrid**	**Mother**	**Father**	**N**	**Hybrid**	**Mother**	**Father**	**N**	**Hybrid**	**Mother**	**Father**	**N**	**Hybrid**	**Mother**	**Father**	**N**
Pear SNP	**Lg0**	81	37	0	1	78	41	0	0	81	37	0	1	84	29	2	4	87	32	0	0
Apple SNP	**Lg1**	27	16	0	0	27	16	0	0	27	16	0	0	33	7	2	1	25	18	0	0
	Lg2	64	29	0	0	64	29	0	0	64	29	0	0	61	17	8	7	70	23	0	0
	Lg3	43	18	0	0	48	13	0	0	43	18	0	0	52	6	2	1	51	10	0	0
	Lg4	30	13	0	0	20	22	1	0	30	13	0	0	34	4	4	1	24	19	0	0
	Lg5	56	25	0	0	57	24	0	0	56	25	0	0	56	10	10	5	72	9	0	0
	Lg6	23	23	0	0	33	13	0	0	23	23	0	0	43	3	0	0	30	16	0	0
	Lg7	20	16	0	0	20	16	0	0	20	16	0	0	17	15	4	0	18	18	0	0
	Lg8	29	21	0	0	34	16	0	0	29	21	0	0	39	9	2	0	33	17	0	0
	Lg9	57	11	0	0	54	14	0	0	57	11	0	0	51	13	3	1	44	24	0	0
	Lg10	52	19	0	0	54	17	0	0	52	19	0	0	46	17	2	6	51	20	0	0
	Lg11	40	9	0	0	39	10	0	0	40	9	0	0	33	11	4	1	31	18	0	0
	Lg12	36	19	0	0	40	15	0	0	36	19	0	0	39	9	2	5	42	13	0	0
	Lg13	35	10	0	1	29	15	1	1	35	10	0	1	23	15	4	4	26	20	0	0
	Lg14	24	15	0	0	26	13	0	0	24	15	0	0	31	7	1	0	22	17	0	0
	Lg15	69	22	0	0	58	33	0	0	69	22	0	0	64	26	1	0	58	33	0	0
	Lg16	23	12	0	0	30	5	0	0	23	12	0	0	20	9	5	1	31	4	0	0
	Lg17	28	31	0	0	30	29	0	0	28	31	0	0	35	19	1	4	35	24	0	0

S 3.33: Number of SNPs supporting the hybridity (Hybrid), have same results as the mother (Mother) and father (Father) or have a completely different result compared with the parent (N) for the 'Fuji' x P125R095T002 population for the apple and pear Infinium® II 9K SNP array by chromosome for the apple SNPs, while the pear SNPs were all put in one group, Lg0.

		FP12 1, FP12 4 (PN), FP12 6 (PN), FP12 7 (PN)				FP12 2				FP12 3			
		Hybrid	**Mother**	**Father**	**N**	**Hybrid**	**Mother**	**Father**	**N**	**Hybrid**	**Mother**	**Father**	**N**
Pear SNP	**Lg0**	117	3	0	0	118	2	0	0	79	7	8	26
Apple SNP	**Lg1**	42	1	0	0	41	2	0	0	26	1	6	10
	Lg2	94	0	0	0	93	1	0	0	66	4	11	13
	Lg3	61	0	0	0	60	1	0	0	49	2	5	5
	Lg4	43	0	0	0	39	4	0	0	27	4	4	8
	Lg5	81	0	0	0	70	11	0	0	49	16	10	6
	Lg6	45	1	0	0	46	0	0	0	26	3	8	9
	Lg7	37	0	0	0	37	0	0	0	21	2	2	12
	Lg8	50	0	0	0	46	4	0	0	29	7	5	9
	Lg9	66	2	0	0	67	1	0	0	40	10	2	16
	Lg10	69	3	0	0	70	2	0	0	39	7	4	22
	Lg11	49	0	0	0	41	8	0	0	28	11	6	4
	Lg12	47	8	0	0	54	1	0	0	35	7	4	9
	Lg13	42	2	2	0	41	3	2	0	25	4	5	12
	Lg14	39	0	0	0	39	0	0	0	21	8	2	8
	Lg15	88	3	0	0	91	0	0	0	60	8	13	10
	Lg16	35	0	0	0	35	0	0	0	24	1	7	3
	Lg17	58	0	0	0	58	0	0	0	27	6	3	22

S 3.34: Number of SNPs supporting the hybridity (Hybrid), have same results as the mother (Mother) and father (Father) or have a completely different result compared with the parent (N) for the 'Fuji' x P266R231T015 population for the apple and pear Infinium® II 9K SNP array by chromosome for the apple SNPs, while the pear SNPs were all put in one group, Lg0.

		FP26 1- FP26 2				FP26 3			
		Hybrid	**Mother**	**Father**	**N**	**Hybrid**	**Mother**	**Father**	**N**
Pear SNP	**Lg0**	86	30	0	1	90	27	0	0
Apple SNP	**Lg1**	33	9	0	0	28	14	0	0
	Lg2	75	19	0	0	59	35	0	0
	Lg3	47	14	0	0	43	18	0	0
	Lg4	27	16	0	0	27	15	1	0
	Lg5	70	11	0	0	55	26	0	0
	Lg6	23	23	0	0	28	18	0	0
	Lg7	20	17	0	0	24	13	0	0
	Lg8	32	18	0	0	28	22	0	0
	Lg9	43	26	0	0	52	17	0	0
	Lg10	42	30	0	0	52	20	0	0
	Lg11	37	12	0	0	35	14	0	0
	Lg12	41	14	0	0	36	19	0	0
	Lg13	29	17	0	0	27	19	0	0
	Lg14	32	7	0	0	27	12	0	0
	Lg15	66	25	0	0	76	15	0	0
	Lg16	30	5	0	0	32	3	0	0
	Lg17	26	32	0	0	34	24	0	0

S 3.35: Number of SNPs supporting the hybridity (Hybrid), have same results as the mother (Mother) and father (Father) or have a completely different result compared with the parent (N) for the 'Fuji' x P354R200T138 population for the apple and pear Infinium® II 9K SNP array by chromosome for the apple SNPs, while the pear SNPs were all put in one group, Lg0.

		FP35 1				FP35 2				FP35 3				FP35 4				FP35 5-FP35 6			
		Hybrid	**Mother**	**Father**	**N**	**Hybrid**	**Mother**	**Father**	**N**	**Hybrid**	**Mother**	**Father**	**N**	**Hybrid**	**Mother**	**Father**	**N**	**Hybrid**	**Mother**	**Father**	**N**
Pear SNP	**Lg0**	85	35	0	0	84	36	0	0	81	39	0	0	75	33	6	6	86	34	0	0
Apple SNP	**Lg1**	22	21	0	0	30	13	0	0	25	18	0	0	20	16	6	1	30	13	0	0
	Lg2	70	24	0	0	66	28	0	0	65	29	0	0	54	27	6	7	74	20	0	0
	Lg3	45	16	0	0	49	12	0	0	50	11	0	0	41	17	1	2	44	17	0	0
	Lg4	27	16	0	0	31	12	0	0	28	14	1	0	32	10	1	0	34	9	0	0
	Lg5	61	19	0	0	56	24	0	0	51	29	0	0	43	16	11	10	62	18	0	0
	Lg6	27	19	0	0	27	19	0	0	34	12	0	0	31	11	3	1	34	12	0	0
	Lg7	23	14	0	0	24	13	0	0	17	20	0	0	17	14	4	2	25	12	0	0
	Lg8	40	10	0	0	28	22	0	0	31	19	0	0	32	15	3	0	32	18	0	0
	Lg9	48	20	0	0	52	16	0	0	42	26	0	0	43	16	6	3	49	19	0	0
	Lg10	41	29	0	0	44	26	0	0	46	24	0	0	42	24	0	4	46	24	0	0
	Lg11	33	16	0	0	34	15	0	0	35	14	0	0	27	16	3	3	39	10	0	0
	Lg12	40	15	0	0	36	19	0	0	40	15	0	0	32	15	1	7	37	18	0	0
	Lg13	37	9	0	0	23	23	0	0	30	15	0	1	25	18	1	2	29	17	0	0
	Lg14	24	15	0	0	26	13	0	0	30	9	0	0	25	10	3	1	26	13	0	0
	Lg15	69	22	0	0	67	24	0	0	60	31	0	0	55	25	7	4	75	16	0	0
	Lg16	29	5	0	0	23	11	0	0	31	3	0	0	21	5	7	1	30	4	0	0
	Lg17	36	24	0	0	32	28	0	0	34	26	0	0	17	30	4	9	33	27	0	0

S 3.36: Number of SNPs supporting the hybridity (Hybrid), have same results as the mother (Mother) and father (Father) or have a completely different result compared with the parent (N) for the 'Fuji' x P354R200T138 population for the apple and pear Infinium® II 9K SNP array by chromosome for the apple SNPs, while the pear SNPs were all put in one group, Lg0.

		FP35 7				FP35 8 (PN), FP35 12 (PN), FP35 9 (PN) and FP35 13 (PN)				FP35 20 (PN)				FP35 22 (PN)- FP35 10 (PN)			
		Hybrid	**Mother**	**Father**	**N**	**Hybrid**	**Mother**	**Father**	**N**	**Hybrid**	**Mother**	**Father**	**N**	**Hybrid**	**Mother**	**Father**	**N**
Pear SNP	**Lg0**	82	38	0	0	89	31	0	0	85	35	0	0	87	27	2	4
Apple SNP	**Lg1**	29	14	0	0	23	20	0	0	30	13	0	0	33	7	2	1
	Lg2	72	22	0	0	71	23	0	0	66	28	0	0	61	17	9	7
	Lg3	44	17	0	0	45	16	0	0	49	12	0	0	52	6	2	1
	Lg4	30	13	0	0	29	14	0	0	31	12	0	0	34	4	4	1
	Lg5	60	20	0	0	58	22	0	0	56	24	0	0	55	10	10	5
	Lg6	31	15	0	0	28	18	0	0	27	19	0	0	43	3	0	0
	Lg7	19	18	0	0	17	20	0	0	24	13	0	0	19	14	4	0
	Lg8	37	13	0	0	36	14	0	0	28	22	0	0	38	10	2	0
	Lg9	48	20	0	0	48	20	0	0	52	16	0	0	50	14	2	2
	Lg10	43	27	0	0	45	25	0	0	44	26	0	0	45	17	2	6
	Lg11	35	14	0	0	33	16	0	0	34	15	0	0	32	12	4	1
	Lg12	41	14	0	0	40	15	0	0	36	19	0	0	38	10	3	4
	Lg13	29	16	0	1	28	17	0	1	23	23	0	0	22	16	4	4
	Lg14	19	20	0	0	24	15	0	0	26	13	0	0	31	7	1	0
	Lg15	69	22	0	0	73	18	0	0	67	24	0	0	63	27	1	0
	Lg16	31	3	0	0	31	3	0	0	23	11	0	0	20	9	4	1
	Lg17	26	34	0	0	33	27	0	0	32	28	0	0	38	17	1	4

S 3.37: Number of SNPs supporting the hybridity (Hybrid), have same results as the mother (Mother) and father (Father) or have a completely different result compared with the parent (N) for the 'Abate' x 'Fuji' population for the apple and pear Infinium® II 20K SNP array by chromosome for the apple SNPs.

		AF			
		Hybrid	**Mother**	**Father**	**N**
Apple SNP	**Lg0**	617	2	9	0
	Lg1	336	2	5	0
	Lg2	547	6	14	0
	Lg3	514	1	11	0
	Lg4	408	4	5	0
	Lg5	509	5	22	0
	Lg6	413	1	11	0
	Lg7	357	2	9	0
	Lg8	416	3	11	0
	Lg9	447	0	11	0
	Lg10	547	1	8	0
	Lg11	548	1	15	0
	Lg12	533	2	10	0
	Lg13	443	2	12	0
	Lg14	425	1	14	0
	Lg15	631	5	26	0
	Lg16	435	1	12	0
	Lg17	479	1	10	0

S 3.38: Number of SNPs supporting the hybridity (Hybrid), have same results as the mother (Mother) and father (Father) or have a completely different result compared with the parent (N) for the 'Decana' x 'Murray' population for the apple and pear Infinium® II 20K SNP array by chromosome for the apple SNPs.

		DM1				DM2			
		Hybrid	**Mother**	**Father**	**N**	**Hybrid**	**Mother**	**Father**	**N**
Apple SNP	**Lg0**	800	76	0	0	805	78	0	0
	Lg1	275	48	4	1	281	48	1	0
	Lg2	467	65	2	1	481	64	1	2
	Lg3	434	65	1	1	443	66	1	0
	Lg4	374	43	2	0	369	39	1	1
	Lg5	467	47	1	1	470	48	2	1
	Lg6	343	42	5	2	358	47	2	1
	Lg7	326	43	0	0	331	42	0	0
	Lg8	380	52	0	0	386	49	0	1
	Lg9	408	39	0	1	409	38	0	1
	Lg10	489	51	4	0	496	51	2	0
	Lg11	482	59	1	1	482	61	0	0
	Lg12	462	45	1	0	468	47	2	0
	Lg13	409	42	1	0	412	43	0	0
	Lg14	389	31	0	0	390	32	0	0
	Lg15	578	48	0	3	602	51	1	1
	Lg16	380	37	0	0	376	38	0	0
	Lg17	433	29	0	2	434	32	0	2

CHAPTER IV

Disease evaluation

4 Disease evaluation

4.1 Abstract

Apple scab (*Venturia inaequalis*) and pear scab (*V. pyrina* and *V. nashicola*) are fungal diseases specific to apple and pear, respectively. Together with fire blight (*Erwinia amylovora*), these diseases cause major losses to apple and pear production worldwide if untreated.

We investigated apple scab, pear scab (*V. pyrina*) and fire blight resistance in the apple-pear hybrids by inoculating replicate trees of the putative hybrids from The New Zealand Institute for Plant and Food Research Limited. Inoculation was by spray and/or bag methods for scab, and by cut-leaf method for fire blight. Macroscopic, complemented with microscopic observation on selected trees, observations were performed after 3 weeks for apple and pear scab. Fire blight progress was observed in the period from 2 to 6 weeks after inoculation, with disease expressed as a percentage of the total shoot length.

The results from this study showed that FP18, FP12 2, FP26 and FP35 4 of the progeny were susceptible and CO, P26A19, IP26, IP12, FP12 3 and three FP35 resistant to apple scab, but all resistant to *V. pyrina*.

All 'Cox's Orange Pippin' x 'Old Home' apple x pear and P26A19 4 pear x apple hybrids exhibited low to no fire blight infection. Most 'Fuji' progeny in our work are moderately susceptible or susceptible to fire blight. IP12, IP26, P26A19 3 and P26A17 are susceptible to fire blight.

Future work is needed to confirm these results by repeating this study. Furthermore, mapping the recombination events during the crossing of apple and pear more precisely along the chromosomes of the apple x pear hybrids can give more explanations of these data.

Keywords: disease, apple scab, pear scab, fire blight, apple-pear hybrid

4.2 Introduction

Scab

Apple and pear are two of the most cultivated fruit crops throughout the temperate regions of the world. Their production faces continual new challenges, such as a constant change in consumer demand based on a variation of tastes and flavours, and, from an agronomic point of view, climate change and harmful biotic agents (insects or bacterial, fungal and viral pathogens).

Apple is attacked by a number of pathogens, resulting in inferior quality of fruit and in some instances a drastic reduction in overall production. Apple scab is the most devastating of these pathogens, and therefore the biggest challenge faced by apple growers all over the world (MacHardy, 1996). Equally, pear scab, caused by two species of *Venturia*, viz., *V. nashicola* and *V. pyrina*, is one of the most serious fungal pathogen diseases of Asian and European pears, respectively. The fungi infect leaves, fruit and twigs, each with a mostly mutually exclusive host range (Janick *et al.*, 1997; Ishii *et al.*, 2000) as *V. nashicola* infects Asian pears throughout their natural range, and *V. pyrina* occurs in most regions where European pears are grown (Terakami *et al.*, 2006).

Fire blight

Fire blight disease is indigenous to North America and has spread to more than 50 countries around the world, including Europe, North Africa, Middle East, Oceania and Asia (Figure 4.1). *Erwinia amylovora* is the causal agent of fire blight, affecting most species of the subfamily Maloideae in the family Rosaceae. Fire blight is probably the most serious bacterial pathogen disease affecting *Pyrus* spp. and *Malus* spp. cultivars in many countries. Although copper compounds and antibiotics are used for controlling fire blight (Psallidas and Tsiantos, 2000), the success of these treatments are variably effective against this disease. In addition, with the pathogen becoming resistant to these compounds and antibiotics increasingly being banned in many countries, growers have few options for disease control. The main long-term alternative is to create new resistant or at least less susceptible apple and pear cultivars (Kellerhals *et al.*, 2017; Bell, 2019; Kostick *et al.*, 2019; Zurn *et al.*, 2020).

A number of loci for control of resistance to these diseases have been located on genetic maps of both apple and pear. Bus *et al.* (2011) summarized the global positions on the apple genome of 17 *Rvi* scab resistance genes (LG1, LG2, LG3, LG4, LG6, LG8, LG10, LG12 and LG17). In 2007, Pierantoni *et al.* (2007) identified two major quantitative trait loci (QTLs) on LG3 and LG7 associated with resistance to pear scab.

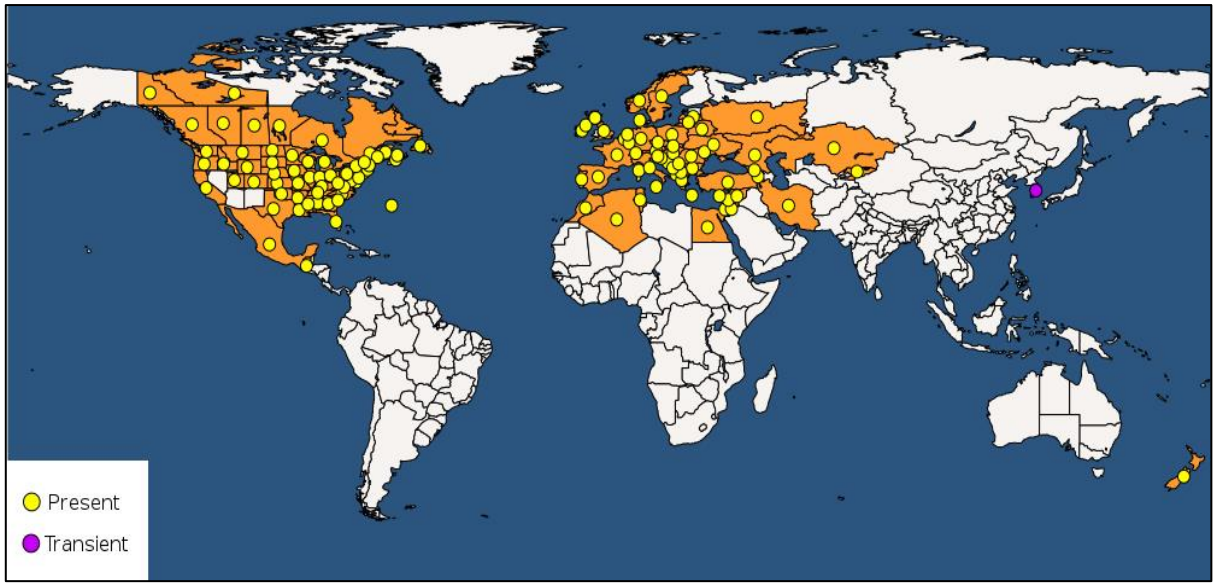

Figure 4.1: Fire Blight distribution in the world (EPPO 2021).

Major fire blight resistance have been identified on LG7 of the apple cultivar 'Fiesta' (Khan *et al.*, 2007), which descends from 'Cox's Orange Pippin'; on LG3 of the wild apple cultivar 'Robusta 5' (Gardiner *et al.*, 2012); on LG10 of the American wild species *Malus fusca* (Emeriewen *et al.*, 2014), and on LG2 of pear cultivar 'Old Home' (Dondini *et al.*, 2005; Le Roux *et al.*, 2012) and 'Moonglow' (Montanari *et al.*, 2016). Many more minor QTLs have been mapped, particularly in apple (Peil *et al.*, 2020).

In this study, we investigated the resistance of the putative apple-pear hybrids and their parents to apple scab, pear scab and fire blight.

4.3 Materials and Methods

4.3.1 Scab

The plant material used to assess the resistance or susceptibility to *V. inaequalis* and *V. pyrina* was located at the PFR research orchard in Havelock North (NZ). The putative hybrids with apple as female parent and apple reference accessions ('Cox's Orange Pippin', A174R01T204, A199R45T055, 'Fuji', 'Imperial Gala', 'Red Delicious', 'Robusta 5' and 'Splendour') were grafted onto M9. Those with pear as the female parent and reference pear accessions ('Old Home', P265R23T018, P125R095T002, P037R048T081, P186R125T002, P354R200T138, P266R231T015 and 'Williams Bon Chretien') were grafted onto *P. calleryana*.

To determine the scab resistance of each genotype to the *Venturia* pathogens present in New Zealand, three replicate trees each were inoculated with conidia of the two species. Inoculation with *V. inaequalis* was done with the spray and bag methods, and *V. pyrina* with the bag method.

For *V. inaequalis*, the plants were inoculated with isolate MNH120 in 2018 and 2019 with spray method. The inoculum was prepared using infected leaves from seedlings harvested in the previous year and stored at -18°C. The leaves were soaked in water and the concentration of the resulting spore suspension was adjusted to 3 $\times 10^5$ spores/mL. The inoculum was spray-inoculated onto the leaves till run-off (Figure 4.2). The spore germination rates were ~80% in 2018 and ~90% in 2019. The relative humidity in the glasshouse was maintained at 100% for the first 2 days, ten at 80% for 3 weeks till visual observation was performed and samples were harvested for microscopic observation.

For *V. inaequalis*, the plants were inoculated with isolate MNH120 in 2018 with bag method. The germination rate was about ~80% in 2018 (Figure 4.2).

For *V. pyrina*, inoculum of isolate P35.2 at 10^5 spores/mL was prepared as described above from dried leaves stored at -18°C since 2011, showing germination rates of ~85% in 2018 and in 2019. For the inoculation, the youngest unfolded leaf was placed in a zip-lock bag and inoculum was placed on the adaxial surface of the leaf using a pipette, after which the zip was locked around the petiole, ensuring that the leaf was covered with a film of inoculum between leaf and bag (Figure 4.2). After two days the bag was removed and after 3 weeks at 80% relative humidity, visual observation was performed and leaf samples were harvested for microscopic observations.

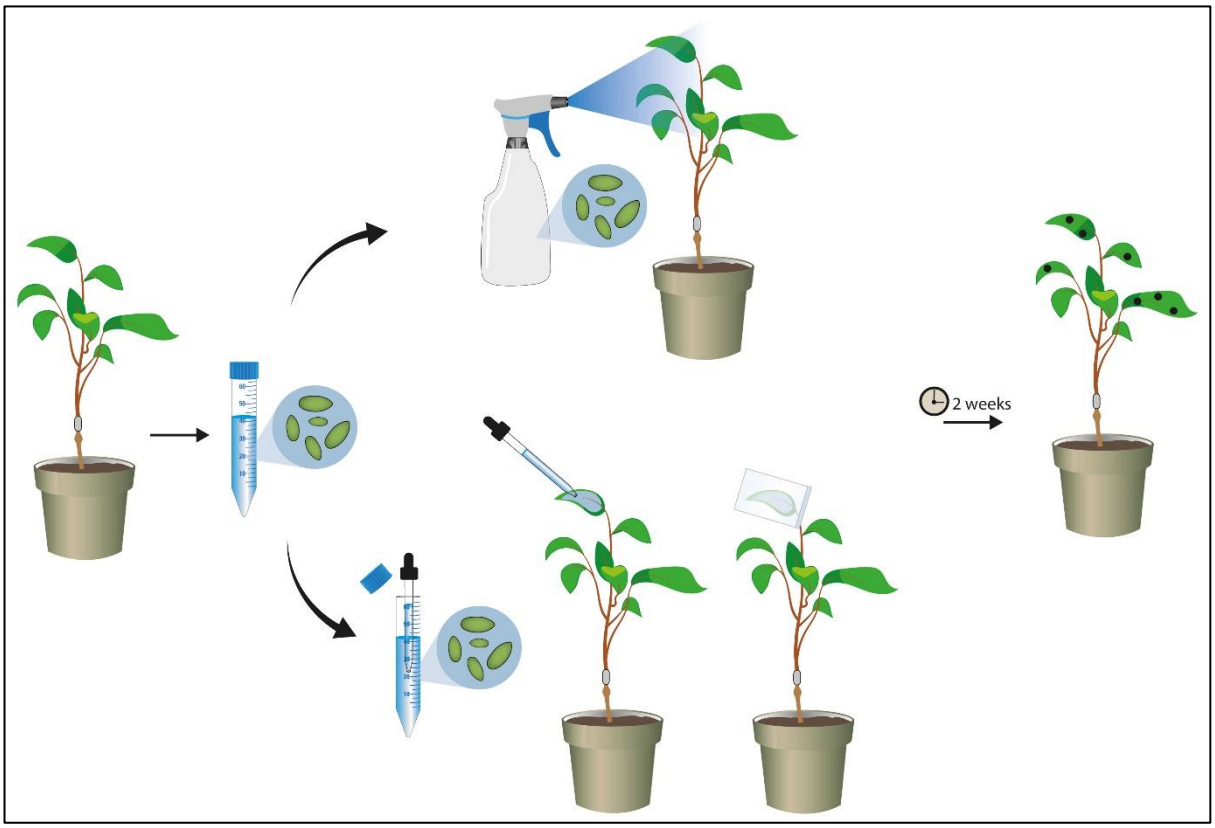

Figure 4.2: Steps in the spray (top) and bag (bottom) inoculation methods for *V. inaequalis* and *V. pyrina* for the assessment of disease resistance levels (Tony Corbett, 2019).

The leaves were prepared for microscopic observation as follows: the leaves were cut into portions of up to 2x2 cm^2 and were immersed in a clearing and staining solution for 48 h at room temperature (20-25°C) after which the leaves were placed for 12-24 h in chloral hydrate (chloral hydrate 2.5 g/mL water) (Bruzzese and Hasan, 1983). The leaves were then rinsed rapidly in distilled water, dabbed dry on tissue paper and mounted on microscope slides in a mounting solution (Cunningham, 1972). The leaves were dried for a month after mounting prior to making observations (Figure 4.3). The slides were observed for the presence of scab susceptible and resistance symptoms. Images of slides were taken using an Axio Imager 2 microscope (ZEISS), DAPI fluorescence filter set, and charge-coupled device camera (AxioCam MRm, Zeiss).

Value corresponds of symptoms were given (0 to 4): 0 indicated resistance/no macroscopic symptoms; 1 = resistance/hypersensitive response; 2 = resistance/(stellate) necrosis; 3 = resistance/chlorosis, including stellate chlorosis (SC), with limited sporulation; 4 = susceptibility. If a symptom showed sporulation, the amount was rated on a 1-5 scale and included as a decimal in the symptom score.

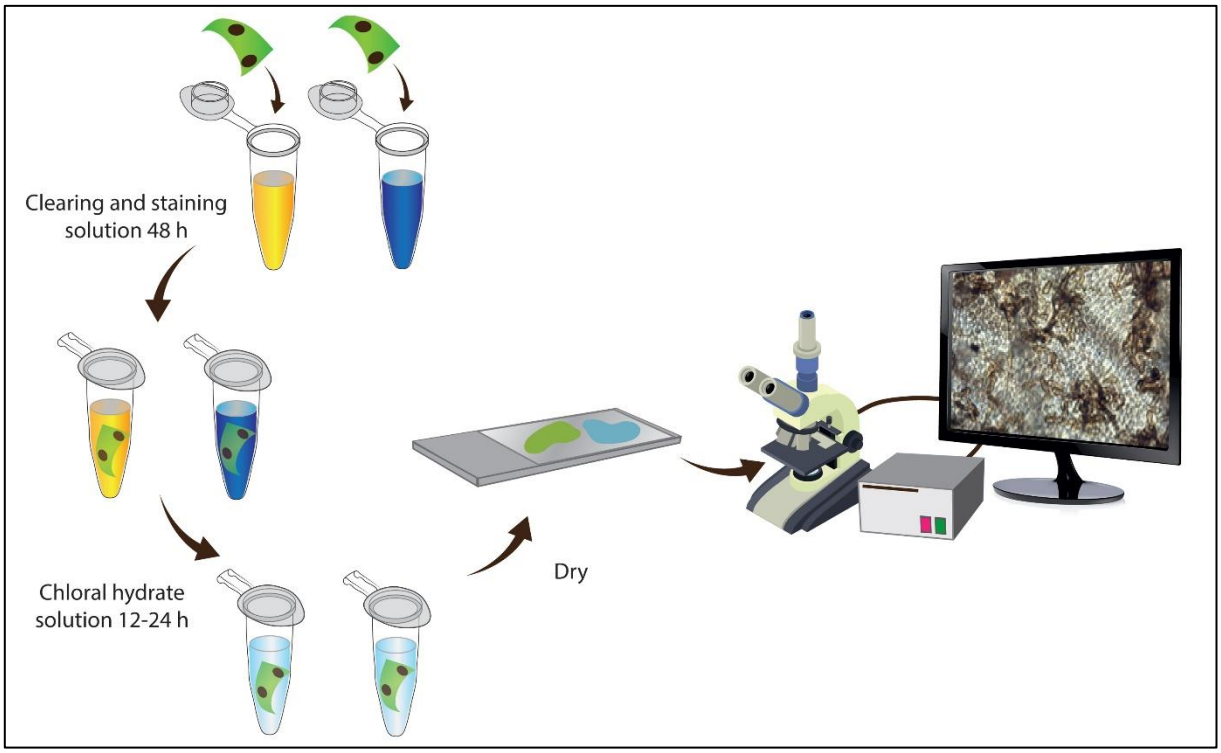

Figure 4.3: Steps in preparing microscopic slides for microscope observation of *V. inaequalis, and V. pyrina* (Tony Corbett, 2020).

4.3.2 Fire blight

The plant material used to assess the resistance to *E. amylovora*, as well as the tree preparation (up to eight replicates each) was the same as described for the scab experiments above.

Prior to inoculation, trees were placed in the greenhouse with a temperature regime of 25°C in the daytime and 20°C at night, and 80% relative humidity (RH) for 14 days, after which the plants were acclimated to 26°C and 95% RH for inoculation, which was by the cut-leaf method (Maas Geesteranus and Heyting, 1980). Shoots about 25 cm long that were still actively growing, were inoculated by cutting off 2/3 of the two youngest expanding leaves with scissors dipped in an aqueous buffer suspension of the strain Ea236 of *E. amylovora* at 1 x 10^9 cfu/ml (Figure 4.4). The plants were maintained at 26°C and 95% RH for 7 days, then a further 21 days at 25°C and 80% RH until observations were made.

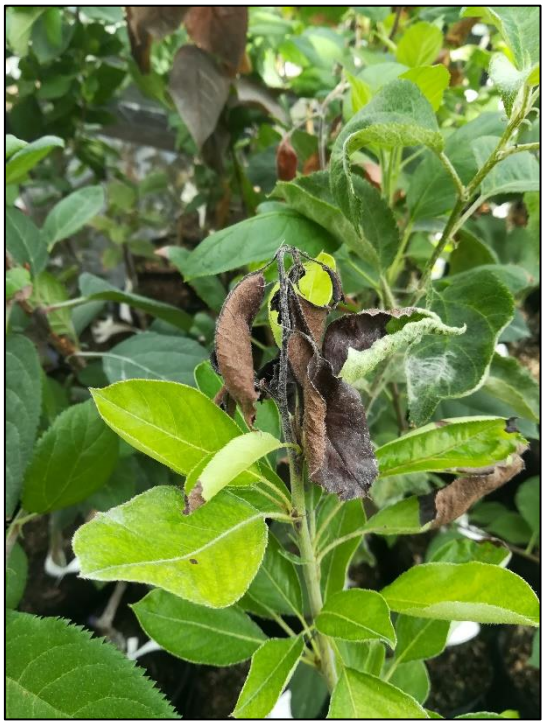

Figure 4.4: Example of fire blight infection 27 days after inoculation (seedling).

Disease progress was observed in the period from 12 to 37 days after inoculation (four time). The degree of disease was quantified by expressing necrosis length as a percentage of the total shoot length, both measured downwards from the point of inoculation (Figure 4.5). The mean percent necrosis was then calculated for each genotype at each observation date and the area-under-the-disease-progress-curve (AUDPC) was calculated using the trapezoidal rule.

We used a non-linear scale for the percentage of necrosis to determine the degree of resistance/susceptibility to fire blight: 0% = immune; 1-5% = highly resistant; 6-15% = resistant; 16-30% = moderately resistant, 31-50% = moderately susceptible; and 51-100% = (highly) susceptible (Le Lezec and Paulin, 1983).

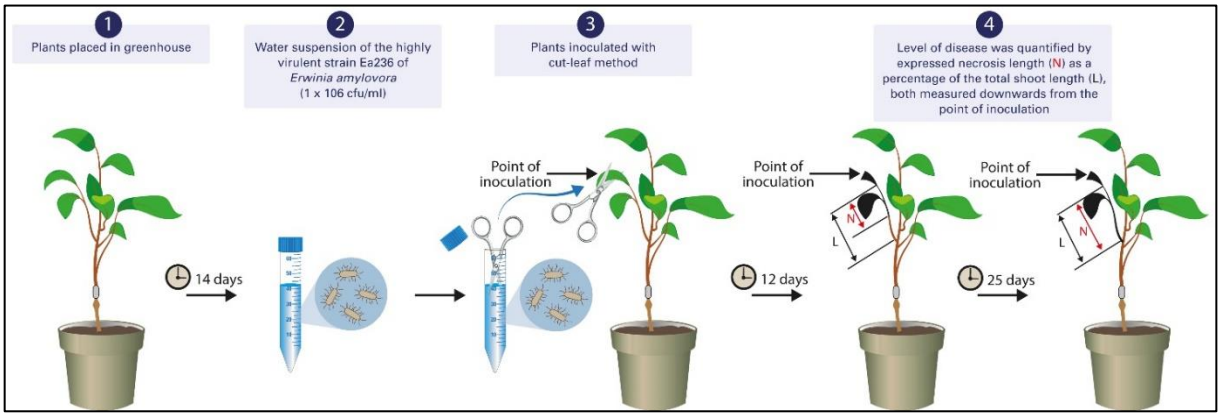

Figure 4.5: Steps during plant inoculation with *E. amylovora* and measurement of disease level (Tony Corbett, 2019).

Statistical variance analysis was performed on the AUDPC data to detect the significantly different resistance/susceptibility. The data were randomized with a Fisher randomization test.

4.4 Results

4.4.1 Scab

The results for apple scab inoculated with the bag and spray methods as well as those for pear scab inoculated with the bag method in 2018, did not give clear results in our study, therefore are not presented here.

The inoculated plants showed more distinctive symptoms for *V. inaequalis* (spray-inoculated) and *V. pyrina* (bag-inoculated) in 2019, with the maximum symptom score on any of the three replicates of a genotype presented from these preliminary exploratory observations. The maximum score of 'Cox's Orange Pippin' apple was used as the reference for susceptibility, which was 3.3 rather than 4, indicating that the infection conditions may have been sub-optimal. Similarly for pear, the generally susceptible cultivar 'Williams Bon Chretien' showed a necrotic reaction rather than the expected sporulation.

All of the CO hybrids were resistant to apple scab, with most showing chlorosis, as did the pear parent 'Old Home', too, with a few progeny showing limited sporulation, while the female apple

parent 'Cox's Orange Pippin' showed considerable sporulation. The findings for *V. pyrina* showed that all CO progeny were resistant with either no macroscopic symptoms or with a hypersensitive response (HR), although the pear parent 'Old Home' did show some chlorosis (Table 4.1).

Table 4.1: Maximum scores for value corresponds to *Venturia inaequal*s and *V. pyrina* symptoms on inoculated 'Cox's Orange Pippin' x 'Old Home' apple/pear hybrids in 2019. See Materials & Materials for scale; N/C = missing data.

	V. inaequalis	***V. pyrina***
CO 1	3	1
CO 2	3.1	1
CO 3	SC	1
CO 4	3	1
CO 5	3	1
CO 7	3.1	1
CO 8	3	1
CO 9	3	1
CO 10	3	0
CO 11	3	1
CO 13	3.1	1
CO 14	3	0
CO 15	0	1
CO 17	3	1
CO 19	3	1
CO 20	3	1
CO 22	3.1	1
CO 25	3	1
CO 26	SC	0
CO 27	3	1
CO 29	3	1
CO 31	3	1
CO 33	3	1
CO 34	3.1	1
'Cox's Orange Pippin'	3.3	0
'Old Home'	3	0
'Williams Bon Chretien'	3	2
'Fuji'	N/C	0
'Splendour'	N/C	0
'Robusta 5'	N/C	0

Brightfield microscopic observations of the Class 3 (resistance – chlorosis with limited sporulation) apple scab symptoms of progeny CO 1 showed dense sporulation (Figure 4.6).

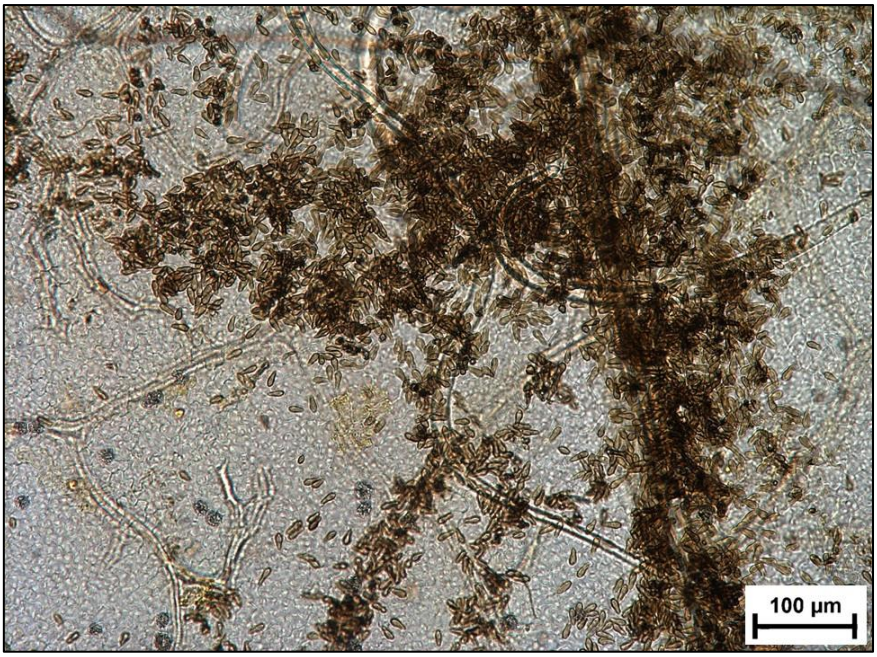

Figure 4.6: Brightfield microscopic observations of *V. inaequalis* reactions on leaves with chlorotic resistance reaction with limited sporulation in progeny CO 1.

Microscopic observations of the Class 3 symptoms of *V. inaequalis* infection on progeny CO 33 showed some necrotic browning (brightfield (Figure 4.7A) interspersed with areas of light-blue fluorescence when incited by fluorescence ultraviolet light (Figure 4.7B)

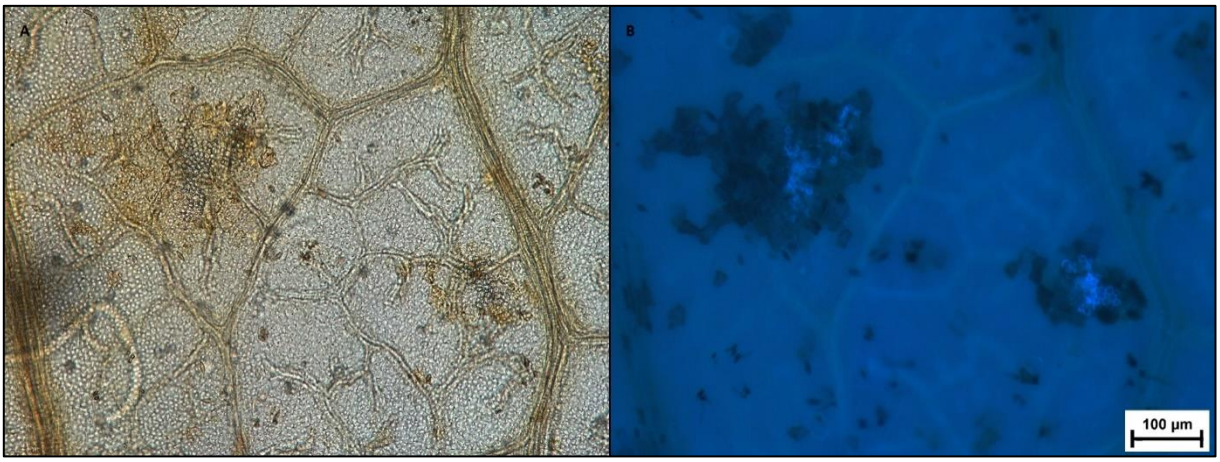

Figure 4.7: Brightfield (A) and DAPI fluorescence (B) microscopic observations of *V. inaequalis* reactions on leaves of CO 33, whose macroscopic symptoms were rated Class 3 (chlorosis, sometimes with limited sporulation).

Brightfield microscopic observations of *V. pyrina* infection on leaf samples from CO 1 and CO 33 with Class 0 for CO 1 and Class 1 (resistance – hypersensitive response) for CO 33 in the glasshouse screen showed that both progeny showed hypersensitive responses. (Figure 4.8).

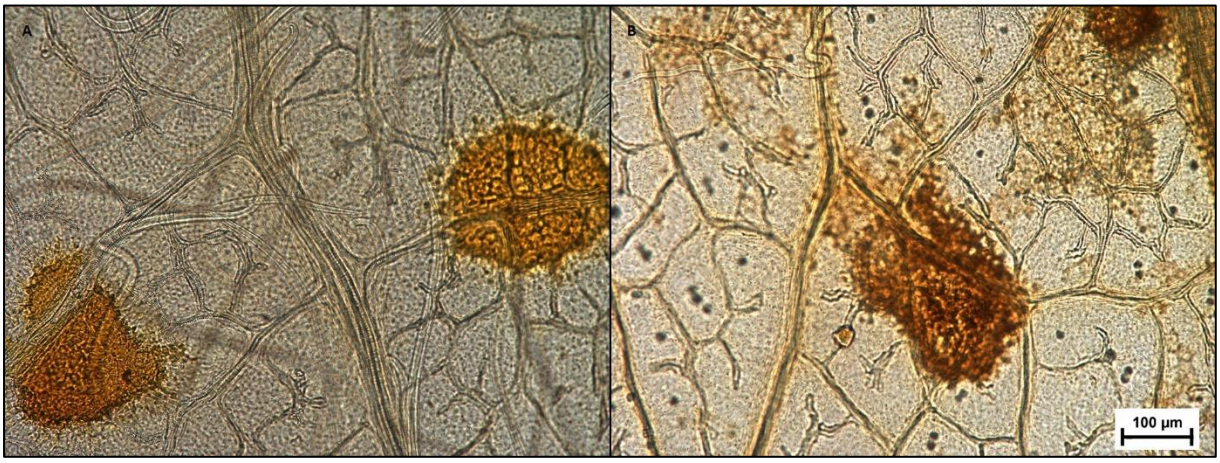

Figure 4.8: Brightfield microscopic observations of *V. pyrina* hypersensitive response reactions on leaves of CO 1 (A) and CO 33 (B) samples.

P26A19 3 and P26A19 4 showed resistance with no macroscopic symptoms to both apple and pear scab, like their female pear parent P265R23T018 (Table 4.2).

IP26 and its male pear parent P266R231T015 showed chlorotic resistance with and without limited sporulation, respectively, for apple scab and resistance with no macroscopic symptoms for pear scab.

The two IP12 progeny showed resistance with limited sporulation for apple scab, with IP12 1 tending to susceptibility. Both were resistant to pear scab (Table 4.2).

Both FP18 progeny were regarded susceptible to apple scab because of their high levels of sporulation, but immune to pear scab, like their female apple parent 'Fuji' as it is a nonhost for *V. pyrina*. Unfortunately, we do not have a result for apple scab for 'Fuji', but it is generally regarded as highly susceptible (Sheikh *et al.*, 2020) (Table 4.2). The FB18 progeny were immune to pear scab, like their female apple parent 'Fuji' as it is a nonhost for *V. pyrina*.

FP12 2 and FP12 3 showed chlorosis with considerable and limited sporulation, respectively, for apple scab, with FP12 2 showing sufficient sporulation to be regarded susceptible. Both were highly resistant without macroscopic or HR symptoms for pear scab (Table 4.2).

All three FP26 progeny are regarded susceptible to apple scab as they all showed high levels of sporulation, while none of them, like their male pear parent P266R231T015, showed macroscopic symptoms for *V. pyrina* infection (Table 4.2).

Of the four FP35 progeny tested, one with score 4 were clearly susceptible and three, FP35 2, 5 and 6, being regarded resistant to apple scab based on its limited sporulation. None of them showed any symptoms of pear scab, nor did their male pear parent P354R200T138 (Table 4.2).

Table 4.2: Maximum scores for value corresponds to *Venturia inaequal*s and *V. pyrina* symptoms on inoculated P265R232T018 x A199R45T055, 'Imperial Gala' x P266R231T015, 'Imperial Gala' x P125R095T002, 'Fuji' x P186R125T002, 'Fuji' x P125R095T002, 'Fuji' x P266R231T015 and 'Fuji' x P354R200T138apple/pear or pear/apple hybrids in 2019. See Materials & Materials for scale; N/C = missing data.

	V. inaequalis	***V. pyrina***
P26A19 3	0	0
P26A19 4	0	0
IP26 2	3.2	0
IP12 1	3.3	0
IP12 2	3.2	0
FP18 1	3.5	0
FP18 3	3.4	0
FP12 2	3.4	1
FP12 3	3.2	0
FP26 1	4	0
FP26 2	4	0
FP26 3	3.5	0
FP35 2	3.2	0
FP35 4	4	0
FP35 5	3.2	0
FP35 6	3.1	0
P354R200T138	0	0
P266R231T015	3	0
'Williams Christ'	0	necrosis
P125R095T002	0	0
P186R125T002	0	
P265R23T018	0	0
'Fuji'	N/C	0
'Splendour'	N/C	0
'Robusta 5'	N/C	0

4.4.2 Fire Blight

The results clearly show that all of the 31 putative CO hybrids were resistant to fire blight, while the parents and controls more or less exhibited the expected range of resistance/susceptibility according to previous research, with 'Old Home' being more resistant (Dondini *et al.*, 2005; Le Roux *et al.*, 2012) than 'Cox's Orange Pippin', which was moderately susceptible in our experiment (Kostick *et al.*, 2019) (Figure 4.9). Strikingly, the degree of observed fire blight resistance in all progeny was greater than that for the 'Cox's Orange Pippin' apple parent. Resistance ranged from 0% necrosis exhibited by 25 hybrids, the same as for the pear parent 'Old Home', with the remaining six progeny showing 12.5% to 25.0% necrosis, and AUDPCs from 3.27 u^2 to 7.25 u^2 at the final measurement. In the latter plants, the necrosis lengths had reached their maximum by 19 days after inoculation (Figure 4.9 B). Although 'Imperial Gala' and 'Williams Bon Chretien' both were susceptible controls, their resistance profiles differed over time (Figure 4.9 A). At 28 days after inoculation, necrosis in 'Imperial Gala' had plateaued, whilst in 'Williams Bon Chretien', the necrosis was still increasing at 37 days and might be predicted to increase further with time.

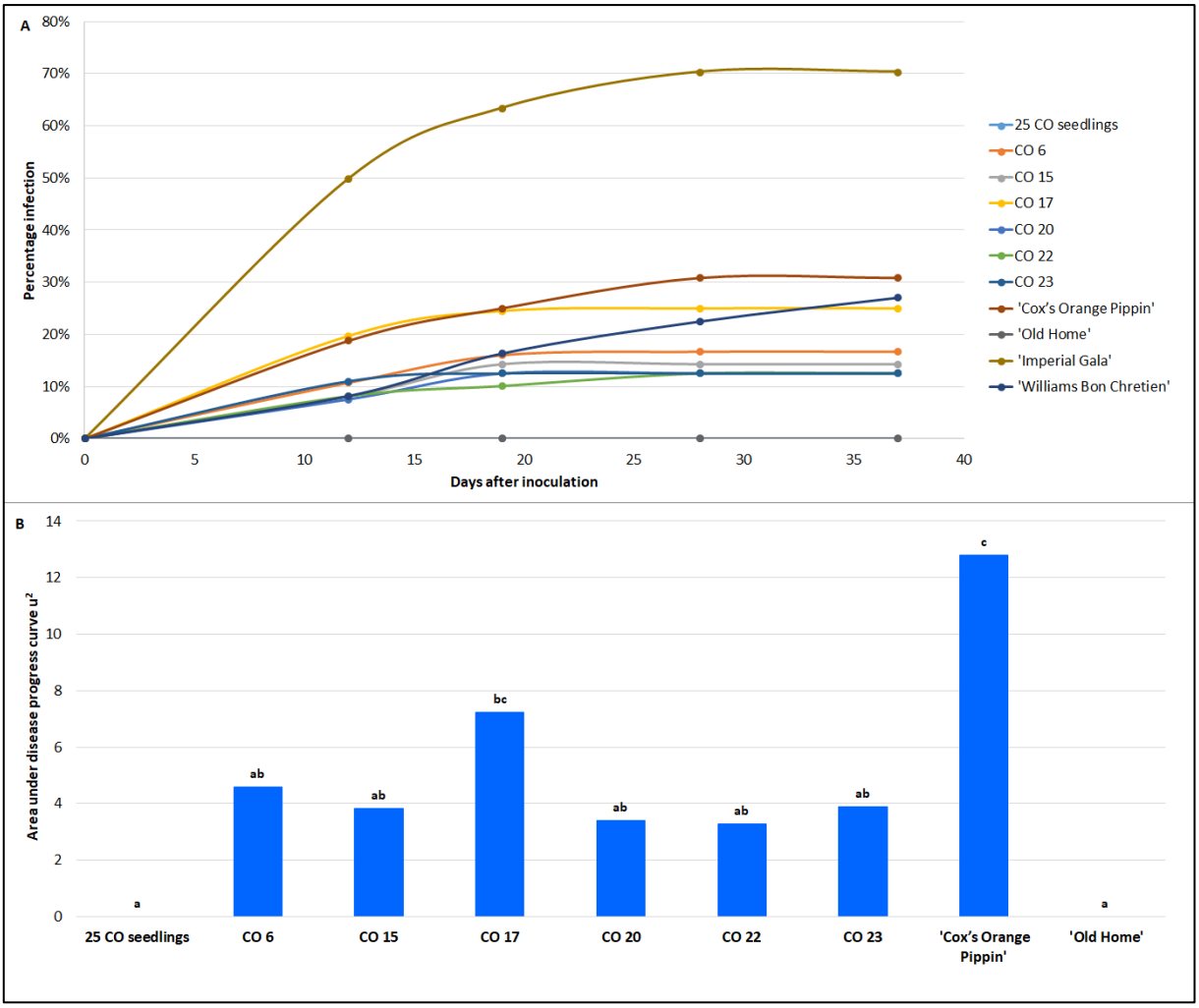

Figure 4.9: Fire blight necrosis progress in 'Cox's Orange Pippin' x 'Old Home' population and reference accessions after inoculation with *E. amylovora* using the cut-leaf method (A). Area under disease progress curves from Figure 4.9 A (B).

The P26A17 progeny was susceptible, with the parents and controls more or less exhibiting the expected range of resistance to fire blight. The degree of observed fire blight resistance in P26A17, both parents were resistant with 0% necrosis in this trial, but heterozygous for the resistance. P26A17 showed 50.0% necrosis at 37 days after the inoculation with an AUDPC of 2.25 u^2 (Figure 4.10).

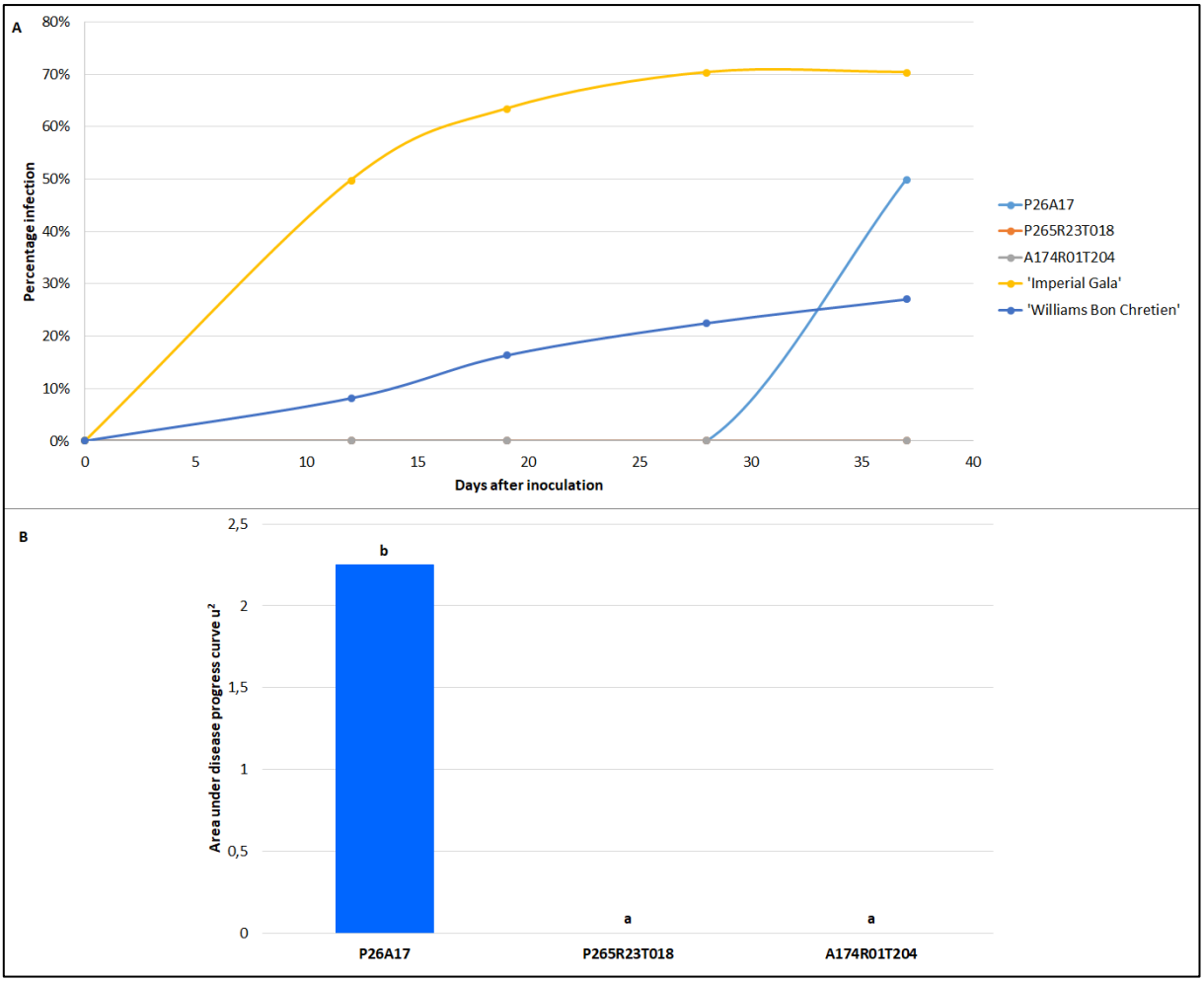

Figure 4.10: Fire blight necrosis progress in P265R232T018 x A174R01T204 population and reference accessions after inoculation with *E. amylovora* using the cut-leaf method (A). Area under disease progress curves from Figure 4.10 A (B).

The results of the two P26A19 accessions showed progeny P26A19 3 as susceptible to fire blight, similar to its father (A199R45T055), while P26A19 4 was highly resistant like its mother (P265R232T018). At 28 days after the inoculation, necrosis in 'Imperial Gala', A199R45T055 and P26A19 3 had plateaued, whilst in 'Williams Bon Chretien', the necrosis was still increasing at 37 days, as noted above. With 1.07% necrosis and AUDPC of 0.05 u^2, P26A19 4 exhibited a high level of resistance, similar to that of its pear parent P265R232T018. In contrast, with 50.0% necrosis and AUDPC of 8.75 u^2 at the final measurement, P26A19 3 was susceptible, similar to its apple parent A199R45T055 (Figure 4.11).

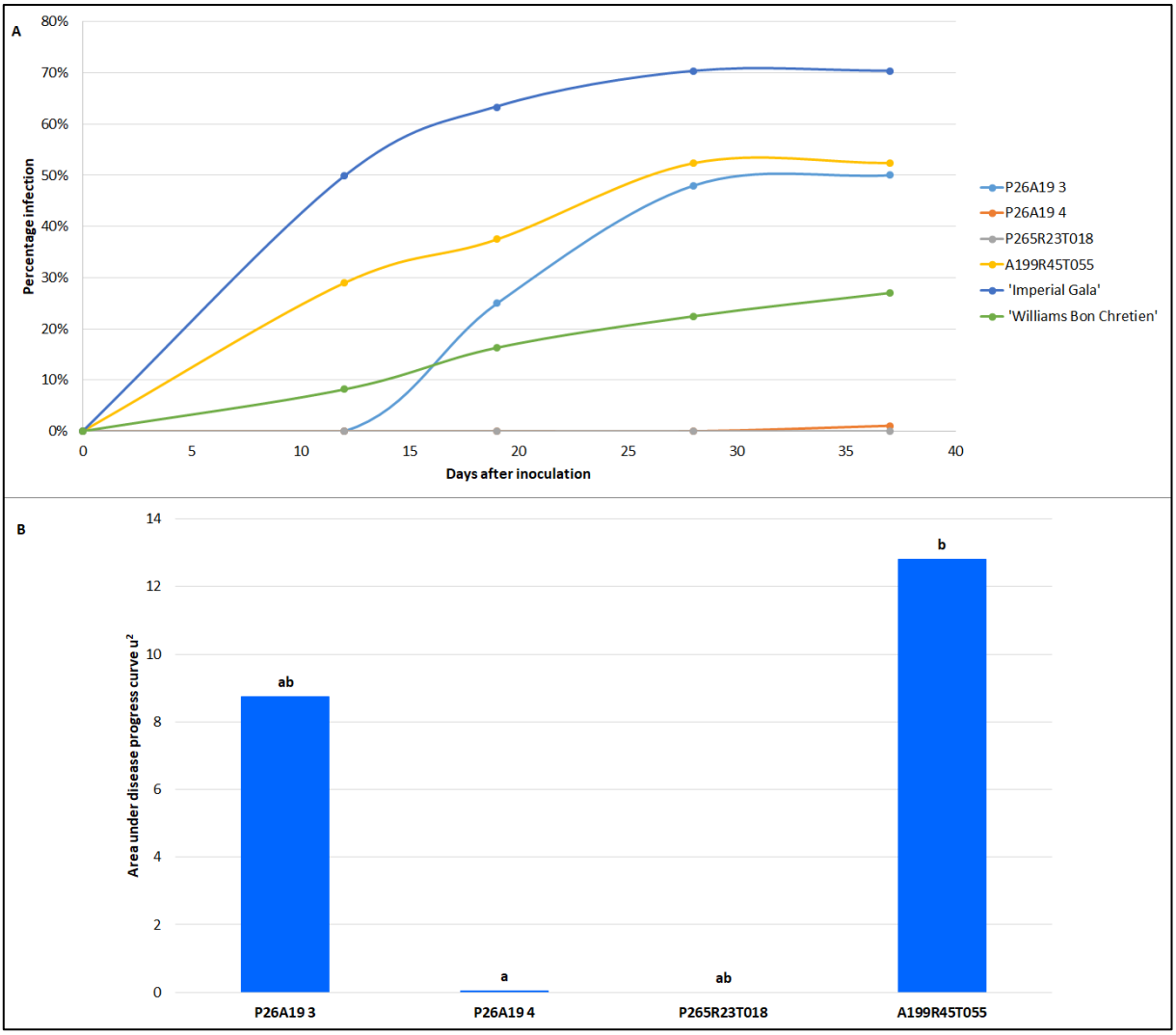

Figure 4.11: Fire blight necrosis progress in P265R232T018 x A199R45T055 population and reference accessions after inoculation with *E. amylovora* using the cut-leaf method (A). Area under disease progress curves from Figure 4.11 A (B).

The results clearly show that both IP26 1 and IP26 2 were susceptible to fire blight, with the latter similar to that of the 'Imperial Gala' apple parent. The necrosis lengths were 57% to 100%, and the AUDPCs 16.9 u^2 to 28.2 u^2, respectively, at the final measurement for both progeny (Figure 4.12).

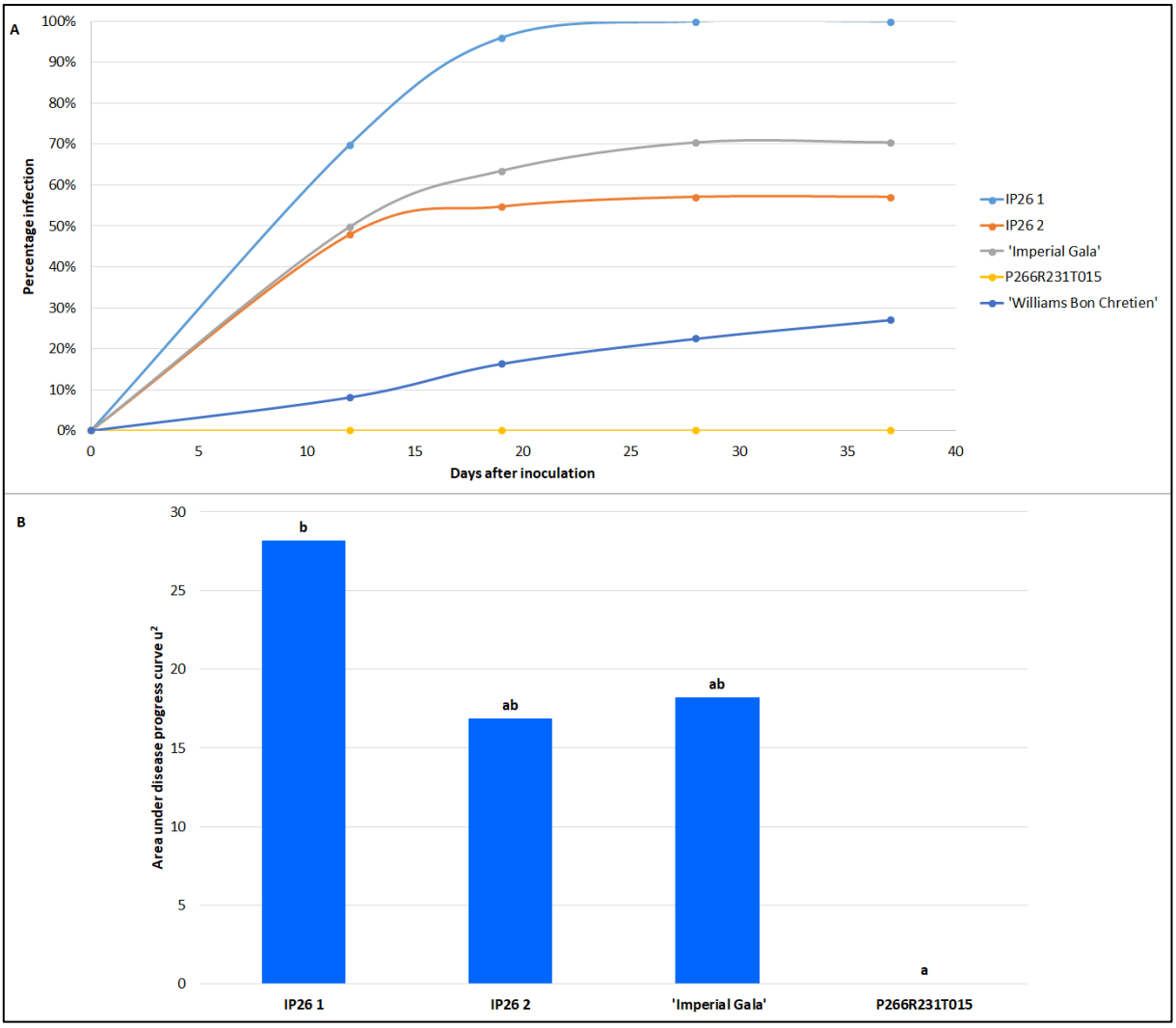

Figure 4.12: Fire blight necrosis progress in 'Imperial Gala' x P266R231T015 population and reference accessions after inoculation with *E. amylovora* using the cut-leaf method (A). Area under disease progress curves from Figure 4.12 A (B).

Similarly, both IP12 progeny were susceptible to fire blight, same as the apple mother 'Imperial Gala', with 50% and 67% of necrosis at 37 days after inoculation and with AUDPCs of 11.21 u^2 and 13.59 u^2 for IP12 1 and IP12 2, respectively (Figure 4.13).

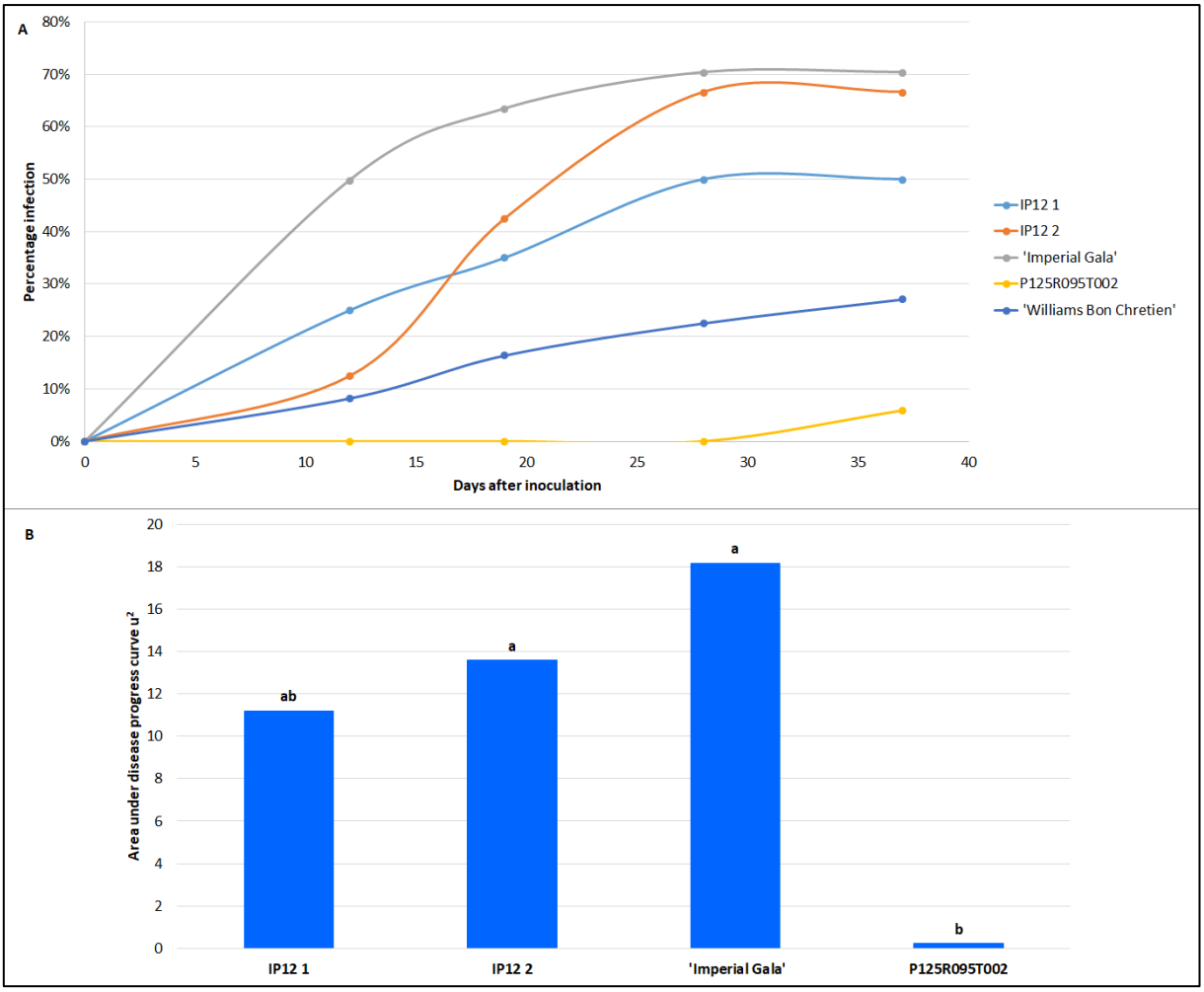

Figure 4.13: Fire blight necrosis progress in 'Imperial Gala' x P125R095T002 population and reference accessions after inoculation with *E. amylovora* using the cut-leaf method (A). Area under disease progress curves from Figure 4.13 A (B).

The three FP18 progeny were moderately susceptible with the necrosis having plateaued at 37 days after the inoculation, while the parents 'Fuji' and P186R125T002 were resistant in this study. The FP18 progeny showed 36.0% to 41.0% necrosis at the final measurement and AUDPCs from 7.28 u^2 to 10.2 u^2 (Figure 4.14).

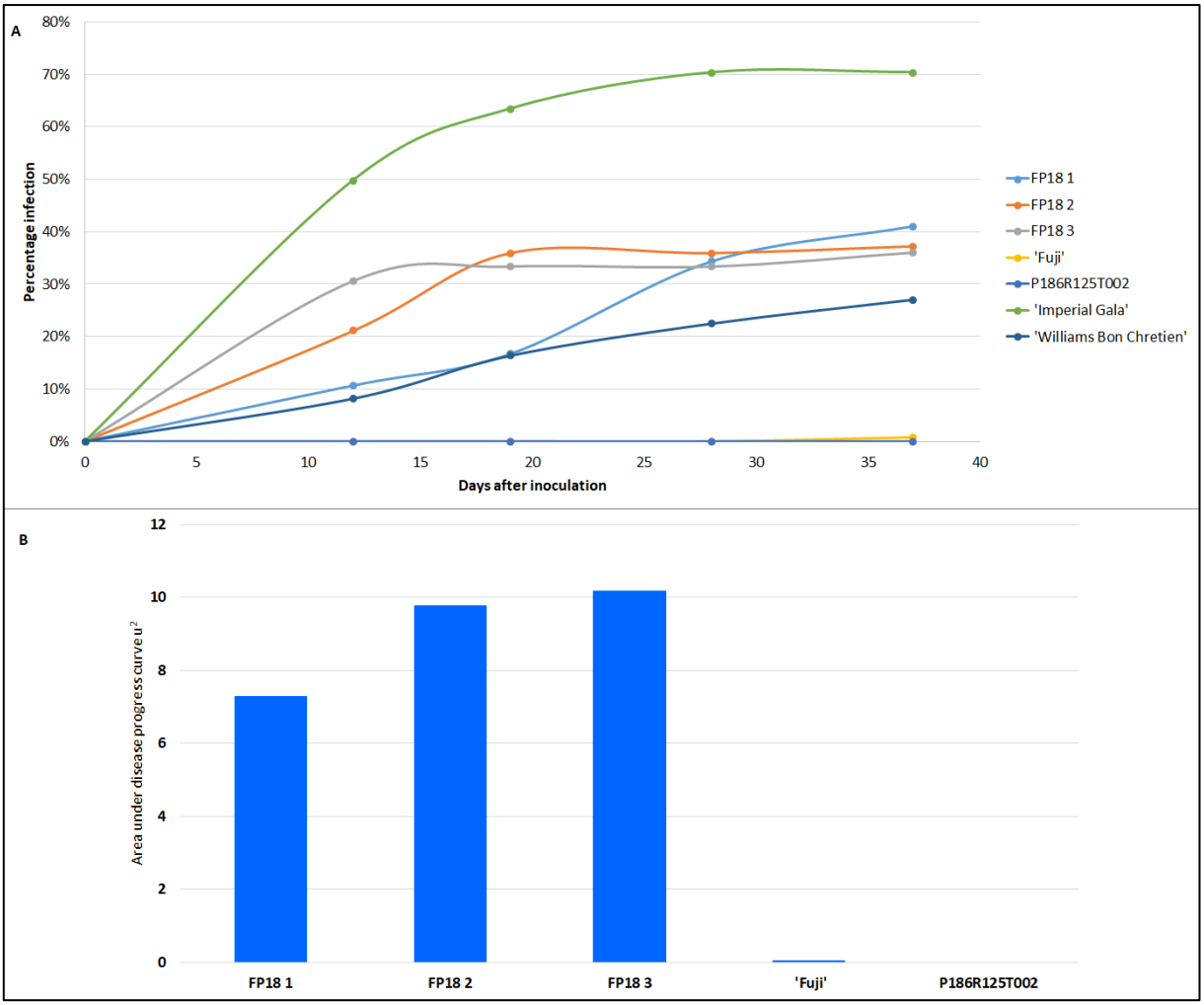

Figure 4.14: Fire blight necrosis progress in 'Fuji' x P186R125T002 population and reference accessions after inoculation with *E. amylovora* using the cut-leaf method (A). Area under disease progress curves from Figure 4.14 A (B).

Of the three FP12 progeny, two were (highly) susceptible like the reference cultivar 'Imperial Gala', while FP12 1 was moderately resistant with 15.8% necrosis at the final measurement and AUDPC of 2.51 u^2, which was less than its pear parent P125R095T002 (Figure 4.15).

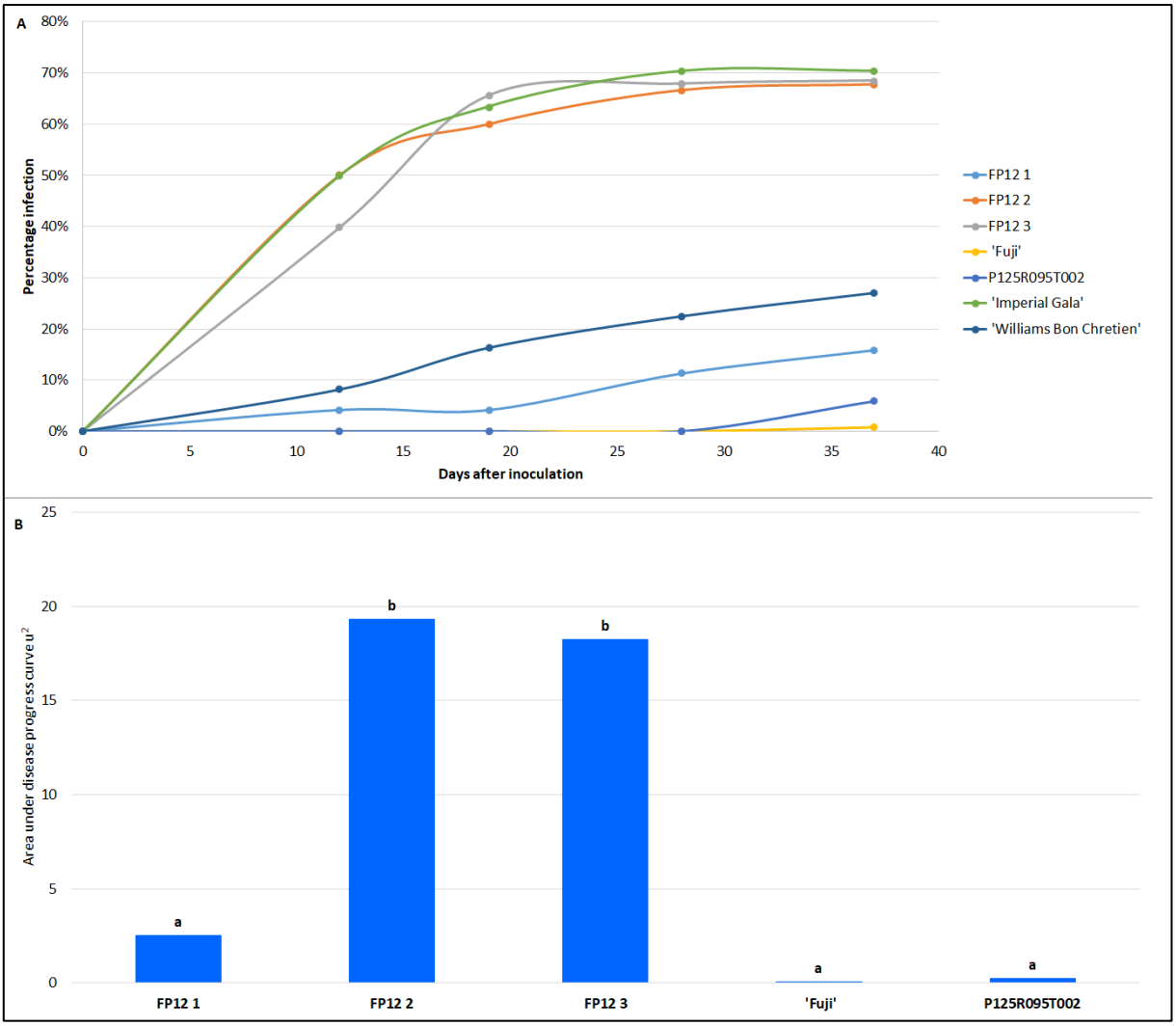

Figure 4.15: Fire blight necrosis progress in 'Fuji' x P125R095T002 population and reference accessions after inoculation with *E. amylovora* using the cut-leaf method (A). Area under disease progress curves from Figure 4.15 A (B).

The results clearly showed that FP26 1 and 2 were highly resistant like both parents, while FP26 3 was moderately susceptible to fire blight (Figure 4.16).

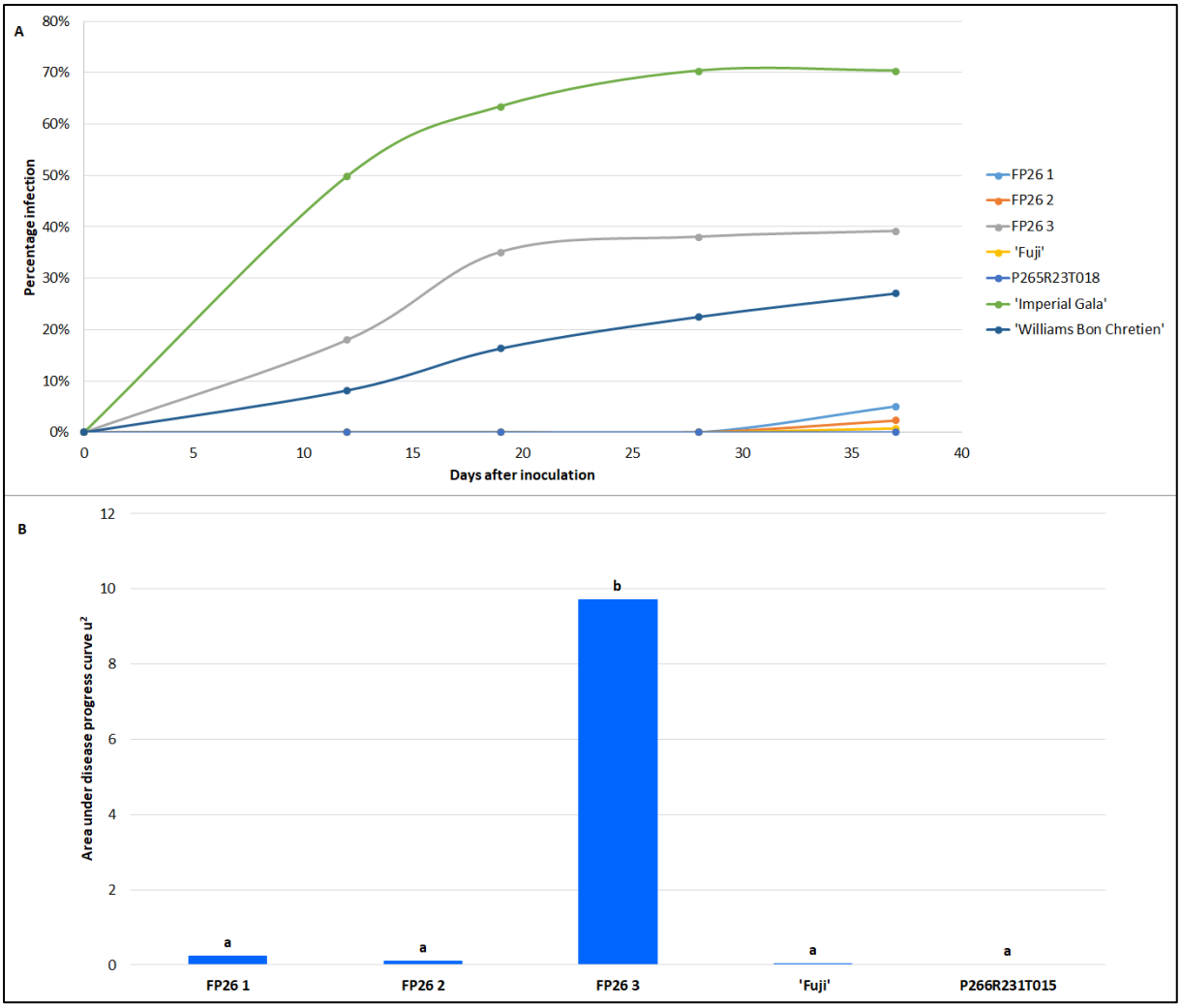

Figure 4.16: Fire blight necrosis progress in 'Fuji' x P265R23T018 population and reference accessions after inoculation with *E. amylovora* using the cut-leaf method (A). Area under disease progress curves from Figure 4.16 A (B).

In contrast, it was clearly shown that all FP35 progeny were (highly) susceptible to fire blight, much more so than both parents 'Fuji' and P354R200T138. Their susceptibility ranged from to 42.6% to 66.7% necrosis, and AUDPCs from 11.8 u^2 to 17.7 u^2 at the final measurement (Figure 4.17).

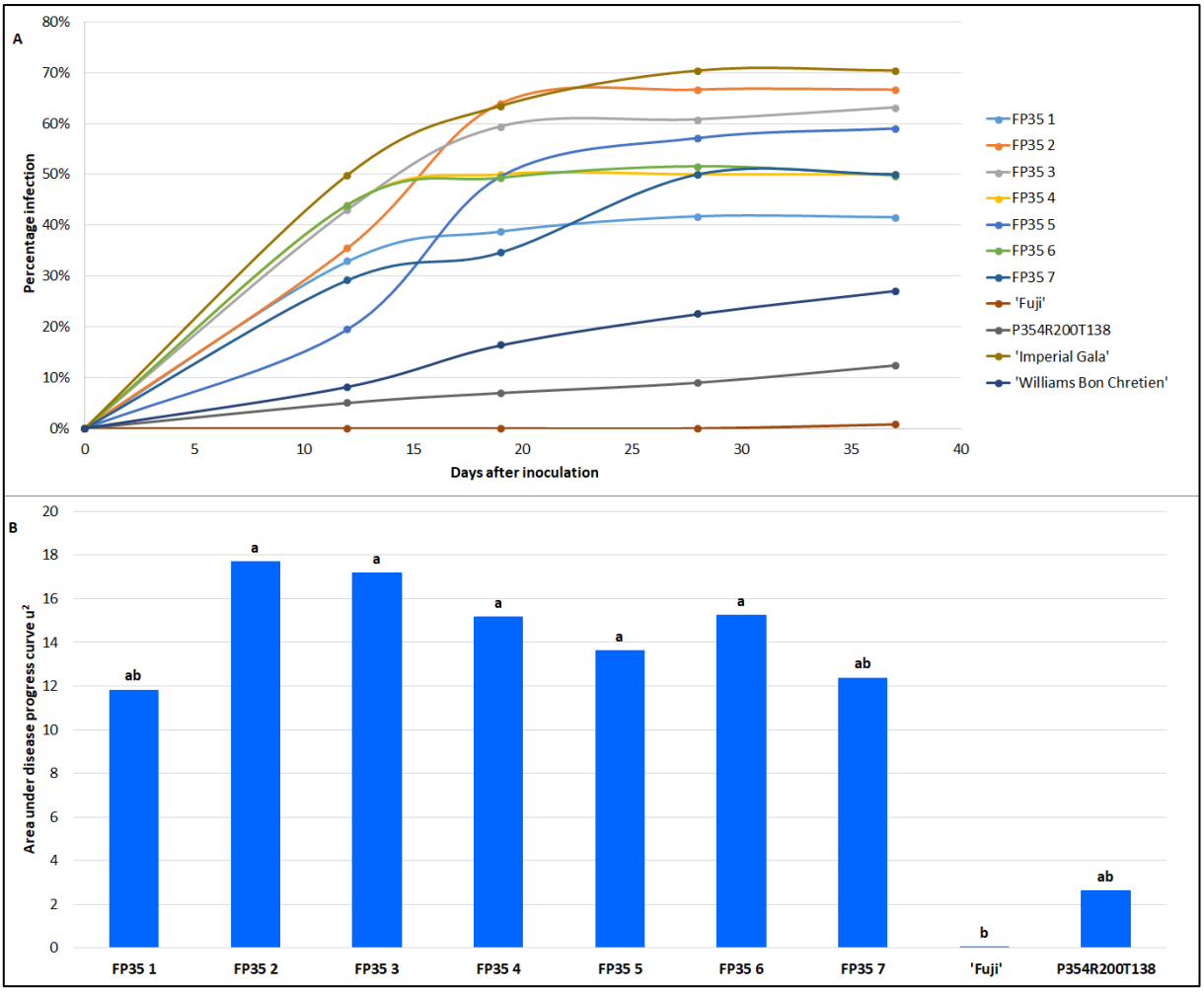

Figure 4.17: Fire blight necrosis progress in 'Fuji' x P354R200T138 population and reference accessions after inoculation with *E. amylovora* using the cut-leaf method (A). Area under disease progress curves from Figure 4.17 A (B).

4.5 Discussion

In this part of the study, resistance evaluations of the available putative apple-pear or *vice versa* hybrids were performed for two common diseases of apple and pear: apple/pear scab, and fire blight.

Almost all the apple-pear hybrids were resistant to apple scab, with only some progeny in the populations with 'Fuji' as the female parent showing susceptibility, indicating that not any possible introgressions from pear effectuated resistance into the 'Fuji' progeny. This cultivar is generally regarded highly susceptible to this disease (Barbara *et al.*, 2008; Bus *et al.*, 2011; Bogo *et al.*, 2012; Sheikh *et al.*, 2020).

All the progeny inoculated with pear scab showed resistance symptoms, which can be attributed to the non-host resistances as the result of introgressions from both apple and the Asian pear

parents (Won *et al.*, 2014; Bus *et al.*, 2013). This seems to be supported by the microscopic auto-fluorescence observation, which did not show the production of (yellow fluorescing) polyphenols usually associated with active defence responses common to gene-for-gene relationships in the *V. inaequalis-Malus* pathosystem (Bus *et al.*, 2005, 2010). That is, perhaps necrosis involved in nonhost resistance is passive, but may involve production of different (light-blue fluorescing) polyphenols that indicate tissue damage as the result of the infected plant cells not expanding with the surrounding cells, causing the tissues to tear.

The scab observations being exploratory, further research is required to understand the genetics of the scab resistance in the apple/pear hybrids, but indications are that resistance has been conferred across generic barriers.

It is noteworthy that all CO apple x pear hybrids exhibited resistance to fire blight, with the least resistant hybrid, CO 17, exhibiting both a %necrosis and AUDPC value that were about 3/4 those of 'Cox's Orange Pippin'. This progeny is moderately resistant and the other five are resistant.

Preliminary results using HRM marker analysis of the seedling genomes, with three apple/pear SNP markers per LG, indicated that each exhibit a hybrid apple/pear genomic region on LG2, while LG7 is represented by apple DNA. These regions correlate with the fire blight resistance QTLs reported on LG2 of pear 'Old Home' (Montanari *et al.*, 2016) and on LG7 of the apple cultivar 'Fiesta', which inherited it from 'Cox's Orange Pippin' (Khan *et al.*, 2007).

All IP26 and IP12 progeny were susceptible to fire blight, i.e. none inherited resistance from their respective male pear parents, P266R231T015 and P125R095T002, since their female parent, 'Imperial Gala', is susceptible (Norelli *et al.*, 2003).

'Fuji' is susceptible to fire blight (Norelli *et al.*, 2003), but in this study and in some other cases appeared to be resistant (Yoder and Biggs, 2011; Kostick *et al.*, 2019) or rather may have been an escape. There are several possible reasons for 'Fuji' not being in optimal condition for infection in our experiment in light of the observation that the susceptible parent 'Imperial Gala' showed a large necrosis as expected. Lower average temperatures and relative humidity during the inoculation period could partially account for some cultivars appearing more resistant than previously reported. Susceptibility of the shoots is highly dependent on their vigour, with actively growing, vigorous, succulent shoots being the most susceptible to fire blight (Van der Zwet *et al.*, 2012). Most 'Fuji' progeny in our work were moderately susceptible or susceptible to fire blight in contrast with our directly parent results. Hence, the study needs repeating to validate all findings described here.

4.6 Conclusion

In conclusion, in this study all the CO, P26A19, IP26, IP12, FP12 3 and three FP35 progeny exhibited resistance to apple scab and pear scab, while the FP18, FP12 2, FP26 and FP35 4 of the progeny were susceptible to apple scab. The presence of resistance in crosses with susceptible apple parents suggests that sufficient pear introgressions have taken place to effectuate non-host resistance. However, apple parent 'Fuji' was the exception with most of its progeny being susceptible to *V. inaequalis*.

All the progeny being resistant to pear scab indicates that hybridity was only partial at best since not any pear introgressions into the non-host female apple parents enabled susceptibility. Then, same applies for the introgression of Asian pear genomes since these *Pyrus* species are non-hosts for *V. pyrina*, too.

The scab findings only are preliminary, hence the research needs to be confirmed by repeating it.

The observation that all CO apple x pear hybrids appeared to be resistant to fire blight, with the preliminary marker analysis indicating that this may be attributed to the presence of the 'Moonglow' QTL on LG2. The high number of progeny suggested to carry the QTL might also indicate preferential introgression of the genomic pear region into apple.

Most 'Fuji' progeny in our work are moderately susceptible or susceptible to fire blight in contrast to their parental results, which in one case varied from reported results.

Hence, the research will need to be repeated to validate all findings. Future work is to map recombination events during the crossing of apple and pear more precisely along the chromosomes of the apple x pear hybrids. This will enable us to further investigate the possible relationship of these reported QTL resistances for control of fire blight infection. If confirmed, these hybrids will provide an opportunity to develop cultivars with a pear fire blight resistance novel to apple.

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CHAPTER V

An attempt to understand and explain the arbutin biosynthesis pathway by metabolomics and gene expression analysis

5 An attempt to understand and explain the arbutin biosynthesis pathway by metabolomics and gene expression analysis

5.1 Abstract

The subfamily of *Pomoideae* (family Rosaceae) comprises a number of genera known as "pome fruits", which are valuable fruit crops for human nutrition and health (Espley and Martens, 2013). Apple (*Malus* x *domestica* Borkh.) is the major crop with respect to global consumption, followed by pear (*Pyrus communis* L.) (Cornille *et al.*, 2019).

The secondary metabolites that are species specific for apple and pear are phloridzin and arbutin, respectively. Phloridzin is a dihydrochalcone glucoside (phloretin 2'-*O*-glucopyranoside), while arbutin is a glycosylated phenol (hydroquinone-*β*-d-glucopyranoside). In this work, we used three sources of apple-pear and pear-apple hybrid plants, Zwintzscher's Hybrid (Fischer *et al.*, 2014), its F2 progeny held at FEM, putative F1 and F2 hybrids developed at PFR as well as hybrids obtained at UniBo. Our objective was first to determine the presence/absence of arbutin and phloridzin in the hybrids using metabolomics analysis. These two secondary metabolites are species specific, so it is possible to detect true hybrids by the pattern of these two phenolics. Metabolic fingerprinting by LC-MS/MS coupled with multiple MRM quantification was used to characterize leaf tissue from the putative hybrids on the metabolite level. The second objective was to use the available RNA-seq dataset to characterize expression profiles for putative arbutin pathway genes in apple, pear and Zwintzscher's Hybrid, to detect the genes involved in the arbutin biosynthetic pathway.

Using Zwintzscher's Hybrid as a positive control, the results confirmed that the putative F1 hybrids P26A19 4 and AF, and the F2 hybrid F2-FP12 1-1.2-OP accumulate metabolites typical of both pear and apple in their leaves.

Three candidate genes involved in arbutin pathway, 4-coumarate CoA ligase, 4-hydroxybenzaldehyde synthase and chorismate-pyruvate lyase (CPL), were differentially expressed in pear and apple-pear, as compared to apple. The presence and expression of a CPL orthologue in Zwintzscher's Hybrid and the pears 'André Desportes' and 'Williams Christ' needs to be further evaluated, as CPL has been described only in bacteria to date.

The results from this study provide an important resource for understanding the arbutin biosynthesis in *P. communis* L. and apple-pear hybrid Zwintzscher's Hybrid, but also in other arbutin synthesizing plant species.

Keywords: Arbutin, phloridzin, biosynthetic pathway, MRM, RNA-seq

5.2 Introduction

Phenolic compounds are secondary metabolites, which are the most common and widespread substances in plants (Lattanzio, 2013). Apple and pear are rich in phenolic compounds (Kolniak-Ostek and Oszmiański, 2015; Mushtaq *et al.*, 2020). Phloridzin is the main phenolic compound in apple, present in apple flesh, peel and seeds (Zielińska and Turemko, 2020). Arbutin is the main phenolic compounds in pear fruit, which also occur in leaf and floral buds, and flowers (Dong *et al.*, 2018). The genus-specific accumulation of high concentrations of phloridzin in *Malus* and arbutin in *Pyrus* is well established (Hofsommer, 1999; Schieber *et al.*, 2001).

Arbutin (*p*-hydroquinone-β-d-*O*-glucopyranoside) is a natural phenolic glucoside found in various plant species of diverse families such as Ericaceae (*Vaccinium* spp., *Arctostaphylos* spp.), Asteraceae (*Achillea millefolium*), Betulaceae (*Betula alba*) and Rosaceae (*Pyrus communis* L.) (Lee and Eun, 2012). Arbutin has a great capacity to inhibit melanin formation and is therefore used to remove, for example sunspots and age spots. It is commonly used in medical, healthcare and cosmetic industries due to its anti-oxidant, anti-microbial and anti-inflammatory activities (Wang *et al.*, 2018). The presence of arbutin in pear has been correlated with the biochemical processes that operate as defence mechanisms against bacterial invasion (Petkou *et al.*, 2002). It may be involved in fire blight protection in resistant pear cultivars (Gunen *et al.*, 2005). In addition, the degradation of arbutin has been considered as a possible cause of graft incompatibility between pear and quince (*Cydonia oblonga* Mill.) (Hudina *et al.*, 2014).

Arbutin biosynthesis has been suggested to involve 4-hydroxybenzoic acid synthesis as described in *Piper gaudichaudianum* (Batista *et al.*, 2018). The possible microbial synthesis of arbutin was demonstrated in *E. coli* by evaluating the active 4-hydroxybenzoate 1-hydroxylase encoded by MNX1 and a glucosyltransferase encoded by arbutin synthase (AS) with high specificity using the versatile platform intermediate 4-hydroxybenzoate (4-HBA) as a precursor (Shen *et al.*, 2017; Wang *et al.*, 2018). However, it is questionable if the microbial biosynthesis described above can be directly transferred to the plant system. Grisdale and Towers (1960) showed that the aromatic ring of hydroquinone derives from phenylpropanoids like cinnamic acid. Zenk (1964) proposed that hydroquinone is formed by an oxidative decarboxylation of 4-hydroxybenzoic acid; 4-hydroxybenzoic acid was identified in apple, pear and in Zwintzscher's Hybrid (Fischer *et al.*, 2014). An oxidative decarboxylation reaction is necessary to obtain hydroquinone from 4-hydroxybenzoic acid. This reaction, most probably catalysed by dehydrogenases, oxidises a carboxyl group and forms carbon dioxide while reducing NAD^+, the cofactor of the enzyme, to NADH.

In this study, metabolic fingerprinting by liquid chromatography-tandem mass spectrometry (LC-MS/MS) coupled with multiple reactions monitoring (MRM) quantification was used to characterize the different plant materials of putative hybrids on the metabolite level and we utilized RNA-seq data to characterize expression profiles of putative arbutin pathway genes in apple, pear and Zwintzscher's Hybrid transcriptomes.

5.3 Materials and Methods

5.3.1 Multiple reaction monitoring

Freeze dried leaf samples from putative hybrids between apple and pear held at FEM and PFR (F1 and F2 progeny; Chapter II) (100 mg) were accurately weighed into a 15 ml plastic tube and extracted with 4 ml of 80% methanol in three biological replicates. Fresh leaf materials from *in vitro* plants of three hybrids from UniBo (200 mg) were accurately weighed into a 15 ml plastic tube and extracted with 1 ml of 80% methanol in three biological replicates. In this work we used different sources of plant material (fresh vs. freeze dried; outdoor vs. *in vitro*) because some of these materials are not present in the field (UniBo) and different methanol/plant material ratios because of the variation of quantity of metabolites in the different tissues origins. Generally, *in vitro* plant material has a lower overall concentration of polyphenols compared to plants from greenhouse or field cultivation (Gosch *et al.*, 2010).

Samples were rotated with a vertical multi-function rotator for 20 min and sonicated for 30 min. After 48 h in the dark at 4°C, samples were centrifuged at 1000g and 4°C for 10 min. The resulting supernatants were collected and filtered through a 0.22 µm PVDF filter. The targeted analysis of polyphenol compounds was performed using the LC-MS/MS method coupled with MRM quantification with a slightly modified method according (Vrhovsek *et al.*, 2012) optimised for Rosaceae tissues including the expected species specific metabolites.

Statistical variance analysis was performed on the phloridzin and arbutin data, which were randomized with a Fisher randomization test.

5.3.2 RNA extraction and Illumina sequencing

RNA was extracted from three samples ('André Desportes' pear, 'Kalco' apple and Zwintzscher's Hybrid) using the total RNA extraction kit (Sigma). For each accession three technical replicates were produced. The RNA concentration ranged between 97 and 324 ng/ul.

The RNAs were used to obtain the libraries for the RNA seq analysis, following the KAPA Library Preparation Kit for Illumina platforms® (Kapa Biosystems). The respective transcriptomics datasets

were generated using HiSeq2000 facilities and PE100 bp mRNA stranded libraries with the support of sequencing and bioinformatics units at FEM.

5.3.3 Analysis of the RNA-seq data

The analyses of the RNA-seq data were carried out on the Galaxy platform (www. galaxyproject.org) (Afgan *et al.,* 2018). Quality reads of the raw RNA-Seq data were processed by the fastp tool. Fastp is designed to provide quick all-in-one pre-processing for FASTQ files. This tool was developed in C++ with multi-threading supported to afford high performance (Chen *et al.*, 2018). Afterward, paired-end clean reads of 'Kalco' and Zwintzscher's Hybrid were aligned to the available reference genome of apple (GCF_002114115.1_ASM211411v1) (Daccord *et al.*, 2017), while paired-end clean reads of 'André Desportes' and Zwintzscher's Hybrid were aligned to the available reference genome of pear (*P. communis* cv. Bartlett DH Genome v2.0 transcripts) (Linsmith *et al.*, 2019) using the Bowtie2 tool (www. galaxyproject.org). Bowtie is an ultrafast and memory-efficient tool for aligning sequencing reads to long reference sequences. It is particularly good at aligning reads of about 50 up to 100s or 1,000s of characters to relatively long genomes. Bowtie 2 supports gapped, local, and paired-end alignment modes. Bowtie2 outputs alignments in SAM format, enabling interoperation with a large number of other tools. The Htseq-count tool (www. galaxyproject.org) was employed to count the number of reads mapped to each gene and quantify the gene expression level. Salmon is a wicked-fast program tool (www. galaxyproject.org) and was used to produce highly-accurate, transcript-level quantification estimates from RNA-seq data. Salmon achieves its accuracy and speed via a number of different innovations, including the use of quasi-mapping (accurate but fast-to-compute proxies for traditional read alignments), and massively-parallel stochastic collapsed variational inference. The result is a versatile tool that fits nicely into many different pipelines.

The bibliography was screened for genes coding putative enzymes involved in the arbutin pathway. Candidate genes were identified for each enzyme involved in the putative arbutin biosynthesis from the pear genome description. Next, the multiple reads for each gene and replicate for 'André Desportes' and Zwintzscher's Hybrid were aligned to the pear genome, and the 'Kalco' and Zwintzscher's Hybrid reads aligned to the apple genome, which led to the identification of eight candidate genes.

5.3.4 Detection of differentially expressed genes with qRT-PCR validation

The expression pattern of the differentially expressed genes (DEGs) retrieved from the RNAseq analysis was validated by qRT-PCR. The RNA was extracted from two pear cultivars, 'André Desportes' and 'Williams Christ' (grandfather and great-grandfather of Zwintzscher's Hybrid,

respectively), two apple cultivars 'Kalco' and 'Cox's Orange Pippin' (mother and grandmother of Zwintzscher's Hybrid, respectively), and Zwintzscher's Hybrid. RNAs were extracted from three biological replicates using the cetyltrimethylammonium bromide (CTAB) method (Chang et al., 1993). The RNA quality and quantity were evaluated with the NanoDrop™ 8000 Spectrophotometer (Thermo Scientific™). The preparation of RNA for RT-PCR for each sample was done following the Sigma-Aldrich® kit (www.sigmaaldrich.com).

The primers of the selected genes were designed using Primer 3 software (www.primer3.ut.ee). Two primers per candidate gene were designed and blasted to the pear and apple genomes. These primers were tested with a sample mix of a 1:10 dilution of cDNA in water and eight primers were selected for the qRT-PCR analysis (Table 5.1). The reactions were carried out with three biological replicates with two technical replicates using ubiquitin as an internal control in a 96-well plate on a Bio-Rad CFX Real-Time PCR Thermocycler (Bio-Rad). The total volume of 12.5 µL contained 2 µL of a 1:4 dilution of cDNA in water, 6.25 µL of SYBR Master Mix, 1 µL of each primer and 2.25 µL of water. The thermal cycling program was follows: 98°C for 5 s, then 39 cycles of 98°C for 5 s, 58°C for 5 s, 60°C for 5 s and 76°C for 10 s; 98°C for 30 s and 65°C to 95°C in increments of 0.2°C every 10s. The data acquisitions and data analyses were performed using Bio-Rad CFX Manager, v 3.0. The determination of Ct values was done using the regression mode of the software, which applies a multivariable, nonlinear regression model to individual well traces and then uses this model to compute an optimal Ct value (Bio-Rad, 2008).

Table 5.1: A list of eight gene primers used for qRT-PCR analysis.

Enzyme	Gene_id	Forward_primer	Reverse_primer
CPL –chorismate-pyruvate lyase (UbiC)	pycom03g18150	TACCTTCCGAATGTCCTTGCAA	GCGACATTCACATTTGGACCTT
PAL – phenylalanin ammonium lyase	pycom04g09130	GCTACTCCGGCATAAGATTCGA	ATCCAGCAATGTAGGATAGCGG
C4H – cinnamate 4-hydroxylase	pycom11g04310	ATACTGGTGAATGCTTGGTGGT	GAGATACCTGAAGTCGTTCCCG
4CL – 4-coumarate CoA ligase	pycom02g20960	TCCACATTGCGGGAAGTACTAC	TATGCTTGGCGGGGATTTTGTA
HBS – 4-hydroxybenzaldehyde synthase	pycom15g19350	CAGGCATTCGGAAAGCAAATCT	AGACCGCCATTGTACTTGATGT
HCHL – 4-hydroxycinnamoyl-CoA hydratase/lyase	pycom15g16410	CTTGGGCTTTGGAAATTGCAGA	GAGCAGTCTTCTTGGCTTGTTG
HBD – 4-hydroxybenzaldehyde dehydrogenase	pycom06g16790	TCCACGAATGACCCTTTTCACT	TCATGAACGTCAGGCCCTAAAA
MNX1 – 4- hydroxybenzoate 1-hydroxylase	pycom14g07580	TCCATCTCCAGCTCAAAATCGT	ACCAGACTCCTTATTCTCCCGA

5.4 Results

5.4.1 Multiple reaction monitoring

The CO populations accumulated only phloridzin from the female apple parents 'Cox's Orange Pippin' (Figure 5.1).

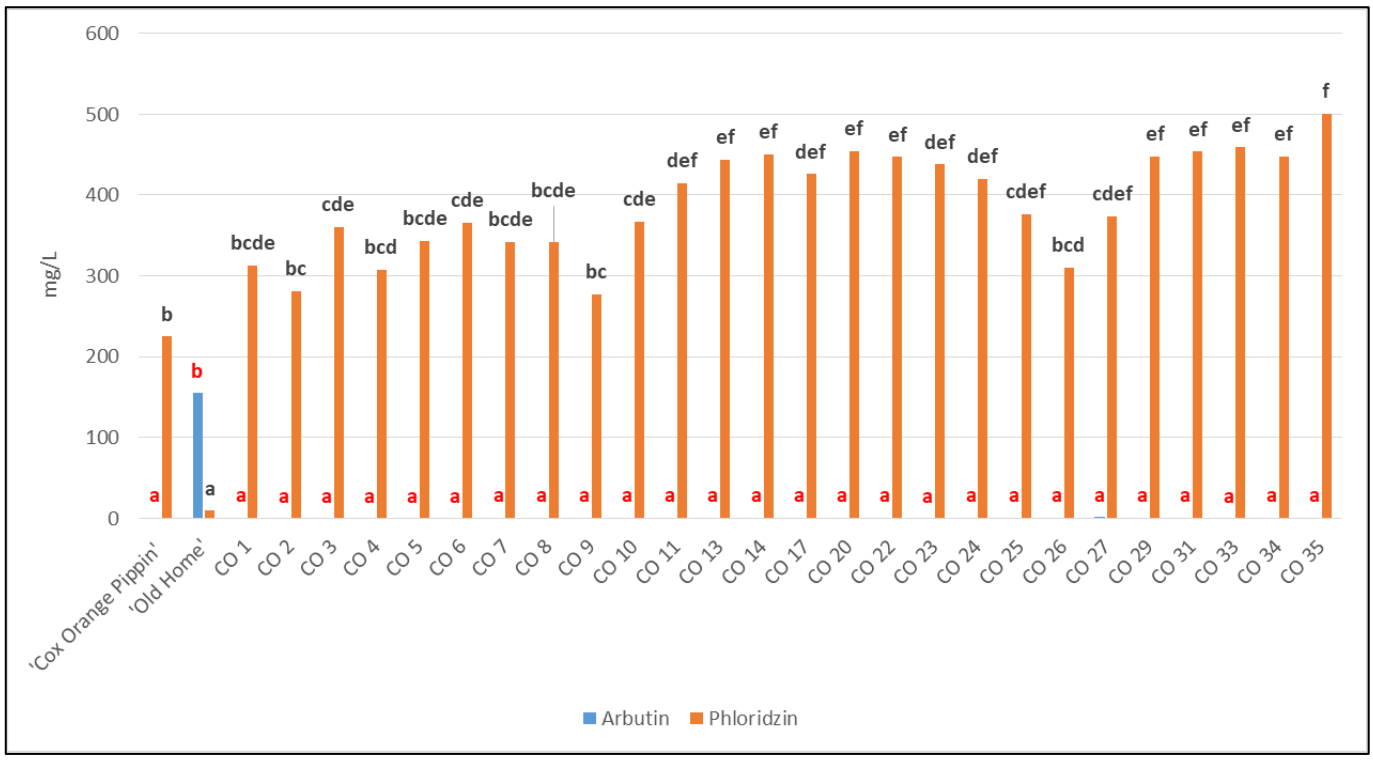

Figure 5.1: Mean concentrations of arbutin (blue) and phloridzin (orange) in 'Cox's Orange Pippin', 'Old Home' and their progeny.

In the three progeny with pear P265R232T018 as the female parent crossed with A199R45T055, and A174R01T204 only arbutin from *Pyrus* was detectable in P26A17 and P26A19 3, but P26A19 4 accumulated high concentrations of metabolite compounds typical of both pear and apple in its leaves (Figure 5.2 and Figure 5.3).

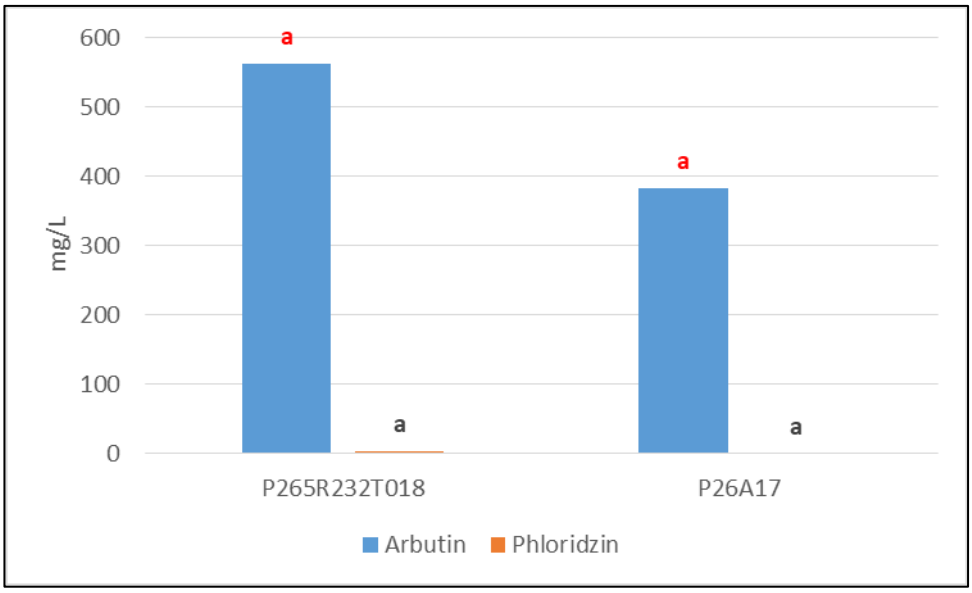

Figure 5.2: Mean concentrations of arbutin (blue) and phloridzin (orange) in pear parent P265R232T018 and its progeny P26A17.

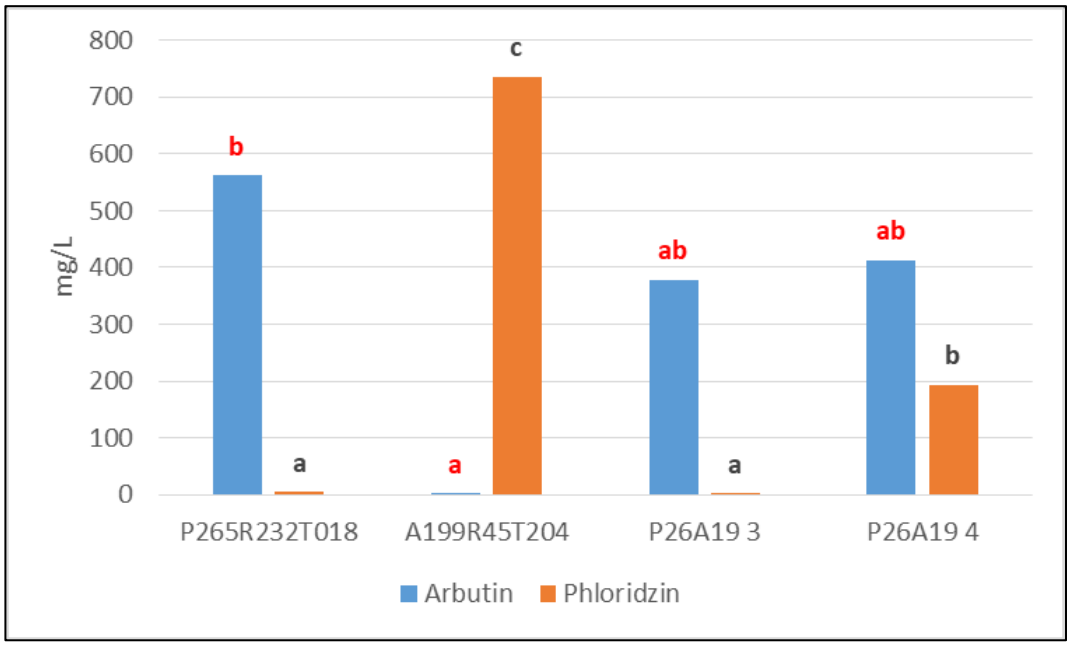

Figure 5.3: Mean concentrations of arbutin (blue) and phloridzin (orange) in P265R232T018, A199R45T055 and their progeny.

For IP26, the two 'Imperial Gala' progeny from a cross with P265R231T015 accumulated only the female *Malus* secondary metabolite phloridzin Figure 5.4. 16 F2 progeny from the open-pollinated F1 parents IP26-1 and IP26-2.

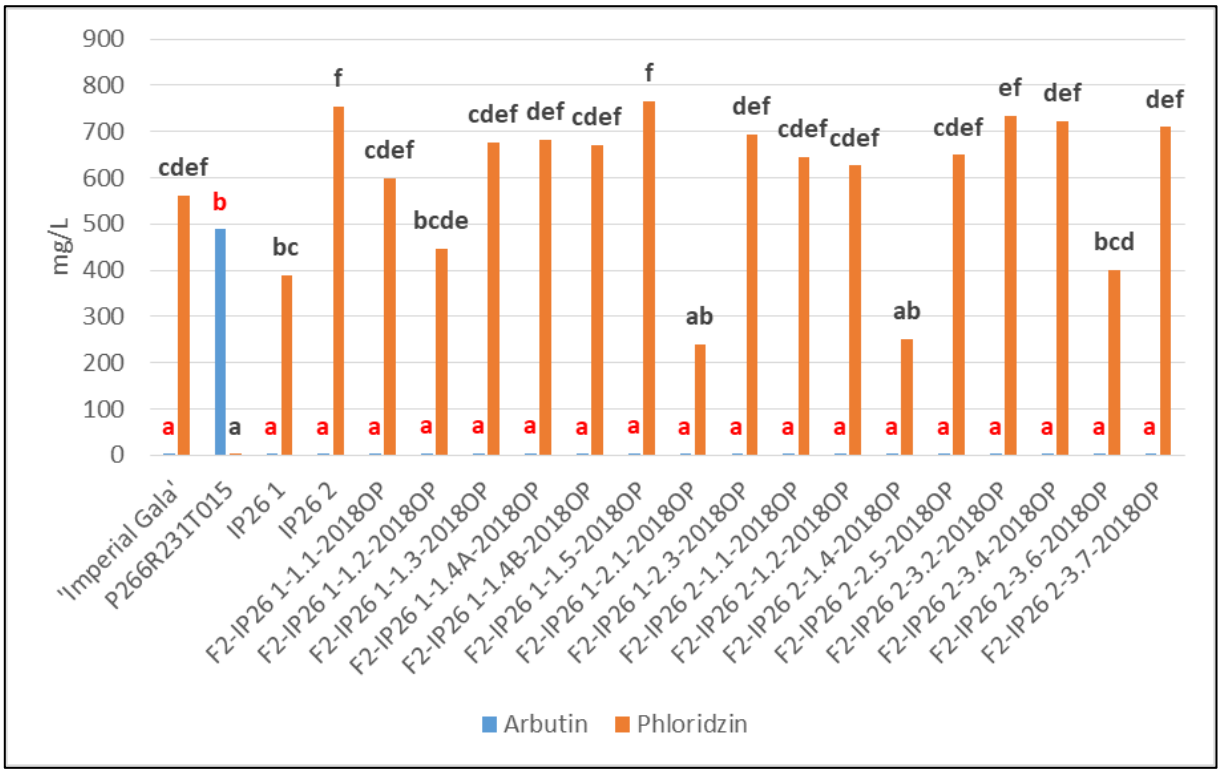

Figure 5.4: Mean concentrations of arbutin (blue) and phloridzin (orange) in 'Imperial Gala', P266R231T015, their progeny IP26 1 and IP26 2, and their open-pollinated F2 offspring from both IP26 1 and IP26 2.

Progeny IP12 1 accumulated only phloridzin from the female apple parent, 'Imperial Gala' (Figure 5.5), as did all the FP18 progeny from their 'Fuji' apple parent (Figure 5.6).

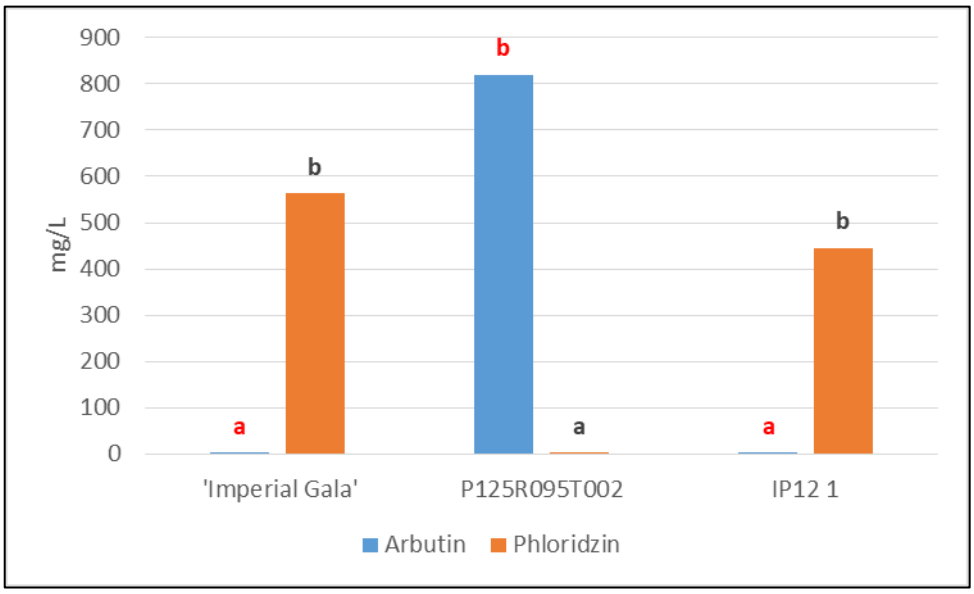

Figure 5.5: Mean concentrations of arbutin (blue) and phloridzin (orange) in 'Imperial Gala', P125R095T002, and their progeny IP12 1.

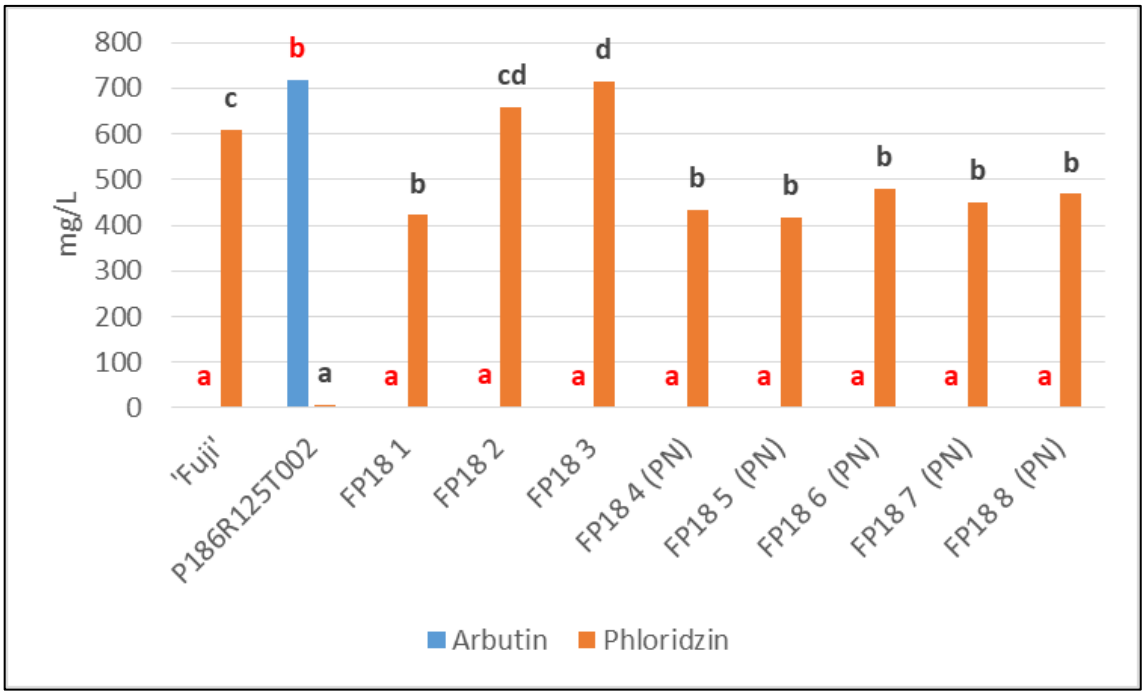

Figure 5.6: Mean concentrations of arbutin (blue) and phloridzin (orange) in 'Fuji', P186R125T002 and their FP18 progeny.

All five FP12 progeny of female apple parent 'Fuji' crossed with P125R095T002 and the subsequent F2-FP12 1 population accumulated phloridzin, but F2-FP12 1-1.2-OP accumulated significantly less than the others, while also accumulating arbutin in significant amounts (Figure 5.7).

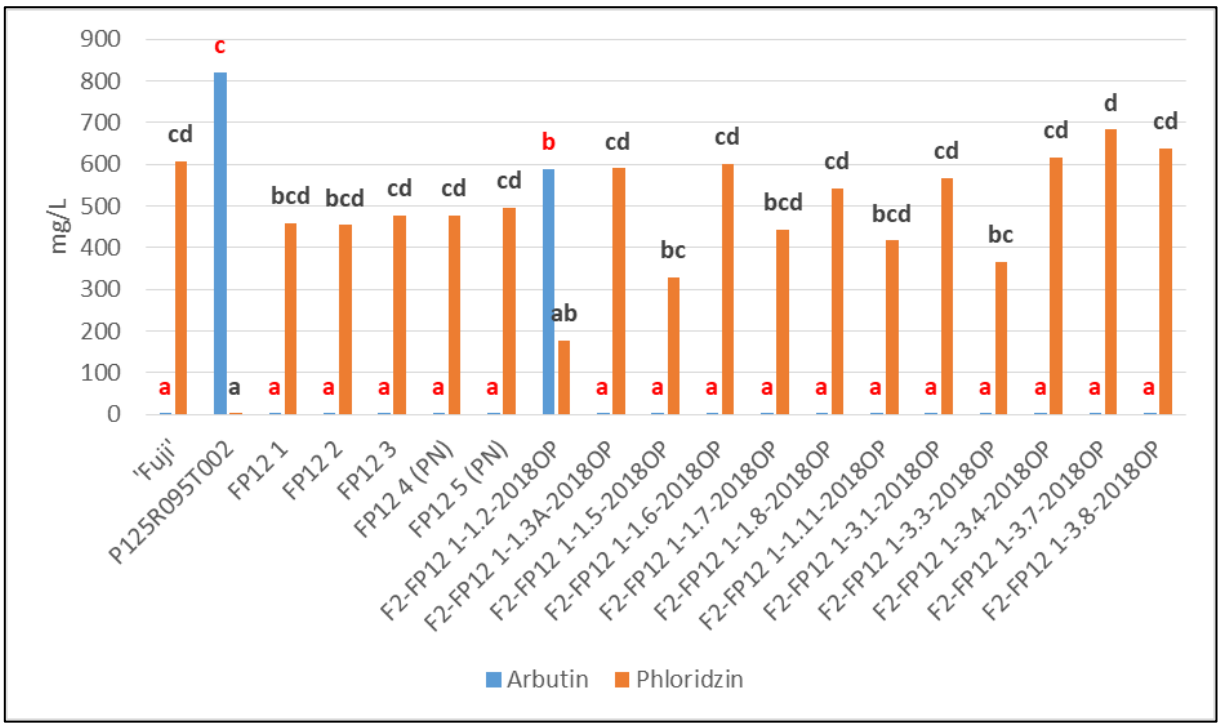

Figure 5.7: Mean concentrations of arbutin (blue) and phloridzin (orange) in 'Fuji', P125R095T002 their progeny FP12, and their open-pollinated F2 offspring from FP12 1.

The 'Fuji' progenies (FP26 and FP35) accumulated only the phloridzin from the female *Malus* parents (Figure 5.8 and Figure 5.9).

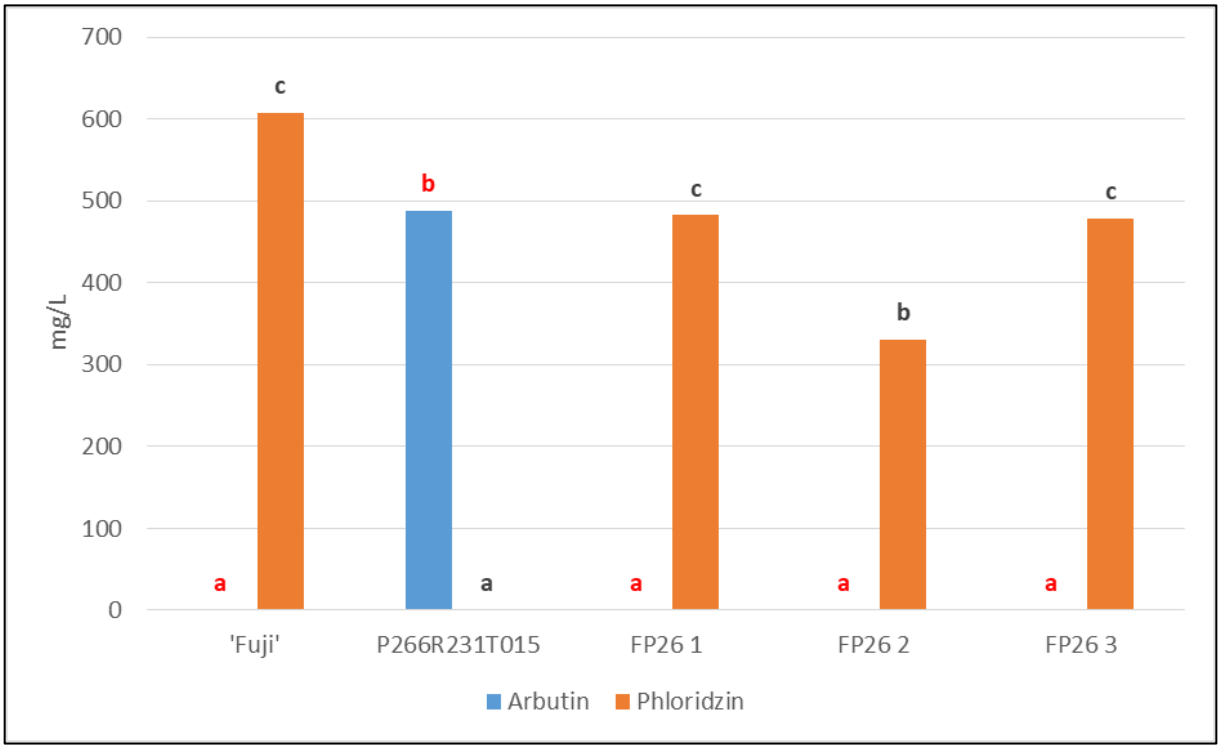

Figure 5.8: Mean concentrations of arbutin (blue) and phloridzin (orange) in 'Fuji', P265R23T018 and their progeny.

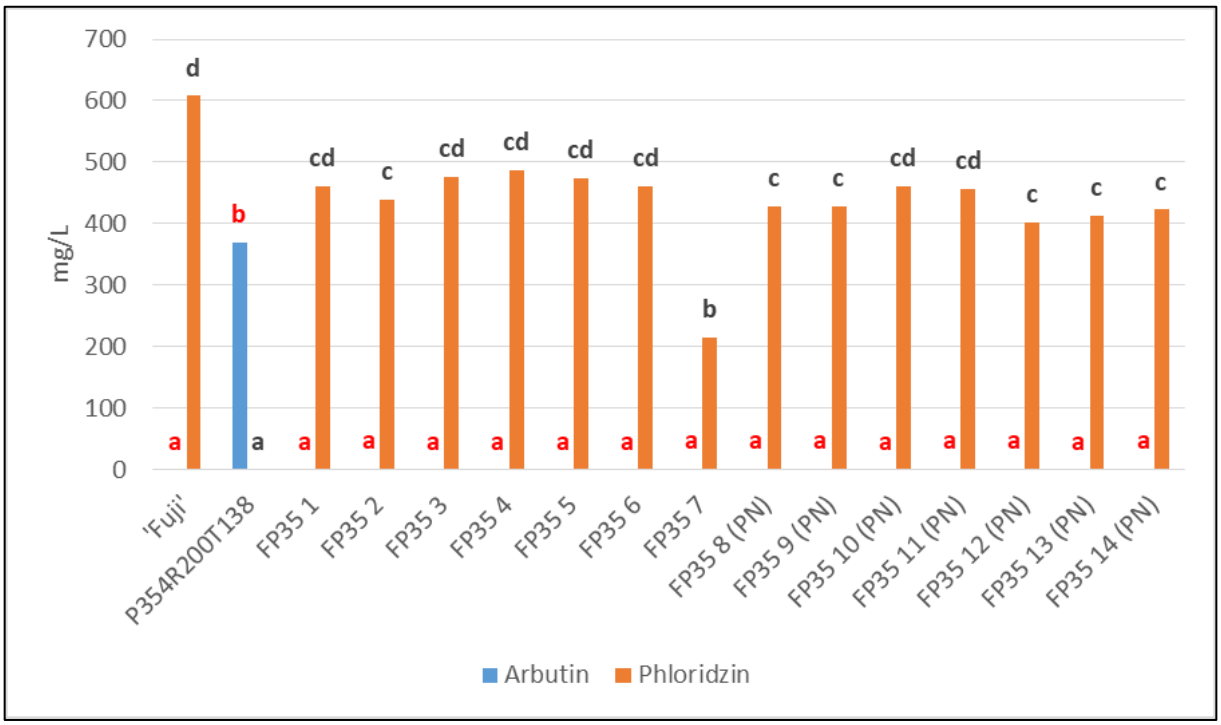

Figure 5.9: Mean concentrations of arbutin (blue) and phloridzin (orange) in 'Fuji', P354R200T138 and their progeny.

Metabolomics analysis confirmed that the F1 hybrid, Zwintzscher's Hybrid, used as a positive control, accumulates metabolites typical of both pear and apple in its leaves (Figure 5.10), while only one or the other was detectable in the respective parents and (great) grandparents. Secondary metabolites from both genera were present in Zwintzscher's Hybrid, while 'Williams Christ' (pear) accumulated *p*-hydroquinone and arbutin specific to *Pyrus* sp., and 'Cox's Orange Pippin' (apple) exhibited phloretin and phloridzin typical of *Malus* sp. It is well established that the aglycon is usually accumulated in a significant lower amount compared with the respective glycosylated metabolites, which are considered the end-product of the biosynthesis.

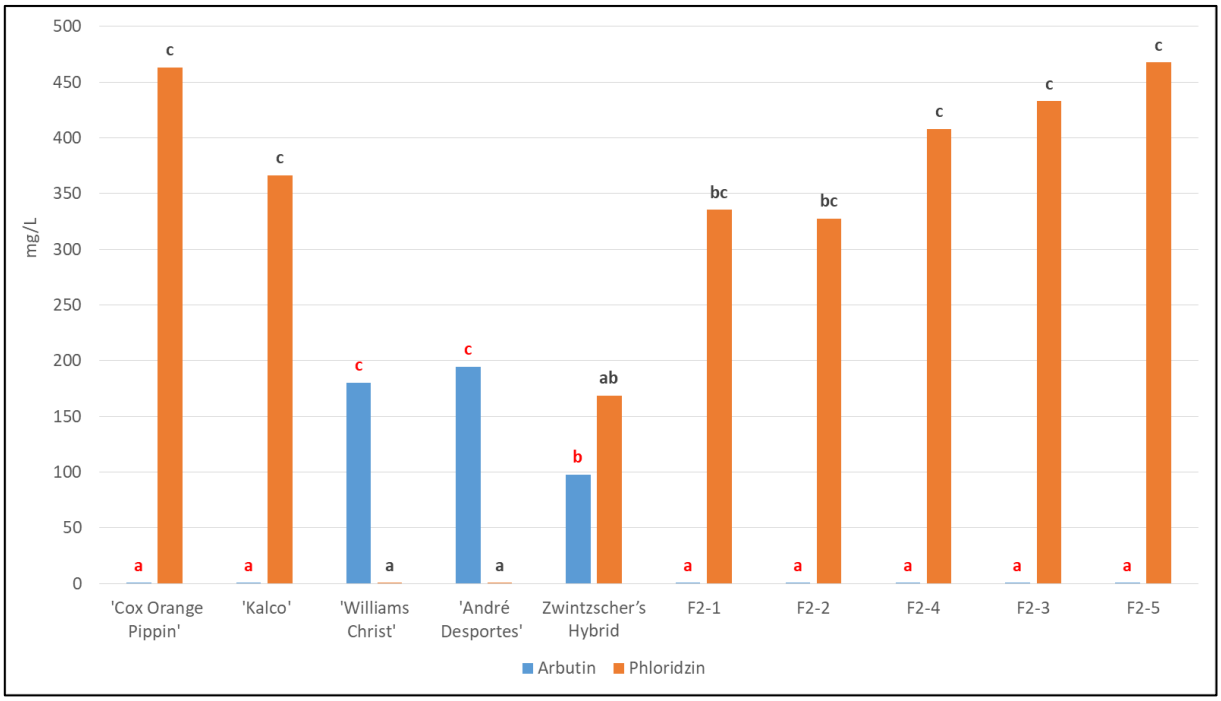

Figure 5.10: Mean concentrations of arbutin (blue) and phloridzin (orange) in F2 apple/pear hybrids and their parent and (great) grandparents of the Fondazione Edmund Mach population.

The putative 'Decana' x 'Murray' hybrid samples DM1 and DM2 from UniBo only produced one compound, arbutin, corresponding to the female pear parent (Figure 5.11). In contrast, the putative hybrid 'Abate' x 'Fuji' accumulated genus-specific secondary metabolite phloridzin from the paternal *Malus* line.

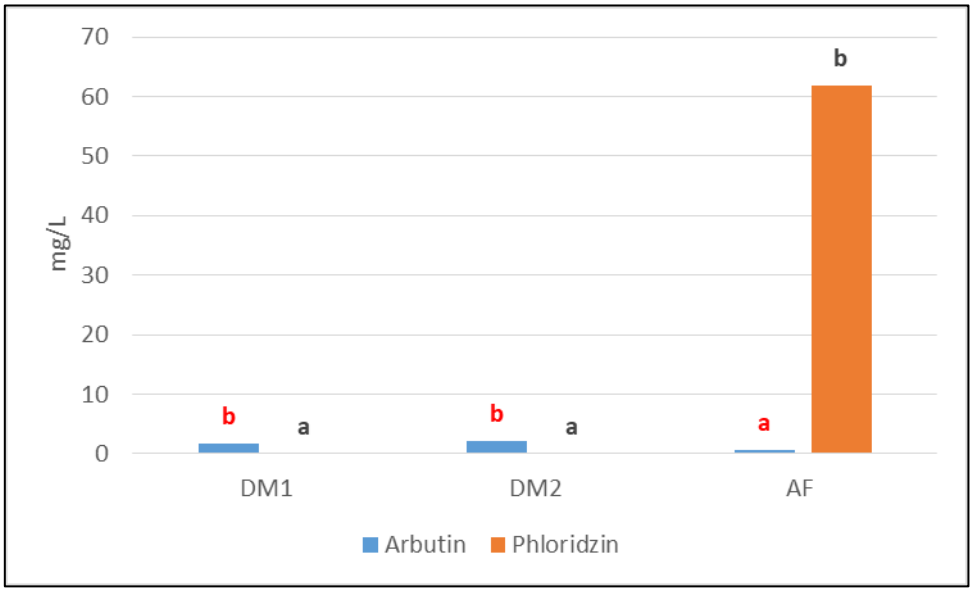

Figure 5.11: Mean concentrations of arbutin (blue) and phloridzin (orange) in F1 apple/pear hybrids of the University of Bologna population. DM = 'Decana' x 'Murray'; AF = 'Abate' x 'Fuji'.

5.4.2 Arbutin biosynthesis pathway

The genomes of pear and apple were screened for candidate genes encoding enzymes of the biosynthesis for each putative step of the predicted arbutin pathway (Figure 5.12).

For each candidate gene derived from pear, an orthologue was identified also in apple. The reads obtained from the RNA-seq analysis of 'André Desportes' and Zwintzscher's Hybrid were aligned to the pear genome, while those of 'Kalco' and Zwintzscher's Hybrid were aligned to the apple genome to get a first impression of the transcript level. As arbutin is known as a genus-specific secondary metabolite found in pear (Hofsommer, 1999; Schieber *et al.*, 2001), it could be predicted that the number of reads of at least one candidate gene (if not more) involved in the arbutin biosynthesis to be very low in 'Kalco' apple, and significantly reduced in Zwintzscher's Hybrid compared to pear. To determine the DEGs, the means of the three biological replicates of the normalized expression data were used, with statistical variance analyses performed to detect genes that were significantly differentially expressed in a Fisher randomization test.

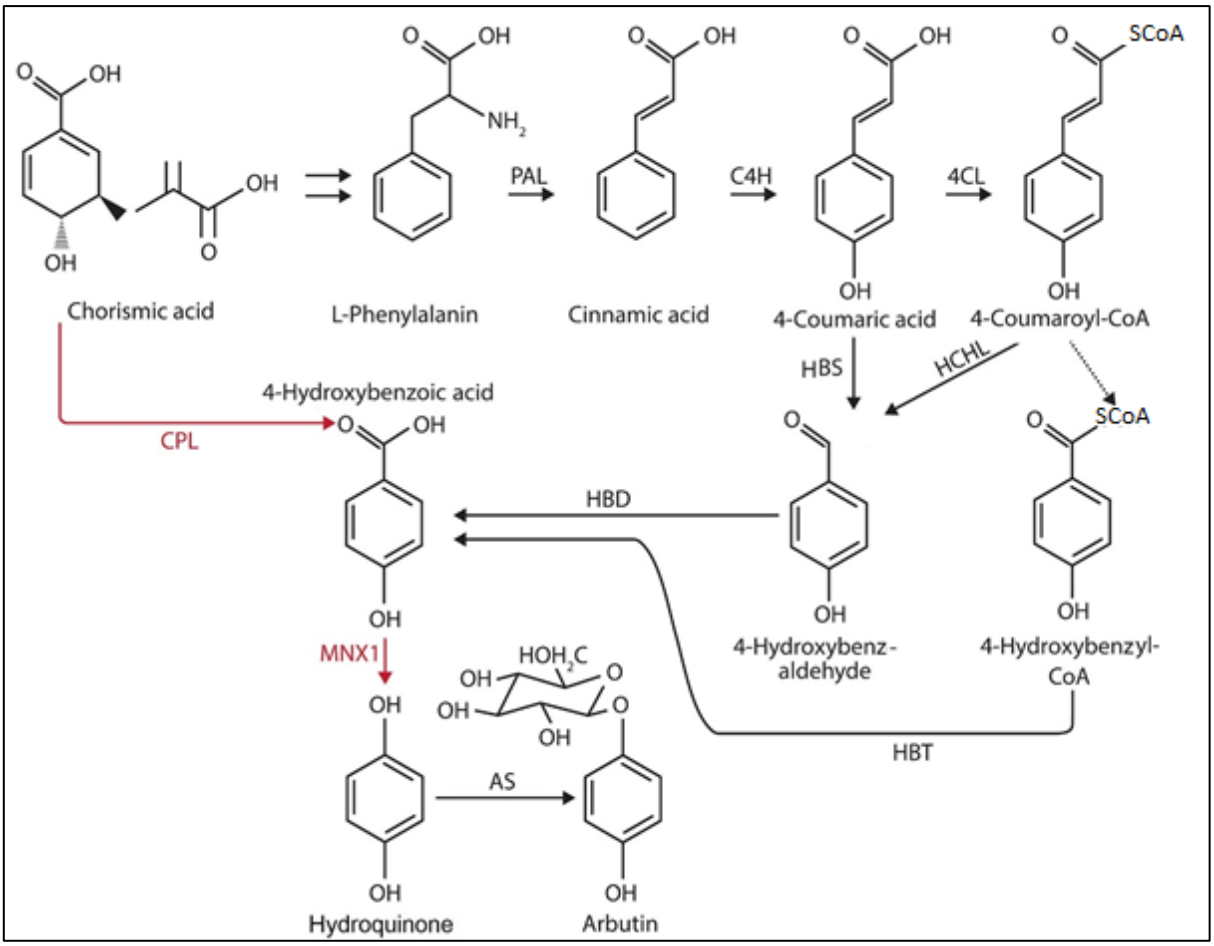

Figure 5.12: Putative steps of the predicted arbutin pathway. In red a microbial pathway. Chorismate-pyruvate lyase (CPL), phenylalanin ammonium lyase (PAL), cinnamate 4-hydroxylase (C4H), 4-coumarate CoA ligase (4CL), 4-hydroxybenzoyl-CoA thioesterase (HBT), 4-hydroxybenzaldehyde synthase (HBS), 4-hydroxycinnamoyl-CoA hydratase/lyase (HCHL), 4-hydroxybenzaldehyde dehydrogenase (HBD), 4-hydroxybenzoate 1-hydroxylase (MNX1, *Candida parapsilosis*), arbutin synthase (AS), multiple step reaction (----) (Tony Corbett, 2020).

The Fisher randomization test showed that the expression levels were not statistically significant for cinnamate 4-hydroxylase (C4H) (Figure 5.13B) and 4-hydroxybenzaldehyde dehydrogenase (HBD) (Figure 5.13F). All four other genes showed statistical differences in their expression (Figure 5.13A, C, D and E). The phenylalanine ammonium lyase (PAL) expression (Figure 5.13A) is statistically different with a group a for 'Cox's Orange Pippin', 'Kalco'; Zwintzscher's Hybrid and 'André Desportes', group b for Zwintzscher's Hybrid, 'André Desportes' and 'Williams Christ'. As can be seen the Zwintzscher's Hybrid and the two pear parents, 'André Desportes' and 'Williams Christ' were found in the same group b for 4-coumarate CoA ligase (4CL) (Figure 5.13C). The expression of 4-hydroxybenzaldehyde synthase (HBS) (Figure 5.13D) show statistically significant differences with group b 'Kalco', Zwintzscher's Hybrid and 'Williams Christ'; and in group a the two apples and 'André Desportes'.

Statistical analysis for 4-hydroxycinnamoyl-CoA hydratase/lyase (HCHL) (Figure 5.13E) placed this gene in a group with all the samples except 'Williams Christ' and in the group b all the samples except 'Cox Orange Pippin'.

For chorismate-pyruvate lyase (CPL) the apple-pear hybrid Zwintzscher's Hybrid and pear 'Williams Christ' were found to be significantly different from COP (Figure 5.13G). For 4-hydroxybenzoate 1-hydroxylase (MNX1), the expression in all samples was not significantly different (Figure 5.13H).

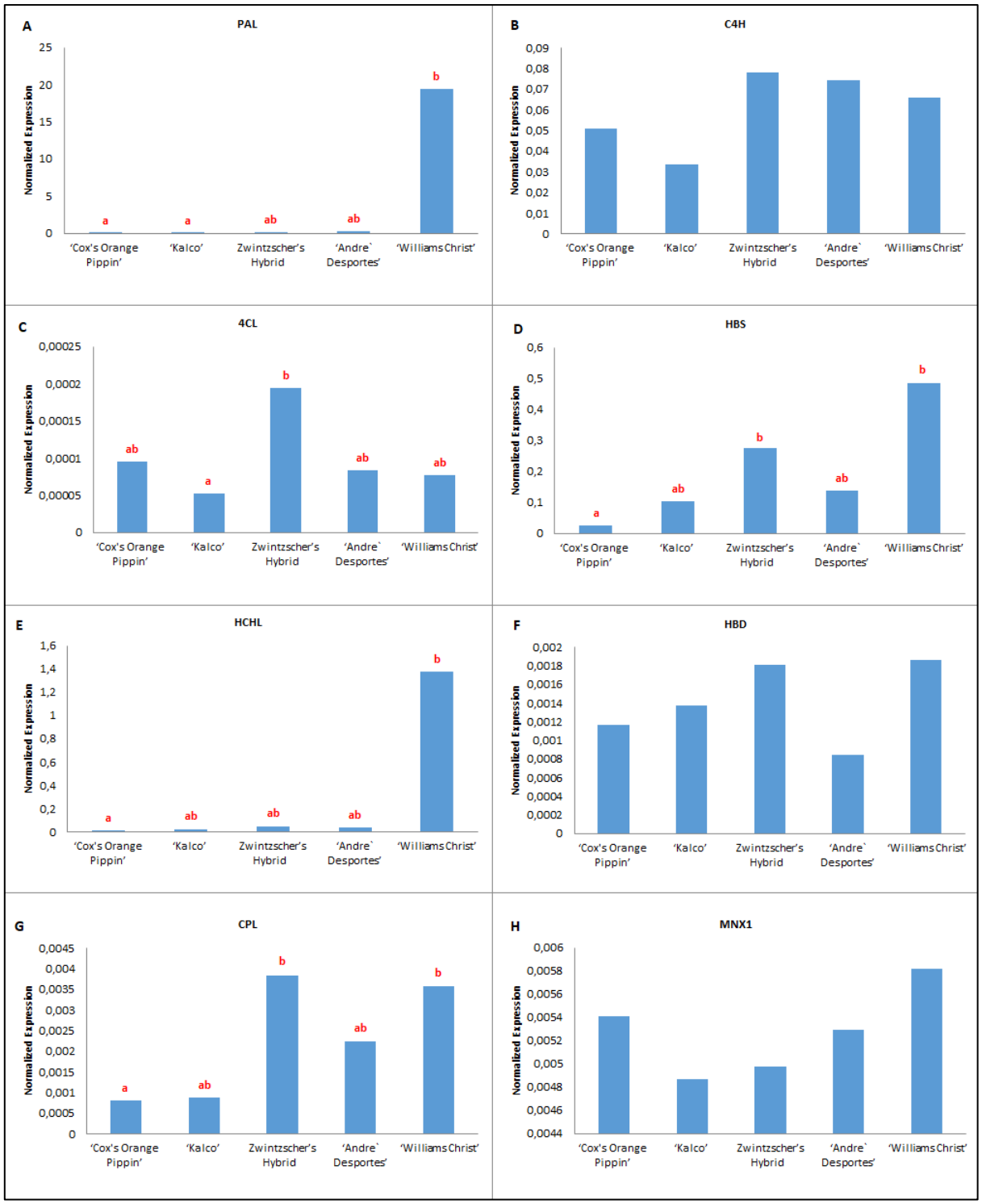

Figure 5.13: Expression profile plots obtained for the candidate genes of the predicted arbutin biosynthesis pathway in a Fisher randomization test. Phenylalanine ammonium lyase (PAL) (A), cinnamate 4-hydroxylase (C4H) (B), 4-coumarate CoA ligase (4CL) (C), 4-hydroxybenzaldehyde synthase (HBS) (D), 4-hydroxycinnamoyl-CoA hydratase/lyase (HCHL) (E), 4-hydroxybenzaldehyde dehydrogenase (HBD) (F), chorismate-pyruvate lyase (CPL) (G), 4-hydroxybenzoate 1-hydroxylase (MNX1) (H).

5.5 Discussion

A metabolomics analysis was performed on putative apple/pear hybrids to detect the combined presence of genus-specific compounds phloridzin for *Malus* and arbutin for *Pyrus* as a measure of hybridity. In general terms, the most of the putative hybrids showed the same compound as their female parent, which could indicate that their genome is predominantly inherited from that parent, i.e. that the meiosis was imperfect and resulted in segregation distortion attributed to e.g., lethality (Gonai *et al.*, 2006). This aspect will be investigated further by genotyping the hybrids (Chapter III).

This study confirmed the presence of both metabolites in the true hybrid reference accession Zwintzscher's Hybrid (Fischer *et al.*, 2014). Out of the 101 F1 and F2 hybrids tested, three putative new ones were found: AF, P26A19 4 and F2-FP12 1-1.2-OP. The latter two accumulated both phloridzin and arbutin, while, surprisingly, AF only produced phloridzin, at a low level, in spite of 'Abate' pear being the female parent. With the apple parents 'Fuji' and A199R45T204 showing high concentrations of phloridzin as expected (Dong *et al.*, 2007; Gosch *et al.*, 2009; Gosch *et al.*, 2010), and the pear parents 'Abate' and 265R232T018 only of arbutin (Petkou *et al.*, 2002; Gunen *et al.*, 2005; Hudina *et al.*, 2014), we conclude that all three therefore are maybe true hybrids which need to be further confirmed by genetic methods (see Chapter III).

Really interesting is the presence of both arbutin and phloridzin in F2-FP12 1-1.2-OP, because this F2 is from open pollination of FP12 1, which accumulated phloridzin only (Figure 5.2). The concentration of arbutin in the F2 progeny was considerably higher than that of phloridzin, indicating a greater activity of the biosynthesis for this compound.

From previous research it was predicted that several enzymes are potentially involved in the arbutin biosynthesis in *Piper gaudichaudianum*. These are PAL, C4H, 4CL, HBT, HBS, HCHL and HBD, which are involved in the 4-hydroxybenzoic acid biosynthesis (Batista *et al.*, 2018). The biosynthetic pathway of arbutin in a bacterial system was identified as via CPL and MNX1 (Shen *et al.*, 2017; Wang *et al.*, 2018). These preliminary results combined with the results presented in this research work, helped to identify most of the genes of the hypothetical pathway of biosynthesis of arbutin.

The expression of 4CL, HBS and CPL candidate genes were confirmed. Of outstanding interest is the differential expression of orthologues of the bacterial CPL in the Zwintzscher's Hybrid, 'André Desportes' and 'Williams Christ' and the absence of differential expression of PAL in the samples. The CPL was identified only in the bacterial system different from the PAL gene, because PAL is involved in the 4-hydroxybenzoic acid biosynthesis (Batista *et al.*, 2018). This result need

to be further confirmed with heterologous systems by cloning candidate genes into expression vectors and following biochemical characterisation of obtained recombinant proteins.

5.6 Conclusion

In conclusion, this study presents a chemical method for a fast identification of putative true hybrids by analysing the presence or absence of the genus-specific secondary metabolites phloridzin for *Malus* and arbutin for *Pyrus* (Petkou *et al.*, 2002; Gutierrez *et al.*, 2018). The three true hybrid F1 and F2 progeny together with Zwintzscher Hybrid can be used in controlled pollination to generate new offspring for future genetic studies, e.g., to map recombination events during the hybridization of pear and apple more precisely. A first step towards that will be the genotyping of the hybrids using SSR markers, HRM point markers, the 9K SNP-chip (Montanari *et al.*, 2013), the 20K apple SNP array (Bianco *et al.*, 2014) and/or 70K pear SNP array (Montanari *et al.*, 2020) to delineate their chromosomal structure (Chapter III). Also, pedigree analysis can be used to identify the pear or pear x apple parent of F2-FP12 1-1.2-OP. As it happens in the field the 3 FP12 selections are growing right next to the 5 (3 left) pear x apple selections, so with other pears at 100 m or so distance, the chances are high that the other parent is one of these pear x apple accessions.

The combination of a bibliographic search, RNA-seq data mining and gene expression analysis has proven to be a powerful method for identifying candidate genes encoding putative enzymes responsible for the biosynthesis of secondary metabolites, such as the genus-specific arbutin in pear. Eight candidate genes were selected as potential candidates to be involved in arbutin biosynthesis. Out of them, three candidates, 4CL, HBS and CPL, were differentially expressed in pear and apple-pear hybrid as compared to apple. The presence and expression of a microbial CPL orthologue in Zwintzscher's Hybrid and the pears 'André Desportes' and 'Williams Christ' needs to be further evaluated, as CPL has been described only in bacteria to date. The results of this study provide an important resource for understanding the arbutin biosynthesis in *P. communis* L. and apple-pear hybrid Zwintzscher's Hybrid, but also in other arbutin synthesizing plant species.

To understand the arbutin pathway better, it would be interesting to repeat this analysis with more pear varieties and apple-pear hybrids, for example the 'Abate' x 'Fuji' hybrids, P26A19 4 and F2-FP12 1-1.2-OP, because these samples accumulated both arbutin and phloridzin.

Future analysis by cloning genes into expression vectors and the functional characterisation of encoded proteins can give more information about the arbutin pathway.

5.7 References

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CHAPTER VI

General conclusion

6 General conclusion

In this research work different putative pear-apple hybrids were characterized by the use of different "Omics". SSR markers, SNP markers, DNA content, and the presence of arbutin and phlorizin were successfully used to determine the cross hybridity. Moreover the phenotyping was done to assess the resistance or susceptibility to fire blight, apple scab and pear scab derived from the different species (apple or pear) used to obtain the hybrids. Finally for the first time was done a confirmation/characterization of the e candidate genes involved in the arbutin pathway. Of the molecular markers used, the HRM method resulted the most successful and robust probably due to marker analysis. The HRM analysis, as the primers were designed and tested specifically for the apple and pear hybrids available in this study. These markers can maybe be used in future work in combination with the 20K (Bianco *et al.*, 2014) and 70K (Montanari *et al.*, 2019) arrays to provide improved results for the apple-pear or vice versa hybrids. The HRM method looks more sensitive and effective to SSR as reported in Distefano *et al.*, 2012.

The genetic analysis clearly determined that the CO progeny are all hybrids, but the parents did not contribute equally to the hybrid genome as can be seen from the marker analysis. The hybridity of these progeny was confirmed by their resistance to apple scab. Nevertheless, we speculate that some of the seedlings from this cross may carry non-host resistances from both the parents as the interspecific pears carry non-host resistance, too from the Asian pears. The same may to be applied the fire blight resistance as both the putative LG2 QTL of pear 'Moonglow', with has the same haplotype as the 'Old Home' resistance (Montanari *et al.*, 2016) and the LG7 QTL of the apple cultivar 'Fiesta', which it inherited the resistance from 'Cox's Orange Pippin' (Khan *et al.*, 2007), were segregating in this family. However, these results were not confirmed by the metabolomics analysis, because all the CO progeny inherited only the female parent's, *Malus*, genus-specific secondary metabolites phloridzin. While, it was assessed that in the progeny with P265R232T018 as the female, the parents did not contribute equally to the hybrid genomes either. It happens probably, because the parents were not compatible and showed less chromosome recombination than the other hybrids, or because the markers used in the HRM method resulted more specific for other progeny than the P26A19, as we know the HRM markers were tested and designed with other populations. On the other hand, P26A19 was resistant to *V. inaequalis* and *V. pyrina*. Apple scab can not infect *Pyrus*, and pear scab can not infect *Malus*, but in this case if the P26A19 was hybrid the *V. inaequalis* and *V. pyrina* can, both, infect the plants and P26A19 progeny can be resistance to both scab. P26A17 and P26A19 3 were susceptible to fire blight; P26A19 4 was highly resistant to fire blight, like its pear mother. The hybridity of the P265R232T018 progeny was

partially confirmed in the metabolomics analysis, because while P26A17 and P26A19 3 accumulated only arbutin different from the P26A19 4 accumulated both arbutin and phloridzin. Others New Zealand population, 'Imperial Gala' and 'Fuji' progeny are partially hybrids based on the obtained marker results, but were resitantece (IP26, IP12, FP12 3 and three FP35) and susceptible to apple scab and resistant to pear scab as were the apple parents. The 'Imperial Gala' progeny were susceptible to fire blight, i.e. none inherited resistance from their respective male pear parents, P266R231T015 and P125R095T002, since their female parent, 'Imperial Gala', is susceptible. The 'Fuji' progenies are moderately susceptible or susceptible to fire blight which is in contrast to their parental behaviour, noting that the low rating for 'Fuji' in our study varied from reported results indicating that it is moderately resistant to the disease (Kostick *et al.*, 2019). The 'Imperial Gala' and 'Fuji' progenies inherited only the maternal phloridzin genus-specific secondary metabolites. What it is highly significant is F2-FP12 1-1.2-O, offspring of FP12 1 because it accumulates both genus-specific secondary metabolites for *Malus* and *Pyrus* (Petkou *et al.*, 2002; Gutierrez *et al.*, 2018). A far as the Italian crosses concerned, the Zwintzscher's Hybrid and the 'Abate' x 'Fuji' progeny are true hybrids where the parents did contribute equally to the hybrid's genomes, as measured by the nuclear DNA content like for Zwintzscher's Hybrid reported by Fischer, *et al.* 2014 and accumulated both arbutin and phloridzin. Unfortunately, the five F2 Zwintzscher's Hybrid offspring, and the 'Decana' x 'Muray' 1 and 'Decana' x 'Murray' 2 progeny are not hybrids as proven from the markers analysis, DNA content and metabolomics analysis. As it happened in the field Zwintzscher's Hybrid progeny were made from open pollination, and maybe the mother of these F2 was a *Malus*. Maybe the incompatibility of 'Decana' and 'Muray' led to the formation of progeny none hybrid.

Finally, Zwintzscher's Hybrid, was used to characterise the putative genes involved in the arbutin pathway. Three candidates out of eight selected genes hypothesized to be involved in arbutin biosynthesis, 4CL, HBS and CPL, were differentially expressed in pear and apple-pear as compared to apple. Future analysis with heterologous expression systems might give more information about the function of the encoded proteins and their potential involvement in the arbutin pathway. In such future work AF, P26A19 4 and F2-FP12 1-1.2-OP plants should be utilized, as these lines accumulated both arbutin, from *Pyrus*, and phloridzin, from *Malus,* and would give a better overview of genes involved in the arbutin pathway. More research can be used to detect the difference between the arbutin pathway in pear and in the hybrids, as we know the hybrids accumulate both secondary metabolites. The presence of a CPL in Zwintzscher's Hybrid and the pears 'André Desportes' and 'Williams Christ' needs to be further evaluated in other hybrids and pears, as CPL has been described only in bacteria to date.

Moreover, future works on F2-FP12 1-1.2-O with molecular markers, especially an extended HRM analysis, would provide a better understanding of the introgression of apple into pear and to delineate their chromosomal structures. Also, pedigree analysis could be used to identify the pear or pear x apple parent.

A complication in assigning hybridity to the putative hybrids compared with resistance or susceptibility to apple and pear scab is, that it is difficult to determine whether the disease resistance is inherited from the parents or because they are not hybrids or partial hybridits so they are non-host of *V. pyrina* or *V. inaequalis*. As we know, pear scab can not infect apple and apple scab can not infect pear.

Future work on mapping recombination events during the crossing of apple and pear is more required to precisely characterise the chromosomes of the apple x pear hybrids. Additionally, further studies about the diseases, e.g. repeating this analysis or the genetic analysis, e.g., design primers for the more HRM markers will give more relevant informative data and can be interesting to see which parts of the genomes of pear or apple are introgressed in each hybrid.

Finally, future research on disease resistance analysis on Zwintzscher's Hybrid, 'Abate' x 'Fuji' and F2-FP12 1-1.2-O would give more data regarding their resistance or susceptibility to apple/pear scab and fire blight and maybe other diseases.

In conclusion, this research demonstrated that SSR and SNP markers, the metabolomics analysis as well as DNA content can be useful tools to detect hybridity in progeny derived from crosses between apple and pear or *vice versa*. The true hybrids found with the methods used in this study can be used for breeding to introduce more traits of interest, such as flavour or texture in apple or pear. This research can be the know how for the study of the genes involved in the arbutin biosynthetic pathway.

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Appendices

Appendix I

Oral presentation at: Student flash talk at the McGinty's (New Zealand conference).
January 31st and February 1st, 2019 (Auckland, New Zealand)
This work received the Second Place Award for the Student flash talks competition.

Biochemical, metabolic and molecular characterization of pear-apple hybrids: PEARAPPLE-Omics

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Appendix II

Poster presentation at: XV EUCARPIA Fruit Breeding and Genetics Symposium 2019 (conference).
June 3-7, 2019 (Prague, Czech Republic).
This work received the First Place Award for the poster competition.

Confirmation of intergeneric hybrids between apple and pear

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Abstract

Malus and *Pyrus* are closely related, with highly co-linear genomes. However, the two genera are characterized by many specific differences, including disease resistances, secondary metabolites, fruit texture, flavour and shape. Hence, intergeneric hybrids between apple and pear provide a unique germplasm resource for genomic analysis, the application of advanced breeding strategies, and new cultivar development.
We utilized two sources of apple-pear hybrid plants, Zwintscher's Hybrid (Fischer *et al.*, 2014) and its F2 progeny held at FEM, plus putative apple-pear hybrids developed at PFR. We determined which of the putative apple-pear hybrid genotypes were true hybrids by characterising the genomes for hybridity. We scanned the genomes of 47 putative hybrids with High Resolution Melting analysis (HRM) 'apple/pear' primers, developed following bioinformatics analysis, to detect SNP variants unique to the apple or pear genomes, respectively.
The initial primer set was optimised in a preliminary screen of DNA samples that included 'Cox's Orange Pippin', 'Old Home', Zwintscher's Hybrid, its parents and various mixtures. A set of 39

useful primers distributed along the genome was identified and used to screen the 47 putative hybrids. Results indicated these were true hybrids: 9-15 primers per genotype provided evidence for this. Interestingly, the apple and pear parents did not appear to contribute equally to the genomes of the progenies. Screening with a set of SSRs, as well as a preliminary screen of 6 samples over the 20K SNP array (Bianco *et al.*, 2014) validated these results. We plan to screen all hybrid seedlings with the 9K apple/pear SNP array (Chagné *et al.*, 2012; Montanari *et al.*, 2013) to delineate their chromosomal structure.

Keywords: intergeneric hybrids, *Malus*, *Pyrus*, apple-pear hybrid, HRM

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Appendix III

Poster and oral presentations at: 6th International Horticulture Research Conference.
September 30th - Oct 5th, 2019 (Venice, Italy).

Fire blight resistance in apple/pear hybrids may be related to their genomic structure

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Abstract

Although *Malus* and *Pyrus* are closely related, with highly co-linear genomes, the two genera are characterised by many specific differences, including disease resistances, secondary metabolites, fruit texture, flavour and shape. Hence, intergeneric hybrids between apple and pear provide a unique germplasm resource for genetic analysis, as well as new cultivar development, using advanced genomic breeding strategies.

Fire blight, caused by the Gram-negative bacterium *Erwinia amylovora* (Enterobacterales; Erwiniaceae), affects apple and pear production worldwide. A number of resistance loci against this disease have been located on genetic maps of both apple and pear.

We investigated fire blight resistance in apple-pear hybrids, by studying 31 putative hybrids raised from a 'Cox's Orange Pippin' x 'Old Home' cross at The New Zealand Institute for Plant and Food Research Limited. We inoculated up to eight replicates of each hybrid grafted on 'M9' rootstock and compared these with 'Cox's Orange Pippin' and 'Imperial Gala' grafted on 'M9' rootstock and 'Old Home' and 'Williams' grafted with *Pyrus calleryana*, using the cut-leaf method

(Maas Geesteranus and Heyting, 1981) for inoculation with *E. amylovora* (Ea 236 at 1 x 106 cfu/ml), as it is widely applied in both apple and pear. Disease progress was observed in the period from 2 to 6 weeks after inoculation. Level of disease was quantified by expressed necrosis length as a percentage of the total shoot length, both measured downwards from the point of inoculation. The result clearly showed that all of the 31 putative hybrids were resistant to fire blight, while the parents and controls exhibited the expected range of resistance and susceptibility according to previous work.

Preliminary results using high-resolution melting marker analysis of the seedling genomes, indicate there is a hybrid apple/pear genomic region on LG2, while LG7 is represented by apple DNA. Interestingly, fire blight resistance has been reported on LG2 of pear 'Old Home' (Montanari *et al.*, 2016), while Khan et al. (2007) have located fire blight resistance on LG7 of the apple 'Fiesta', which is related by descent from 'Cox's Orange Pippin'.

Our next step is to analyse recombination events during the crossing of apple and pear, using the IRSC 9K apple/pear SNP array: this will enable us to further investigate the relationship of these reported QTL resistances to fire blight infection within the genomic structure of our 31 apple/pear hybrids.

Keywords: Fire blight, apple-pear hybrid, *Malus*, *Pyrus*

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Appendix IV

Poster presentations at: 7th International Horticulture Research Conference, Online.
July 1st-30th, 2020.

Characterization of putative pear-apple hybrids

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Appendix V

Oral presentations at: 10th International Rosaceae Genomics Conference (RGC10), Virtual. December 9-10-11-16-17-18, 2020.

To be or not to be: apple-pear intergeneric hybrids characterization with molecular and biochemical markers

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Abstract

Malus and *Pyrus* are closely related, with highly co-linear genomes. However, the two genera are characterized by many specific differences, including disease resistances and secondary metabolite composition. Intergeneric hybrids between apple and pear provide a unique germplasm resource for new cultivar development.

We utilized two sources of apple-pear hybrid plants, Zwintscher's Hybrid (Fischer *et al.*, 2014), its F2 progeny held at FEM and 71 putative hybrids developed at PFR. Our objective was to determine which of the putative hybrids are true hybrids by characterising their genomes, determining their chromosomal structure and assessing resistance to apple and pear diseases. For that, we scanned the genomes of putative hybrids with High Resolution Melting analysis (HRM) markers, a set of SSRs and the 9K apple/pear SNP array. A preliminary work was carried out infecting with *Erwinia amylovora*, *Venturia inaequalis* and *Venturia pyrina* some clones of these new plants to assess the resistance/susceptibility to these pathogens.

All the data obtained with the different analysis methods were used to carry out a parentage analysis to test the relationships among the individuals and to assess the intra- and inter-population genetic

variability. Moreover we did a preliminary work focused to correlate our genetic data with data related to secondary metabolite production and resistance or/and susceptibility to fire blight and scab by the use of the Canonical Correspondence Analysis (CCA).
The characterized plant materials can be used in future breeding programs.

Keywords: apple-pear hybrid, HRM, SNP-chip, SSR, fire blight, scab

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