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Genetic mapping and phenotyping plant characteristics, fruit quality and disease resistance traits in octoploid strawberry (*Fragaria* × *ananassa*)

Thesis submitted for the degree of Doctor of Philosophy

School of Biological Sciences

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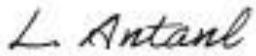
Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.



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Abstract

The cultivated strawberry (*Fragaria* × *ananassa* Duch.) is the third most economically important fruit crop. In recent years the withdrawal of many fungicides and soil fumigants have made the sustainability and profitability of this crop more challenging. To overcome these challenges, plant breeders aim to improve upon existing cultivars and to release new ones with higher yield, better fruit quality and more disease resistance.

Through Quantitative Trait Mapping, markers linked to genetic variants associated with traits of economic and agronomic importance can be identified through and molecular markers such as simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs), can be used to improve plant breeding efficiency at the molecular level, which significantly reduces the breeding time and cost of phenotyping.

In this thesis the following work is described: a correlation analysis of plant characteristics and fruit quality traits; the saturation of an existing SSR-based linkage map; the development of a high density consensus SNP-based octoploid strawberry linkage map, and the identification of quantitative trait loci (QTL) linked to two key plant attributes, fruit quality and powdery mildew resistance. In addition, the most closely linked SSR markers were identified and validated in a wider strawberry germplasm using firmness as an example study. Moreover, the physical locations of expansin genes and SNP markers associated with firmness QTLs were investigated. The purpose of this analysis was to find out if QTLs associated with fruit firmness overlapped the positions of expansins, genes known to be important in controlling fruit firmness.

Overall the results provide new insights into how complex traits are correlated in octoploid strawberry and valuable information about QTL location and relationships. The information generated will be important for future analyses, such as identification of genes linked to traits of importance, and novel molecular marker development for marker-assisted breeding. Finally, the data from this study will contribute towards genomic selection studies conducted in cultivated strawberry and other closely related rosaceous crops.

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List of Abbreviations

ABI	Applied Biosystems
AFLP(s)	Amplified Fragment Length Polymorphism(s)
BLAST	Basic Local Alignment Search Tool
bp	Base pair(s)
ºBrix	Sugar content of an aqueous solution
ºC	Celsius degrees
cDNA	Complementary DNA
cM	Centi-Morgans
cm	Centimeter
DNA	Deoxyribonucleic acid
dNTP	Deoxyribo Nucleotide Triphosphate
dsDNA	Double-stranded DNA
EMR	East Malling Research
EST(s)	Expressed Sequence Tag(s)
ETI	Effector-Triggered Immunity
g	Gram
GDR	Genome Database for Rosaceae
GS	Genomic selection
GWA	Genome-wide Association
H^2	Broad-sense heritability coefficient
Hi-Di	Formamide
HR	Hypersensitive Response
IM	Interval Mapping
K	Thousand
K*	Kruskal-Wallis test statistics value
km/h	Kilometres per hour

KW	Kruskal-Wallis test
LG(s)	Linkage Group(s)
LOD	Logarithm of the Odds
m	Number of years
MAB	Marker-Assisted Breeding
MADCE	Microsatellite Allele Dose Configuration and Establishment
MAS	Marker-Assisted Selection
Mb(p)	Mega-base(s) (pair) (1 × 106 bp)
$MgCl_2$	Magnesium Chloride
min	Minute(s)
mlo	Mutation-induce recessive alleles
mm	Milimeter
mM	Mili-Mole
mRNA	Messenger Ribonucleic acid
n	Number of tunnels
NCBI	National Center for Biotechnology Information
ng	Nanogram(s)
NGS	Next Generation Sequencing
nm	Wavelength absorbance units
No	Number of individuals
PAMP	Pathogen-Associated Molecular Pattern
PCR	Polymerase Chain Reaction
PRR(s)	Plant Recognition Receptors
PTI	Primary defence response
QTL(s)	Quantitative Trait Loci
r	Pair-wise correlation coefficients
R	Plant disease resistance proteins
RAPD(s)	Random Amplification of Polymorphic DNA(s)
REML	Restricted Maximum Likelihood

RFLP(s)	Restriction Fragment Length Polymorphism(s)
R*Pa*	Resistance to *Podosphaera aphanis*
R*Vd*	Resistance to *Verticillium dahliae*
SC	Self-compatible
SCARs	Sequence Characterized Amplified Regions
SCRI	Specialty Crop Research Initiative
SD	Standard Deviation
SE	Standard Error
sec	Seconds
SI	Self-incompatible
SNP(s)	Single Nucleotide Polymorphism(s)
SSR(s)	Simple Sequence Repeats
U	Unit
USDA	United States Department of Agriculture
X^2	Chi-square
W	Shapiro-Wilko statistics value
*var*G	Genetic variance
*var*GT	Genotype by tunnel interaction variance
*var*GY	Genotype by year interaction variance
*var*P	Phenotypic variance
*var*R	Residual error variance
µl	Microlitre(s)
µM	Micro-Mole(s)

Chapter 1

General introduction

1.1 The Rosaceae family

1.1.1 Taxonomy of the Rosaceae

The Rosaceae is a major group of morphologically diverse flowering plants, consisting more than 3,000 species from approximately 100 genera (Shulaev et al. 2008; Cabrera et al. 2009; Jung et al. 2012). The family encompasses plants of many different types and architectural forms including woody shrubs, herbs and trees (Judd et al. 1999). Members of the family are rhizomatous, climbing or thorny and most are deciduous. Rosaceous species are distributed throughout the world and are especially common in North America, Europe and Asia (Judd et al. 1999). Members of the family can be found in forests, marshes and fields, demonstrating the range of different environments and climates to which rosaceous species are adapted.

The Rosaceae family was originally divided into four subfamilies: Rosoideae, Amygdaloideae (Prunoideae), Spiraeoideae and Maloideae (Pomoideae) (Shulaev et al. 2008), mainly grouped by fruit structure and chromosome number. For example, the chromosome number is x=7, 8 or 9 in Rosoideae; x=8 in Amygdaloideae; x=9 in Spiraeoideae; and x=17 in Maloideae (Potter et al. 2002). More recent phylogenetic studies have demonstrated that Maloideae and Rosoideae are monophyletic groups, whereas Spiraeoideae is polyphyletic. These findings and new phylogenetically-based classification strongly suggested that the family should be divided into three subfamilies: Dryadoideae, Rosoideae and Spiraeoideae (Potter et al. 2007). More recent comparison of the genomes of *Fragaria* (Rosoideae), *Malus* and *Prunus* (Spiraeoideae), confirmed that these genomes have undergone different modes of evolution (Jung et al. 2012). This study concluded that *Fragaria* had more chromosomal rearrangements than

Malus or *Prunus*, and thus that these three genera do not share a common ancestor as was previously believed.

Economically, the Rosaceae is the third most important plant family in temperate regions after the Poaceae (grass family) and Fabaceae (legume family) (Bennet, 2010; Dirlewanger et al. 2002). The family includes important crops such as apple, cherry, strawberry, pear, nut (almond) and ornamentals (e.g. roses) (Shulaev et al. 2008). In Spiraeoideae and Dryadoideae, *Prunus* and *Malus* are the most economically important genera, whilst genus *Fragaria* is the most important within the Rosoideae subfamily (Potter et al. 2002). The economically important members of Rosaceae are shown in Table 1.1.

Table 1.1 A summary of the most economically important genera of the Rosaceae and crop production values for 2013 (http://faostat.fao.org)

Subfamily	Genus	Common name	Worldwide production in 2013 (million tonnes)
Dryadoideae	*Prunus*	Sweet cherry	2.3
		Sour cherry	1.3
		Plum	11.5
		Almond	2.9
		Peach and nectarine	21.6
		Apricots	4.1
Spiraeoideae	*Malus*	Apple	80.8
	Pyrus	Pear	25.2
Rosoideae	*Fragaria*	Strawberry	7.7
	Rosa	Rose	-[a]
	Rubus	Raspberry	0.6

[a] Data are not available at http://faostat.fao.org

1.1.2 The genus *Fragaria*

Strawberry, genus *Fragaria L.*, belongs to the Rosoideae subfamily of the Rosaceae family (Harrison et al. 1997). The use of the latin name '*Fragaria'* predates

Linnaean literature (Hummer and Hancock, 2009; Hummer et al. 2011), and is derived from the Latin word ‘Fraga’ meaning fragrance. The genus *Fragaria* is closely related to the genera *Potentilla* and *Duchesnea* (Harrison et al. 1997; Potter et al. 2007; Hummer et al. 2009). Based on morphological data, it has been proposed to merge *Fragaria* and *Potentilla* due to their similarities (Mabberley, 2002), but further genetic-based research has indicated that both genera should remain distinct, and this is now accepted by the majority of botanical associations. The morphological structure of the strawberry plant is presented in Figure 1.1.

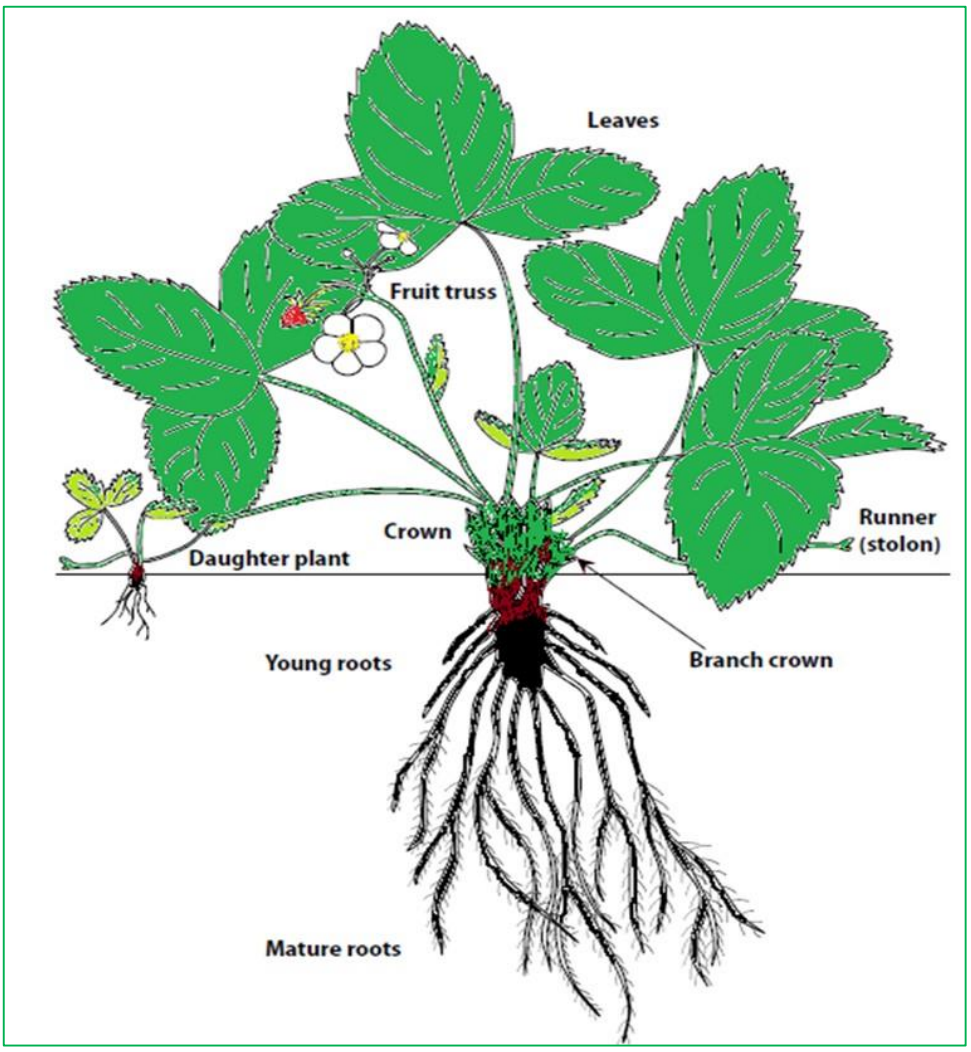


Figure 1.1 The morphological structure of the strawberry plant. Modified from the Mid-Atlantic Berry Guide, 2013-2014 (http://extension.psu.edu/publications/agrs-097).

As perennials, *Fragaria* species typically survive for several years and can reproduce both sexually and asexually; most are monoecious, having hermaphrodite flowers (with both male and female floral organs), and produce fruits with fertile seeds (Eriksson et al. 2003). Cross-pollination and self-pollination can both occur; Roselino et al. (2009) demonstrated that cross-pollination produces stronger plants with better quality fruit compared to self-pollinated plants and fruits. In the octoploid strawberry, self-pollinated or closely related plants suffer from inbreeding depression with less fit individuals that have recessive deleterious traits (Botham et al. 2009). It is worth noting that a number of diploid and hexaploid strawberry species are self-incompatible; this will be discussed in more detail in the next section of this chapter.

Asexual reproduction in strawberry occurs through the production of stolons (runners arising from the crown of the plant) (Figure 1.1). Runners usually grow laterally along the surface of the soil and give rise to daughter plants. Once a daughter plant comes into contact with the soil, it produces its own root system and develops into an independent plant. Propagators of commercial strawberry typically utilise runners as this allows the propagation of genetically identical (clonal) plants with all the favourable characteristics of the mother plant. Other *Fragaria* species can be propagated using different methods; for example, some species do not produce many runners and are propagated by separating branch crowns. Compared to other rosaceous plants, strawberry plants are easy and quick to propagate, especially through runners and using micropropagation techniques (Mir et al. 2010).

1.1.3 Strawberry species, distribution and self-incompatibility

Currently, twenty-five species, including four hybrid species, are recognised in the genus *Fragaria* and are distributed throughout the world (Staudt, 2008; Hummer et

al. 2011). *Fragaria* species have a basic chromosome number of seven (x=7) and there is extensive natural polyploidy variation ranging from diploids to decaploids (Hummer et al. 2011; Njuguna et al. 2013). There are six ploidy levels known in *Fragaria* species: diploid (2n=2x=14), tetraploid (2n=4x=28), pentaploid (2n=5x=35), hexaploid (2n=6x=42), octoploid (2n=8x=56) and decaploid (2n=10x=70). Table 1.2 summarises the currently described strawberry species, their ploidy levels, compatibility rates and their geographical distribution.

Table 1.2 The twenty-five *Fragaria* species (including 4 hybrids), ploidy level, compatibility (SI: self-incompatible, SC: self-compatible) and their geographical distribution (Hummer et al. 2011; Liston et al. 2014)

Species	Ploidy	Compatibility	Geographical distribution
F. bucharica	2×	SI	Western Himalayas
F. chinensis		SI	China
F. daltoniana		SC	Himalayas
F. iinumae		SC	Japan
F. mandshurica		SI	North China
F. nilgerrensis		SC	Southeastern Asia
F. nipponica		SI	Japan
F. nubicola		SI	Himalayas
F. pentaphylla		SI	North China
F. vesca		SC	Europe, Asia, North America
F. viridis		SI	Europe, Asia
F. yezoensis		SI	Japan
F. × *bifera*		SC	France, Germany
F. corymbosa	4×	-[1]	East Russian, China
F. gracilis		-[1]	Northwestern China
F. moupinensis		-[1]	Northen China
F. orientalis		-[1]	East Russian
F. tibetica		-[1]	China
F. × *bringhurstii*	5×	-[1]	California, China
F. moschata	6×	SI[1,3]	Euro-Siberia
F. chiloensis	8×	SC[2]	Western N. America, Hawaii, Chile
F. virginiana		SC[2]	North America
F. × *ananassa*		SC[2]	Cultivated worldwide

Table 1.2 Continued

Species	Ploidy	Compatibility	Geographical distribution
F. iturupensis	10×	SC[2]	Iturup and Kurile Islands, North America
F.× vescana		SC[2]	Cultivated in Europe

[1]Dioecious (hermaphroditic, but distinct male and female flowers on separate plants)
[2]Sub-dioecious species (transitional state towards dioecy)
[3]http://www.upcscavenger.com/wiki/Musk%20Strawberry/#page=wiki

The biggest group of wild strawberries is the diploids, with 13 species that can be found from the north temperate to Holarctic zones; however all species are restricted to single continents or specific areas, except *Fragaria vesca* (Rousseau-Gueutin et al. 2009). *Fragaria vesca* has a wide distribution and has the largest native range among *Fragaria* species. It is native in Europe, Asia and is the only diploid common in North America. In contrast, the remaining 12 diploid species such as *Fragaria viridis*, *Fragaria chinensis* and *Fragaria nipponica* are mainly native to Europe and Asia (Hummer and Hancock, 2009; Hummer et al. 2011).

There are five tetraploid wild species native in Siberia and Russia. It is believed that the ancestor of the tetraploids are diploid species due to their similar distribution and morphological characteristics as well as natural chromosome polyploidization (Staudt, 2003; Hummer and Hancock, 2009; Hummer et al. 2011; Rho et al. 2012). For example, according to Staudt (2003), *Fragaria corymbosa* and *Fragaria moupimemsis* may have been derived from the diploid *F. chinensis*. Similarly, it is proposed that tetraploid *Fragaria orientalis* is derived from diploid *Fragaria mandshurica* due to its similar distribution (Staudt, 2003). The only known pentaploid species (*Fragaria* × *brinhurstii*) is a hybrid derived from diploid *Fragaria vesca* and octoploid *Fragaria chiloensis* and is native in California and China (Hummer and Hancock, 2009). The only known wild hexaploid species (*Fragaria moschata*) is native to Europe and is

known as the musk strawberry. It is not known for certain, although it is likely that the ancestry of *Fragaria moschata* may have been various diploid and tetraploid species (http://strawberrygenes.unh.edu/history.html). According to Staudt (2003) *F. moschata* was one of the favourite consumed strawberries in the past due to its pleasant flavour and strong aroma. Two wild octoploid species (*Fragaria chiloensis* and *Fragaria virginiana*) are native across North and South America (Staudt, 1999a, b). The ancestry of these two species is yet to be confirmed, although it is clear that because they are diploidized allopolyploids they have descended from ancestors such as *F. vesca*, *F. iinumae*, *F. bucharica* and *F. mandshurica*, (Folta and Davis, 2006). *Fragaria chiloensis* is known as the beach strawberry and is mainly found along sandy beaches in California (Hummer et al. 2011).

The most important octoploid *Fragaria* and most widely cultivated worldwide is *Fragaria* × *ananassa* Duch. This species, which is the focus of the research conducted here, is an accidental hybrid between two wild octoploid species *F. chiloensis* (brought into France from Chile) and *F. virginiana* (imported from North America) (Darrow, 1966; Hummer and Hancock, 2009). The decaploid *Fragaria* species (*F. iturupensis*) has a limited distribution and is common in the Kurile and Iturup Islands in Russia, as well as in North America (Hummer et al. 2011). It has been reported that *F. iturupensis* has similar morphological characteristics to *F. vesca* and one of the *F. virginiana* subspecies (*glauca*) (Hummer and Hancock, 2009).

In addition to differences in ploidy level, *Fragaria* species range in sexual compatibility systems (Staudt, 1988). *Fragaria* species and subspecies can be self-compatible (SC) or self-incompatible (SI) and dioecious or sub-dioecious (Table 1.2). Self-incompatibility reduces inbreeding in plants by preventing self-fertilization, thus resulting in a wider heterozygosity in wild populations (Bosković et al. 2010). However,

self-incompatible strawberry species must be planted in close proximity to plants with which they are compatible in order to produce fruit through cross pollination. In contrast, self-compatibility mechanisms allow the formation of fruits through self-fertilisation resulting to more homozygous wild populations. Compatibility studies in *Fragaria* show high interfertility levels between diploids and between diploid and higher ploidy *Fragaria* species (Sargent et al. 2004a; Bors and Sullivan, 2005). This suggests that *Fragaria* species have not diverged greatly during evolution.

1.1.4 Economic importance and strawberry production

Among the *Fragaria* species, the cultivated strawberry *Fragaria* × *ananassa* (2n=8x=56) is the most economically valuable. The nutritional value, flavour and aroma of strawberry, in addition to being an excellent source of vitamin C, iron, antioxidants and other nutritional qualities make it an important fruit crop (Schwab et al. 2011; Tulipani et al. 2011). The largest producer of strawberry fruits is the United States of America with a total production of more than 1.36 million tonnes of fruit in 2013, followed by Turkey and Spain with a total production of the fruit more than 0.37 and 0.31 million tonnes respectively (http://faostat.fao.org).

World strawberry production has been increasing for many years, with the exception of 2010 (Figure 1.2). In 2010, on average, worldwide strawberry production decreased approximately 0.3% compared to 2009 mainly due to unfavourable weather conditions during the harvesting season (Figure 1.2). However, since 2003 strawberry production has increased by nearly 2.70 million tonnes to 7.73 million tonnes in 2013 (Figure 1.2; http://faostat.fao.org). During the last decade, the largest increases in world strawberry production were observed between 2008 and 2009 (an increase of 0.61

million tonnes), between 2011 and 2012 (an increase of 0.54 million tonnes) and between 2012 and 2013 (an increase of 0.44 million tonnes).

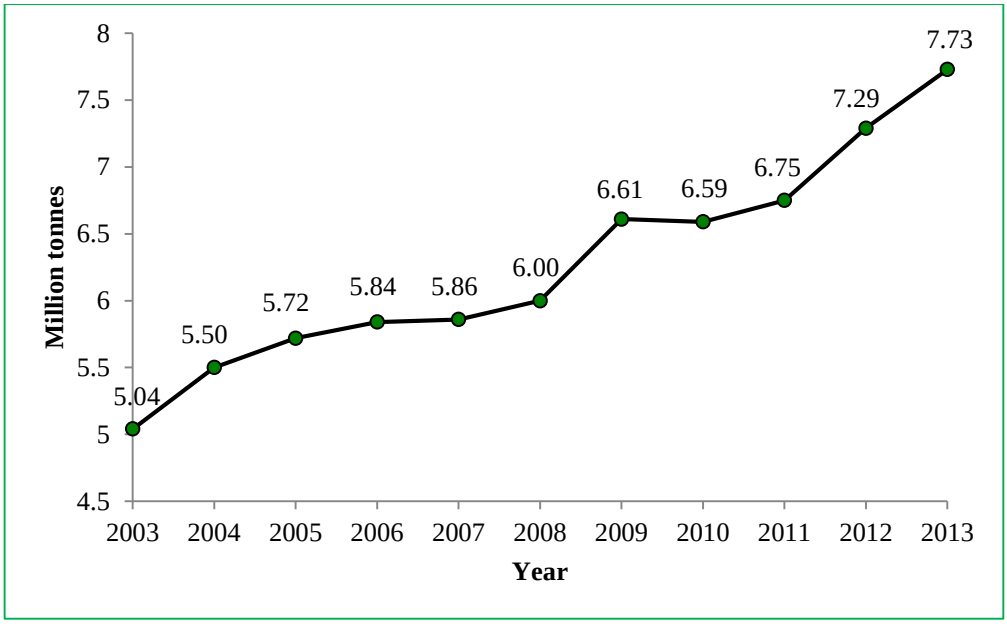


Figure 1.2 World strawberry production in million tonnes per annum (http://faostat.fao.org).

Strawberry production in the UK has decreased every year since 2009. The average strawberry production decreased by 15.5 thousand tonnes from 2009 to 2013 (http://faostat.fao.org). In 2013 the market value of UK strawberry fruit was more than £190 million with a total production of nearly 94.5 thousand tonnes. Thus the UK is one of the major strawberry growers and producers in Europe.

1.2 Strawberry breeding

The established strawberry breeding programmes worldwide are usually very similar in structure and have common goals (though are often optimised for specific

climatic conditions). Details of the breeding process, including the number of seedlings and genotypes produced and used in the programmes have been previously reported (Chandler et al. 2012). Briefly, the strawberry breeding process is based on crossing different genotypes exhibiting valuable characteristics. The most desirable genotypes are selected from the F_1 seedlings, which are further crossed and trialled for several years to confirm the plant characteristics (phenotype). Only the best plants, which express the target traits, are selected and further assessed before release as a new variety (Gil-Ariza et al. 2009; Chandler et al. 2012). For example, at East Malling Research the strawberry cultivar 'Elegance' was developed by crossing two East Malling breeding lines, EM834 and EM1033, and EM834 and EM1033 were themselves developed from a series of crosses involving a number of breeding lines and cultivars including 'Eros', 'Elsanta', 'Holiday', 'Honeoye' and 'Alice' (Figure 1.3; A. Whitehouse, personal communication).

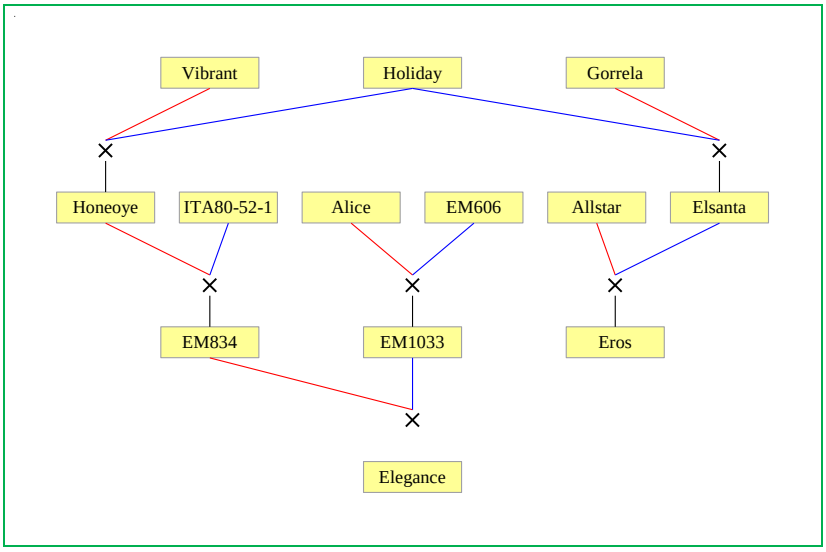


Figure 1.3 An example of the cultivated strawberry breeding process and the pedigree used for making crosses (A.Whitehouse, personal communication). Maternal parentages are presented in red lines and paternal parentages are in blue. The figure was produced using Pedimap 1.2 software (Voorrips et al. 2012).

The process may appear straight forward, but it may take up to 7 years (and sometimes up to 20 years, Gallardo et al. 2012) to release a new strawberry cultivar (Shaw, 2004). A number of replicated trial-based tests for multiple years are carried out to confirm the performance of the new potential variety under different environmental conditions and grower trials, as well as the level of susceptibility and/or resistance to pests and different diseases (Shaw, 2004; Chandler et al. 2012).

The majority of newly released strawberry varieties belong to the octoploid species *Fragaria* × *ananassa* and only a few to other ploidy level *Fragaria* species such as diploid and decaploid (Faedi et al. 2002). This has resulted in the reduced diversity observed in modern strawberry cultivars and a limited germplasm available for use in future breeding programmes (Gil-Ariza et al. 2009). For example, a study of the genetic diversity of octoploid strawberry by Degani et al. (2001), revealed that the majority of octoploid strawberry cultivars grown in the United States and Canada share either a common parent or more than one ancestor. Moreover, it has been reported that the repeated use of favourable parental lines in breeding programmes, has resulted in a loss of genetic diversity and reduced the level of heterozygosity among cultivars. This has been demonstrated in the study by Gil-Ariza et al. (2009) which reported that the genetic similarity among 92 widely diverse strawberry cultivars bred between pre-1949 to post-2000, significantly increased with time. A similarity coefficient of more than 70% was observed among modern cultivars, reflecting their low genetic diversity.

In the UK, Thomas A. Knight was the first to develop a formal strawberry breeding program in 1817 (Darrow, 1966). He used clones of ancestors of *Fragaria* × *ananassa* (*F. virginiana* and *F. chiloensis*) in his crosses. Unsurprisingly, novel strawberry cultivars were produced (‘Downton’ and ‘Elton’) with large, firm fruits (Hummer and Hancock, 2009). Later, strawberry breeding became more active and

during the last 200 years many different strawberry cultivars have been released. For example, the most popular cultivars of the 20th century were 'Nobel' (early cropping, firm and disease resistant); 'Royal Sovereign' (early cropping, excellent flavour, high productivity); 'Hovey' (the first North American strawberry) and 'Ettersburg 80' ('Huxley') (drought resistant, high productivity, attractive colour) (Hummer and Hancock, 2009). Many of these and other old varieties are still used in modern strawberry breeding programmes for their excellent fruit qualities.

The national strawberry breeding programme in the UK is based at East Malling Research (Kent). Since 1988, 39 new strawberry varieties have been released of which 32 are being propagated and grown not only in the UK but also in North Europe, USA and South Korea (A. Whitehouse, personal communication; www.emr.ac.uk). The cultivars exhibit improved fruit appearance (Simpson et al. 1996, 2004, 2012a), high yield (Simpson et al. 1996, 2012a), an extended cropping season (Simpson et al. 2004; Johnson et al. 2008) and show moderate resistance to a variety of diseases including wilt, crown rot and powdery mildew (Simpson et al. 1994, 1996; Johnson et al. 2008). In addition, several private companies, such as those of Edward Vinson Ltd, Driscoll's Genetics Ltd and Angus Soft Fruits, are successfully running strawberry breeding programmes in the UK (Simpson, 2012b).

The range in ploidy level, inbreeding depression, a limited germplasm base and the complexity of genomes all conspire to make octoploid strawberry breeding a challenging process (Shaw, 1997; Comai, 2005; Gil-Ariza et al. 2009). The genetics of polyploids is complicated due to the multiple alleles associated with a single locus, which changes segregation and inheritance ratios (Acquaah, 2007). However, the advantages of being polyploid are that these species are more likely to have increased allelic diversity and heterozygosity, therefore a higher rate of novel phenotypic

variations may arise from the gene duplication when compared to the diploid genomes (Udall and Wendel, 2006).

The cultivated strawberry is highly heterozygous and it is sensitive to inbreeding (Shaw, 1997). Inbreeding has a negative effect in crop breeding. It reduces the survival and fertility of offspring's, as well as the expression of a desirable trait in the progeny due to a high level of mutations (Charlesworth and Willis, 2009; Botham et al. 2009; Kacsmarska et al. 2014). As a result, these progenies are more likely to be affected by diseases, have slow growth rates, small plant and fruit size. For example, it has been reported that strawberry fruit yield can be severely affected by inbreeding depression even if a moderate level of inbreeding is applied (Shaw, 1997).

To avoid excessive inbreeding, genetically distant and unrelated cultivars (pedigree selections) are required (Gil-Ariza et al. 2009). However, the availability of strawberry germplasm collections remains narrow worldwide, thus breeding through pedigree cultivars remains a complicated process (Hancock et al. 2001). A number of studies have been performed to improve diversity of the cultivated strawberry through introducing some of the wild *Fragaria* species to the breeding programmes (Stegmeir et al. 2010; Diamanti et al. 2012). Wild clones of *F. virginiana* and *F. chiloensis* were introduced into the *Fragaria* × *ananassa* crosses resulting in the development of novel families and individuals with exceptional fruit quality attributes (Hancock et al. 2000, 2001, 2010).

1.3 Molecular markers in plant breeding

1.3.1 Marker types

To speed up the traditional plant breeding approaches and to cut down the costs, molecular technologies have been harnessed for the improvement of crop breeding programmes. Today, the most widely used molecular tools for breeding rosaceous crops are molecular markers (Whitaker, 2011, Sargent et al. 2009, 2012; Antanaviciute et al. 2012). Molecular markers are specific locations on a chromosome that can be used as landmarks for a range of purposes including the construction of genetic linkage maps, comparative mapping analysis, understanding germplasm relationships, detection of economically important genes, MAS (marker-assisted selection) and map-based cloning (Kumar, 1999).

There are three main groups of markers (morphological, biochemical and molecular markers) that have different characteristics and are used for different purposes (Kumar, 1999; Caligari, 2001; Semagn et el. 2006). It is crucial to select the right type of marker for the particular analysis to succeed. Morphological markers are monitored visually, whereas biochemical markers, similar to molecular markers, express polymorphism at the protein or DNA level and require electrophoresis for visualisation (Kumar, 1999). Molecular markers can be further classified as hybridization-based and PCR-based DNA markers (Semagn et al. 2006). At present, the most valuable and widely used DNA-based molecular markers are PCR-based.

Hundreds of different types of PCR-based molecular markers have been developed for species within the Rosaceae, including, amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), sequence characterized amplified regions (SCARs), expressed sequence tags (EST), simple

sequence repeats (SSR or microsatellite) and single nucleotide polymorphism (SNP) markers (Semagn et al. 2006; Hummer et al. 2011). However not all molecular markers have the desired characteristics to make them useful to both applied plant breeders and research scientists (Hummer et al. 2011). The most valuable DNA-based molecular markers must be highly polymorphic, multi-allelic, inexpensive to develop and easy and fast to test using a small amount of DNA for the assay. For example, restriction fragment length polymorphisms (RFLPs) are hybridization-based polymorphism markers that are used for the identification of DNA sequence polymorphism and are time consuming, expensive to develop and require a large amount of DNA for the assay (Semagn et al. 2006). Similarly, AFLP markers, which reveal restriction fragment polymorphism as present or absent are time consuming and expensive to use. Other markers may require different methods for their development, such as cloning and DNA sequencing (sequence-characterized amplified regions (SCARs)) resulting in a higher cost in their design (Hummer et al. 2011). In contrast, SSR and SNP markers are well-distributed throughout the genome and can be detected using rapid automated procedures at significantly lower costs (Hummer et al. 2011). The advantages of these molecular markers are that they require small amounts of DNA to function, are highly polymorphic, which allows development of genetic markers within a short time, and also have the ability to screen many genes (Semagn et al. 2006). It is likely that SNP markers based on DNA sequencing will become the dominant markers among other commonly-used DNA-based PCR markers.

SSR and SNP markers have been developed for many rosaceous crops including apple (Guilford et al. 1997; Liebhard et al. 2002; Chagné et al. 2008; Celton et al. 2009; Gasic et al. 2009), pear (Yamamoto et al. 2002), raspberry (Graham and Smith, 2001; Graham et al. 2004; Castillo et al. 2010; Palmieri and Giongo, 2012), cherry (Aranzana

et al. 2003), peach (Aranzana et al. 2002; Dirlewanger et al. 2002). In strawberry, SSR markers are mainly used, not only for cultivar identification and fingerprinting but also for genetic diversity analysis and linkage mapping (Hadonou et al. 2004; Sargent et al. 2004b, 2006, 2009; Davis et al. 2006; Govan et al. 2008; Njuguna, 2010; Spigler et al. 2010). Currently, over 900 *Fragaria* derived SSR markers are available. These markers were mainly developed for use with the diploid species *F. vesca* and *F. viridis* (James et al. 2003; Sargent et al. 2003; Hadonou et al. 2004; Monford et al. 2005), but they have also been developed for use with the octoploid species *F. virginiana* and *F.* × *ananassa* (Ashley et al. 2003; Gil-Ariza et al. 2006, Spigler et al. 2010). SSRs may be transferable not only within species but also in different genera and subfamilies (Yamamoto et al. 2001; Mnejja et al. 2010; Zorrilla-Fontanesi et al. 2011a). For example, SSR markers developed for apple screening are informative and can identify polymorphism in pear (Yamamoto et al. 2001), and some *Fragaria* SSRs are informative in raspberry (Zorrilla-Fontanesi et al. 2011a).

The use of molecular markers improves the efficiency of breeding techniques by allowing the pre-selection of superior genotypes at a very early stage in the breeding process. Molecular markers can also be used for linkage map development, which is discussed in more detail in Section 1.3.2, and are important for use in marker-assisted selection, which is discussed in Section 1.3.3.

1.3.2 Linkage mapping in strawberry

The development of a genetic linkage map is a challenging process and a number of important factors must be kept in mind. It is important to consider the right selection of parents for the cross, the population size, and the aim of study, because each of these has a significant influence on the developed map (Young, 2001). In order to identify all

linkage groups (chromosomes) on the map, a large number of polymorphic markers have to be identified in the parents of the cross, because non-segregating markers do not provide any useful information. Furthermore, the ploidy level is an important factor in map development. Usually, it is easier to identify linkage groups in crops with a lower ploidy level (e.g. diploids) compared to higher ploidy crops such as the cultivated strawberry (octoploid).

Genetic linkage maps have been created for most of the important cultivated species including apple (Hemmat et al. 1994; Maliepaard et al. 1998; Liebhard et al. 2003a, 2003b; Silfverberg-Dilworth et al. 2006; Fernández-Fernández et al. 2008), pear (Yamamoto et al. 2007), peach (Yamamoto et al. 2005; Dirlewanger et al. 2006), raspberry (Graham et al. 2004) and cherry (Stockinger et al. 1996; Wang et al. 1998). The first diploid strawberry linkage map was developed and reported by Davis and Yu in 1997. The map was constructed using an F_2 population from an intra-specific cross of diploid *F. vesca* ('Baron Solemacher') × 'WC6' (a wild accession)). The expected seven linkage groups of diploid strawberry were identified and the map contained a total of 79 markers, mainly RAPD (75). Later, a second diploid map, now utilized as the reference map for diploid strawberry was reported by Sargent et al. (2004b). The map was based on a *F. vesca* × *F. bucharica* (FV × FB) inter-specific cross and contained mainly SSR markers (68), six gene-specific markers and one SCAR marker. The map was further saturated using a bin-mapping technique, which allows the development of SSR markers from both coding and non-coding regions of the genome, resulting in increased marker density. A total of 257 markers (243 SSRs, 12 gene-specific and two SCAR markers) were eventually mapped to the reference linkage map of diploid strawberry (Sargent et al. 2006). The first linkage map in cultivated strawberry was developed by Lerceteau-Köhler et al. (2003). The map was constructed in a population

of diverse parentage [‘Capitola’ × ‘CF1116’ (‘Pajaro’ × (‘Earliglow’ × ‘Chandler’))] using AFLP markers. Although the map was incomplete, 43 linkage groups in each parent of the cross were successfully identified. One of the most comprehensive octoploid linkage maps to date is that of ‘Redgauntlet’ × ‘Hapil’, containing 549 loci (490 transferrable SSR or gene-specific markers) (Sargent et al. 2012).

Many of reported linkage maps were constructed for particular aims resulting in the use of different parental genotypes, mapping population size, different molecular markers and developing variable genetic map lengths and level of saturation. The linkage maps of diploid and octoploid strawberry are invaluable tools not only for future comparative mapping studies, but also in generating transferable maps within the *Fragaria* genus which will allow the identification and mapping of genes linked to economically important traits.

1.3.3 Marker-assisted selection

During the last decade, the identification of genes linked to important traits in rosaceous crops has been increasing and the methods used for molecular marker development has progressed rapidly, reducing the time and screening costs. To date, 21 different database resources containing genes, whole genome sequences, transcriptome data, large sets of molecular markers, numerous high density genetic linkage maps, and pedigree information for the various species in the Rosaceae are freely available (Jung and Main, 2014). The National Center for Biotechnology Information (NCBI) and the Genome Database for Rosaceae (GDR) are two large and commonly used databases among the Rosaceae community and contain enormous amount of genotypic and phenotypic information (Jung and Main, 2014).

The genotypic and phenotypic information available for rosaceous crops enabled the development of a novel, efficient method for molecular plant breeding known as marker-assisted selection (MAS) or marker-assisted breeding (MAB) (Xu and Crouch, 2008; Whitaker, 2011). As the name suggests, MAS is an indirect pre-selection process where genotypes expressing a trait of interest are selected indirectly based on marker information only (Xu and Crouch, 2008). The use of MAS in plant breeding facilitates the selection of progeny which carry desirable alleles of genes of interest and eliminates undesirable progeny at a very early stage of plant development. This makes MAS a helpful technique, especially when used to screen large number of seedlings, resulting in cost, time and space savings (Collard and Mackill, 2008; Shulaev et al. 2008; Jannink et al. 2010). Currently, MAS is most effective for identification of traits that are linked to a single major controlling gene and it is poorly suited to crop improvement when the trait of interest is controlled by several small effect genes; this latter situation is common in resistance to major strawberry diseases (Heffner et al. 2009). This disadvantage results in the need to identify quantitative trait loci (QTL) and then estimate the size of their effects (Jannink et al. 2010). A further complication is that due to continuous changes in the environment, plant pathogens are able to adapt or/and overcome host-plant resistance. Numerous new species of pathogens and pests arise constantly, so that previously developed molecular markers may not be useful and novel molecular markers need to be continuously developed (Collard and Mackill, 2008). Perhaps for these, amongst other reasons, although the use of marker-assisted selection has made it possible to improve and speed up the selection process in plant breeding programmes, the approach has not been widely used within fruit crop breeders and remains more of an ambition than a practical method.

1.4 Mapping quality traits in strawberry

1.4.1 Multiple models for linking traits of interest in octoploid strawberry

The detection of linkages between markers and genes is a challenging and time-consuming process because most traits such as yield, fruit quality and maturity are likely to be controlled by more than one major gene (Kumar, 1999). Furthermore, environmental changes can affect fruit quality and yield, resulting in modification in gene expression. In this case, a QTL approach can be used for the identification of linkages between markers and a large number of loci (Lerceteau-Köhler et al. 2012). The approach is based on both genotypic and phenotypic data analysis generated over several years or seasons to maximise the final phenotypic expression. The software commonly used for QTL analysis is MapQTL reported by van Ooijen (2004), although other software such as FlexQTL (which uses pedigree-based association) is becoming a more favoured tool in QTL analyses (Bink et al. 2008). These software models are capable of estimating and identifying the positions of QTLs which influence traits of interest on genetic maps (Asins et al. 2009). Once QTLs are mapped, molecular markers can be detected, assigning linkage groups (chromosomal positions) to QTLs of interest.

Interval mapping (IM) is a different technique for the identification of QTL that are controlled by a major single gene (Kao et al. 1999; Li et al. 2007). The method is based on the information provided by a genetic linkage map and the method of a maximum likelihood which estimates genetic parameters (Kao et al. 1999). Interval mapping analyses linkages between markers and estimates the most likely QTLs within these flanking marker groups. The likelihood of QTL being present is expressed as a LOD (a logarithm of the odds) score which varies depending on the recombination frequency between analysed markers (Kumar, 1999).

The success of linking traits using molecular tools depends on a number of factors: size of population, quality of linkage map, the magnitude of the QTL's effect on the trait and the distance between the marker and identified candidate gene (Kumar, 1999). A number of studies have identified major genes controlling fruit quality traits in apple (Patocchi et al. 2009; Bus et al. 2010; Longhi et al. 2012), peach (Dirlewanger et al. 2004; Gillen and Bliss, 2005; Ogundiwin et al. 2009; Eduardo et al. 2011), sour cherry (Wang et al. 2000) raspberry (Sargent et al. 2007) and other rosaceous crops. Markers have been developed for fruit quality traits in *Fragaria x ananassa*, including fruit flavour, size, firmness, shape, metabolites (Lerceteau-Köhler et al. 2012) and the yellow fruit colour (Deng and Davis, 2001).

1.4.2 Next generation sequencing approaches to understand fruit quality traits

Recently, next generation sequencing approaches have been used for a range of purposes including genome sequencing, re-sequencing, sequence assembly and functional annotation, microarray platform development for high-throughput gene expression, and most importantly single nucleotide polymorphism (SNP) detection and the identification of genes linked to economically important traits (Edwards and Batley, 2010). Advances in next generation sequencing have permitted easier and more cost efficient genomic studies not only within model systems such as *Arabidopsis thaliana* but also within plant species of agronomic importance, with larger and more complicated genomes. Today, more than 20 plant species have had their genomes sequenced (Kumar et al. 2012; Jung and Main, 2014). Among these, are the publicly available genome sequences of the economically important crops rice (*Oryza sativa L.*) (International Rice Genome Sequencing Project, 2005), cotton (*Gossypium spp*.) (Wang et al. 2012), papaya (*Carica papaya L.*) (Ming et al. 2008) and banana (*Musa*

acuminate) (Global Musa Genomics Consortium, 2012). In the Rosaceae, the genomes of apple (*Malus* × *domestica* Borkh.) (Velasco et al. 2010), pear (*Pyrus Bretschneideri Rehd*.) (http://peargenome.njau.edu.cn:8004; Wu et al. 2013), peach (*Prunus persica L.*) (http://www.rosaceae.org; Ahmad et al. 2011), and diploid strawberry (*Fragaria vesca*) (Shulaev et al. 2011) have been sequenced. In addition, four other genomes are being sequenced: red raspberry, black raspberry, apricot and plum (Jung and Main, 2014).

To date, several next generation sequencing (NGS) technologies have been developed (reviewed in El-Metwally et al. 2014). Among these, Roche 454, ABI SOLiD and the Illumina MiSeq and HiSeq platforms have been widely used for high-resolution and high-throughput sequencing of complex genomes for a range of plant species (Varshney et al. 2009; Edwards and Batley, 2010; Minoche et al. 2011; Shulaev et al. 2011; Chagné et al. 2012; Verde et al. 2012). These novel approaches are able to sequence plant genomes rapidly resulting in significant reduction in time. The method also enables researchers to screen and genotype thousands of markers within a plant genome within hours. For example, the consensus linkage map of apple rootstock reported by Antanaviciute et al. (2012) containing 2,272 SNPs and 306 SSRs required approximately nine hours of 'hands on' researcher time, whereas the SSR-based apple rootstock linkage map of Fernández-Fernández et al. (2012) containing 324 SSR genetic loci spanned a time-scale of approximately four months.

Genome sequence information is invaluable for a range of further studies. It enables the development of novel markers for saturation of linkage maps and detection of novel genes which could not be discovered using traditional molecular tools. Thus, the identification of novel gene or QTLs linked to a specific phenotype expressing traits of interest become more precise and easier (Nordborg and Weigel, 2008). Moreover, much higher resolution techniques for linkage analysis have been reported, such as

genome-wide association (GWA) mapping, advanced back-cross QTL analysis and functional genomics (Varshney et al. 2009). For example, GWA permits hundreds of thousands of single nucleotide polymorphisms (SNPs) to be screened within a selected plant genome which can be used for association analysis between phenotypes of interest and DNA sequence variants of genotypes (Nordborg and Weigel, 2008).

Next generation sequencing (NGS) methods enable novel marker development, not only in major crops for which genome sequence data already exist, but also for less characterized species for which genome resources are limited (Varshney et al. 2009). For example, the transcriptome of the butterfly (*Melitaea cinxia*) (with no previous genomic data available) was successfully characterised using the 454 pyrosequencing method (Vera et al. 2008). This demonstrates that NGS has had a profound impact on molecular and genetic analysis of model and non-model species and it can assist in numerous studies in crop improvement programmes. However, the large amount of data generated by high throughput sequencing/genotyping still remains a challenge for satisfactory analysis.

1.5 Pathogens infecting cultivated strawberry

1.5.1 Diseases caused by different types of pathogens

Strawberry breeders and growers around the world face plant breeding and strawberry production challenges against pest and serious plant diseases that can affect the plant root system, crown, leaves, flowers and fruits resulting in damage to the host, significant reduction of the yield, and poor fruit quality. Among these, some diseases cause serious plant damage and are lethal in strawberry cultivation, whereas others have a minor affect to the plant (Maas, 1998; Duncan, 2000). Thus, the development of

disease resistant strawberry cultivars is an alternative approach for control of strawberry diseases and is a major goal for strawberry breeders and growers.

The main pathogens affecting strawberry plants can be grouped into five groups: insects, bacterial diseases, viruses, nematodes and fungal diseases (Figure 1.4; Hancock, 1999).

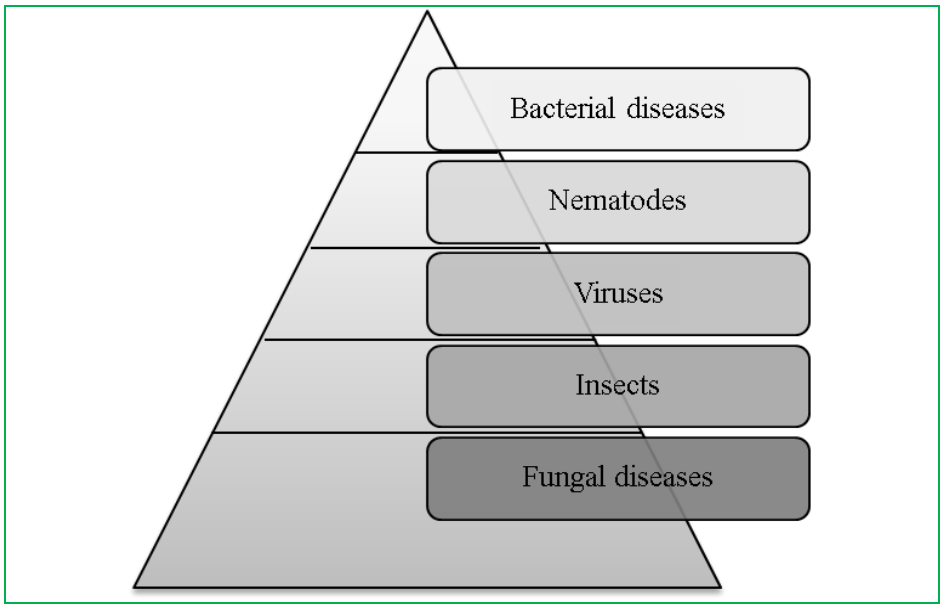


Figure 1.4 Pathogen groups by size affecting strawberry plants.

Insects are the largest pest group of strawberry that has not only highly localized but also widespread distribution (Hancock, 1999). It has been reported that around 200 different insect species attack strawberry plants either directly or indirectly (www.virginiafruit.ento.vt.edu). Some of the most common are aphids, spider mites and plant bugs that cause extreme damage (deformation) to strawberry fruit (Hancock, 1999). Other insects such as strawberry bud weevil, flower thrips, strawberry rootworm, slugs, strawberry crown moth and sap beetles damage not only the plant but also flowers and unripe and ripe fruits (Hancock, 1999).

Angular leaf spot, caused by *Xanthomonas fragariae* Kennedy & King is the only known bacterial disease in strawberry (Peres et al. 2004). Usually, it is common during the wet season and causes water-soaked lesions on the lower leaf surfaces (Hancock, 1999; Peres et al. 2004). These lesions develop into larger spots as the disease spreads and becomes necrotic (Peres et al. 2004). Bacterial diseases are difficult to control due to the ability to overwinter in dead, dry or buried in the soil and their ability to spread easily through infected plants (Pooler et al. 1996).

Strawberry diseases caused by viruses are difficult to identify because the symptoms may be confused with those of other pathogens; viruses are also small and invisible using light microscopy (Moyer et al. 2010). Although more than 30 viruses have been reported to infect strawberry plants worldwide, viral infections are not of economic importance (Martin and Tzanetakis, 2006; Moyer et al. 2010). Usually, plant seedlings expressing viruses are eliminated at very early stages of propagation. The most common virus diseases in strawberry include strawberry mottle, crinkle, mild yellow-edge and latent ring spot viruses (Hancock, 1999).

Nematodes mainly affect strawberry plants indirectly, however direct damage to strawberries is common (Hancock, 1999). The main damage caused by nematodes is that they feed directly on plant roots making roots more susceptible to other root diseases. Other symptoms may appear including low plant vigour, plants showing water stress, fruit and leaf deformation and even plant death (Brown et al. 1993; Hancock, 1999).

The largest group of pathogens in strawberry are the fungi (Figure 1.4). According to Garrido et al. (2011), more than 50 different genera of fungi can affect cultivated strawberry plants. Fungal diseases affect the whole plant from roots to fruits and cause significant economic losses in crop production (Santos et al. 2004). Fungal

diseases can be grouped into those caused by soil-borne pathogens and those caused by aerial pathogens (Santos et al. 2004). These pathogens can be grouped further depending on which part of the strawberry plant they affect: root, crown, leaf and fruit fungal diseases (Hancock, 1999). The most common fungal diseases in strawberry are leaf diseases. Leaf blight, leaf spot, leaf scorch and powdery mildew are common diseases that are caused by aerial pathogens and can be easily noticed by eye (Sikora, 2004). Among these, the most widespread leaf disease of strawberries is leaf spot caused by the fungus *Mycosphaerella fragariae*. The pathogen affects not only plant leaves but also fruits, runners and berry calyces (Hancock, 1999). Powdery mildew is another common disease caused by the fungus *Podosphaera aphanis*. The fungus affects plant leaves which become curly and dry with patches of powdery fungus mycelium usually on the upper surface of the leaves (Hancock, 1999). The disease causes losses in yield by producing leaf damage, necrosis and defoliation. If disease is severe, white mycelium can be found on the fruits, making them unmarketable (Santos et al. 2004).

The second most common and serious fungal diseases in strawberry are fruit diseases. These diseases can cause a direct loss of the harvested product. Strawberry anthracnose is caused by a group of *Colletotrichum* species (*Colletotrichum fragariae* Brooks, *Colletotrichum acutatum, Colletotrichum gloeosporioides* and *Colletotrichum dematium*). It is very common and affects not only fruits but also almost all parts of the plant (Damm et al. 2012). *Colletotrichum acutatum* species is predominant causing fruit rot, black spots of fruits, death of blossom clusters, brown cap conditions, lesions on stolon and crown discoloration (Damm et al. 2012). In addition, grey mould and leather rot, caused by *Botrytis cinerea* and *Phytophthora cactorum* respectively, are other common strawberry fruit rot diseases (Santos et al. 2004). The pathogen for grey mould

disease is airborne and can cause crown rot and blossom infections that spreads into infections of the fruits. In contrast, *Phytophthora cactorum* is a soil-borne pathogen that causes leather rot on fruits, crown rot and wilt (Santos et al. 2004).

Root and crown diseases caused by fungal pathogens are mainly soil-borne primary attacking plant root system and later spreading infection to plant crown (Santos et al. 2004). Among these, red stele and *Verticillium* wilt diseases caused by the oomycete *Phytophthora fragariae* and the fungus *Verticillium dahliae* respectively are major diseases of economic importance throughout the world (Santos et al. 2004). Infected susceptible varieties usually die and most importantly, pathogens can survive for many years even in the absence of strawberries.

1.5.2 Difficulties in breeding disease resistance

Disease resistance is the most effective technique for controlling disease (Shaw et al. 1997; Amil-Ruiz et al. 2011). However, breeding cultivars that are disease resistant is challenging, because strawberry resistance to the pathogens is mostly polygenic and quantitatively inherited (Shaw et al. 1996, 1997; Lewers et al. 2003; Zorrilla-Fontanesi et al. 2011b). As a result, the identification of molecular markers linked to resistance genes is very difficult. In addition, the octoploid genome of the cultivated strawberry makes the process even more challenging. However, the high level of transferability of molecular markers between the different ploidy level strawberry species (Davis et al. 2006; Gil-Ariza et al. 2006; Sargent et al. 2009) and the co-linearity between the diploid and the octoploid strawberry genomes (Rousseau-Gueutin et al. 2008, 2009), has permitted the successful identification of some resistance genes. The best known major genes involved in disease resistance including red stele (red core) root rot caused by soil-borne fungus *Phytophthora fragariae* (van de Weg, 1997) and resistance to

anthracnose caused by *Colletotrichum acutatum* (Deoyes-Rotham et al. 2004; Lerceteau- Köhler et al. 2005) have been reported.

Breeding strawberry cultivars that are pest and disease resistant is a long, time consuming and costly process as was previously discussed in Section 1.2. In addition, the focus on disease resistance traits in strawberry breeding potentially resulted in new strawberry varieties losing other economically important traits such as flavour and nutritional qualities (Amaya, 2012). The availability of a narrow strawberry germplasm (e.g. the study by Gil-Ariza et al. 2009, which showed that modern strawberry cultivars with Californian pedigree share 73% genetic similarity), and the high tendency to inbreeding (Shaw et al. 1997; Kaczmarska et al. 2014), also contributed to the deleterious effects of breeding and genetic vulnerability to pests and disease (Section 1.2). Therefore, a combination of chemical, biological and cultural methods are essential to fight pests and diseases caused by different type of pathogens in strawberry (Pinkerton et al. 2002; Martin and Bull, 2002; Amil-Ruiz et al. 2011).

Some diseases can be avoided by employing pesticide and fungicide spray programmes, and regular soil fumigation (Santos et al. 2004; Molay, 2005; Adaskaveg et al. 2013). However, because regular applications of pesticides are harmful to the environment and human health, their indiscriminate use is not considered appropriate or possible (Fernandes et al. 2011). Moreover, these applications can provide only temporary disease and pathogen control and are not long lasting protection solutions. For example, strawberry cultivars have a wide phenotypic diversity of varying susceptibility to diseases; the variety of soil type and location of production fields makes pathogen control even more challenging (Amil-Ruiz et al. 2011). One of the best ways to control diseases is to plant disease-free and resistant varieties that maintain essential components for natural plant defence responses against pests and diseases.

1.5.3 Defence mechanisms against infections in strawberry

Pathogens such as bacteria, fungi, viruses and nematodes have specific techniques to infect, multiply and successfully exist in their hosts (Maas, 1998). For example, bacteria usually infect their host through wounds on leaves, stems, roots, stomata, and water pores (hydathodes) (Vidaver and Lambrecht, 2004; Jones and Dangl, 2006; Melotto et al. 2006), whereas fungi can directly enter the plant through the epidermal cells (Jones and Dangl, 2006). Nematodes and aphids, similar to fungi, attack their host directly by inserting the stylet into plant cells (Jones and Dangl, 2006). In contrast, viruses can successfully attack and survive in their host only if they have a biological vector such as nematode or insect and are transferred directly to plant tissues (Martin and Tzanetakis, 2006).

Strawberry species exhibit vast phenotypic diversity, and thus the susceptibility level varies towards different pathogens (Shaw et al. 1996; Maas, 1998). It has been reported that the resistance level to diseases is greater among the wild strawberry species (Gooding et al. 1981; Maas, 1998), although some octoploid (*Fragaria* × *ananassa*) cultivars also have natural defence mechanisms against the majority of pests and diseases described in Section 1.5.1 (Nelson et al. 1996; Mori et al. 2005; Particka and Hancock, 2005).

Similar to animals, the plant immune system is able to recognize pathogens and use defence mechanisms to prevent pathogen infections and pest attacks (Jones and Dangl, 2006; Amil-Ruiz et al. 2011). However, plant defence mechanisms differ from that of animals, because plants do not have mobile defender cells and a somatic adaptive immune system and rely on the innate immunity of each cell and signals within infection sites (Jones and Dangl, 2006, Amil-Ruiz et al. 2011). There are several defence mechanisms against pests and diseases in strawberry plants, listed in Figure 1.5.

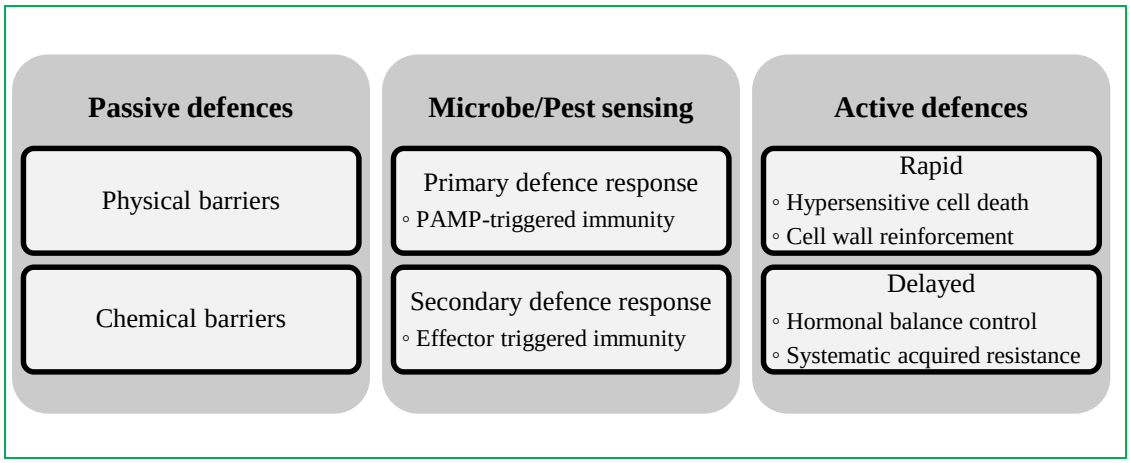


Figure 1.5 Strawberry plant defence mechanisms against pests and diseases (reviewed in Amil-Ruiz et al. 2011). Adapted with minor changes from Amil-Ruiz et al. 2011.

The passive defence mechanism is based on physical (cell wall and cuticle) and chemical (toxic chemicals, antifungal proteins and enzymatic inhibitors) barriers which can naturally prevent pathogen and pest attacks (Nüernberger and Lipka, 2005; Amil-Ruiz et al. 2011). According to Nüernberger and Lipka (2005), plants also have an inducible defence system and are able to respond to pathogens which pass the physical and chemical barriers. This usually leads to the activation of the primary defence response (PTI), known as PAMP-triggered immunity (Pathogen-Associated Molecular Pattern). The plant recognition receptors (PRRs) enable early and rapid recognition of pathogen molecular patterns, which triggers primary defence responses (Jones and Dangl, 2006). Thus, the plant cell wall becomes more protected by inducing molecular complexes including suberin, lignin and proteins, and pathogens cannot invade the plant (Amil-Ruiz et al. 2011). However, in some cases, pathogens are able to suppress primary defence responses in plants by expressing effector proteins (Jones and Dangl, 2006). If that happens, plant disease resistance proteins (R) then become active providing a second layer of defence (secondary defence response), which are able to suppress pathogen effectors (Amil-Ruiz et al. 2011). The process is known as effector-

triggered immunity (ETI), usually resulting in a hypersensitive response (HR). It is clear that primary and secondary defence responses are controlled by a complex system including receptors, signalling pathways and cell changes which are coordinated by specific genes (Jones and Dangl, 2006; Amil-Ruiz et al. 2011).

Correlations have been reported between defence mechanisms against pathogens and fruit quality traits. According to Miles and Schilder (2013), as the fruits start to ripen their susceptibility and vulnerability level to pests and diseases increase. For example, in strawberry, fruit firmness relies on the structure of the cell wall and has a clear relationship with skin strength and susceptibility to pathogen infections (Barritt, 1980; Table 1, reviewed by Amil-Ruiz et al. 2011). This means that firm fruits are less susceptible to the diseases due to the different composition and structure of the cell wall. Indeed, during fruit ripening a number of physiological changes occur including cell wall expansion, fruit softening, changes in pH and increase in soluble sugars (Miles and Schilder, 2013). As the fruit cell wall degrades, pathogens can release cell wall degrading enzymes to speed up the process and to overcome plant defence mechanism. This has been reported in cultivated strawberry, where fruit ripening and cell wall changes resulted in higher susceptibility to *Colletotrichum acutatum* (Guidarelli et al. 2011). In addition, strawberry cultivars differ in fruit ripening period and softening range, thus the susceptibility to pathogens also varies (Chandler et al. 2004).

1.6 Genomic selection in plant breeding

Recent advantages in next generation sequencing approaches have resulted in the availability of genome sequence information and the ability to perform high throughput genotyping studies. These resources are transforming methods for genetic improvement

(Kumar et al. 2012). Genomic selection (GS) is a technique widely used for genetic improvement in animal and some arable plant breeding programmes (Meuwissen et al. 2001; Calus, 2010; Jannink et al. 2010). The detection of QTLs controlled by many genes with a small effect is challenging when applying the traditional molecular biology and biotechnology methods. To overcome these barriers GS can be used. In contrast to QTL analysis, which is based on identification of individual loci associated with a trait, GS analyses phenotypes by estimating all locus and marker data across the whole genome at once within the population, and calculates genomic estimated breeding values, hence predicting superior breeding individuals (Meuwissen et al. 2001).

In order for GS to be successful, high quality phenotypic and genotypic data must be collected from a population of individuals known as the training population (Heffner et al. 2009). The data are then used to estimate model parameters and using statistical models the estimated breeding values are calculated of selected candidate genotypes (Heffner et al. 2009). These breeding values are used for selection of the best genotypes for further use in the breeding process. A schematic illustration representing relationships between GS, phenotype and genotype in a plant breeding program is shown in Figure 1.6.

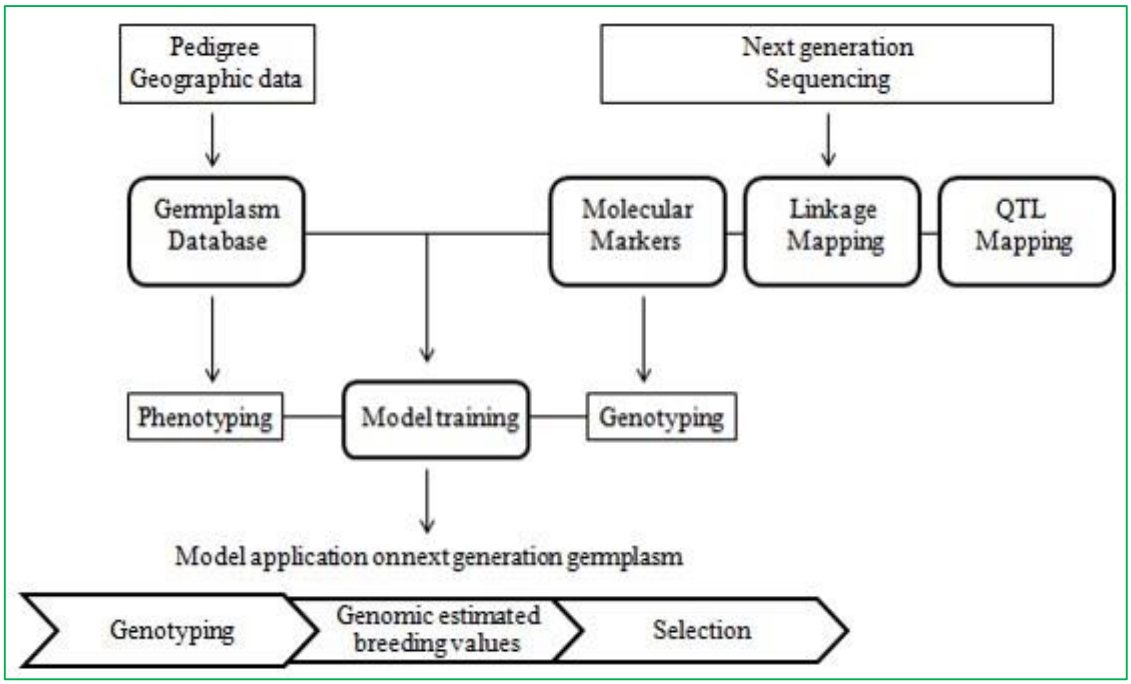


Figure 1.6 Steps involved in the use of genomic selection, in which high throughput genotypic and phenotypic data are combined. Adapted with minor changes from Cabrera-Bosquet et al. (2012).

Genomic selection methods are capable of analysing a large amount of data resulting in the identification of a large number of minor QTLs. For example, in human research, 40 significant markers were linked to the variability in height representing 5% of the total number of participants, whereas using computer based models (GS) more than 300,000 markers were screened simultaneously resulting in a total of 45% variability within the same patients (Yang et al. 2010).

Although GS was first proposed more than 10 years ago and is widely used in human and animal research (Calus et al. 2008; Legarra et al. 2008; Yang et al. 2010), not many studies have been reported using GS in plants (Meuwissen et al. 2001). The use of GS has been reported in maize (Bernardo and Yu, 2007; Lorenzana and Bernardo, 2009; Albrecht et al. 2011), barley (Lorenzana and Bernardo, 2009) and *Arabidopsis thaliana* (Lorenzana and Bernardo, 2009) and screening for fruit quality

traits of apple (Kumar et al. 2012). In the study of Kumar et al. (2012), 1120 apple seedlings were used to evaluate the accuracy of genomic selection. All seedlings were genotyped using next generation sequencing and phenotyped for a range of quality traits. The study demonstrated that through GS, molecular markers that are most likely to be linked to the trait of interest can be detected and that the approach is an invaluable tool for plant breeding programmes. Similar conclusions were reported for other GS studies in plants, indicating that GS is superior to marker-assisted selection (Bernardo and Yu, 2007).

It has been predicted that molecular marker technology will change plant breeding practices through marker-assisted selection, however most current MAS approaches failed to significantly improve successful pre-selection process (Babu et al. 2004; Xu and Crouch, 2008; Ragimekula et al. 2013). Therefore, genomic selection may fulfil these predictions by analysing genome wide marker coverage combined with phenotypic data to estimate breeding values, accelerate the breeding cycle and introduce novel approaches for linking phenotype, genotype and seedling selection.

1.7 The aims of this investigation

The overall aims of this PhD project were to: 1) describe correlations between plant characteristics and fruit quality traits in cultivated strawberry (*Fragaria* × *ananassa*); 2) saturate an SSR-based genetic linkage and develop a novel SNP-based linkage map for the octoploid strawberry mapping population 'Redgauntlet' × 'Hapil'; 3) identify QTLs linked to plant characteristics-related traits, fruit quality-related traits and powdery mildew resistance; 4) validate SSR markers associated with fruit firmness

QTLs; 5) investigate relationships between plant characteristics, fruit quality and disease resistance QTLs.

An octoploid strawberry mapping population segregating for a number of plant characteristics, fruit quality and disease resistance traits was used for phenotypic data collection across three consecutive years (2013, 2014 and 2015). Correlation analysis was performed on a total of 30 different traits in order to better understand how complex traits are correlated in octoploid strawberry (Chapter 2).

A set of previously published SSR markers was tested using fluorescent PCR and electrophoresis on an ABI 3130× genetic analyser, firstly on the parental genotypes of selected mapping populations; secondly, markers that show polymorphism were scored in whole progenies. The purpose of this was to improve the quality of the existing SSR-based genetic linkage map (Chapter 3). In addition, recently available, IStraw90® 90K SNP genotyping array was used for high density SNP-based linkage map construction for the 'Redgauntlet' × 'Hapil' mapping population (Chapter 3).

Phenotypic data (Chapter 2) and genotypic data (Chapter 3) were combined for quantitative trait loci (QTL) detection (Chapter 4). SSR markers closely linked to firmness QTLs were validated in the wider strawberry germplasm as an example study. Furthermore, the physical locations between SNPs linked to fruit firmness and expansin genes (genes linked to fruit softening) were compared, in order to know if markers linked to firmness QTLs overlapped exapansin gene locations (Chapter 5).

Finally, QTLs associated with powdery mildew (*Podosphaera aphanis*) resistance were identified for the first time in cultivated strawberry using the existing phenotypic data for 3-years and genotypic data generated in Chapter 3 (Chapter 6). In addition, the relationship between plant characteristics, fruit quality, powdery mildew and wilt (*Verticillium dahliae*) disease resistance QTLs was investigated (Chapter 6).

Chapter 2

The investigation of correlations between morphological and fruit quality traits using phenotypic and genotypic data in *Fragaria* × *ananassa*

2.1 Introduction

The cultivated strawberry (*Fragaria* × *ananassa*), similarly to other rosaceous crops, such as apple, pear, cherry and raspberry, has a long history of artificial selection for improved cultivars (Hummer et al. 2011). Breeding for fruit quality traits such as yield, fruit size and recently flavour as well as resistance to diseases and pests are the primary aims for strawberry breeders (Capocasa et al. 2008; Klee, 2010; Zorrilla-Fontanesi et al. 2011b; Lerceteau-Köhler et al. 2012; Emdad et al. 2013). However, the majority of fruit quality traits in cultivated strawberry are quantitatively expressed and complex due to it being an octoploid species and the fact that traits are controlled by many genes at different locations on different chromosomes (Zorrilla-Fontanesi et al. 2011b). Moreover, fruit quality traits can be highly influenced by a number of other factors such as environmental, genotype by environment interactions, genetic drift, epistasis and pleiotropy, making strawberry breeding even more challenging (Wang and Summers, 2010; Fellahi et al. 2013). In addition, due to the limited strawberry germplasm available and to the fact that cultivated strawberry is highly heterozygous, and thus sensitive to inbreeding, the correct parent selection for the cross is a crucial first step in a successful breeding program (Gil-Ariza et al. 2009).

Traditional plant breeding is usually based on cross pollination of selected parental genotypes which are selected on the phenotypic information available for traits of interest. In order to avoid some of the breeding challenges listed above, pedigree information of the parents of the cross is essential because genetically distant parental genotypes must be selected to be used in breeding programmes (Ukalska et al. 2006). The selection of diverse parents also increases genetic and phenotypic diversity (Ukalska et al. 2006; Gil-Ariza et al. 2009). In addition to the right parent selection in a

breeding program, understanding of the association between phenotype and genotype and knowledge of how different traits correlate and which traits have direct or indirect effect is important when determining the selection.

The relationship between different traits can be determined by correlation analysis using statistical models. The estimation of correlation coefficients (index between -1.0 and 1.0) between traits of interest, indicates which two variables are linearly related and if traits are affected by genes and/or environmental conditions (Shaw and Larson, 2005; Ukalska et al. 2006; Ezeaku et al. 2015). Correlation analysis between different traits is important due to the possibility of predicting breeding gains between the traits. For example, one study showed that number of flowers per plant had a positive direct effect on strawberry yield per plant, whereas number of fruits per plant had a negative effect on yield (Emdad et al. 2013). Similarly, according to Ukalska et al. (2006), a positive correlation existed between skin color and flesh color, whereas a negative correlation was observed between skin color and fruit glossiness. In order to study correlations between different phenotypic values, high quality phenotypic data must be collected, while a lack of reliable phenotypic data can prevent the association study and lead to lack of understanding of the genetic basis of biological processes (Bassil and Volk, 2010).

The heritability coefficient estimates the proportion of phenotypic variation that is due to genetic and genetic-environmental factors (Wray and Visscher, 2008). High heritability coefficients suggest high similarities between parental genotypes and seedlings for a particular trait, while low heritability suggests low resemblance between individuals (Shaw and Larson, 2005; Wray and Visscher, 2008). Thus, phenotypic variation in the population can be affected not only by measurement errors or environmental effects but also by heritability (Bloom et al. 2013).

In strawberry, some studies have been conducted reporting correlation and/or heritability analysis in order to better understand the genetic basis controlling fruit quality and plant developmental traits (Ukalska et al. 2006; Capocasa et al. 2008; Zorrilla-Fontanesi et al. 2011b; Lerceteau-Köhler et al. 2012; Emdad et al. 2013). However, the majority of these studies were conducted using a relatively small number of genotypes, ranging from six (Emdad et al. 2013) to 117 (Ukalska et al. 2006). Other studies have been conducted using strawberry mapping populations containing four replicates of 95 (Zorrilla-Fontanesi et al. 2011b), and one replicate of 193 individuals (Lerceteau-Köhler et al. 2012). Here, a correlation and heritability analysis is described. This was conducted on a large scale using more replicates of individuals of a strawberry mapping population. Furthermore, more plant characteristics and fruit quality traits were evaluated than in previous studies.

The objective of this study was to investigate how different complex plant characteristics and fruit quality traits correlate in octoploid strawberry (*Fragaria* × *ananassa*), to determine which traits are easiest to measure in an open field experimental plot, and to assess if there are any potential environmental correlations among traits. In addition, broad-sense heritability coefficients (H^2) defined here were estimated to determine which traits are under a strong parental-seedling interaction. Six replicates of an octoploid strawberry mapping population (‘Redgauntlet’ × ‘Hapil’) containing a total of 120 seedlings and parental genotypes was used for phenotyping plant characteristics and key fruit quality traits for three consecutive years (2013-2015). The phenotypic values were used to calculate trait means and estimate correlation and heritability coefficients for each trait for each year. This information identified the most important traits and may provide significant cost and time savings in future breeding.

Furthermore, phenotypic data combined with the genotypic data can be used for identification of quantitative trait loci (QTL) linked to traits (Chapter 4).

2.2 Materials and methods

2.2.1 Plant material

The F_1 mapping population used in this study was raised in the glasshouse from a cross between the two octoploid strawberry (*Fragaria* × *ananassa*) cultivars 'Redgauntlet' and 'Hapil'. A total of 188 seedlings were raised from the cross and of those 120 seedlings randomly were selected and further clonally propagated twice (once during summer 2012 and once during summer 2014) by pinning down the runners of the mother plants. A total of six replicates of the 122 seedlings and parental genotypes ('Redgauntlet' and 'Hapil') were produced, thus 732 plants (including parents) were planted in the open field at East Malling Research in late September 2012 and mid-August 2014. Seedlings were randomly distributed within three tunnels, where each tunnel had three beds and two rows per bed (Figure 2.1). Seedlings were planted in a double row in zig-zag (40 cm between plants) on raised beds, 35 cm high and 50 cm wide.

Plants in the field trial were allowed to grow and establish naturally over winter. All runners and dead material was removed in spring for ease of phenotyping. The field trial plots were covered with polyethylene while plant phenotyping was on-going; this was later (late July) removed in order to avoid disease (Figure 2.1). An irrigation system was installed in each row, and plants were watered and fertilized following conventional practices and depending on weather conditions. Plants were sprayed against common pests (aphid), insects (spotted wing drosophila) and diseases (mildew and botrytis)

before, during and after the phenotyping season. The spraying programme for the season was as follows: once a week for 23 weeks for mildew (March - September), once a week for ten weeks for Botrytis (May - September), a single spray for spotted wing drosophila (in August) and five sprays for aphid (March - June).

Figure 2.1 Seedlings of the 'Redgauntlet' × 'Hapil' mapping population and parental genotypes planted in the field at East Malling Research; a) seedlings without cover before phenotyping, photograph was taken on 17.05.2013; b) seedlings under cover while collecting phenotypic data, photograph was taken on 25.06.2013; c-e) seedlings in each tunnel, photographs were taken on 12.08.2013.

2.2.2 Phenotypic data collection – plant characteristics

A total of thirteen plant characteristic traits were measured in the field in this study. Of those, 11 traits were recorded for three consecutive years (2013, 2014, 2015), whereas the remaining two traits (plant height and width) were recorded for one year (2013). The list of traits measured is presented in Table 2.1.

Table 2.1 The list of a total 30 physical traits evaluated within 'Redgauntlet' × 'Hapil' mapping population. Traits recorded in 2013, 2014 and 2015 are highlighted in black. Traits recorded in 2013 are highlighted in blue. Trait recorded in 2013 and 2015 is highlighted in green and traits recorded in 2014 and 2015 are highlighted in red

Traits			
Plant characteristics-related traits	**Units**	**Fruit quality-related traits**	**Units**
Flower number		Yield	g
Flower diameter	mm	Unmarketable fruit	g
Petal number		Marketable fruit	g
Pedicel length	mm	Firmness	g/mm
Leaflet number		Soluble solids content	Brix^{o}
Vigour	scale (1-5)	pH	
Height	cm	Achene position	scale (1-3)
Width	cm	Seediness	scale (1-3)
Runner number		Skin brightness	
Runner length	cm	Cap size	scale (1-3)
Truss number		Shape	scale (1-5)
Truss length	mm	Outline	scale (1-5)
Truss width	mm	Redness	scale (1-5)
		Glossiness	scale (1-5)
		Neck line	scale (1-5)
		Skin strength	scale (1-5)
		Internal fruit colour	scale (1-5)

The collection of flowering-related data began when at least one flower was opened per plant and data were recorded three times a week (Monday, Wednesday and Friday) for 9, 8 and 7 weeks in 2013, 2014 and 2015 respectively. The total number of flowers per plant was calculated by adding together the number of flowers counted throughout the recording period for each year. When at least 50% of flowers per plant were opened, the flower diameter of three randomly selected flowers was noted using manual fruit-sizing rings ranging from 20 mm to 45 mm (Figure 2.2, d). In addition, the same three flowers were used to record the number of petals per flower (Figure 2.2, g-i).

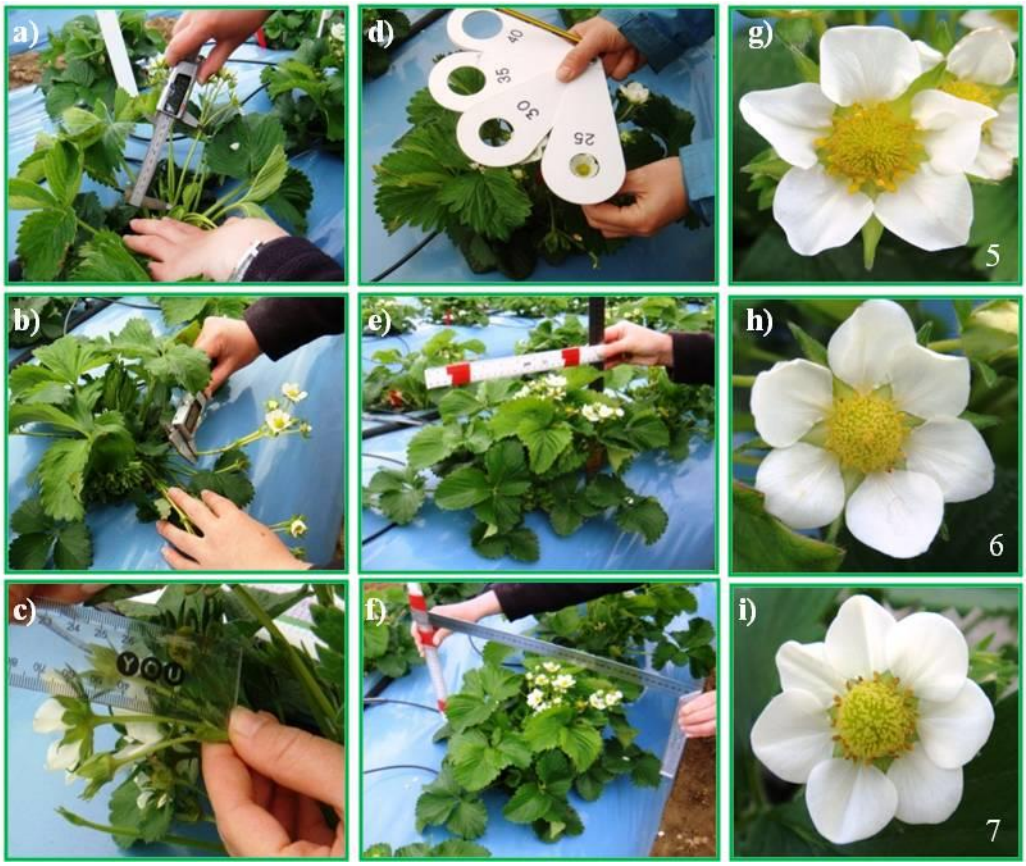


Figure 2.2 Measuring plant characteristics-related traits using different instruments of the 'Redgauntlet' × 'Hapil' mapping population; a) measuring truss length using digital calliper; b) measuring truss width using digital calliper; c) measuring pedicel length using ruler or digital calliper; d) measuring flower diameter using fruit-sizing rings; e) measuring plant height using ruler; f) measuring plant width using ruler; g-i) counting petals per flower. Photographs were taken between May – July 2013.

The length of pedicel was measured by randomly selecting the longest pedicel per plant using ruler or digital calliper (Figure 2.2, c). The length and width of a randomly selected truss was recorded using digital callipers. One truss per plant was measured in 2013 and 2014, whereas three randomly selected trusses per plant were measured in 2015 when at least 50% of flowers per plant were opened (Figure 2.2, a-b). The total number of trusses was also noted. The number of leaflets per plant was

counted whiles noting if each leaf had three leaflets (as expected in strawberry) or had more than three leaflets per leaf. Plant vigour was determined using a scale of 1 – 5, where 1 meant plant being weak and 5 meant plant being very vigorous, and was recorded once a week for 5, 6 and 5 weeks in 2013, 2014 and 2015 respectively.

Plant height was measured using a 50 cm ruler at least once a week for a total of 5 weeks. Using the same ruler, plant width was measured three times from different angles; horizontally, vertically and diagonally in order to capture the distribution of leaves and shape of the plant (Figure 2.2, e-f). The trait was recorded for 5 weeks during the same period as plant vigour and height in 2013 only. The number of runners per plant was counted once each plant had at least five ripe fruits. The length of the three longest runners per plant was also recorded.

2.2.3 Phenotypic data collection – fruit quality

A total of 17 fruit quality-related traits were recorded within the 'Redgauntlet' × 'Hapil' population (Table 2.1). Of those, eight traits were evaluated for three consecutive years (2013, 2014 and 2015), whereas the remaining nine traits were evaluated for two years (2013 and 2015 or 2014 and 2015). Fruit-related phenotypic data for 2013 were collected by A. Llorente. Fruit quality traits were measured after harvesting ripe fruits. Strawberries were harvested by picking all ripe fruits twice in 2013, whereas ripe strawberries were picked twice a week for 6 and 5 weeks in 2014 and 2015 respectively to minimize environmental effects.

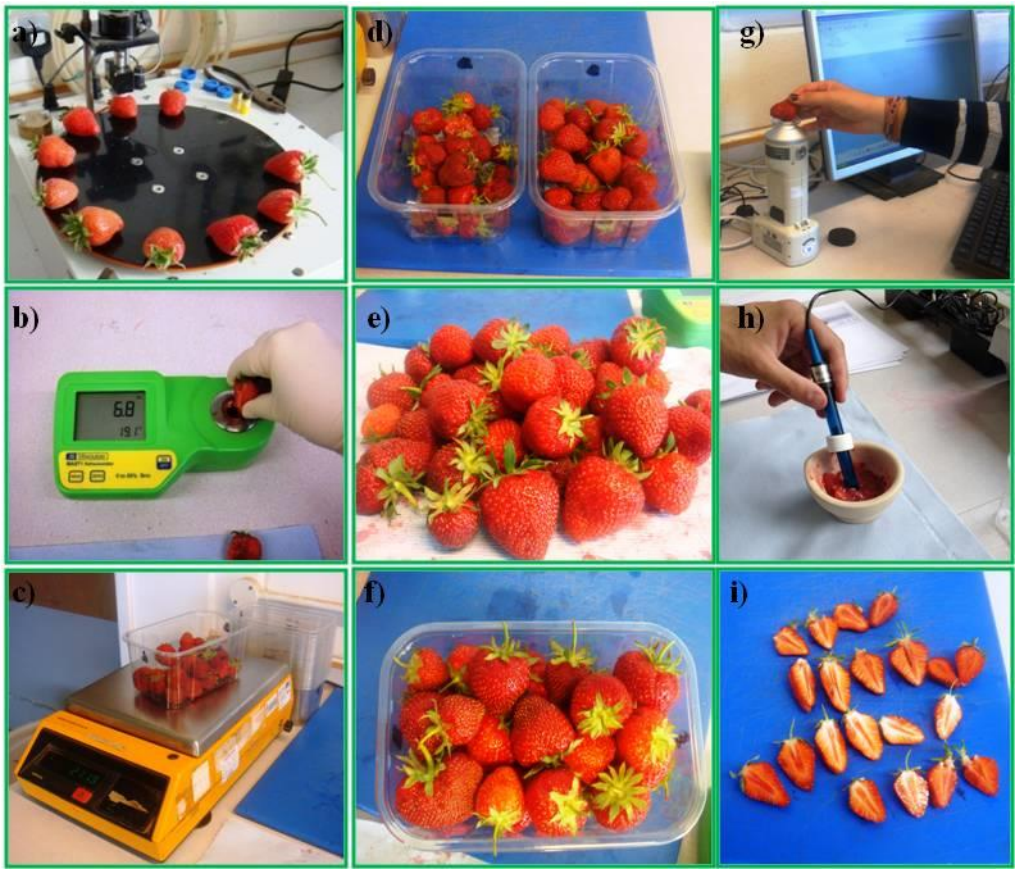


Figure 2.3 Recording strawberry fruit quality-related traits using different instruments of the 'Redgauntlet' × 'Hapil' mapping population; a) measuring fruit firmness (g/mm); b) measuring sugar content (ºBrix); c) weighing marketable fruits; d) unmarketable and marketable fruits before weighing; e) fruits during the assessment; f) marketable fruits before the assessment; g) measuring skin color of the fruit; h) measuring pH level of the fruit; i) the assessment of the internal fruit color of the strawberry fruit.

Marketable fruits (1st and 2nd class, undamaged fruits) and unmarketable fruits (damaged fruits) were picked separately and weighed individually for each seedling and parental genotype for six replicates (Figure 2.3, c, d). Yield was calculated by adding all weights of harvested marketable and unmarketable fruits for each seedling for each year. Fruit firmness was measured on ten marketable fruits (if available) per seedling using Firmtech (Umweltanalytische Produkte GmbH) in g/mm (the values observed

were the force which is required to deflect the fruit by 1 mm) (Figure 2.3, a). Soluble solids content (ºBrix) was measured in ten marketable fruits (if available) per seedling with a digital refractometer (MA871, Milwaukee instruments) by squeezing a few drops of strawberry fruit juice onto the lens (Figure 2.3, b). The pH of each seedling was measured for each year with pH meter by mashing 2-3 strawberry fruits per individual (Figure 2.3, h). External fruit skin brightness was determined for three marketable fruits per seedling using a chromameter (CR-400, Konica Minolta) (Figure 2.3, g).

The achene position, fruit seediness and cap size (scored as greater than, less than or equal to the width of the fruit) of the fruits were visually rated on marketable fruits on a scale of 1-3 (Table 2.2). The same fruits were further used to visually rate fruit shape, outline, redness, glossiness and neck line (scored as raised, even or sunken relative to the shoulder of the fruit) on a scale of 1-5 (Table 2.2). The skin strength was evaluated by gently rubbing ten marketable fruits (if available) with the thumb ten times and rating on the scale of 1-5 (Table 2.2). Ten randomly selected marketable fruits were cut into halves to record internal fruit colour on a scale of 1-5 (Table 2.2, Figure 2.3, i). Fruit quality traits were rated according to the previously reported Strawberry Phenotyping Protocol developed by RosBREED (www.rosbreed.org).

Table 2.2 Strawberry phenotypic scale rates adapted from RosBREED strawberry phenotyping protocol

Phenotyping scale		
Achene position	**Outline (Appearance)**	**Neck line**
1 Sunken	1 Very irregular	1 Sunken
2 Even	2 Irregular	2 Between sunken and flat
3 Protruding (sticking out)	3 Medium	3 Flat
Seediness	4 Even	4 Between flat and raised
1 Few	5 Very even	5 Raised
2 Medium	**Redness**	**Internal fruit colour**
3 Many	1 White	1 White
Cap size	2 Fairly pale	2 Fairly pale
1 Smaller than fruit width	3 Medium	3 Medium
2 The same size as fruit width	4 Dark	4 Dark
3 Larger than the fruit width	5 Very dark	5 Very dark
Shape	**Glossiness**	**Skin strength**
1 Round	1 Very dull	1 Weak[1]
2 Roundish	2 Dull	2 Between weak and moderate[2]
3 Blunt conical	3 Medium	3 Moderate[3]
4 Conical	4 Bright / shiny	4 Between moderate and strong[4]
5 Long conical	5 Very bright / very shiny	5 Strong[5]

[1]Skin broke for 8-10 fruits out of 10 fruits tested

[2]Skin broke for 6-7 fruits out of 10 fruits tested

[3]Skin broke for 3-5 fruits out of 10 fruits tested

[4]Skin broke for 1-2 fruits out of 10 fruits tested

[5]Skin was not damaged for all 10 fruits tested

2.2.4 Weather data

Weather data for the duration of phenotypic data collection (April – July) for three consecutive years (2013, 2014 and 2015) was obtained from a weather station located at East Malling Research, Kent. Five different weather attributes were selected: data for air temperature (°C), air temperature at the grass level (°C) and daily sunshine (hours) were available for three years (2013, 2014 and 2015), whereas data for wind speed (km/h) and relative humidity (%) were available for two years (2013 and 2014).

2.2.5 Statistical data analysis

Different functions and models of the R statistical package (R Development Core Team (2011), Austria) and GenStat software (Payne et al. 2011) were used to analyze the phenotypic data. The phenotypic mean values were calculated across six replicates for parental genotypes and seedlings for each trait and year in R using the 'aggregate ()' function. The standard deviation (SD), standard error (SE) and phenotypic range were calculated and were used for the comparative study of trait expression between parental genotypes and individuals within the population. In addition, Student's *t*-test was applied to estimate pairwise significance level of the means between parental genotypes ('Redgauntlet' and 'Hapil').

The Shapiro-Wilk ('shapiro.test()') function was applied in R to investigate the normality of the trait distributions (Shapiro and Wilk, 1965). Traits expressing significant and high skewed phenotypic value distributions were log and square root transformed using 'log()' and 'sqrt()' functions respectively. Significant and/or least-skewed transformations were used to calculate the phenotypic mean values across six replicates for all individuals for each year. The same means were used to estimate the Spearman rank correlation coefficients between traits by applying 'chart.Correlation()' function under the 'PerformanceAnalytics' library in R. In addition, normally and/or near normally distributed mean values of the progeny were further used in QTL analysis (Chapter 4).

Pairwise comparisons among parental genotypes and seedlings were analyzed for significant differences and were presented as scatterplot correlation matrices. The distribution of the phenotypic means as histograms, kernel density overlays, correlation coefficients and significance asterisks were also presented within matrices.

Furthermore, differences among tunnels and years for the progeny were estimated by the REML random model using GenStat software package.

The normally and/or close to normally distributed mean values were further standardized to a scale of 0 - 1 for each trait and year, and were used for heat map hierarchical clustering analysis in R ('heatmap.2()' function in gplots library).

Non-transformed phenotypic values were used to calculate the variance components using 'lmer()' function in the 'lme4' R package. The estimates of the variance components were further used to calculate broad-sense heritability (H^2) coefficients of genotypic means across years. Broad-sense heritability was calculated by adapting the formula of Piepho and Möhring (2007) as follows:

$$H^2 = var(G)/var(P)$$

where: $var(G)$ - the genetic variance, and $var(P)$ – the phenotypic variance.

$$var(P) = var(G) + \frac{var(GT)}{n} + \frac{var(GY)}{m} + \frac{var(R)}{n * m}$$

where: $var(GT)$ – the genotype by tunnel interaction variance, $var(GY)$ – the genotype by year interaction variance, $var(R)$ – the residual error variance, n – number of tunnels, and m – number of years.

2.3 Results

2.3.1 Phenotypic data analysis

The mean phenotypic values of the two parental genotypes ‘Redgauntlet’ and ‘Hapil’, and F_1 progeny were calculated by combining the data collected across six replications for 30 traits for three years (2013, 2014 and 2015) (Table 2.3).

Under the tunnel conditions, the two parental genotypes (‘Redgauntlet’ and ‘Hapil’) exhibited consistently different behaviours throughout the phenotyping season (April-July) in all years for some traits. When phenotypic means for each trait were compared between parental genotypes for each year, ten traits showed no significant differences: the length of the pedicel (Pdc.L.) was on average 66.4 ± 8.6 and 76.9 ± 7.3 mm for ‘Redgauntlet’ (‘Rg’) and ‘Hapil’ (‘H’) respectively; the height of the plant (Hgh.) was identical (26.1 cm) for both parents; the width (Wdt.) of the plants was similar, 37.8 ± 0.8 in ‘Rg’ and 39.5 ± 1.3 in ‘H’; the length of the longest truss (Tr.L.) (which was only 7.5 mm longer in ‘Rg’); the yield ranged from 455 g to 771.9 g in ‘Rg’ and from 426 g to 1,000 g in ‘H’ (see also Figure 2.4); the unmarketable fruit weight (Unmark.) on average was only 21.4 g different between the parents; fruit shape (Shp.), outline (Outl.) and redness (Rdn.) was rated on the scale of 1-5 and ranged on average between 3.0 ± 0.2 and 3.4 ± 0.2 in ‘Rg’, and between 3.2 ± 0.2 and 3.4 ± 0.3 in ‘H’; skin strength (Sk.Str.) was identical for both parents (4.7 ± 0.2) (Table 2.3).

Table 2.3 Phenotypic variation of 30 plant characteristics and fruit quality traits recorded in parental genotypes ('Redgauntlet' and 'Hapil') and F_1 progeny for three consecutive years (2013, 2014 and 2015). The number of individuals analysed (No.), mean values, standard deviations (SD), range and *t*-test significance between parental genotypes ('Rg' vs 'H') among six replicates are presented

Traits[1]	**Year**	**'Redgauntlet'**				**'Hapil'**					**F_1 progeny**			
		No.	Mean	SD	Range	No.	Mean	SD	Range	'Rg' vs 'H'[2]	No.	Mean	SD	Range
Fl.N.	2013	6.0	85.7	21.1	69.0-118.0	6.0	41.5	26.1	11.0-85.0	**	717	52.1	29.0	3.0-159.0
	2014	6.0	89.2	31.3	55.0-135.0	6.0	60.0	13.4	48.0-82.0	*	715	78.2	32.9	2.0-237.0
	2015	6.0	101.8	36.9	29.0-132.0	6.0	55.2	17.8	33.0-83.0	**	718	57.6	28.2	4.0-164.0
Fl.D.	2013	6.0	25.3	3.2	20.0-30.0	6.0	32.8	6.8	20.0-38.3	*	710	29.4	4.8	20.0-41.7
	2014	6.0	25.8	4.3	20.0-31.6	6.0	37.2	6.0	26.6-43.3	**	711	30.2	4.1	20.0-45.0
	2015	6.0	28.1	1.9	25.0-30.0	6.0	34.4	3.9	30.0-40.0	**	715	31.5	3.8	21.7-43.3
Pt.N.	2013	6.0	6.2	0.5	5.7-7.0	6.0	6.1	1.1	5.0-8.0	n.s.	709	6.0	1.2	4.7-12.3
	2014	6.0	6.1	0.7	5.3-7.0	6.0	5.9	0.7	5.0-7.0	n.s.	712	6.1	0.9	4.0-10.7
	2015	6.0	6.6	0.3	6.3-7.0	6.0	5.6	0.9	5.0-6.7	**	715	6.0	0.9	5.0-11.0
Pdc.L.	2013	6.0	88.6	30.9	31.5-116	6.0	95.6	27.0	62.3-135	n.s.	710	85.9	27.9	27.0-183.0
	2014	6.0	54.2	14.6	39.1-72.2	6.0	65.6	7.4	56.0-75.1	n.s.	712	73.3	21.5	8.5-144.8
	2015	6.0	56.3	17.6	36.9-85	6.0	69.7	19.6	44.9-89.6	n.s.	718	73.0	18.4	19.9-156.4
Lf.N.	2013	6.0	3.5	0.5	3.0-4.0	6.0	4.0	0.0	4.0-4.0	*	720	3.4	0.6	3.0-5.0
	2014	6.0	3.2	0.4	3.0-4.0	6.0	3.0	0.0	3.0-3.0	n.s.	715	3.1	0.3	3.0-5.0
	2015	6.0	3.0	0.0	3.0-3.0	6.0	4.2	0.8	3.0-5.0	**	720	3.2	0.5	3.0-6.0
Vig.	2013	6.0	4.9	0.2	4.6-5.0	6.0	4.3	0.6	3.7-4.3	*	720	4.4	0.6	2.4-5.0
	2014	6.0	4.1	0.5	3.5-4.7	6.0	4.5	1.0	2.5-5.0	n.s.	716	4.2	0.8	1.0-5.0
	2015	6.0	4.6	0.5	3.8-5.0	6.0	4.7	0.3	4.4-5.0	n.s.	720	4.2	0.8	1.0-5.0
Hgh.	2013	6.0	26.1	2.1	24.1-29.9	6.0	26.1	2.9	22.7-30.8	n.s.	720	24.8	3.3	15.8-36.2
Wdt.	2013	6.0	37.8	1.9	34.7-39.7	6.0	39.5	3.2	35.8-43.9	n.s.	720	38.3	3.7	26.7-62.2

Table 2.3 Continued

Traits[1]	Year	'Redgauntlet'				'Hapil'					F_1 progeny			
		No.	Mean	SD	Range	No.	Mean	SD	Range	'Rg vs 'H'[2]	No.	Mean	SD	Range
Run.N.	2013	6.0	14.0	2.6	10.0-17.0	6.0	10.3	2.2	7.0-13.0	*	720	13.7	5.3	3.0-41.0
	2014	6.0	17.8	4.4	12.0-23.0	6.0	21.8	8.9	10.0-37.0	n.s.	715	23.2	9.8	3.0-74.0
	2015	5.0	3.2	3.0	1.0-7.0	5.0	3.4	2.1	1.0-6.0	n.s.	679	10.9	6.3	1.0-37.0
Run.L.	2013	6.0	55.1	5.2	47.0-61.1	6.0	48.0	7.0	39.3-53.9	*	720	53.6	9.4	24.4-93.0
	2014	6.0	47.3	7.2	40.5-57.3	6.0	45.1	9.5	33.0-58.3	n.s.	715	43.4	10.6	13.8-78.0
	2015	5.0	52.2	11.3	34.0-65.0	5.0	49.5	15.6	31.1-64.2	n.s.	680	56.7	11.8	21.2-94.1
Tr.N.	2013	6.0	8.2	1.2	7.0-10.0	6.0	4.8	2.8	1.0-9.0	*	715	6.0	3.2	1.0-19.0
	2014	6.0	18.0	4.6	13.0-23.0	6.0	11.5	3.8	7.0-16.0	*	714	15.6	6.4	1.0-40.0
	2015	6.0	19.2	10.4	6.0-36.0	6.0	9.2	4.8	5.0-18.0	*	716	11.3	6.2	1.0-42.0
Tr.L.	2013	6.0	145.0	53.0	81.9-216.4	6.0	115.7	37.7	77.9-180.4	n.s.	717	121.9	34.0	22.8-274.2
	2014	6.0	134.3	27.6	97.6-180.5	6.0	148.3	27.5	110.3-184.5	n.s.	697	117.6	33.1	19.1-246.8
	2015	6.0	155.4	23.5	123.8-196.7	6.0	148.2	21.0	125.4-173.7	n.s.	716	138.0	31.1	36.0-368.4
Tr.Wdt.	2013	6.0	3.8	0.5	2.9-4.1	6.0	5.1	1.0	3.4-6.2	*	716	4.5	1.1	1.6-9.3
	2014	6.0	2.6	0.8	1.7-3.9	6.0	3.7	0.8	2.5-4.9	*	691	3.1	0.7	1.3-6.3
	2015	6.0	2.9	0.5	2.0-3.5	6.0	4.1	0.5	3.6-5.1	**	716	3.6	0.7	1.1-6.9
Yield	2013	6.0	455.0	145.0	243.0-669.0	6.0	426.2	181.8	229.0-765.0	n.s.	712	456.0	256.8	6.0-2169.0
	2014	6.0	771.9	271.0	264.2-982.9	6.0	1000.4	364.1	561.2-1345.9	n.s.	701	783.8	376.9	12.3-2432.7
	2015	6.0	559.8	362.4	187.6-1162.1	6.0	681.6	178.5	464.1-901.4	n.s.	690	531.1	332.8	4.4-1827.6
Unmark.	2013	6.0	85.2	59.9	20.3-191.7	3.0	184.5	199.1	45.8-412.6	n.s.	618	173.5	197.0	7.0-1442.0
	2014	6.0	317.6	133.5	81.5-473.7	6.0	222.5	128.1	83.3-426.3	n.s.	701	301.2	183.2	5.0-1347.0
	2015	6.0	252.5	138.3	91.1-362.3	6.0	184.1	67.5	102.7-289.2	n.s.	673	181.2	123.9	4.5-656.9
Mark.	2013	6.0	369.8	145.1	222.8-628.0	5.0	401.6	228.5	183.2-765.0	n.s.	664	325.5	222.7	5.3-1563.7
	2014	6.0	454.3	162.6	182.7-563.4	6.0	777.9	328.0	304.3-1127.4	*	699	483.6	267.2	7.3-1683.5
	2015	6.0	307.3	243.9	81.1-747.1	6.0	497.6	168.8	283.0-668.8	*	678	360.6	247.8	4.4-1378.5

Table 2.3 Continued

Traits[1]	Year	'Redgauntlet'				'Hapil'					F_1 progeny			
		No.	Mean	SD	Range	No.	Mean	SD	Range	'Rg vs 'H'[2]	No.	Mean	SD	Range
Firm.	2013	6.0	169.3	33.8	134.3-231.7	6.0	184.5	29.4	126.4-205.2	n.s.	715	187.2	36.7	82.6-314.9
	2014	6.0	151.8	11.3	141.6-173.6	6.0	171.0	19.0	145.7-198.2	*	699	187.1	27.3	77.1-308.5
	2015	6.0	175.2	12.9	156.2-188.1	6.0	211.4	29.6	173.5-239.3	*	675	193.8	33.7	73.7-346.5
Brix	2013	6.0	7.5	0.7	6.6-8.5	6.0	7.6	0.4	6.9-7.9	n.s.	713	8.3	1.2	4.1-12.7
	2014	6.0	7.4	0.8	6.6-8.6	6.0	8.3	1.2	6.5-9.9	*	691	8.3	1.2	5.1-13.0
	2015	6.0	5.6	1.1	4.3-7.3	6.0	8.1	0.8	7.2-9.1	***	669	7.6	1.3	4.2-13.0
pH	2013	6.0	3.1	0.1	3.0-3.3	5.0	3.3	0.1	3.3-3.4	**	705	3.3	0.2	2.9-3.9
	2014	5.0	3.9	0.4	3.4-4.4	5.0	4.1	0.2	3.8-4.3	n.s.	456	3.3	0.4	3.1-5.3
	2015	6.0	3.4	0.1	3.3-3.6	6.0	3.7	0.2	3.3-3.9	*	617	3.6	0.2	3.1-4.6
Ach.Ps.	2013	6.0	1.4	0.5	1.0-2.0	6.0	1.7	0.6	1.0-2.5	**	712	1.8	0.6	1.0-3.0
	2014	6.0	1.5	0.3	1.0-2.0	6.0	2.6	0.4	2.0-3.0	n.s.	691	1.8	0.6	1.0-3.0
	2015	6.0	2.0	0.6	1.3-3.0	6.0	2.7	0.3	2.3-3.0	n.s.	668	2.3	0.7	1.0-3.0
Sdn.	2013	6.0	2.2	0.4	2.0-3.0	6.0	2.5	0.4	2.0-3.0	n.s.	711	2.1	0.6	1.0-3.0
	2014	6.0	2.7	0.4	2.0-3.0	6.0	2.5	0.4	2.0-3.0	*	691	2.4	0.4	1.0-3.0
	2015	6.0	2.5	0.4	2.0-3.0	6.0	2.6	0.4	2.0-3.0	***	667	2.4	0.4	1.0-3.0
Sk.Brg.	2013	6.0	34.1	2.4	30.3-36.2	6.0	36.8	2.3	34.3-40.1	n.s.	714	38.9	3.7	30.2-52.9
	2015	6.0	33.1	1.5	31.3-35.6	6.0	36.4	0.8	35.6-37.8	***	679	37.0	3.0	28.7-48.3
Shp.	2014	6.0	3.3	0.5	3.0-4.0	6.0	3.5	0.4	3.0-4.0	n.s.	691	2.5	0.7	1.0-5.0
	2015	6.0	3.1	0.3	2.7-3.5	6.0	3.3	0.8	2.0-4.0	n.s.	668	3.3	0.8	1.0-5.0
Cap.Sz.	2014	6.0	2.2	0.5	1.7-3.0	6.0	2.8	0.6	1.5-3.0	*	691	2.5	0.5	1.0-3.0
	2015	6.0	1.6	0.8	1.0-3.0	6.0	2.7	0.4	2.0-3.0	**	668	2.3	0.8	1.0-3.0
Outl.	2014	6.0	2.8	0.2	2.7-3.0	6.0	3.0	0.4	2.5-3.5	n.s.	691	2.9	0.4	1.0-3.0
	2015	6.0	3.1	0.7	2.0-4.0	6.0	3.4	0.4	3.0-4.0	n.s.	668	3.1	0.6	1.0-5.0

Table 2.3 Continued

Traits[1]	Year	'Redgauntlet'				'Hapil'					F_1 progeny			
		No.	Mean	SD	Range	No.	Mean	SD	Range	'Rg' vs 'H'[2]	No.	Mean	SD	Range
Rdn.	2014	6.0	3.3	0.4	3.0-4.0	6.0	3.4	0.6	2.7-4.0	n.s.	691	3.1	0.5	1.0-4.0
	2015	6.0	3.6	0.5	3.0-4.0	6.0	3.3	0.4	3.0-4.0	n.s.	668	3.1	0.6	2.0-5.0
Gls.	2014	6.0	3.1	0.3	2.7-3.5	6.0	3.6	0.4	3.0-4.0	*	691	3.2	0.5	2.0-5.0
	2015	6.0	2.7	0.4	2.0-3.0	6.0	3.7	0.3	3.3-4.0	***	668	3.3	0.6	2.0-5.0
Nck.Ln.	2014	6.0	1.8	0.3	1.3-2.0	6.0	2.7	0.7	1.5-3.3	**	691	2.5	0.9	1.0-5.0
	2015	6.0	1.3	0.3	1.0-1.7	6.0	4.0	1.0	3.0-5.0	***	668	2.6	1.1	1.0-5.0
Sk.Str.	2014	6.0	4.6	0.4	4.0-5.0	6.0	4.5	0.5	4.0-5.0	n.s.	691	4.5	0.5	1.0-5.0
	2015	6.0	4.8	0.3	4.5-5.0	6.0	4.9	0.2	4.5-5.0	n.s.	668	4.6	0.7	1.0-5.0
In.Fr.Col.	2014	6.0	3.2	0.2	3.0-3.3	6.0	4.1	0.7	3.5-5.0	**	691	3.3	0.6	2.0-5.0
	2015	6.0	3.4	0.7	2.7-4.5	6.0	3.6	0.4	3.0-4.0	n.s.	668	3.2	0.6	1.0-5.0

[1]Traits are the following plant characteristics (values are per plant for all quantitative traits): Fl.N., flower number; Fl.D., flower diameter; Pt.N., petal number; Pdc.L., pedicel length; Lf.N., leaflet number; Vig., plant vigour; Hgh., plant height; Wdt., plant width; Run.N., runner number; Run.L., runner length; Tr.N., truss number; Tr.L., truss length; Tr.Wdt., truss width. Fruit quality: Unmark., unmarketable fruits; Mark., marketable fruits; Firm., firmness; Ach.Ps., achene position; Sdn., seediness; Sk.Brg., skin brightness; Shp., fruit shape; Cap.Sz., cap size; Outl., outline; Rdn., redness; Gls., glossiness; Nck.Ln., neck line; Sk.Str., skin strength; In.Fr.Col., internal fruit colour.

[2]*t*-test significance level between parental genotypes 'Redgauntlet' ('Rg') and 'Hapil' ('H'): *, significant at $p < 0.05$; **, significant at $p < 0.01$, ***, significant at $p < 0.001$; n.s., not statistically significant.

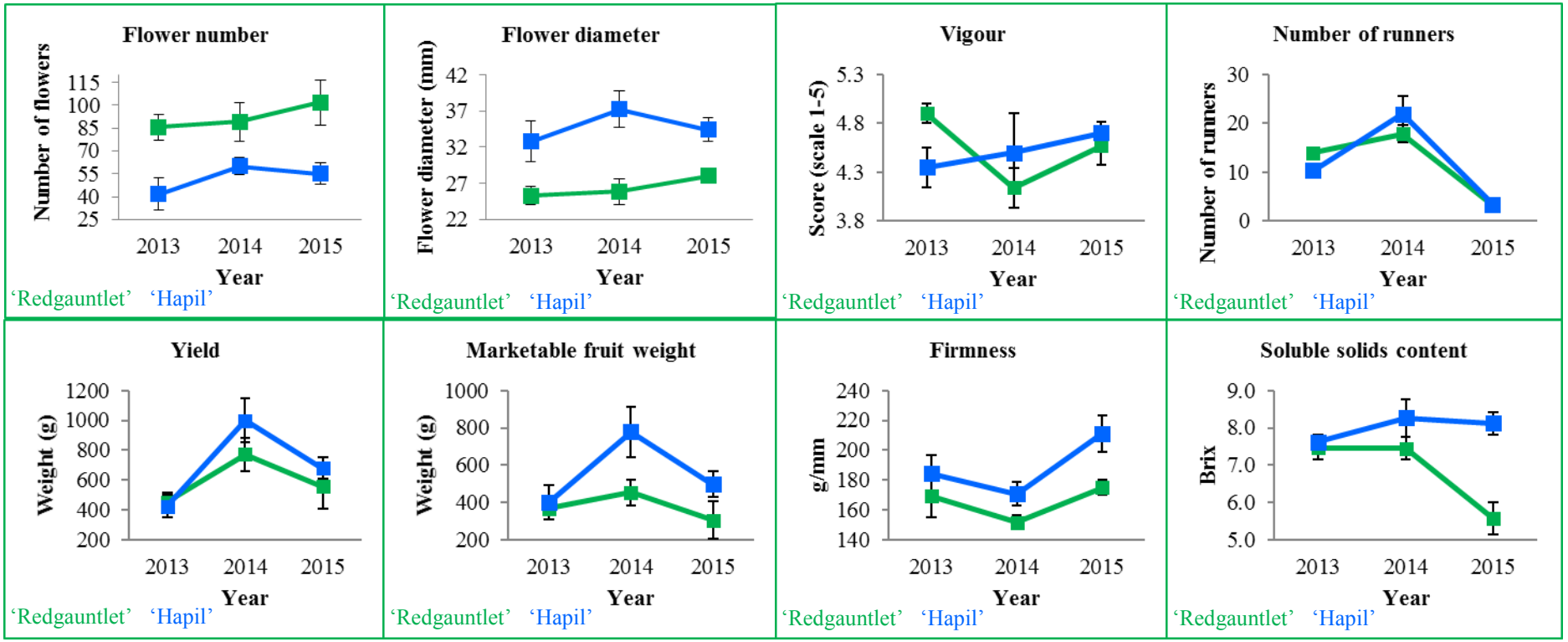


Figure 2.4 Plant characteristics and fruit quality related traits expressing differences between parental genotypes ('Redgauntlet' in green and 'Hapil' in blue) for 3-year phenotypic values recorded. The average mean values and standard errors are shown.

Seven traits showed significant differences between parental genotypes for one year only out of three-year's data. In 2013, 'Redgauntlet' had higher scores than 'Hapil' in four traits, plant vigour, the number of runners per plant (Figure 2.4), runner length and achene position ($p < 0.05$). However, the same traits in 2014 and 2015 were not statistically significant between the parents (Table 2.3). The petal number per flower (6.6 ± 0.1 in 'Rg' and 5.6 ± 0.4 in 'H') and skin brightness of the marketable fruits (33.1 ± 0.6 in 'Rg' and 36.4 ± 0.3 in 'H') were significantly different ($p < 0.05$) between the parents in 2015, whereas no significant differences were observed in 2013 and 2014. The internal fruit colour was also significantly different ($p < 0.05$) in 2014 between 'Rg' and 'H' and ranged from 3.2 ± 0.1 to 4.1 ± 0.3 respectively (Table 2.3).

Six traits showed significant differences between phenotypic means of the parental genotypes for two years of the three-year data collecting period. These traits were the number of leaflets per leaf, marketable fruit weight, firmness, soluble solids content, pH and fruit seediness. Of those, four traits (Mark., Firm., ºBrix, and Sdn.) showed significant differences in 2014 and 2015, and no significance in 2013 (Figure 2.4 except Sdn.), whereas the remaining traits (Lf.N. and pH) were significantly different in 2013 and 2015 but not in 2014 (Table 2.3).

The remaining seven traits (flower number, flower diameter, truss number, truss width, cap size, glossiness and neck line) exhibited common or fairly similar (statistically significant for three years) differences among parental genotypes for all years (Table 2.3). The most important differences between parental genotypes were observed for flowering time, number of flowers per plant, flower diameter and fruit glossiness (Figure 2.4 except Gls.). Compared to 'Hapil', 'Redgauntlet' began flowering earlier (on average ten days before 'Hapil') and developed more flowers (on average 94 ± 13.1 flowers in 'Rg' and 52 ± 7.8 flowers in 'H', Table 2.3, Figure 2.4,

Figure 2.5) consistently for all years analysed. Furthermore, 'Rg' developed more trusses than 'H' (on average 15.1 ± 2.2 and 8.5 ± 1.6). In contrast, 'Hapil' developed less but larger flowers (on average 8.4 ± 1.8 mm wider), wider trusses (on average 1.2 ± 0.3 mm wider) and more glossy fruits, which ranged from 2.9 ± 0.1 in 'Rg' to 3.7 ± 0.2 in 'H'.

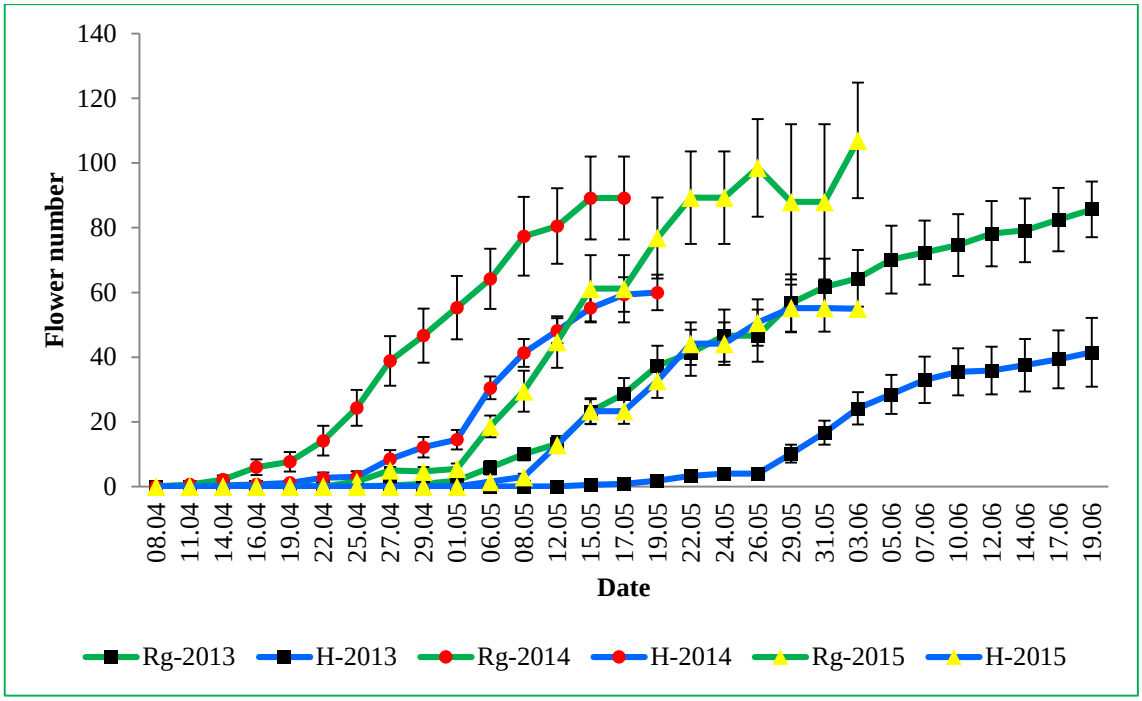


Figure 2.5 Flowering pattern of 'Redgauntlet' (Rg) and 'Hapil' (H) between April and June for 2013, 2014 and 2015.

When phenotypic mean values were compared between parental genotypes and the progeny, no significant differences were found for all traits and years analysed. However, greater mean values were seen for six traits in 2014 when compared to 2013 and 2015 phenotypic means within the progeny. This can be explained by the fact that the progeny were two year's old in 2014, whereas plants were one year old in 2013 and 2015 (individuals were planted in 2013 and replanted in 2015). The most significant differences within the progeny among the years analysed were yield, unmarketable and

marketable fruit weight as expected (second year strawberry plants produce more fruits) (Table 2.3). However, progeny means were inconsistent when values in 2014 were compared to 2013 and 2015 for all traits (Table 2.3). The distributions of the phenotypic values for the progeny are presented as boxplot and whisker diagrams for all traits and years in Appendix 2.1.

2.3.2 Weather data analysis

A total of five weather-related parameters were selected and analysed for the duration of the phenotypic data collection (April – July) in 2013, 2014 and 2015. The results showed that the air temperature ($p < 0.05$) and wind speed ($p < 0.05$) were statistically significantly different among the years analysed. The distributions of the remaining parameters (temperature at the grass level, daily sunshine and humidity) were not statistically different. Graphical data distributions among the years are presented in Figure 2.6.

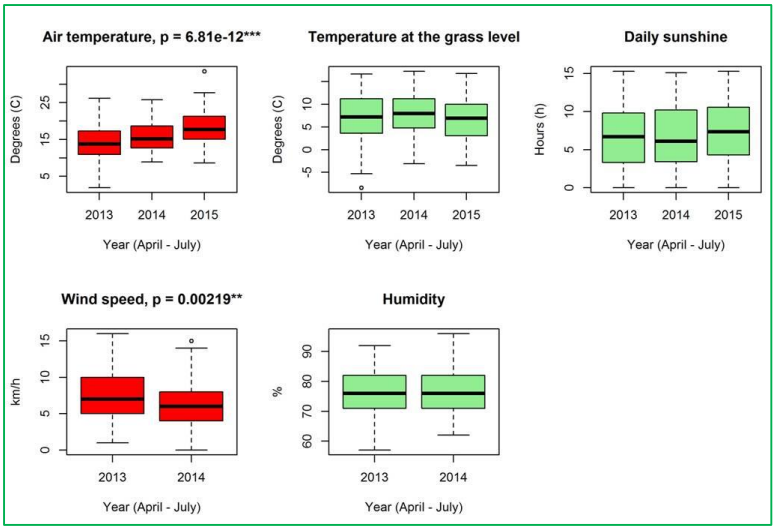


Figure 2.6 The distribution of the weather-related data between April and July in 2013, 2014 and 2015. Box plots highlighted in red are significantly different trait variations between the years ($p < 0.05$).

2.3.3 Correlation analysis of plant characteristics and fruit quality traits

To investigate how different complex traits are correlated and if variables are related, scatterplot matrices representing 30 traits for each year, showing pair wise correlations of phenotypes were produced (Supplementary Figure 2.7). The correlation chart combines a large amount of information; the diagonal line presents phenotypic variables plotted as histograms and kernel density plots, the pair-wise correlation coefficients (r) and red stars signifying significance level (*** = $p < 0.001$, ** = $p < 0.01$ and * = $p < 0.05$) were plotted on the right of the diagonal line; the bigger font of the correlation coefficients represents closer correlations between two variables observed and vice-versa; the scatterplot matrix with smoothers in red illustrates the underlying relationship were plotted on the left side of the diagonal line.

The correlation matrices and/or histograms of the phenotypic mean distributions showed continuous variations in most trait values in the progeny (Appendix 2.2). A total of 25 traits (83.3%) showed high variations in trait values; the remaining five traits were recorded on a scale-basis (1 – 3 or 1 – 5) and were expected to show low variation. The traits exhibiting low variations were the number of petals per plant, leaflet number per plant, the cap size of the fruit, fruit seediness and the skin strength of the fruit.

The majority of traits (13) analysed for all years were not normally distributed. Twelve traits showed near to normal distributions for three or two years analysed. The remaining three traits (plant height (2013), ºBrix and skin brightness) displayed normal distributions for all years (Appendix 2.3).

The Spearman correlation coefficients between different physical traits are presented in Supplementary Figure 2.7. The correlation coefficients for most of the correlations observed were high (i.e. < 0.30) suggesting statistically significant relationships between the variables. For example, a correlation coefficient of 0.90 was

observed between flower number and truss number in 2013, indicating that flower number was closely related to truss number; whereas flower number and petal number had a correlation coefficient of 0.15 in 2014, indicating a weak relationship between these traits.

The correlations observed in this study were highly statistically significant (*** p = < 0.001, ** = p < 0.01), although the majority of them correlated only in one or two years; thus they were year dependent. For example, a negative correlation was observed between the number of flowers per plant and ºBrix only between the data collected in the same year among both traits. No correlations were observed between the data collected in 2013 for ºBrix and the data collected in 2014 and 2015 for the number of flowers per plant, and vice-versa.

Highly significant, consistent correlations over the three years were observed for 11 traits (Figure 2.8). Among the plant characteristics traits, the strongest significant positive correlation was found between flower number and truss number, and had a correlation coefficient ranging from 0.26 to 0.90 among the years. Flower number was also positively correlated with yield and marketable fruits. Plant vigour showed positive correlations with plant height (on average r = 0.44) and width (on average r = 0.60). The yield exhibited significant positive correlation with plant vigour, width and number of trusses (as also reported by Ukalska et al. (2006), Singh et al. (2010) and Zorrilla-Fontanesi et al. (2011b)), although the correlations observed in this study were year dependent. No correlation was found between yield and plant height, which is in agreement with previously reported study of Zorrilla-Fontanesi et al. (2011b). A negative correlation was found between truss number and flower diameter, suggesting that the number of trusses affect the flower size and smaller flowers are likely to be developed if the plant develops many trusses.

Among the fruit quality traits, the most significant and closely related were negative correlations between skin brightness and redness, and internal fruit colour, with r = -0.71 and r = -0.64 respectively. In contrast, positive correlations were observed between redness and internal fruit colour (r = 0.76), and glossiness (r = 0.41). Highly significant positive correlations were also found between neck line and fruit shape (r = 0.37), and the cap size (r = 0.34), and were consistent over the three years (Figure 2.8).

Interestingly, a positive correlation between fruit weight and fruit shape was previously reported by Lerceteau-Köhler et al. (2012), however no correlation was found between these traits in this study (Supplementary Figure 2.7).

A few traits expressed unusual relationships between variables. For example, negative correlations can be seen between the ºBrix and the number of flowers per plant, and yield. Moreover, a highly significant correlation between yield and ºBrix is previously unreported. In contrast, firmness, pH, runner number, leaflet and petal number, as well as runner length did not show correlations over the three years (Supplementary Figure 2.7).

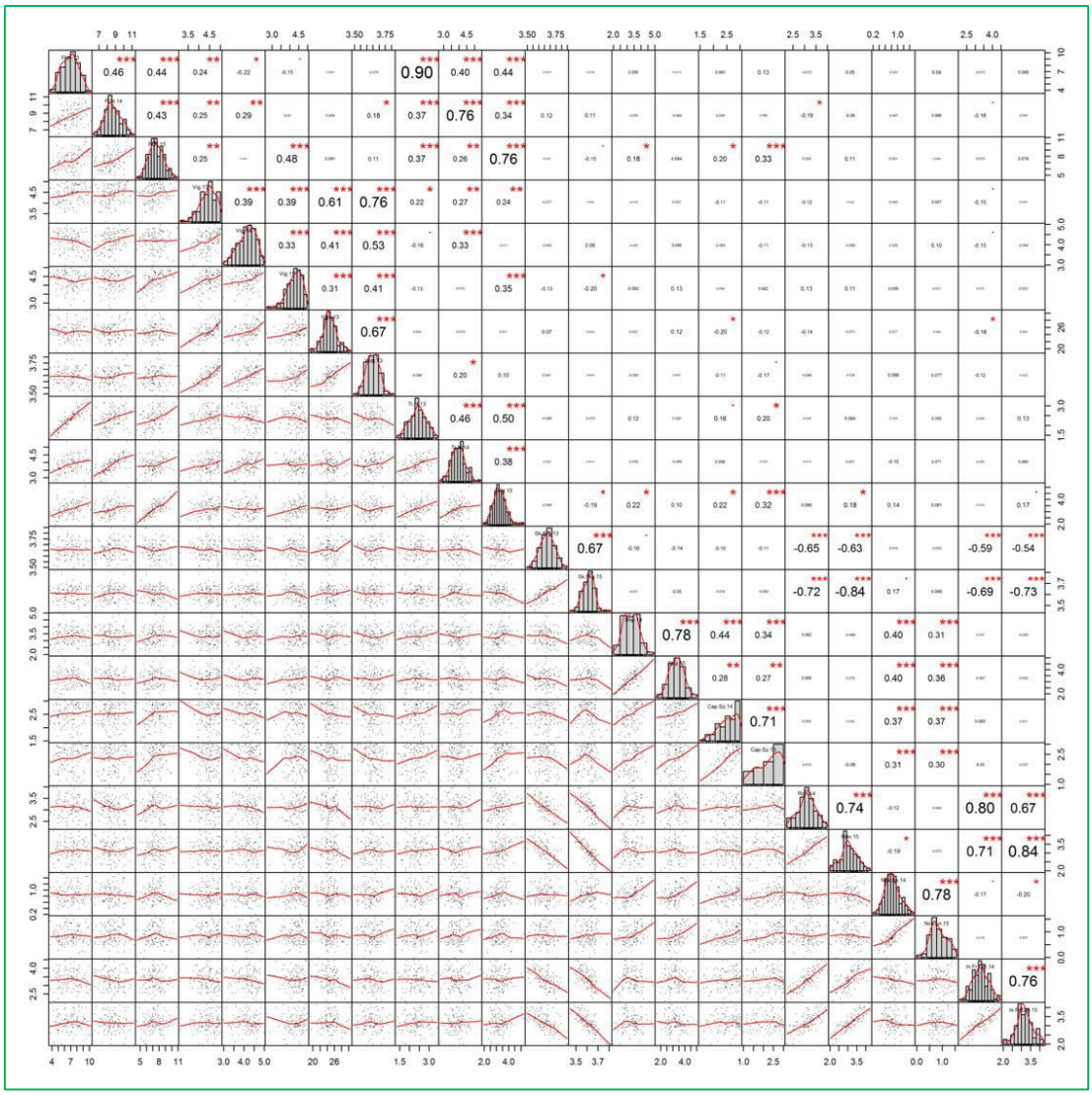


Figure 2.8 Scatterplot matrices of phenotypic correlations among 11 different traits phenotyped within 'Redgauntlet' × 'Hapil' mapping population for three consecutive years. Histograms, kernel density overlays, Spearman rank correlations and significance asterisks are presented (*** = p < 0.001, ** = p < 0.01 and * = p < 0.05). The traits are from the top as follow: Fl.N.2013, Fl.N.2014, Fl.N.2015, Vig.2013, Vig.2014, Vig.2015, Hgh.2013, Wdt.2013, Tr.N.2013, Tr.N.2014, Tr.N.2015, Sk.Brg.2013, Sk.Brg.2015, Shp.2014, Shp.2015, Cap.Sz.2014, Cap.Sz.2015, Rdn.2014, Rdn.2015, Nck.Ln.2014, Nck.Ln.2015, In.Fr.Col.2014 and In.Fr.Col.2015. Number 13, 14 or 15 after the trait, represents year (2013, 2014 or 2015) when the trait was recorded. For abbreviations see legend to Table 2.3.

2.3.4 Clustering analysis of the phenotypic data

The phenotypic means used for correlation analysis were also used for a clustering analysis in this study. Due to the large size of the data set, a sub-set of 11 traits which showed consistent correlations over the years was selected for analysis. The variables for these traits were standardized to a scale of 0 – 1 before carrying out a hierarchical clustering analysis. The heat map of the traits analysed in this study is presented in Figure 2.9.

The hierarchical distribution of the traits showed mostly expected similarities among the same traits recorded over two or three years. Skin brightness (2013 and 2015), soluble solid content (2013, 2014 and 2015), cap size (2014 and 2015), shape (2014 and 2015), neck line (2014 and 2015), firmness (2013, 2014 and 2015) and glossiness (2014 and 2015) were the traits showing similarities over the two or three years. However, a number of the same traits recorded for different years were dissimilarly distributed. Among the plant characteristic traits, vigour, flower number and truss number were distributed on different clusters. Similarly, for fruit quality traits, yield, redness, seediness and internal fruit colour showed dissimilarities (Figure 2.9). Interestingly, a consistent pattern of the distributions can be seen for dissimilar traits. These traits were clustered as similar by the year recorded. For example, flower number, truss number and yield recorded in 2013 were clustered together. The same traits were clustered together for 2014 and 2015, with the exception of vigour (2015) joining the same cluster. The same pattern can be seen for redness and internal fruit colour (Figure 2.9). However, one trait (seediness) displayed random clustering. Phenotypic variables for seediness in 2014 were similar to seediness data collected in 2015 but different to the data collected in 2013.

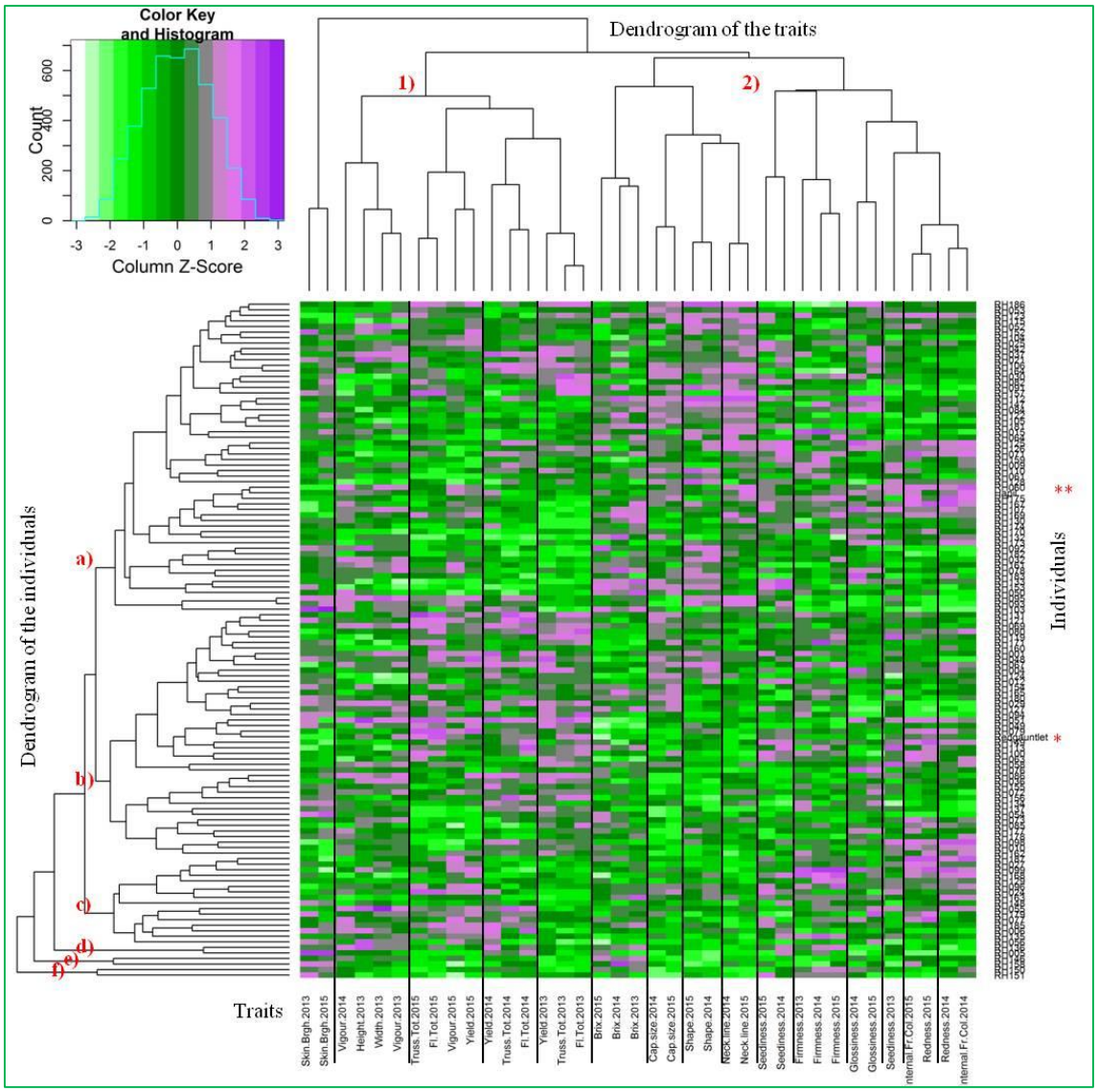


Figure 2.9 Heat map and hierarchical clustering analysis between 'Redgauntlet' (*), 'Hapil' (**) and progenies of phenotypic means for 11 different physical traits. The row dendrogram (a, b, c, d, e and f) shows the similarity between rows, representing individuals. The column dendrogram (1 and 2) shows similarities between the variables. Different colours for each cell summarises the phenotypic mean between each individual for each trait.

The dendrogram of the traits showed two main clusters. The first cluster (1) grouped plant architectural traits with the exception of yield, which was also grouped

under the same cluster, whereas the second cluster (2), grouped fruit quality related traits only (Figure 2.9).

The hierarchical distribution of the parental genotypes and individuals showed three main clusters. Parental genotypes ('Redgauntlet' and 'Hapil') were distributed on different, far from each other, clusters and showed strong dissimilarity (Figure 2.9). Furthermore, a clear pattern can be seen in clustering analysis of individuals of the mapping population. A total of 55 individuals were grouped on the same cluster as 'Hapil' (cluster a), suggesting that these individuals were expressing high similarity to 'Hapil'. Forty three individuals were grouped on the same cluster as 'Redgauntlet' (cluster b) and expressed similarities to 'Redgauntlet'. The remaining 22 individuals were grouped on three smaller clusters (clusters c, d, e and f) expressing dissimilarities to the parental genotypes.

A transgressive segregation pattern was observed among some individuals, as can be seen on the heat map. For example, an extremely high level of firmness can be seen observed for individual RH099 (dark purple colour), whereas parental genotypes showed lower levels of firmness (green to light purple colour) across three years (Figure 2.9).

2.3.5 Broad-sense heritability

The heritability coefficients were calculated for 30 traits analysed. Of those, 20 (66.7%) traits showed high heritability coefficients ($H^2 \geq 0.5$) and only two traits (Tr.L. and Sk.Str.) showed low values ($H^2 \leq 0.3$) (Figure 2.10). The same threshold for high and low heritability coefficients was used as previously reported by Lerceteau-Köhler et al. (2012).

The highest heritability coefficients were observed within fruit quality traits, such as neck line ($H^2 > 0.88$), fruit shape ($H^2 > 0.84$), internal fruit colour ($H^2 > 0.80$), skin brightness ($H^2 > 0.79$), redness ($H^2 > 0.77$) and cap size ($H^2 > 0.72$). Interestingly, these traits exhibited high correlation coefficients and suggest high agreement between their phenotypic and genotypic values for the progeny. The lowest heritability coefficient ($H^2 = 0.02$) was also recorded among fruit quality traits, for skin strength. Other fruit quality traits including soluble solids content ($H^2 > 0.68$), glossiness ($H^2 > 0.67$) and firmness ($H^2 > 0.55$) had high heritability coefficients.

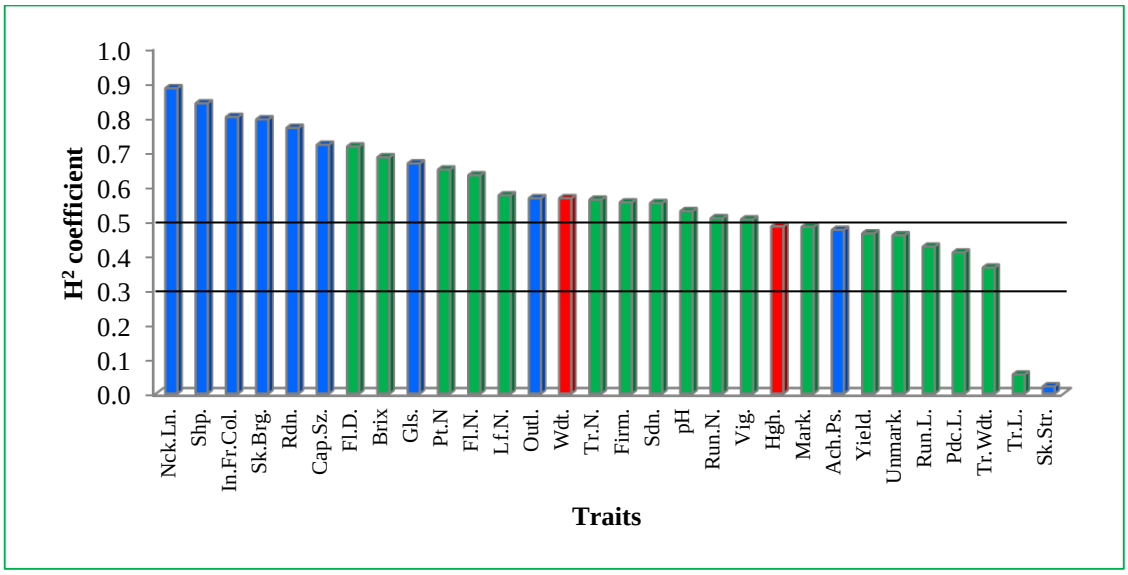


Figure 2.10 Broad-sense heritability coefficients for 30 traits analysed. Correlation coefficients for traits in green were calculated among the 3-year data, for traits in blue for 2-year data and traits in red for 1-year data. The two horizontal black lines represents the threshold of the high ($H^2 \geq 0.5$) and low ($H^2 \leq 0.3$) heritability coefficient values.

The highest heritability coefficient among plant characteristics traits were for flower-related traits, such as flower diameter, petal number and flower number ($0.63 \leq H^2 \geq 0.71$) (Figure 2.10). Similarly to the fruit quality-related traits, phenotypic and genotypic values were in agreement for flower diameter and flower number. However, petal number did not show any correlation among the traits and years analysed, thus suggesting a weak relationship between phenotypic and genotypic values. The lowest heritability coefficient among plant characteristics traits analysed was observed for truss length ($H^2 = 0.05$) only.

2.4 Discussion

The aim of this study was primarily to investigate phenotypic correlations between complex plant characteristics and fruit quality traits of the octoploid strawberry (*Fragaria* × *ananassa*). The data were analysed to have a better understanding of how traits correlate at the phenotypic and genotypic level (see also Chapters 4 and 5), and to provide new insight into enhancing strawberry breeding for improved fruit quality. In addition, the information obtained in this study is a valuable tool, especially for phenotyping a large number of individuals. For example, the study showed that runner number and runner length per plant, and petal number per flower were the least correlated traits among the data set analysed. Therefore, these traits can be excluded from the breeding process resulting in reduced phenotyping time and cost.

2.4.1 Phenotypic variation among parental genotypes and the progeny

The parental genotypes (‘Redgauntlet’ and ‘Hapil’) studied here showed consistent significant differences in a number of traits, such as flowering time, number

of flowers per plant (over a limited period), flower diameter, truss width and glossiness (Table 2.3). The results showed that 'Redgauntlet' is an early, high cropping strawberry cultivar. This may suggest that 'Redgauntlet' cultivar is more likely to produce higher yields. However, the results obtained in this study suggested this is not the case. In fact, 'Hapil' produced higher yields in 2014 and 2015, and consistently higher yields of marketable fruits for all years than 'Redgauntlet' (Table 2.3, Figure 2.4). In addition, most of the key fruit quality traits were exceptional in 'Hapil'. Higher yield, firmer, glossy and more attractive fruits containing higher soluble solids content were produced by 'Hapil' consistently for all years analysed (Table 2.3, Figure 2.4).

The results obtained from the hierarchical clustering analysis between the traits and the progeny (including parental genotypes), provided further evidence that parental genotypes exhibited different behaviours among the phenotypic variables (Figure 2.9). Both parental genotypes were distributed on distant clusters, indicating genotypic differences between them (clusters a and b). In addition, clustering analysis showed that 45.8% and 35.8% of individuals expressed similarities to 'Hapil' and 'Redgauntlet' respectively, and were grouped on the same two clusters as the parental genotypes. However, the remaining 18.3% of individuals expressed dissimilarities compared to parental genotypes. Transgressive segregation type can be seen among individuals grouped on different clusters (Figure 2.9 c, d, e and f) than those of parental genotypes. As an example, a consistent pattern was observed among progeny expressing transgressive behaviour for fruit firmness. The majority of individuals grouped on different clusters showed higher level of firmness than 'Redgauntlet' or 'Hapil'.

Finally, 'Redgauntlet' and 'Hapil' expressed differences in root architecture (data not shown) and strawberry disease resistance, such as *Verticillium* wilt (*Verticillium dahliae*) (Antanaviciute et al. 2015) and powdery mildew (*Podosphaera*

aphanis) (Chapter 6). As a result, this progeny is a good candidate population for further analysis, especially to investigate whether disease resistance QTL are overlapping QTLs associated with physical traits.

2.4.2 Correlation analysis

When relationships between variables were compared during the correlation analysis for all 30 different phenotypic traits, a number of traits did not show any correlations between phenotypes (Supplementary Figure 2.7). These results were expected because all traits cannot be related and correlate with each other. Traits such as runner number, leaflet and petal number, as well as runner length did not show correlation with any other traits over the three years. Moreover, most of plant characteristics and fruit quality traits in cultivated strawberry are complex traits, which are controlled by many genes, resulting in potential trade-offs' between the relationships of variables. In contrast, some economically important fruit quality traits expressed correlations among the progeny. The majority of important fruit quality traits, such as yield, redness, glossiness and soluble solid content, as well as some plant characteristics traits, such as plant vigour, height, width and flower number were correlated in agreement with previously reported studies in strawberry (Ukalska et al. 2006; Singh et al. 2010 and Lerceteau-Köhler et al. 2012).

A few traits expressed unusual relationships between variables. For example, negative correlations were found between the soluble solids content and the number of flowers per plant, and yield. This suggests that plants with lower number of flowers are likely to produce fruits with lower sugar content, and flower number also has a direct effect on a lower yield. Moreover, a highly significant negative correlation between yield and soluble solids content is previously unreported. Furthermore, according to

Zorrilla-Fontanesi et al. (2011b), no correlations were found between yield, plant height and width, however this study showed that yield was positively correlated with plant vigour, and due to the fact that a highly significant correlation was observed between plant vigour and plant height, yield is most likely to be affected by plant height and width.

Some discrepencies among correlations observed in this study and previously reported studies were observed. For example, yield positively correlated with fruit shape in the study of Lerceteau-Köhler et al. (2012), however no correlation was found between the same traits in this study (Supplementary Figure 2.7). These disagreements may be due to the presence of the enviromental factors, which affected phenotypic variables among the 3-year analysis reported here. Furthermore, observed measurement errors within the phenotypic data may have contributed to the discrepancies, although the phenotypic data collected in this study was accurately measured using six replicates and recording the measurements several times during the phenotyping season for each year. Thus, the results reported in this chapter are unlikely to be inaccurate. Indeed, the statistical analysis of the weather-related data (Figure 2.6) confirmed that significant differences were observed between the air temperature (for all years) and the wind speed (for 2-year data). In addition, clustering analysis revealed that similarities among the phenotypic data between three years were not consistent for all traits and were distributed within different clusters (Figure 2.9). These findings support the presence of the environmental effects which are likely controlling variables.

The correlation study reported here, provided crucial information towards understanding the associations between phenotypes and genotypes. This information is essential for plant breeders when selecting for optimal cultivars. In addition, good quality phenotypic information collected here will be used for quantitative trait loci

(QTL) mapping approaches and enhance breeding efficiency through marker-assisted selection (MAS). Traits that are closely related can be merged into groups, and traits, which are least important can be ignored, resulting in significant time and cost savings.

2.4.3 Heritability analysis

Broad-sense heritability coefficients were calculated for all traits analysed. The results showed that the highest heritability coefficients were observed for fruit quality traits. Among those, neck line of the fruit had the highest heritability coefficient (0.88), following by fruit shape (0.84), internal fruit colour (0.80), skin brightness (0.79) and redness (0.77) (Figure 2.10). Interestingly, clustering analysis showed similar results in terms of phenotypic value distributions among the fruit quality traits, which were generally closely distributed. This suggests that genetically diverse variables are in a close agreement between their phenotypic and genotypic values. The results also suggests that variation of the majority of the traits analysed, especially fruit quality-related traits, were influenced by genetic differences between individuals. The low heritability values observed for truss length and skin strength demonstrated that different genotypes had the same phenotype (no genetic variation) in the environment where the population was kept. However, in a different environment the same traits within the same population are likely to be heritable.

In general, heritability coefficients observed in this study were similar to those previously reported for the same traits in strawberry (Ukalska et al. 2006 and Lerceteau-Köhler et al. 2012). For example, the heritability coefficient for plant vigour and glossiness were reported as 0.61 and 0.53 by Ukalska et al. (2006) and as 0.53 for pH by Lerceteau-Köhler et al. (2012), whereas the heritability value of 0.50, 0.67 and 0.53 were observed in this study for the same traits respectively.

2.5 Conclusions

This work reports a correlation, clustering and heritability analysis of a large number of different plant characteristics and fruit quality traits in cultivated strawberry. The analysis of phenotypic data collected in this study showed a number of highly significant differences between parental genotypes (‘Redgauntlet’ and ‘Hapil’). The segregating traits among the genotypes will be mapped through QTL analysis in octoploid strawberry. This information is a valuable tool for further studies such as the development of novel molecular markers for use in marker-assisted selection.

The results provided new information on the correlations between physiological and fruit quality traits at the phenotypic level among the octoploid strawberry mapping population. Correlation coefficients were very low for a large number of traits analysed suggesting that many traits were not correlated to each other. The least and the most correlated traits were identified.

The heritability analysis provided a better understanding between the genotypic and phenotypic associations among the progeny for the traits analysed. The high heritability coefficients observed for the majority of the traits revealed that phenotypic variations were affected by substantially large genotypic variations.

Chapter 3

The development of a high density SNP-based genetic linkage map of an octoploid strawberry mapping population

3.1 Introduction

The cultivated strawberry (*Fragaria* × *ananassa*) is an economically important soft fruit, which is grown throughout the world. Strawberry is a member of a diverse Rosaceae plant family that also includes fruit crops such as apple (*Malus*), cherry, plum, apricot and peach (*Prunus*), raspberry and blackberry (*Rubus*), and pear (*Pyrus*) (Sargent et al. 2009; van Dijk et al. 2014). Although cultivated strawberry has been well studied, the complex allo-octoploid genome (2n = 8x = 56), means that genetic analysis of quantitative traits and the development of molecular markers linked to important fruit quality and disease resistance traits for use in enhancing the breeding programmes through marker-assisted selection (MAS), is a challenging process (Folta et al. 2005; Sargent et al. 2009; Whitaker, 2011). To overcome the complexity of cultivated strawberry genetics, great effort has been put into the diploid strawberry (*Fragaria vesca*) genomics studies, from the development of the molecular markers and genetic linkage maps (Davis and Yu, 1997, James et al. 2003; Sargent et al. 2003, 2004b, 2006) to genome sequencing (Shulaev et al. 2011).

Several studies reported comparative genetics analyses between diploid and octoploid strawberry species, which revealed high level of similarity between their genomes and the transferability of molecular markers between them (Davis et al. 2006; Rousseau-Gueutin et al. 2008, 2009; Sargent et al. 2009). These achievements permitted further genomic research in octoploid *Fragaria* and led to the construction of a first genetic linkage map derived from the cross ‘Capitola’ × ‘CF1116’ (Lerceteau-Köhler et al. 2003) and a reference genetic linkage map for octoploid strawberry (Rousseau-Gueutin et al. 2008) using the same statistical methods developed for diploid *Fragaria*.

The first linkage map (‘Capitola’ × ‘CF1116’) contained a total of 789 AFLP and two putative gene markers and spanned 1,604 cM and 1,496 cM genetic distance for the female and male map respectively (Lerceteau-Köhler et al. 2003). The quality of the ‘Capitola’ × ‘CF1116’ linkage map was significantly improved by mapping novel AFLP, SCAR and SSR markers. The female and male maps contained a total of 367 and 440 loci and covered genetic distances of 2,582 cM and 2,165 cM respectively (Rousseau-Gueutin et al. 2008). The improved map was the first comprehensive reference genetic linkage map for octoploid strawberry.

Soon after, other linkage maps for octoploid strawberry were developed using different molecular markers. The linkage map for the ‘Tribute’ × ‘Honeoye’ progeny containing only AFLP markers and spanning genetic distance of 1,541 cM was developed by Weebadde et al. (2008). The first SSR-based linkage map containing 210 SSR markers and spanning 2,373 cM was reported by Spigler et al. (2008). This map was later further saturated, resulting in an improved quality linkage map (Spigler et al. 2010). The genetic linkage map derived from the cross ‘Redgauntlet’ × ‘Hapil’ containing 170 loci and 182 loci and covering 1,675 cM and 1,440 cM for the female and male linkage map respectively, was reported by Sargent et al. (2009). Furthermore, a genetic linkage map derived from the cross between two octoploid strawberry selection lines (‘232’ × ‘1392’) was developed and improved by Zorrilla-Fontanesi et al. (2011b, 2012). The map contained a total of 363 SSR markers and covered a genetic distance of 1,400 cM. In contrast, the existing ‘Redgauntlet’ × ‘Hapil’ linkage map was extended by further mapping 330 loci, resulting in a linkage map containing a total of 549 loci and spanning the genetic distance of 2,140 cM (Sargent et al. 2012). Most recently, a high quality integrated SSR-based linkage map developed for an octoploid

strawberry progeny ‘Holiday’ × ‘Korona’, which contained 508 SSR loci and covered genetic distance of 2,050 has been reported (van Dijk et al. 2014).

High quality, saturated linkage maps are crucial for the successful deployment of molecular markers in plant breeding programmes and genomic analysis. Furthermore, it is important to understand the genome structure of the cultivated strawberry in order to be able to identify the locations of QTL linked to economically important fruit quality and disease resistance traits (Sargent et al. 2012). Genetic linkage maps can be used not only for the development of a novel molecular markers but also for other purposes, such as genome evolution and comparative genomics studies, map-based cloning of genes, quantitative trait loci (QTL) analysis and genome assembly (Diaz et al. 2011; Sargent et al. 2012).

To date, several genetic linkage maps have been developed for octoploid strawberry using different molecular markers (Lerceteau-Köhler et al. 2003; Rousseau-Gueutin et al. 2008; Weebadde et al. 2008; Spigler et al. 2008, 2010; Zorrilla-Fontanesi et al. 2011b; Sargent et al. 2009, 2012; Isobe et al. 2013, van Dijk et al. 2014). Simple sequence repeats (SSRs) were the preferred, dominant markers in these studies. SSRs are extremely valuable and highly suitable for map development and for genotyping any crop. SSRs are particularly suitable for map development, not only because of their high polymorphisms and even distribution through the genome, but also because of their wide transferability among closely related species in Rosaceae such as apple and pear (Dirlewanger et al. 2002, Zhang et al. 2014). However, despite the advantages, SSR-based linkage map construction requires high investment and is a time consuming process. In addition, regions with low marker density are common within the genetic map developed using SSR markers, making them insufficient and ineffective for some purposes, such as QTL identification and gene cloning.

Recent advantages in next generation high-throughput sequencing and genotyping technologies have permitted the rapid development of high quality genetic linkage maps of various crops in the Rosaceae family using genotyping arrays. The approach allows researchers to sequence and genotype thousands of single nucleotide polymorphism (SNP) markers in a single array.

To date, the genotyping arrays have been developed and are available for several economically important crops within Rosaceae. These include a 9 K Illumina Infinium array for peach (Verde et al. 2012), 8 K and 20 K arrays for apple (Chagne et al. 2012, Bianco et al. 2014), a 6 K array for cherry (Peace et al. 2012) and 1 K array for pear (Montanari et al. 2013). Most recently, a 90 K Affymetrix Axiom® SNP array, named as IStraw90® array, has been developed for octoploid strawberry through joint effort of the USDA-SCRI and RosBREED project and Affymetrix Ltd. (van Dijk et al. 2014; Bassil et al. 2015). The genotyping array contained a total of 95,063 SNP markers within 138,099 probe sets. These probe sets were obtained from the alignment of short-read sequences from 1 diploid and 19 octoploid strawberry accessions to the publically available diploid *Fragaria vesca* 'Hawaii 4' genome sequence.

The evaluation of the IStraw90® array was based on genotyping a total of 384 individuals, including 27 accessions of diploid and 357 accessions of octoploid strawberries. Of the 357 octoploids, 306 strawberry accessions were *Fragaria* × *ananassa* and the remaining 51 were non *F. ananassa* samples (*Fragaria chiloensis*, *Fragaria virginiana* and pedigree-linked populations) (Bassil et al. 2015). Interestingly, the transferability of SNP markers between octoploid and diploid germplasm was very low. The study demonstrated that more than 80% of SNPs identified from diploids were possibly mapped to the diploid *Fragaria iinumae* linkage map, whereas only 199 of 85,663 loci developed from octoploid germplasm could be placed to the *F. iinumae*

linkage map. This suggests that the applicability of the array is primarily for studies in the octoploid strawberry species with the exception of diploid *F. iinumae*.

Since the first appearance of genotyping arrays for rosaceous species, a number of saturated linkage maps have been developed within the family. For example, high density SNP-based linkage maps have been reported for apple (Clark et al. 2014), apple rootstock (Antanaviciute et al. 2012), cherry (Klagges et al. 2013) and peach (Zeballos et al. 2013; Frett et al. 2014). The availability of IStraw90® array for octoploid strawberry, will certainty boost the construction of high quality genetic maps, leading to more effective validation of QTL controlling complex fruit quality and disease resistance traits. Moreover, the availability of the high-throughput platform will be a valuable tool for genome-wide association studies and will contribute greatly towards the assembly of an octoploid strawberry genome sequence.

In this chapter, the saturation of the regions with low marker density (gaps) and development of a consensus linkage map for an octoploid strawberry mapping population using previously mapped SSR markers and 90 K Affymetrix Axiom® SNP genotyping array is described. The aim of the study was to construct a high density linkage map that could be used for candidate gene identification, molecular marker development and QTL mapping. The map was constructed using a progeny derived from the cross 'Redgauntlet' × 'Hapil' described in Chapter 2 and Sargent et al. (2009, 2012). A set of 111 previously reported SSR marker pairs was selected for targeted mapping and the markers tested in the parental genotypes for identification of polymorphisms. Polymorphic primer pairs were screened in the whole mapping population of 173 offspring for segregation analysis. Segregating SSR primer pairs were combined with SSR data developed previously (Sargent et al. 2012), which resulted in a linkage map comprising of 824 loci mapped. Furthermore, a first high-throughput

genotyping array for octoploid *Fragaria*, the Affymetrix IStraw90® Axiom array, described by Bassil et al. (2015) was used for genotyping 'Redgauntlet' × 'Hapil' mapping progeny consisting of 140 individuals. The novel SNP-based linkage map contained a total of 3,933 unique SNPs and spanned 28 linkage groups of the octoploid strawberry, covering a genetic distance of 2,624.7 cM. All 28 linkage groups were well covered by SNP markers with the largest number of markers in one linkage group totalling 272 and the smallest 72. However, a small number of low marker density regions greater than 20 cM were observed.

3.2 Materials and methods

3.2.1 Plant material and DNA extractions

An F_1 mapping population of 188 individuals obtained from a cross between 'Redgauntlet' and 'Hapil' was raised as described in Chapter 2. The targeted mapping of the existing Rg × H genetic linkage map was based on screening 188 individuals and parental genotypes, however due to propagation errors 15 seedlings (rogues) were excluded for the analysis, thus a total of 173 seedlings remained. DNA was isolated from young and healthy leaf tissue of 173 Rg × H individuals and parental genotypes using DNeasy plant miniprep kit (Qiagen) according to the manufacturer's handbook. The concentration and purity of DNA was measured using a NanoDrop ND-1000 spectrophotometer (Thermo-Scientific, Loughborough, UK). All DNA samples were diluted to ~3 ng/µl for the use in PCR (polymerase chain reaction).

A total of 140 seedlings and parental genotypes were selected out of 173 Rg × H individuals for genotyping using 90 K Affymetrix Axiom® SNP array. The DNAs were further purified for the samples which had concentrations 10 ng/µl or lower and the

absorbance at 260 nm (A260) and at 280 nm (A280) rates were lower than 1.65, before sending DNAs for genotyping.

3.2.2 SSR marker selection for SSR-based genetic linkage map saturation

A total of 111 simple sequence repeat (SSR) markers were selected for saturation of the gaps of the existing SSR-based Rg × H genetic map. Thirty four gaps greater than 10 centi-Morgans (cM) were identified for targeted mapping in this study. SSR primers and primer sequences were selected from previously mapped high density *Faragaria* × *ananassa* genetic linkage map, reported by Isobe et al. (2013) and data available on-line (http://marker.kazusa.or.jp/Strawberry). At least one SSR marker was selected from all the 28 linkage groups (LGs) with the exception of LG3C and LG6B. No gaps (< 10 cM) were present on these two linkage groups on the Rg × H map, thus no markers were selected from the same linkage groups from the map reported by Isobe et al. (2013). Although no gaps greater than 10 cM were present on the other three linkage groups (LG1D, LG2A and LG3A), two novel SSRs for each LG were selected for mapping. The selected SSR markers were previously developed using publicly available genome sequence data at NCBI EST database (www.ncbi.nlm.nih.gov) and were named with the prefix FVES (*F*. *vesca* expressed sequence tag (EST) - derived markers), FAES (*F*. × *ananassa* EST – derived markers) and FATS (*F*. × *ananassa* transcriptome-derived markers) (Isobe et al. 2013). One marker, with the prefix UFF (*F*. × *ananassa* EST – derived marker) was developed by Spigler et al. (2008). A list of SSR marker acronyms, for all selected and tested markers in this study and targeted regions is presented in Appendix 3.1.

3.2.3 PCR conditions

All SSR markers were first tested in the 'Redgauntlet' and 'Hapil' parental genotypes, and in three, or in some cases four randomly selected seedlings of the progeny for polymorphisms. Secondly, markers that showed clear polymorphisms or amplified many bands were screened in a whole mapping population. Forward marker sequences were re-ordered for polymorphic markers with either a fluorescently labelled dye molecule 6-FAM™ (blue) or VIC™ (green).

Firstly, single primer pair PCR reactions were performed because of unknown amplification regions of markers selected. The final volume for each PCR reaction was 12.5 µl. Each reaction consisted of 1.25 µl 10× PCR buffer (Molzym), 1 µl $MgCl_2$ (2.5 mM) (Applied Biosystems), 1 µl dNTP mix (2 mM) (Life Technologies Ltd), 1.25 µl of forward and reverse unlabelled primer (2 µM), 0.05 µl MolTaq polymerase (1 U) (Molzym), 2.5 µl of genomic DNA (diluted to ~3 ng/µl) and 3.55 µl of sterile distilled water (Sigma). The PCR reactions were performed at the following cycling conditions: the initial denaturation step at 94 ºC for 3 min, followed by 30 cycles of denaturation at 94 ºC for 1 min 30 sec, an annealing temperature of 55-50 ºC for 1 min, decreasing by 0.5 ºC for the first 10 cycles, extension at 72 ºC for 1 min 30 sec, and followed by a final extension step at 72 ºC for 5 min.

Secondly, SSR primer multiplexes (containing two different primer pairs) were developed by combining different fluorescent dyes (blue and green). The PCR amplification reactions were performed using the "Type-it" microsatellite kit (Qiagen) following the manufacturer's protocol in a final volume of 12.5 µl. Due to unique combination of salts and additives, "Type-it" buffer allows the amplification of multiple PCR products. Each multiplexed PCR reaction contained 6.25 µl "Type-it" buffer (Qiagen), 1.25 µl primer mix (2 µM of forward and reverse primer), 2 µl genomic DNA

(diluted to ~3 ng/µl) and 3.5 µl sterile distilled water (Sigma). The PCR cycling conditions for the multiplexed PCR reactions were slightly different from those of the single primer PCR conditions and were as follows (as previously reported by Sargent et al. (2012)): an initial denaturation step of 95 ºC for 5 min was followed by 28 cycles of 95 ºC for 30 sec, an annealing temperature of 55-50 ºC for 1 min 30 sec, decreasing by 0.5 ºC for the first ten cycles, then 72 ºC for 30 sec, followed by a final extension step of 30 min at 60 ºC. PCR protocols for the amplification of the single and multiple primer pairs used in this study are listed in Table 3.1.

Table 3.1 PCR reaction protocol and cycling conditions for amplification of single and multiplex primer pairs

Single primer pair PCR reaction and amplification protocol							
10 × PCR buffer (Molzym)	×	1.25 µl	Initialization	3 min	at	94 ºC	
$MgCl_2$ (25 mM) (Applied Biosystems)	×	1.00 µl		0.30 min	at	94 ºC	
dNTPs (2.5 mM) (Life Technologies Ltd.)	×	1.00 µl	Denaturation	1.30 min	at	55 - 50 ºC	× 10
MolTaq (Molzym)	×	0.05 µl		1.00 min	at	72 ºC	
SSR primer (F + R, 2 µM)	×	1.25 µl		0.30 min	at	94 ºC	
H_2O (Sigma)	×	5.45 µl	Annealing	1.30 min	at	50 ºC	× 25
DNA (~3 ng/µl)	×	2.50 µl		1.00 min	at	72 ºC	
Total		**12.50 µl**	Extension	30 min	at	60 ºC	

Multiple primer pair PCR reaction and amplification protocol							
2 × Type-it master mix (Qiagen)	×	6.25 µl	Initialization	5 min	at	95 ºC	
10 × SSR primer mix (2 µM of each primer)	×	1.25 µl	Denaturation	0.30 min	at	95 ºC	× 28
H_2O (Sigma)	×	3.50 µl		1.30 min	at	55 - 50 ºC	
DNA (~3 ng/µl)	×	2.00 µl	Annealing	0.30 min	at	72 ºC	
Total		**12.50 µl**	Extension	30 min	at	60 ºC	

3.2.4 Product visualization

Single primer PCR reactions products were separated using fragment analyser (Advanced Analytical Technologies, GmbH). A total of 2 µl of PCR product was analysed together with 22 µl 1 × TE buffer (dsDNA Reagent Kit, 35 bp – 1,500 bp; Advanced Analytical Technologies, GmbH). 24 µl of ladder (dsDNA Reagent Kit, 35 bp – 1,500 bp) was loaded to each first well of each row followed by 11 PCR reactions per row. The outputs from the fragment analyser were analysed visually. An example of PCR amplification products separated using fragment analyzer is presented in Figure 3.1.

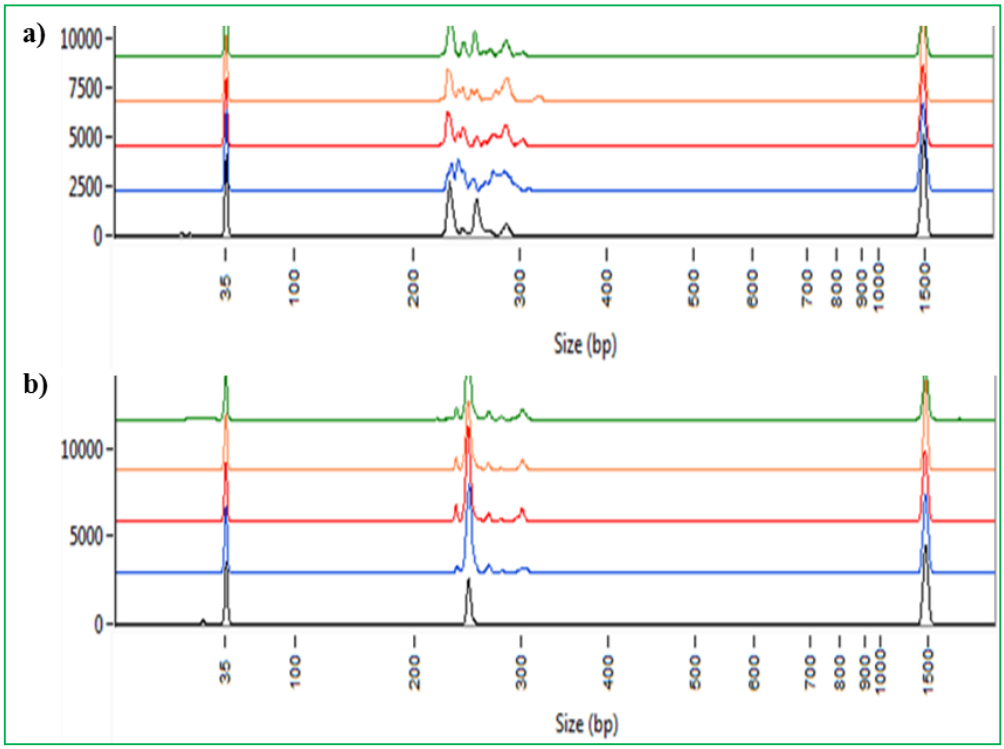


Figure 3.1 PCR product separation using fragment analyzer for two SSR primer pairs of three seedlings of the progeny and parental genotypes (‘Redgauntlet’ and ‘Hapil’): a) fragment analyzer traces of RH001, RH002, RH003, ‘Hapil’ and ‘Redgauntlet’ for FVES1672 primer (from the top); b) fragment analyzer traces of RH009, RH010, RH011, ‘Hapil’ and ‘Redgauntlet’ for FVES3720 primer (from the top).

The SSR markers which showed clear polymorphisms or amplified many bands among the samples tested were further selected to screen in a whole mapping population using an ABI Prism 3130× capillary automated genetic analyser using 36-cm capillary array with POP-7 polymer (Applied Biosystems).

Multiplexed PCR products were diluted 3:10. A total of 1.3 µl of the diluted PCR reaction was analysed together with 7.45 µl of Hi-Di formamide (Life Technologies Ltd.) and 0.25 µl GeneScan 500 Liz™ size standard (Life Technologies Ltd) per reaction. The allocated diluted PCR reaction mixed with other reagents (Hi-Di and Liz 500) were denatured at 95 ºC for 3 min using Thermo cycler just before placing samples to be analysed. The generated electropherograms were analysed using GENESCAN® version 3.7 (Applied Biosystems) and amplification products were visualized graphically using GENOTYPER® version 3.7 (Applied Biosystems) software. An example of genetic analyzer traces of three seedlings and parental genotypes for 2 SSR markers is presented in Figure 3.2.

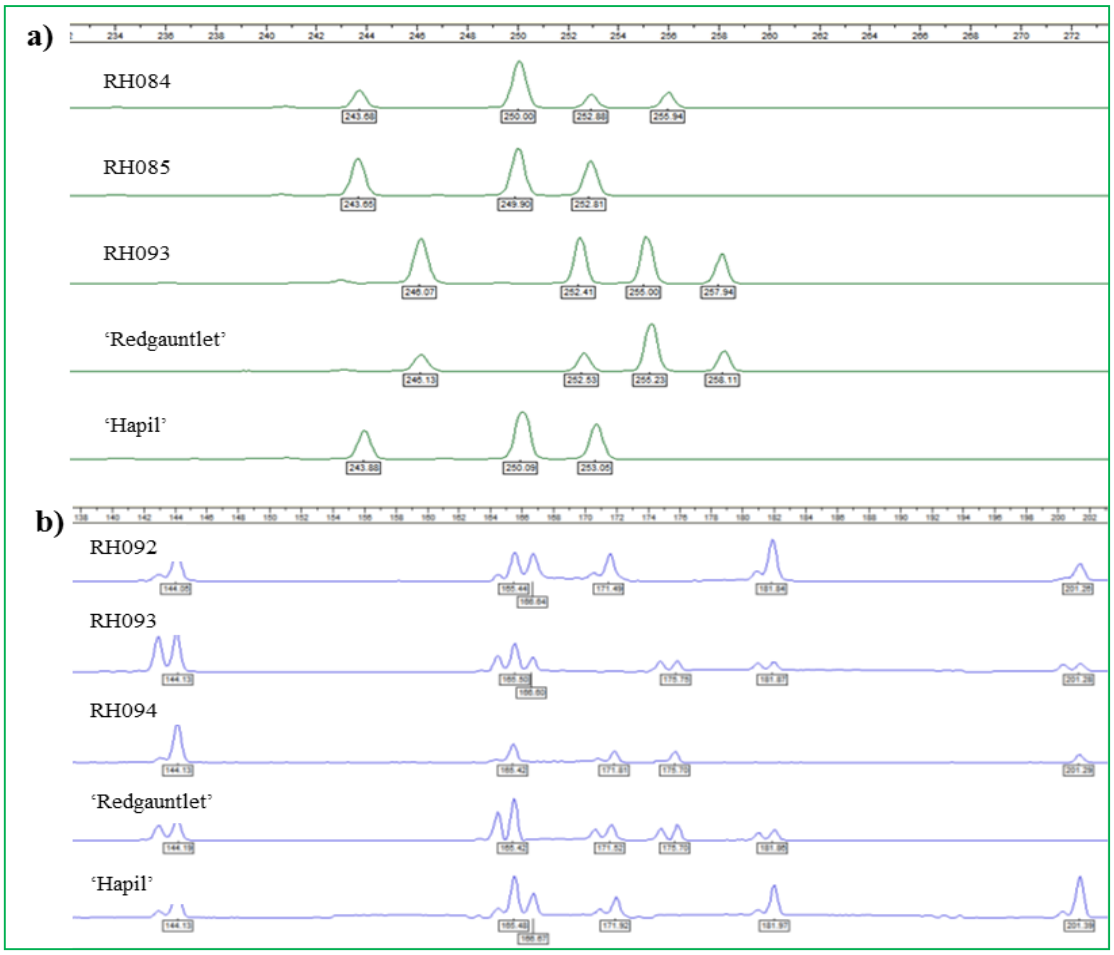


Figure 3.2 An ABI 3100 automated electrophoresis traces for 3 seedlings of the progeny and parental genotypes ('Redgauntlet' and 'Hapil') for FVES1687 in green (a) and FAES0001 in blue (b) SSR markers.

3.2.5 SSR marker amplification and genetic linkage map development

The mapping of novel SSR markers to the targeted regions of the existing SSR-based genetic linkage map was based on combining segregating marker data observed in this study with previously mapped marker data for Rg × H mapping population. Alleles showing segregation types AB × AA (present in female parent 'Redgauntlet'), AA × AB (present in male parent 'Hapil') and AB × AB (present in both parents) were selected for mapping. Alleles at the segregating loci were re-coded, depending on the segregation type, for use in SSR mapping as follows: primers with segregation type AB

× AA were re-coded to lm × ll (1:1 Mendelian ratio), AA ×AB segregations were re-coded as nn × np (1:1 Mendelian ratio) and segregating alleles present in both parental genotypes with segregation type AB × AB were re-coded as hk × hk (3:1 Mendelian ratio).

The targeted mapping of the existing SSR-based genetic map was performed using JoinMap 4.0 (Kyazma, NL) software applying the Haldane mapping function. The function is capable of calculating and converting the recombination frequencies to genetic distances. Novel SSR markers were mapped by analysing a single linkage group at a time. The parameters used for map construction were modified using a more stringent analysis and were as follows: a minimum of a logarithm of the odds (LOD) score of 1.0 used to assign markers to linkage groups and a maximum recombination fraction of 0.4, goodness-of-fit jump threshold of 5.0 and a triplet threshold of 1.0. Markers exhibiting segregation distortions were identified applying the Chi-square (X^2) test. Graphical presentation of an improved SSR-based genetic linkage map of the Rg ×H progeny consisting of 28 linkage groups was generated using MapChart version 2.2 software (Voorrips, 2002). Marker genetic distances on the linkage groups were presented in centi-Morgans (cM).

3.2.6 The genotyping a 90 K Axiom® SNP array in octoploid strawberry

DNA samples for a total of 140 Rg × H progenies and parental genotypes were extracted and purified to the required standards (DNA concentration of ≤ 10 ng/µl and DNA purity of ≤ 1.65). A total of 30 µl of each DNA sample were aliquoted into 96 well skirted plates (ThermoFisher) and were sealed using adhesive seals before samples were submitted to Affymetrix UK Ltd. (High Wycombe, UK) for genotyping. DNA concentrations and purity observed for all individuals are presented in Table 3.2.

The Affymetrix GeneTitan® system was applied to genotype samples as described by Affymetrix (http://media.affymetrix.com/support/dowloads/manuals/axiom_2_assay_auto_workflow_user_guide.pdf). Data files (cell intensity files .CEL) were generated by the Affymetrix GeneTitan® system and were provided for genotypic data analysis.

Table 3.2 DNA concentration (ng/µl) and DNA purity (A_{260}/A_{280}) for all individuals of Rg × H progeny and parental genotypes used for high-throughput genotyping

Individual	DNA concentration (ng/µl)	DNA purity (A260/A280)	Individual	DNA concentration (ng/µl)	DNA purity (A260/A280)
Hapil	23.48	1.65	RH043	129.54	1.81
Redgauntlet	45.83	1.87	RH047	43.69	1.80
RH004	62.47	1.84	RH049	41.32	1.87
RH005	26.53	1.71	RH050	70.27	1.89
RH006	45.03	1.78	RH053	28.83	1.85
RH007	77.39	1.86	RH055	71.69	1.84
RH008	32.37	1.76	RH056	51.67	1.84
RH009	24.68	1.77	RH057	58.17	1.83
RH011	97.67	1.85	RH060	55.00	1.83
RH012	46.81	1.82	RH061	67.61	1.85
RH014	20.08	1.75	RH062	46.30	1.75
RH017	34.14	1.79	RH068	19.98	1.78
RH018	61.49	1.87	RH069	20.63	1.99
RH019	33.45	1.73	RH071	36.93	1.73
RH020	96.88	1.84	RH072	56.75	1.93
RH021	38.66	1.72	RH074	35.93	1.83
RH022	14.32	1.81	RH075	34.16	1.80
RH024	41.75	1.77	RH076	28.78	1.76
RH026	21.28	2.07	RH077	8.64	1.74
RH027	47.30	1.79	RH078	49.00	1.76
RH031	74.40	1.81	RH079	34.58	1.72
RH033	26.91	1.78	RH080	84.48	1.82
RH034	23.84	1.61	RH081	27.25	1.87
RH035	53.54	1.76	RH083	20.27	1.79
RH037	34.62	1.81	RH085	27.92	1.73
RH038	47.61	1.86	RH086	85.49	1.76
RH039	32.54	1.72	RH087	26.60	1.75
RH041	30.30	1.86	RH088	47.79	1.83
RH042	109.57	1.82	RH089	59.55	1.82

Table 3.2 Continued

Individual	DNA concentration (ng/µl)	DNA purity (A260/A280)	Individual	DNA concentration (ng/µl)	DNA purity (A260/A280)
RH090	29.24	1.74	RH142	50.88	1.96
RH091	109.89	1.85	RH143	36.45	1.86
RH093	63.06	1.83	RH144	25.66	1.80
RH094	51.18	1.75	RH145	58.97	1.88
RH095	30.78	1.80	RH146	27.43	1.84
RH096	255.59	1.82	RH147	24.23	2.00
RH097	21.54	1.69	RH148	27.97	2.00
RH100	53.17	1.75	RH149	8.29	1.93
RH101	75.42	1.85	RH150	53.66	1.81
RH102	52.24	1.87	RH152	28.52	1.70
RH106	98.72	1.85	RH153	24.06	1.94
RH107	11.47	1.76	RH154	54.86	1.79
RH108	58.85	1.77	RH155	90.49	1.87
RH109	22.22	1.59	RH156	69.40	1.82
RH110	17.16	1.76	RH157	35.81	1.66
RH111	18.51	1.76	RH158	50.29	1.78
RH112	39.16	1.75	RH159	52.31	1.85
RH115	35.37	1.78	RH160	57.09	1.69
RH116	33.63	1.83	RH161	86.26	1.81
RH117	49.38	1.82	RH162	106.51	1.78
RH118	52.22	1.79	RH163	55.22	1.82
RH119	36.18	1.88	RH164	43.81	1.79
RH120	47.16	1.79	RH165	63.78	1.75
RH121	37.47	1.76	RH167	20.62	1.65
RH122	55.61	1.81	RH168	53.83	1.79
RH123	19.07	1.94	RH169	37.62	1.75
RH124	27.33	1.78	RH170	51.33	1.74
RH125	86.98	1.85	RH172	25.13	1.74
RH127	20.34	1.67	RH173	35.36	1.73
RH128	46.44	1.92	RH174	40.99	1.81
RH129	18.83	1.90	RH175	38.45	1.72
RH131	13.10	2.01	RH176	52.29	1.77
RH132	16.11	1.88	RH177	12.98	2.01
RH133	112.71	1.85	RH178	84.18	1.87
RH134	42.54	1.91	RH179	12.48	1.67
RH135	81.68	1.82	RH180	28.24	1.82
RH136	26.11	1.88	RH182	24.11	1.86
RH137	33.62	1.59	RH184	12.80	1.67
RH138	45.44	2.08	RH185	26.29	1.77
RH139	51.38	1.59	RH186	32.62	1.77
RH140	29.34	1.97	RH187	49.60	1.80
RH141	25.54	1.86	RH188	15.80	1.66

3.2.7 90 K Axiom array data analysis

A large set of SNP marker genotypic data generated using 90 K SNP genotyping array was analysed using a set of Affymetrics suggested softwares and customs scripts written in Python (www.python.org). The data analysis of the genotypic data was performed by a bioinformatician (Dr Robert Vickerstaff, East Malling Research). The generated data files were firstly converted to genotype calls using Genotyping Console™, version 4.1 software. Secondly, the quality of the genotypic data was checked for all samples and SNPs observed. SNP markers with low call rates and/or below the standard passing threshold of 95% were excluded from the dataset. Markers which passed the standard quality check procedure were further analysed by grouping markers into one of six quality categories using Affymetrix's 'SNPolisher' package in R. The categories used to group SNPs according to their clustering performance were as follows: Poly High Resolution (three clusters of polymorphic SNPs), No Minor Homozygote (two clusters of polymorphic SNPs), Off-Target variant (three clusters with an additional cluster), Mono High Resolution (monomorphic SNPs), Call Rate Below Threshold (well clustered but call rate was below 97% SNPs) and Other (clusters, which did not fall into other 5 group clustering properties). An example of SNP quality clustering observed for genotyping Rg × H progeny in this study is presented in Figure 3.3.

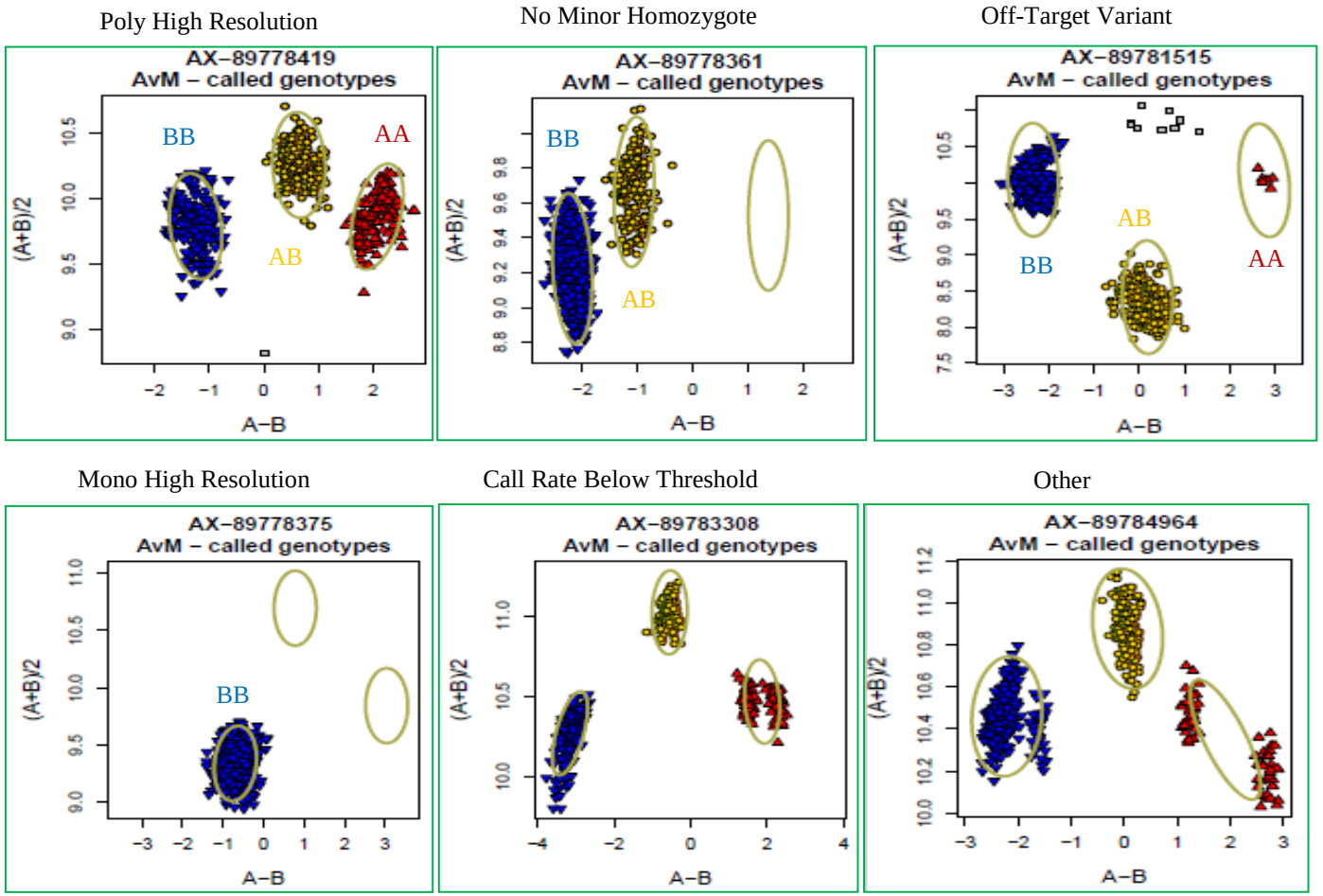


Figure 3.3 An example of six SNP quality clusters revealed following genotypic data analysis of genotyping 'Redgauntlet' × 'Hapil' mapping progeny.

Two different types of SNP quality classes (Poly High Resolution and No Minor Homozygote) were selected for the construction of the SNP-based linkage map. Of those, a further set of SNP markers which failed to produce any amplification products or failed in at least one of the parental genotypes in the assay were excluded from the analysis. SNP markers for which the number of missing genotypes was greater than 5% were not considered for map construction.

Identical SNP markers observed in the assay were grouped into single bins with the purpose of complexity reduction for the linkage analysis. A single SNP containing no missing data for a progeny was used for linkage analysis from each bin.

3.2.8 SNP-base map construction

The consensus SNP-based genetic linkage map was constructed using personally developed scripts, due to the large data set generated. The scripts were developed and linkage groups were identified by Dr Richard Harrison (East Malling Research). Once SNP markers were assigned to each of 28 linkage groups using the perl scripting language, data was further analysed using JoinMap 4.0 (Kyazma, NL) software with the same parameters set as described in 3.2.5 Section of this Chapter. Linkage groups were identified and named accordingly by comparing each chromosome to the previously reported high density SNP-based linkage map constructed for an octoploid strawberry mapping population 'Holiday' × 'Korona' (van Dijk et al. 2014). Graphical presentation of SNP-based genetic map was performed using MapChart 2.2 software (Voorrips, 2002), as described in 3.2.5 Section of this Chapter.

3.3 Results

3.3.1 Simple sequence repeat marker polymorphisms

A total of 111 SSR primer pairs, previously reported by Isobe et al. (2013), were selected and tested in the parental genotypes and three or four progenies for the initial polymorphisms using fragment analyser. Of those, four SSR primer pairs failed to amplify any PCR product, fourteen amplified products that were not polymorphic, six amplified PCR products larger than 500 bp (base pairs) size and were excluded from further analysis (ABI genetic analyser is only capable of separating PCR products up to 500 bp size, due to the specific size standard used). Additionally, seven SSRs showed amplification but were too weak or complex to score and were not considered for progeny screening. The remaining 80 SSR primer pairs showing possible but not guaranteed segregating products were further investigated and were reduced to 43 SSRs, which were screened in a whole Rg × H mapping population.

Of the 43 SSR primer pairs which were screened for polymorphism in all Rg × H mapping progenies, six primer pairs failed to amplify products or the amplification products were too weak to score for all or the majority of the progenies, six amplified non-segregating PCR products, three were too complex to score and were excluded from the analysis. The remaining 28 primer pairs amplified polymorphic PCR products in the population and were used for mapping (Appendix 3.1).

Each segregating allele for each of the 28 polymorphic primer pairs was scored individually as 1 if the allele was present in the genotype, and as 0 if the allele was absent. A total of 107 segregating loci were generated in the Rg × H progeny. Of those, 70 loci representing 26 SSR markers were successfully allocated to the map positions and mapped, whilst the remaining 37 loci (2 SSR markers) were unlinked and excluded.

The largest number of loci per marker (seven loci) was mapped for two SSR markers (FVES1687 and FVES1414), followed by six and five loci amplified by FVES1672 and FVES1409 markers respectively. The majority of SSRs amplified two or one loci per marker and were mapped to the genetic linkage map.

The largest number of mapped SSRs (19) were developed from diploid *Fragaria* expressed sequence tags (EST) (FVES). Five mapped SSRs were designed using octoploid *Fragaria* EST (FAES) and only two SSRs were developed from octoploid *Fragaria* transcriptome (FATS).

The number of loci mapped for each of the three segregation types (AB × AA, AA × AB and AB × AB) was distributed nearly evenly with a minor dominance of AB × AA markers. A total of 41 mapped loci (38.3%) segregated in the female ('Redgauntlet') parent, 35 loci (32.7%) were heterozygous in the male ('Hapil') parent and the remaining 31 loci (29%) were heterozygous in both parents.

3.3.2 Novel SSR mapping and loci distribution

A total of 28 SSR primer pairs of the 111 tested amplified 107 loci and were used for saturation of the gaps of an existing linkage map of the Rg × H mapping population. Of those, 70 loci representing 26 SSR markers were mapped (Figure 3.4). The reconstructed SSR-based linkage map consisted of 28 linkage groups (LG), the number of chromosomes in octoploid strawberry genome. In addition, the existing LG6B split into two parts in this study due to more stringent parameters used for mapping analysis and were named as LG6Ba and LG6Bb respectively. Additionally, two unlinked linkage groups were identified and named as Un1 and Un2.

Linkage map reconstruction resulted in mapping more loci. A total of 824 loci were mapped to the new linkage map, whereas 598 loci were mapped to the existing

map, resulting in coverage of longer genetic distance. The largest number of markers mapped on a single linkage group was 63 loci and were mapped on LG3A, and the least number of markers mapped was 14 loci, mapped on LG5A and LG7C. Linkage group 5A was the shortest LG on the map spanning only 36.7 cM, whilst LG6A was the longest and spanned 154.1 cM. The two split linkage groups (LG2D and LG6B), and two unlinked LGs (Un1 and Un2) were excluded for the comparison analysis. The reconstructed SSR-based octoploid strawberry linkage map of the Rg × H population is presented in Figure 3.4.

Marker positions for each of the 28 linkage groups of the newly developed linkage map were compared to the marker order of the existing SSR-based linkage map. In general, the marker order of the new map corresponded to nearly exact positions of the existing map. Only minor changes were observed for a small number of SSRs on some linkage groups (Figure 3.4). The most number of discrepancies in marker order were observed among LG7D and FG7C, and FG7D. The results show that some markers mapped to LG7D in this study were previously mapped to FG7C and FG7D. This can be explained by the more stringent parameters used for map construction in this study.

Marker saturation within 28 linkage groups was good, although eleven regions with low marker density (gaps) greater than 20 cM and 39 regions greater than 10 cM were observed. Seven linkage groups (LG1C, LG2A, LG2B, LG2C, LG4A, LG5A and LG7C) were well covered with SSR markers and had no gaps greater than 10 cM. However, one linkage group (LG6C) showed six, one linkage group (LG6D) showed five and one linkage group (LG3C) showed four gaps greater than 10 cM. The largest gap with no markers was 30.7 cM in length on LG7B. In addition, five linkage groups (LG3B, LG4D, LG6A, LG6C and LG6D) had gaps greater than 26 cM in length.

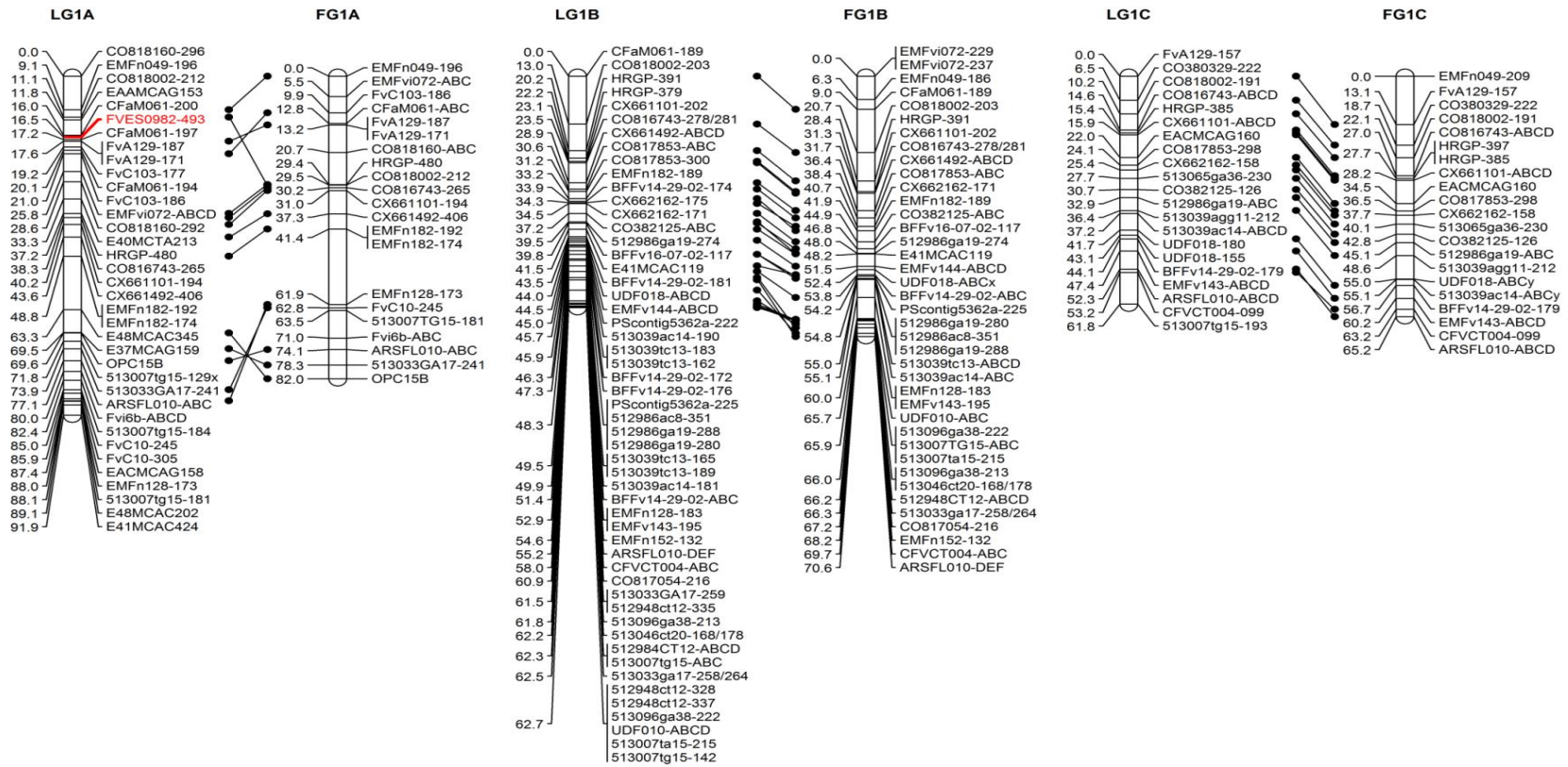


Figure 3.4 A comparison of the genetic positions of SSR markers in an octoploid strawberry (Rg × H) progeny. Marker positions on linkage groups LG1 – LG7 were determined in this study, whilst those named FG1 – FG7 were determined from the existing linkage map. Novel SSR map contained 824 loci and spanned a total genetic distance of 2,486.3 cM. SSR loci mapped in this study are highlighted in red. Two unlinked LGs are named Un1 and Un2.

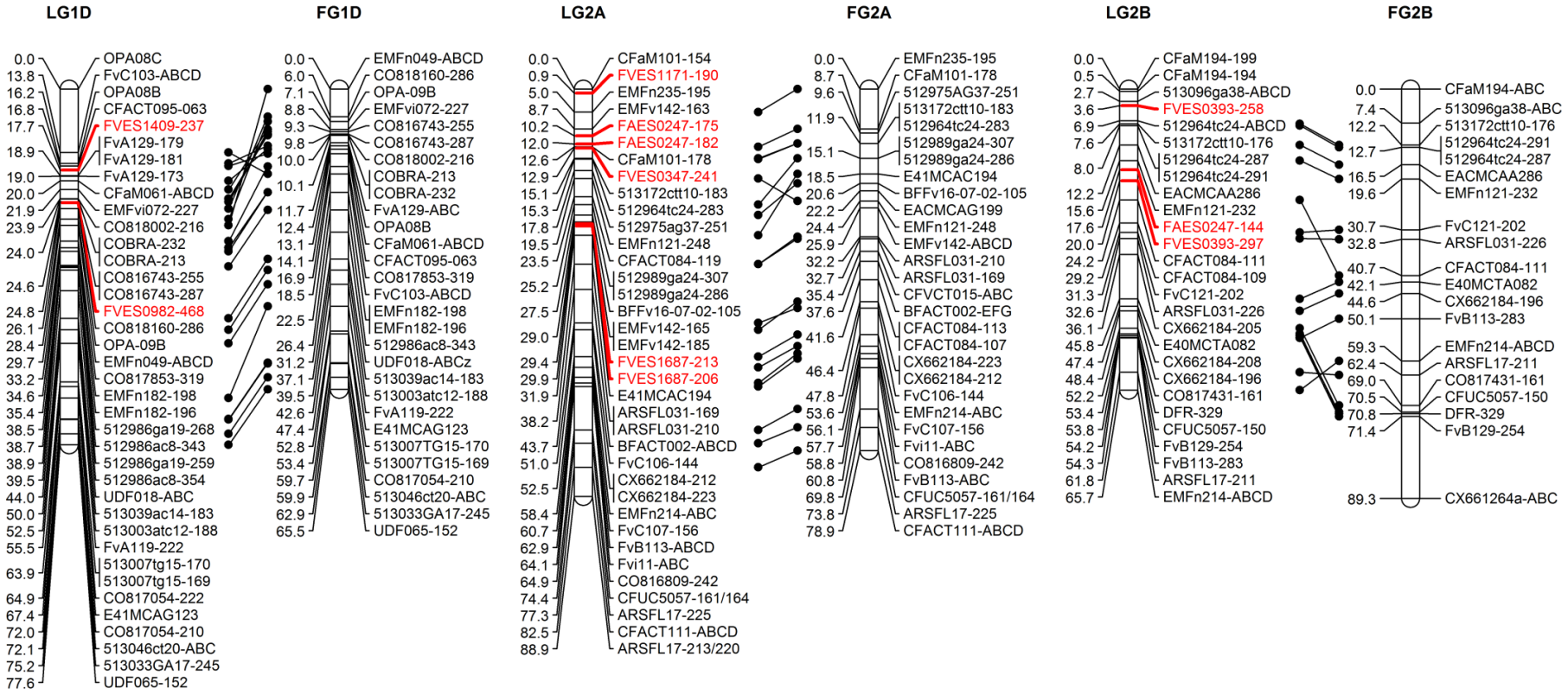
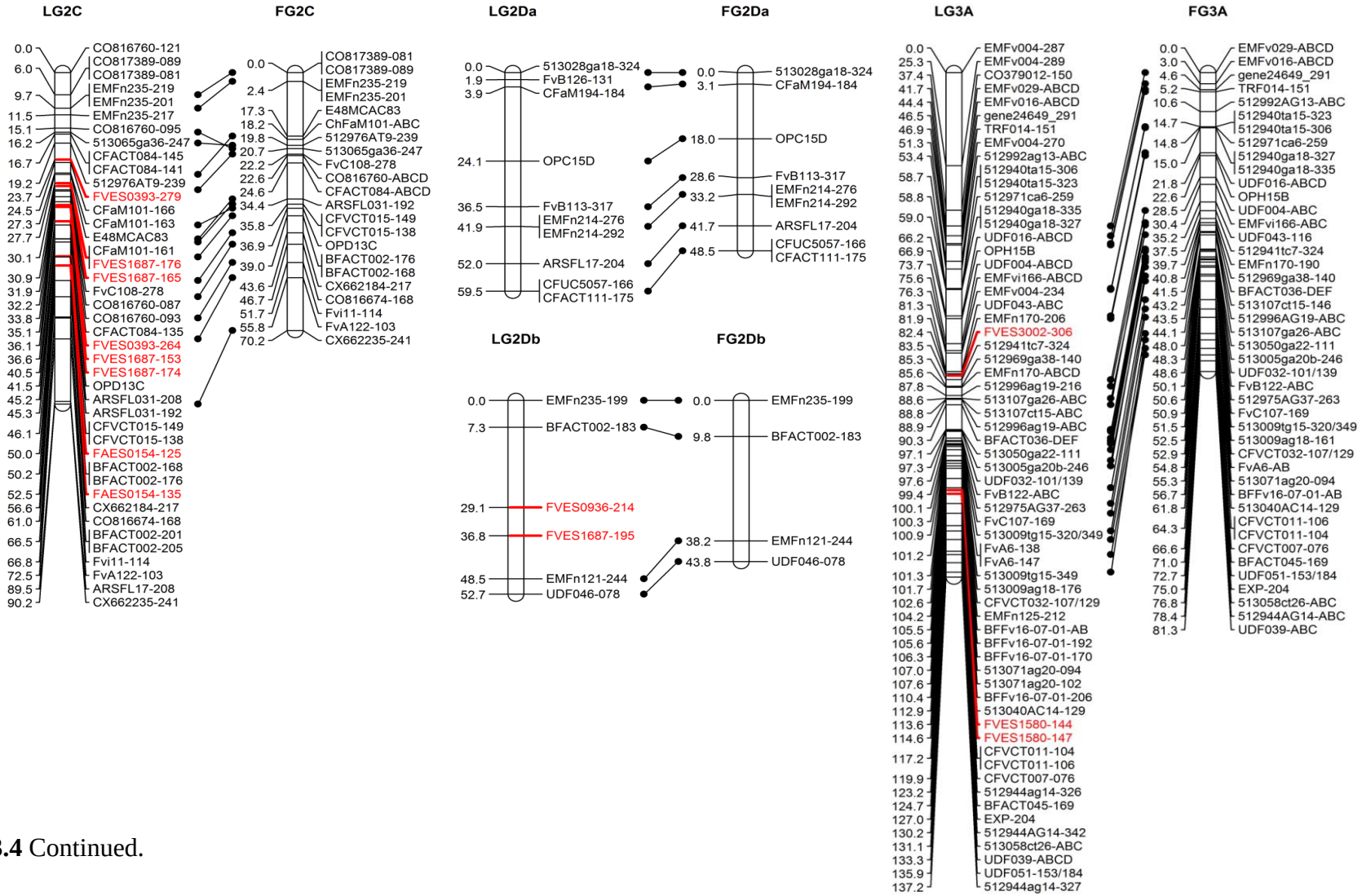


Figure 3.4 Continued.



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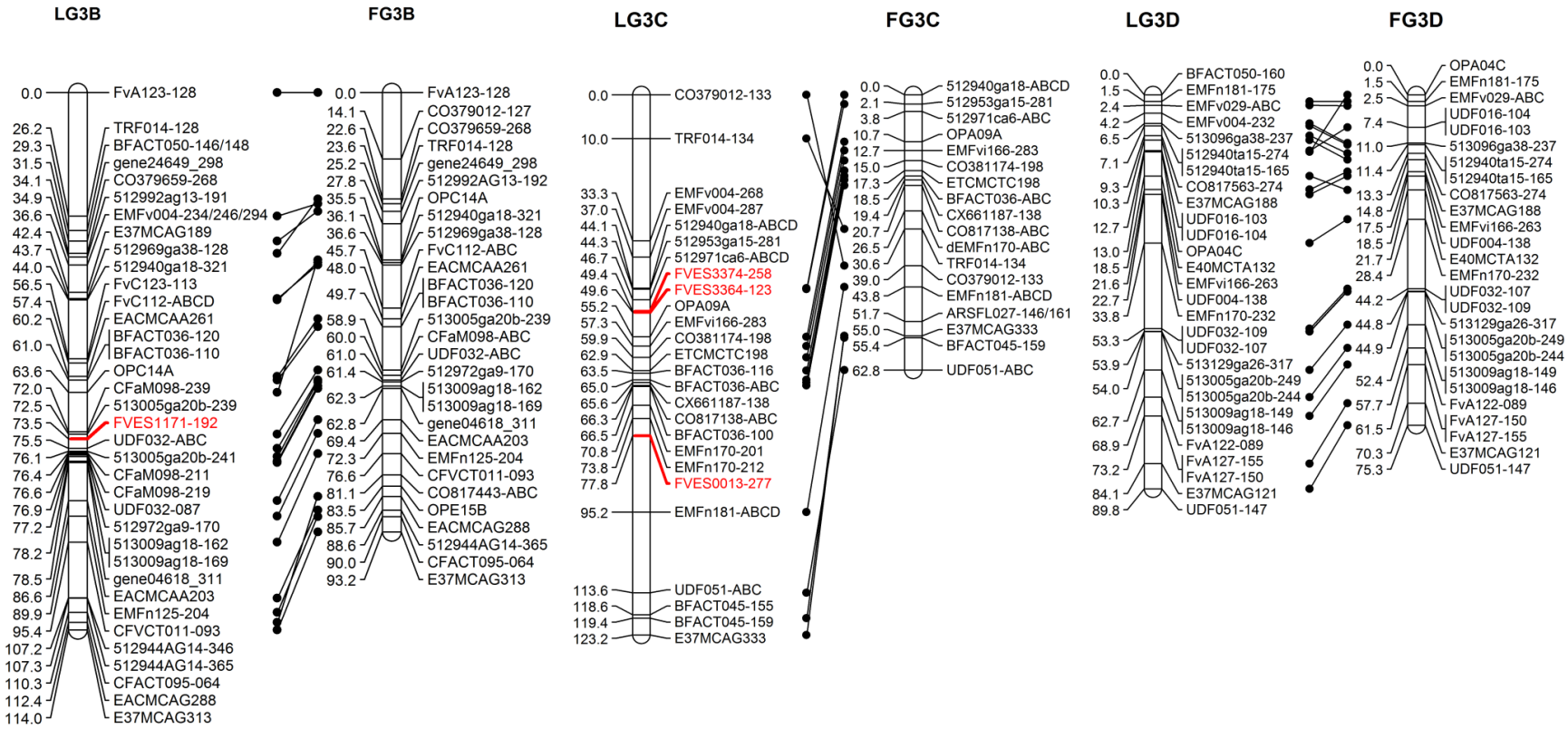

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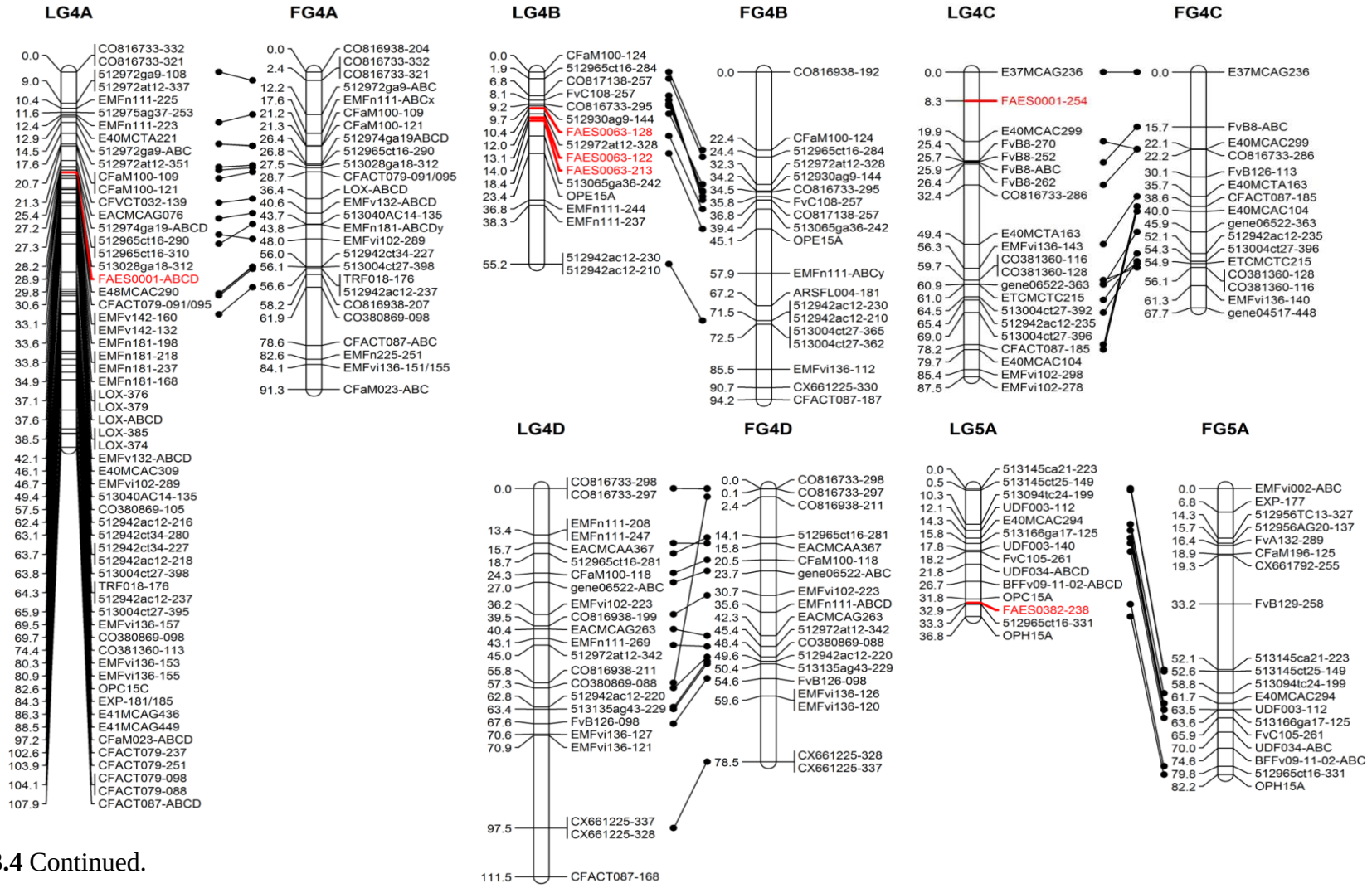

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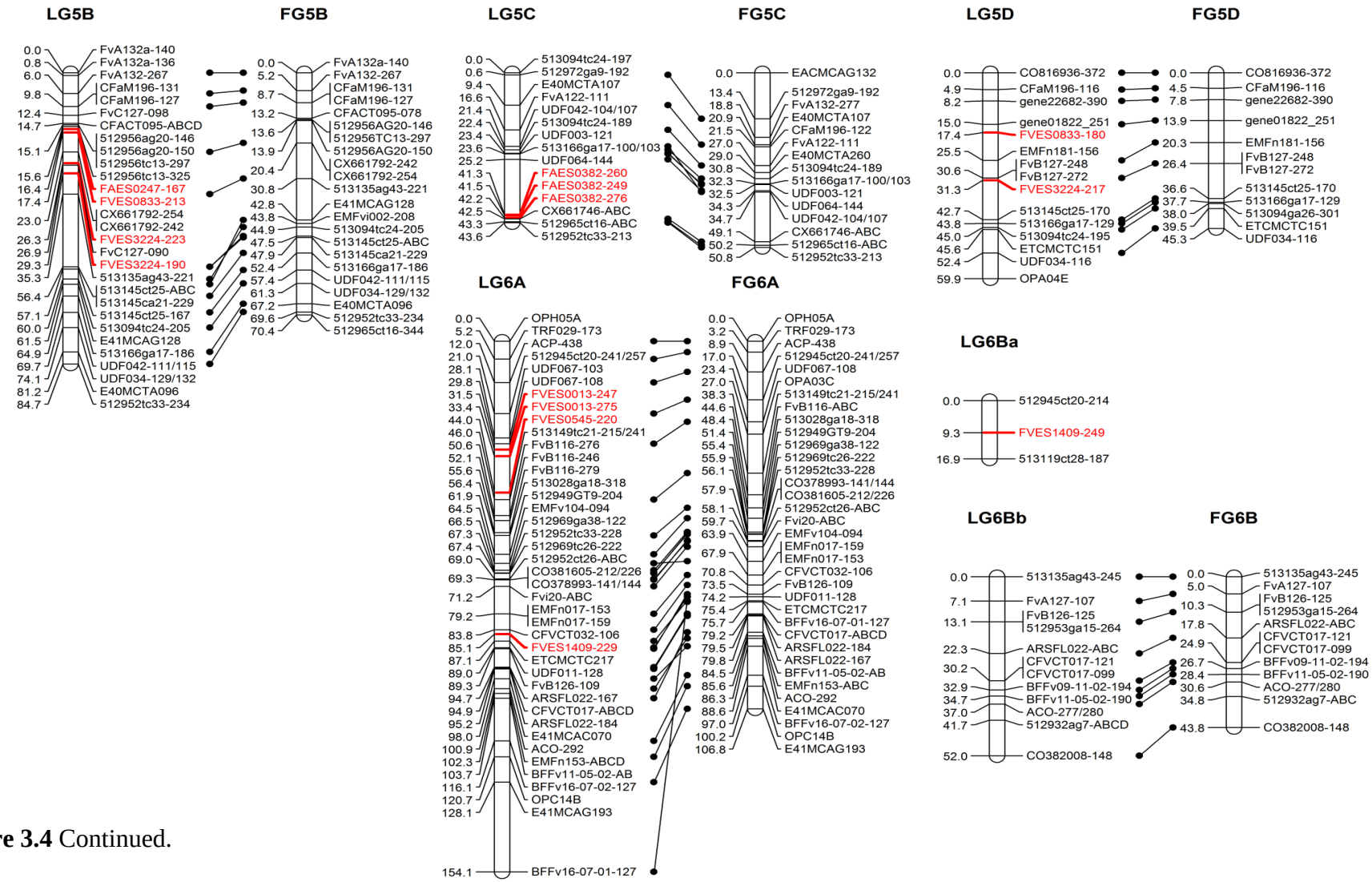

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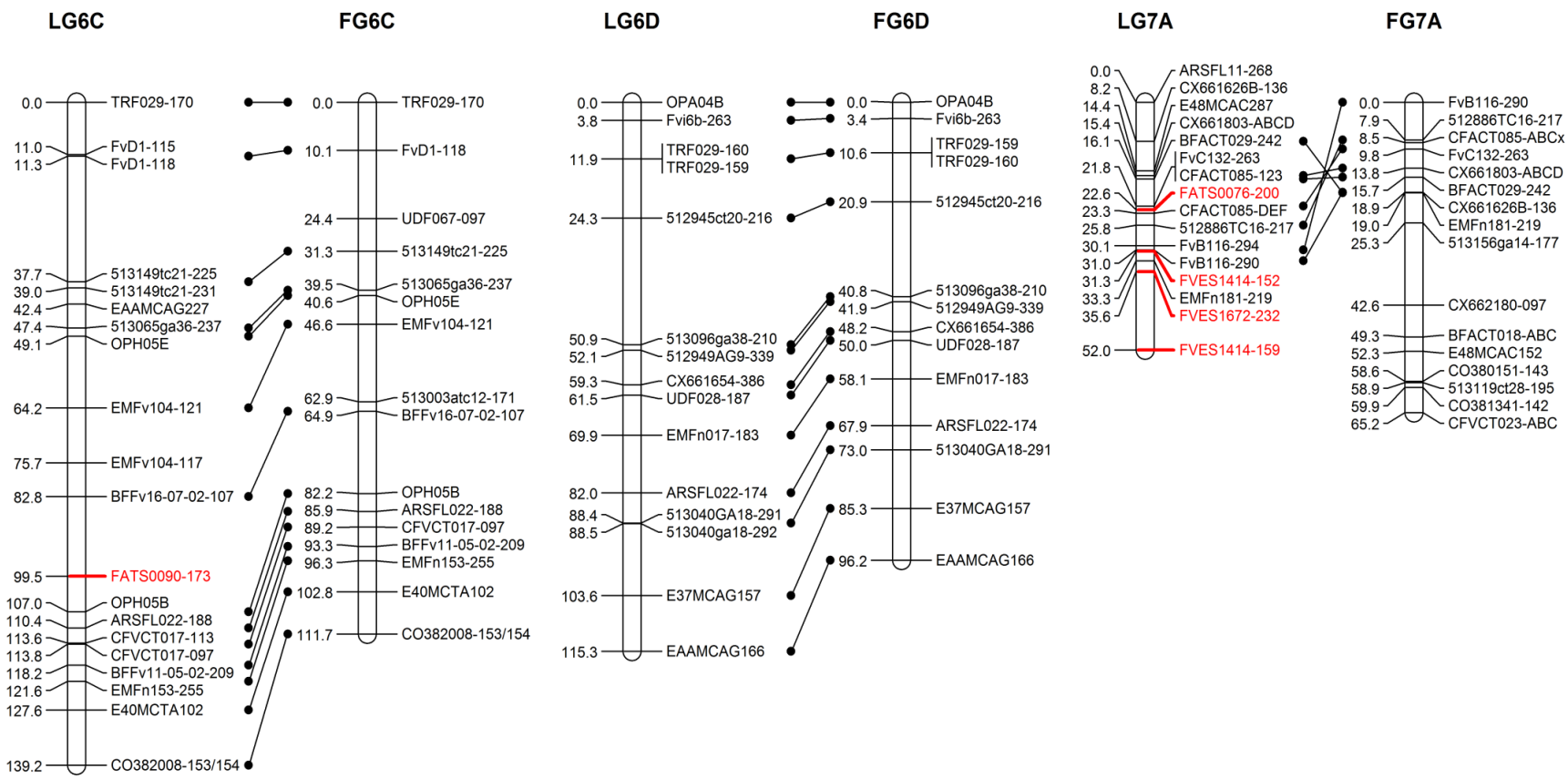

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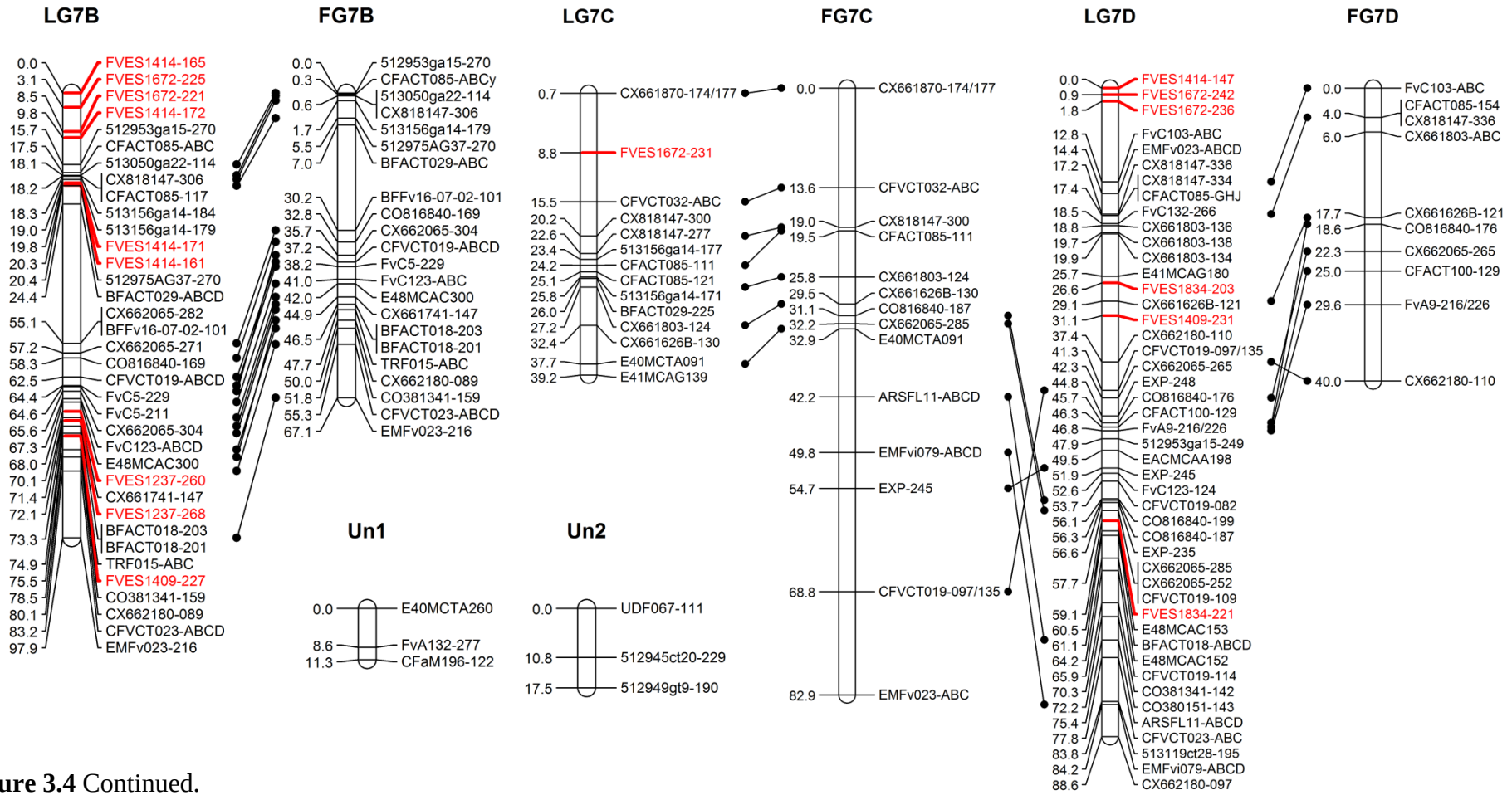

Figure 3.4 Continued.

Of the novel 26 SSRs mapped to the Rg × H map, five were mapped on the same linkage groups as previously reported by Isobe et al. (2013). Of those, only three markers (FVES3224, FVES0013 and FVES1672) were mapped within targeted regions. The majority of new loci mapped within homoeologous linkage groups. For example, marker FAES0154 mapped to LG2C, FVES0347 to LG2A and FVES0982 mapped two loci to LG1A and LG1D, instead of to LG2B, LG2C and LG1B mapped previously. Three markers (FVES1409, FVES0545 and FVES1580) were mapped to unexpected linkage groups based on the positions mapped in the Isobe et al. (2013) genetic map. Marker FVES1409 mapped four loci to LG1D, LG6A, LG6Bd and LG7D instead of to linkage group 4D; marker FVES0545 mapped to LG6A and marker FVES1580 mapped to LG3A, instead of to LG5A and LG7A. In addition, one locus for three markers (FAES0247, FVES1171 and FVES0013) was mapped to an unexpected linkage group (Table 3.3, loci highlighted in red).

Table 3.3 Summary of SSR markers mapped in this study, details of their fluorescently labelled primer names, previously mapped linkage groups and mapped positions of selected markers, linkage groups and map positions to which SSRs were mapped in this study, targeted region and number of loci per SSR mapped. Linkage groups highlighted in bold represent markers which mapped to the same linkage groups and targeted regions. Loci in red were mapped to unexpected linkage groups

Marker	Isobe et al. (2013)		Mapped in this study		Target region	Loci mapped
	LG	cM	LG	cM		
FVES0982	1B	18.3	1A, 1D	16.5, 24.8	9.0 - 20.7	2
FAES0154	2B	20.4	2C	49.9/52.5	19.6 - 30.7	2
FVES1687	2B	29.3	2A, 2C, 2Db	29.4/29.9, 30.1/30.9/36.6/40.5, 36.8	19.6 - 30.7	7
FAES0247	2C	84.2	2A, 2B, 5B	10.2/12.0, 17.6, 16.4	55.8 - 70.2	4
FVES0347	2C	56.6	2A	12.9	55.8 - 70.2	1
FVES0393	**2C**	10.3	2B, **2C**	3.6/19.9, **23.7/36.1**	**2.4 - 17.3**	4
FVES0936	2C	17.9	2Db	29.1	2.4 - 17.3	1
FVES1171	3A	67.5	2A, 3B	0.9, 73.5	-[1]	2
FVES3374	3B	0.0	3C	49.4	0.0 - 14.1	1
FVES3002	3D	32.6	3A	82.4	28.4 - 44.2	1
FVES3364	3D	41.2	3C	49.6	28.4 - 44.2	1
FAES0001	4D	8.9	4A, 4C	28.9, 8.3	2.4 - 14.1	2
FAES0063	4D	5.4	4B	10.4/13.1/14.0	2.4 - 14.1	3
FVES1409	4D	11.3	1D, 6A, 6Ba, 7D	17.7, 85.0, 9.2, 31.1	2.4 - 14.1	5
FVES0545	5A	34.5	6A	43.9	33.2 - 52.1	1
FVES3224	**5B**	39.6	**5B**, 5D	**26.3/29.3**, 31.3	**20.4 - 30.8**	3
FVES0833	5C	0.0	5B, 5D	17.4, 17.4	0.0 - 13.4	2
FAES0382	5D	34.4	5A, 5C	32.9, 41.3/41.5/42.2	26.4 - 36.6	4
FVES0013	**6A**	35.3	3C, **6A**	77.8, **31.5/33.4**	**27.0 - 38.3**	3
FATS0090	6D	27.9	6C	99.5	20.9 - 40.8	1
FVES1580	7A	28.5	3A	113.6/114.6	25.3 - 42.6	2
FATS0076	7B	63.3	7A	22.6	55.3 - 67.1	1
FVES1834	7B	72.4	7D	26.6/59.1	55.3 - 67.1	2
FVES1672	**7C**	6.7	7A, 7B, **7C**, 7D	35.6, 3.1/8.5, **8.8**, 0.9/1.8	**0.0 - 13.6**	6
FVES1237	7D	9.7	7B	70.1/72.1	6.0 - 17.7	2
FVES1414	**7D**	38.0	7A, 7B, **7D**	31.3/52.0, 0.0/9.8/19.8/20.3, **0.0**	**29.6 - 40.0**	7

[1]No target region was selected

3.3.3 SNP marker amplification in 'Redgauntlet' × 'Hapil' progeny

A total of 140 seedlings and parental genotypes were genotyped using the 90 K IStraw® (International Strawberry) genotyping array containing a total of 95,062 SNP markers interrogated with 138,099 probe sets. Of those, 85,663 (128,099 probe sets) SNPs were from the octoploid genome, 3,751 (4,000 probe sets) were target sites from the diploid *Fragaria iinumae* genome, and 5,648 (6,000 probe sets) were codon-based sites (Bassil et al. 2015). Following the initial quality control analysis of the genotypic data, 58,919 (42.7%) probe sets failed standard Affymetrix quality control analysis, 42,057 (30.5%) probe sets were monomorphic and the remaining 37,123 (26.8%) probe sets were non-monomorphic (includes no minor homozygote and polymorphic hi resolution clusters) in the Rg × H progeny.

The non-monomorphic probe sets were further quality controlled and checked based on call rates. Of those, 20,704 probe sets failed to pass the threshold of 97% and were excluded from the further analysis, the remaining 16,419 probe sets passed the quality check and were used for SNP-based linkage map construction.

Polymorphic SNPs revealing identical scores for all individuals in the progeny and parents of the cross were collapsed into bins for an easier map construction and representation. Of the 16,419 heterozygous probe sets, 2,946 (17.9%) were polymorphic in both parents of mapping progeny and were grouped into 1,564 unique bins, 7,129 (43.4%) probe sets were polymorphic in the 'Redgauntlet' parental genotype and were collapsed into 1,600 genotyping bins, and the remaining 6,344 (38.6%) were heterozygous in the 'Hapil' parent and were grouped into 1,615 bins (Figure 3.5). As a result, a total of 16,419 probe sets observed in the data were collapsed into 4,779 bins and were used for linkage map construction.

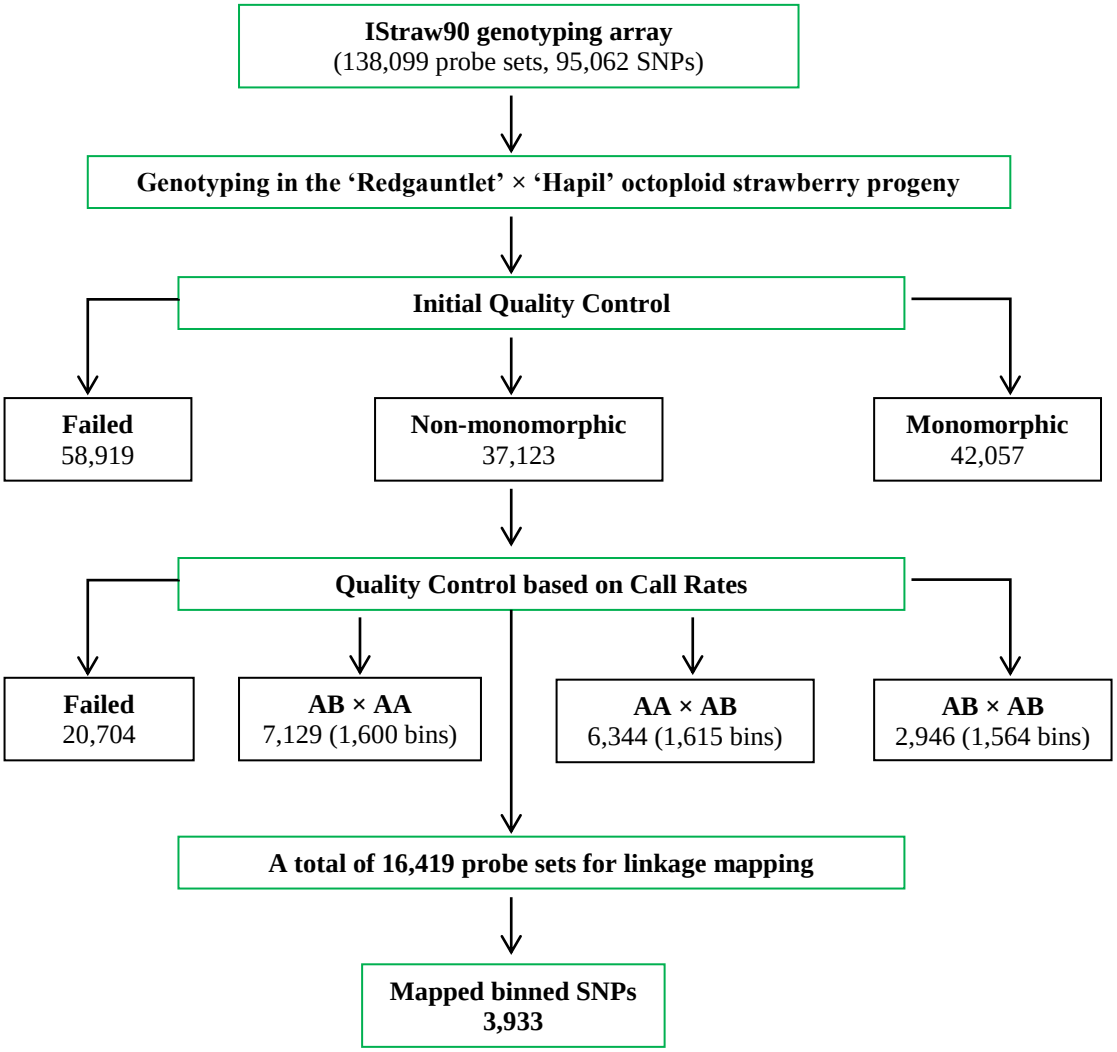

Figure 3.5 The IStraw90 genotyping array amplification results in the octoploid strawberry mapping progeny 'Redgauntlet' × 'Hapil'. Out of 95,062 SNPs, 3,933 binned markers were successfully mapped to the Rg × H linkage map.

3.3.4 SNP-based linkage map construction

The construction of the consensus SNP-based linkage map was based on heterozygous SNPs observed in this investigation. The linkage map of the Rg × H octoploid strawberry progeny spanned a total genetic distance of 2,624.7 cM and contained 3,933 binned SNP markers. SNP marker density per linkage group ranged from 0.4 to 1.3 cM per SNP. A schematic picture of the integrated linkage map is presented in Figure 3.6.

The distributions of all mapped markers in linkage groups, map length in centi-Morgans (cM) and marker density for each LG for all 28 linkage groups are presented in Table 3.4.

Linkage group 2A (LG2A) spanned 162.8 cM and was the longest on the SNP-based Rg × H map, whilst LG5D was the shortest on the map, spanning 58.3 cM. The highest number of SNPs mapped to the single linkage group was 272 on LG6A, the least was 72 on LG5B. The average mapped SNP marker density on the map was one marker every 0.7 cM, resulting in high map saturation and only three regions of low marker density greater than 20 cM were observed. The largest region to which no markers were mapped was 31 cM length on LG6C. Interestingly, the same linkage group (LG6C) had the most regions with no markers mapped on the SSR-based linkage map (five regions greater than 10 cM and one region greater than 20 cM with no markers mapped). Two other linkage groups (LG4D and LG7B) had gaps of 24 cM and 12 cM, and 20 cM with no markers mapped respectively. In addition, 13 regions greater than 10 cM with no markers were observed on the SNP-based linkage map. Of those, two gaps were observed on a single linkage group for LG5B (18 cM and 12 cM), LG6B (14 cM and 12 cM) and LG7A (14 cM and 13 cM) each. The remaining six gaps were observed on different linkage groups once: LG1D (19 cM), LG2D (12 cM), LG3C (12 cM), LG4A (13 cM), LG4B (10 cM) and LG7C (11 cM) (Table 3.4).

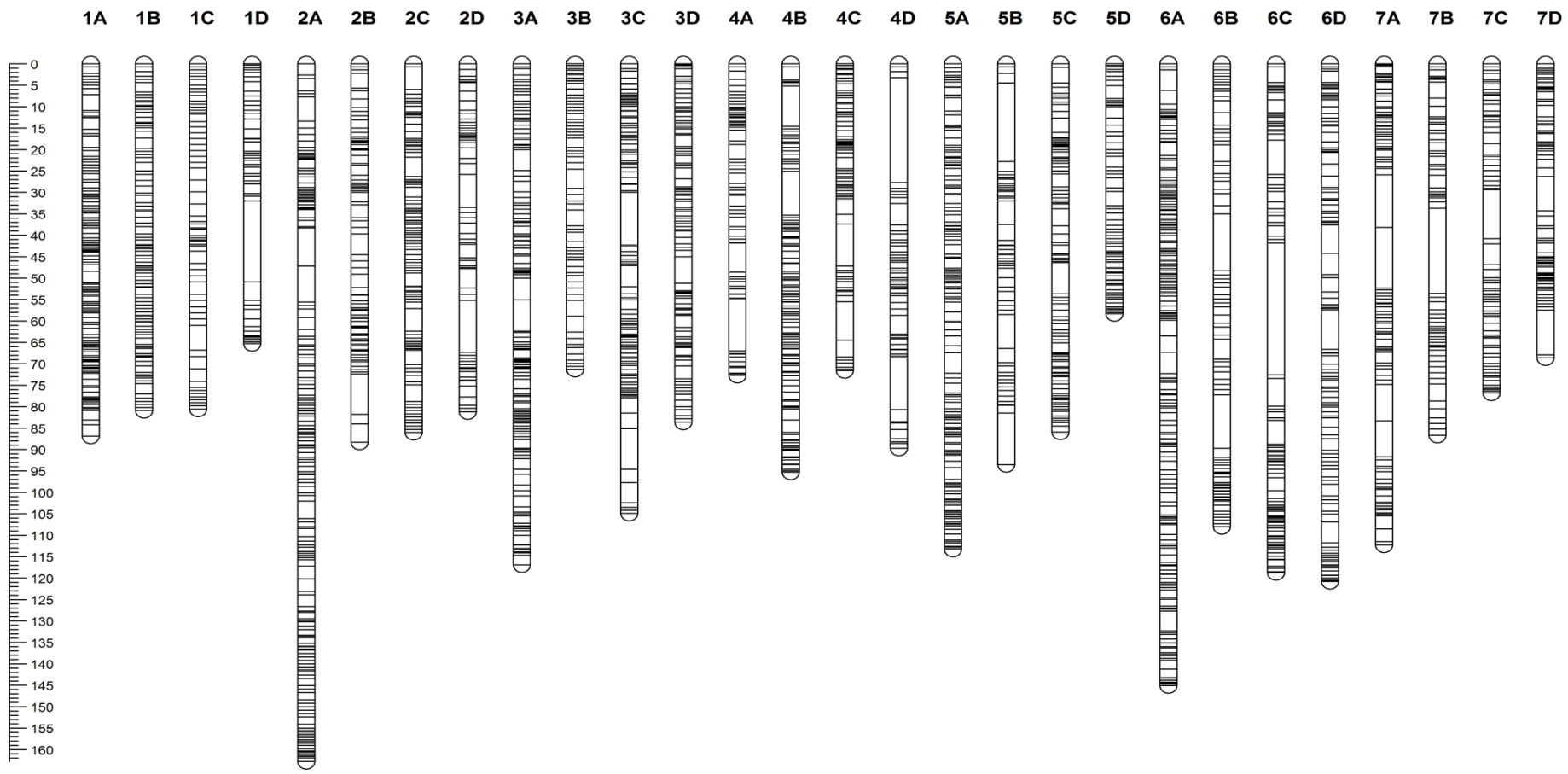

Figure 3.6 An integrated SNP-based consensus linkage map of an octoploid strawberry mapping progeny Rg × H composed of 3,933 binned SNP markers, generated with the IStraw90 array in this study. Map spans all 28 linkage groups of *F. ananassa* and a total genetic distance of 2,624.7 cM. The scale in centi-Morgans is given at the edge of the figure.

Table 3.4 Total number of SNP markers mapped in the 'Redgauntlet' × 'Hapil' octoploid strawberry mapping progeny. The number of markers mapped per linkage group (LG), the length of the linkage group (cM), marker density per linkage group and the length of the regions with no markers mapped per linkage groups (gaps)

LG	SNPs	Length (cM)	SNP density	Gaps
1A	204	86.8	0.4	
1B	148	80.9	0.5	
1C	94	80.5	0.9	
1D	82	65.2	0.8	19 cM
2A	230	162.8	0.7	
2B	137	88.2	0.6	
2C	145	85.9	0.6	
2D	89	81.1	0.9	12 cM
3A	235	116.9	0.5	
3B	99	71.3	0.7	
3C	152	104.9	0.7	12 cM
3D	149	83.5	0.6	
4A	99	72.7	0.7	13 cM
4B	169	95.3	0.6	10 cM
4C	100	71.6	0.7	
4D	105	89.6	0.9	24 cM and 12 cM
5A	216	113.3	0.5	
5B	72	93.5	1.3	18 cM and 12 cM
5C	137	85.9	0.6	
5D	107	58.3	0.5	
6A	272	145.0	0.5	
6B	103	107.9	1.0	14 cM and 12 cM
6C	141	118.6	0.8	31 cM
6D	157	120.8	0.8	
7A	162	112.3	0.7	13 cM and 14 cM
7B	107	86.6	0.8	20 cM
7C	107	76.7	0.7	11 cM
7D	115	68.6	0.6	
Total	**3933**	**2624.7**	**Average 0.7**	**16 gaps**

3.4 Discussion

A targeted mapping of SSRs within regions of low marker density of the previously developed linkage map of the octoploid strawberry mapping progeny (Rg × H) was conducted in this study. A total of 26 novel SSRs representing 70 loci were successfully mapped to the existing linkage map. The reconstruction of the existing linkage map resulted in mapping more SSR loci, increased marker saturation and the genetic distance covered. However, a large number of regions with low marker density were observed on the reconstructed SSR-based linkage map. The targeted mapping of gaps with no markers mapped by selecting previously mapped SSR markers within the regions, proved to be ineffective in this study. Only three SSRs were mapped within targeted regions.

The IStraw90® genotyping array for octoploid strawberry was used to genotype 'Redgauntlet' × 'Hapil' mapping progeny. The generated genotypic data was used for novel high density SNP-based linkage map development of the Rg × H population. The genotyping array contained a total of 95,062 SNP markers, of which 16,419 were heterozygous in Rg × H progeny. High quality SNP-based genetic linkage map for an octoploid strawberry containing a total of 3,933 binned SNP markers and spanning genetic distance of 2,624.7 cM was developed. Only three regions greater than 20 cM with no markers mapped were identified. This study demonstrates the usefulness of the IStraw90® array for a rapid high density linkage map construction, which is a valuable tool for further studies, such as QTL detection, candidate gene identification and genomic selection.

3.4.1 Targeted mapping of novel SSR markers

The targeted mapping of regions with low marker density of the existing SSR-based linkage map (Sargent et al. 2012) was based on selecting previously mapped SSR markers within the gaps from the different octoploid strawberry progeny reported by Isobe et al. (2013). A total of 26 novel SSR markers were mapped to the SSR-based linkage map of Rg × H mapping progeny (Figure 3.4). Of those, three were successfully mapped to the targeted regions of low marker density. However, newly mapped markers within the gaps did not reduce the gap length due to the chromosomal rearrangements observed in this study.

Novel SSR marker mapping was performed using more stringent mapping parameters, thus the existing linkage groups were reconstructed and significantly more loci were mapped in this study. Indeed, 824 loci were mapped to the reconstructed SSR-based linkage map, whereas 598 loci were mapped to the existing SSR-based map previously reported by Sargent et al. (2012). It is worth commenting that all loci (individually segregating alleles for each and both parental genotypes) were used for the reconstruction of the existing map, and in some cases duplication of the same loci was present. For example, marker CFaM061-ABC was mapped to the linkage group FG1A, whilst three loci of the same marker (CFaM061-194, 197 and 200) were mapped on LG1A in this study (Figure 3.4). The individual loci mapping is useful, especially for quantitative trait loci (QTL) analysis, because it can provide important information on how QTL is controlled and which allele(s) is associated with the gene(s) linked to the trait of interest.

The selected markers were developed either from diploid or octoploid strawberry expressed-sequence tag (EST) information and overall, the polymorphism of the novel SSRs tested was high (78.4%) as expected. The majority of novel markers

were located on the homoeologous linkage groups. This suggests that the collinearity between the octoploid genetic map reported by Isobe et al. (2013) and the reconstructed SSR map in this study was good. Only three markers were located to unexpected non-homoeologous regions of the Rg × H map (Table 3.3). These minor inconsistencies are likely due to genotyping errors, missing values, the use of different octoploid strawberry individuals and differences in the recombination frequencies between different individuals.

This study demonstrates that direct selection of the SSRs mapped to the specific locations on a different linkage map is ineffective for targeted mapping. As rightly suggested by Sargent et al. (2007), other approaches, such as gene-specific marker development, are more likely to be more efficient for saturation of the target regions.

3.4.2 IStraw90 genotyping array evaluation in 'Redgauntlet' × 'Hapil' progeny

A total of 95,062 marker loci were genotyped in the octoploid strawberry Rg × H mapping progeny. Of those, only 3,933 (4.1%) binned markers were successfully mapped to the SNP-based genetic map in this study. Following closer investigation of the unmapped loci using the Genotyping Console software package (Affymetrix Ltd.) and SNPolisher, showed that the majority of probe sets (62%) failed to pass quality control test, and of those that passed the initial quality control, only 17.3% of probe sets were heterozygous in the Rg × H mapping progeny.

Filtering analysis based on SNP call rates observed for all individuals revealed that cluster separation calls were low for most of the probe sets. In addition, some of loci generated several sub-clusters in each genotype causing some discrepancies in the data. The sub-clustering was likely due to the identification of more than one locus per SNP by the probes. For example, the indel or allele containing SNPs within the probe

sequence may be recognised as individual marker and non-uniform clustering can be observed of the detected genotypes. Similar events were observed in 8 K and 20 K genotyping arrays for *Malus* (Antanaviciute et al. 2012; Bianco et al. 2014).

Out of 16,419 most amenable probe sets, which were clustered either to the poly high resolution or no minor homozygote groups, 3,933 (4.1%) binned marker loci were mapped to the Rg × H progeny using custom written scripts and novel developed pipeline for data analysis (R. Vickerstaff and R. Harrison, East Malling Research). The results obtained in this study are in close agreement with the initial IStraw90 array evaluation analysis based on octoploid strawberry mapping progeny 'Holiday' × 'Korona' (Bassil et al. 2015). A total of 6,594 (4.8%) markers were successfully incorporated to the genetic linkage map of 'Holiday' × 'Korona', which is only 0.7% greater than it was obtained in this study. In addition, the ongoing SNP data analysis indicates that a further set of SNPs could be likely mapped to the Rg × H progeny, once genotypic data observed have been scrutinized further following filtering analysis.

A large number of markers that were mapped to the Rg × H linkage map in this study provided coverage of all 28 expected linkage groups, with an average marker density one marker per 0.7 cM, resulting in a high quality genetic map.

3.5 Conclusions

In this chapter targeted mapping of regions of the existing SSR-based linkage map with low marker density has been reported. In addition, the 90 K Affymetrix Axiom genotyping array has been tested in an octoploid strawberry ('Redgauntlet' × 'Hapil') mapping progeny with the purpose of developing a high density novel SNP-based linkage map.

This study demonstrated that mapping of the novel previously reported SSR markers within the targeted regions was ineffective and represents a relatively costly strategy because only three markers mapped to the targeted regions. Furthermore, the process is relatively slow and labour intensive.

A total of 3,933 binned SNP markers were successfully mapped to the consensus linkage map resulting in the development of a high density linkage map for octoploid strawberry mapping progeny. The ongoing SNP marker data analysis will likely increase the number of loci which could be mapped, thus increasing the quality of the linkage map even further. The development of the SNP-based linkage map will facilitate further studies, such as QTL associated with disease resistance and fruit quality traits identification.

This application of the Affymetrix IStraw90® Axiom genotyping array is the first high-throughput genotyping platform for rapid, reliable and cost-effective method for linkage map development in the cultivated strawberry (*Fragaria* × *ananassa*).

Chapter 4

QTL analysis linked to plant characteristics and fruit quality traits in octoploid strawberry (*Fragaria* × *ananassa*)

4.1 Introduction

Strawberry, as well as other rosaceous crops, such as apple, pear and raspberry, has a long history of artificial selection for improved cultivars which has traditionally been very time-consuming (Hummer et al. 2011). Only recently, new technologies such as marker-assisted selection (MAS) or marker-assisted breeding (MAB) have been integrated into traditional approaches to identify desirable phenotypes resulting in increased breeding efficiency (Collard and Mackill, 2008; Whitaker, 2011).

MAS, as the name suggests is an indirect pre-selection process where phenotypes expressing desirable traits are selected based on molecular marker information only (Xu and Crouch, 2008; Jannik et al. 2010; Whitaker, 2011). Molecular markers for fruit quality and disease resistance traits are developed by a process known as quantitative trait loci (QTL) mapping. The process involves associating phenotypic variation with genetic markers, using the progeny most commonly from a biparental cross. However, it is well known that fruit quality and disease resistance traits in cultivated strawberry are complex traits and are controlled by a large number of loci, thus multiple markers need to be developed in order to identify the degree of fruit quality or level of resistance (Sargent et al. 2012; Antanaviciute et al. 2015).

During the last decade, an enormous amount of research has been conducted to look at the development of MAS in plant breeding (Xu and Crouch, 2008; Collard and Mackill, 2008; Iezzoni et al. 2010). However, despite advances in molecular biology, such as next-generation sequencing technologies and high-throughput genotyping platforms, MAS strategies have had limited success in improving the pre-selection process in rosaceous crops (Collard and Mackill, 2008; Iezzoni et al. 2010; Bink et al.

2014). The main reasons why MAS approach is considered to be inefficient in crop breeding includes: the majority of QTL detections have been based on a single progeny (narrow genetic basis of the germplasm) leading to a small proportion of significant QTLs identified; the majority of fruit quality and disease resistance traits are controlled by several genes instead of a single gene, and thus many alleles with useful information are potentially missed; environmental factors affect the allelic expressions; the identified QTL regions are usually large; many available markers are monogenic and therefore a large number of molecular markers associated with the genes controlling traits of interest must be identified (Collar and Mackill, 2008; Heffner et al. 2009; Bink et al. 2014). In addition, due to the range in ploidy level, QTL identification in polyploid species is even more challenging. For example, octoploid cultivated strawberry (*F. ananassa*) has eight sets of the seven common chromosomes (56 chromosomes in total) and is very complex at the genetic level.

Despise the challenges listed above, which make MAS an unreliable approach for enhancing crop breeding in practise, research scientists have successfully developed a number of molecular markers for use in plant breeding programmes based on broad and comprehensive genetics and genomics information available (Whitaker, 2011). Pedigree-based information (pedigree genotyping) and determination of the allelic configuration through haplotype information (MADCE – microsatellite allele dose configuration and establishment) are just few important approaches adopted for the identification of significant QTLs. These approaches permit more reliable development of novel molecular markers in rosaceous crops including octoploid strawberry (van de Weg et al. 2004; van Dijk et al. 2014).

To date, a number of QTLs linked to fruit quality and disease resistance traits were identified in different polyploid species. For example, QTLs controlling fiber

quality traits in tetraploid cotton (Zhang et al. 2009; Tang et al. 2015), resistance to *Fusarium* wilt in triploid watermelon (Levi, 2015) and resistance to *Fusarium* head blight in hexaploid wheat (Löffler et al. 2009; Buerstmayr and Buerstmayr, 2015) have been identified.

In the Rosaceae plant family a large number of QTLs linked to agronomical, fruit quality and disease resistance traits have been reported in different species. In apple, QTL regions have been reported for key fruit quality, physiological traits and phenolic compounds, such as firmness, soluble sugar level, fruit size, growth habit, aroma, flesh and peel related phenolic compounds (Liebhard et al. 2003a; Kenis and Keulemans, 2007; Kenis et al. 2008; Dunemann et al. 2009; Khan et al. 2012; Chang et al. 2014; Guan et al. 2015), and for resistance to fire blight (Calenge et al. 2005; Papp et al. 2015) and powdery mildew (Calenge and Durel, 2006). Furthermore, fruit quality, physiological and disease resistance QTLs have been reported in peach (Foulongne et al. 2003; Eduardo et al. 2013; Martínez-García et al. 2013; Zeballos et al. 2013), raspberry (Graham et al. 2009, 2015; Kassim et al. 2009; Woodhead et al. 2013), sour and sweet cherry (Wang et al. 2000; Sooriyapathirane et al. 2010; Quero Garcia, 2012) and pear (Dondini et al. 2005; Pierantoni et al. 2007; Zhang et al. 2013).

It is only recently that a series of molecular markers has been developed for fruit quality, metabolites, agronomical and disease resistance traits in cultivated strawberry (*Fragaria* × *ananassa* Duch.). These include genes linked to seasonal flowering (Sugimoto et al. 2005), day-neutrality (Weebadde et al. 2008; Castro et al. 2015), fruit flavour, colour, size, firmness, shape, soluble sugars, organic acids and phenolic compounds (Zorrilla-Fontanesi et al. 2011b, 2012; Lerceteau-Köhler et al. 2006, 2012). In contrast, due to the nature of the complex octoploid strawberry (2n=8x=56) genome, only a few studies have reported molecular markers or QTLs associated with disease

resistance in cultivated strawberry (van de Weg, 1997; Lerceteau-Köhler et al. 2005; Antanaviciute et al. 2015). These studies will be discussed in more detail in Chapter 6.

In this chapter, QTL identification associated with plant characteristics and fruit quality traits in octoploid strawberry ('Redgauntlet' × 'Hapil') mapping progeny, previously reported in Chapter 2, will be discussed. Some of the key plant characteristic traits included flower number, flower size, number of trusses and runners, plant height, width and vigour. Fruit quality traits included yield, firmness, sugar and pH level, fruit colour, appearance, skin strength, seediness and fruit shape. A consensus, high density SNP-based genetic linkage map (previously discussed in Chapter 3) was used for QTL mapping. A total of 179 potential QTLs for all traits analysed were identified in this study. However, the majority of QTLs identified (69.8%) were year-dependent and only 30.2% of QTLs were stable over the two or three year period.

4.2 Materials and methods

4.2.1 Plant material

An octoploid strawberry mapping progeny derived from the cross of 'Redgauntlet' and 'Hapil' was used for phenotypic data collection (Chapter 2, Section 2.2.1) and the construction of SNP-based genetic linkage map (Chapter 3, Section 3.3.4). A total of 120 individuals were randomly selected for phenotypic data collection over the three consecutive years (2013, 2014 and 2015), whereas 140 individuals were used for high-throughput genotyping. SNP-based linkage map development was based on 173 individuals where individuals not included in phenotypic and genotypic data collected were treated as missing values.

4.2.2 Phenotypic data

A total of 13 different plant characteristics traits were recorded. Of those, 11 traits were evaluated over the three consecutive years (2013, 2014 and 2015), whereas two traits (plant height and width) were recorded in 2013 only. Out of 17 fruit quality related traits recorded, eight were evaluated for three years and the remaining nine traits were recorded for two years. Phenotypic data collection and statistical analyses are discussed in more detail in Chapter 2, Sections 2.2.2, 2.2.3 and 2.2.5.

4.2.3 Linkage map development

The consensus SNP-based genetic linkage map containing a total of 3,933 unique markers over the 28 linkage groups of the 'Redgauntlet' × 'Hapil' mapping progeny was developed using high-throughput ISraw90® genotyping array. The linkage map constructed and discussed in Chapter 3, Section 3.3.4 was used for QTL analysis in this study.

4.2.4 QTL identification

The mean values of phenotypic traits reported in Chapter 2 (Section 2.3.3) were used for the analysis. A consensus SNP-based linkage map was used for QTL mapping. QTL identification was performed for all phenotypic traits for each year individually using MapQTL® 5 software package (van Ooijen, 2004). As a result of phenotypic means not being normally distributed after the performance of two types of transformations (log and square root), the non-parametric test of Kruskal-Wallis (KW) was used for the identification of significant associations between phenotypic traits and molecular markers. The KW test sorts individuals according to the single-dose quantitative trait loci and classifies them according to their marker genotype (van

Ooijen, 2004). The closest SNP markers linked to QTLs were identified based on the significance level and the KW test statistics (K^*) value.

Due to the large data set analysed in this study, only five clusters of QTLs (hotspots) identified were graphically presented on the linkage map using MapChart 2.2 software (Voorrips, 2002). In addition, unique SNP markers located to the identical positions and closely mapped SNPs on the same linkage groups on the SNP-based linkage map were removed for graphical presentation of QTL positions.

4.3 Results

4.3.1 QTLs linked to plant characteristics traits in 'Redgauntlet' × 'Hapil' progeny

The analysis of SNP markers and phenotypic data resulted in the identification of QTLs linked to the plant characteristic traits recorded. QTLs were detected for all the 13 plant characteristic traits using the KW test. QTLs detected were distributed over 24 homoeologous linkage groups and ranged from one QTL on LG1B, LG4B, LG6B and LG7A to six QTLs on LG2A, LG2C, LG3A and LG7C for different traits. Four homoeologous linkage groups did not have any QTL identified (LG2D, LG4A, LG5B and LG7B) in this study (Table 4.1, Figure 4.1). The largest number of QTLs identified per four homoeologous linkage groups together (A, B, C and D) was LG3 with a total number of 19 QTLs. The lowest number of QTLs detected per four homoeologous linkage groups was LG4 with a total number of five QTLs (Table 4.1).

Table 4.1 Summary of the number of QTLs detected per homoeologous linkage groups for plant characteristic-related traits

LG	QTL number per homoeologous LG				Total
	A	B	C	D	
1	2	1	3	4	10
2	6	5	6	0	17
3	6	5	3	5	19
4	0	1	2	2	5
5	4	0	3	3	10
6	4	1	3	2	10
7	1	0	6	2	9
Total	23	13	26	18	**80**

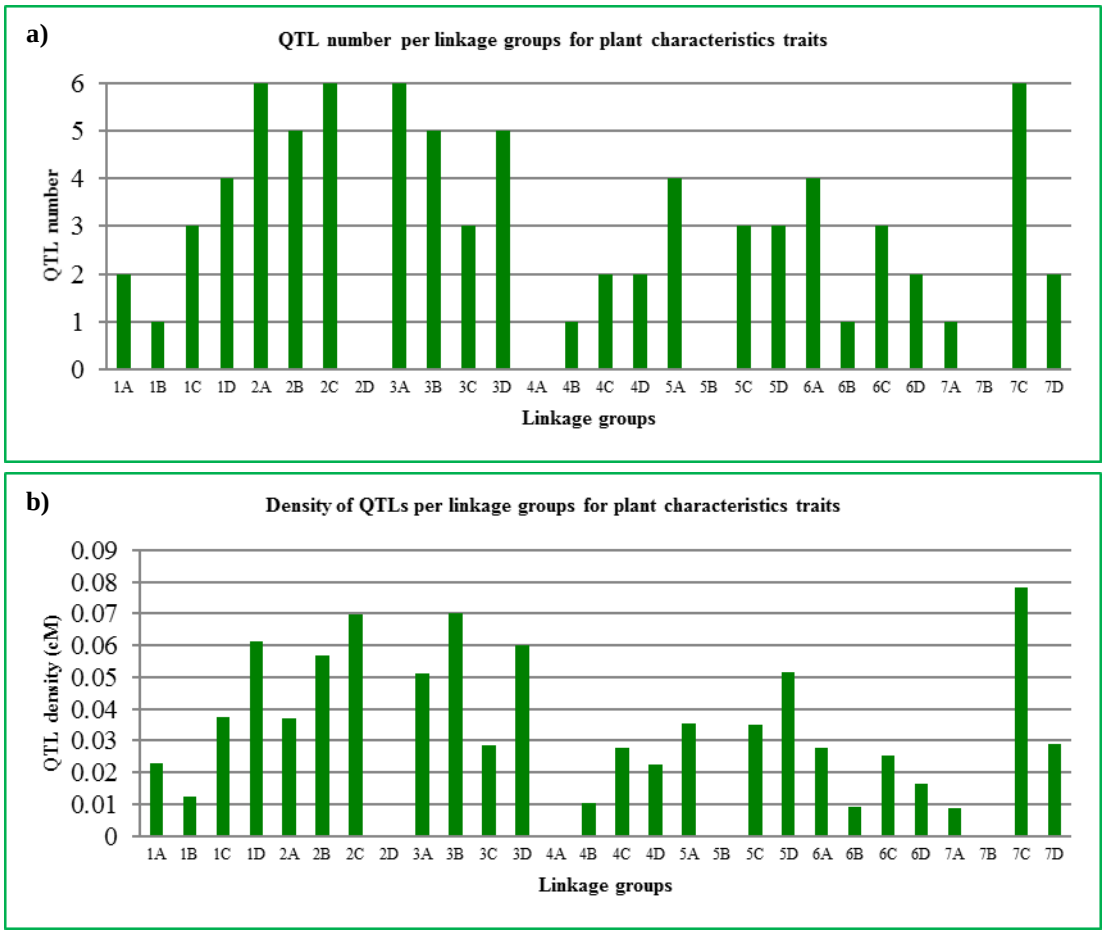

Figure 4.1 Number of QTLs per linkage group detected for plant characteristics traits over three consecutive years in the Rg × H progeny (a), and QTL density (cM) per linkage groups (b).

A total of 80 QTL locations associated with plant characteristic traits were detected taking into consideration all observations (present in one year, two years and three years) and are listed in Table 4.2. Of those, the majority 57 (71.3%) QTLs were present in one year on different locations across 28 linkage groups, and were considered as a minor effect QTLs. These findings also suggest that loci were strongly affected by environmental factors. The remaining 23 (28.7%) QTLs were present in two or three years, and were considered stable among the years analysed. Among them, the most stable QTLs, which had at least 50% of QTLs linked to a particular trait were traits linked to flower diameter, petal number and truss width. This suggests that potentially the same gene(s) control the variation of quantitative characters of these traits. In contrast, QTL positions for four traits were present on different homoeologous linkage groups for all loci observed over the three years. These traits were plant vigour (eight loci), plant height (two loci), plant width (three loci) and number of runners per plant (seven loci) (Table 4.2).

The results of this study showed that runner length was controlled by the largest number of loci (nine), followed by flower number, plant vigour and truss width, which had eight loci each. Plant height (two loci) and width (three loci) had the least QTLs as expected, because these two traits were recorded only in one year (2013). Out of the traits which were recorded for three years, petal and leaflet number were controlled by the fewest QTLs, four loci each (Table 4.2).

Table 4.2 A summary of QTLs identified for plant characteristics related traits in 'Redgauntlet' × 'Hapil' mapping progeny using Kruskal-Wallis test. Trait recorded, number of years trait evaluated, number of QTLs identified and QTL positions on the SNP-based genetic linkage map are presented. Numbers in the brackets correspond to the year that QTL was detected and indicate 2013 (13), 2014 (14) and 2015 (15). QTLs observed for the female ('Redgauntlet') are highlighted in red, for the male ('Hapil') in blue and for both parental genotypes in pink

Trait	Years	No. of QTL	Location of QTL
Plant characteristics related traits			
Flower number	3 years	8	1A (14), 1C (15), 1D (14), 2C (15), 3A (13, 15), 3B (13, 14), 3D (15), 4C (14, 15)
Flower diameter	3 years	6	2A (13), 2C (14, 15), 4B (14), 4D (13), 6C (14, 15), 7A (13, 14, 15)
Petal number	3 years	4	2A (13, 14, 15), 2B (13), 2C (15), 6A (13, 14, 15)
Pedicel length	3 years	7	2C (15), 3A (13, 15), 3C (15), 3D (14), 5A (15), 5C (13), 6A (13, 14, 15)
Leaflet number	3 years	4	3A (13, 14, 15), 5A (13), 5D (13), 7C (15)
Vigour	3 years	8	1C (14), 1D (14), 2A (15), 2B (14), 3B (13), 5C (14), 5D (13), 7D (13)
Height	1 year	2	3D (13), 7C (13)
Width	1 year	3	2A (13), 2C (13), 3B(13)
Runner number	3 years	7	2B (14), 3B (13), 6A (13), 6C (15), 6D (13), 7C (13), 7D (14)
Runner length	3 years	9	1C (14), 1D (15), 2A (15), 3A (13), 3C (13, 14), 3D (13), 5A (13, 14), 6C (13, 14), 7C (13)
Truss number	3 years	7	1A (13, 14, 15), 2C (15), 3A (13), 3B (14), 5D (15), 6D (14), 7C (13, 14, 15)
Truss length	3 years	7	2B (15), 3A (13, 14), 3C (13, 15), 3D (14), 4C (14), 5C (14, 15), 6A (13)
Truss width	3 years	8	1B (14, 15), 1D (14), 2A (13, 15), 2B (15), 4D (14), 5A (13, 14, 15), 6B (13, 15), 7C (14)
Total		**80**	

The QTL locations of some closely correlated plant characteristics traits, previously discussed in Chapter 2 (Section 2.3.3), were identified on the same or homoeologous linkage groups. This can be seen between QTL locations identified for flower number, truss number, flower size, plant vigour, width and height. For example, a positive correlation was observed between flower number and truss number, where seven out of eight QTLs controlling flower number were identified on the same or homoeologous linkage groups as for truss number. Similarly, all (six) loci linked to flower size were mapped on the same or homoeologous linkage groups as QTLs mapped controlling truss number. A significant negative correlation was observed between these two traits (reported in Chapter 2, Section 2.3.3). Furthermore, positive correlations were observed between plant vigour and height, as well as plant vigour and width. QTL locations linked to plant height and width were identified on a homoeologous linkage group as QTLs associated to plant vigour.

4.3.2 QTLs linked to fruit quality traits in 'Redgauntlet' × 'Hapil' progeny

At least a single QTL was detected for all the 17 fruit quality traits taking into consideration QTLs present in one year, two years and three years. QTLs were identified across 28 homoeologous linkage groups with the exception of two linkage groups (LG2B and LG5B), which did not have any QTL present. The number of QTLs linked to fruit quality traits ranged from one QTL on LG1B and LG7B to eight QTLs on LG2A and LG6C (Table 4.3, Figure 4.2).

When the identified QTL locations were compared among all 28 linkage groups, LG6 and its four homoeologous had the largest number of QTLs (20). The lowest number of QTLs observed (10 QTLs) were among the four homoeologous linkage groups of LG7 (Table 4.3).

Table 4.3 Summary of the number of QTLs detected per homoeologous linkage groups for fruit quality related traits

LG	QTL number per homoeologous LG				Total
	A	B	C	D	
1	4	1	5	3	13
2	8	0	3	2	13
3	7	3	3	3	16
4	4	4	2	4	14
5	6	0	3	4	13
6	6	3	8	3	20
7	5	1	2	2	10
Total	40	12	26	21	**99**

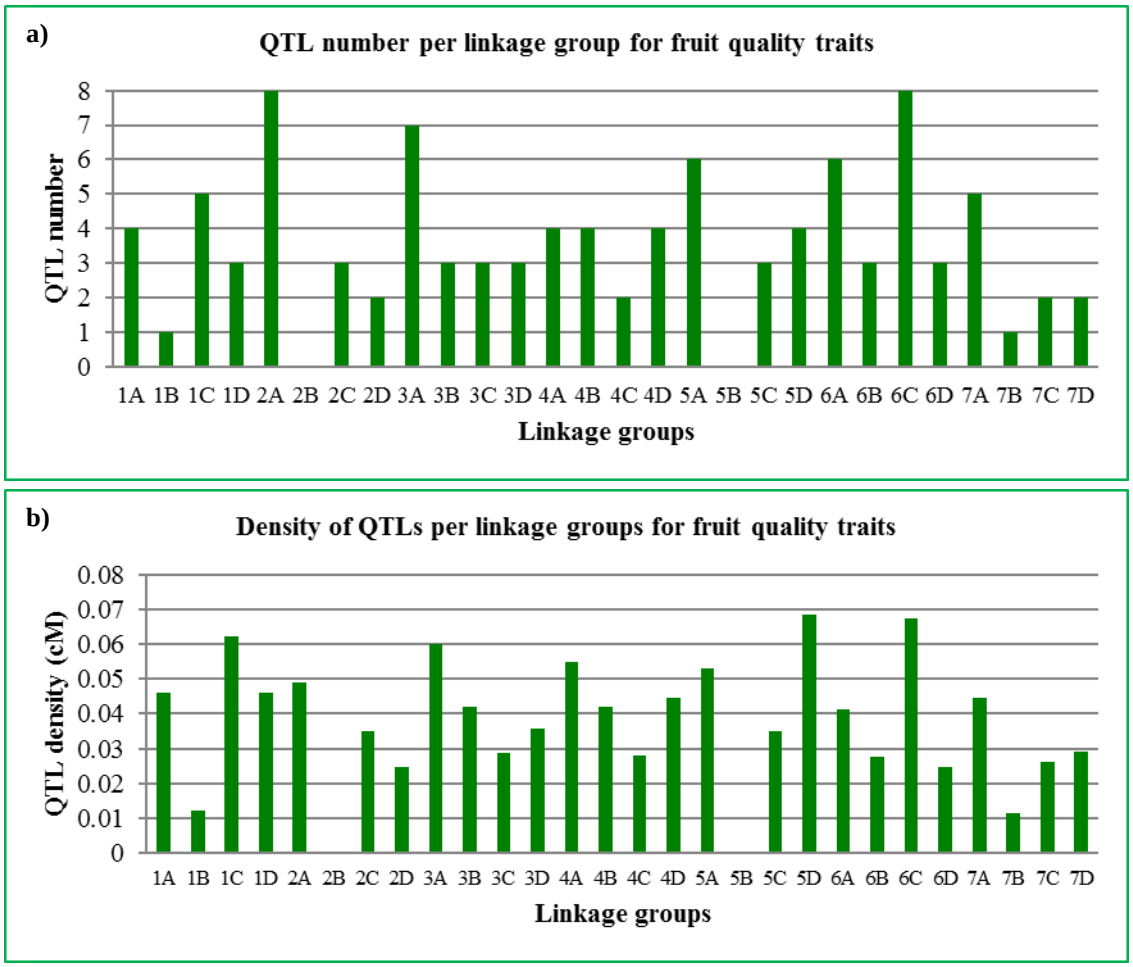

Figure 4.2 Number of QTLs per linkage group detected for fruit quality related traits over the three consecutive years in the 'Redgauntlet' × 'Hapil' progeny (a), and density of QTLs (cM) per linkage group (b).

A total of 99 QTLs were detected for fruit quality traits. Of those, the majority of QTLs 68 (68.7%) were present in one year (in 2013, in 2014 or in 2015). The remaining 31 (31.3%) were present in two or three years. Only three QTLs identified on LG1C, LG3A and LG6C linked to achene position, soluble solids content (ºBrix) and unmarketable fruit weight respectively, were stable over the three years. Several QTLs had at least 50% of QTLs identified as stable in two or three years and were linked to firmness, soluble sugar level, fruit shape and neck line. In addition, QTLs controlling fruit shape were detected on LG2A, LG3A and LG6C consistently over the two years. Similarly, four out of five QTL regions detected linked to the neck like were identified on the same linkage groups for two years. This suggests that the same gene(s) are likely to be controlling the variation of these traits. In contrast, eight QTL loci detected for marketable fruit weight and four loci linked to skin brightness were detected on different homoeologous linkage groups for all loci observed. This suggests that many independent loci of small effects control these traits. Furthermore, the identified inconsistencies among QTL locations for these traits could be explained by the genetic heterogeneity, meaning that several loci control the same trait.

Although, at least three QTLs were identified controlling each of 17 fruit quality traits analysed, the results showed that firmness was controlled by the largest number of loci (nine), followed by marketable fruit weight, sugar level and achene position which had eight loci each. It is worth noting that seven QTLs controlling skin strength were detected over the two years, thus the trait is genetically complex and is controlled by a large number of genes. Fruit shape and outline had the fewest QTLs, three loci each, suggesting that these traits are controlled by genes located on fewer chromosomal locations (Table 4.4).

Table 4.4 A summary of QTLs identified for fruit quality related traits in 'Redgauntlet' × 'Hapil' mapping progeny using Kruskal-Wallis test. Trait recorded, number of years trait evaluated, number of QTLs identified and QTL positions on the SNP-based genetic linkage map are presented. Numbers in the brackets correspond to the year of present QTL detected and indicate 2013 (13), 2014 (14) and 2015 (15). QTLs observed for the female ('Redgauntlet') are highlighted in red, for the male ('Hapil') in blue and for both parental genotypes in pink

Trait	Years	No. of QTL	Location of QTL
Fruit quality related traits			
Yield	3 years	6	1D (14), 3A (15), 5A (14, 15), 6C (15), 6D (13), 7A (14)
Unmarketable fruit	3 years	7	1C (14), 1D (14), 5A (14, 15), 6A (13), 6C (13, 14, 15), 7A (15), 7B (13)
Marketable fruit	3 years	8	2A (14), 3A (15), 3B (13), 4B (13), 5A (14), 5D (15), 6C (15), 7A (14)
Firmness	3 years	9	1A (13, 14), 1D (13), 2C (14, 15), 2D (13), 3A (13, 14), 4A (13), 4B (14, 15), 4C (13), 7A (13, 15)
°Brix	3 years	8	1A (14, 15), 3A (13, 14, 15), 3C (13, 15), 3D (15), 4D (14), 5C (13, 14), 6A (15), 6C (13)
pH	3 years	7	1C (14), 3B (14), 3C (13), 4D (14), 5A (14), 5C (14, 15), 6B (13)
Achene position	3 years	8	1A (13), 1C (13, 14, 15), 3A (14, 15), 4A (13), 4D (13), 5A (13, 15), 6D (14), 7A (14)
Seediness	3 years	6	1A (14), 2A (14), 3A (15), 3B (13), 6A (14, 15), 6B (13)
Skin brightness	2 years	4	2A (13), 4B (13), 6A (15), 7D (13)
Shape	2 years	3	2A (14, 15), 3A (14, 15), 6C (14, 15)
Cap size	2 years	4	2D (14), 3D (14), 5C (14, 15), 5D (15)
Outline	2 years	3	2A (14), 4D (15), 6C (14, 15)
Redness	2 years	4	1C (14), 2C (14, 15), 6A (15), 7C (14)
Glossiness	2 years	5	2A (14, 15), 6B (15), 6C (14, 15), 6D (14), 7D (14)
Neck line	2 years	5	1C (14, 15), 2A (15), 4A (14, 15), 5D (14, 15), 6A (14, 15)
Skin strength	2 years	7	1B (14, 15), 2A (14), 3D (14), 4A (15), 5A (14), 5D (15), 6C (15)
Internal fruit colour	2 years	5	2C (14), 3C (15), 4B (14, 15), 4C (15), 7C (14)
Total		**99**	

Similar to plant characteristics related traits, some QTL positions that showed significant correlations among fruit quality traits were identified on the same or homoeologous linkage groups as has previously been reported by Lerceteau-Köhler et al. (2012). For example, a positive correlation was observed between redness and glossiness (Chapter 2, Section 2.3.3), where four QTLs were identified for redness and were mapped to LG1C, LG2C, LG6A and LG7C, whereas five QTLs associated with glossiness were mapped to homoeologous linkage groups 2A, 6B, 6C, 6D and 7D (Table 4.4).

In some cases, not all QTLs identified showing significant correlations among the fruit quality traits were mapped to the same or homoeologous linkage groups. For example, positive correlations were observed between neck line and shape, and neck line and cap size. However, shape and cap size did not have any QTL identified on LG1C and LG4A, whereas two loci, both present for two years (2014 and 2015), linked to neck line were mapped on those linkage groups. A similar situation can be found for closely related correlations between skin brightness and internal fruit colour, and yield and soluble sugars. A single locus was identified on LG6A (present in 2015) related to skin brightness, whereas no QTLs were identified linked to internal fruit colour on the same or homoeologous linkage group. Five out of six loci associated with yield were identified on the same or homoeologous linkage groups as seven out of eight QTLs linked to soluble sugars; only one locus present in one year (2014) for both traits was mapped to unlinked linkage groups (LG7A for yield and LG4D for sugar).

4.3.3 QTL positions detected for plant characteristics and fruit quality traits

A total of 179 QTLs were identified controlling plant characteristics and fruit quality traits in this study. Of those, 80 loci were linked to plant characteristics (Table

4.2) and 99 loci were linked to fruit quality traits (Table 4.4). When locations for all QTLs detected were further investigated, five significant QTL clusters (hotspots) linked to different traits which were stable in two or three years were found (Figure 4.3). The phenomenon has been previously reported in other rosaceous crops including peach (Romeu et al. 2014), apple (Khan et al. 2012) and octoploid strawberry (Zorrilla-Fontanesi et al. 2011b; Lerceteau-Köhler et al. 2012).

The smallest clusters of QTLs identified in this study, controlling four different traits were identified on the LG2A and LG6A of the SNP-based linkage map of 'Redgauntlet' × 'Hapil' progeny. QTL locations controlling petal number, truss width and fruit glossiness were located in overlapping chromosomal region on LG2A. QTLs linked to fruit shape were also identified on LG2A, however the positions of these QTLs did not overlap the regions of other QTLs on the same linkage group. QTLs linked to pedicel length, neck line, seediness and a second stable QTL region linked to petal number were identified on LG6A. All QTLs located on LG6A overlapped the same region with the exception of seediness (Figure 4.3).

Another cluster of QTLs controlling five different traits was identified on the LG5A. These traits were runner length, truss width, yield, unmarketable fruit weight and achene position and had QTL regions closely distributed on the same linkage group.

Thirteen QTLs linked to six different traits were identified on LG6C. Interestingly, two overlapping regions can be seen on this linkage group. This could be due to the region of low marker density of more than 31 cM present in the middle of the linkage group. QTLs controlling flower size, runner length and unmarketable fruit weight had inconsistent locations over two or three years. In contrast, QTL regions associated with fruit shape, outline and glossiness were on overlapping chromosomal regions (Figure 4.3).

The largest cluster of QTLs (18 loci) controlling four different plant characteristics and four fruit quality related traits was identified in a region of approximately 83 – 114 cM on LG3A. Two overlapping regions of nine QTLs and eight QTLs, and unlinked single QTL were observed (Figure 4.3). One locus for flower number (2015) and ºBrix (2014) were located on the top of the LG3A, whereas pedicel length (2013) was identified in the middle of the linkage group and did not overlap the same QTLs observed for different years. QTLs controlling leaflet number, truss length and fruit shape were located to near the same chromosomal positions for three, two and two years respectively. Closely located QTLs for two years were linked to firmness and achene position.

Inconsistencies were observed among the QTLs mapping to the same regions on the same linkage groups depending on whether QTL was from 'Redgauntlet', 'Hapil' or both parental genotypes. For example, QTLs controlling fruit shape and glossiness were identified on LG2A and were from both parental genotypes and from 'Hapil' respectively (Figure 4.3). In contrast, QTL linked to truss width was from 'Hapil' for 2013 but it was from 'Redgauntlet' in 2015, although both loci were identified on the same overlapping region of the linkage group 2A. Only 10 QTLs linked to four traits on LG6A showed consistent presence of the allelic distributions for all years analysed.

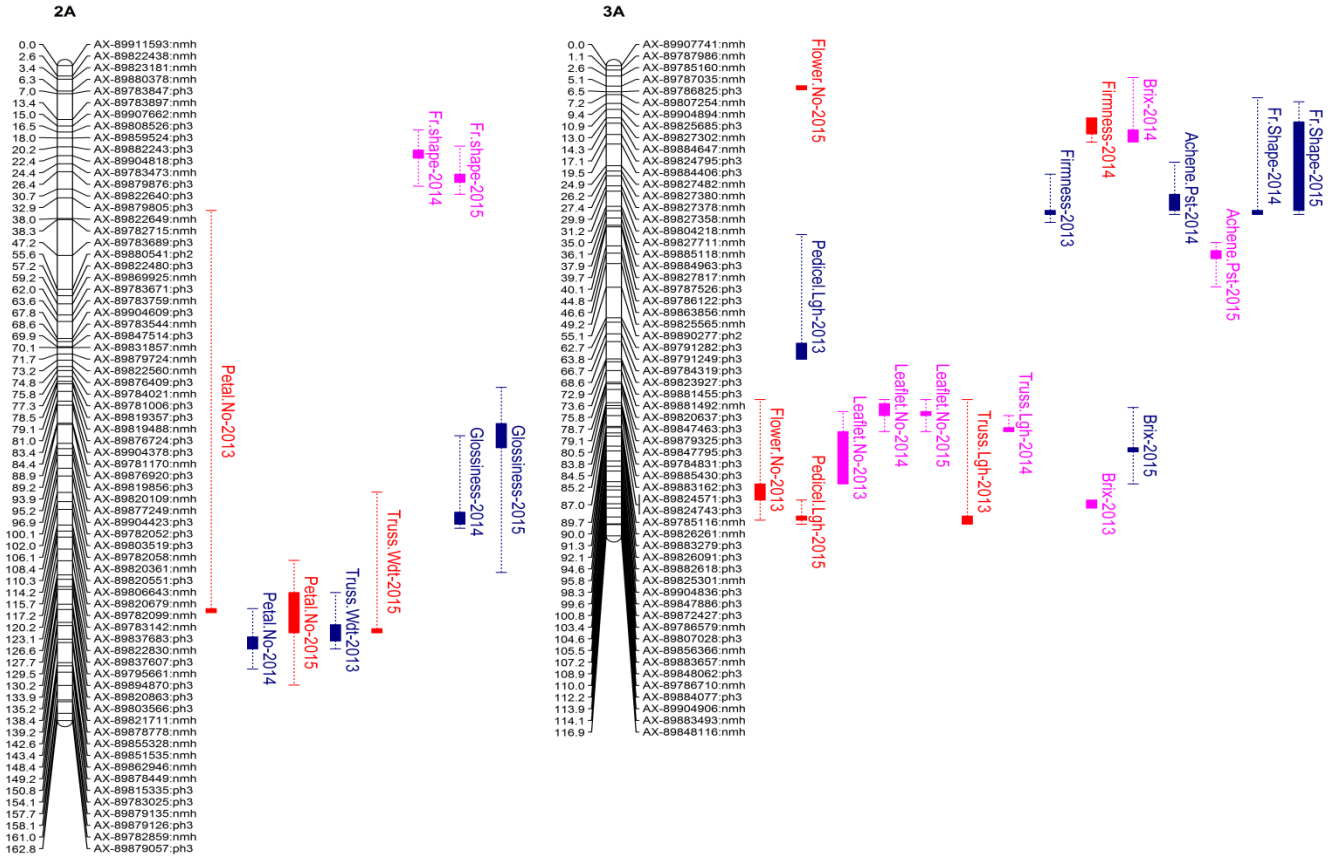

Figure 4.3 Locations of QTL linked to plant characteristics and fruit quality traits analysed for three consecutive years (2013, 2014 and 2015) in the 'Redgauntlet' × 'Hapil' mapping progeny. Only four linkage groups which show QTL clusters are presented. Thick bars represents the closest linked SNPs (p = < 0.0001), whereas the dotted lines are the whiskers of the significance intervals (between p = < 0.0001 and p = < 0.05). QTLs from 'Redgauntlet' are highlighted in red, from 'Hapil' are in blue and from both parental genotypes are in pink.

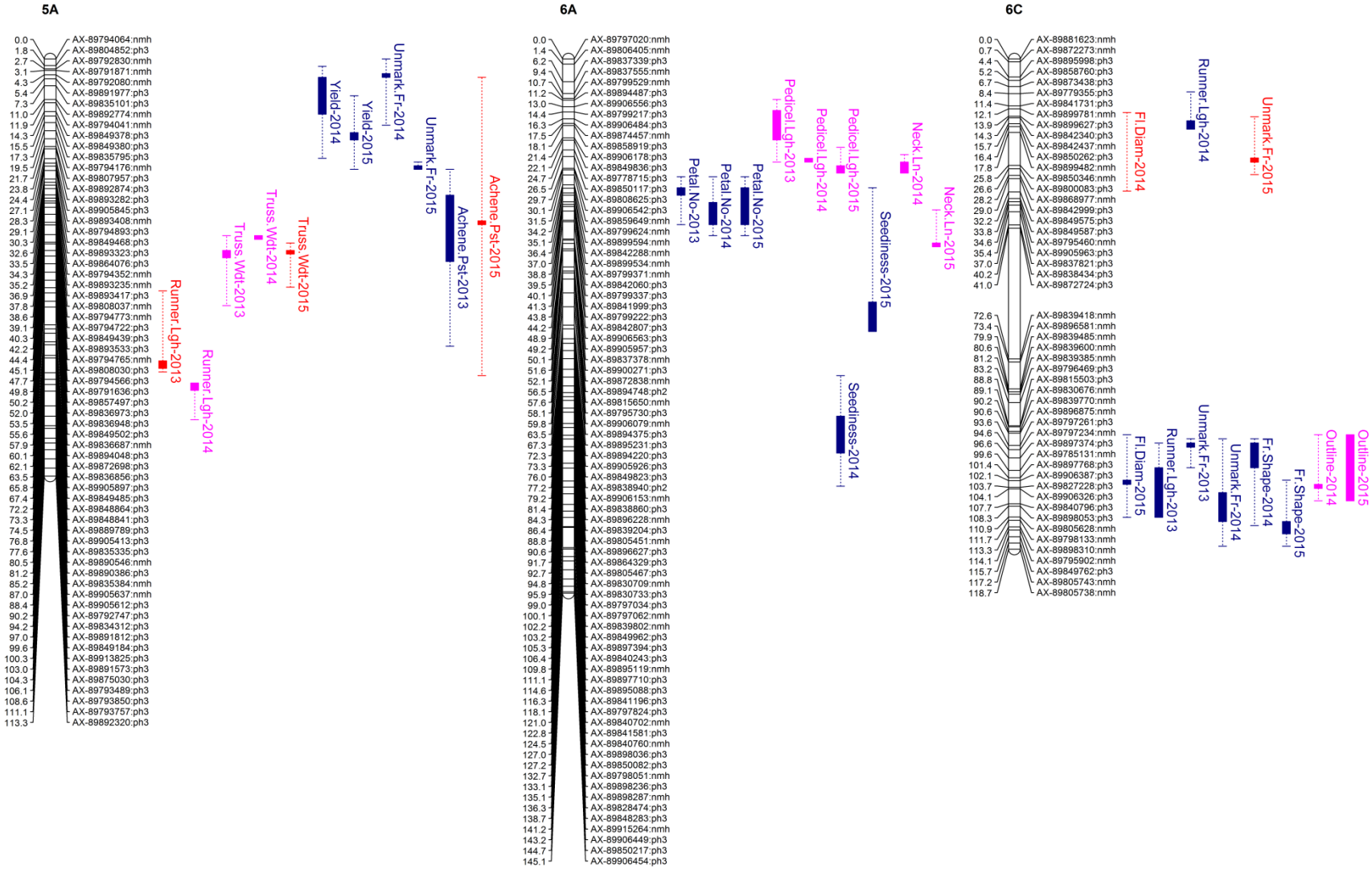

Figure 4.3 Continued.

4.3.4 Association of SNP markers with QTLs

At least one significant SNP associated with the QTL region was identified for all traits analysed. The selection of the closest linked SNPs to the QTL regions was based on the Kruskal-Wallis test value (K*) and the significance level. The K* value observed in this study ranged from 5.757 for petal number on LG2A to 20.967 for neck line on LG4A. The significance level of selected SNPs ranged from two stars (**), $p < 0.05$ to seven stars (*******), $p < 0.0001$.

A total of 339 significant SNP markers were identified associated with QTL regions for the traits analysed (Appendix 4.1). Of those, 16 SNPs were highly significant with significance level of seven stars ($p < 0.0001$), 65 SNPs had significance level of six stars ($p < 0.0005$) and 52 SNPs had five stars ($p < 0.001$). The majority of SNPs selected (176) had the significance level of four stars ($p < 0.005$). The 28 SNPs had three stars ($p < 0.01$) and the remaining two SNPs had significance level of two stars ($p < 0.05$).

4.4 Discussion

In this chapter, the identification of QTLs linked to plant characteristics and fruit quality traits in octoploid strawberry ‘Redgauntlet’ × ‘Hapil’ mapping progeny is reported. The analyses were conducted using a high quality SNP-based genetic linkage map and phenotypic data collected for three consecutive years for a total of 30 different traits.

A total of 179 potential QTLs were identified for the traits using a non-parametric Kruskal-Wallis test. The majority of QTLs identified were year-dependent, suggesting that QTLs were minor effect, many genes controlled the traits over the

different genetic regions of the genome, and that strong environmental factors affected phenotypic variations over the years. Indeed, the findings observed in this study support the results obtained for the correlation study previously reported in Chapter 2, Section 2.4.2. Significant differences were observed for air temperature and wind speed over the years studied, presumably resulting in inconsistent QTL locations for a majority of traits over the three years.

However, five hotspots of stable QTLs (present in two or three years) were identified on chromosomal regions indicating that the same genes may consistently control a group of different traits. The clusters of QTLs were associated with plant characteristic traits, such as flower number, pedicel length, truss and runner number, and fruit quality traits, such as shape, glossiness, sugar level, firmness and achene position.

Finally, the majority of QTL locations for a number of traits, which showed highly significant correlations, were identified on the same or homoeologous linkage groups. However, in some cases no QTL co-locations were identified.

4.4.1 QTLs linked to plant characteristics and fruit quality traits in octoploid strawberry

A total of 179 potential QTLs associated with 13 plant characteristics and 17 fruit quality traits distributed across 28 linkage groups of octoploid strawberry were identified in this study. Of those, 30.2% were stable in two or all three years. Two QTLs for shape and neck line were most stable and were present in all two years evaluated. Similar results were obtained in other studies reporting QTLs associated to plant architectural and fruit quality traits in octoploid strawberry. According to Zorrilla-Fontanesi et al. (2011b), 33.3% of QTLs identified linked to 14 agronomical and fruit

quality traits were stable in two or all three years. Similarly, 27% of 60 unique QTLs detected were present in two years and only three loci linked to fruit length, and the ratio of fruit diameter and fruit length were detected in all three years in the study of Lerceteau-Köhler et al. (2012). These findings suggest that strong environmental factors affect QTL stability over the years resulting in changes in allelic expressions, and thus phenotypic and genotypic variations. It is worth noting that QTL identification was performed using different tests among QTLs identified in this study and two previously reported studies by Zorrilla-Fontanesi et al. (2011b) and Lerceteau-Köhler et al. (2012). Furthermore, the differences in population size and different parental backgrounds restrict direct comparison of QTL locations between these studies and potentially could lead to false results.

Although a large number of QTLs was present on different unlinked linkage groups and those QTLs were considered year-dependent, 17 stable QTLs (present in two or three years) were identified on five chromosomal regions of LG2A, LG3A, LG5A, LG6A and LG6C. Among them, LG3A had the most loci mapped linked to eight different traits, whereas LG2A and LG6A had the least (four) loci detected each. Hotspots (genomic regions affecting many traits) of QTL regions overlapping the same chromosomal regions have been reported previously in different crops, suggesting that potentially the same genes control many different traits and/or more genes are located in certain regions of the genome than others (Zorrilla-Fontanesi et al. 2011b; Khan et al. 2012; Lerceteau-Köhler et al. 2012; Romeu et al. 2014). Furthermore, common genomic regions with QTLs linked to different traits could reflect the pleiotropic effects of single genes and/or that physical locations of different genes are close to each other (Yang et al. 2010).

4.4.2 Genetic control of QTLs identified in octoploid strawberry

The study conducted here demonstrated that the majority of plant characteristics and fruit quality traits are under complex genetic and physiological control and that a large number of genes contribute to the phenotypic and genotypic variation. No clear difference was observed between the variation in the number of loci observed for plant characteristics and fruit quality related traits. Although a lower number of loci were identified for nine of 17 fruit quality traits. This can be explained by the fact that these traits were evaluated for two instead three years.

It is well known that QTL mapping is influenced by a number of factors, including the heritability of the trait. The results showed that fruit firmness and runner length had the most (nine) loci each. These QTLs are likely to be correct because high ($H^2 > 0.55$) and medium ($H^2 > 0.42$) heritability coefficients were observed for firmness and runner length respectively. Medium to high heritability results in a good QTL detection rate and reduces false positives. In addition, similar results were previously reported by Lerceteau-Köhler et al. (2012), where firmness had most (seven) loci. Moreover, fruit firmness depends on a number of factors, such as cell wall degradation and strength at different stages of fruit ripening as previously reported in strawberry and tomato (Santiago-Domenech et al. 2008; Gilbert et al. 2009), thus skin strength (the elasticity) is likely to be directly linked to fruit firmness, although the correlation analyses did not show any correlation between these two traits in this study. As a result, a large number of genes may be linked to the skin strength. Indeed, a large number of QTLs (seven loci) linked to skin strength over two years were identified in this study. However, the large number of loci associated with skin strength may be partly erroneous due to false positives. Indeed, a very low heritability coefficient ($H^2 = 0.02$) was observed for skin strength (previously reported in Chapter 2, Section 2.3.4), which

supports the suggestion that QTLs linked to skin strength may be erroneous. Similarly, a large number of QTLs (seven loci) were associated with truss length which might also be false positives due to the fact that a low heritability coefficient (H^2 = 0.05) was observed for this trait (Chapter 2, Section 2.3.4).

Interestingly, a significant difference was observed between the number of QTLs linked to soluble solids content when the results obtained in this study were compared to those reported by Lerceteau-Köhler et al. (2012). This study confirms that soluble solids content is a complex trait (eight loci identified, $H^2 > 0.68$), whereas only three loci were reported by Lerceteau-Köhler et al. (2012).

4.5 Conclusions

At least two potential QTL associated with plant characteristics and fruit quality traits were successfully identified for all 30 traits analysed in this study. The results obtained here demonstrated that 30.2% of loci detected were present in two or three years and were stable over the years. However, the majority of loci were present in one year only and were year-dependable. Five QTL clusters (hotspots) of overlapping chromosomal regions were detected. These findings are significant and could be considered as targeted regions for further analysis, such as candidate gene(s) identification. The results also suggest that the number of QTLs identified for two traits (truss length and skin strength) might be erroneous based on heritability analysis conducted in Chapter 2 (Section 2.3.5). This demonstrates the importance of heritability analysis of the traits prior to QTL mapping. Although a large number of potential QTLs was identified for all traits studied, further analysis, such as interval mapping using

individual linkage maps for female and male, as well as permutation tests are essential to build on these results.

The results obtained here will be a valuable tool for future studies including candidate gene identification and novel molecular marker development. The molecular markers linked to traits of economic importance will be crucial for marker-assisted breeding in strawberry breeding programmes.

Chapter 5

Validation of QTLs detected linked to fruit firmness and expansin genes in octoploid strawberry

5.1 Introduction

Strawberry fruits are mainly sold for the fresh market, as well as for the food processing industries worldwide. Fruit firmness is important for consumers and therefore directly influences producers, suppliers and commercial retailers. In addition, firmness is an essential attribute for fruit production and allows easier harvesting, handling and storage. Firm fruits usually have better fruit qualities than soft fruits, including better postharvest storage. This has been reported in a number of stone and soft rosaceous crops including cherry (Kappel, 2008; Sansavini and Lugli, 2008; Oraguzie, 2010), apple (Johnston et al. 2002; Rocha and Morais, 2003; Zude et al. 2006), peach (Gorny et al. 1999; Manganaris et al. 2007; Bianchi et al. 2013), pear (Gorny et al. 2000) and strawberry (Vicente et al. 2002). Furthermore, the disadvantages of soft fruits include increased pathogen susceptibility, significantly reduced postharvest shelf life and quality loss of fresh fruits (Dotto et al. 2006). As a result, breeding for fruit firmness in strawberry is a major goal.

Fruit firmness is a complex trait and is associated with the fruit ripening process. The process involves multiple developmental factors and includes environmental signals, age, auxins, enzymes and proteins controlling modification of cell wall and fruit maturity (Civello et al. 1999; Harrison et al. 2001). Fruit ripening changes not only fruit firmness, texture, flavour and coloration but also increases the susceptibility to microbial infections (Harpster et al. 1998). It has been reported that fruit firmness constantly declines during fruit development, mainly as a result of cell wall changes (Harrison et al. 2001). These involve changes in the composition and the structure of the cellulose microfibrils, polysaccharides and structural proteins located within the cell wall (Carpita and Gibeaut, 1993; Harpster et al. 1998). Indeed, for a plant to grow, the

cell wall must continuously modify and expand. According to Knee et al. (1977), the cell volume can increase up to 1000-fold by the extreme swelling of the cell walls, resulting in fruit softening.

A group of genes, named expansins, associated with fruit softening have been identified. Expansins are present in cell walls and are linked to cell wall expansion, loosening and metabolism, as well as in changes in the ripening fruit tissue (Harrison et al. 2001; Dotto et al. 2006). Expansins have been identified in many different plant tissues in a wide range of species. For example, expansin mRNAs or cDNAs have been identified in *Arabidopsis thaliana,* tomato and maize leaves (Keller and Cosgrove, 1995; Cho and Cosgrove, 2000; Cosgrove, 2000; Muller et al. 2007), *Arabidopsis*, maize and rice roots (Wu et al. 1996; Cho and Cosgrove, 2002; ZhiMing et al. 2011), and tomato, apricot and cherry fruits (Brummell et al. 1999; Civello et al. 1999).

In strawberry, seven expansin genes, named *FaEXP1* to *FaEXP7* have been reported to regulate fruit softness (Civello et al. 1999; Harrison et al. 2001; Dotto et al. 2006). Of these, two genes (*FaEXP2* and *FaEXP5*) are fruit specific and regulate ripening, resulting in increased expression of these genes during the fruit ripening stages (Dotto et al. 2006). The remaining five genes (*FaEXP1*, *FaEXP3*, *FaEXP4*, *FaEXP6* and *FaEXP7*) are expressed in other plant tissues such as leaves, roots and runners, and thus are thought to be not fruit specific (Civello et al. 1999; Harrison et al. 2001; Dotto et al. 2006). Indeed, the study of Dotto et al. (2006), showed that although *FaEXP1* gene is expressed in fruits, the highest mRNA accumulation is found in strawberry roots. Harrison et al. (2001) demonstrated that genes *FaEXP3* and *FaEXP4* are expressed in leaves, runners and roots during fruit development, whereas the expression of genes *FaEXP6* and *FaEXP7* is enhanced in unripe fruits and these genes are associated with fruit ripening. Interestingly, three expansins (*FaEXP1*, *FaEXP2* and

FaEXP5) showed correlations between their mRNA expression level and fruit firmness (Dotto et al. 2006). This work also suggests that although not all of the expansin genes are fruit specific, they may all be involved in fruit softening.

The aims of this study were therefore (1) to investigate the transferability of SSR markers significantly associated with fruit firmness QTLs; and (2) to determine whether previously reported expansin genes are underlying the QTL regions linked to firmness previously described in Chapter 4, Section 4.3.2. The validation analysis was performed using a cultivated strawberry germplasm producing strawberry fruits of different known firmness levels. Four SSRs were identified as good predictors of differences in fruit firmness in strawberry cultivars. In addition, mRNA sequences of seven expansin genes (*FaEXP1*, *FaEXP2*, *FaEXP3*, *FaEXP4*, *FaEXP5*, *FaEXP6* and *FaEXP7*) were obtained from the on-line database (the National Center for Biotechnology Information) and were aligned to the diploid *Fragaria* reference genome sequence. Of those, three genes (*FaEXP1*, *FaEXP2* and *FaEXP5*) were the main focus in this study because these genes were reported to be correlated with fruit firmness in cultivated strawberry (Dotto et al. 2006), although the positions of the remaining genes were also investigated. The aligned gene positions were compared to the positions of the SNP markers associated with QTLs linked to firmness (identified in Chapter 4, Section 4.3.2).

5.2 Materials and methods

5.2.1 Strawberry germplasm for marker transferability analysis

Octoploid strawberry cultivars, selections and a number of individuals from the 'Redgauntlet' × 'Hapil' (Rg × H) mapping progeny were selected for the validation of molecular markers linked to fruit firmness QTLs. Cultivars were chosen to represent

firm and soft fruit variability among the material tested. The sample set contained 16 plants representing fruit softness (three cultivars, two selections and eleven progenies of the Rg × H population) and 17 plants representing fruit firmness (ten cultivars, one selection and six individuals from the Rg × H progeny). Strawberry samples selected and tested in this study are summarised in Table 5.1.

Table 5.1 The list of strawberry plants selected for the transferability analysis of SSR markers. Plants were identified as producing either soft or firm fruits. The names of the individuals of 'Redgauntlet' × 'Hapil' mapping progeny start with two capital letters 'RH', followed by three numbers

Soft fruits		**Firm fruits**	
1.Earliglow	9.RH105	1.Albion	9.Seascape
2.Gorrela	10.RH115	2.Argentera	10.Selva
3.Osmanli	11.RH122	3.Buddy	11.SDBL122
4.EM1792	12.RH130	4.Diamante	12.RH050
5.P85	13.RH153	5.Elegance	13.RH135
6.RH006	14.RH158	6.Flamenco	14.RH137
7.RH051	15.RH164	7.Florence	15.RH163
8.RH093	16.RH179	8.Holiday	16.RH168
			17.RH061

5.2.2 Firmness

Fruit firmness of individuals from Rg × H mapping population was evaluated by measuring up to ten marketable fruits using Firmtech, as previously described in Chapter 2, Section 2.2.3. The level of fruit firmness for the strawberry cultivars and selections was already known from the phenotypic data collected from strawberry breeding projects at East Malling Research (EMR).

5.2.3 Expansin genes and mRNA alignment to the *Fragaria vesca* genome sequence

Seven mRNA sequences of the expansin genes were obtained from the National Center for Biotechnology Information (NCBI) database. These sequences were aligned to the diploid *Fragaria vesca* genome sequence, in order to identify the physical locations of the genes. The BLAST (Basic Local Alignment Search Tool) function was applied to compare the mRNA gene sequences to the *F. vesca* genome sequence, and was performed using the on-line available genome browser - The Plant Genome portal (Phytozome 10.3) (http://phytozome.jgi.doe.gov/pz/portal.html). An example of the three expansin gene mRNA sequences aligned against the *F. vesca* genome is presented in Figure 5.1. All expansin gene names, GenBank accessions, mRNA sequences and physical positions on the *F. vesca* genome are summarized in Appendix 5.1.

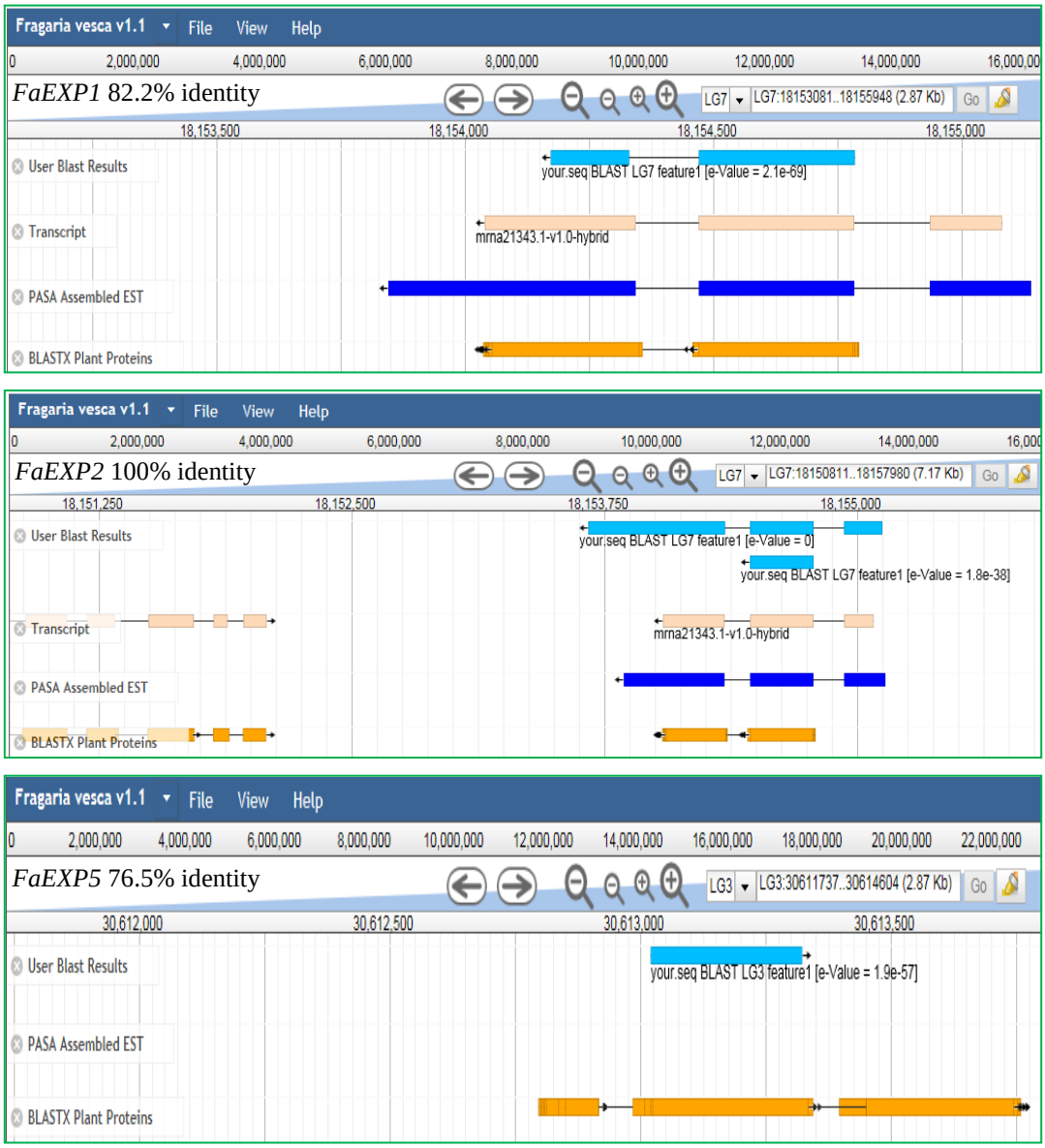

Figure 5.1 The expansin proteins coding gene positions aligned to the diploid strawberry (*Fragaria vesca*) genome sequence using Phytozome 10.3 genome browser. The *FaEXP1* gene had 82.2% identity and was located on LG7; the *FaEXP2* gene had 100% identity and was located on LG7; the *FaEXP5* gene had 76.5% identity and was located on LG3.

5.3 Results

5.3.1 Fruit firmness

Phenotypic mean values for fruit firmness were compared between parental genotypes ('Redgauntlet' and 'Hapil') over the three years. 'Hapil' consistently exhibited firmer fruits than 'Redgauntlet' (Figure 5.2). The firmness of fruits produced by 'Hapil' ranged from 171 (g/mm) to 211 (g/mm), whereas the firmness of 'Redaguntlet' fruits ranged from 152 (g/mm) to 175 (g/mm). Statistically significant differences in fruit firmness between the parental genotypes were observed in 2014 ($p < 0.029$) and 2015 ($p < 0.010$). Fruit firmness observed between parental genotypes in 2013, was not statistically significant ($p < 0.213$) (Figure 5.2).

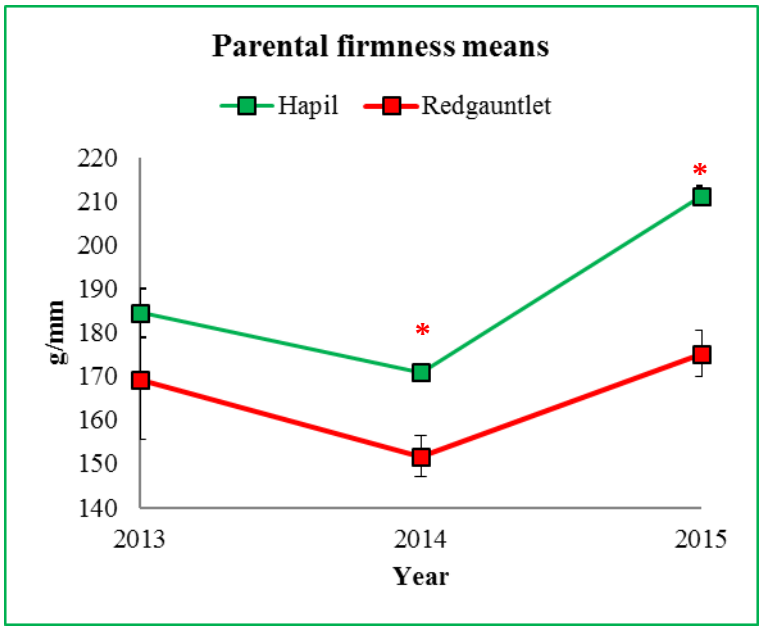

Figure 5.2 Phenotypic means for fruit firmness of 'Redgauntlet' and 'Hapil' for three consecutive years. Red stars indicate differences ($p < 0.05$) between parental genotypes based on Student *t*-Test in 2014 and 2015.

Phenotypic means for fruit firmness were compared between parental genotypes and the progeny. In general, the softest fruits were observed in 2014, whereas the most firm fruits were harvested in 2015 (Figure 5.3). The variance within the progeny was not equally distributed among the individuals, and was transgressive. A large number of individuals displayed higher or lower values than parental genotypes and this was consistent across three years.

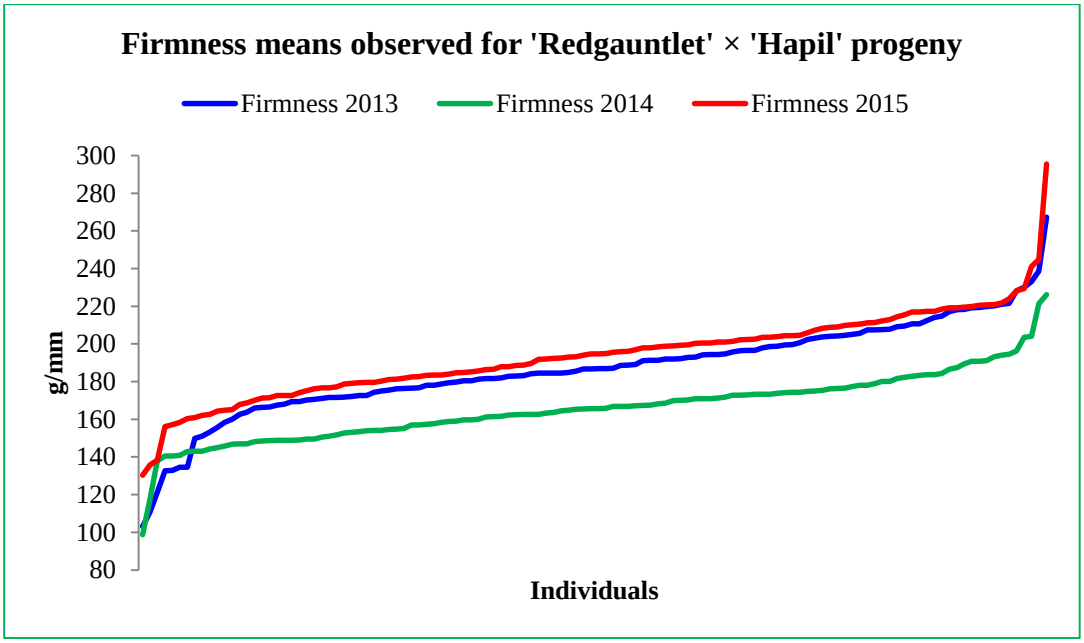

Figure 5.3 Distribution of the phenotypic means calculated across six replicates for fruit firmness observed among 'Redgauntlet' × 'Hapil' mapping progeny for 2013, 2014 and 2015.

5.3.2 QTL identification linked to fruit firmness using SSR-based linkage map and the comparison of the locations to the QTLs identified using SNP-based map

The combined phenotypic (Chapter 2, Section 2.3.1) and genotypic (Chapter 3, Section 3.3.2) information was used for mapping QTLs linked to fruit firmness. QTL identification using the SSR-based linkage map was performed as previously described in Chapter 4, Section 4.2.4. A total of eight QTLs associated with fruit firmness were detected on the SSR-based genetic linkage map. When the mapping positions of QTLs were compared between mapping positions on SSR and SNP linkage maps, some inconsistencies were observed (Table 5.2, a). The results showed that five loci were identified on the same linkage groups (LG1A, LG1D, LG3A, LG4A and LG4B) between the two maps, whereas the remaining three loci were mapped to the homoeologous linkage groups (LG1B, LG2B and LG7B).

Table 5.2 (a) QTL positions mapped to the linkage groups of SNP and SSR genetic linkage maps. (b) Comparison of linkage group name annotations between SNP and SSR linkage maps. (c) QTL positions on the SSR linkage map after linkage group re-naming

a)	Year	Firmness		b)	The comparison of the linkage group names		c)	Year	Firmness	
		SNP map	SSR map[1]		SNP map	SSR map			SNP map	SSR map[2]
	2013	1A	1A		1A	1B		2013	1A	1A
	2014	1A	1A		1B	1C		2014	1A	1A
	2015		1A		1C	1D		2015		1A
	2013		1B		1D	1A		2014		1C
	2014		1B		**2A**	**2A**		2015		1C
	2015		1B		2B	2C		2013	1D	1D
	2013	1D			2C	2B		2014		1D
	2014		1D		**2D**	**2D**		2015		1D
	2015		1D		**3A**	**3A**		2014	2C	2C
	2014		2B		3B	3D		2015	2C	2C
	2015		2B		3C	3C		2013	2D	
	2014	2C			3D	3B		2013	3A	3A
	2015	2C			4A	4B		2014	3A	3A
	2013	2D			4B	4A		2015		3A
	2013	3A	3A		**4C**	**4C**		2013	4A	4A
	2014	3A	3A		**4D**	**4D**		2014		4A
	2015		3A		5A	5B		2015		4A
	2013	4A	4A		5B	5C		2013		4B
	2014		4A		5C	5A		2014	4B	4B
	2015		4A		**5D**	**5D**		2015	4B	4B
	2013		4B		**6A**	**6A**		2013	4C	
	2014	4B	4B		6B	6D		2013	7A	7A
	2015	4B	4B		6C	6B		2014		7A
	2013	4C			6D	6C		2015	7A	7A
	2013	7A			7A	7B				
	2015	7A			7B	7C				
	2013		7B		7C	7A				
	2014		7B		**7D**	**7D**				
	2015		7B							

[1]QTL locations on the SSR-based linkage map

[2]QTL locations on the SSR-based linkage map after the re-naming of the linkage groups according to the SNP-based linkage map

The inconsistencies among QTL positions between the two linkage maps could have arisen because of incorrect annotation of the SSR-based linkage group names. The linkage groups of the SNP-based linkage map were named according to the high density octoploid strawberry genetic linkage map 'Holiday' × 'Korona', previously reported by van Dijk et al. (2014). The linkage group naming of the SSR-based linkage map was based on common molecular markers between the diploid *Fragaria* reference map (*Fragaria vesca* × *Fragaria bucharica*) and the octoploid strawberry linkage map (Sargent et al. 2009). As a result, seven main linkage groups (LG1 to LG7) were identified correctly, however the homoeologous linkage groups (A, B, C and D) were named randomly. Indeed, when each of the 28 linkage groups of the SSR-based map were annotated according to the SNP-based map, only eight linkage group names (highlighted in green in Table 5.2, b) were in agreement with the linkage groups of the SNP linkage map. The remaining 20 linkage groups showed inconsistent homoeologous linkage group naming.

After the re-naming of the linkage groups of the SSR-based linkage map according to the SNP-based linkage map, seven out of eight QTLs linked to firmness were mapped to the same linkage groups. Only one QTL (LG1C) was mapped to the homoeologous linkage group (Table 5.2, c). Nine loci were detected on the SNP-based linkage map, instead of eight identified on the SSR-based map.

QTLs identified on the SSR linkage map were more stable than those detected on the SNP map. Out of eight loci, six were present in all three years and the remaining two loci were present in two of three years. In contrast, out of nine loci identified on the SNP linkage map, four loci were present in two of three years and the remaining four loci were present in one year only. No QTLs were present in all three years on the SNP-based linkage map (Table 5.2, c).

5.3.3 Markers closely linked to the QTLs associated with fruit firmness

The most closely linked molecular markers were identified for each QTL associated with fruit firmness using SSR-based genetic linkage map, as previously described in Chapter 4, Section 4.3.4. Sixteen molecular markers were identified for each of eight QTLs; these are summarised in Table 5.3.

Table 5.3 A summary of the 16 most closely linked molecular markers associated with fruit firmness loci using the re-named SSR-based genetic linkage map. The year the QTL was detected, marker name, linkage group and position (cM) of the most closely linked marker, K* value, significance level and parent in which the QTL was present are presented

Year	Linkage group	Position (cM)	Marker	K*	Significance[1]	Parent
2013	LG1A	38.23	**CO817853-300**	8.386	****	Redgauntlet
2014	LG1A	38.23	**CO817853-300**	8.245	****	Redgauntlet
2015	LG1A	77.276	ARSFL010-228	4.386	**	Redgauntlet
2014	LG1C	31.493	OPA-09B	6.639	***	Redgauntlet
2015	LG1C	22.304	FvA129-173	5.683	**	Redgauntlet
2013	LG1D	98.525	**Fvi6b-276**	18.24	*******	Hapil
2014	LG1D	52.737	EMFn182-192	8.379	****	Redgauntlet
2015	LG1D	98.525	**Fvi6b-276**	6.844	***	Hapil
2014	LG2C	83.778	CFUC5057-150	9.361	****	Redgauntlet
2015	LG2C	83.422	DFR-329	6.845	***	Redgauntlet
2013	LG3A	28.4	UDF016-109	7.932	****	Hapil
2014	LG3A	14.35	512992ag13-211	8.957	****	Redgauntlet
2015	LG3A	100.225	512944ag14-327	9.152	****	Redgauntlet
2013	LG4A	12.347	**FAES0063-213**	8.401	****	Hapil
2014	LG4A	12.347	**FAES0063-213**	4.722	**	Hapil
2015	LG4A	12.347	**FAES0063-213**	7.18	***	Hapil
2013	LG4B	17.035	512972at12-351	7.472	***	Hapil
2014	LG4B	49.914	**513040ac14-135**	8.042	****	Redgauntlet
2015	LG4B	49.914	**513040ac14-135**	7.943	****	Redgauntlet
2013	LG7A	62.115	CFVCT019-105	16.198	*******	Redgauntlet
2014	LG7A	66.525	**FvC123-112**	7.249	***	Redgauntlet
2015	LG7A	66.525	**FvC123-112**	7.867	***	Redgauntlet

[1]Significance level were calculated using the Kruskal-Wallis test in MapQTL and are indicated as follows: ** = 0.05, *** = 0.01, **** = 0.005, ***** = 0.001, ****** = 0.0005 and ******* = 0.0001

Out of 16 molecular markers identified, two were amplified fragment length polymorphism (AFLP) markers (OPA and DFR), whereas the remaining 14 markers were simple sequence repeats (SSRs). In four cases, the same SSR marker was identified in two out of three years for the same QTL. These QTLs were mapped to LG1A, LG1D, LG4B and LG7A (Table 5.3, markers in bold). In one case, the same SSR marker (FAES0063) was identified for all years on LG4A.

5.3.4 QTL effects across years

The 16 molecular markers identified that had the strongest association with firmness QTLs were used to investigate the effect of QTLs across the three years (2013, 2014 and 2015) analysed. The results showed increasing linear distributions of fruit firmness with the increase number of molecular markers linked to fruit firmness. This is demonstrated across the three years by the gradient of slope (Figure 5.4).

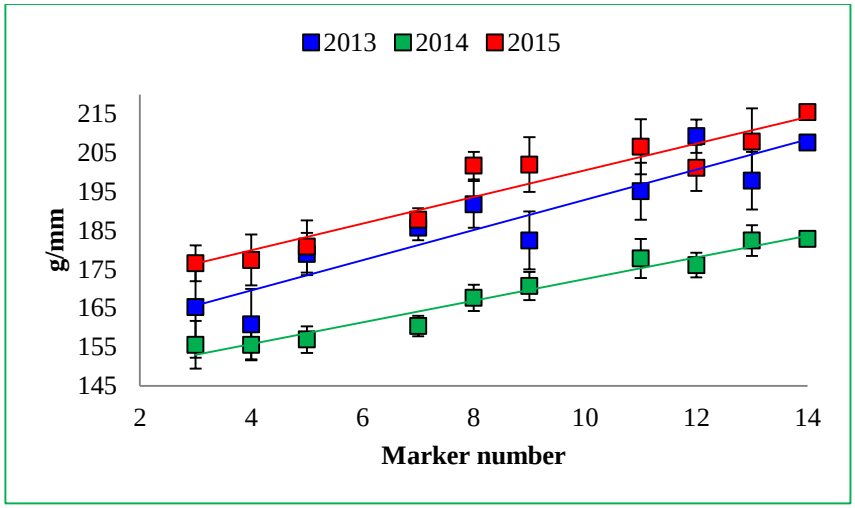

Figure 5.4 The relationship between QTLs identified and phenotypic firmness score among individuals of 'Redgauntlet' × 'Hapil' mapping progeny for the three consecutive years analysed. A consistent increase in firmness can be seen over the years. Error bars are 95% confidence intervals.

The steepest slope was observed in 2015, which also represents the most firm fruits were harvested. In contrast, the slope was least steep in 2014, which was the year of the softest fruits harvested (Figure 5.4 and Figure 5.5).

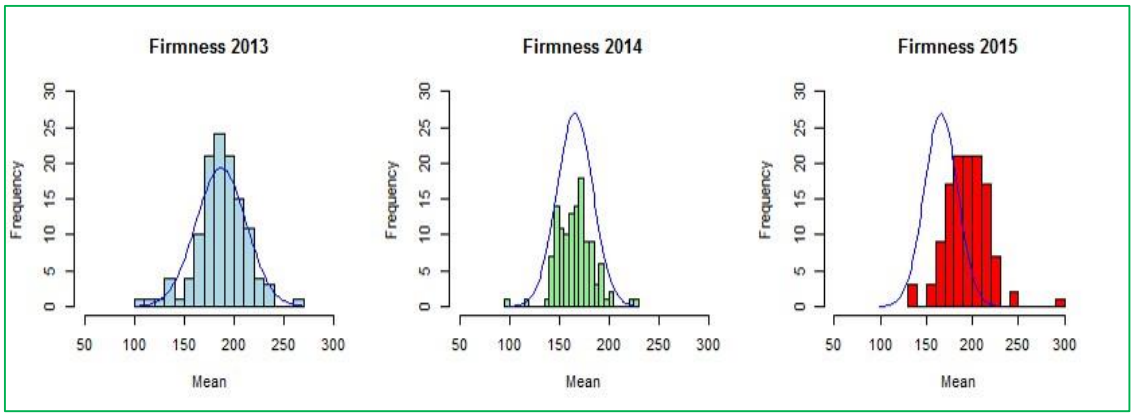

Figure 5.5 Distribution of the firmness means of the 'Redgauntlet' × 'Hapil' progeny for three years.

5.3.5 Validation of markers in strawberry germplasm

A set of strawberry cultivars, selections and individuals from the 'Redgauntlet' × 'Hapil' progeny with known firmness level were chosen for the marker validation study (Table 5.1). Two markers out of 16 identified were AFLPs and were excluded from further analysis. The majority of markers (nine) identified explained incorrect phase group association with fruit firmness. These markers were associated with fruit softness instead the firmness and were excluded for further analysis. Only five SSR markers, which showed alleles in coupling with the QTL, were screened in plant material because only these markers could potentially be suitable for use in marker-assisted breeding.

A total of 58.8% of the firm plant material tested consistently had higher number of markers amplified (Table 5.4). For example, three strawberry cultivars ('Earliglow',

‘Gorrela’ and ‘Osmanli’) with soft fruits had only a small number (one or two) of SSR markers amplified, whereas the majority of strawberry cultivars with firm fruits had a higher number (two to four) of markers amplified. Individuals from the ‘Redgauntlet’ × ‘Hapil’ mapping progeny (names starting with RH) exhibited the most reliable results. Seven out of ten individuals with soft fruits amplified PCR products for only one SSR marker, while all five individuals producing firm fruits had higher allele frequencies (PCR products were amplified for four to five SSR markers).

Despite the expected trends for the majority of the plant material tested, there were some inconsistencies among the lines. For example, some lines producing soft fruits (e.g., P85 and RH093) amplified three SSR markers, while other lines producing firm fruits (e.g., ‘Holiday’, ‘Selva’ and ‘SDBL122’) only amplified one SSR marker (Table 5.4). However, these results were based on five out of 16 markers, thus inconsistencies were expected because it is likely that some of the markers identified may not be the closest linked to the QTL associated with firmness.

Of the five SSRs tested, four (512972at12, EMFn182, ARSFL010 and 512992ag13) expressed better amplification patterns (higher allele frequency among the lines with firm fruits tested); therefore marker UDF016 was not considered suitable for use in MAS. Moreover, the results showed that fruit firmness is associated with SSR markers amplifying alleles from paternal (‘Hapil’) individual, thus the 512972at12 SSR is the most reliable marker linked to firmness QTL identified in this study. The remaining SSRs were amplifying alleles from maternal (‘Redgauntlet’) individual (Table 5.4). Further marker validation analysis in a larger set of plant material, especially strawberry cultivars, is essential in order to confirm the associations between these markers and QTLs linked to firmness, and their potential to be used in MAS.

Table 5.4 Validation of QTLs in wider strawberry germplasm and mapping progeny using most closely linked SSR markers. The presence of the markers is indicated by a number 1 (firmness QTL). Alleles from 'Redgauntlet' are indicated by R, whereas those from 'Hapil' are indicated by H

Phenotype	Cultivar	UDF016	512972at12	EMFn182	ARSFL010	512992ag13	Sum
		111 H	351 H	194 R	232 R	213 R	
Soft	EM1792						0
Soft	Earliglow					1	1
Soft	RH006		1				1
Soft	RH051				1		1
Soft	RH122				1		1
Soft	RH130					1	1
Soft	RH158				1		1
Soft	RH164					1	1
Soft	RH179	1					1
Soft	Gorrela		1			1	2
Soft	Osmanli			1		1	2
Soft	RH105		1	1			2
Soft	RH115		1	1			2
Soft	RH153		1	1			2
Soft	P85	1		1	1		3
Soft	RH093	1	1			1	3
Firm	Holiday					1	1
Firm	Selva		1				1
Firm	SDBL122		1				1
Firm	Buddy				1	1	2
Firm	Flamenco				1	1	2
Firm	Florence		1	1			2
Firm	RH050		1			1	2
Firm	Argentera		1	1	1		3
Firm	Seascape	1		1	1		3
Firm	Albion		1	1	1	1	4
Firm	Diamante		1	1	1	1	4
Firm	Elegance		1	1	1	1	4
Firm	RH135		1	1	1	1	4
Firm	RH137	1	1	1		1	4
Firm	RH168		1	1	1	1	4
Firm	RH061		1	1	1	1	4
Firm	RH163	1	1	1	1	1	5

5.3.6 Physical positions of SNPs and comparison to the strawberry expansin gene locations aligned to *Fragaria vesca* genome sequence

In order to investigate whether QTL positions associated with fruit firmness overlapped expansin gene locations, mRNA sequences of the three expansin genes (*FaEXP1*, *FaEXP2* and *FaEXP5*) were aligned to the *Fragaria vesca* genome sequence v1.1. The physical positions of the expansin genes were compared to the physical positions of the closest SNPs linked to fruit firmness QTLs identified in Chapter 4, Appendix 4.1. The list of SNP markers selected and the comparison of their physical positions against expansin gene physical positions are summarised in Table 5.5.

When the physical locations of 19 SNP markers (Appendix 4.1) were compared to the actual locations, one marker (AX-89795942:nmh) mapped on LG1D had an incorrect physical linkage group (Table 5.5, highlighted in red). This marker was excluded from further analysis. This suggests that potentially the *F. vesca* genome assembly has errors. Physical linkage groups were in agreement with the actual mapped linkage groups for the remaining 18 SNPs.

Table 5.5 List of SNP markers most closely linked to firmness QTLs, their physical and actual linkage groups, position on the SNP-based linkage map and QTL locations on linkage groups. SNP marker physical positions were compared to the physical positions (aligned to the *Fragaria vesca* genome sequence v1.1) of the three genes encoding expansin proteins. One SNP (in red) had an incorrect physical location

	Linkage group						**Expansin genes**			
SNP	Physical	Mapped	Position (cM)	QTL region (cM)	Parent	SNP physical position	Name	LG	Physical position	Identity
AX-89780703:nmh	LG1	1A	43.527	43 - 44	Rg	8091967				
AX-89904305:ph3	LG1	1A	43.527	43 - 44	Rg	8134908				
AX-89847157:ph3	LG1	1A	43.887	43 - 44	Rg	8231059				
AX-89780660:nmh	LG1	1A	42.448	42 - 43	Rg	7867898				
AX-89795942:nmh	LG6	1D	64.548	63 - 64	H	16171921				
AX-89878587:nmh	LG2	2C	78.788	66 - 80	Rg	20241367	***FaEXP2***	**LG2**	**19165616..19166515**	**66.2%**
AX-89820344:ph3	LG2	2D	2.942	2 - 4	H	15946662				
AX-89877470:ph3	LG2	2D	2.942	2 - 4	H	15933049				
AX-89877426:ph3	LG2	2D	3.661	2 - 4	H	15757720				
AX-89820302:ph3	LG2	2D	3.661	2 - 4	H	15756774				
AX-89856683:nmh	LG3	3A	36.984	36 - 37	H	6986698	*FaEXP1*	LG3	20228990..20229307	80.0%
AX-89785507:ph3	LG3	3A	13.398	13 - 17	Rg	2179493	*FaEXP2*	LG3	20228821..20229735	87.9%
AX-89824842:ph3	LG3	3A	15.207	13 - 17	Rg	1952281	*FaEXP5*	LG3	30613024..30613750	76.5%
AX-89824795:ph3	LG3	3A	17.066	13 - 17	Rg	1919703				
AX-89889023:nmh	LG4	4A	11.418	9 - 11	H	6588568				
AX-89887459:nmh	LG4	4B	38.17	36 - 39	Rg	21021764				
AX-89829872:nmh	LG4	4C	31.532	20 - 31	H	18898079				
AX-89801084:nmh	LG7	7A	20.126	19 - 21	Rg	16938915	*FaEXP1*	LG7	18154043..18154793	82.2%
AX-89800994:ph3	LG7	7A	53.426	53 - 54	Rg	16004624	*FaEXP2*	LG7	18154043..18154793	100%
							FaEXP5	LG7	18154043..18154793	78.7%

Comparison of the physical positions between the SNP markers and expansin genes showed that three SNPs were located close to the expansin gene locations (Table 5.5). The physical position of a single SNP (AX-89878587:nmh) mapped to LG2C was 20.2 Mb, while the position of the *FaEXP2* expansin was 19.2 Mb. Moreover, this SNP marker (AX-89878587:nmh) was closely linked to a firmness QTL on the same linkage group (LG2C) over two years (2014 and 2015). Moreover, the *FaEXP2* expansin located on LG2C had only 66.2% identity, and therefore this gene is likely to be homologous to the *FaEXP2* expansin (Table 5.5 in bold). Similarly, the physical positions of AX-89801084:nmh and AX-89800994:ph3 SNPs mapped on LG7 were 16.9 Mb and 16 Mb respectively, whereas three expansin genes were located approximately at 18.2 Mb (Table 5.5). Interestingly, *FaEXP1*, *FaEXP2* and *FaEXP5* were located at the identical position in the diploid *Fragaria* genome sequence (v1.1), the linkage group seven.

The same three expansin genes were identified on LG3 with identity ranging from 76.5% to 87.9%. Four SNPs were also linked to firmness loci on LG3, although the physical locations between expansins and SNPs were far away from each other (a difference of more than 18 Mb was observed) (Table 5.5).

In general, BLAST analysis yielded six linkage groups with the locations of the expansin genes. These linkage groups were mainly common among the expansions, although their positions were different (Appendix 5.1). For example, all expansins showed a match on LG5, and of those, three expansins (*FaEXP4*, *FaEXP5* and *FaEXP6*) had the highest percentage identity (82.5% - 98.4%). Similarly, all expansins had a match on LG3, LG6 and LG7. In contrast, four expansins (*FaEXP2*, *FaEXP3*, *FaEXP4* and *FaEXP7*) were located on LG2 and only one gene (*FaEXP3*), most likely to be homologous to *FaEXP3*, was located on LG4 (the percentage identity was 69.8%).

5.4 Discussion

In this chapter the validation of QTLs linked to fruit firmness is reported as a case study. In addition, physical positions between the SNPs closest to these QTLs and expansin genes were compared to investigate whether SNP markers overlap the positions of expansin genes reported to control fruit firmness.

A total of 16 SSRs most closely linked to firmness QTLs were identified across eight loci on the SSR-based genetic linkage map, previously described in Chapter 3, Section 3.3.2. Validation of the most strongly associated SSRs was performed using wider strawberry germplasm including soft and firm strawberry material. The results showed that four markers produced the most reliable pattern of allele frequency among soft and firm lines tested, therefore four SSRs potentially associated with firmness were identified.

The study also reports discrepancies of the linkage group names between SNP-based and SSR-based linkage maps. The majority of linkage groups (20) of the SSR-based linkage map were incorrectly named previously and were re-named here, according to the SNP-based linkage groups. Correct linkage group naming is very important, especially for the comparative analysis carried out here. Furthermore, the physical locations of 19 SNP markers linked to firmness QTLs and three expansin genes were compared. Three SNPs were closely located to the expansins and these findings provide supporting evidence that the locations of several QTLs associated with firmness identified on the SNP-based map are correct. However, genes exhibiting the highest percentage identity to expansins were mostly located on linkage groups five and six, but no QTLs linked to firmness were detected on these linkage groups on either SNP or SSR-based maps. This could be explained by the large number of regions with low

marker density observed on these linkage groups on both genetic linkage maps. A high degree of homozygosity was observed in the mapping population (‘Redgauntlet’ × ‘Hapil’), therefore the saturation of some regions of the genome, in this case linkage group five and six, was difficult.

5.4.1 Comparison of QTL positions linked to fruit firmness on SSR and SNP maps

Nine QTLs associated with fruit firmness were identified on the SNP-based linkage map (Chapter 4, Section 4.3.2), while eight QTLs linked to fruit firmness were identified on the SSR-based linkage map reported in this chapter. However, the results showed that only five QTL locations mapped to the same linkage groups and the remaining six loci were mapped to the homoeologous linkage groups when QTL locations were compared between the SSR and the SNP maps (Table 5.2, a).

Two possible hypotheses may explain these inconsistencies. First of all, the quality between the two linkage maps used for QTL mapping is significantly different. More regions with low marker density were present and lower number of molecular markers was mapped on the SSR linkage map, and therefore the quality of the map was poorer than that of SNP map. A low density genetic linkage map is not suitable for QTL analysis and potentially important QTLs might have been missed due to the low QTL detection power. Secondly, annotation of some linkage group names, either on the SSR map or the SNP map, is incorrect. It is more likely that linkage group naming is incorrect for the SSR map because linkage groups on the SNP map were named according to a different *Fragaria* × *ananassa* high density linkage map derived from the cross ‘Holiday’ × ‘Korona’ (van Dijk et al. 2014). Indeed, the previously reported SSR-based linkage map, constructed for the ‘Redaguntlet’ × ‘Hapil’ mapping population,

was compared to a diploid *Fragaria* reference map (*Fragaria vesca* × *Fragaria bucharica*). This suggests that linkage group naming was based on common molecular markers detected between diploid and octoploid strawberry populations (Sargent et al. 2009).

After the re-naming the linkage groups on the SSR-based map according to the SNP-based map, seven out of eight QTLs linked to fruit firmness were detected on the same linkage groups between the two maps, and only one QTL was mapped to the homoeologous linkage group (Table 5.2, c). These results suggest that incorrect linkage group names were the main reason why QTL locations between the SNP and SSR maps were not in agreement.

This study demonstrated the importance of the consistent linkage group naming, especially for comparative analyses.

5.4.2 Transferability of SSRs linked to fruit firmness

The marker validation study showed, in general, consistent patterns of allele amplification among soft and firm lines tested. However, some discrepancies were observed among plant material. In some cases, lines producing soft fruits had more markers amplified instead of less, and vice versa. One of the reasons behind this could be that firmness is controlled by multiple loci and that markers linked to firmness were selected based on the biallelic cross between 'Redgauntlet' and 'Hapil', thus only a portion of fruit firmness was detected in a wider germplasm. Similar findings were previously reported by Antanaviciute et al. (2015). The transferability of the identified molecular markers associated with *Verticillium* wilt resistance was partial among the strawberry germplasm tested. The study also reports several other hypotheses to explain transgressive-like behaviour among the lines tested.

In addition, a relatively small number of individuals were selected for the validation analysis and this may contribute to the false positive results. A further validation analysis needs to be performed to confirm the significance of the four SSRs identified as showing consistent allele frequencies among plant material tested.

This study also demonstrated that SSR markers associated with fruit firmness are often on a wrong phase. Nine out of 16 SSRs identified were on a wrong phase, and therefore were excluded from the validation analysis and were not suitable for use in marker-assisted breeding (MAB), because these markers will have an inverse sign (in this case the association with fruit softening). The marker phase explains whether marker/allele came from maternal or paternal genotype. It is crucial to identify the association between the trait of interest and molecular marker correctly because markers associated with a positive effect (in this case fruit firmness) usually are preferable for use in MAB. Markers associated with a negative effect (in this case fruit softening) must be avoided due to the inverse association. This is a significant issue in the development of molecular markers for MAB. To overcome it, use of a genotyping array containing thousands of SNPs may provide more reliable molecular markers associated with traits of interest because a large number of markers have a higher potential to be closely linked to the QTLs. As a result, SNPs are likely to become the most preferable molecular markers for a wide range of studies.

5.4.3 Expansin genes associated with fruit firmness in strawberry

The analysis conducted here, based on three expansin genes (*FaEXP1*, *FaEXP2* and *FaEXP5*) (Appendix 5.1) and 19 SNP markers (Appendix 4.1), demonstrated that two QTLs on linkage groups 2C and 7A are closely located to the expansin genes and/or homologues of the expansin genes. These genes were previously reported to show

correlation between their expression level and fruit firmness (Dotto et al. 2006). An additional analysis of all seven expansins showed that the most significant regions associated with these genes were located on LG5 and LG6. However, no QTLs linked to fruit firmness were identified on these linkage groups using SNP-based and SSR-based linkage maps in this study. However, a large number of regions with low marker density were observed on LG5 and LG6 on the SSR-based linkage map (Chapter 3, Figure 3.4), and therefore the power to detect QTL was low. A similar situation was observed on the SNP-based linkage map: two gaps greater than 10 cM were observed on LG5B and LG6B, in addition to a 31 cM gap on LG6C (Chapter 3, Table 3.4). This could be because of a high degree of homozygosity observed in 'Redgauntlet' × 'Hapil' progeny, resulting in difficulty in saturation of the specific regions of the genome. The low marker density observed on these linkage groups might be the reason why no QTLs linked to fruit firmness were associated with them.

5.5 Conclusions

A case study focusing on validation of the fruit firmness QTLs successfully provided novel information on SSR markers linked to firmness for use in marker-assisted breeding programmes. Four potential SSR markers associated with fruit firmness QTLs have been identified, although further validation is necessary to confirm the significance of the markers identified. The study also emphasizes the importance of consistent linkage group naming on genetic linkage maps, especially those developed for the same mapping progeny. Twenty linkage groups of the SSR-based linkage map were named inconsistently when compared with the SNP-based map. The re-named

linkage groups on the SSR-based map show that seven out of eight QTLs on the SSR map were located on the same linkage groups as on the SNP map.

The comparison of the physical positions between expansin genes aligned to the diploid *Fragaria* genome sequence (v1.1) and QTLs associated with firmness provided supporting evidence that two QTLs are located close to the locations of expansin genes. These findings suggest that the expansin gene family, are linked to fruit firmness and that two QTLs identified on the SNP-based genetic linkage map are significant.

Chapter 6

Mapping QTLs linked to powdery mildew in cultivated strawberry and the relationship between wilt, plant characteristics and fruit quality QTLs

6.1 Introduction

Powdery mildew is a common and widespread disease caused by fungal pathogens belonging to the family Erysiphales, Ascomycota (Takamatsu, 2004; Heffer et al. 2006). The majority of these pathogenic species have strict specificity and can infect only a narrow range of host plants (Lipka et al. 2008). However, a large number of different species of the pathogen infect more than 9,800 host plant species, which range from trees and shrubs to grasses, and include a large number of economically important crops (Heffer et al. 2006).

Powdery mildew of cultivated strawberry (*Fragaria* × *ananassa* Duch.), caused by the fungal pathogen *Podosphaera aphanis* (formerly known as *Sphaerotheca macularis*), is one of the most common fungal diseases of strawberry and is widespread (Maas, 1998; Carisse and Bouchard, 2010). The pathogen is capable of infecting all above-ground plant tissues, especially leaves, which become curly and dry with patches of powdery fungus mycelium usually on the upper surface of the leaves (Hancock, 1999; Pessina et al. 2014). Severe damage to the leaves results in reduction of photosynthesis leading to leaf necrosis and defoliation (Maas, 1998). In addition, the pathogen is capable of infecting strawberry flowers, stems, runners and even fruits (Maas, 1998; Santos et al. 2004; Amsalem et al. 2006). The infected flowers usually develop deformed, unmarketable fruits. In other, more severe cases, where flowers are completely covered in powdery fungus mycelium, no fruits are developed and flowers are killed. This results in significant yield reduction (Spencer, 1978; Pertot et al. 2008).

It has been reported that powdery mildew infections in strawberry have become more severe compared to those in the past (Hukkanen et al. 2007). Strawberries for commercial production have increasingly been grown under polytunnel or greenhouse

conditions which also create an ideal environment for the mildew pathogen to develop and spread (Hukkanen et al. 2007; Xu et al. 2008). According to Amsalem et al. (2006), high levels of humidity and air temperature (15 – 25 ºC) increase the level of infection of powdery mildew. These ideal conditions may also increase resistance of the pathogen to fungicides: the powdery mildew pathogen expresses a high degree of tolerance to fungicides by developing new fungicide tolerant strains (Hukkanen et al. 2007).

To date, there are no strawberry cultivars resistant to powdery mildew available, although some short-day and day-neutral cultivars show a higher level of tolerance to the disease compared to long-day cultivars (Simpson et al. 1994; Davik and Honne, 2005). Therefore, breeding new strawberry cultivars which are resistant to powdery mildew is a major goal for plant breeders. However, breeding for disease resistance in strawberry is a challenging process due to the complex octoploid strawberry genetics and the fact that disease resistance traits are usually controlled by more than one gene.

Breeding new cultivars that are disease resistant usually focuses on the deployment of dominant plant resistance genes, named R-genes (Pessina et al. 2014; van Schie and Takken, 2014). These genes encode proteins which activate plant defence signalling network not only within the infection site but also throughout the plant, in order to prevent pathogen invasion (Hammond-Kosack and Kanyuka, 2007). Resistance (R) genes recognise pathogen effectors and trigger defence mechanisms known as effector-triggered immunity (ETI), following by the hypersensitive response (HR) (van Schie and Takken, 2014). However, breeding for R-gene inheritance is challenging, because usually these genes originate from wild-relatives and their introgression is difficult due to the interspecific barriers (Fu et al. 2009). Moreover, according to Pessina et al. (2014), R-genes are associated with the incorporation of undesirable traits and repeated backcrossing is necessary to eliminate those traits, therefore breeding

becomes a time-consuming process, especially within rosaceous crops. In addition, it has been reported that pathogens can overcome R-gene resistance in just a few years, due to the fact that resistance is based on recognition on a single pathogen infection pathway (Parlevliet, 1993; van Schie and Takken, 2014). In contrast to R-genes, plant susceptibility genes (S-genes) or loss-of-function mutations are broader spectrum genes and have been showed to have higher potential in breeding for disease resistance (van Schie and Takken, 2014). These genes accommodate and support pathogen infection, therefore the mutation or loss of S-genes lead to the reduction of the infection because the compatible interaction between the host and the pathogen is no longer supported, and the pathogen cannot survive within the host (Pessina et al. 2014; van Schie and Takken, 2014).

The S-gene resistance has been well studied in a number of different crops and plant species. These include barley (Büschges et al. 1997), pea (Humphry et al. 2011; Pavan et al. 2011), tomato (Bai et al. 2008), pepper (Zheng et al. 2013) and *Arabidopsis* (Consonni et al. 2006). The mutation-induced recessive alleles (mlo) of the Mlo locus associated with powdery mildew resistance were first reported in 1942 in barley (Pessina et al. 2014) and followed by the release of the first barley varieties resistant to powdery mildew in 1979 (Jørgensen, 1992; Lyngkjær et al. 2000). Interestingly, since then, the same recessive barley mutant, expressing resistance to all powdery mildew races, has been used in barley breeding (van Schie and Takken, 2014). Thus, S-genes or mlo genes are more reliable and potentially are more important than R-genes in breeding resistance to powdery mildew.

Despite the challenges in breeding disease resistant cultivars, quantitative trait loci (QTL) associated with disease resistance have been reported in a number of rosaceous crops, as discussed in Chapter 4 (Introduction). Briefly, QTLs linked to

fireblight and powdery mildew resistance in apple (Calenge et al. 2005, Calenge and Durel, 2006; Khan et al. 2013; Papp et al. 2015), powdery mildew and bacterial spot in peach (Foulonge et al. 2003; Gasic et al. 2013; Yang et al. 2013), scab and fire blight resistance in pear (Dondini et al. 2005; Pierantoni et al. 2007; Bouvier et al. 2012), *Phytophthora* root rot in red raspberry (Pattison et al. 2007; Graham et al. 2011), and leaf spot in cherry (Stegmeir et al. 2014) have been discovered. In contrast, to date, only a few studies have reported inheritance of resistance, molecular markers or QTLs associated with disease resistance in octoploid strawberry. The inheritance of resistance to a single strain of the root rot fungus (*Phytophthora fragariae*) was reported by van de Weg (1997). The study was based on analyzing 12 different segregating populations derived from susceptible and resistant *F.* × *ananassa* cultivars. The results showed that a single dominant resistance gene (R*pf*2) controls disease susceptibility among resistant cultivars. About eight years later, two sequence characterized amplified regions (SCAR) markers were identified linked to the anthracnose (*Colletotrichum acutatum*) resistance gene (R*ca*2) in strawberry (Lerceteau-Köhler et al. 2005). This study analysed a single progeny derived from the resistant ('Capitola') and susceptible ('Pajaro') *F.* × *ananassa* cultivars. The two SCARs were further validated in 43 resistant and susceptible octoploid strawberry cultivars. The study demonstrated a successful prediction of resistant (81.4%) and susceptible (62.8%) genotypes using two SCAR markers which will greatly contribute selection of anthracnose resistance in strawberry breeding programmes.

Recently, QTLs linked resistance to *Verticillium* wilt (caused by the vascular wilt pathogen *Verticillium dahlia*) were identified in cultivated strawberry (Antanaviciute et al. 2015). The study was based on analyzing wilt disease scores collected on a single field-based octoploid strawberry population ('Redgauntlet' ×

‘Hapil’) and a further simple sequence repeat (SSR) marker validation in 49 resistant, susceptible and intermediate strawberry genotypes. In contrast to the two previously discussed studies (van de Weg, 1997; Lerceteau-Köhler et al. 2005), resistance to *Verticillium* wilt is controlled by multiple QTLs, implying that more than one gene is linked to the trait (Antanaviciute et al. 2015). A total of eleven QTL regions were associated with wilt resistance in at least one year of a three-year analysis. The study also demonstrated that a single QTL region liked to wilt resistance has a small effect and that additive control by multiple QTLs is present.

The main aim of the study described in this chapter was to identify QTL associated with powdery mildew in an octoploid strawberry (‘Redgauntlet’ × ‘Hapil’) mapping population. The disease scores collected over the three-year (2012, 2013 and 2014) field trials and the SNP-based genetic linkage map developed in this study (Chapter 3) was used for the analyses. A total of nine loci linked to powdery mildew were detected. Of those, five were present in one year, and the remaining four loci were present in two or three years and were considered to be stable.

In addition, the relationships between QTL regions linked to powdery mildew identified in this study, loci linked to plant characteristics and fruit quality traits, previously discussed in Chapter 4, and QTL regions linked to *Verticillium* wilt previously reported by Antanaviciute et al. (2015) was investigated. The purpose of this was to establish any potential overlapping chromosomal regions between disease resistance, plant attribute and fruit quality traits. Interestingly, two loci linked to wilt resistance and two loci linked to fruit neck line were mapped to the same overlapping regions.

6.2 Materials and methods

6.2.1 Plant material

An octoploid strawberry progeny consisting of 173 individuals generated from the cross between 'Redgauntlet' and 'Hapil' (Chapter 2, Section 2.2.1) was used for screening powdery mildew disease susceptibility over three consecutive years (2012, 2013 and 2014). Individuals were clonally propagated by pinning down the runners from mother plants in the polytunnel. Three replicates of the progeny were planted in the field in a randomized block design in late August in 2011 for phenotyping disease susceptibility in 2012. Four replicates of the progeny were pinned down in summer 2012 and were planted in late summer of the same year for phenotyping in 2013. The progeny phenotyped in 2013 was kept for a second year and was used for phenotyping disease susceptibility in 2014. A new field trial was planted in 2012 and 2013 only, whereas plants for phenotyping in 2014 were the same plants from 2013 and were two years old.

6.2.2 Evaluation of disease resistance

Field screening of the powdery mildew symptoms among the 'Redgauntlet' × 'Hapil' (Rg × H) progeny was carried out for three years in 2012, 2013 and 2014 as part of an on-going research project at East Malling Research (EMR). The experimental trial site contained three replicates of a total of 155 Rg × H seedlings and parental genotypes in 2012, whereas four replicates of 163 seedlings and parental genotypes were planted and assessed in 2013 and 2014. The disease symptoms were assessed twice for each year in the field-based trial as follows: the first assessments were carried out on 08.08.2012, 22.08.2013 and 09.07.2014; the second assessments were carried out on

29.08.2012, 05.09.2013 and 04.09.2014. The time of each assessment was determined by the development of disease. The severity of the disease was scored on a scale of 1 – 5, with 1 being no symptoms and 5 being severe disease infection.

Phenotypic data related to powdery mildew resistance was collected by research scientists at EMR, and was kindly provided for data analysis and QTL mapping which is discussed in detail in this chapter. Powdery mildew resistance data used for QTL analysis in this study are presented in Supplementary Table 6.1.

6.2.3 Statistical analysis

As described in Chapter 2 (Section 2.2.5), standard deviation (SD), standard error (SE) and phenotypic range were estimated and were used for comparative study of disease susceptibility between parental genotypes and the progeny. Student's *t*-test was applied to estimate pairwise significance level of the means between parental genotypes ('Redgauntlet' and 'Hapil') for each measurement for each year. The normality distributions of the disease scores were also investigated by applying the Shapiro-Wilk test. Non-normally distributed values were further transformed using log and square root transformations. Phenotypic means of the powdery mildew values were calculated for each measurement across three or four replicates for each year individually using the statistical package R as described in Chapter 2 (Section 2.2.5).

6.2.4 QTL identification

Non-transformed phenotypic means of the second disease evaluations for all years were used for further analysis. The second disease evaluations for each year were selected for QTL analysis because better disease symptom representation was observed when compared to the first assessments over the three years. This may have been

because of different weather conditions and the poor homogeneity of inoculum spread between the two assessments in each year. A consensus SNP-based genetic linkage map was used for QTL mapping as was previously described in Chapter 4. A non-parametric test using Kruskal-Wallis (KW) was applied from the MapQTL® 5 software package (van Ooijen, 2004), but interval mapping was not used. This was because phenotypic data observed for disease symptoms were not normally distributed and KW testing is more suitable for identifying associations between molecular markers and powdery mildew resistance for this type of data (Kruskal and Wallis, 1952; Antanaviciute et al. 2015). MapChart 2.2 software (Voorrips, 2002) was used for graphical presentation of identified QTL regions on the genetic linkage map. Unique SNPs mapped to identical regions were removed for graphical presentation of QTL regions on the linkage map.

QTLs linked to plant characteristics, fruit quality, powdery mildew and *Veritcillium* wilt resistance were used to investigate if there were clear, significant QTL overlapping chromosomal regions among these traits. Only stable QTLs (present in two or three years) linked to all traits were used in the analysis.

6.3 Results

6.3.1 Distribution of the phenotypic data for powdery mildew

The distribution of phenotypic variation was not normal for all the assessments for all years. A p-value of 2.2×10^{-16} was observed for non-transformed, log and square root transformed data (Table 6.2). This could be explained by the insufficient number of different values observed among the replicates. Indeed, disease symptoms were evaluated on a scale (1 – 5), resulting in a low level of dominant phenotypic variation in the data set.

Table 6.2 Results observed for all phenotypic values using the Shapiro-Wilko normality test. Shapiro-Wilko statistics value (W) and p-value are presented

Trait	Phenotypic data		
	Non-transformed	Log transformed	Square root transformed
2012-1	W = 0.8068, p-value < 2.2e-16	W = 0.7773, p-value < 2.2e-16	W = 0.7974, p-value < 2.2e-16
2012-2	W = 0.8529, p-value < 2.2e-16	W = 0.8278, p-value < 2.2e-16	W = 0.853, p-value < 2.2e-16
2013-1	W = 0.8413, p-value < 2.2e-16	W = 0.8126, p-value < 2.2e-16	W = 0.833, p-value < 2.2e-16
2013-2	W = 0.8985, p-value < 2.2e-16	W = 0.8469, p-value < 2.2e-16	W = 0.8837, p-value < 2.2e-16
2014-1	W = 0.6804, p-value < 2.2e-16	W = 0.6837, p-value < 2.2e-16	W = 0.6849, p-value < 2.2e-16
2014-2	W = 0.7619, p-value < 2.2e-16	W = 0.7548, p-value < 2.2e-16	W = 0.7622, p-value < 2.2e-16

When phenotypic means between two measurements for each year were plotted as distribution histograms, disease symptoms were close to normally distributed for all years (Figure 6.1). The distribution of means for 2014 showed the most skewed levels of resistance.

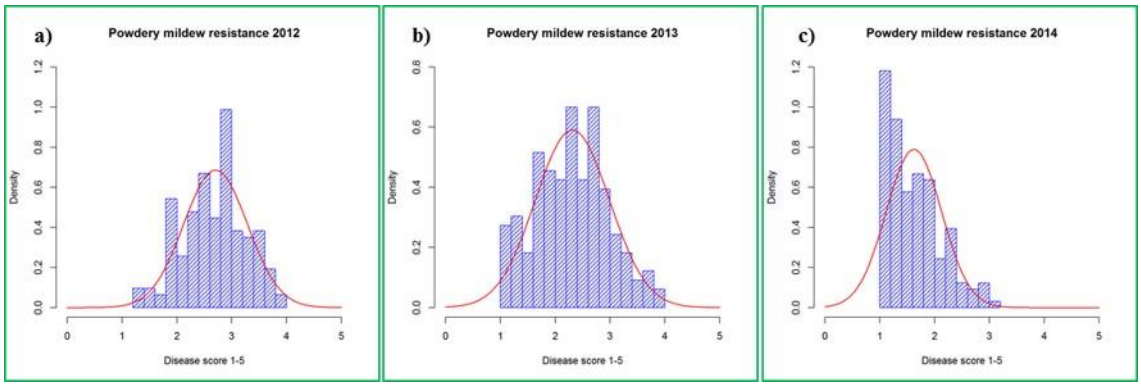

Figure 6.1 Histograms of the distributions of resistance to powdery mildew in 'Redgauntlet' × 'Hapil' progeny in 2012 (a), 2013 (b) and 2014 (c). The normal distribution curve in red was calculated for the mean of the phenotypic variation.

6.3.2 Phenotypic evaluation of powdery mildew resistance

The assessment of the disease symptoms observed in parental genotypes ('Redgauntlet' and 'Hapil') and the progeny for each evaluation and each year was based on calculated phenotypic means across three (2012) and four (2013 and 2014) replications. A summary of phenotypic means, standard deviation, the range and significance level (*t*-test, p-value) in parental genotypes and progeny are presented in Table 6.3.

Table 6.3 Phenotypic variation of powdery mildew susceptibility in parental genotypes ('Redgauntlet' and 'Hapil') and F_1 progeny for three consecutive years (2012, 2013 and 2014) for all measurements. The number of individuals analysed (No.), mean values, standard deviations (SD), range and *t*-test significance between parental genotypes ('Rg' vs 'H') for three in 2012, and four in 2013 and 2014, replicates are presented. One and two after the year represents the first and the second evaluation of the disease symptoms

Year	'Redgauntlet'				'Hapil'					F_1 progeny			
	No.	Mean	SD	Range	No.	Mean	SD	Range	'Rg' vs 'H'	No.	Mean	SD	Range
2012-1	3	2.7	0.6	2 - 3	3	2.3	0.6	2 - 3	ns[1]	420	2.1	0.8	1 - 4
2012-2	3	3.0	0.0	3 - 3	3	4.7	0.6	4 - 5	**[1]	416	3.2	0.8	1 - 5
2013-1	4	1.8	0.5	1 - 2	4	3.0	0.8	2 - 4	*[1]	645	2.1	0.9	1 - 4
2013-2	4	2.5	1.0	1 - 3	4	3.3	0.5	3 - 4	ns	645	2.6	1.0	1 - 5
2014-1	4	1.8	1.0	1 - 3	4	2.5	1.0	1 - 3	ns	645	1.5	0.7	1 - 3
2014-2	4	2.0	0.8	1 - 3	4	2.3	1.0	1 - 3	ns	643	1.7	0.8	1 - 4

[1]*t*-test significance level between parental genotypes 'Redgauntlet' ('Rg') and 'Hapil' ('H'): *, significant at $p < 0.05$; **, significant at $p < 0.01$, ***, significant at $p < 0.001$; n.s., not statistically significant

'Redgauntlet' and 'Hapil' expressed different level of disease resistance for all years assessed (Table 6.3, Figure 6.2). 'Hapil' consistently showed a higher level of susceptibility to the powdery mildew than 'Redgauntlet', with the exception of the first

measurement in 2012 (Figure 6.2). On average, disease scores of 3.0 ± 0.4 and 2.3 ± 0.2 were observed for 'Hapil' and 'Redgauntlet' respectively. The range of the disease score was 1 to 5 in 'Hapil' and 1 to 3 in 'Redgauntlet'. However, significant differences were observed for only two measurements between parental genotypes, whereas the remaining four observations were not statistically different (Table 6.3, Figure 6.2).

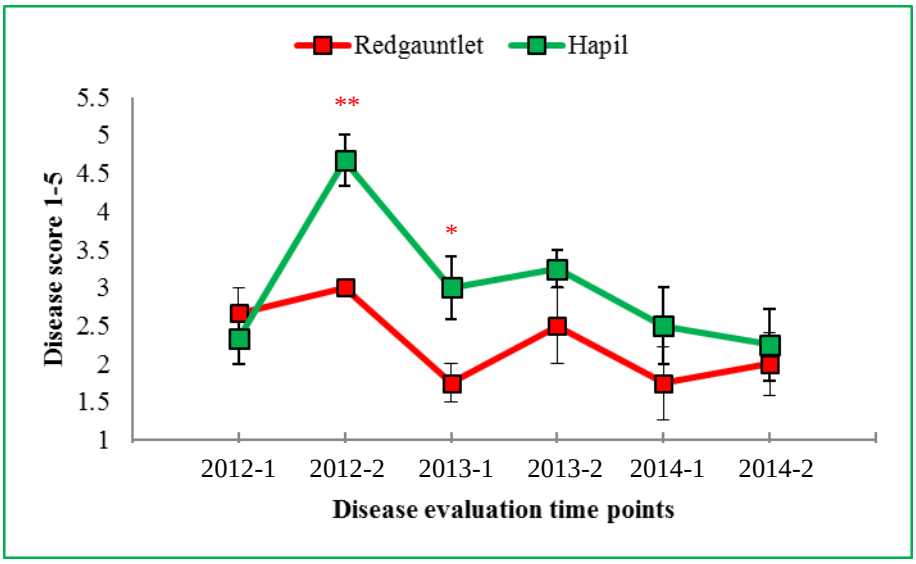

Figure 6.2 Comparison between 'Redgauntlet' and 'Hapil' of disease susceptibility on six occasions over three years. Red asterisks represent significant differences between parental genotypes observed based on *t*-test, where * = $p < 0.05$ and ** = $p < 0.01$.

Disease symptoms evaluated on 29th August 2012 (2012-2) showed the most significant differences between parents ($p < 0.01$), following by the evaluation on 8th August 2013 (2013-1) ($p < 0.05$). When phenotypic (mildew disease severity) mean values were compared between parental genotypes and progeny, some extreme phenotypic variations were seen (Table 6.3). Progeny phenotype means were lower than those of the parents in 2012 (first assessment) and in 2014 (both assessments). Average phenotypic means of 2.7 and 2.3 were observed for 'Redgauntlet' and 'Hapil'

respectively, whereas the mean value of the progeny was 2.1 in 2012 for the first assessment (2012-1). In the first 2014 assessment (2014-1), a phenotypic mean of 1.8 was observed in 'Redgauntlet' and 2.5 in 'Hapil', whereas it was 1.5 for the progeny. For the second assessment in 2014 (2014-2), the phenotypic values ranged from 2 to 2.3 for the parents, whereas 1.7 was observed in the progeny (Table 6.3). This suggests that some individuals of the progeny expressed a higher level of disease resistance than the parents of this population, and that the segregation of disease resistance among the progeny was transgressive. Interestingly, the same disease resistance patterns were reported for *Verticillium* wilt resistance in 'Redgauntlet' × 'Hapil' mapping progeny compared to the parents (Antanaviciute et al. 2015).

Phenotypic mean values of the progeny for the remaining three assessments (2012-2, 2013-1 and 2013-2) were within the disease severity range of the parental genotypes (Table 6.3). However, seedlings had more severe disease symptoms than parental genotypes. For example, disease severity ranged from 3 to 5 for the parents, whereas from 1 to 5 was observed in the progeny. Therefore, the segregation of disease resistance in the progeny was transgressive in all years analysed.

6.3.3 QTL mapping associated with powdery mildew resistance in strawberry

QTL were detected using average data of the last evaluations (2012-2, 2013-2 and 2014-2) over the three years separately, using Kruskal-Wallis (KW) method. A total of nine potential QTLs associated with powdery mildew resistance were identified and mapped on the SNP-based genetic linkage map of the 'Redgauntlet' × 'Hapil' mapping progeny (Table 6.4, Figure 6.3). QTL naming associated with powdery mildew resistance was based on the pathogen name *Podosphaera aphanis* and QTLs were given the prefix R*Pa* (**R**esistance to ***P****odosphaera* ***a****phanis*). Three loci (R*Pa*3, R*Pa*7 and

R*Pa*9) linked to mildew resistance were present in all three years, one locus (R*Pa*1) was present in two out of three years and the remaining five loci (R*Pa*2, R*Pa*4, R*Pa*5, R*Pa*6 and R*Pa*8) were highly significant but present only in one year (Table 6.4). More than half (55.6%) of QTLs identified are likely to have been strongly affected by environmental factors.

Table 6.4 QTLs associated with powdery mildew (*Podosphaera aphanis*) resistance across years in the 'Redgauntlet' × 'Hapil' mapping progeny. QTL name, year QTL was present, linkage group (LG), closest linked SNP marker and marker position (cM) on the linkage map, K* statistics and p-value, and parent are presented

QTL	Year observed	LG	Position (cM)	Closest SNP	K* value	p value[1]	Parent
R*Pa*1	2013	1A	41.234	AX-89876098:nmh	8.537	***	Hapil
R*Pa*1	2014	1A	20.207	AX-89875098:ph3	7.522	**	Hapil
R*Pa*2	2014	2A	137.612	AX-89862889:ph3	9.583	***	Hapil
R*Pa*3	2012	2B	53.794	AX-89819492:nmh	4.312	*	Hapil
R*Pa*3	2013	2B	39.739	**AX-89819696:ph3**	8.516	***	Hapil
R*Pa*3	2014	2B	39.739	**AX-89819696:ph3**	14.535	*****	Hapil
R*Pa*4	2012	2D	47.845	AX-89867822:ph3	17.428	*****	Both
R*Pa*5	2013	3A	65.999	AX-89828381:ph3	12.897	*****	Redgauntlet
R*Pa*6	2014	4C	55.547	AX-89790619:nmh	13.665	*****	Hapil
R*Pa*7	2012	6D	0	AX-89850201:ph3	16.504	*****	Both
R*Pa*7	2013	6D	5.829	AX-89841661:ph3	16.111	*****	Both
R*Pa*7	2014	6D	0	AX-89915462:nmh	10.489	***	Redgauntlet
R*Pa*8	2014	7C	18.605	AX-89801200:ph3	9.62	***	Redgauntlet
R*Pa*9	2012	7D	45.015	**AX-89802341:ph3**	8.178	***	Hapil
R*Pa*9	2013	7D	45.015	**AX-89802341:ph3**	11.39	****	Hapil
R*Pa*9	2014	7D	20.323	AX-89844102:ph3	11.406	***	Both

[1]Significance level as observed using Kruskal-Wallis test in MapQTL and are as follows: * = 0.05, ** = 0.01, *** = 0.005, **** = 0.001, ***** = 0.0005 and ****** = 0.0001

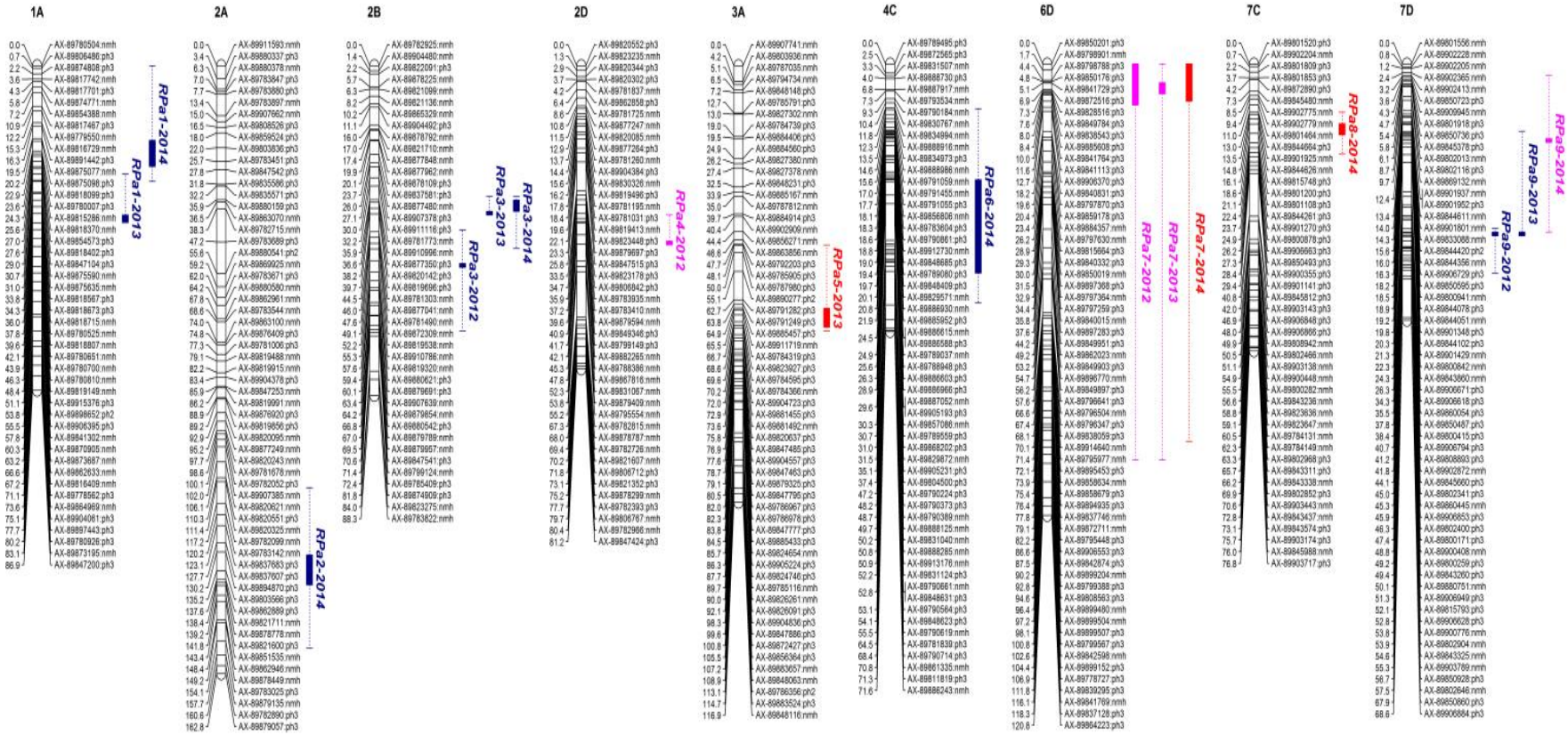

Figure 6.3 QTL regions linked to powdery mildew resistance mapped to linkage groups of the consensus linkage map of 'Redgauntlet' × 'Hapil'. Thick bars represent the markers with significance level of $p < 0.005$, whereas the dotted lines represent significance intervals of $p < 0.005$ and $p < 0.05$. QTLs from 'Redgauntlet' are highlighted in red, from 'Hapil' in blue and from both parental genotypes in pink.

The 14 most closely linked SNP markers for each QTL locus associated with disease resistance were identified, based on Kruskal-Wallis K* statistics and p-value. In two cases, the same highly significant SNP marker linked to the same locus was present in two out of three years. For example, SNP marker AX-89819696:ph3 was identified as most closely linked to the R*Pa*3 locus in 2013 and 2014, whereas SNP marker AX-89802341:ph3 was identified as most closely linked to the R*Pa*9 locus in 2012 and 2013 (Table 6.4, SNPs in bold).

6.3.4 QTL mapping linked to *Verticillium* wilt resistance using the SNP linkage map and comparison of QTL positions identified between SSR and SNP maps

QTLs associated with *Verticillium* wilt resistance in 'Redgauntlet' × 'Hapil' progeny were mapped on the SSR-based linkage map previously discussed in Chapter 3, and were reported by Antanaviciute et al. (2015). However, in order to be able to identify overlapping chromosomal regions between mildew, plant characteristics, fruit quality and wilt QTLs, it was essential that all loci were mapped on the same genetic linkage map. Thus, QTL analysis of *Verticillium* wilt resistance was carried out using the SNP-based linkage map from the very beginning in this study.

The number of loci identified linked to *Verticillium* wilt using the SNP linkage map was the same as the number of loci identified using the SSR map, as expected. QTLs were named according to the QTL positions previously reported by Antanaviciute et al. (2015), and were given the prefix R*Vd* (**R**esistance to ***V****eriticillium* ***d****ahliae*). The list of QTLs detected on the SNP map and consistent naming of QTL regions in this study are presented in Table 6.5.

Table 6.5 QTLs associated with *Verticillium* wilt (*Verticillium dahliae*) resistance across years in the 'Redgauntlet' × 'Hapil' mapping progeny using the SNP linkage map. QTL name, year QTL was present, linkage group, closest linked SNP and marker position (cM) on the linkage map, K* statistics and p-value, and parent are presented

QTL	Year	LG	Position (cM)	Closest SNP	K* value	p-value[1]	Parent
R*Vd1*	2009	3B	71.312	AX-89826831:nmh	8.746	***	Hapil
R*Vd1*	2010	3B	4.039	AX-89883614:ph3	14.169	*****	Hapil
R*Vd1*	2011	3B	7.973	AX-89907882:nmh	7.42	**	Hapil
R*Vd3*	2009	7A	74.849	AX-89801698:ph3	11.75	***	Both
R*Vd3*	2010	7A	66.481	AX-89808764:ph3	11.181	***	Both
R*Vd3*	2011	7A	66.481	AX-89808764:ph3	20.972	******	Both
R*Vd4*	2009	2B	72.441	AX-89799119:nmh	8.99	***	Redgauntlet
R*Vd4*	2010	2B	72.441	AX-89799119:nmh	8.639	***	Redgauntlet
R*Vd4*	2011	2B	61.589	AX-89822478:ph3	6.968	**	Redgauntlet
R*Vd5*	2009	6C	26.641	AX-89800083:ph3	9.705	***	Redgauntlet
R*Vd5*	2011	6C	25.848	AX-89850346:nmh	9.423	***	Redgauntlet
R*Vd7*	2009	2D	75.239	AX-89878299:nmh	7.798	**	Redgauntlet
R*Vd7*	2011	2D	75.239	AX-89878299:nmh	10.994	****	Redgauntlet
R*Vd9*	2010	2A	86.188	AX-89819991:nmh	10.897	****	Redgauntlet
R*Vd9*	2011	2A	108.364	AX-89820361:nmh	8.568	***	Hapil
R*Vd10*	2010	3A	110.031	AX-89786710:nmh	10.624	***	Hapil
R*Vd12*	2011	3D	29.806	AX-89882869:nmh	7.858	**	Redgauntlet
R*Vd13*	2009	4A	9.4	AX-89892153:ph3	12.56	*****	Hapil
R*Vd13*	2010	4A	10.479	AX-89888955:nmh	10.053	***	Hapil
R*Vd14*	2009	5A	90.592	AX-89833764:nmh	15.011	*****	Hapil
R*Vd15*	2009	6A	50.119	AX-89837378:nmh	8.736	***	Redgauntlet
R*Vd15*	2011	6A	43.456	AX-89841986:ph3	12.074	***	Both

[1]Significance level as observed using Kruskal-Wallis test in MapQTL and are as follow: * = 0.05, ** = 0.01, *** = 0.005, **** = 0.001, ***** = 0.0005 and ****** = 0.0001

Eleven potential QTLs (the number of QTLs identified previously) were mapped across the years (Table 6.5). Of those, three loci (R*Vd*1, R*Vd*3 and R*Vd*4) were detected in all three years, five loci (R*Vd*5, R*Vd*7, R*Vd*9, R*Vd*13 and R*Vd*15) were significant in two out of three years and a further three loci (R*Vd*10, R*Vd*12 and R*Vd*14) were present in one out of three years. QTL positions associated with wilt resistance on the SNP linkage map are presented graphically in Appendix 6.1.

The locations of some QTLs were inconsistent with those previously reported on the SSR map (Table 6.6). Seven out of 11 loci identified on the SNP map mapped to the same linkage groups as previously reported. Two loci mapped to the homoeologous linkage groups and the remaining two loci were identified on different linkage groups when compared between SSR and SNP maps. These results are based on the comparison of QTL locations on the re-named SSR-based linkage map. As previously discussed (Chapter 5, Sections 5.3.2 and 5.4.1) that some linkage groups of the SSR-based linkage map were annotated incorrectly when compared to the SNP-based map. Therefore a large number of QTL positions do not agree between their locations on SSR-based linkage map reported by Antanaviciute et al. (2015) and the SNP-based map developed in this study (Chapter 3).

QTL stability across the years was similar between SSR and SNP maps, although more stable loci were reported on the SSR map than were detected in this study using the SNP map. For example, seven out of 11 loci were present in all three years on the SSR map, whereas only four out of 11 loci were present across the years on the SNP map (Table 6.6).

The inconsistencies and stability of QTL locations associated with *Veriticillium* wilt resistance identified on SSR and SNP-based linkage maps were similar to those found for fruit firmness (Chapter 5, Section 5.3.2).

Table 6.6 Comparison of QTL locations associated with *Verticillium* wilt resistance in the 'Redgauntlet' × 'Hapil' progeny in the study of Antanaviciute et al. (2015) (SSR map) with those obtained in the present study (SNP map). QTLs mapping on the same linkage groups (LG) in both studies are highlighted in green, on homoeologous LG in blue and QTLs mapping on different LGs between the two studies are in red

SSR-based linkage map				SNP-based linkage map[1]		
QTL	LG[2]	Re-named LG[3]	Years present	QTL	LG	Years present
R*Vd*1	3D	3B	3	R*Vd*1	3B	3
R*Vd*2	1D	1C	3	-	-	-
R*Vd*3	7B	7A	3	R*Vd*3	7A	3
R*Vd*4	2C	2B	3	R*Vd*4	2B	3
R*Vd*5	6B	6C	3	R*Vd*5	6C	2
R*Vd*6	5D	5D	1	-	-	-
R*Vd*7	2D-A	2D	3	R*Vd*7	2D	2
R*Vd*8	1D	1C	1	-	-	-
R*Vd*9	2A	2A	2	R*Vd*9	2A	2
R*Vd*10	3A	3A	3	R*Vd*10	3A	1
R*Vd*11	4A	4B	1	-	-	-
				R*Vd*12	3D	1
				R*Vd*13	4A	2
				R*Vd*14	5A	1
				R*Vd*15	6A	2

[1]This study

[2]Antanaviciute et al. (2015)

[3]See Chapter 5, Section 5.3.2

6.3.5 Relationship between mildew, wilt, plant characteristics and fruit quality QTLs

An analysis of QTL positions linked to powdery mildew resistance (identified in this chapter), *Verticillium* wilt resistance (previously reported by Antanaviciute et al. (2015) and re-mapped on the SNP map in this study), plant characteristics and fruit quality traits (identified in Chapter 4) was performed. The purpose of this analysis was mainly to investigate if there were any overlapping chromosomal regions between

disease resistance and plant attribute traits. Stable QTL positions linked to disease resistance, plant attribute and fruit quality traits on the SNP linkage map are presented in Figure 6.4. When the QTL positions linked to mildew and wilt resistance were compared, two closely mapped regions were identified, although the majority of QTLs linked to both diseases did not have common overlapping regions. Four QTLs controlling both disease resistances were mapped to the same linkage groups, three loci (on LG2A, LG2D and LG3A) were at least 22 cM apart and one locus (on LG2B) was 7 cM apart. A single close relationship between mildew and wilt QTLs was found on linkage group 2B. Two stable QTLs associated with mildew (R*Pa*3) and wilt (R*Vd*4) resistance, were closely mapped on the same linkage group and had overlapping chromosomal locations.

When the QTL positions for disease resistance and plant characteristics were compared, along with disease resistance and fruit quality traits, there was a significant relationship between wilt resistance and neck line. The most significant chromosomal region linked to *Verticillium* wilt resistance was on LG4A (R*Vd*13-2009 and R*Vd*13-2010) between 7 - 18 cM, whereas the significant region of QTL linked to neck line (Neck line-2014 and Neck line-2015) was between 10 - 25 cM on the same linkage group. Stable QTL locations linked to these two traits were therefore present on an overlapping chromosomal location. Furthermore, further loci linked to wilt resistance (present in 2009) and neck line (present in 2015), also mapped to approximately the same region (between 50 - 51 cM) on LG6A. However, QTLs linked to the same traits but identified in different years (R*Vd*15-2011 and Neck line-2014) mapped approximately 10 cM apart on the same linkage group 6A (Figure 6.4). The remaining loci linked to different traits showed some overlapping regions on different linkage groups but were inconsistent over the years.

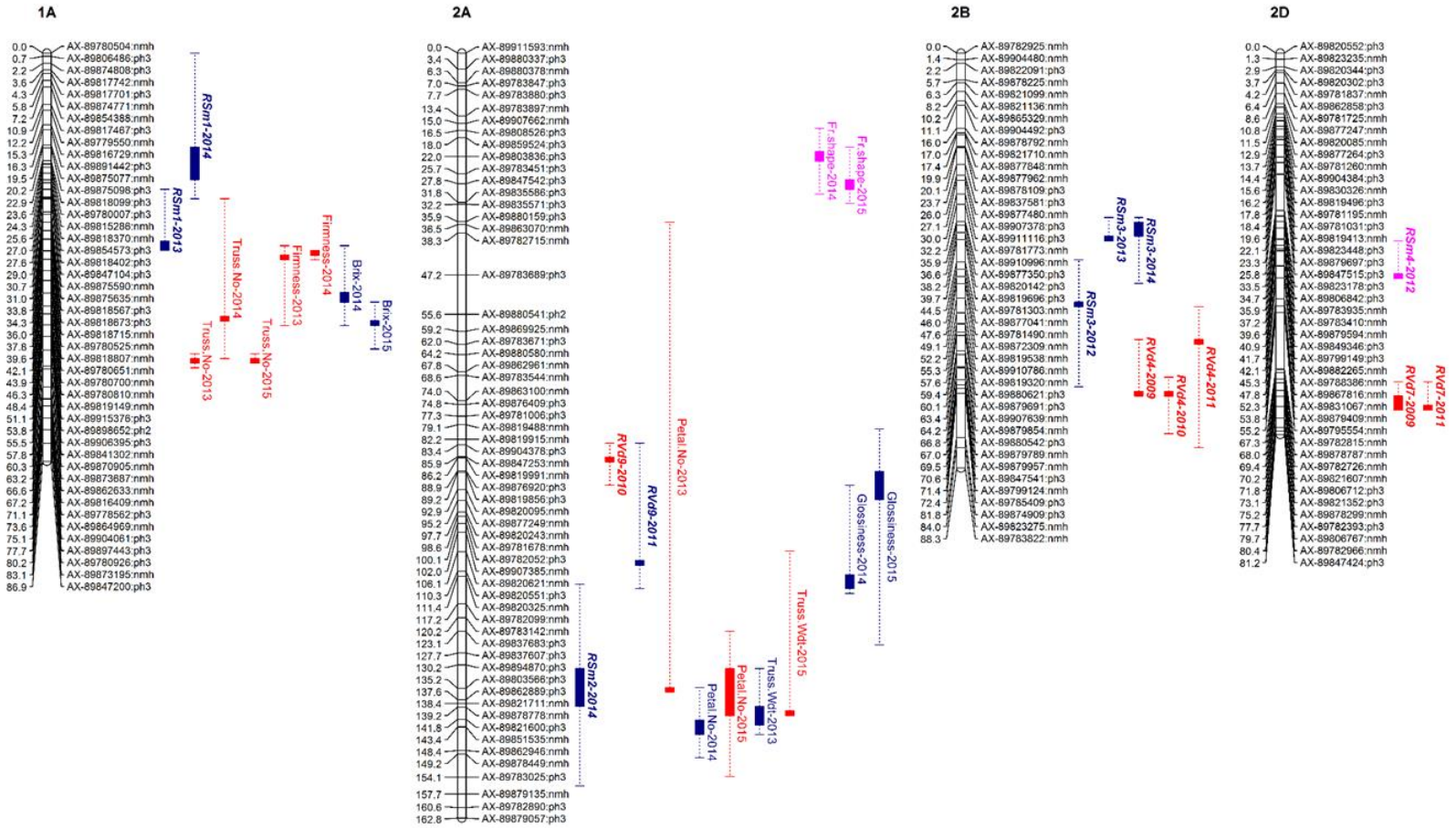

Figure 6.4 QTL regions linked to powdery mildew (R*Pa*) and *Verticillium* wilt (R*Vd*) resistance as well as plant characteristics and fruit quality traits mapped to linkage groups of the consensus linkage map of 'Redgauntlet' × 'Hapil'. QTLs from 'Redgauntlet' are highlighted in red, from 'Hapil' in blue and from both parental genotypes in pink.

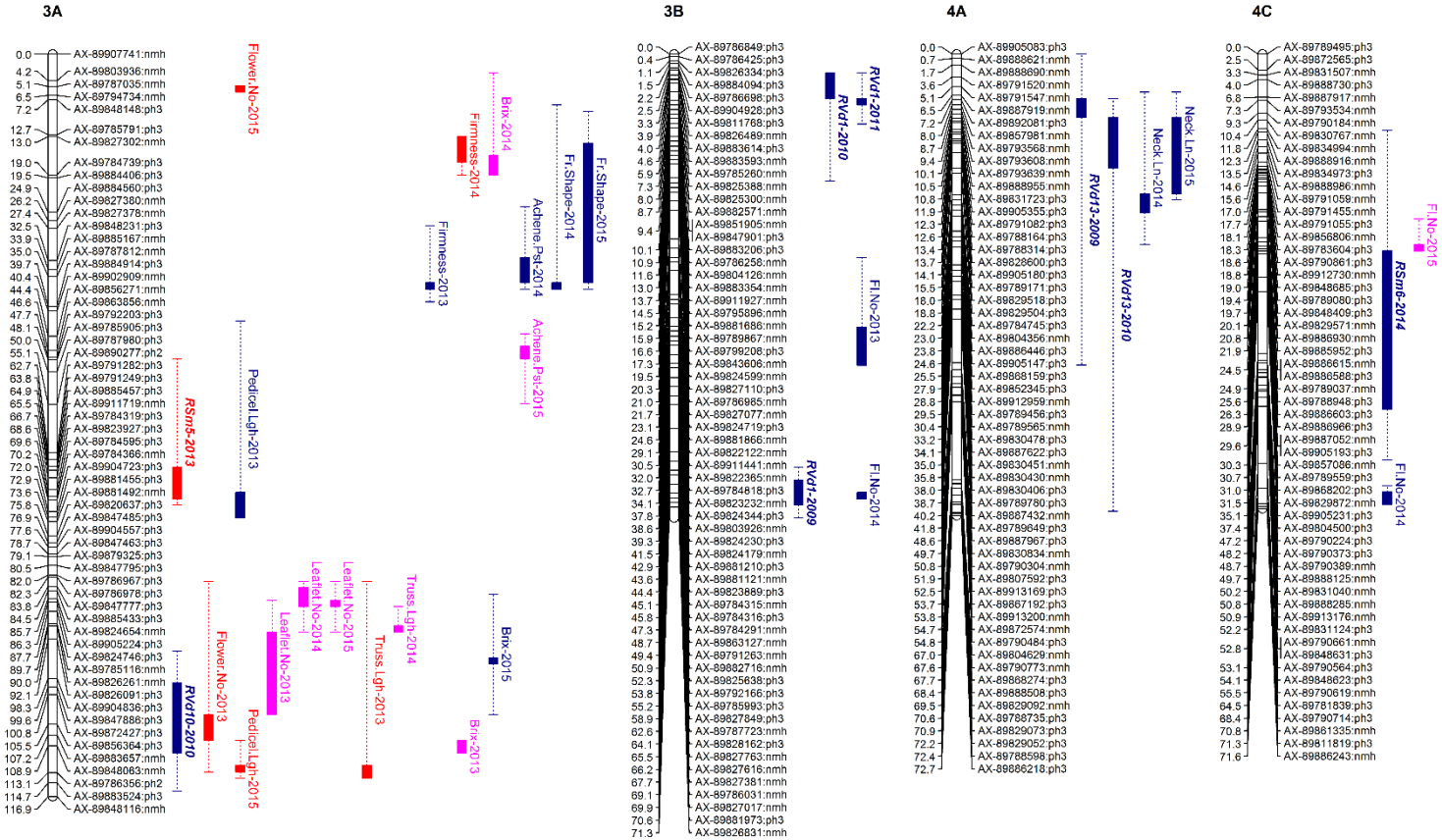

Figure 6.4 Continued.

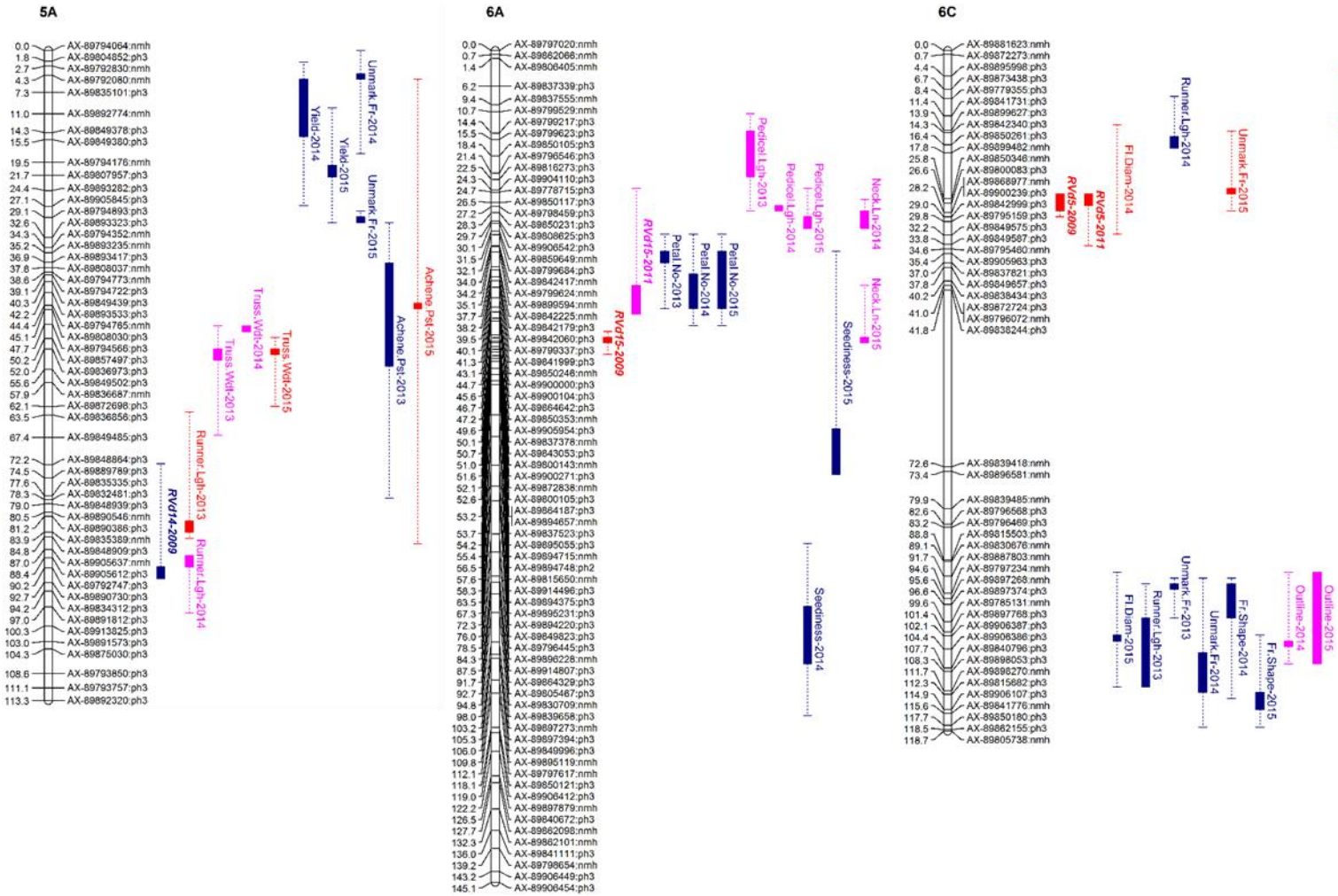

Figure 6.4 Continued.

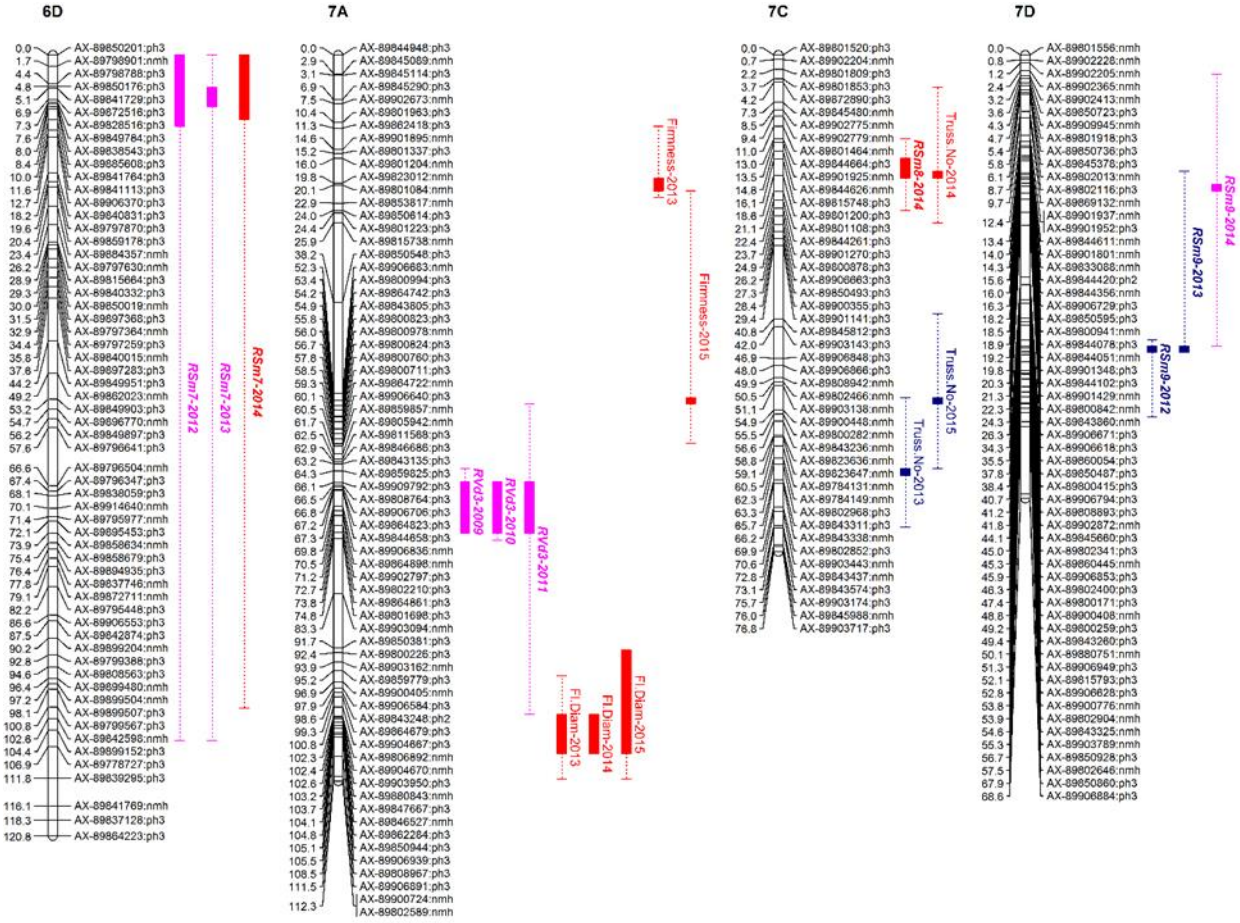

Figure 6.4 Continued.

6.4 Discussion

6.4.1 Phenotypic variation of powdery mildew resistance in octoploid strawberry

The ‘Redgauntlet’ × ‘Hapil’ mapping progeny showed high segregation of powdery mildew resistance in a field-based experiment. The phenotypic variation among the individuals showed non-normal distribution of the disease symptoms over the three-year period (Table 6.2). This might have been due to the fact that disease evaluation was based on a scale of 1-5, resulting in low phenotypic variation. In contrast, the phenotypic means for each year provided more data points in the data set, and the distribution of the disease symptoms was close to normal in all years (Figure 6.1). Similar results were reported in the study by Antanaviciute et al. (2015), where the same octoploid strawberry mapping population (‘Redgauntlet’ × ‘Hapil’) was used to detect QTLs associated with *Verticillium* wilt resistance in the field-based plants. Disease evaluation was based on a scale of 1-9, and low variations were observed among individuals and close to normal distributions were shown for the means plotted as distribution histograms (Figure 3, Antanaviciute et al. 2015). In addition, phenotypic variation of some of the plant characteristics and fruit quality traits (described in Chapter 2) which were phenotyped on the scale rates showed non-normal distributions.

Interestingly, the distribution of the mean of the phenotypic variation was skewed towards powdery mildew resistance in 2014 (Figure 6.1, Table 6.2). The number of susceptible individuals was reduced in 2014 when compared to 2012 and 2013 disease evaluations. This suggests that older strawberry plants might be more resistant to powdery mildew, because two-year old strawberry plants were evaluated in 2014, whereas one-year old individuals were assessed in 2012 and 2013 (Section 6.2.1). Thus, the increased resistance to the disease might be an age effect rather than a genetic

change in the resistance. This link between age and resistance has also been reported in apple, where resistance to powdery mildew was seen to increase in older plants over a four-year period (Calenge and Durel, 2006). The age of a plant may therefore be an important factor to consider when looking at resistance of different crops to specific pathogens. The increased resistance to the powdery mildew seen in this study could also have been due to other factors, such as: (1) pathogenic inoculum not being spread homogeneously in the orchards over the study period, and (2) environmental factors may have affected disease severity over the evaluation period. It has been previously reported that dramatic changes in disease severity may be seen, when favourable environmental conditions for the pathogen develop over several seasons (Amsalem et al. 2006; Calenge and Durel, 2006).

6.4.2 QTL linked to powdery mildew in octoploid strawberry

Although several previous studies have reported markers or QTLs linked to different disease resistance in strawberry such as resistance to red stele root rot (*Phytophthora fragariae*) (Haymes et al. 1997; van de Weg et al. 1997), crown rot (*Phytophthora cactorum*) (Lerceteau-Köhler et al. 2003), anthracnose (*Colletotrichum acutatum*) (Lerceteau-Köhler et al. 2005), and recently to *Verticilium* wilt (*Verticilium dahlia*) (Antanaviciute et al. 2015), resistance to powdery mildew (*Podosphaera aphanis*) has not been previously reported in octoploid strawberry.

Fourteen closely linked SNP markers were successfully detected linked to powdery mildew resistance using a QTL mapping approach in this study. However, it is not yet known if these SNPs linked to mildew resistance QTL locations are in fact linked to resistance or susceptibility genes. A recent study on the characterisation of the Mlo gene family (susceptibility genes with a lost-of-function mutation in **M**ildew **l**ocus

o) in Rosaceae by Pessina et al. (2014), reported the chromosome locations of a total of 18 Mlo gene homologs predicted on the *Fragaria vesca* genome sequence. Interestingly, eight out of nine QTL locations associated with powdery mildew resistance detected in this study were on the same chromosomes as the 13 out of 18 Mlo gene homologs in the diploid strawberry (Pessina et al. 2014). One QTL linked to mildew resistance was detected on linkage group 4C in *Fragaria* × *ananassa* instead of linkage group 5 where two Mlo genes were predicted to be located in *Fragaria vesca* genome sequence. Further analysis such as investigation of the phenotypic variation explained by the QTL locations, candidate gene identification and marker validation is necessary to determine the usefulness of molecular markers identified here for use in marker-assisted breeding (MAB).

Multiple QTLs linked to powdery mildew resistance in cultivated strawberry suggests that the trait is polygenic and genetically complex. Indeed, nine potential QTLs were detected linked to mildew resistance in cultivated strawberry. These results were expected, because recent findings reported 11 QTLs associated with *Verticilium* wilt disease resistance in the same 'Redgauntlet' × 'Hapil' progeny (Antanaviciute et al. 2015). Therefore, powdery mildew resistance is controlled by more than one gene, and the more QTLs that are present in a single individual, the higher the tolerance level to powdery mildew. In addition, these individuals have a better chance of survival in field-based scenarios. Each single QTL associated with powdery mildew resistance is likely to be a small effect. Five out of nine QTLs were present only in one year suggesting a minor effect and that it is important to combine them with stable QTLs (four) in order to achieve significant powdery mildew resistance in new strawberry cultivars. Minor effect QTLs may greatly contribute to the overall quantitative variation in the phenotype, as well as being linked to important genes. This has been previously reported in rice

(Andaya and Mackill, 2003; Wan et al. 2006), maize (Yang et al. 2010), apple (Calenge and Durel, 2006; Peil et al. 2007) and peach (Dhanapal and Crisosto, 2013; Frett et al. 2014).

This study also demonstrates that field-based disease evaluation is an effective method for selecting individuals showing the highest level of disease resistance and to discard individuals with low disease resistance.

6.4.3 Relationships between fruit quality, plant characteristics and disease resistance QTLs

A combined analysis of QTLs linked to mildew and wilt disease resistance, plant characteristics and fruit quality traits was reported here for the first time in octoploid strawberry. The aim of this analysis was to investigate if different traits are co-regulated in cultivated strawberry. The majority of disease resistance and fruit quality or plant characteristics traits were mapped to different locations and no consistent overlapping chromosomal regions were detected among the traits analyzed. These results show that disease resistance and fruit quality traits are independent in this species and traits are unlikely to be pleiotropic. Therefore breeding for disease resistance in strawberry should not be affected by potential loss of other important genes, for example linked to economically important fruit quality traits, through breeding process. However, this is not the case for the fruit quality and plant characteristics traits. Significant correlations were detected among some of these traits (Chapter 2) and overlapping chromosomal regions were observed (Figure 6.4). Fruit quality and plant characteristics traits may therefore be co-regulated and breeding focusing on a specific trait may eliminate other important trait(s). This problem has been reported in peach, where a set of volatile compounds were reported to be co-regulated and associated with fruit quality traits

(Sánchez et al. 2012). The study showed that lactones were strongly correlated with fruit colour and flesh firmness. Another study in sweet orange revealed that highly connected genes usually cluster together; as a result these genes tend to regulate the same biological processes (Du et al. 2015). The analyses by Du et al. (2015), showed that 25 homologues of a citrus canker susceptibility gene (*CsLOB1*) were involved in the citrus canker network and were co-regulated. Another example was reported in *Arabidopsis*, which suggested that transcription factors are able to regulate the expression of different genes (heat stress factors and shock proteins) within the same cluster (Guan et al. 2013).

Interestingly, significant overlapping regions were found for two loci linked to *Verticillium* wilt resistance and fruit neck line on LG4A and LG6A. It is well known that *Veriticillium* wilt attacks strawberry plants through roots, and that the infection then spreads through vascular tissue (xylem vessels) around the plant (Fradin and Thomma, 2006; Schubert et al. 2008). The infection can also spread through damaged or wounded fruits. No reports are available on the formation of the neck line during the development stages in strawberry plants which could reveal potential information as to why neck line is linked to *Verticillium* wilt resistance in strawberry as identified in this study. Therefore, although the relationship of QTL positions between wilt resistance and neck line was significant, it is not known if strawberry plants with sunken, flat or raised fruit neck line are more susceptible or resistant to wilt disease at this point. It would be useful to phenotype wider strawberry germplasm for this trait in order to identify consistencies between wilt resistance and neck line position. For example, the same resistant, susceptible and intermediate strawberry cultivars and selections used by Antanaviciute et al. (2015) could be used in a validation analysis of the relationship found in this study.

6.5 Conclusions

In this chapter the identification of multiple QTLs and closely linked SNP markers associated with powdery mildew resistance in an octoploid strawberry mapping progeny (‘Redgauntlet’ × ‘Hapil’) was reported. The results observed in this study provide valuable information and a foundation for further analysis to estimate precise QTL locations, effects of each individual QTL, epistatic interactions and phenotypic variations. The characterisation of the closest SNPs identified for each locus using populations with wider genetic background is crucial and if successful, might offer novel molecular tools to predict potential resistance to powdery mildew in strawberry cultivars through marker-assisted selection.

The novel relationship between QTLs linked to wilt resistance and neck line in cultivated strawberry needs to be investigated further because it is not known what causes disease prevention and thus resistance at this point. Furthermore, further investigation of whether flat, sunken or even neck line prevents powdery mildew disease infection is necessary in order to better understand the relationship between wilt resistance and neck line shape.

Chapter 7

General Discussion

General Discussion

As the consumption of strawberry fruits has been rapidly increasing for the last 10 years worldwide, the sustainability and competitiveness of strawberry production requires the breeding of new cultivars with improved fruit quality and disease resistance traits. A better understanding of the associations between phenotype and genotype, the identification of disease resistant germplasm and knowledge of the biological processes involved at the genetic level are all essential for a successful strawberry breeding program. Thus, the main aim of this research was to provide novel information which will assist breeders more effectively to develop superior new cultivars with combined fruit quality and disease resistance (in this case resistance to powdery mildew) traits.

This thesis reports new insights on: 1) how different plant characteristics and fruit quality traits correlate in octoploid strawberry; 2) the complexity of the majority of economically important traits analyzed at the genetic level; 3) the potential locations of genes linked to plant characteristics, fruit quality and disease resistance traits in cultivated strawberry; and 4) the identification and validation of molecular markers associated with fruit firmness and powdery mildew resistance. The knowledge and new information at the phenotypic and genetic level obtained in this study will contribute to the improvement of strawberry breeding process by reducing time and cost through the application of a marker-assisted breeding. In this chapter, the results observed and their potential implications in strawberry breeding will be discussed.

7.1 Heritability, environmental and age effects in octoploid strawberry

The correlation analysis among a total of 30 different plant characteristics and fruit quality traits revealed that not all traits were related (and therefore not correlated) in the 'Redgauntlet' × 'Hapil' mapping progeny over the three-year analysis (Chapter 2, Section 2.3.3, Supplementary Figure 2.7). In addition, some discrepancies were observed among some correlations obtained in this study when compared to previous results (Lerceteau-Kohler et al. 2012). For example, yield positively correlated with fruit shape in the study of Lerceteau-Köhler et al. (2012), yet no correlation was found between the same traits in this study. The reasons why all traits were not correlated and some discrepancies were observed could be due to 1) potential trade-offs between different traits (such that if one trait is overexpressed then another trait could be suppressed); and 2) environmental factors affecting the variation of observed traits over the three-year time period. Differences from year to year in, for example, air temperature and wind speed could have influenced allelic expression, phenotypic and genotypic variations across the multi-year observations (Figure 2.6).

An environmental effect can also be seen in the QTL analysis (based on the three-year phenotypic data). A total of 69.8% of all QTLs detected in this study were present only in one year, and thus were year-dependent. This suggests that the majority of QTLs either were small effect and the power to detect them weak, or were influenced by strong environmental factors (Chapter4, Section 4.3.3). QTL stability may have been affected by variation in temperature over the three years. As an example, this can be seen across QTLs associated with fruit firmness. Four out of nine loci linked to fruit firmness were present in 2013 only (Table 4.4), which was also the coolest year

compared to 2014 and 2015. Year-dependent QTLs linked to strawberry fruit quality traits were reported in other studies. For example, only 27% and 37% of all QTLs identified linked to fruit quality traits in octoploid strawberry were present in all years analysed in studies by Lerceteau-Köhler et al. (2012) and Zorrilla-Fontanesi et al (2011b) respectively.

Additive genetic factors (broad-sense heritability) and measurement errors can affect phenotypic variation in the population and the genetic control of QTLs. This study demonstrated that high heritability coefficients (<0.5) were observed for 20 out of 30 traits (Figure 2.10) suggesting that genetic factors contributed more than environmental factors to these correlations and QTLs. Highly heritable traits and the large number of QTLs detected in this study represent consistent associations between phenotype and genotype, although further testing is necessary to confirm the significance of the QTLs. In addition, results supports the evidence that plant characteristics and fruit quality traits in octoploid strawberry are complex and a large number of genes control each trait (Chapter 4, Section 4.3.3).

Plant age may have contributed to the overall lack of correlations of the traits studied and the year-dependent QTLs identified among the 'Redgauntlet' × 'Hapil' mapping progeny. The strawberry plants were two-years old in 2014, whereas in 2013 and 2015 the plants used for analysis were one-year old. Interestingly, the highest yield, largest diameter flowers, most runners, highest soluble solids content and softest fruits were observed among the older strawberry plants (Figure 2.4). The results also showed that older strawberry plants had greater resistance to powdery mildew (Table 6.3). Plant age may therefore be an important factor in breeding for fruit quality and disease resistance traits in cultivated strawberry. As previously discussed in Chapter 6, Section 6.4.1, plant age has been reported to influence the powdery mildew resistance in apple

(Calenge and Durel, 2006), supporting the argument that increased resistance to disease might be an age effect rather than a genetic change in resistance. Despite the environmental and age effects seen in this study, the overall correlations between traits analysed were in agreement with results previously reported in strawberry (Ukalska et al. 2006; Singh et al. 2010; Zorrilla-Fontanesi et al. 2011b; Lerceteau-Köhler et al. 2012).

7.2 Transferability of SSR markers between two different octoploid strawberry populations and their application for targeted mapping

Simple sequence repeat **(**SSR) markers are co-dominant, highly polymorphic and abundant making them the most useful markers for genetics and breeding studies (Hemmat et al. 2003; Gasic et al. 2009; Celton et al. 2009; Sargent et al. 2009). Several studies have previously reported high transferability level of SSRs among closely related species in Rosaceae. For example, high SSR marker transferability has been demonstrated between SSRs developed in *Fragaria vesca* and other diploid *Fragaria* species including *Fragaria* × *ananassa*, and vice versa (Monfort et al. 2005; Gil-Ariza et al. 2006; Gasic et al. 2009), between SSRs developed in apple and pear (Pierantoni et al. 2004; Gasic et al. 2009 Celton et al. 2009), and SSRs developed in rose and diploid and octoploid strawberry (Park et al. 2010).

In this study, targeted saturation of regions with low marker density on the previously reported SSR-based linkage map derived from the 'Redgauntlet' × 'Hapil' progeny (Sargent et al. 2012) was conducted by selecting and screening previously mapped SSR markers from within the same positions on the linkage groups of a

different octoploid strawberry mapping population (Isobe et al. 2013) (Chapter 3). In general, the success rate of the SSR marker transferability between the two different octoploid strawberry populations was high (78.4%). These results were expected because markers were tested in the same species (octoploid strawberry). Eighty out of 111 SSR markers selected and tested amplified at least one PCR product (Appendix 3.1).

However, the success rate of mapping selected SSRs within 26 targeted regions was very low (23.4%). In fact, only three SSRs were mapped within targeted gaps and additional 23 SSRs were mapped randomly across 28 linkage groups. A low success rate of a targeted mapping of novel SSRs in this study might be due to the amplification of a different locus, genotyping errors, missing values, the use of different octoploid strawberry individuals, or differences in recombination frequencies between different individuals. Additionally, SSR markers were selected from regions of low marker density on the existing SSR-based linkage map, which may explain the high levels of homozygosity observed and difficulty in saturating them.

The incorrect linkage group annotation of the existing SSR-based 'Redgauntlet' × 'Hapil' linkage map (Sargent et al. 2012), which was discovered later in this study (Chapter 5, Section 5.3.2), may have also influenced the low number of markers mapped within the targeted regions. Normally, linkage group annotations of a novel genetic linkage map are based on common markers mapped (Sargent et al. 2009; Fernández-Fernández et al. 2012), and usually no errors are observed in linkage group naming. But this study demonstrated that 71.4% of linkage group names of the existing SSR-based linkage map were annotated incorrectly based on common markers mapped between the diploid and octoploid strawberry (Sargent et al. 2009). This suggests that

potential errors in the diploid strawberry genome sequence assembly might still be present and may lead to erroneous results.

The overall low success rate of mapping novel SSRs demonstrates that the approach used in this study for targeted saturation of gaps is ineffective, costly and comparably slow. Other approaches, such as gene-specific marker development may be more efficient for saturation of the target regions (Sargent et al. 2007), although genotyping arrays are fast becoming the preferred approach for saturation of genetic linkage maps, given the rapidly decreasing cost of high-throughput genotyping. (Antanaviciute et al. 2012; Peace et al. 2012; Verde et al. 2012; Bianco et al. 2014; Bassil et al. 2015).

A novel high density SNP-based map containing a total of 3,933 binned SNPs was developed as part of the work carried out for this thesis (described in Chapter 3) by genotyping 90 K single nucleotide polymorphism (SNP) markers incorporated on a genotyping array (IStraw90®). The results showed that the technique is very quick and cost-efficient and is an excellent method for high density linkage map development within *Fragaria* species.

7.3 Markers linked to fruit firmness QTLs in octoploid strawberry and expansin genes

Breeding for fruit firmness in strawberry is an important aim because excessive fruit softening is an undesirable trait which causes poor postharvest storage, limiting the transportability and shelf-life, and eventually causing economical losses in fruit production. Therefore, a case study of a validation of SSR markers closely linked to fruit firmness QTLs in cultivated strawberry was conducted with the purpose of

providing novel information on molecular markers linked to fruit firmness, which could be potentially used in strawberry breeding programmes.

Sixteen markers were identified as closely linked to eight fruit firmness QTLs (Chapter 5). However, validation analysis of the markers in strawberry germplasm revealed that only four SSRs were reliable for use in enhancing breeding efficiency through marker-assisted breeding (MAB) (Table 5.4). The majority of markers linked to fruit firmness QTLs were on a wrong phase, and if they were used further an inverse sign of fruit firmness (in this case association with fruit softening) was likely to be observed. This is an important issue in the development of molecular markers for MAB, because it makes the marker development process more challenging. Similar issues were reported in the study by Antanaviciute et al. (2015), where SSR markers were associated with *Verticillium* wilt resistance. Despite the identification of SSRs linked to fruit firmness, further validation of these markers in a larger strawberry germplasm is necessary to confirm the significance level of markers detected in this study.

Fruit firmness is associated with fruit ripening and is a complex trait (Harpster et al. 1998; Civello et al. 1999; Harrison et al. 2001). The large number of QTLs linked to fruit firmness (eight loci on the SSR-based linkage map and nine QTLs on the SNP-based map) and high heritability coefficient (0.55) identified in this study also confirm the complexity of this trait. Strawberry fruit ripening has been well studied and a huge amount of information is available on the processes which takes place during the fruit ripening stages (Medina-Escobar et al. 1997; Civello et al. 1999; Bombarely et al. 2010; Csukasi et al. 2011, 2012). However, further investigations to better understand the regulation of genetically programmed events is essential (Merchante et al. 2013). In strawberry, seven expansin genes have been reported to be linked to fruit softening (Civello et al. 1999; Harrison et al. 2001; Dotto et al. 2006). The functions of this gene

family associated with fruit softening include the regulation of cell wall expansion, loosening and metabolism (Harrison et al. 2001; Dotto et al. 2006). Further evidence on expansin gene function (a correlation between mRNA expression levels and fruit firmness for expansions *FaEXP*1, *FaEXP*2 and *FaEXP*5) linked to fruit firmness was reported by Dotto et al. (2006). Therefore, physical locations of the expansin genes (*FaEXP1* to *FaEXP7*) were compared to the physical positions of the SNP markers most closely linked to firmness QTLs. Interestingly, two loci were closely located to the expansin gene locations based on the diploid strawberry genome sequence (Chapter 5, Section 5.3.6). The associations between firmness QTLs and expansin gene locations are significant findings in this research and provide further evidence that SNP markers underlying those QTL regions are likely to be linked to expansin genes. It would be worth checking if SSR markers linked to firmness QTLs also map to the expansin gene locations in addition to further validation analysis using larger cultivated strawberry germplasm.

7.4 Co-regulation of different traits in octoploid strawberry

The understanding of how different traits are regulated in octoploid strawberry is important when breeding new cultivars. Breeding focusing on a specific trait may eliminate other economically important traits because of co-regulation and/or trade-offs between different traits (Sánchez et al. 2012; Guan et al. 2013; Du et al. 2015). To investigate whether plant characteristics, fruit quality and disease resistant traits are co-regulated in strawberry, a combined analysis of QTLs associated with these traits was conducted for the first time in octoploid strawberry (Chapter 6, Section 6.3.4).

The results revealed a single consistent overlapping region between QTLs associated with *Verticillium* wilt resistance and fruit neck line (Figure 6.4). Despite many studies describing how *Verticillium* wilt attacks and spreads through strawberry plants (Fradin and Thomma, 2006; Schubert et al. 2008), no information is available on the relationship between wilt and fruit neck line during development of strawberry plants. This study demonstrates that breeding for a raised, flat or sunken fruit neck line could lead to individuals expressing higher level of wilt resistance, although it is not known yet which neck line form is associated with *Veritcillium* wilt resistance. Further phenotyping and validation analysis for wilt resistance and neck line would provide important information about the possibility to combining these two traits in strawberry breeding; it is also necessary to investigate further the relationship between them.

7.5 Concluding remarks

This work reports novel information on correlations between different traits in octoploid strawberry, the construction of a high quality consensus genetic linkage map which was later used for the QTL identification linked to plant characteristics, fruit quality traits and powdery mildew resistance, marker validation linked to fruit firmness and powdery mildew resistance, and co-regulation analysis of different traits in octoploid strawberry. Several different approaches were employed to achieve the above goals with significant results observed in the QTL mapping and marker validation analysis. Reliable molecular markers were found to be associated with fruit firmness in addition to the closest linked SNP markers linked to powdery mildew resistance. This work will have practical applications in the identification and mapping of candidate genes linked, for example, to yield, fruit size and sugar level, in addition to molecular

marker development for use in breeding new, improved strawberry cultivars. The phenotypic and genotypic data reported in this thesis will be a valuable resource for future studies, including the application of a genomic selection approach in cultivated strawberry.

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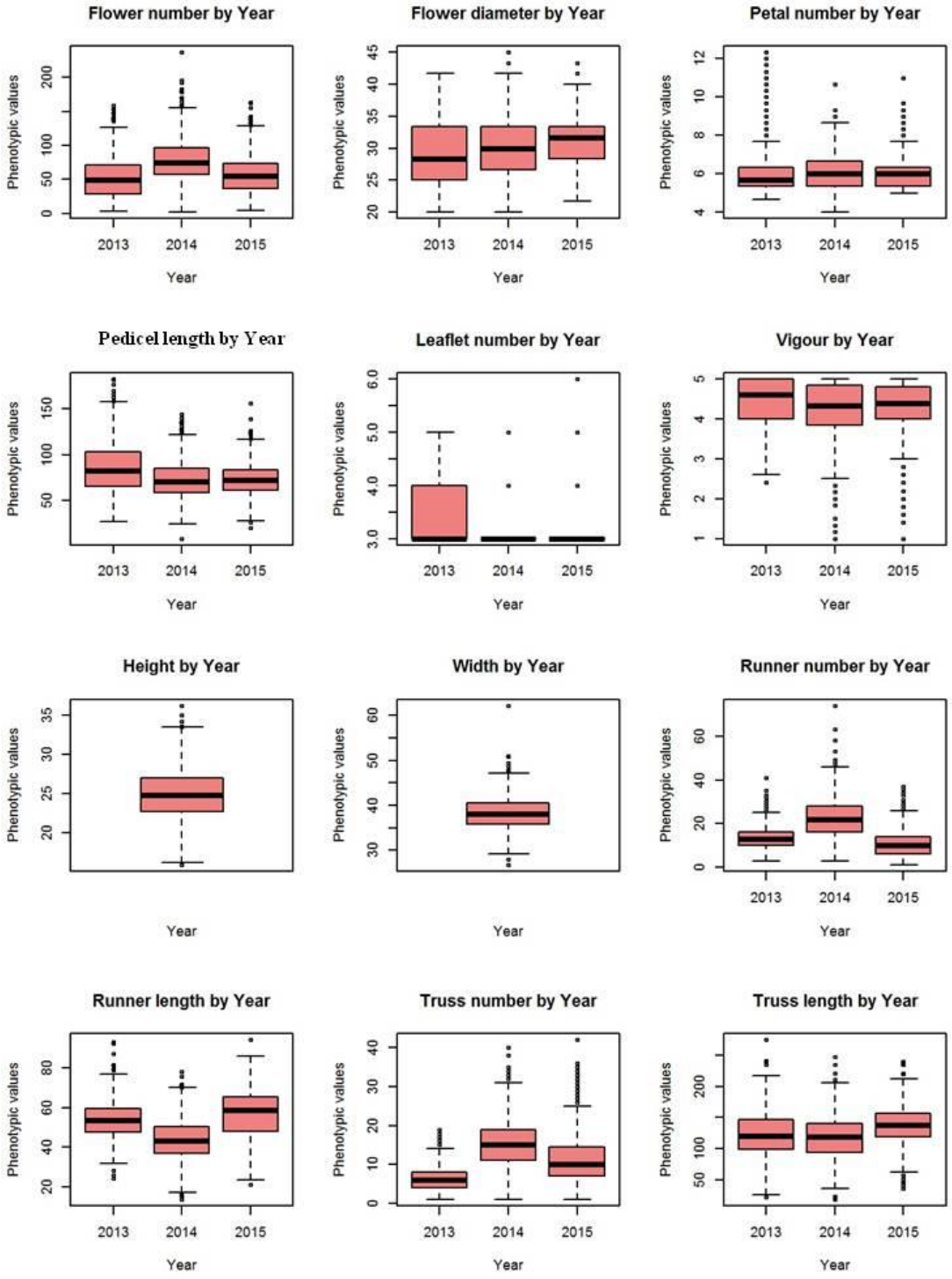
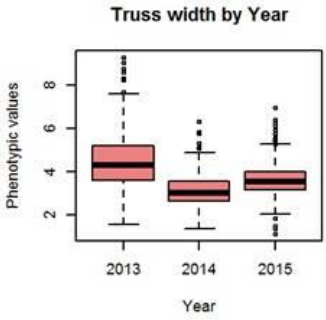
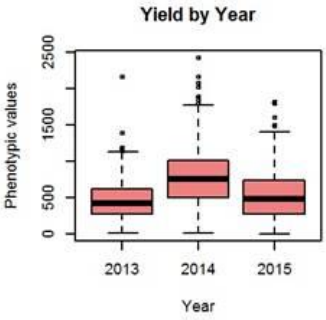
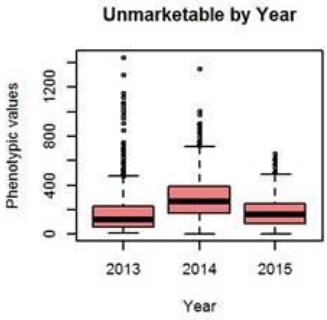
Appendices

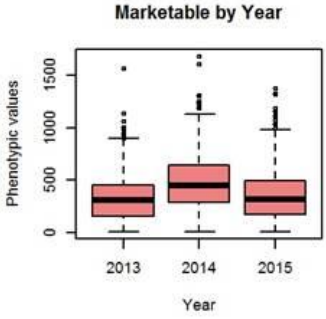
Appendix 2.1 The distributions of phenotypic values, minimum, maximum and median of individuals of the 'Redgauntlet' × 'Hapil' progeny for 30 plant characteristics and fruit quality traits over the three years.

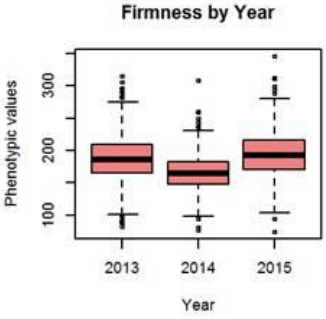


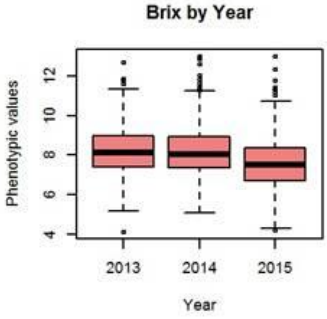

Truss width by Year
Phenotypic values
2
4
6
8
2013
2014
2015
Year

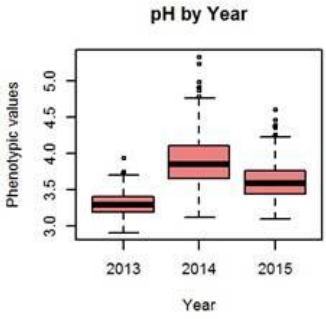

Yield by Year
Phenotypic values
0
500
1500
2500
2013
2014
2015
Year

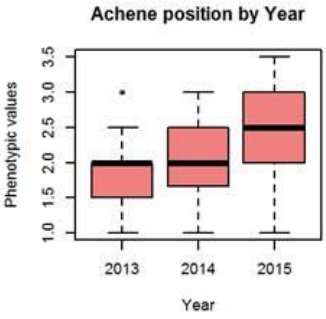

Unmarketable by Year
Phenotypic values
0
400
800
1200
2013
2014
2015
Year

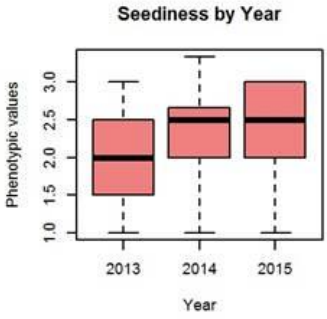

Marketable by Year
Phenotypic values
0
500
1000
1500
2013
2014
2015
Year

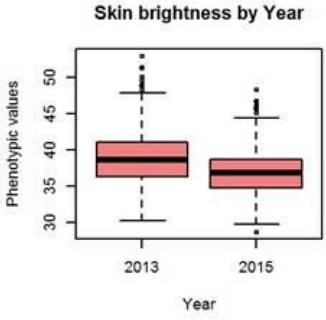

Firmness by Year
Phenotypic values
100
200
300
2013
2014
2015
Year

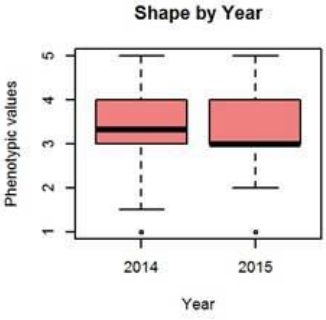

Brix by Year
Phenotypic values
4
6
8
10
12
2013
2014
2015
Year

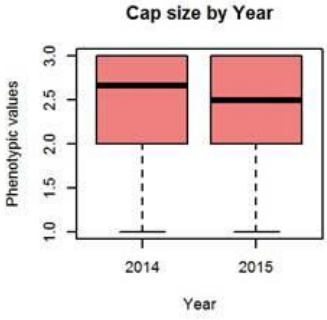

pH by Year
Phenotypic values
3.0
3.5
4.0
4.5
5.0
2013
2014
2015
Year

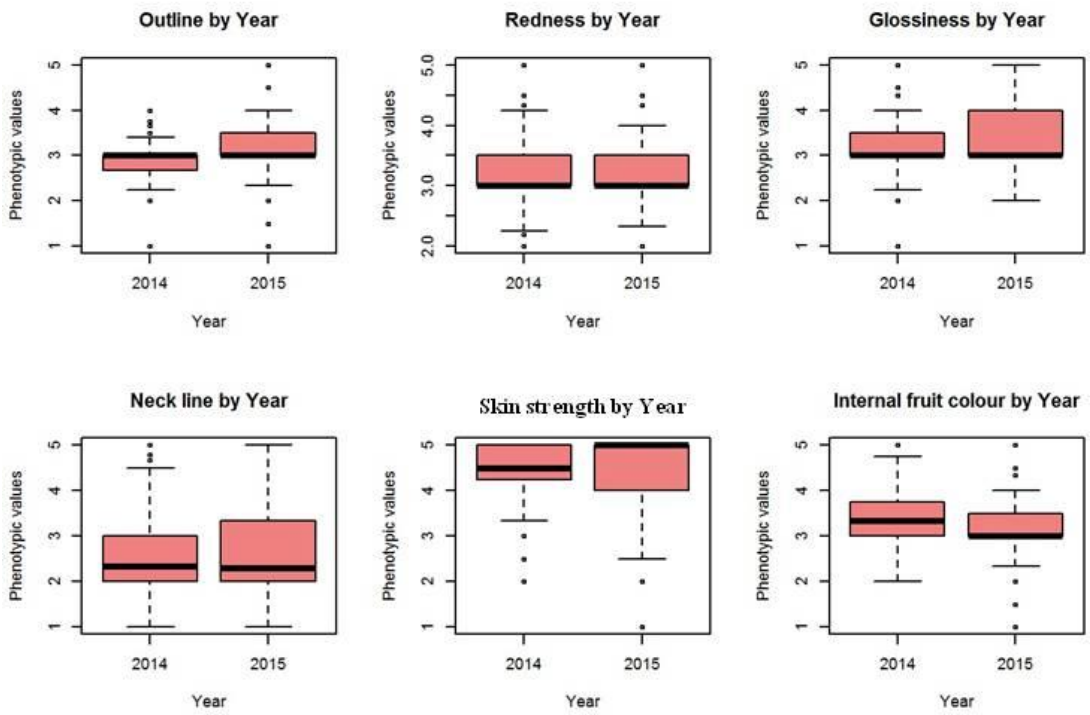

Achene position by Year
Phenotypic values
1.0
1.5
2.0
2.5
3.0
3.5
2013
2014
2015
Year


Seediness by Year
Phenotypic values
1.0
1.5
2.0
2.5
3.0
2013
2014
2015
Year

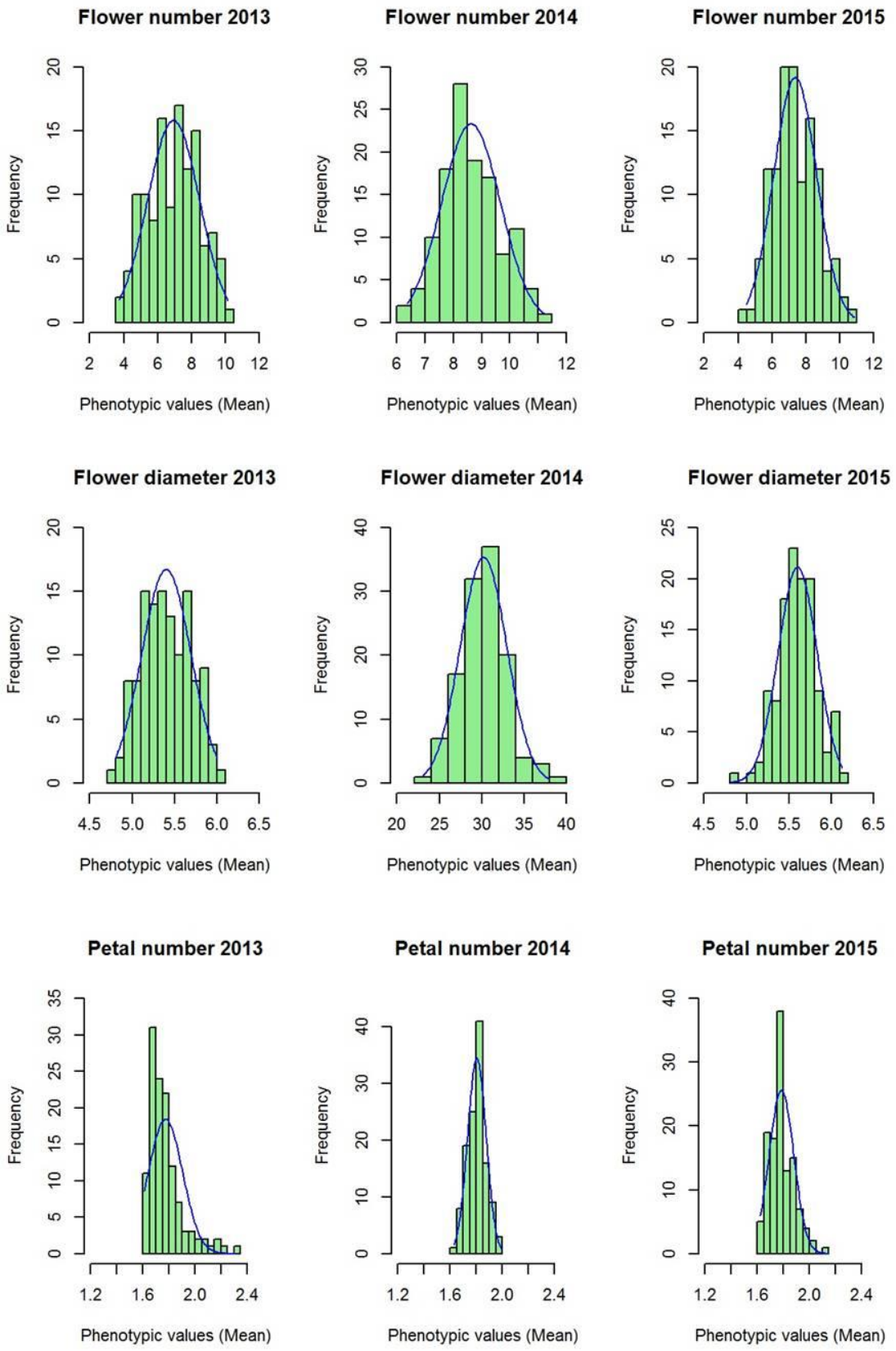

Skin brightness by Year
Phenotypic values
30
35
40
45
50
2013
2015
Year

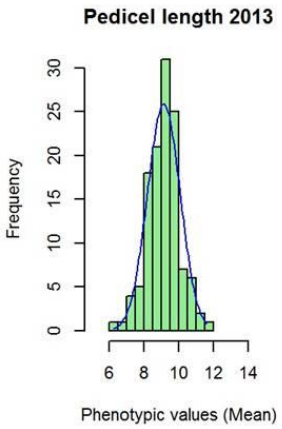

Shape by Year
Phenotypic values
1
2
3
4
5
2014
2015
Year

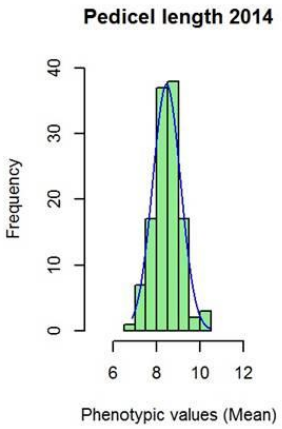

Cap size by Year
Phenotypic values
1.0
1.5
2.0
2.5
3.0
2014
2015
Year

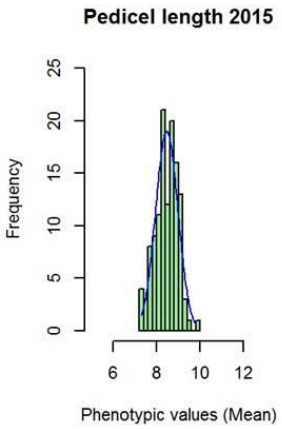

Outline by Year
Redness by Year
Glossiness by Year
Neck line by Year
Skin strength by Year
Internal fruit colour by Year
Phenotypic values
Year
2014
2015

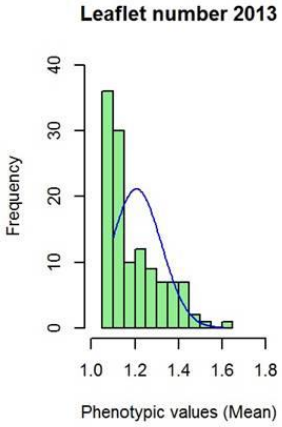
Appendix 2.2 Phenotypic mean value distributions among the progeny for 30 plant characteristics and fruit quality traits over the three year-data.

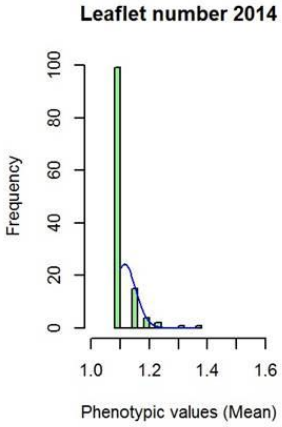


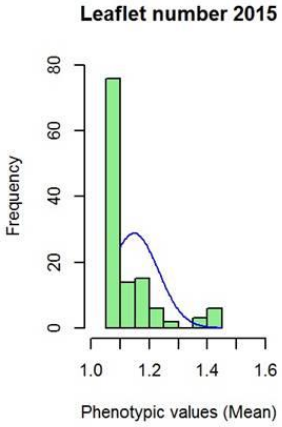

Pedicel length 2013
Frequency
Phenotypic values (Mean)

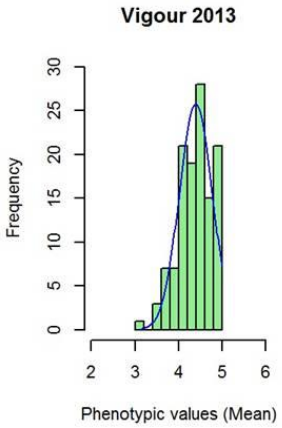

Pedicel length 2014
Frequency
Phenotypic values (Mean)

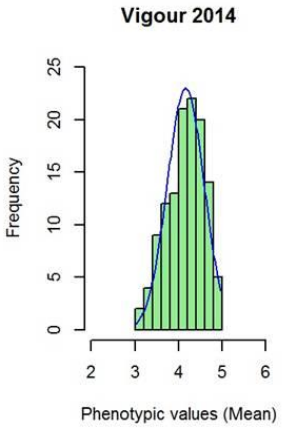

Pedicel length 2015
Frequency
Phenotypic values (Mean)

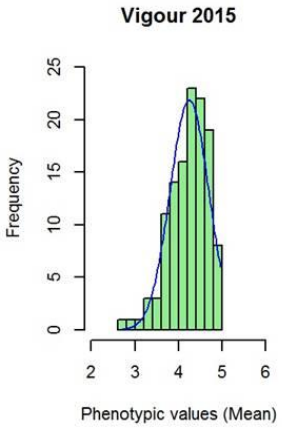

Leaflet number 2013
Frequency
Phenotypic values (Mean)

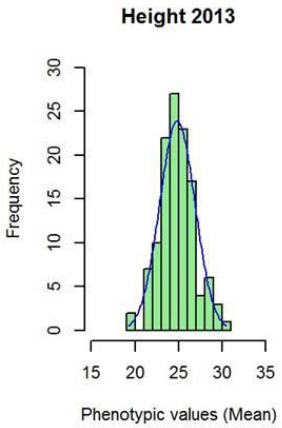

Leaflet number 2014
Frequency
Phenotypic values (Mean)

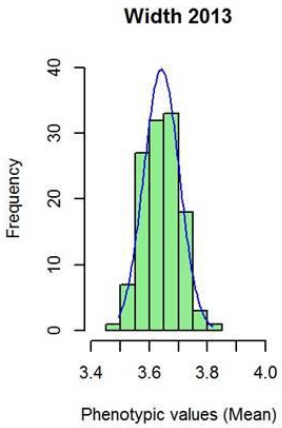

Leaflet number 2015
Frequency
Phenotypic values (Mean)

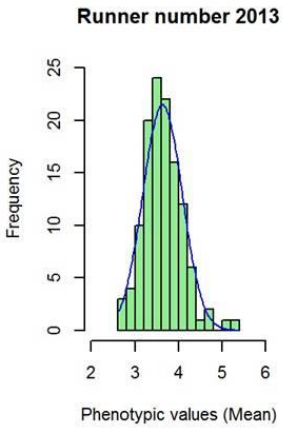

Vigour 2013
Frequency
Phenotypic values (Mean)

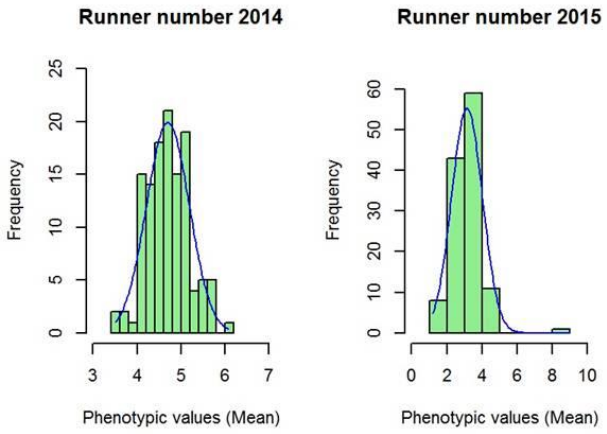

Vigour 2014
Frequency
Phenotypic values (Mean)


Vigour 2015
Frequency
Phenotypic values (Mean)

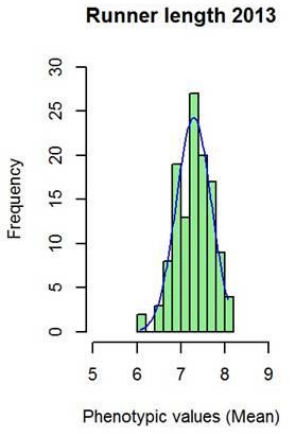

Height 2013
Frequency
Phenotypic values (Mean)

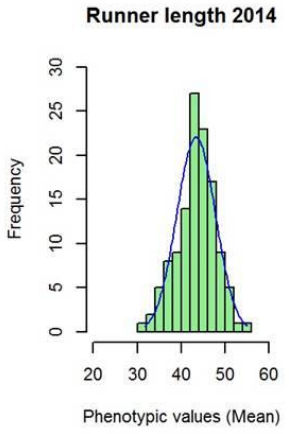

Width 2013
Frequency
Phenotypic values (Mean)

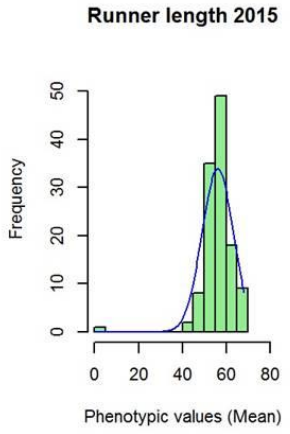

Runner number 2013
Frequency
Phenotypic values (Mean)

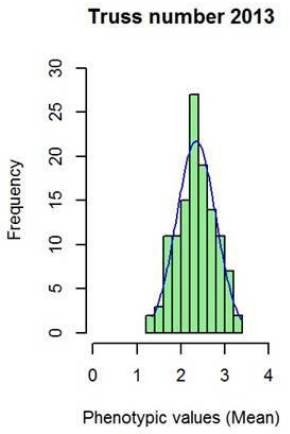

Runner number 2014
Frequency
Phenotypic values (Mean)

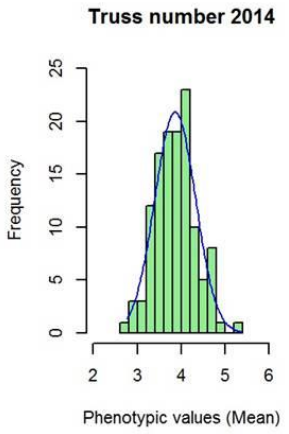
Runner number 2015
Frequency
Phenotypic values (Mean)

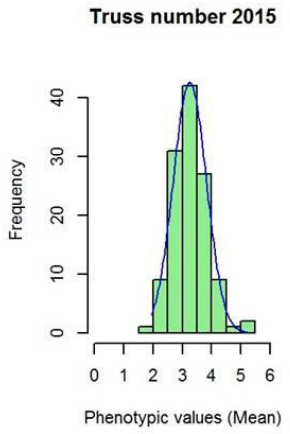

Runner length 2013
Frequency
Phenotypic values (Mean)

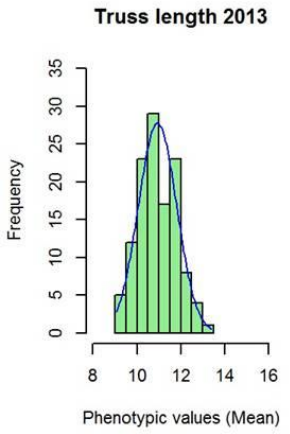

Runner length 2014
Frequency
Phenotypic values (Mean)

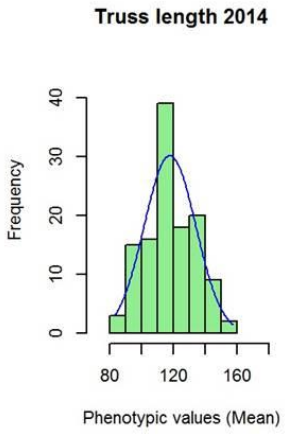

Runner length 2015
Frequency
Phenotypic values (Mean)

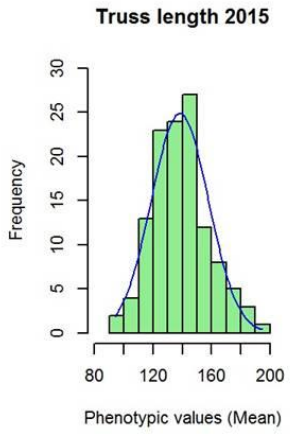

Truss number 2013
Frequency
Phenotypic values (Mean)

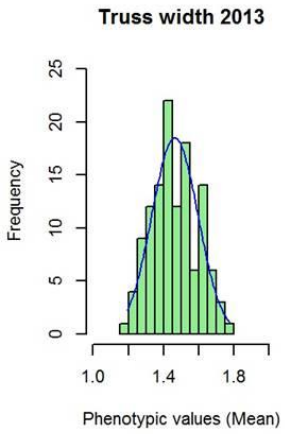

Truss number 2014
Frequency
Phenotypic values (Mean)

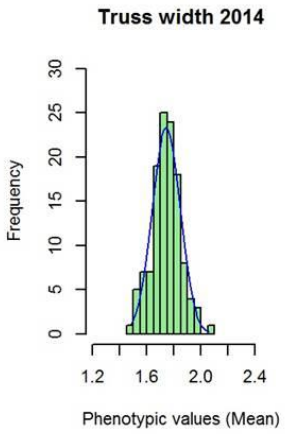

Truss number 2015
Frequency
Phenotypic values (Mean)

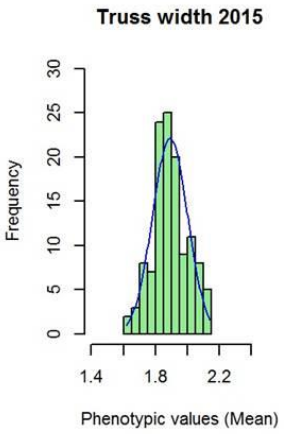

Truss length 2013
Frequency
Phenotypic values (Mean)


Truss length 2014
Frequency
Phenotypic values (Mean)


Truss length 2015
Frequency
Phenotypic values (Mean)


Truss width 2013
Frequency
Phenotypic values (Mean)


Truss width 2014
Frequency
Phenotypic values (Mean)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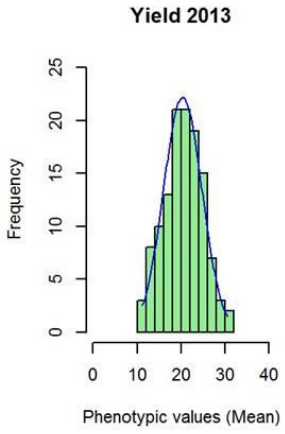
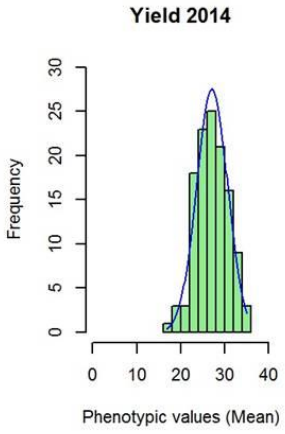
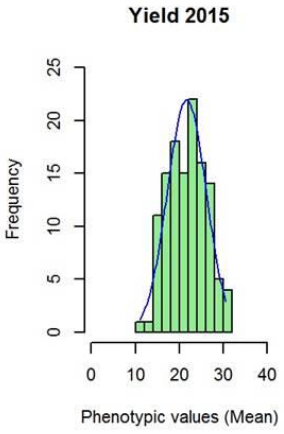
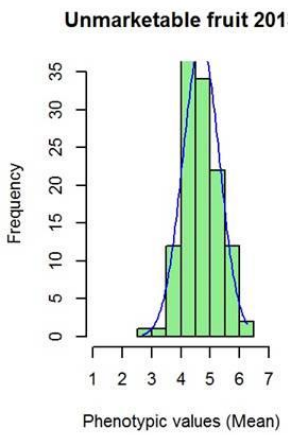
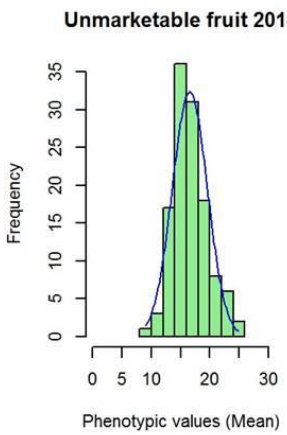
Frequency
Phenotypic values (Mean)

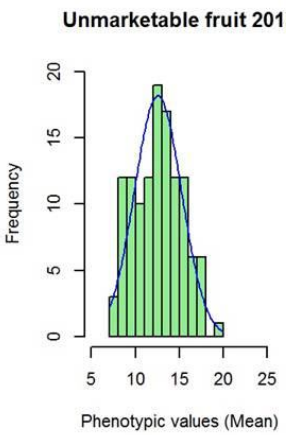

Yield 2013
Frequency
Phenotypic values (Mean)

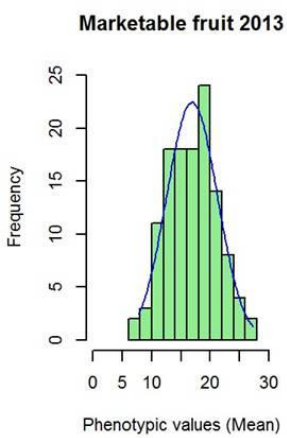

Yield 2014
Frequency
Phenotypic values (Mean)

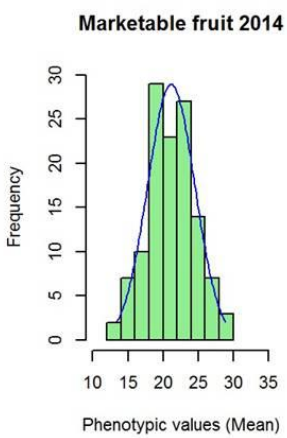

Yield 2015
Frequency
Phenotypic values (Mean)

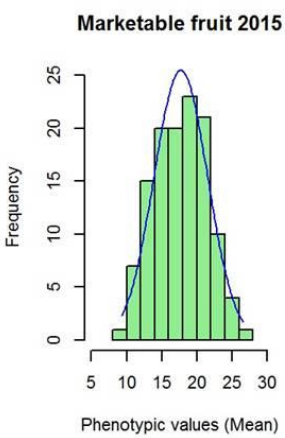

Unmarketable fruit 2013
Frequency
Phenotypic values (Mean)

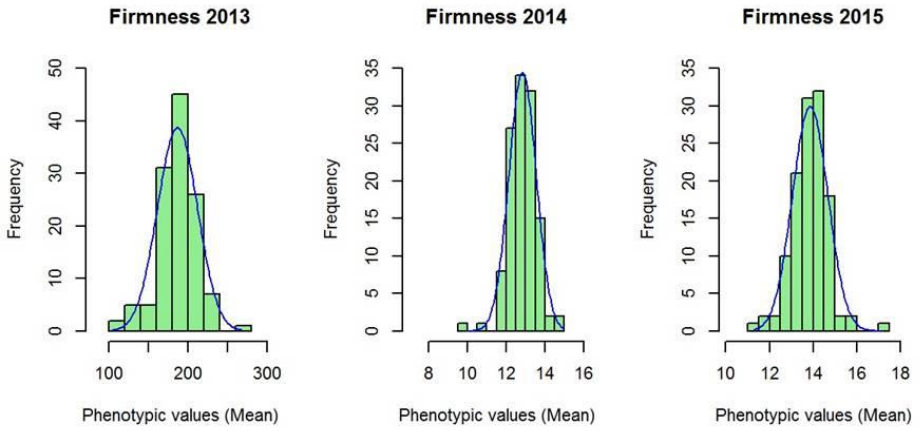

Unmarketable fruit 2014
Frequency
Phenotypic values (Mean)


Unmarketable fruit 2015
Frequency
Phenotypic values (Mean)


Marketable fruit 2013
Frequency
Phenotypic values (Mean)

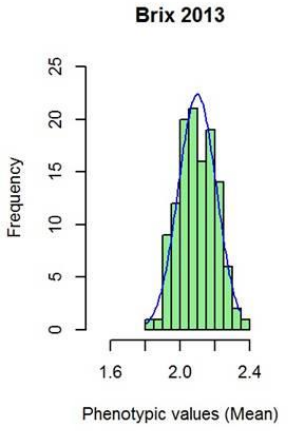

Marketable fruit 2014
Frequency
Phenotypic values (Mean)

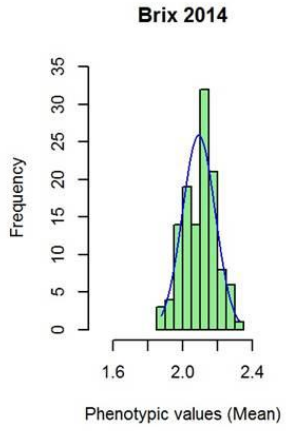

Marketable fruit 2015
Frequency
Phenotypic values (Mean)

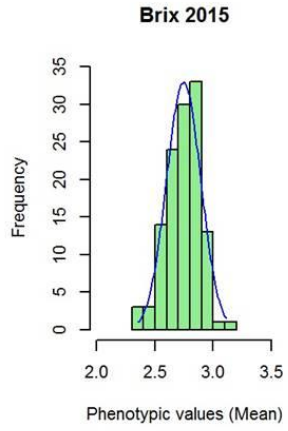

Firmness 2013
Frequency
Phenotypic values (Mean)

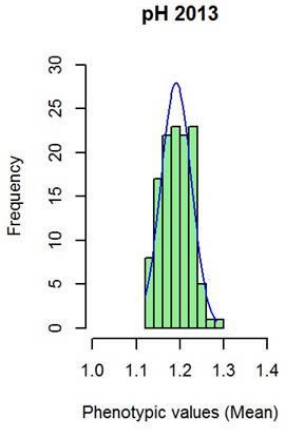
Firmness 2014
Frequency
Phenotypic values (Mean)

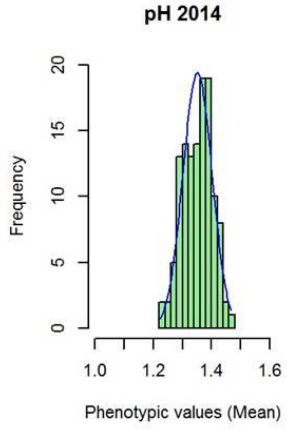
Firmness 2015
Frequency
Phenotypic values (Mean)

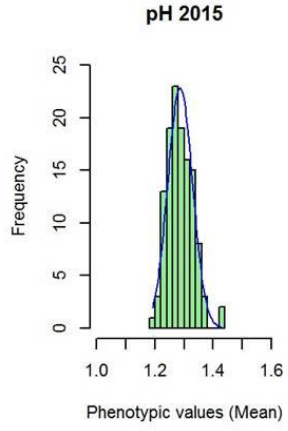

Brix 2013
Frequency
Phenotypic values (Mean)

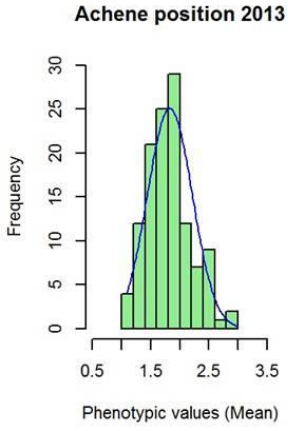

Brix 2014
Frequency
Phenotypic values (Mean)

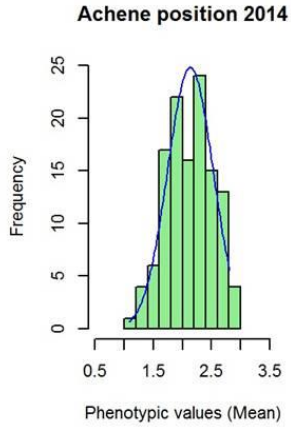

Brix 2015
Frequency
Phenotypic values (Mean)

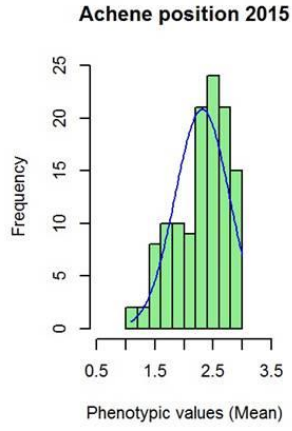

pH 2013
Frequency
Phenotypic values (Mean)

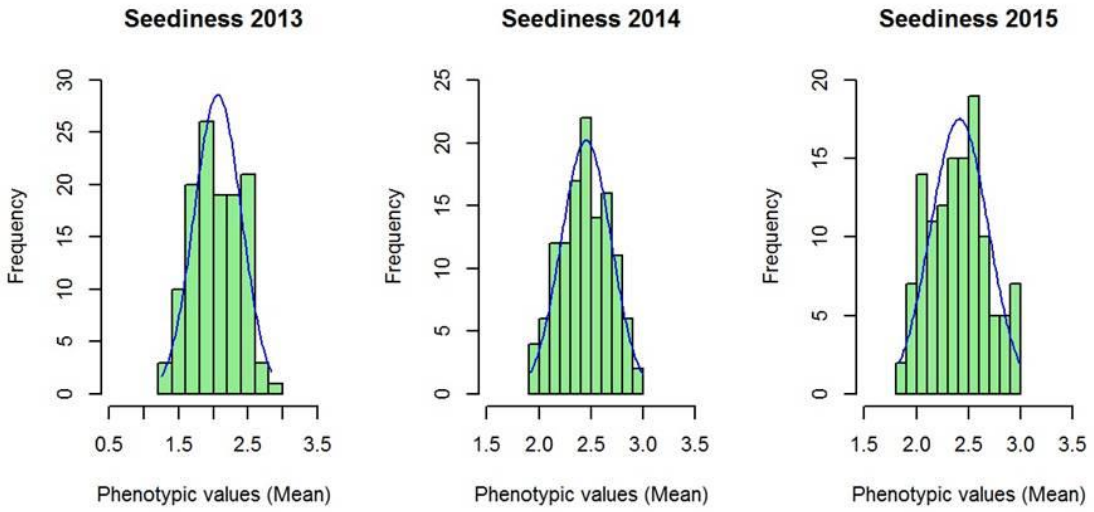

pH 2014
Frequency
Phenotypic values (Mean)


pH 2015
Frequency
Phenotypic values (Mean)


Achene position 2013
Frequency
Phenotypic values (Mean)

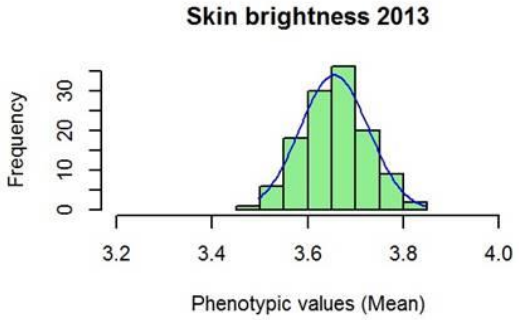

Achene position 2014
Frequency
Phenotypic values (Mean)

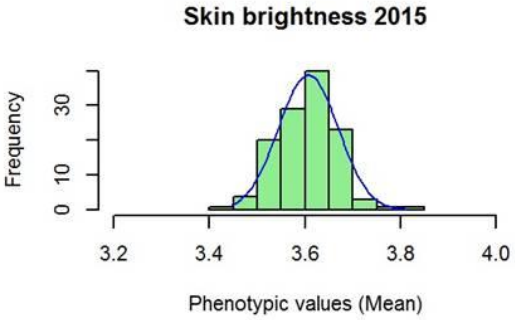

Achene position 2015
Frequency
Phenotypic values (Mean)

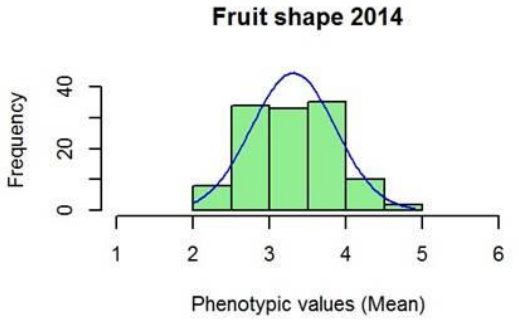

Seediness 2013
Frequency
0 5 10 15 20 25 30
0.5 1.5 2.5 3.5
Phenotypic values (Mean)

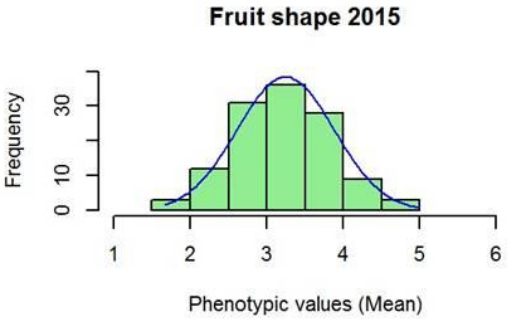
Seediness 2014
Frequency
0 5 10 15 20 25
1.5 2.0 2.5 3.0 3.5
Phenotypic values (Mean)

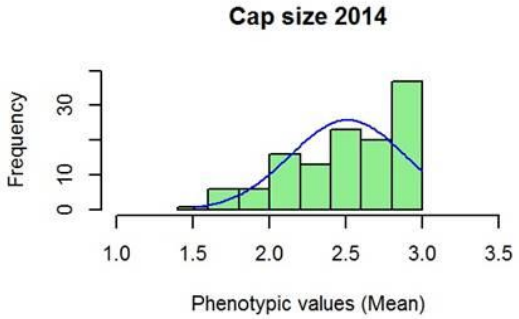
Seediness 2015
Frequency
0 5 10 15 20
1.5 2.0 2.5 3.0 3.5
Phenotypic values (Mean)

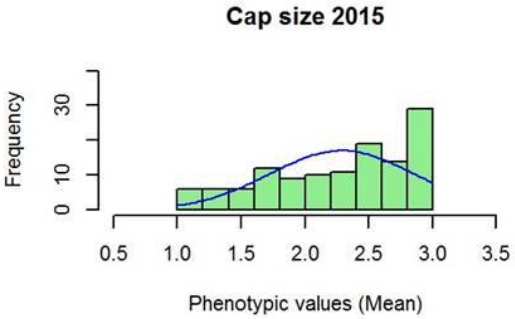

Skin brightness 2013
Frequency
0 10 20 30
3.2 3.4 3.6 3.8 4.0
Phenotypic values (Mean)

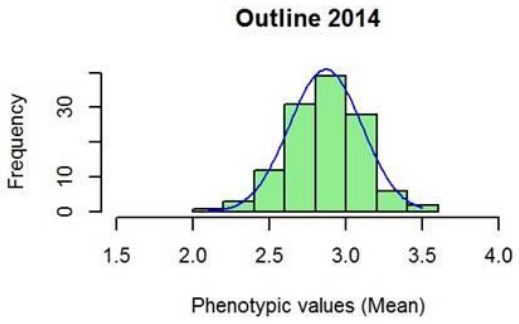

Skin brightness 2015
Frequency
0 10 30
3.2 3.4 3.6 3.8 4.0
Phenotypic values (Mean)

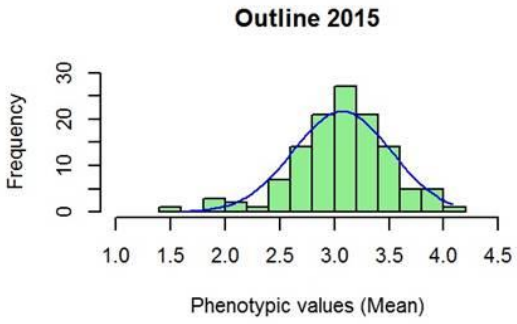

Fruit shape 2014
Frequency
0 20 40
1 2 3 4 5 6
Phenotypic values (Mean)

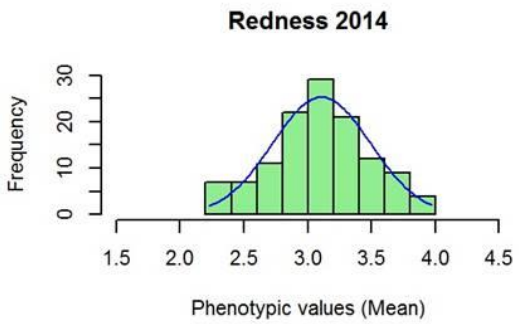

Fruit shape 2015
Frequency
0 10 30
1 2 3 4 5 6
Phenotypic values (Mean)

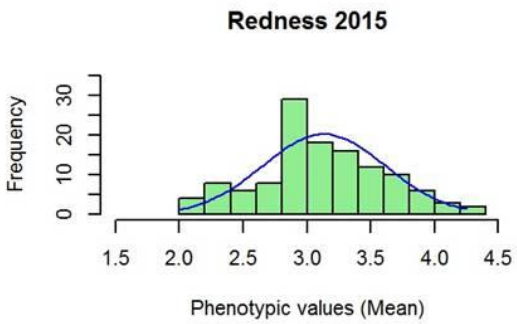

Cap size 2014
Frequency
0 10 30
1.0 1.5 2.0 2.5 3.0 3.5
Phenotypic values (Mean)

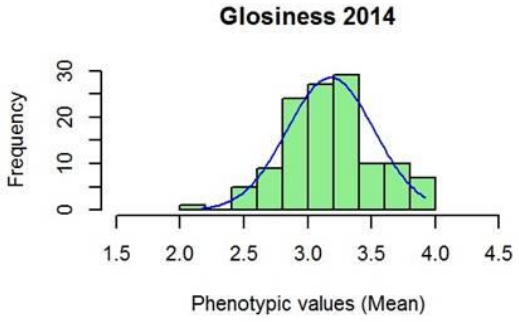

Cap size 2015
Frequency
0 10 30
0.5 1.0 1.5 2.0 2.5 3.0 3.5
Phenotypic values (Mean)

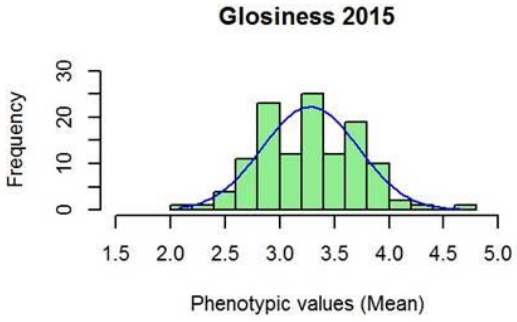

Outline 2014
Frequency
0
10
30
1.5
2.0
2.5
3.0
3.5
4.0
Phenotypic values (Mean)

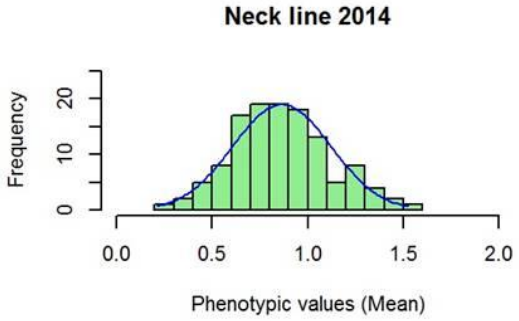

Outline 2015
Frequency
0
10
20
30
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
Phenotypic values (Mean)

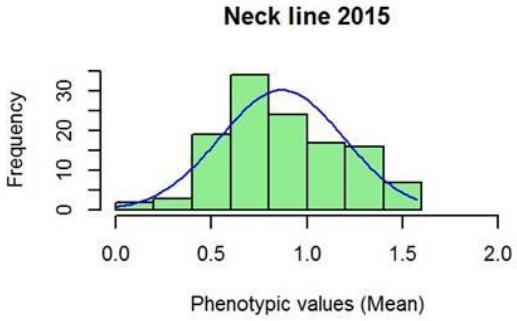

Redness 2014
Frequency
0
10
20
30
1.5
2.0
2.5
3.0
3.5
4.0
4.5
Phenotypic values (Mean)

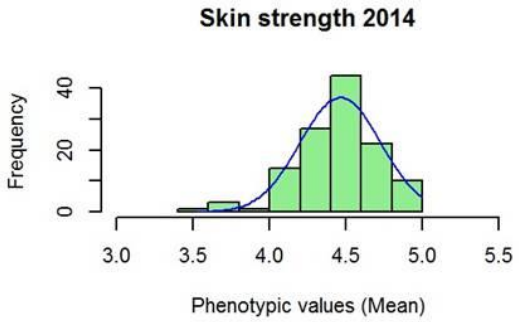

Redness 2015
Frequency
0
10
20
30
1.5
2.0
2.5
3.0
3.5
4.0
4.5
Phenotypic values (Mean)

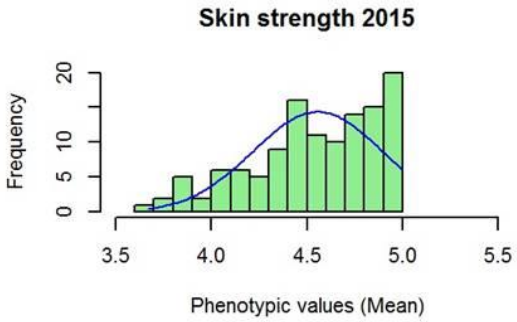

Glosiness 2014
Frequency
0
10
20
30
1.5
2.0
2.5
3.0
3.5
4.0
4.5
Phenotypic values (Mean)

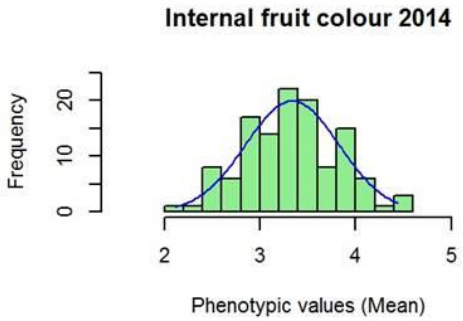

Glosiness 2015
Frequency
0
10
20
30
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
Phenotypic values (Mean)

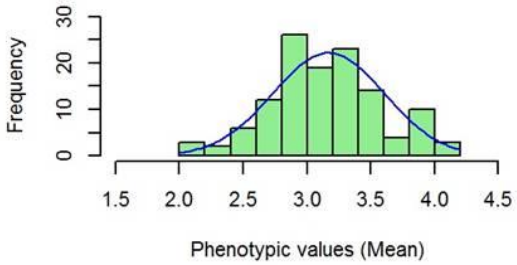

Neck line 2014
Frequency
0
10
20
0.0
0.5
1.0
1.5
2.0
Phenotypic values (Mean)


Neck line 2015
Frequency
0
10
20
30
0.0
0.5
1.0
1.5
2.0
Phenotypic values (Mean)


Skin strength 2014
Frequency
0
20
40
3.0
3.5
4.0
4.5
5.0
5.5
Phenotypic values (Mean)


Skin strength 2015
Frequency
0
5
10
20
3.5
4.0
4.5
5.0
5.5
Phenotypic values (Mean)


Internal fruit colour 2014
Frequency
0
10
20
2
3
4
5
Phenotypic values (Mean)


Internal fruit colour 2015
Frequency
0
10
20
30
1.5
2.0
2.5
3.0
3.5
4.0
4.5
Phenotypic values (Mean)

Appendix 2.3 The Shapiro-Wilk test results for non-transformed, log transformed and square root transformed traits for each year analysed. The transformations in bold were selected and used for further analysis. Year highlighted in bold represents normally distributed trait values.

Trait	Year	Raw data	Log - transformed	Square root - transformed
Fl.N.	2013	W = 0.9573, p-value = 1.025e-13	W = 0.9673, p-value = 1.089e-11	**W = 0.9922, p-value = 0.0007314**
	2014	W = 0.9829, p-value = 1.7e-07	W = 0.865, p-value < 2.2e-16	**W = 0.9848, p-value = 7.105e-07**
	2015	W = 0.9645, p-value = 2.634e-12	W = 0.9586, p-value = 1.775e-13	**W = 0.9978, p-value = 0.4422**
Fl.D.	2013	W = 0.9749, p-value = 8.356e-10	W = 0.9772, p-value = 3.508e-09	**W = 0.9781, p-value = 6.224e-09**
	2014	**W = 0.9839, p-value = 3.66e-07**	W = 0.9767, p-value = 2.468e-09	W = 0.9829, p-value = 1.732e-07
	2015	W = 0.9749, p-value = 7.741e-10	W = 0.978, p-value = 5.328e-09	**W = 0.9784, p-value = 7.162e-09**
Pt.N.	2013	W = 0.7685, p-value < 2.2e-16	**W = 0.8444, p-value < 2.2e-16**	W = 0.8097, p-value < 2.2e-16
	2014	W = 0.935, p-value < 2.2e-16	**W = 0.9603, p-value = 4.382e-13**	W = 0.9498, p-value = 5.583e-15
	2015	W = 0.8908, p-value < 2.2e-16	**W = 0.9294, p-value < 2.2e-16**	W = 0.9127, p-value < 2.2e-16
Pdc.L.	**2013**	W = 0.9858, p-value = 1.8e-06	W = 0.985, p-value = 9.539e-07	**W = 0.9976, p-value = 0.3855**
	2014	W = 0.9825, p-value = 1.294e-07	W = 0.9682, p-value = 1.897e-11	**W = 0.9939, p-value = 0.005315**
	2015	W = 0.9911, p-value = 0.0002114	W = 0.9776, p-value = 3.975e-09	**W = 0.9956, p-value = 0.03667**
Lf.N.	2013	W = 0.6355, p-value < 2.2e-16	**W = 0.6407, p-value < 2.2e-16**	W = 0.6389, p-value < 2.2e-16
	2014	W = 0.2064, p-value < 2.2e-16	**W = 0.2105, p-value < 2.2e-16**	W = 0.2087, p-value < 2.2e-16
	2015	W = 0.3908, p-value < 2.2e-16	**W = 0.3975, p-value < 2.2e-16**	W = 0.3946, p-value < 2.2e-16
Vig.	2013	**W = 0.8724, p-value < 2.2e-16**	W = 0.8526, p-value < 2.2e-16	W = 0.8638, p-value < 2.2e-16
	2014	**W = 0.8639, p-value < 2.2e-16**	W = 0.7286, p-value < 2.2e-16	W = 0.8083, p-value < 2.2e-16
	2015	**W = 0.8523, p-value < 2.2e-16**	W = 0.762, p-value < 2.2e-16	W = 0.8141, p-value < 2.2e-16
Hgh.	**2013**	**W = 0.9976, p-value = 0.3858**	W = 0.9943, p-value = 0.007117	W = 0.9983, p-value = 0.6755
Wdt.	2013	W = 0.9814, p-value = 4.913e-08	**W = 0.9937, p-value = 0.003862**	W = 0.9897, p-value = 5.065e-05
Run.N.	2013	W = 0.9471, p-value = 1.649e-15	W = 0.9869, p-value = 4.102e-06	**W = 0.9884, p-value = 1.479e-05**
	2014	W = 0.9659, p-value = 5.751e-12	W = 0.9667, p-value = 8.337e-12	**W = 0.9955, p-value = 0.0335**
	2015	W = 0.9544, p-value = 9.015e-14	W = 0.9282, p-value < 2.2e-16	**W = 0.9905, p-value = 0.0002002**

Run.L.	2013	W = 0.991, p-value = 0.000202	W = 0.9867, p-value = 3.405e-06	**W = 0.9944, p-value = 0.008029**
	2014	**W = 0.9959, p-value = 0.054**	W = 0.9477, p-value = 2.341e-15	W = 0.9836, p-value = 2.92e-07
	2015	**W = 0.9822, p-value = 1.995e-07**	W = 0.9389, p-value = 3.191e-16	W = 0.9656, p-value = 1.185e-11
Tr.N.	2013	W = 0.9369, p-value < 2.2e-16	W = 0.9472, p-value = 1.966e-15	**W = 0.9805, p-value = 2.844e-08**
	2014	W = 0.9786, p-value = 8.383e-09	W = 0.9162, p-value < 2.2e-16	**W = 0.9884, p-value = 1.715e-05**
	2015	W = 0.9192, p-value < 2.2e-16	W = 0.9803, p-value = 2.565e-08	**W = 0.9862, p-value = 2.217e-06**
Tr.L.	2013	W = 0.9905, p-value = 0.0001305	W = 0.9688, p-value = 2.521e-11	**W = 0.9947, p-value = 0.01255**
	2014	**W = 0.9958, p-value = 0.05181**	W = 0.9284, p-value < 2.2e-16	W = 0.9831, p-value = 2.678e-07
	2015	**W = 0.9958, p-value = 0.04833**	W = 0.9494, p-value = 4.295e-15	W = 0.9855, p-value = 1.261e-06
Tr.Wdt.	2013	W = 0.9659, p-value = 5.718e-12	**W = 0.9955, p-value = 0.03328**	W = 0.9876, p-value = 8.077e-06
	2014	W = 0.9864, p-value = 3.712e-06	W = 0.9871, p-value = 6.681e-06	**W = 0.9955, p-value = 0.03902**
	2015	W = 0.9692, p-value = 3.027e-11	W = 0.9738, p-value = 3.795e-10	**W = 0.9822, p-value = 9.875e-08**
Yield	2013	W = 0.9636, p-value = 1.988e-12	W = 0.8645, p-value < 2.2e-16	**W = 0.9872, p-value = 5.663e-06**
	2014	W = 0.9785, p-value = 9.79e-09	W = 0.9042, p-value < 2.2e-16	**W = 0.9958, p-value = 0.05236**
	2015	W = 0.9576, p-value = 2.342e-13	W = 0.9017, p-value < 2.2e-16	**W = 0.9955, p-value = 0.03962**
Unmark.	**2013**	W = 0.7275, p-value < 2.2e-16	**W = 0.9955, p-value = 0.06824**	W = 0.921, p-value < 2.2e-16
	2014	W = 0.9211, p-value < 2.2e-16	W = 0.9537, p-value = 3.452e-14	**W = 0.9939, p-value = 0.005623**
	2015	W = 0.9347, p-value < 2.2e-16	W = 0.9451, p-value = 3.027e-15	**W = 0.9937, p-value = 0.005694**
Mark.	2013	W = 0.9476, p-value = 1.019e-14	W = 0.906, p-value < 2.2e-16	**W = 0.993, p-value = 0.00313**
	2014	W = 0.9611, p-value = 8.676e-13	W = 0.9254, p-value < 2.2e-16	**W = 0.9988, p-value = 0.9309**
	2015	W = 0.9356, p-value < 2.2e-16	W = 0.9386, p-value = 2.966e-16	**W = 0.9948, p-value = 0.01989**
Firm.	2013	**W = 0.9914, p-value = 0.0003221**	W = 0.9696, p-value = 3.762e-11	W = 0.9882, p-value = 1.411e-05
	2014	W = 0.9902, p-value = 0.0001131	W = 0.988, p-value = 1.446e-05	**W = 0.9946, p-value = 0.01282**
	2015	W = 0.9913, p-value = 0.0004632	W = 0.9862, p-value = 4.498e-06	**W = 0.995, p-value = 0.02509**
Brix	**2013**	W = 0.9906, p-value = 0.0001355	**W = 0.9966, p-value = 0.1225**	W = 0.9966, p-value = 0.128
	2014	W = 0.9766, p-value = 3.556e-09	**W = 0.997, p-value = 0.2132**	W = 0.9898, p-value = 8.628e-05
	2015	W = 0.991, p-value = 0.0003795	W = 0.9936, p-value = 0.005545	**W = 0.9968, p-value = 0.2034**
pH	2013	W = 0.9919, p-value = 0.0005837	**W = 0.9957, p-value = 0.04259**	W = 0.994, p-value = 0.006213

	2014	W = 0.9779, p-value = 1.615e-06	**W = 0.9912, p-value = 0.007141**	W = 0.9856, p-value = 0.0001456
	2015	W = 0.9698, p-value = 4.242e-10	**W = 0.9823, p-value = 6.739e-07**	W = 0.9766, p-value = 1.704e-08
Ach.Ps.	2013	**W = 0.8793, p-value < 2.2e-16**	W = 0.8592, p-value < 2.2e-16	W = 0.8766, p-value < 2.2e-16
	2014	**W = 0.9462, p-value = 2.639e-15**	W = 0.9095, p-value < 2.2e-16	W = 0.9346, p-value < 2.2e-16
	2015	**W = 0.861, p-value < 2.2e-16**	W = 0.8193, p-value < 2.2e-16	W = 0.8463, p-value < 2.2e-16
Sdn.	2013	**W = 0.9067, p-value < 2.2e-16**	W = 0.8778, p-value < 2.2e-16	W = 0.8993, p-value < 2.2e-16
	2014	**W = 0.9011, p-value < 2.2e-16**	W = 0.8946, p-value < 2.2e-16	W = 0.9005, p-value < 2.2e-16
	2015	**W = 0.8028, p-value < 2.2e-16**	W = 0.8, p-value < 2.2e-16	W = 0.8043, p-value < 2.2e-16
Sk.Brg.	**2013**	W = 0.9869, p-value = 4.311e-06	**W = 0.9968, p-value = 0.1616**	W = 0.993, p-value = 0.001728
	2015	W = 0.9905, p-value = 0.0001953	**W = 0.9972, p-value = 0.2905**	W = 0.9947, p-value = 0.0178
Shp.	2014	**W = 0.9768, p-value = 4.064e-09**	W = 0.943, p-value = 8.704e-16	W = 0.968, p-value = 2.903e-11
	2015	**W = 0.94, p-value = 6.201e-16**	W = 0.9205, p-value < 2.2e-16	W = 0.9357, p-value < 2.2e-16
Cap.Sz.	2014	**W = 0.8324, p-value < 2.2e-16**	W = 0.7904, p-value < 2.2e-16	W = 0.8161, p-value < 2.2e-16
	2015	**W = 0.803, p-value < 2.2e-16**	W = 0.7696, p-value < 2.2e-16	W = 0.7903, p-value < 2.2e-16
Outl.	2014	**W = 0.9234, p-value < 2.2e-16**	W = 0.8761, p-value < 2.2e-16	W = 0.9075, p-value < 2.2e-16
	2015	**W = 0.9084, p-value < 2.2e-16**	W = 0.842, p-value < 2.2e-16	W = 0.8872, p-value < 2.2e-16
Rdn.	2014	**W = 0.9565, p-value = 1.433e-13**	W = 0.9389, p-value < 2.2e-16	W = 0.9515, p-value = 1.953e-14
	2015	**W = 0.9017, p-value < 2.2e-16**	W = 0.8811, p-value < 2.2e-16	W = 0.896, p-value < 2.2e-16
Gls.	2014	**W = 0.9495, p-value = 9.209e-15**	W = 0.922, p-value < 2.2e-16	W = 0.9428, p-value = 8.036e-16
	2015	**W = 0.9205, p-value < 2.2e-16**	W = 0.8999, p-value < 2.2e-16	W = 0.9149, p-value < 2.2e-16
Nck.Ln.	2014	W = 0.9472, p-value = 3.803e-15	**W = 0.976, p-value = 2.539e-09**	W = 0.9723, p-value = 2.903e-10
	2015	W = 0.9278, p-value < 2.2e-16	**W = 0.9481, p-value = 1.042e-14**	W = 0.9495, p-value = 1.744e-14
Sk.Str.	2014	**W = 0.8641, p-value < 2.2e-16**	W = 0.8094, p-value < 2.2e-16	W = 0.8401, p-value < 2.2e-16
	2015	**W = 0.694, p-value < 2.2e-16**	W = 0.5916, p-value < 2.2e-16	W = 0.6538, p-value < 2.2e-16
In.Fr.Col.	2014	**W = 0.9682, p-value = 3.139e-11**	W = 0.9655, p-value = 8.2e-12	W = 0.9713, p-value = 1.625e-10
	2015	**W = 0.8983, p-value < 2.2e-16**	W = 0.8729, p-value < 2.2e-16	W = 0.8923, p-value < 2.2e-16

Appendix 3.1 Summary of the amplification of 111 SSR primer pairs tested in the 'Redgauntlet' × 'Hapil' progeny in this study. Amplification results for parental genotypes and mapping population, range of the amplification products, linkage groups (LG) and marker positions (cM) of the primers selected from the previously reported genetic map, targeted regions in this study, forward and reverse primer sequences are listed.

Marker name	Polymorphism in parents	Polymorphism in Rg × H progeny	Range	Isobe et al. (2013)		Targeted region (cM)	Forward primer sequence	Reverse primer sequence
				LG	Position (cM)			
FVES0983	N.S.[2]		150-180	1A	47.3		gctctactctccgcctctca	ggaaaatccggaagcgtaat
FAES0271	< 500 bp[3]		-	1A	50.5	41.4-61.9	accttgtgacctggcgttac	ttctttgacgcccaacttct
FVES2012	N.S.		310-360	1A	57.7		aggcatccgacctaatgttg	gacacccaaaatgcacaaga
FVES2280	Failed[4]		-	1B	13.2		caatgcttccaatgggactt	gtcaccaacatcagcccttt
FVES0982	PolyM.[5]	PolyM.	480-550	1B	18.3	9.0-20.7	tcttcaaagccacaaccctc	gatctcattgcatgcttaggg
FVES1738	PolyM.		110-200	1B	19.4		ggcttttcggattgatttga	gaaaaagccattgtgcccta
FVES0670	N.S.		220-440	1C	0.8	0.0-13.1	cgtgcctgtttgatttctga	caagcatcaagggaaccact
FAES0378	PolyM.	N.S.	129-146	1D	16.2		ggtgggaatttttctgggat	gccaacctaacctccctttc
FVES3126	W. or C.[6]		-	1D	17.2		aaccccaaagtgatcaccaa	ctccgaacacctcggagtag
FAES0053	PolyM.	F. or W.[7]	113-116	2A	45.6		ccatcatcgtctctcgttttt	cgtcgatatcgcacatcaag
FVES0901	PolyM.		100-300	2A	47		gccaccatctcctctgaaac	catagccaatgctgtcctca
FAES0154	PolyM.	PolyM.	100-180	2B	20.4	19.6-30.7	cgccaaaacttggtagatgg	atcaggcaccaattgacctc
FVES3275	PolyM.		200-220	2B	22.5		cgaagcattcatggcttttt	ctcaagatgacgacagcgaa
FVES1687	PolyM.	PolyM.	160-280	2B	29.3		acgaagggtgaagggtctct	cccaaaaacccaaatcctct
FVES1292	PolyM.	N.S.	250-300	2B	81.3	71.4-89.3	gtcccaccatccagttatgg	actccacttggctgagcagt
FVES0623	PolyM.		300-400	2B	95.1		cacaagcctctctcctcacc	aagctcttgctctgcaccat
UFFa11A11[1]	PolyM.	F. or W.	221-272	2C	7.7		acgaggctccaatagagttctg	ctgagcagaagccatagtatcac
FVES0393	PolyM.	PolyM.	260-360	2C	10.3		aagccatctcattcaccgat	gcgacaaaggcaagaatagc
FVES1747	PolyM.		300-480	2C	15.9	2.4-17.3	tcgttcgtcataattagcagaga	cggctttgagctcgtaaatc
FVES0936	PolyM.	PolyM.	214-220	2C	17.9		cacatatataaaccccagtcgg	tgaaggaagggatggagttg
FVES0129	PolyM.		100-300	2C	17.9		acagggacattagggcacag	ccactcgctcaattcgtgta
FVES0347	PolyM.	PolyM.	100-490	2C	56.6	55.8-70.2	aactcctcctcctcctcgtc	gtaaggagcagagccactcg
FVES3470	PolyM.		200-250	2C	68.8		caaatccctcttctcctctcc	cctcagagaccatcaccgat

FAES0247	PolyM.	PolyM.	180-220	2C	84.2		acgccttcgatccttttctt	caaggcagtaaagctcccag
FAES0326	PolyM.		250-320	2C	93.8		gcaatttcagcaatccctgt	tctttttggggcgtacaaat
FVES1917	N.S.		120-130	2D	7.9		ccattccaggtacatcctgc	acaaatcgctcgtatcggtc
FATS0033	PolyM.		240-300	2D	14.5		tacatgcaaacctgctccag	acgcccagacttactggtgt
FVES2137	Failed		-	2D	20.2	3.0-18.0	ttcagcagatgatgcagacc	agtcccaatcgttgacgaag
FATS0034	< 500 bp		-	2D	24.4		ctcaacctacacaccctctgc	aaatttcatgccccacattc
FAES0276	PolyM.	F. or W.	180-400	2D	25.9		cgctagcttttctgctcgat	acactccaccggcttacatc
FVES1726	< 500 bp		-	2D	48		ctgaccgatcagggattcac	ccttcctagctgcaatctgg
FVES1525	W. or C.		-	2D	49		ctccaccttcttcttcttcaca	tatggttgaggctgaggagc
FAES0226	PolyM.		120-180	2D	50.1		actccacttggctgagcagt	cacacagcctgctgcatatt
FVES0673	N.S.		280-380	2D	54.3		aacaaagaggccaggagctt	agaatcctcccatccgattt
FAES0582	PolyM.		250-350	2D	57.5	9.8-38.2	ggcaatgcctacctttgtgt	cattgcaacaagcattcaca
FAES0277	PolyM.	N.S.	210-290	2D	59.6		gaactccctttctgggtcc	caatgagtgggagaggaagg
FAES0380	PolyM.	Complex[8]	280-500	2D	71.5		cattgcccaccttgtaacct	ctcggcgctcctatattcaa
FVES0256	PolyM.		200-280	2D	81.1		gagtctcagacatctcgccc	atatgaggacgcagaatcgg
FAES0151	PolyM.	F. or W.	275-286	3A	65.7		gcctccaaaggttgttacttctt	actttgctcgagccatcaat
FVES1171	PolyM.	PolyM.	192-210	3A	67.5		tgaatacatgcatcgctggt	gtgggaaacaaagtctggga
FVES3374	PolyM.	PolyM.	250-310	3B	0	0.0-14.1	agttcctcccttctcgcttc	gttgatgtagctgtacgccg
FVES0398	PolyM.	N.S.	289-300	3D	12.5		gagatttctcactcgtcccg	aacaaagggtcgatcaccag
FVES3002	PolyM.	PolyM.	310-420	3D	32.6	28.4-44.2	actcggagtaggaaatgcca	ctgttgatggtggtagggct
FAES0241	PolyM.		250-350	3D	36.8		gactaaggggtgggagcttt	taagttggccaggtcgagat
FVES3364	PolyM.	PolyM.	120-150	3D	41.2		gaccaccgccactctctaaa	ggtgttgaggaaggcgtaga
FVES2235	PolyM.		310-450	4A	72.9	61.9-78.6	gatcttgatgcccacttcgt	ttcatcgaccaacgtttaca
FVES2289	PolyM.		230-290	4A	75.1		acaacaatggaaccctgagc	gcacaaaattacgagccaaa
FVES2722	PolyM.		200-220	4B	46.8	45.1-57.9	cgaggttgaggagttcttcg	cgcattcaaatcaaagtcca
FAES0108	PolyM.		280-380	4B	53.4		cctgcagagtgcaagagaga	gtgcacacatgaatccacaa
FVES2278	PolyM.		120-180	4B	76.8	72.5-85.5	caggggaatggagaaagtga	attcctgggcttttcgtctt
FVES3219	PolyM.		300-360	4B	79.9		atttgcgattccagcagatt	cccccacaatgcttcagtag
FVES3039	PolyM.	N.S.	120-160	4C	7.6	0.0-15.7	gagtgtgacggatggtgttg	ccacgtgtacggctcctaat
FAES0296	PolyM.		180-210	4C	11.1		tctgtcattgctcaacctcg	ggctcccaaactgtggttag
FAES0063	PolyM.	PolyM.	90-300	4D	5.4		aacccagatgaaattgctgc	cccagtgacaaacaagcaga
FAES0001	PolyM.	PolyM.	200-350	4D	8.9	2.4-14.1	gggctcaaaagatgtggaaa	tttatttgggaaggcatcgt
FVES1409	PolyM.	PolyM.	210-300	4D	11.3		tcggtttctcgctttctttc	gtgatccgatacgttggctt

FVES0688	W. or C.		-	5A	27.6	19.3-33.2	aatcaaacctaacccgtccc	gttggagtccggtctgttgt
FVES1122	PolyM.		220-260	5A	31.8		tcacttccattcctaacgcc	ttccctcactgttccgattc
FVES0545	PolyM.	PolyM.	220-270	5A	34.5		gcaagtccatatccacttctttg	tcaaattccgtttcgtcctc
FVES0847	N.S.		220-300	5A	38.4		aaaccggtcatcagttacgg	gaagctctcgaagctggtgt
FVES3720	PolyM.		100-300	5A	40.9	33.2-52.1	atcccatttctattggggga	attgcgccatacaaacacaa
FAES0018	PolyM.	PolyM.	90-140	5A	47.7		tgtaagtgcctctaaagatggtagg	tgtcgtgtgttttagttcacaatg
FVES1537	PolyM.		280-400	5A	50.5		ggagacatccaacgatcagg	ccatggggttgagcttagag
FAES0394	N.S.		210	5B	23		ccaaatgcagaaacccagat	actccattttggctcccttt
FVES0536	PolyM.		170-200	5B	25.7	20.4-30.8	gatcatgtttttggatagagaagaa	tgggagacaactgaggctct
FAES0318	N.S.		140-210	5B	33.5		aggctctaggcgacaacaaa	caaatgactggattgatatattgttag
FVES3224	PolyM.	PolyM.	200-400	5B	39.6		taacttccctcccgattcct	cctcttgaagctccgatcac
FVES0833	PolyM.	PolyM.	180-280	5C	0	0.0-13.4	agccaagaagccagaagaca	cctgctctcgtcatccattt
FVES3434	PolyM.		200-260	5C	38.3	34.7-49.1	cattattgcacacaccagcc	cgttgattggtcgtagcctt
FVES0639	N.S.		210-290	5C	41.8		gttcaagcaaatctccgagg	tctcggcgtaaatatcgtcc
FVES3096	PolyM.	F. or W.	198-254	5D	20.4		ctctctccgatcgtgtctcc	gtccacgacccgttttcata
FVES0618	PolyM.		250-400	5D	28.9	26.4-36.6	cttcctcccaaaaaccttcc	tagtcaatgtgcttcaccgc
FAES0382	PolyM.	PolyM.	240-380	5D	34.4		ataccagaacccaccaccaa	gtggttcccagagctgaaag
FVES2960	PolyM.	F. or W.	250-360	6A	34.9	27.0-38.3	gtcttgcgggaagcagttag	gatgtcgtcggagagaggag
FVES0013	PolyM.	PolyM.	200-300	6A	35.3		tctcctcctctcttcccgat	gaaatgctctcttcggttcg
FVES3346	PolyM.		310-360	6C	0		ttgcttttagatggctgctg	cttcggtttagctgctttgg
FVES3450	W. or C.		-	6C	14.1	10.1-24.4	tgagtgcagagatcccagtg	cctcctcatcagccaaatgt
FVES1143	PolyM.		200-240	6C	16.5		gatgtgcagttcatgcatcc	aaggcctggaacagagatga
FVES0775	N.S.		410-430	6C	21.1		gccaacctcttgttcttgga	aattgggtgcttggagtttg
FVES0669	< 500 bp		-	6C	50.6	46.6-62.9	ctgcctggtttggtaatgct	gagaccaagccctctgtttg
FVES2192	N.S.		250-320	6C	54.7		gtgatcagcatggggactct	aggaggaggggtgaagatgt
FAES0078	PolyM.		250-400	6C	58.6		ctaagctcgtcatcaagccc	agtcccattttccagtggtg
FATS0047	PolyM.		180-270	6C	60.8		ggaatgtcgatctgggtctg	tgatcagcttatacgacggc
FAES0243	< 500 bp		-	6C	65.5	64.9-82.2	gaagcagaaactgaggacgg	tcaggtttaagatcccggtg
FATS0014	N.S.		310-410	6C	75		cctgaaccagcttctgggta	ctcatgctgaagaagctccc
FVES1154	PolyM.		280-320	6D	13		ctcagtgacctccacagcaa	agggtcccgaatgagtttct
FATS0090	PolyM.	PolyM.	170-220	6D	27.9	20.9-40.8	agagccggtttagctgagtg	cgtcgtcgttttctctcctc
FAES0023	N.S.		100-150	6D	32.2		actgccctccatgtctcaac	gtgcagagaatgagcaacga
FAES0381	PolyM.		280-350	6D	37.8		aatacaaactcggcgcaatc	tggaatccaccatcaaggtt
FVES1271	W. or C.		-	6D	38.8	73.0-85.3	aactggccaccacactttc	ggcgtcacgggtatgttact

FVES0463	PolyM.		180-210	6D	76.4		gctacgtgttcgggtttgat	tcatgttcccatattgccct
FVES1580	PolyM.	PolyM.	140-180	7A	28.5	25.3-42.6	taaacgacatcggcgacata	agaatcagatggtgttgccc
FVES3344	W. or C.		-	7A	40.6		caacagtcgtccatgtcctg	acaattctcgtctccgttgg
FVES0814	PolyM.	Complex	170-210	7B	12.8	7.0-30.2	attagggtttcgttccccac	atggcgatgaagaaaacgac
FAES0410	Failed		-	7B	16.4		taacagctgtcttgcttggc	atcttgacgaatgagggtgc
FVES0104	PolyM.	Complex	292-296	7B	40.1		ctgccttctgggtcgttaaa	aagacgtcgacgagtcccta
FVES3503	PolyM.	N.S.	280-380	7B	57.8	55.3-67.1	gccaacgtactcctggtgat	aggatcaacttcatcacgcc
FATS0076	PolyM.	PolyM.	200-240	7B	63.3		caagggaagtggaagtggaa	gctgaggagaaacctggaga
FVES1834	PolyM.	PolyM.	200-250	7B	72.4		gttgaagcagctcccaaaag	gaattgacgaggcggtaaaa
FAES0042	PolyM.		200-320	7C	2.3	0.0-13.6	ccacatttcacacagaccca	tataagctcatgacccgcct
FVES1672	PolyM.	PolyM.	220-300	7C	6.7		acaccctgtcccttcacaag	gaaagatgacttcggcttcg
FVES1722	PolyM.		220-280	7C	9.7		ggcatatgtagatgggtggg	gcaacagcagaagaacctcc
FVES1237	PolyM.	PolyM.	280-300	7D	9.7	6.0-17.7	gtgtcactcacacacacacca	caccttctccattccctgag
FVES1908	Failed		-	7D	10.4		acaacccaaacagcaccttc	ttgtgagctgagaccctgtg
FATS0029	W. or C.		-	7D	13.1		gcctagttcgtcctgggttt	ccaagttgaggatgctggat
FAES0401	< 500 bp		-	7D	14.1		accgtctctggtttcccttt	atatggttcctgcagatggc
FVES0640	N.S.		180-240	7D	16.1		cagcccctcatcttcttctg	gcgtggtagtcatctgggtt
FVES1453	PolyM.	PolyM.	180-240	7D	30.8		ggctatgatcgaaaaatatgacc	gcggttaagatgagaaaatgtg
FVES0144	PolyM.		250-280	7D	34.4	29.6-40.0	catgaggaaggagctcaagg	tcgaacggcatacattttca
FAES0363	PolyM.		220-300	7D	36.2		cacgaggttccagatcatca	cggcatcaaaccattctcat
FVES1414	PolyM.	PolyM.	120-200	7D	38		atctcgaggcttccaagaca	aatcgggatattcgcattaca

[1]SSR marker was developed by Spigler et al. (2008)

[2]Markers amplified non-segregating products

[3]Markers amplified larger than 500 bp size PCR products

[4]Markers failed to amplify any PCR product

[5]Markers amplified polymorphic products

[6]Markers amplified weak or too complex to score products

[7]Markers failed to amplify any PCR product or were too weak to score

[8]Markers amplified PCR products too complex to score

Appendix 4.1 A summary of QTLs detected for 30 plant characteristic and fruit quality traits in the 'Redgauntlet' × 'Hapil' mapping progeny over the three consecutive years (2013, 2014 and 2015). The traits, year, linkage group (LG) and position in cM of the identified QTL, closest SNP marker linked to the QTL, Kruskal-Wallis test value (K*), degree of freedom (Df), significance level (Signif.) and allelic contribution ('Redgauntlet' – Rg, 'Hapil' – H and both parental genotypes – B) of the QTL are presented.

Trait	Year	LG	Position[a]	SNP marker	K*	Df	Signif.[b]	Parent
Flower number	2013	3A	108.381	AX-89848055:nmh	17.788	1	*******	Rg
	2013	3B	49.421	AX-89885512:nmh	11.081	1	*****	H
	2014	1A	31.446	AX-89875617:ph3	8.843	1	****	Rg
	2014	1D	0	AX-89874863:nmh	8.56	1	****	Rg
	2014	3B	69.147	AX-89786031:nmh	6.928	1	***	H
	2014	4C	69.819	AX-89790803:nmh	7.597	1	***	H
	2015	1C	20.207	AX-89875911:ph3	8.27	1	****	Rg
	2015	2C	42.667	AX-89851357:ph3	12.091	2	****	B
	2015	3A	6.527	AX-89786825:ph3	7.63	1	***	Rg
	2015	3D	53.441	AX-89904724:ph3	14.974	2	*****	B
	2015	4C	31.041	AX-89868202:ph3	11.161	2	****	B
Flower diameter	2013	2A	95.175	AX-89877249:nmh	14.735	1	******	Rg
	2013	4D	48.831	AX-89789604:ph3	12.621	2	****	B
	2013	7A	108.521	AX-89808967:ph3	10.779	1	****	Rg
	2014	2C	53.174	AX-89781842:nmh	10.373	1	****	Rg
	2014	4B	45.38	AX-89905235:ph3	13.601	2	****	B
	2014	6C	32.238	AX-89849575:ph3	9.478	1	****	Rg
	2014	7A	108.521	AX-89808967:ph3	11.181	1	*****	Rg
	2015	2C	53.174	AX-89781842:nmh	12.603	1	******	Rg
	2015	6C	91.654	AX-89897027:ph3	10.613	1	****	H
	2015	7A	108.521	AX-89808967:ph3	19.604	1	*******	Rg
Petal number	2013	2A	135.241	AX-89803566:ph3	9.247	1	****	Rg
	2013	2B	28.507	AX-89781929:nmh	9.158	1	****	Rg
	2013	6A	36.416	AX-89842288:nmh	8.994	1	****	H
		6A	37.024	AX-89899534:nmh	8.994	1	****	H
		6A	37.024	AX-89899527:nmh	8.994	1	****	H
	2014	2A	149.989	AX-89806716:nmh	5.757	1	**	H
		2A	150.807	AX-89815335:ph3	5.757	1	**	H
	2014	6A	45.987	AX-89842821:ph3	10.925	1	*****	H
	2015	2A	131.17	AX-89837698:nmh	8.359	1	****	Rg
	2015	2C	34.93	AX-89781451:nmh	12.056	1	*****	Rg

	2015	6A	45.987	AX-89842821:ph3	10.898	1	*****	H
Pedicel length	2013	3A	69.096	AX-89806963:ph2	12.818	1	******	H
		3A	69.455	AX-89881402:ph2	12.818	1	******	
	2013	5C	21.763	AX-89861709:ph3	15.35	2	******	B
	2013	6A	22.091	AX-89896121:ph3	15.796	2	******	B
	2014	3D	34.519	AX-89856176:nmh	8.609	1	****	H
	2014	6A	27.782	AX-89899151:ph3	10.809	2	****	B
	2015	2C	74.169	AX-89782695:ph3	11.12	1	*****	H
		2C	74.169	AX-89904518:ph3	11.12	1	*****	
		2C	74.889	AX-89803635:nmh	11.12	1	*****	
	2015	3A	113.128	AX-89786356:ph2	8.596	1	****	Rg
		3A	113.937	AX-89904906:nmh	8.596	1	****	
		3A	114.747	AX-89884020:ph3	8.596	1	****	
		3A	114.747	AX-89786850:nmh	8.596	1	****	
	2015	3C	45.607	AX-89904738:ph3	17.443	2	******	B
		3C	45.607	AX-89879521:ph3	17.443	2	******	
	2015	5A	60.163	AX-89836822:ph3	12.181	1	******	Rg
	2015	6A	31.174	AX-89799692:ph3	14.569	2	*****	B
Leaflet number	2013	5A	88.785	AX-89891066:nmh	14.57	1	******	H
	2013	3A	104.598	AX-89807028:ph3	14.271	2	*****	B
	2013	5D	15.944	AX-89835722:ph3	14.914	1	******	Rg
	2014	3A	86.278	AX-89905224:ph3	13.563	2	****	B
	2015	3A	87.004	AX-89824743:ph3	16.643	2	******	B
	2015	7C	75.686	AX-89903174:ph3	16.115	2	******	B
Vigour	2013	3B	65.528	AX-89827763:nmh	15.5	1	*******	H
		3B	65.528	AX-89804249:ph3	15.5	1	*******	
	2013	5D	0.719	AX-89892157:nmh	9.327	1	****	Rg
	2013	7D	0	AX-89860131:nmh	10.579	1	****	Rg
		7D	3.608	AX-89862403:nmh	10.579	1	****	
	2014	1C	42.136	AX-89798365:nmh	9.26	1	****	Rg
	2014	1D	56.288	AX-89873516:nmh	11.636	1	*****	Rg
		1D	56.288	AX-89778745:nmh	11.636	1	*****	
	2014	2B	1.449	AX-89878129:ph3	15.429	1	*******	H
	2014	5C	33.803	AX-89891054:ph3	9.469	1	****	Rg
	2015	2A	66.72	AX-89876601:ph3	12.956	2	****	B
		2A	67.804	AX-89904609:ph3	12.956	2	****	
Height	2013	3D	2.91	AX-89848137:ph3	10.948	2	****	B
	2013	7C	14.808	AX-89844626:nmh	10.182	1	****	Rg
Width	2013	2A	79.081	AX-89819488:nmh	8.471	1	****	Rg
	2013	2C	51.943	AX-89867642:nmh	8.211	1	****	Rg
	2013	3B	66.248	AX-89827616:nmh	8.846	1	****	H
		3B	67.697	AX-89827465:nmh	8.846	1	****	
		3B	67.697	AX-89827299:ph3	8.846	1	****	
Runner number	2013	3B	7.973	AX-89907882:nmh	14.572	1	******	H
	2013	6A	75.955	AX-89796432:ph3	9.898	1	****	H
	2013	6D	84.807	AX-89843070:nmh	10.626	1	****	Rg

	2013	7C	18.605	AX-89801200:ph3	8.579	1	****	Rg
	2014	2B	32.166	AX-89781773:nmh	10.889	1	*****	Rg
	2014	7D	57.467	AX-89802646:nmh	9.196	1	****	Rg
	2015	6C	94.617	AX-89797234:nmh	12.185	1	******	H
Runner length	2013	3A	90.63	AX-89847974:ph3	12.042	2	****	B
		3A	90.63	AX-89826164:ph3	12.042	2	****	
	2013	3C	63.445	AX-89786092:nmh	7.157	1	***	H
	2013	3D	12.299	AX-89787854:nmh	12.088	1	*****	H
	2013	5A	82.993	AX-89833178:nmh	10.034	1	****	Rg
	2013	6C	106.591	AX-89909438:nmh	13.071	1	******	H
	2013	7C	64.026	AX-89903841:nmh	10.619	1	****	H
	2014	1C	41.315	AX-89803109:nmh	16.928	1	*******	H
		1C	42.509	AX-89816618:nmh	16.928	1	*******	H
	2014	3C	77.176	AX-89787247:nmh	12.344	1	******	Rg
	2014	3C	77.176	AX-89787256:nmh	12.344	1	******	
	2014	5A	88.785	AX-89849037:ph3	11.404	2	****	B
	2014	6C	15.709	AX-89842437:nmh	11.915	1	*****	H
		6C	17.755	AX-89899482:nmh	11.915	1	*****	
	2015	1D	7.523	AX-89779102:nmh	9.06	1	****	H
		1D	7.523	AX-89779044:nmh	9.06	1	****	
	2015	2A	21.65	AX-89882280:nmh	13.242	1	******	H
Truss number	2013	3A	83.392	AX-89784929:nmh	10.965	1	*****	Rg
	2013	1A	65.728	AX-89854317:nmh	9.507	1	****	Rg
	2013	7C	64.026	AX-89903841:nmh	8.753	1	****	H
	2014	1A	56.318	AX-89873650:nmh	8.976	1	****	Rg
	2014	3B	71.312	AX-89826831:nmh	15.839	1	*******	H
	2014	6D	118.309	AX-89894258:ph3	10.504	2	***	B
	2014	7C	18.605	AX-89801200:ph3	10.236	1	****	Rg
	2015	1A	65.728	AX-89854317:nmh	8.688	1	****	Rg
	2015	2C	53.174	AX-89781842:nmh	13.494	1	******	Rg
	2015	5D	12.676	AX-89864056:nmh	11.889	1	*****	Rg
		5D	12.676	AX-89805071:nmh	11.889	1	*****	
		5D	12.676	AX-89849375:ph3	11.889	1	*****	
	2015	7C	53.69	AX-89843204:nmh	8.52	1	****	H
		7C	53.69	AX-89900504:ph3	8.52	1	****	
Truss length	2013	3A	112.318	AX-89848121:nmh	15.879	1	*******	Rg
	2013	3C	81.476	AX-89784856:nmh	9.258	1	****	Rg
	2013	6A	50.659	AX-89894555:nmh	9.407	1	****	Rg
	2014	3A	91.349	AX-89883261:ph3	9.679	2	***	B
	2014	3D	23.584	AX-89825911:nmh	9.773	1	****	H
	2014	4C	19.008	AX-89848685:ph3	14.983	2	*****	B
	2014	5C	8.936	AX-89793784:ph3	18.111	2	******	B
	2015	2B	28.867	AX-89877646:nmh	12.048	1	*****	Rg
	2015	3C	28.058	AX-89824557:nmh	8.126	1	****	H
	2015	5C	17.078	AX-89905747:ph3	11.426	2	****	B
Truss width	2013	2A	143.419	AX-89851535:nmh	9.484	1	****	H

	2013	5A	54.373	AX-89795084:ph3	14.459	2	*****	B
	2013	6B	95.334	AX-89859678:nmh	9.056	1	****	Rg
		6B	95.334	AX-89842697:ph3	9.056	1	****	
		6B	95.694	AX-89850340:ph3	9.056	1	****	
		6B	95.694	AX-89842702:nmh	9.056	1	****	
	2014	1B	44.682	AX-89818863:nmh	11.628	1	*****	Rg
		1B	45.507	AX-89780608:nmh	11.628	1	*****	
	2014	1D	26.163	AX-89818767:nmh	9.169	1	****	Rg
	2014	4D	51.981	AX-89829941:nmh	7.8	1	***	Rg
		4D	51.981	AX-89829930:nmh	7.8	1	***	
	2014	5A	48.435	AX-89889496:ph3	9.392	2	***	B
	2014	7C	76.045	AX-89845988:nmh	8.596	1	****	H
	2015	1B	78.755	AX-89779600:nmh	8.56	1	****	Rg
		1B	79.474	AX-89803055:nmh	8.56	1	****	
		1B	80.193	AX-89875223:nmh	8.56	1	****	
	2015	2A	140.876	AX-89821536:nmh	10.001	1	****	Rg
	2015	2B	28.148	AX-89847328:nmh	10.077	1	****	Rg
	2015	5A	52.517	AX-89894153:ph3	8.352	1	****	Rg
	2015	6B	33.096	AX-89906253:ph3	10.647	1	****	H
		6B	34.979	AX-89839977:nmh	10.647	1	****	
Yield	2013	6D	82.244	AX-89795448:ph3	11.326	2	****	B
	2014	1D	65.268	AX-89847808:ph3	13.65	2	****	B
	2014	5A	5.437	AX-89891977:ph3	14.089	1	******	H
	2014	7A	112.305	AX-89802589:nmh	9.864	1	****	H
	2015	3A	100.838	AX-89872427:ph3	11.601	1	*****	H
		3A	104.958	AX-89785285:nmh	11.601	1	*****	
	2015	5A	21.868	AX-89835899:ph3	10.237	1	****	H
		5A	21.868	AX-89794308:ph3	10.237	1	****	
	2015	6C	28.238	AX-89868977:nmh	14.744	1	******	Rg
		6C	26.641	AX-89800083:ph3	14.744	1	******	
Unmarketable fruit	2013	6A	106.03	AX-89849996:ph3	12.838	2	****	B
	2013	6C	94.617	AX-89797234:nmh	9.336	1	****	H
	2013	7B	82.613	AX-89845179:ph3	10.374	1	****	H
	2014	1C	58.096	AX-89903507:nmh	7.289	1	***	H
	2014	1D	65.268	AX-89847808:ph3	17.417	2	******	B
	2014	5A	5.437	AX-89891977:ph3	9.178	1	****	H
	2014	6C	112.531	AX-89853513:nmh	11.975	1	*****	H
	2015	5A	29.085	AX-89794893:ph3	8.214	1	****	H
		5A	29.085	AX-89836642:nmh	8.214	1	****	
	2015	6C	28.238	AX-89900239:ph3	10.943	1	*****	Rg
	2015	7A	19.819	AX-89823012:nmh	8.694	1	****	H
		7A	24.018	AX-89850614:ph3	8.694	1	****	
Marketable fruit	2013	3B	69.147	AX-89786031:nmh	8.644	1	****	H
		3B	70.585	AX-89881973:ph3	8.644	1	****	
	2013	4B	5.163	AX-89790696:nmh	7.643	1	***	Rg
	2014	2A	85.247	AX-89876795:nmh	8.64	1	****	H

		2A	85.247	AX-89781233:nmh	8.64	1	****	
		2A	85.855	AX-89847253:nmh	8.64	1	****	
	2014	5A	11.858	AX-89794041:nmh	9.515	1	****	H
	2014	7A	102.433	AX-89904670:nmh	15.41	1	*******	H
	2015	3A	110.031	AX-89811234:ph3	13.82	2	*****	H
	2015	5D	24.928	AX-89858225:nmh	10.517	1	****	Rg
	2015	6C	26.641	AX-89800083:ph3	9.816	1	****	Rg
		6C	28.238	AX-89868977:nmh	9.816	1	****	
Firmness	2013	1A	43.527	AX-89780703:nmh	10.464	1	****	Rg
		1A	43.527	AX-89904305:ph3	10.464	1	****	
		1A	43.887	AX-89847157:ph3	10.464	1	****	
	2013	1D	64.548	AX-89795942:nmh	10.344	1	****	H
	2013	2D	2.942	AX-89820344:ph3	9.967	1	****	H
		2D	2.942	AX-89877470:ph3	9.967	1	****	
		2D	3.661	AX-89877426:ph3	9.967	1	****	
		2D	3.661	AX-89820302:ph3	9.967	1	****	
	2013	3A	36.984	AX-89856683:nmh	10.215	1	****	H
	2013	4A	11.418	AX-89889023:nmh	10.554	1	****	H
	2013	4C	31.532	AX-89829872:nmh	9.207	1	****	H
	2013	7A	20.126	AX-89801084:nmh	12.313	1	******	Rg
	2014	1A	42.448	AX-89780660:nmh	8.821	1	****	Rg
	2014	2C	78.788	AX-89878587:nmh	17.376	1	*******	Rg
	2014	3A	13.398	AX-89785507:ph3	7.782	1	***	Rg
		3A	15.207	AX-89824842:ph3	7.782	1	***	
		3A	17.066	AX-89824795:ph3	7.782	1	***	
	2014	4B	38.17	AX-89887459:nmh	11.345	1	*****	Rg
	2015	2C	78.788	AX-89878587:nmh	14.987	1	******	Rg
	2015	4B	38.17	AX-89887459:nmh	14.394	1	******	Rg
	2015	7A	53.426	AX-89800994:ph3	10.807	1	****	Rg
ºBrix	2013	3A	108.947	AX-89848062:ph3	14.092	2	*****	B
	2013	3C	10.957	AX-89785181:nmh	8.744	1	****	Rg
	2013	5C	44.274	AX-89890436:nmh	9.36	1	****	Rg
	2013	6C	91.294	AX-89871556:nmh	8.979	1	****	H
		6C	91.294	AX-89896975:nmh	8.979	1	****	
	2014	1A	52.575	AX-89808468:nmh	13.471	1	******	H
		1A	52.575	AX-89808467:nmh	13.471	1	******	
	2014	3A	18.826	AX-89787123:ph3	13.786	2	****	B
	2014	4D	83.655	AX-89848770:nmh	14.756	1	******	H
	2014	5C	7.494	AX-89913490:nmh	12.313	1	******	H
	2015	1A	57.397	AX-89873632:ph3	7.614	1	***	H
		1A	58.117	AX-89841264:nmh	7.614	1	***	
	2015	3A	95.829	AX-89825301:nmh	7.681	1	***	H
		3A	95.829	AX-89807064:ph3	7.681	1	***	
	2015	3C	72.84	AX-89787921:ph3	8.955	1	****	Rg
		3C	72.84	AX-89828280:nmh	8.955	1	****	
		3C	74.288	AX-89889894:nmh	8.955	1	****	

	2015	3D	54.723	AX-89784702:ph3	15.386	2	******	B
	2015	6A	1.439	AX-89806405:nmh	11.391	1	*****	Rg
pH	2013	3C	46.155	AX-89824357:nmh	11.691	1	*****	Rg
	2013	6B	52.054	AX-89849864:ph3	14.545	1	******	H
	2014	1C	79.814	AX-89904333:ph3	11.947	1	*****	Rg
		1C	79.814	AX-89807021:nmh	11.947	1	*****	
	2014	3B	1.09	AX-89826334:ph3	13.945	2	*****	B
		3B	1.45	AX-89884094:ph3	13.945	2	*****	
		3B	1.45	AX-89826324:ph3	13.945	2	*****	
	2014	4D	87.493	AX-89889308:nmh	8.309	1	****	H
	2014	5A	5.476	AX-89913725:nmh	11.812	1	*****	Rg
	2014	5C	81.091	AX-89828937:nmh	10.355	1	****	H
	2015	5C	11.119	AX-89807443:nmh	14.951	1	******	Rg
		5C	11.119	AX-89832883:nmh	14.951	1	******	
Achene position	2013	1A	2.169	AX-89874808:ph3	13.115	1	******	H
	2013	1C	43.703	AX-89832793:nmh	9.754	1	****	H
	2013	4A	9.4	AX-89793608:nmh	9.678	1	****	H
	2013	4D	42.474	AX-89887516:nmh	11.455	1	*****	Rg
		4D	42.474	AX-89830393:nmh	11.455	1	*****	
	2013	5A	40.346	AX-89809452:nmh	19.601	1	*******	H
	2014	1C	36.83	AX-89841381:nmh	14.49	1	******	H
	2014	3A	32.954	AX-89885291:nmh	12.946	1	******	H
	2014	6D	73.001	AX-89895571:nmh	10.703	1	****	Rg
	2014	7A	11.881	AX-89901986:nmh	10.388	1	****	Rg
		7A	11.881	AX-89806056:nmh	10.388	1	****	
		7A	12.51	AX-89901916:nmh	10.388	1	****	
	2015	1C	60.966	AX-89778602:nmh	10.362	1	****	H
	2015	3A	46.643	AX-89890244:ph3	13.118	2	****	B
		3A	46.643	AX-89905494:ph3	13.118	2	****	
		5A	44.352	AX-89794765:nmh	11.05	1	*****	Rg
Seediness	2013	3B	37.847	AX-89824344:ph3	13.073	1	******	H
		3B	38.566	AX-89803926:nmh	13.073	1	******	
	2013	6B	98.077	AX-89816268:ph3	10.557	1	****	Rg
	2014	1A	30.676	AX-89875590:nmh	8.625	1	****	H
	2014	2A	73.984	AX-89863100:nmh	8.78	1	****	Rg
	2014	6A	107.201	AX-89915024:nmh	8.752	1	****	H
		6A	107.201	AX-89797345:ph3	8.752	1	****	
	2015	3A	44.812	AX-89856266:nmh	13.687	1	******	H
	2015	6A	67.281	AX-89895231:ph3	10.662	1	****	H
		6A	73.853	AX-89795885:ph3	10.662	1	****	
		6A	73.853	AX-89895190:nmh	10.662	1	****	
Skin brightness	2013	2A	108.364	AX-89820361:nmh	9.543	1	****	H
	2013	4B	38.529	AX-89789839:nmh	11.617	1	*****	Rg
	2013	7D	52.058	AX-89815793:ph3	11.864	2	****	B
	2015	6A	33.358	AX-89842379:nmh	7.505	1	***	H
		6A	33.358	AX-89899618:nmh	7.505	1	***	

Shape	2014	2A	21.29	AX-89785411:ph3	10.578	2	***	B
	2014	3A	36.082	AX-89885118:nmh	9.957	1	****	H
		3A	36.082	AX-89828055:nmh	9.957	1	****	
	2014	6C	93.629	AX-89797261:ph3	14.857	1	******	H
		6C	95.604	AX-89897268:nmh	14.857	1	******	
		6C	96.592	AX-89897374:ph3	14.857	1	******	
		6C	96.592	AX-89859059:nmh	14.857	1	******	
		6C	99.599	AX-89840406:ph3	14.857	1	******	
	2015	2A	28.919	AX-89867788:ph3	12.498	2	****	B
		2A	29.279	AX-89863038:ph3	12.498	2	****	
		2A	29.638	AX-89783416:ph3	12.498	2	****	
		2A	29.638	AX-89783279:ph3	12.498	2	****	
		2A	29.998	AX-89806801:ph3	12.498	2	****	
	2015	3A	36.082	AX-89885118:nmh	14.13	1	******	H
		3A	36.082	AX-89828055:nmh	14.13	1	******	
	2015	6C	112.866	AX-89859302:nmh	7.791	1	***	H
Cap size	2014	2D	45.275	AX-89788386:nmh	12.289	1	******	Rg
	2014	3D	23.584	AX-89825911:nmh	9.222	1	****	H
	2014	5C	20.554	AX-89834444:nmh	11.41	1	*****	H
	2015	5C	20.554	AX-89834444:nmh	15.513	1	*******	H
	2015	5D	54.527	AX-89848878:ph3	15.257	2	******	B
		5D	54.886	AX-89872626:ph3	15.257	2	******	
	2015	6B	103.034	AX-89803178:nmh	9.39	1	****	H
Outline	2014	2A	81.566	AX-89876688:nmh	13.746	1	******	H
		2A	81.566	AX-89876641:nmh	13.746	1	******	
		2A	82.174	AX-89820013:nmh	13.746	1	******	
		2A	82.174	AX-89819915:nmh	13.746	1	******	
	2014	6C	103.35	AX-89787036:ph3	11.598	2	****	B
		6C	104.07	AX-89906326:ph3	11.598	2	****	
		6C	104.429	AX-89841200:ph3	11.598	2	****	
		6C	104.429	AX-89906386:ph3	11.598	2	****	
	2015	4D	48.253	AX-89830092:nmh	10.739	1	****	Rg
	2015	6C	107.676	AX-89840796:ph3	17.652	2	******	B
Redness	2014	1C	40.121	AX-89816497:nmh	8.54	1	****	H
	2014	2C	53.785	AX-89781852:ph3	14.882	2	*****	B
		2C	53.785	AX-89820402:ph3	14.882	2	*****	
	2014	7C	73.866	AX-89906896:ph3	14.465	2	*****	B
	2015	2C	54.325	AX-89877754:ph3	7.948	1	****	H
	2015	6A	33.358	AX-89842379:nmh	8.971	1	****	H
		6A	33.358	AX-89899618:nmh	8.971	1	****	
Glossiness	2014	2A	111.37	AX-89820325:nmh	10.096	1	****	H
	2014	6C	4.425	AX-89895998:ph3	14.481	2	*****	B
	2014	6D	79.718	AX-89837487:nmh	7.867	1	***	Rg
		6D	81.161	AX-89894627:nmh	7.867	1	***	
		6D	82.244	AX-89795435:ph3	7.867	1	***	
		6D	82.611	AX-89894545:nmh	7.867	1	***	

	2014	7D	52.776	AX-89906628:ph3	12.937	2	****	B
	2015	2A	112.33	AX-89877296:nmh	14.592	1	******	H
	2015	6B	48.317	AX-89849876:ph3	12.532	1	******	H
		6B	49.252	AX-89839239:nmh	12.532	1	******	
	2015	6C	6.675	AX-89873438:ph3	12.972	2	****	B
Neck line	2014	1C	41.315	AX-89803109:nmh	10.807	1	****	H
		1C	42.509	AX-89816618:nmh	10.807	1	****	
	2014	4A	22.18	AX-89784745:ph3	20.967	1	*******	H
	2014	5D	41.504	AX-89836859:nmh	10.588	1	****	Rg
	2014	6A	30.814	AX-89899893:ph3	17.186	2	******	B
		6A	30.814	AX-89850324:ph3	17.186	2	******	
	2015	1C	75.487	AX-89876317:nmh	16.942	1	*******	H
	2015	2A	84.424	AX-89781170:nmh	11.763	1	*****	Rg
	2015	4A	22.18	AX-89784745:ph3	15.268	1	*******	H
	2015	5D	53.979	AX-89834112:nmh	10.383	1	****	Rg
		5D	55.794	AX-89792347:nmh	10.383	1	****	
		5D	57.063	AX-89834195:ph3	10.383	1	****	
	2015	6A	50.659	AX-89905947:ph3	12.443	2	****	B
Skin strength	2014	1B	78.035	AX-89779591:ph3	9.466	1	****	H
	2014	2A	84.424	AX-89803445:nmh	8.774	1	****	Rg
	2014	3D	1.079	AX-89852152:ph3	16.66	2	******	B
	2014	5A	3.077	AX-89791871:nmh	10.54	1	****	Rg
	2015	1B	41.146	AX-89780843:nmh	11.798	1	*****	H
	2015	4A	68.374	AX-89888508:ph3	15.064	1	******	Rg
		4A	68.374	AX-89788811:nmh	15.064	1	******	
	2015	5D	48.372	AX-89832605:ph3	10.1	2	***	B
		5D	48.372	AX-89848827:ph3	10.1	2	***	
	2015	6C	95.604	AX-89897268:nmh	10.624	1	****	H
		6C	96.592	AX-89897374:ph3	10.624	1	****	
		6C	96.592	AX-89859059:nmh	10.624	1	****	
		6C	99.599	AX-89840406:ph3	10.624	1	****	
Internal fruit colour	2014	2C	62.425	AX-89837599:nmh	9.003	1	****	H

[a]The mapping position in cM of the SNP marker on the SNP-based linkage map

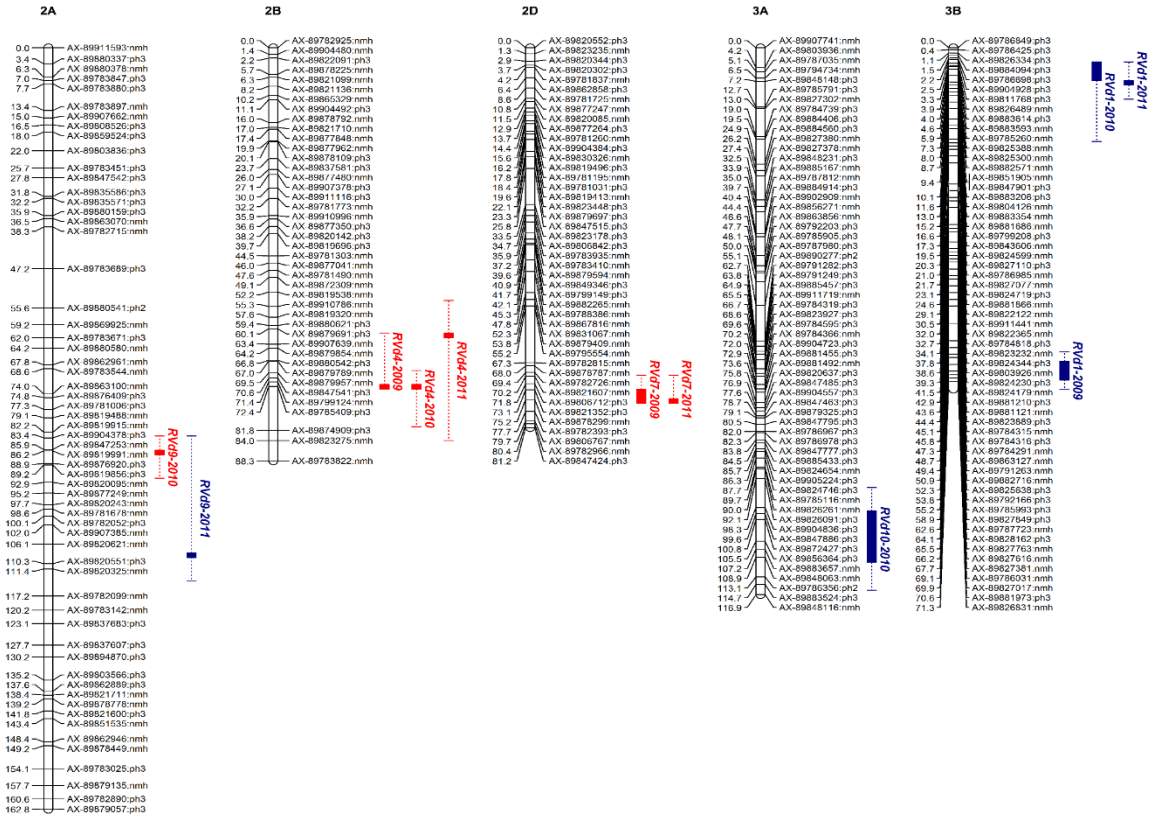
[b]Significance level as observed using Kruskal-Wallis test in MapQTL and are as follow: ** = 0.05, *** = 0.01, **** = 0.005, ***** = 0.001, ****** = 0.0005 and ******* = 0.0001

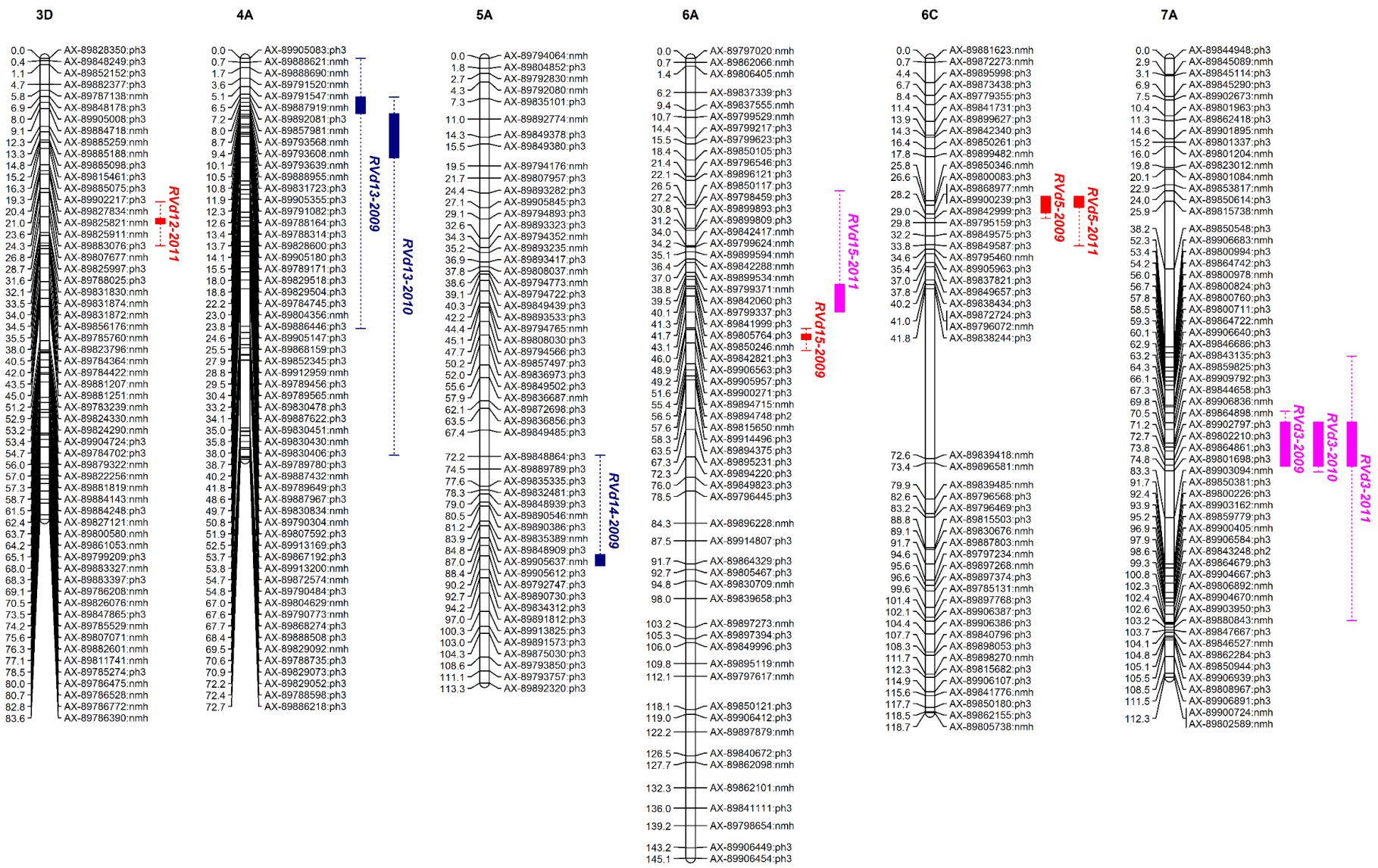
Appendix 5.1 Summary of expansin genes used in this analysis. Gene names, GenBank accessions, mRNA sequences and physical positions of the genes coding proteins aligned to the diploid strawberry (*Fragaria vesca*) genome sequence.

Gene name	GenBank accession	Sequence length (b)	mRNA sequence	Physical location on the *F. vesca* genome		
				LG	Position	Identity (%)
FaEXP1	AF163812	481	ggaaccatgggggtgcttgtggatatggaaacctctacagccagggctacggagtcaacactgctgcgctgagcacggctctgt	LG6	5367020..5368241	97.8
			tcaacaatggcctgagctgcggcgcttgcttcgagatcaagtgcggcgacgacccaaggtggtgcactgccggaaagccctccat	LG5	28194571..28196751	82.1
			tttcgtcaccgccaccaacttctgccctcccaacttcgctcagcccagcgacaatggcggttggtgcaaccctccccggacccacttg	LG7	18154043..18154793	82.2
			gaccttcgccatgcccatgttctcaagatcgccgagtacaaagccggaatcgtccccgtctcttaccgccgggtcccatgcgtaaag	LG3	20228990..20229307	80.0
			aagggtgggatcaggttcacaatcaacggccacaagtacttcaacctggttctgatcaccaacgtggcgggcgcaggggatatcg			
			tgagcgtgagcgtgaaaggcaccaacaccgggtggatgccaatgagccgaaattggggtcaaaactggcag			
FaEXP2	AF159563	1141	tcttctccttctagctagctctcactttctttctcacacaatggcttttacttcatgcttggctattactcttctggtatctgtcctcaacc	LG7	18154043..18154793	100.0
			tctgcatcagaggcacctatgccgactacggcgccggttgggttggtggccatgccactttctatggaggtggtgatgcttctggca	LG3	20228821..20229735	87.9
			caatgggaggtgcatgtggatatggaaacttgtacagccaagggtatggaaccaacactgcagcactaagcacagctctgttcaac	LG6	7648697..7649627	79.7
			gatggcttgagctgcgggtcttgctacgaaatgcgatgtgacaatgaccctagatggtgccttcccggaagcatcatcgtcaccgcc	LG5	7171696..7172655	77.7
			accaacttctgccctcccaactttgctcaggccaatgacaacggtggctggtgcaaccctcccctccagcacttcgatttggccgagc	LG2	19165616..19166515	66.2
			ctgcgttcttgcaaatcgctcagtaccgcgctggtatcgtccccgtctcattcagaagagttgcttgtgtgaaaaagggagggatcag			
			attcacaatcaacgggcactcctacttcaacttggttttgatcacaaacgttgcaggagcaggagatgtgcactcggtttcgatcaaag			
			gctccaagggtggttggcaatccatgtcaaggaactggggacagaactggcagagcaacaactacctcaacggacaagccctgtc			
			ttttcaggtcacaaccagtgacggcaggactgtgaccagcaacaacgttgcccctggtaactggcagtttggtcaaacgttttcaggc			
			ggtcaattctagacttttttcaccgagttactatttgcggtgaaaatgatgatttgtatatgtaatattctgattgggagagaggaggggg			
			atcgagatttgtgagggtagattagggagaggcaacgtgctgaggtggctcattggcacccgctagctaggcctatatatatatataa			
			atatatatatatcatgatataaatatatattatttatagtggaaattgtgagtttatagtttttctagagacaacaattttccatttgatgatgca			
			ttttgcttgaagcaaggaaatgcaagtagtatgtttgaaggtttgtaaaaccagtttggggcagttgctttgttctgtcaccttaatcaaat			
			acttagttgatttcgcagt			
FaEXP3	AF226700	421	atgggggggggcgtgcgggtatggcaatctatacagccaaggctatgggactaacacagcagctctaagcactgctttgttcaacaa	LG6	33647008..33647295	98.3
			tggcttgagctgtggagcatgttatgagctcaggtgtgtgaatgacccacaatggtgcctccctggcaccattgttgtcactgccact	LG5	7171696..7172655	78.9
			aacttctgcccgccgggggggttggtgcgaccctccacagcaacactttgatctctctcagcctgtcttccttaagattgctcagtacag	LG3	30613024..30613750	71.7
			agctggagttgtccctgtatcatacagaagggtgagatgcaggagagcaggaggcataaggttcactataaatggacattcatactt	LG7	18154470..18154781	70.2
			caacctagtgctggtgaccaacgtcggcggtgccggagatgtccaatctgtggccatcaaaggttcaagaacccggtggcaaatg	LG4	23191661..23192236	69.8

			atgtcaagaaattggggtcaaaactggca	LG2	19165616..19166515	75.6
FaEXP4	AF226701	481	atgggggggcttgtggatatggaaacctctacagccaggggtatggaacaaacaccgctgcgctgagcactgctctgttcaaca	LG5	7171696..7172655	82.5
			atgggttggggtgtggttcttgctatgaaattaggtgtgtgaatgacccaaaatggtgcctgcctggctccattgtggtcacagccac	LG3	30613024..30613750	80.0
			taatttctgccctccaaacaatgccctccctaacaatgctgggggatggtgcaaccctccccagcaccactttgacctctctcagcctg	LG6	33647005..33647698	77.8
			tattccagcacattgctcaatacaaagctggagttgtccctgtttcttacagaagggtaccctgcagaagaaggggaggcatcagatt	LG7	18154043..18154793	74.9
			caccatcaatggacactcctacttcaacctggttttgatcacaaacgttggtggtgctggtgatgtgcactctgtttcagtcaaagggtc	LG2	19165616..19166515	67.3
			caaaaccggttggcaagcaatgtccagaaactggggacaaaactggc			
FaEXP5	AF226702	481	atgggggggcatgcgggtatggaaacctatacagccaagggtacggcacaaacacagcagcactaagcacagccttgttcaaca	LG5	7171696..7172655	98.4
			atggcttgagctgcggggcttgctttgagatcaagtgtgtcaatgaccccaaatggtgccttccaggctctattttggtcactgccacc	LG7	18154043..18154793	78.7
			aatttctgccctccaaacaatgcactccctaacaacaacggcggttggtgcaaccctcctcagcaccatttcgatctcgcccagcccg	LG6	33647005..33647698	75.8
			tcttccagcatattgctcaatacagagctggaatcgtccctgtctcctaccgaagagtaccttgccaaaagaagggtggaataaggttc	LG3	30613024..30613750	76.5
			actatcaacggtcactcatacttcaacctggtcctaatcacaaacgttggtggtgctggtgaagttcagtctgtttccatcaaagggtca			
			agaactaattggcaacccatgtcaaggaactggggacaaaactggca			
FaEXP6	AF226703	481	atgggggggcttgtggctacggtaatctttacagccagggatatggagtgagcactgctgctcttagcacggcgctgttcaacaat	LG5	28194571..28196751	98.4
			ggcctcagctgcggtgcctgctttgaaatcaagtgcgcaaacgacccgaactggtgccactctggaagtccttcgattttcatcacag	LG6	5367020..5368241	81.1
			caactaacttttgcccacccaactttgctcagcctagcgacaatggcggatggtgcaaccctcctaggcctcactttgaccttgccatg	LG7	15527230..15528217	77.6
			cccatgtttcttaagatcgctgagtaccgtgccgggattgtccctgtttcctatcgtcgggtgccatgccggaagcgcggagggatca	LG3	20228821..20229735	71.4
			ggttcaccatcaacggcttccgttacttcaacttggttttggtctccaacgtcgcgggtgcaggggatatcgtgagggtgagcgtgaa			
			agggtccaggactggctggatgagcctgagccgtaactggggacagaactggca			
FaEXP7	AF226704	481	atgggggggcgtgtggctatgggaacttgtatagccaagggtatggaactaacactgcagcattgagcacagctttgttcaacgat	LG3	20228821..20229735	97.8
			ggcttgagctgtggctcttgctatgaaatgaggtgtgacaacgatcctaggtggtgccgccctggaagcatcattgtcactgctacca	LG7	18154043..18154793	86.6
			acttctgccctcctaacttcgcgcaggccaatgacaatggcggttggtgcaaccctcccctccagcacttcgatttggccgagcctgc	LG6	7648697..7649005	77.4
			tttcttgcaaatcgcacaataccgtgccggaattgtgcccgttgccttcagaagagtaccatgtgtgaagaaaggaggaataagattc	LG5	7171696..7172655	74.5
			accgtcaatggacactcctacttcaacctggttctgatcactaacgtcgccggtgcaggagacgttcattcagtttcgatcaaggggtc	LG2	19165616..19166515	67.6
			cagaactggatggcaatccatgtcaagaaactggggccaaaactggc			

Appendix 6.1 QTL regions linked to *Verticillium* wilt resistance mapped to linkage groups of the consensus SNP-based linkage map of 'Redgauntlet' × 'Hapil'. Thick bars represent the markers with significance level of $p < 0.005$, whereas the dotted lines represent the significance intervals of $p < 0.005$ and $p < 0.05$. QTLs from 'Redgauntlet' are highlighted in red, from 'Hapil' in blue and from both parental genotypes in pink.

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0.4 AX-89848249:ph3
1.1 AX-89852152:ph3
4.7 AX-89882377:ph3
5.8 AX-89787138:nmh
6.9 AX-89848178:ph3
8.0 AX-89905008:ph3
9.1 AX-89884718:nmh
12.3 AX-89885259:nmh
13.3 AX-89885188:nmh
14.8 AX-89885098:ph3
15.2 AX-89815461:ph3
16.3 AX-89885075:ph3
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24.3 AX-89883076:ph3
26.8 AX-89807677:nmh
28.7 AX-89825997:ph3
31.6 AX-89788025:ph3
32.1 AX-89831830:nmh
33.5 AX-89831874:nmh
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56.0 AX-89879322:nmh
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57.3 AX-89881819:nmh
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76.3 AX-89882601:nmh
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10.5 AX-89888955:nmh
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11.9 AX-89905355:ph3
12.3 AX-89791082:ph3
12.6 AX-89788164:ph3
13.4 AX-89788314:ph3
13.7 AX-89828600:ph3
14.1 AX-89905180:ph3
15.5 AX-89789171:ph3
18.0 AX-89829518:ph3
18.8 AX-89829504:ph3
22.2 AX-89784745:ph3
23.0 AX-89804356:nmh
23.8 AX-89886446:ph3
24.6 AX-89905147:ph3
25.5 AX-89868159:ph3
27.9 AX-89852345:ph3
28.8 AX-89912959:nmh
29.5 AX-89789456:ph3
30.4 AX-89789565:nmh
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34.1 AX-89887622:ph3
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35.8 AX-89830430:nmh
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38.7 AX-89789780:ph3
40.2 AX-89887432:nmh
41.8 AX-89789649:ph3
48.6 AX-89887967:ph3
49.7 AX-89830834:nmh
50.8 AX-89790304:nmh
51.9 AX-89807592:ph3
52.5 AX-89913169:ph3
53.7 AX-89867192:ph3
53.8 AX-89913200:nmh
54.7 AX-89872574:nmh
54.8 AX-89790484:ph3
67.0 AX-89804629:nmh
67.6 AX-89790773:nmh
67.7 AX-89868274:ph3
68.4 AX-89888508:ph3
69.5 AX-89829092:nmh
70.6 AX-89788735:ph3
70.9 AX-89829073:ph3
72.2 AX-89829052:ph3
72.4 AX-89788598:ph3
72.7 AX-89886218:ph3
RVd13-2009
RVd13-2010
5A
0.0 AX-89794064:nmh
1.8 AX-89804852:ph3
2.7 AX-89792830:nmh
4.3 AX-89792080:nmh
7.3 AX-89835101:ph3
11.0 AX-89892774:nmh
14.3 AX-89849378:ph3
15.5 AX-89849380:ph3
19.5 AX-89794176:nmh
21.7 AX-89807957:ph3
24.4 AX-89893282:ph3
27.1 AX-89905845:ph3
29.1 AX-89794893:ph3
32.6 AX-89893323:ph3
34.3 AX-89794352:nmh
35.2 AX-89893235:nmh
36.9 AX-89893417:ph3
37.8 AX-89808037:nmh
38.6 AX-89794773:nmh
39.1 AX-89794722:ph3
40.3 AX-89849439:ph3
42.2 AX-89893533:ph3
44.4 AX-89794765:nmh
45.1 AX-89808030:ph3
47.7 AX-89794566:ph3
50.2 AX-89857497:ph3
52.0 AX-89836973:ph3
55.6 AX-89849502:ph3
57.9 AX-89836687:nmh
62.1 AX-89872698:ph3
63.5 AX-89836856:ph3
67.4 AX-89849485:ph3
72.2 AX-89848864:ph3
74.5 AX-89889789:ph3
77.6 AX-89835335:ph3
78.3 AX-89832481:ph3
79.0 AX-89848939:ph3
80.5 AX-89890546:nmh
81.2 AX-89890386:ph3
83.9 AX-89835389:nmh
84.8 AX-89848909:ph3
87.0 AX-89905637:nmh
88.4 AX-89905612:ph3
90.2 AX-89792747:ph3
92.7 AX-89890730:ph3
94.2 AX-89834312:ph3
97.0 AX-89891812:ph3
100.3 AX-89913825:ph3
103.0 AX-89891573:ph3
104.3 AX-89875030:ph3
108.6 AX-89793850:ph3
111.1 AX-89793757:ph3
113.3 AX-89892320:ph3
RVd14-2009
6A
0.0 AX-89797020:nmh
0.7 AX-89862066:nmh
1.4 AX-89806405:nmh
6.2 AX-89837339:ph3
9.4 AX-89837555:nmh
10.7 AX-89799529:nmh
14.4 AX-89799217:ph3
15.5 AX-89799623:ph3
18.4 AX-89850105:ph3
21.4 AX-89796546:ph3
22.1 AX-89896121:ph3
26.5 AX-89850117:ph3
27.2 AX-89798459:ph3
30.8 AX-89899893:ph3
31.2 AX-89899809:ph3
34.0 AX-89842417:nmh
34.2 AX-89799624:nmh
35.1 AX-89899594:nmh
36.4 AX-89842288:nmh
37.0 AX-89899534:nmh
38.8 AX-89799371:nmh
39.5 AX-89842060:ph3
40.1 AX-89799337:ph3
41.3 AX-89841999:ph3
41.7 AX-89805764:ph3
43.1 AX-89850246:nmh
46.0 AX-89842821:ph3
48.9 AX-89906563:ph3
49.2 AX-89905957:ph3
51.6 AX-89900271:ph3
55.4 AX-89894715:nmh
56.5 AX-89894748:ph2
57.6 AX-89815650:nmh
58.3 AX-89914496:ph3
63.5 AX-89894375:ph3
67.3 AX-89895231:ph3
72.3 AX-89894220:ph3
76.0 AX-89849823:ph3
78.5 AX-89796445:ph3
84.3 AX-89896228:nmh
87.5 AX-89914807:ph3
91.7 AX-89864329:ph3
92.7 AX-89805467:ph3
94.8 AX-89830709:nmh
98.0 AX-89839658:ph3
103.2 AX-89897273:nmh
105.3 AX-89897394:ph3
106.0 AX-89849996:ph3
109.8 AX-89895119:nmh
112.1 AX-89797617:nmh
118.1 AX-89850121:ph3
119.0 AX-89906412:ph3
122.2 AX-89897879:nmh
126.5 AX-89840672:ph3
127.7 AX-89862098:nmh
132.3 AX-89862101:nmh
136.0 AX-89841111:ph3
139.2 AX-89798654:nmh
143.2 AX-89906449:ph3
145.1 AX-89906454:ph3
RVd15-2011
RVd15-2009
6C
0.0 AX-89881623:nmh
0.7 AX-89872273:nmh
4.4 AX-89895998:ph3
6.7 AX-89873438:ph3
8.4 AX-89779355:ph3
11.4 AX-89841731:ph3
13.9 AX-89899627:ph3
14.3 AX-89842340:ph3
16.4 AX-89850261:ph3
17.8 AX-89899482:nmh
25.8 AX-89850346:nmh
26.6 AX-89800083:ph3
28.2 AX-89868977:nmh
AX-89900239:ph3
29.0 AX-89842999:ph3
29.8 AX-89795159:ph3
32.2 AX-89849575:ph3
33.8 AX-89849587:ph3
34.6 AX-89795460:nmh
35.4 AX-89905963:ph3
37.0 AX-89837821:ph3
37.8 AX-89849657:ph3
40.2 AX-89838434:ph3
41.0 AX-89872724:ph3
AX-89796072:nmh
41.8 AX-89838244:ph3
72.6 AX-89639418:nmh
73.4 AX-89896581:nmh
79.9 AX-89839485:nmh
82.6 AX-89796568:ph3
83.2 AX-89796469:ph3
88.8 AX-89815503:ph3
89.1 AX-89830676:nmh
91.7 AX-89887803:nmh
94.6 AX-89797234:nmh
95.6 AX-89897268:nmh
96.6 AX-89897374:ph3
99.6 AX-89785131:nmh
101.4 AX-89897768:ph3
102.1 AX-89906387:ph3
104.4 AX-89906386:ph3
107.7 AX-89840796:ph3
108.3 AX-89898053:ph3
111.7 AX-89898270:nmh
112.3 AX-89815682:ph3
114.9 AX-89906107:ph3
115.6 AX-89841776:nmh
117.7 AX-89850180:ph3
118.5 AX-89862155:ph3
118.7 AX-89805738:nmh
RVd5-2009
RVd5-2011
7A
0.0 AX-89844948:ph3
2.9 AX-89845089:nmh
3.1 AX-89845114:ph3
6.9 AX-89845290:ph3
7.5 AX-89902673:nmh
10.4 AX-89801963:ph3
11.3 AX-89862418:ph3
14.6 AX-89901895:nmh
15.2 AX-89801337:ph3
16.0 AX-89801204:nmh
19.8 AX-89823012:nmh
20.1 AX-89801084:nmh
22.9 AX-89853817:nmh
24.0 AX-89850614:ph3
25.9 AX-89815738:nmh
38.2 AX-89850548:ph3
52.3 AX-89906683:nmh
53.4 AX-89800994:ph3
54.2 AX-89864742:ph3
56.0 AX-89800978:nmh
56.7 AX-89800824:ph3
57.8 AX-89800760:ph3
58.5 AX-89800711:ph3
59.3 AX-89864722:nmh
60.1 AX-89906640:ph3
62.9 AX-89846686:ph3
63.2 AX-89843135:ph3
64.3 AX-89859825:ph3
66.1 AX-89909792:ph3
67.3 AX-89844658:ph3
69.8 AX-89906836:nmh
70.5 AX-89864898:nmh
71.2 AX-89902797:ph3
72.7 AX-89802210:ph3
73.8 AX-89864861:ph3
74.8 AX-89801698:ph3
83.3 AX-89903094:nmh
91.7 AX-89850381:ph3
92.4 AX-89800226:ph3
93.9 AX-89903162:nmh
95.2 AX-89859779:ph3
96.9 AX-89900405:nmh
97.9 AX-89906584:ph3
98.6 AX-89843248:ph2
99.3 AX-89864679:ph3
100.8 AX-89904667:ph3
102.3 AX-89806892:nmh
102.4 AX-89904670:nmh
102.6 AX-89903950:ph3
103.2 AX-89880843:nmh
103.7 AX-89847667:ph3
104.1 AX-89846527:nmh
104.8 AX-89862284:ph3
105.1 AX-89850944:ph3
105.5 AX-89906939:ph3
108.5 AX-89808967:ph3
111.5 AX-89906891:ph3
112.3 AX-89900724:nmh
AX-89802589:nmh
RVd3-2009
RVd3-2010
RVd3-2011

Supporting evidence

Reviewed papers:

Antanaviciute, L., Šurbanovski, N., Harrison, N., McLeary, K.J., Simpson, D.W., Wilson, F., Sargent, D.J. & Harrison, R.J. (2015). Mapping QTL associated with *Verticillium dahliae* resistance in the cultivated strawberry (*Fragaria* × *ananassa*). Horticulture Research, 2: 15009.

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