Project title:	Genetic mapping and phenotyping of fruit quality and disease resistance traits in octoploid strawberry (*Fragaria* × *ananassa*)
Project number:	CP 094
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Location of project:	East Malling Research
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Date project completed (or expected completion date):	01 October 2015

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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CONTENTS

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GROWER SUMMARY

Headline

- Phenotypic and genotypic information can be used for quantitative trait loci (QTL) mapping approaches and enhance breeding efficiency through marker-assisted selection (MAS).

Background and expected deliverables

Strawberry is one of the most economically important fruit crops and it is essential to maintain the profitability and sustainability of this crop. Today, strawberry growers face increased production challenges, such as maintaining yield, fruit size and good fruit quality. These traits rely on good plant architecture and high levels of pest and disease resistance. In order to maintain competitiveness, the extension of cropping season and better adaptation to the particular growing environment is essential.

The objective of this project is to provide both phenotyping and genetic techniques to improve high-throughput trait identification in industry-funded breeding programmes. Currently very little is known about how different plant traits are correlated at the molecular level in strawberry, or which traits are the easiest to measure in field and glasshouse scenarios.

The primary aim of this study is to investigate correlations between different physical traits in cultivated strawberry (*Fragaria* × *ananassa*), in addition to the development of novel methods of linking phenotype to genotype. A second aim is to identify and map novel traits linked to fruit quality and disease resistance in cultivated strawberry.

Once molecular markers are identified, pre-screening process of seedlings for the presence of desired alleles can be done through MAS. This study will also illustrate the usefulness of high resolution phenotyping and genomic-assisted selection techniques for rapid, inexpensive and accurate pre-selection of superior seedlings in economically important crops such as cultivated strawberry.

Summary of the project and main conclusions

This project aims to cut the cost of breeding by developing novel phenotyping methods and identifying the most important traits to measure both linked to fruit quality and disease resistance in cultivated strawberry. A mapping population derived from the cross

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‘Redgauntlet’ × ‘Hapil’ was used for phenotypic data collection over a period of 2 years (2013 and 2014), linkage map construction and saturation, as well as quantitative trai loci (QTL) detection. The same population will be used for fruit-related trait phenotyping for the third year (2015 summer). As previously reported in the Year 1 report, over 20 different traits of ‘Redgauntlet’ × ‘Hapil’ population were recorded and correlation analysis was conducted.

This report is mainly focusing on the saturation of the existing SSR-based linkage map and preliminary QTL analysis using Year 1 (2013) phenotypic data. Twenty six novel markers were mapped to the linkage map which was used for QTL detection. Thirty-three potential QTL associated with both fruit quality and plant characteristics traits were identified. The regions of QTL detected on the linkage map suggest that there is a ‘hotspot’ region of the strawberry genome. The ‘hotspot’ region contains overlapping 9 different QTL covering major fruit quality and plant characteristics traits.

Preliminary study of the comparison of the regions between QTL associated with *Verticillium* wilt resistance obtained from the previous study and QTL detected in this study was carried out. However results are not presented in this report. Overlapping regions between *Verticillium* wilt QTL and fruit quality and plant characteristics traits were identified. Further analysis will be carried out in 2015 to investigate if disease resistance QTL are overlapped using 2 years (2013 and 2014) phenotypic data and these results will be presented in the final year report.

‘Sonata’ × ‘SDBL123’ mapping population will mainly be used to test the applicability of novel molecular markers developed in this study that can be later used in MAS.

Financial benefits

For this interim report it is not appropriate to undertake a cost/benefit analysis.

Action points for growers

There are no action points for growers at this stage of the project.

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SCIENCE SECTION

Introduction

Fruit quality (sensorial and nutritional) and disease resistance traits are major targets in cultivated strawberry (*Fragaria* × *ananassa*) breeding programmes. Consumers and retailers demand high quality strawberry fruits. Among them, fruit size, colour, flavour and texture are the traits that are most important commercially (Callahan, 2002). Consequently, efforts to breed new strawberry varieties with improved fruit quality have intensified.

Similarly to other rosaceous crops, fruit quality and disease resistance traits in strawberry vary and are affected by a number of factors at both molecular and environmental levels. During the last decade, a vast number of studies have been published which reported on the relationships between genes and fruit quality traits in strawberry (Salentijn et al. 2003; Carbone et al. 2009; Zorrilla-Fontanesi et al. 2011[b]). To improve fruit quality in strawberry, numerous methods have been developed to investigate plant genetic architecture and to understand why and how plants produce fruits that vary from year to year. Among these methods, the most important approaches are at the molecular and genetic level and include linkage mapping and quantitative trait loci (QTL) analysis and the development of marker-assisted selection (MAS).

The advantage of QTL analysis is that it can be used for the identification of linkages between markers and a large number of loci. In addition, QTL analysis also provides information on the genetic control of traits and is useful for breeding new cultivars through MAS (Lerceteau-Köhler et al. 2012). However, the detection of QTL is a challenging process because most traits, such as yield, fruit quality and maturity, are likely to be controlled by more than one major gene (gene series) (Kumar, 1999). Moreover, environmental changes affect fruit quality and yield, resulting in modifications in expression of genes and QTL. As a result, the approach is based on both genotypic and phenotypic data analysis generated within several years or seasons in order to maximise the final phenotypic expression.

Moreover, the success of linking traits using molecular tools depends on a number of factors, such as the size of the population, the quality of the linkage map, the magnitude of the QTL's effect on the trait and the distance between the marker and identified candidate gene (Kumar, 1999). Despite these factors, major genes controlling fruit quality traits in apple (Longhi et al. 2012; Bus et al. 2010; Patocchi et al. 2009), peach (Gillen and Bliss, 2005; Dirlewanger et al. 2004; Ogundiwin et al. 2009; Eduardo et al. 2011), sour cherry (Wang et al. 2000) and raspberry (Sargent et al. 2007) have been identified. Moreover, a series of molecular markers has been developed for fruit quality traits in *Fragaria*. These

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include genes linked to seasonal flowering (Sugimoto et al. 2005), fruit flavour, size, firmness, shape, metabolites (Lerceteau-Köhler et al. 2012) and the yellow fruit colour (Deng and Davis, 2001). However, it is well known that fruit quality and disease resistance 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 level of resistance or quality attribute (Sargent et al. 2012).

In this report, the work that has been carried out to improve the existing simple sequence repeat (SSR) based linkage map and to identify QTL for fruit quality and plant characteristics traits in the octoploid strawberry (*Fragaria* × *ananassa*) is summarised. To date a total of 26 additional microsatellites or SSR markers representing 71 loci have been successfully mapped to the previously developed 'Redgauntlet' × 'Hapil' linkage map. An improved linkage map ('Redgauntlet' × 'Hapil') saturated in this study was used for identification of QTL associated with phenotypic data collected in 2013. Thirty-three potential QTL (21 for plant characteristics and 12 for fruit quality traits) have been identified and mapped so far. The results obtained in this study will be used for the identification and functional analysis of candidate genes controlling major fruit traits and molecular marker development which potentially will be used in MAS.

Materials and methods

Plant material

An F_1 mapping population of 188 individuals obtained from a cross between two octoploid strawberry cultivars 'Redgauntlet' and 'Hapil' was developed. The population is kept in a polytunnel and is used for further propagations by pinning down the runners. The saturation of the existing 'Redgauntlet' × 'Hapil' 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. DNA was isolated from young leaf tissue of 188 'Redgauntlet' × 'Hapil' individuals and parental genotypes using the DNeasy plant miniprep kit (Qiagen) according to the manufacturer's protocol and diluted 1:50 (~3 ng/µl) for use in polymerase chain reaction (PCR) from previous studies.

As it was reported in the Year 1 report, 122 seedlings out of 188 randomly were selected and six replications were propagated and planted in the open field at East Malling Research in September 2012. These plants were used for phenotyping fruit quality and plant characteristics traits (Figure 1).

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Figure 1. Seedlings of ‘Redgauntlet’ × ‘Hapil’ mapping population and parental genotypes; a) 2 year old seedlings under cover while recording the phenotypic data in the field, photograph was taken on 30.04.2014; b) and c) seedlings in the polytunnel which were used for further propagation and DNA extractions, photographs were taken on 12.09.2014.

SSR marker selection for linkage map saturation

A total of 111 SSR markers were selected for their distribution across the 28 linkage groups of the octoploid strawberry linkage map reported by Isobe et al. 2012 and on-line available data base (http://marker.kazusa.or.jp/Strawberry). Markers were selected to fill the regions with low marker density (gaps) on the existing ‘Redgauntlet’ × ‘Hapil’ map that were greater than 30 centi-Morgans (cM). The existing linkage map did not have gaps larger than 30 cM on linkage groups 3C and 6B, thus no markers were selected from those linkage groups from the previously reported map. All SSRs were tested for heterozygosity in the parental genotypes and three-four seedlings of the cross and those that were polymorphic and segregating were further screened in the ‘Redgauntlet’ × ‘Hapil’ population. Table 1 lists all SSRs selected, marker names, forward and reverse sequences, linkage groups and positions on the map to which markers were previously mapped.

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Table 1. A summary of 111 previously mapped SSR markers selected from different linkage groups to be tested in this study.

Name	Forward primer sequence	Reverse primer sequence	Isobe et al. 2013	
			LG	Position (cM)
FAES0271	accttgtgacctggcgttac	ttctttgacgcccaacttct	LG1A	50.5
FVES0983	gctctactctccgcctctca	ggaaaatccggaagcgtaat	LG1A	47.3
FVES2012	aggcatccgacctaatgttg	gacacccaaaatgcacaaga	LG1A	57.7
FVES0982	tcttcaaagccacaaccctc	gatctcattgcatgcttaggg	LG1B	18.3
FVES1738	ggcttttcggattgatttga	gaaaaagccattgtgcccta	LG1B	19.4
FVES2280	caatgcttccaatgggactt	gtcaccaacatcagcccttt	LG1B	13.2
FVES0670	cgtgcctgtttgatttctga	caagcatcaagggaaccact	LG1C	0.8
FAES0378	ggtgggaatttttctgggat	gccaacctaacctccctttc	LG1D	16.2
FVES3126	aaccccaaagtgatcaccaa	ctccgaacacctcggagtag	LG1D	17.2
FAES0053	ccatcatcgtctctcgttttt	cgtcgatatcgcacatcaag	LG2A	45.6
FVES0901	gccaccatctcctctgaaac	catagccaatgctgtcctca	LG2A	47
FAES0154	cgccaaaacttggtagatgg	atcaggcaccaattgacctc	LG2B	20.4
FVES0623	cacaagcctctctcctcacc	aagctcttgctctgcaccat	LG2B	95.1
FVES1292	gtcccaccatccagttatgg	actccacttggctgagcagt	LG2B	81.3
FVES1687	acgaagggtgaagggtctct	cccaaaaacccaaatcctct	LG2B	29.3
FVES3275	cgaagcattcatggctttttt	ctcaagatgacgacagcgaa	LG2B	22.5
FAES0247	acgccttcgatccttttctt	caaggcagtaaagctcccag	LG2C	84.2
FAES0326	gcaatttcagcaatccctgt	tctttttggggcgtacaaat	LG2C	93.8
FVES0129	acagggacattagggcacag	ccactcgctcaattcgtgta	LG2C	17.9
FVES0347	aactcctcctcctcctcgtc	gtaaggagcagagccactcg	LG2C	56.6
FVES0393	aagccatctcattcaccgat	gcgacaaaggcaagaatagc	LG2C	10.3
FVES0936	cacatatataaacccagtcgg	tgaaggaagggatggagttg	LG2C	17.9
FVES1747	tcgttcgtcataattagcagaga	cggctttgagctcgtaaatc	LG2C	15.9
FVES3470	caaatccctcttctcctctcc	cctcagagaccatcaccgat	LG2C	68.8
UFFa11A11	acgaggctccaatagagttctg	ctgagcagaagccatagtatcac	LG2C	7.7
FAES0226	actccacttggctgagcagt	cacacagcctgctgcatatt	LG2D	50.1
FAES0276	cgctagcttttctgctcgat	acactccaccggcttacatc	LG2D	25.9
FAES0277	gaactccctttttctgggtcc	caatgagtgggagaggaagg	LG2D	59.6
FAES0380	cattgcccaccttgtaacct	ctcggcgctcctatattcaa	LG2D	71.5
FAES0582	ggcaatgcctacctttgtgt	cattgcaacaagcattcaca	LG2D	57.5
FATS0033	tacatgcaaacctgctccag	acgcccagacttactggtgt	LG2D	14.5
FATS0034	ctcaacctacacaccctctgc	aaatttcatgccccacattc	LG2D	24.4
FVES0256	gagtctcagacatctcgccc	atatgaggacgcagaatcgg	LG2D	81.1
FVES0673	aacaaagaggccaggagctt	agaatcctcccatccgattt	LG2D	54.3
FVES1525	ctccaccttcttcttcttcaca	tatggttgaggctgaggagc	LG2D	49
FVES1726	ctgaccgatcagggattcac	ccttcctagctgcaatctgg	LG2D	48
FVES1917	ccattccaggtacatcctgc	acaaatcgctcgtatcggtc	LG2D	7.9
FVES2137	ttcagcagatgatgcagacc	agtcccaatcgttgacgaag	LG2D	20.2
FAES0151	gcctccaaaggttgttacttctt	actttgctcgagccatcaat	LG3A	65.7
FVES1171	tgaatacatgcatcgctggt	gtgggaaacaaagtctggga	LG3A	67.5
FVES3374	agttcctcccttctcgcttc	gttgatgtagctgtacgccg	LG3B	0
FAES0241	gactaaggggtgggagcttt	taagttggccaggtcgagat	LG3D	36.8
FVES0398	gagatttctcactcgtcccg	aacaaagggtcgatcaccag	LG3D	12.5
FVES3002	actcggagtaggaaatgcca	ctgttgatggtggtagggct	LG3D	32.6
FVES3364	gaccaccgccactctctaaa	ggtgttgaggaaggcgtaga	LG3D	41.2
FVES2235	gatcttgatgcccacttcgt	ttcatcgaccaacgtttaca	LG4A	72.9
FVES2289	acaacaatggaaccctgagc	gcacaaaattacgagccaaa	LG4A	75.1
FAES0108	cctgcagagtgcaagagaga	gtgcacacatgaatccacaa	LG4B	53.4
FVES2278	caggggaatggagaaagtga	attcctgggcttttcgtctt	LG4B	76.8
FVES2722	cgaggttgaggagttcttcg	cgcattcaaatcaaagtcca	LG4B	46.8
FVES3219	atttgcgattccagcagatt	cccccacaatgcttcagtag	LG4B	79.9
FAES0296	tctgtcattgctcaacctcg	ggctcccaaactgtggttag	LG4C	11.1
FVES3039	gagtgtgacggatggtgttg	ccacgtgtacggctcctaat	LG4C	7.6
FAES0001	gggctcaaaagatgtggaaa	tttatttgggaaggcatcgt	LG4D	8.9
FAES0063	aacccagatgaaattgctgc	cccagtgacaaacaagcaga	LG4D	5.4
FVES1409	tcggtttctcgctttctttc	gtgatccgatacgttggctt	LG4D	11.3
FAES0018	tgtaagtgcctctaaagatggtagg	tgtcgtgtgttttagttcacaatg	LG5A	47.7
FVES0545	gcaagtccatatccacttctttg	tcaaattccgtttcgtcctc	LG5A	34.5
FVES0688	aatcaaacctaacccgtccc	gttggagtccggtctgttgt	LG5A	27.6
FVES0847	aaaccggtcatcagttacgg	gaagctctcgaagctggtgt	LG5A	38.4
FVES1122	tcacttccattcctaacgcc	ttccctcactgttccgattc	LG5A	31.8
FVES1537	ggagacatccaacgatcagg	ccatggggttgagcttagag	LG5A	50.5
FVES3720	atcccatttctattggggga	attgcgccatacaaacacaa	LG5A	40.9
FAES0318	aggctctaggcgacaacaaa	caaatgactggattgatatattgttag	LG5B	33.5
FAES0394	ccaaatgcagaaacccagat	actccattttggctccctttt	LG5B	23
FVES0536	gatcatgtttttggatagagaagaa	tgggagacaactgaggctct	LG5B	25.7
FVES3224	taacttccctcccgattcct	cctcttgaagctccgatcac	LG5B	39.6
FVES0639	gttcaagcaaatctccgagg	tctcggcgtaaatatcgtcc	LG5C	41.8
FVES0833	agccaagaagccagaagaca	cctgctctcgtcatccattt	LG5C	0
FVES3434	cattattgcacacaccagcc	cgttgattggtcgtagcctt	LG5C	38.3
FAES0382	ataccagaacccaccaccaa	gtggttcccagagctgaaag	LG5D	34.4
FVES0618	cttcctcccaaaaaccttcc	tagtcaatgtgcttcaccgc	LG5D	28.9
FVES3096	ctctctccgatcgtgtctcc	gtccacgacccgttttcata	LG5D	20.4
FVES0013	tctcctcctctcttcccgat	gaaatgctctcttcggttcg	LG6A	35.3
FVES2960	gtcttgcgggaagcagttag	gatgtcgtcggagagaggag	LG6A	34.9
FAES0078	ctaagctcgtcatcaagccc	agtcccattttccagtggtg	LG6C	58.6
FAES0243	gaagcagaaactgaggacgg	tcaggtttaagatcccggtg	LG6C	65.5
FATS0014	cctgaaccagcttctgggta	ctcatgctgaagaagctccc	LG6C	75
FATS0047	ggaatgtcgatctgggtctg	tgatcagcttatacgacggc	LG6C	60.8
FVES0669	ctgcctggtttggtaatgct	gagaccaagccctctgtttg	LG6C	50.6
FVES0775	gccaacctcttgttcttgga	aattgggtgcttggagtttg	LG6C	21.1
FVES1143	gatgtgcagttcatgcatcc	aaggcctggaacagagatga	LG6C	16.5
FVES2192	gtgatcagcatggggactct	aggaggaggggtgaagatgt	LG6C	54.7
FVES3346	ttgcttttagatggctgctg	cttcggtttagctgctttgg	LG6C	0
FVES3450	tgagtgcagagatcccagtg	cctcctcatcagccaaatgt	LG6C	14.1
FAES0023	actgccctccatgtctcaac	gtgcagagaatgagcaacga	LG6D	32.2
FAES0381	aatacaaactcggcgcaatc	tggaatccaccatcaaggtt	LG6D	37.8
FATS0090	agagccggtttagctgagtg	cgtcgtcgttttctctcctc	LG6D	27.9
FVES0463	gctacgtgttcgggtttgat	tcatgttcccatattgccct	LG6D	76.4
FVES1154	ctcagtgacctccacagcaa	agggtcccgaatgagtttct	LG6D	13
FVES1271	aactggccaccacactttttc	ggcgtcacgggtatgttact	LG6D	38.8
FVES1580	taaacgacatcggcgacata	agaatcagatggtgttgccc	LG7A	28.5
FVES3344	caacagtcgtccatgtcctg	acaattctcgtctccgttgg	LG7A	40.6
FAES0410	taacagctgtcttgcttggc	atcttgacgaatgagggtgc	LG7B	16.4
FATS0076	caagggaagtggaagtggaa	gctgaggagaaacctggaga	LG7B	63.3
FVES0104	ctgccttctgggtcgttaaa	aagacgtcgacgagtcccta	LG7B	40.1
FVES0814	attagggtttcgttccccac	atggcgatgaagaaaacgac	LG7B	12.8
FVES1834	gttgaagcagctcccaaaag	gaattgacgaggcggtaaaa	LG7B	72.4
FVES3503	gccaacgtactcctggtgat	aggatcaacttcatcacgcc	LG7B	57.8
FAES0042	ccacatttcacacagaccca	tataagctcatgacccgcct	LG7C	2.3
FVES1672	acaccctgtcccttcacaag	gaaagatgacttcggcttcg	LG7C	6.7
FVES1722	ggcatatgtagatgggtggg	gcaacagcagaagaacctcc	LG7C	9.7
FAES0363	cacgaggttccagatcatca	cggcatcaaaccattctcat	LG7D	36.2
FAES0401	accgtctctggtttcccttt	atatggttcctgcagatggc	LG7D	14.1
FATS0029	gcctagttcgtcctgggttt	ccaagttgaggatgctggat	LG7D	13.1
FVES0144	catgaggaaggagctcaagg	tcgaacggcatacattttca	LG7D	34.4
FVES0640	cagcccctcatcttcttctg	gcgtggtagtcatctgggtt	LG7D	16.1
FVES1237	gtgtcactcacacacacacca	caccttctccattccctgag	LG7D	9.7
FVES1414	atctcgaggcttccaagaca	aatcgggatattcgcattaca	LG7D	38
FVES1453	ggctatgatcgaaaaatatgacc	gcggttaagatgagaaaatgtg	LG7D	30.8
FVES1908	acaacccaaacagcaccttc	ttgtgagctgagaccctgtg	LG7D	10.4

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PCR conditions and product visualisation

Novel markers were tested at two different stages. Firstly, markers were tested in the parental genotypes ('Redgauntlet' and 'Hapil') and in three, or in some cases four, randomly selected seedlings of the population only, using the fragment analyser (Advanced Analytical). During the second stage, primers that were polymorphic, and/or amplified many bands, were further selected to test in a whole mapping population. Forward primer sequences were re-ordered for these primers with either a fluorescent dye molecule 6-FAM™ (blue) or VIC™ (green).

At the first stage, single primer pair PCR reactions were performed because the amplification region was unknown and primers were unlabeled. At the second stage, PCR multiplexes were developed by combining product sizes and fluorescent dye colour. Due to the use of two different dyes the same product size primers were pooled together into multiplexes. PCR protocols for the amplification of the single and multiple primer pairs used in this investigation are listed in Table 2.

Table 2. PCR reaction protocol and cycling conditions used for amplification of single and multiplex primer pairs.

Single primer pair PCR reaction and amplification protocol							
10 × PCR buffer (Molzym)	×	1.25 µl	Initialisation	5 min	at	95 ºC	
$MgCl_2$ (25 mM) (Applied Biosystems)	×	1.00 µl		0.30 min	at	95 ºC	
dNTPs (2.5 mM) (Applied Biosystems))	×	1.00 µl	Denaturation	1.30 min	at	55 - 50 ºC	× 10
MolTaq (Molzym)	×	0.05 µl		0.30 min	at	72 ºC	
SSR primer (F + R, 2 µM)	×	1.25 µl		0.30 min	at	95 ºC	
H_2O (Sigma)	×	5.45 µl	Annealing	1.30 min	at	50 ºC	× 25
DNA (~3 ng/µl)	×	2.50 µl		0.30 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	Initialisation	5 min	at	95 ºC	
10 × SSR primer mix (2 µM of each primer)	×	1.25 µl	Denaturation	0.30 min	at	95 ºC	
H_2O (Sigma)	×	3.50 µl		1.30 min	at	60 - 50 ºC	× 10
DNA (~3 ng/µl)	×	2.00 µl	Annealing	0.30 min	at	72 ºC	
Total		**12.50 µl**	Extension	30 min	at	60 ºC	

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At the first stage PCR product separation and visualisation was carried out using fragment analyser. An example image of three progenies and parents of the cross for two SSR markers tested are presented in Figure 2.

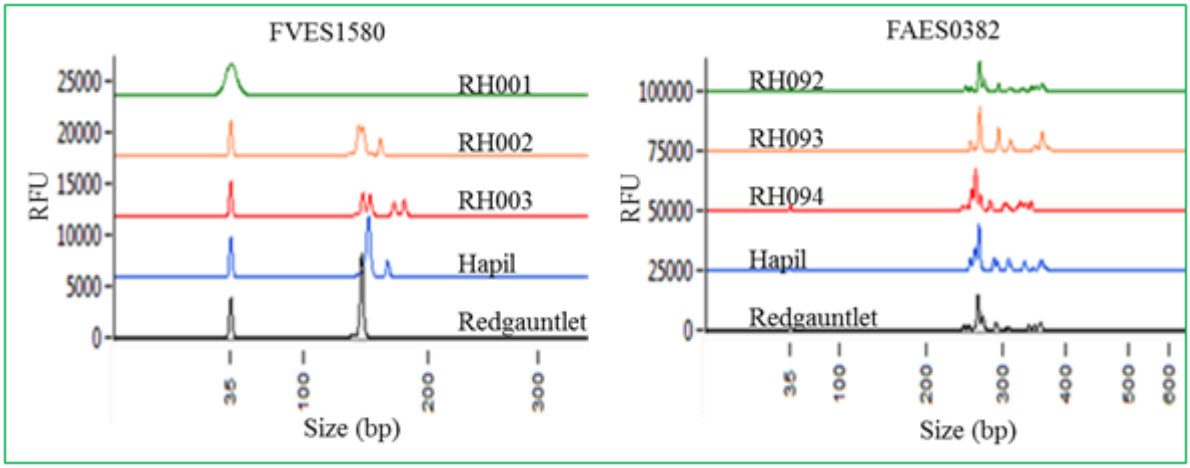

Figure 2. PCR amplification of three seedlings and parents of the cross for FVES1580 and FAES0382 primer pairs. Fragment analyser traces for FVES1580 primer are (from the top) seedlings RH001 (failed), RH002 and RH003 and parental genotypes 'Hapil' and 'Redgauntlet', whereas traces for FAES0382 primer are (from the top) seedlings RH092, RH093 and RH094 and parental genotypes 'Hapil' and 'Redgauntlet' respectively.

During the second stage PCR products of multiplexed primer pairs were screened and visualised using a capillary electrophoresis system on ABI Prism 3100 genetic analyser (Applied Biosystems). The generated electropherograms were collected and analysed using GENESCAN® version 3.7 (Applied Biosystems) and genotype data were scored and visualised graphically with GENOTYPER® version 3.7 (Applied Biosystems) software. An example of a genetic analyser image of three seedlings and parents of the cross for two of the SSR markers is presented in Figure 3.

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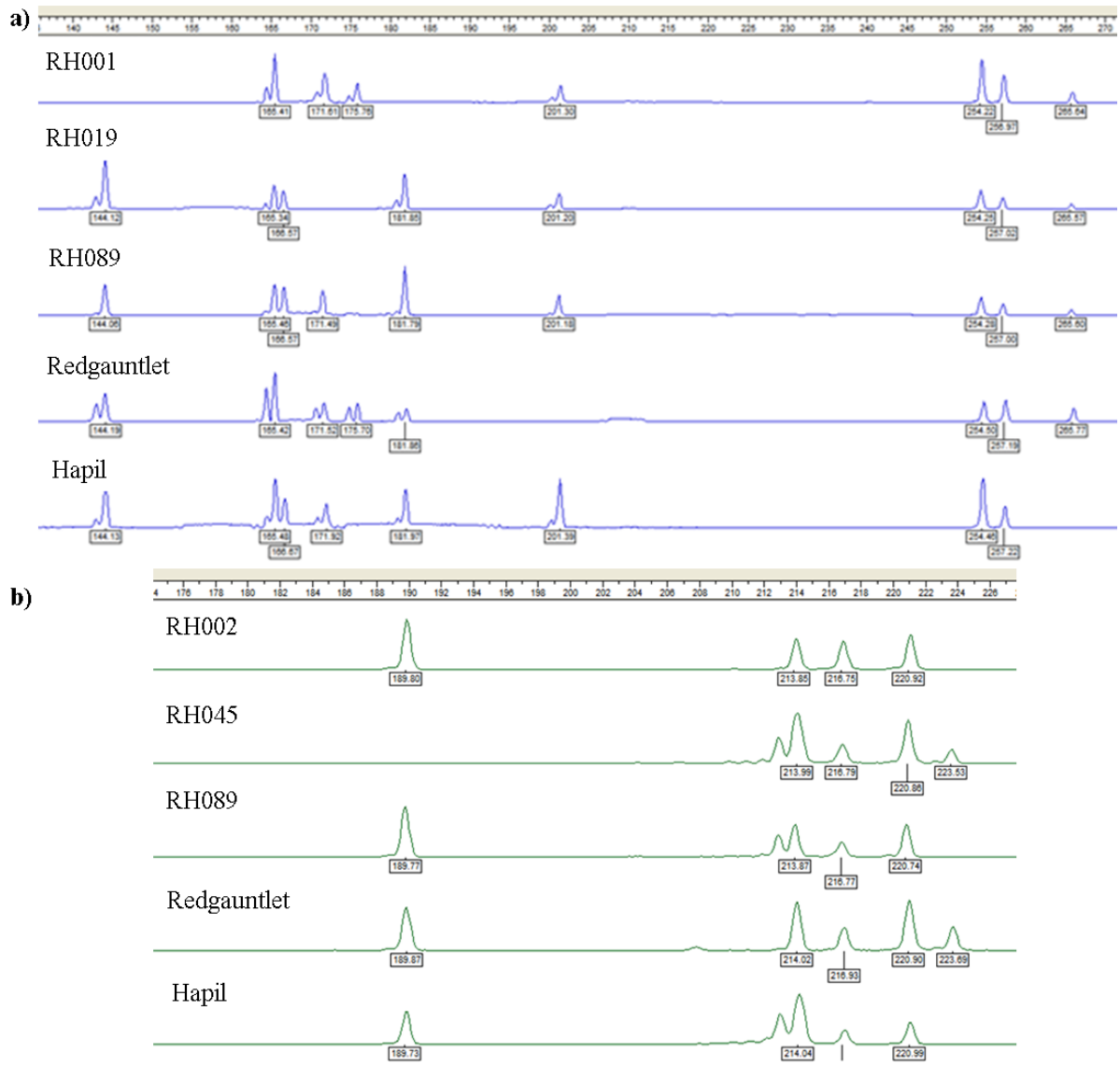

Figure 3. ABI 3100 genotyper traces of PCR amplification of three progenies and parents of the cross; a) GENOTYPER® traces for FAES0001 primer (from the top): RH001, RH019, RH089, 'Redgauntlet' and 'Hapil'; b) GENOTYPER® traces for FVES1453 primer (from the top): RH002, RH045, RH089, 'Redgauntlet' and 'Hapil'.

Data analysis and mapping novel SSR markers

The generated genotypic data in this study was analysed with previously mapped marker data for 'Redgauntlet' × 'Hapil' mapping population. Primer pairs that showed segregation within the population were scored and used for construction of the linkage map using JOINMAP 4.0 (Kyazma, NL) software. Due to a large number of SSR markers mapped previously, new markers tested in this study were mapped by analysing a single linkage

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group at a time. An improved map of each of the 28 linkage groups reported here was plotted using MAPCHART version 2.2 software (Voorrips 2002) and marker distances on the linkage groups presented in centi-Morgans (cM) (Kosambi function).

Phenotypic data analysis

A total of 25 flower-related, fruit-related and plant characteristics traits were phenotyped on the 'Redgauntlet' × 'Hapil' mapping population and parental genotypes for Year 1 (2013) and data analysis for parental genotypes and progeny and this was reported in the Year 1 report. The generated phenotypic data was further analysed for all individuals in the population. Means, standard deviation and range were calculated across six replications for each trait recorded, and mean data distribution histograms were produced using statistical software R (R Development Core Team (2011), Austria). The traits that showed a normal or close to normal data distribution within the population were used in the QTL analysis.

QTL detection

The phenotypic and molecular marker data for each individual of the mapping population was analysed using MapQTL 4 software (Van Ooijen, 2004). A preliminary test to identify regions of the genome linked to traits phenotyped in 2013 was conducted using a Kruskal-Wallis test. In addition, allele fazes were investigated in order to identify if both or one parent of the cross were contributing to the QTL.

Results and Discussion

SSR marker segregation analysis and mapping

A total of 111 primer pairs were selected and tested in parental genotypes and three or four seedlings of the cross in this study (Table 1). Of those, 41 primer pairs that showed the most significant polymorphisms from stage one analysis were further selected for screening in all mapping population (stage two). Five primer pairs amplified non-segregating products, two failed to amplify any PCR product, three showed very weak amplification and were resolved in fewer than 50% of the genotypes and the remaining 31 primer pairs were polymorphic and amplified segregating products. Out of remaining 31 markers, three were further excluded from the analysis due to complex amplification of the products, thus 28 primer pairs were scored for polymorphism in all 188 (15 rogue individuals were treated as failed data) 'Redgauntlet' × 'Hapil' mapping progenies.

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Twenty-eight informative primer pairs amplified one or more loci and generated a total of 89 segregating loci in the mapping progeny. Of those, 71 loci (representing 26 SSRs) were successfully placed on the existing consensus linkage map (Table 3, Figure 4). The rest of the SSR loci were either not linked to any of the recognized linkage groups or were excluded because their segregation pattern conflicted with other markers in the same linkage group.

Table 3. The locus names of the 26 novel SSR markers mapped in this study, the linkage groups and positions on the map to which the markers were mapped previously, along with the linkage groups to which the loci mapped in this study and the number of loci amplified per linkage group. Markers in blue and green represent the fluorescent dye colour used. Linkage groups in bold represent disagreements between marker locations in the original map and the map developed in this study.

	Isobe et al. 2012		This study	
Name	**LG**	**Position (cM)**	**LG**	**Loci Mapped**
FVES0982	LG1B	18.3	LG1A, LG1D	1,1
FAES0154	LG2B	20.4	LG2C	2
FVES1687	LG2B	29.3	LG2A, LG2C, LG2Db	2,4,1
FAES0247	**LG2C**	84.2	LG2A, LG2B, **LG5B**	2,1,1
FVES0347	LG2C	56.6	LG2A	1
FVES0393	LG2C	10.3	LG2B, LG2C	2,2
FVES0936	LG2C	17.9	LG2Db	1
FVES1171	**LG3A**	67.5	**LG2A**, LG3B	1,1
FVES3374	LG3B	0	LG3C	1
FVES3002	LG3D	32.6	LG3A	1
FVES3364	LG3D	41.2	LG3C	1
FAES0001	LG4D	8.9	LG4A, LG4C	1,1
FAES0063	LG4D	5.4	LG4B	4
FVES1409	**LG4D**	11.3	**LG1D, LG6A, LG6Ba, LG7B, LG7D**	1,1,1,1,1
FVES0545	**LG5A**	34.5	**LG6A**	1
FVES3224	LG5B	39.6	LG5B, LG5D	2,1
FVES0833	LG5C	0	LG5B, LG5D	1,1
FAES0382	LG5D	34.4	LG5A, LG5C	1,3
FVES0013	**LG6A**	35.3	**LG3C**, LG6A	1,2
FATS0090	LG6D	27.9	LG6C	1
FVES1580	**LG7A**	28.5	**LG3A**	2
FATS0076	LG7B	63.3	LG7A	1
FVES1672	LG7C	6.7	LG7A, LG7B, LG7C, LG7D	1,2,1,2
FVES1237	LG7D	9.7	LG7B	2
FVES1414	LG7D	38	LG7A, LG7B, LG7D	2,4,1
FVES1834	LG7B	72.4	LG7D	2
Total 26				**71**

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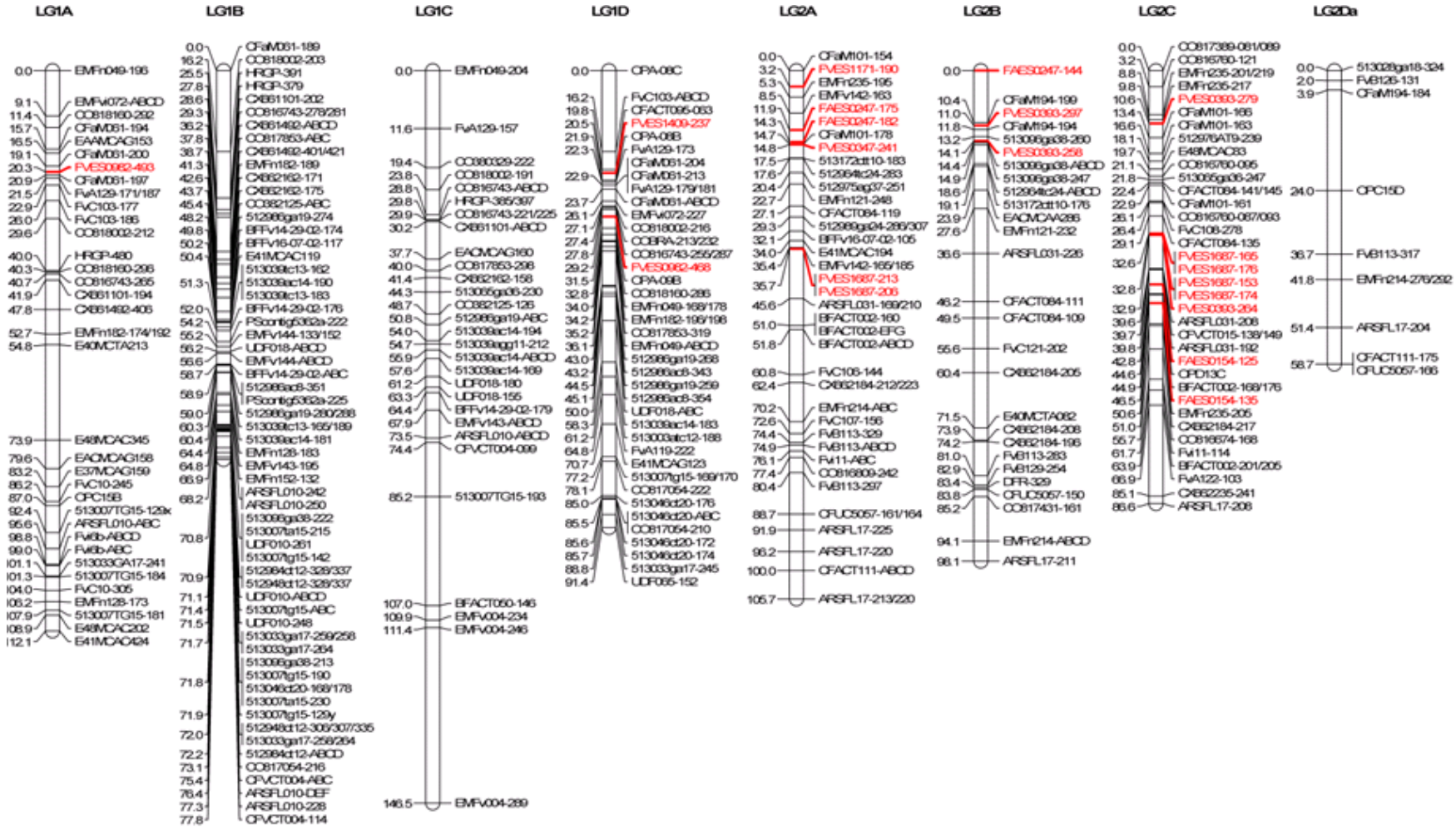

Figure 4. A consensus genetic linkage map of the octoploid strawberry mapping progeny derived from the cross 'Redgauntlet' × 'Hapil'. The map spans 28 linkage groups (LG1-LG7, where each LG has four homologous LGs) as well as two yet unknown regions of linkage groups (Unmapped1 and Unmapped2). Markers highlighted in red were mapped in this study.

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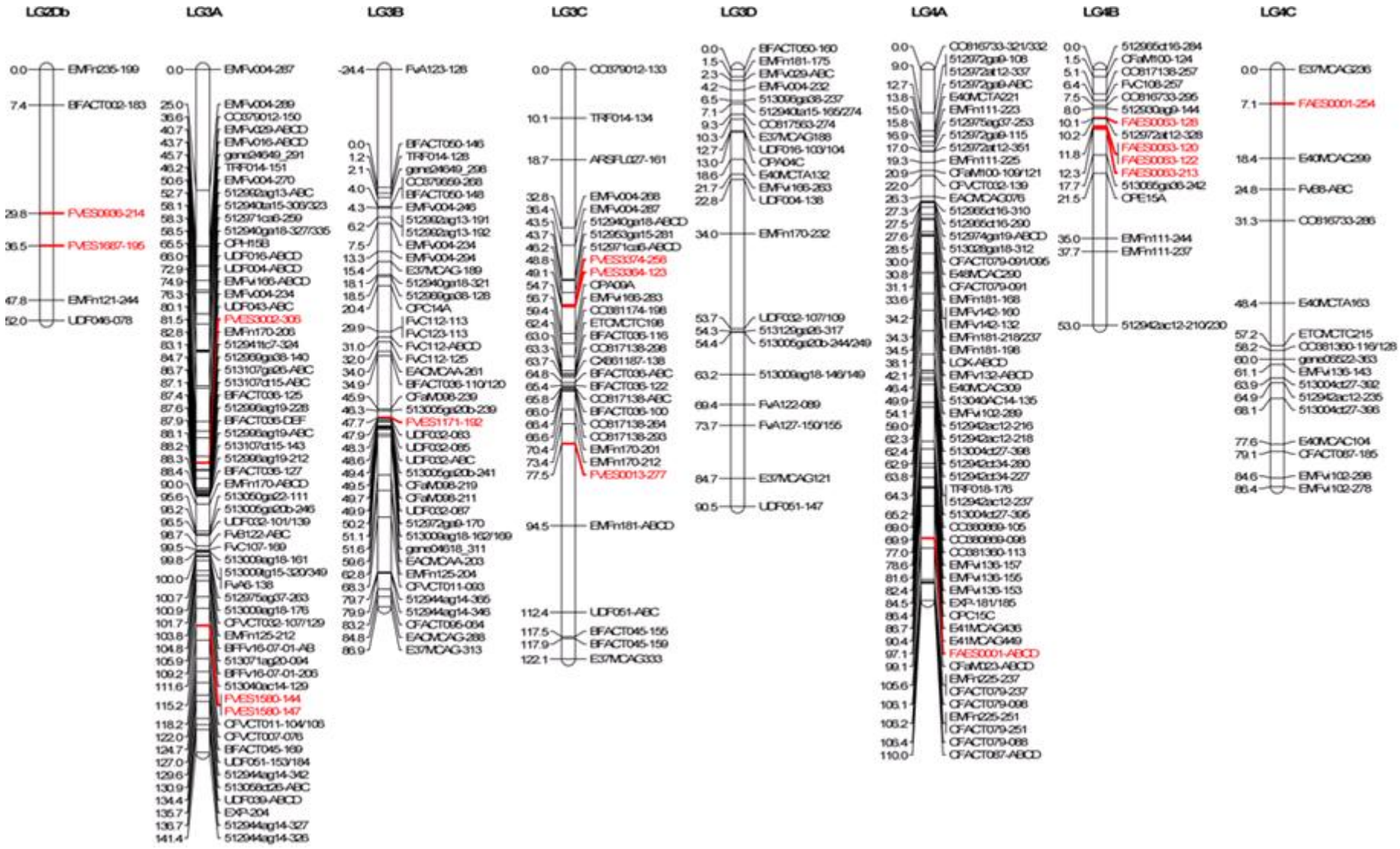

Figure 4. continued

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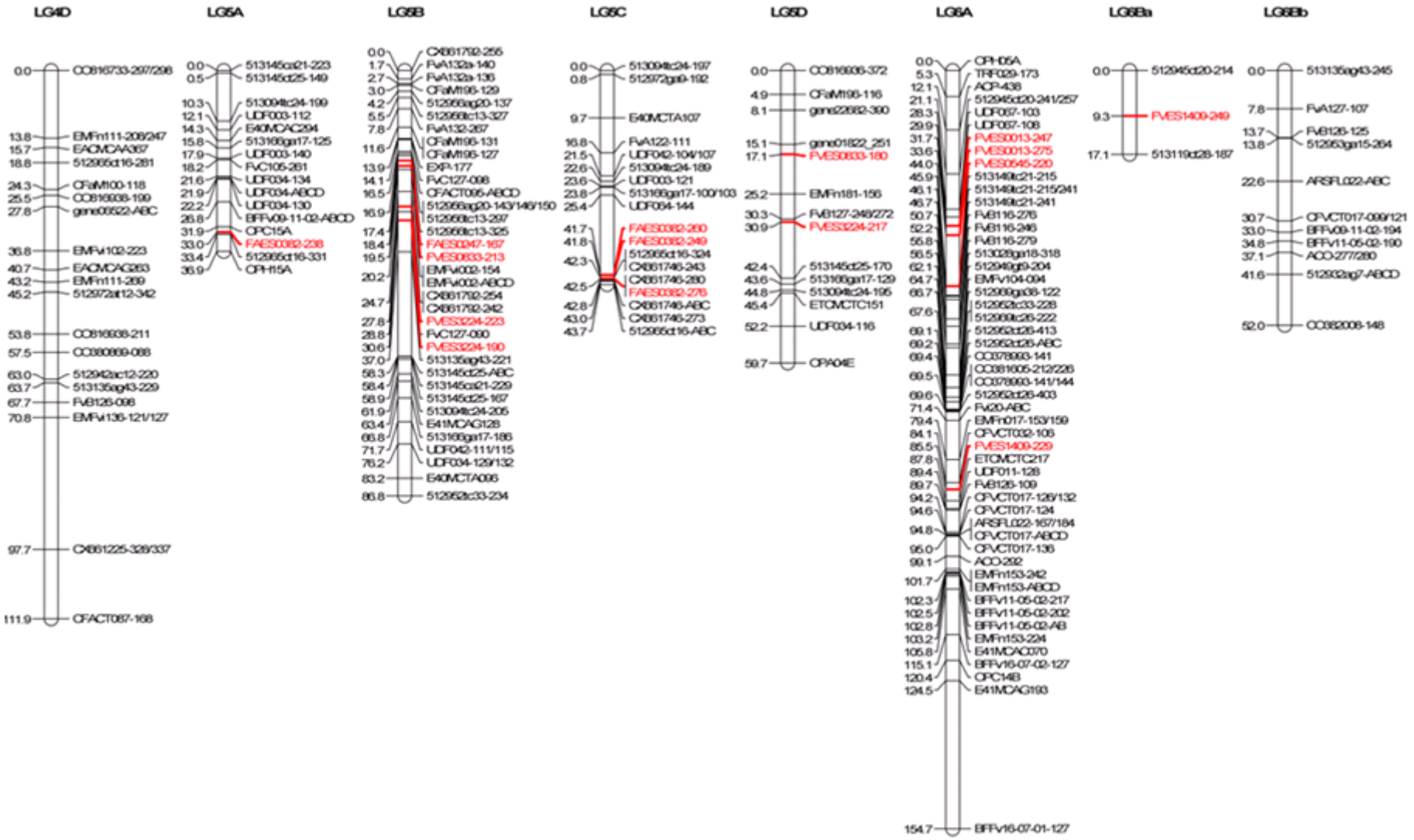

Figure 4. continued

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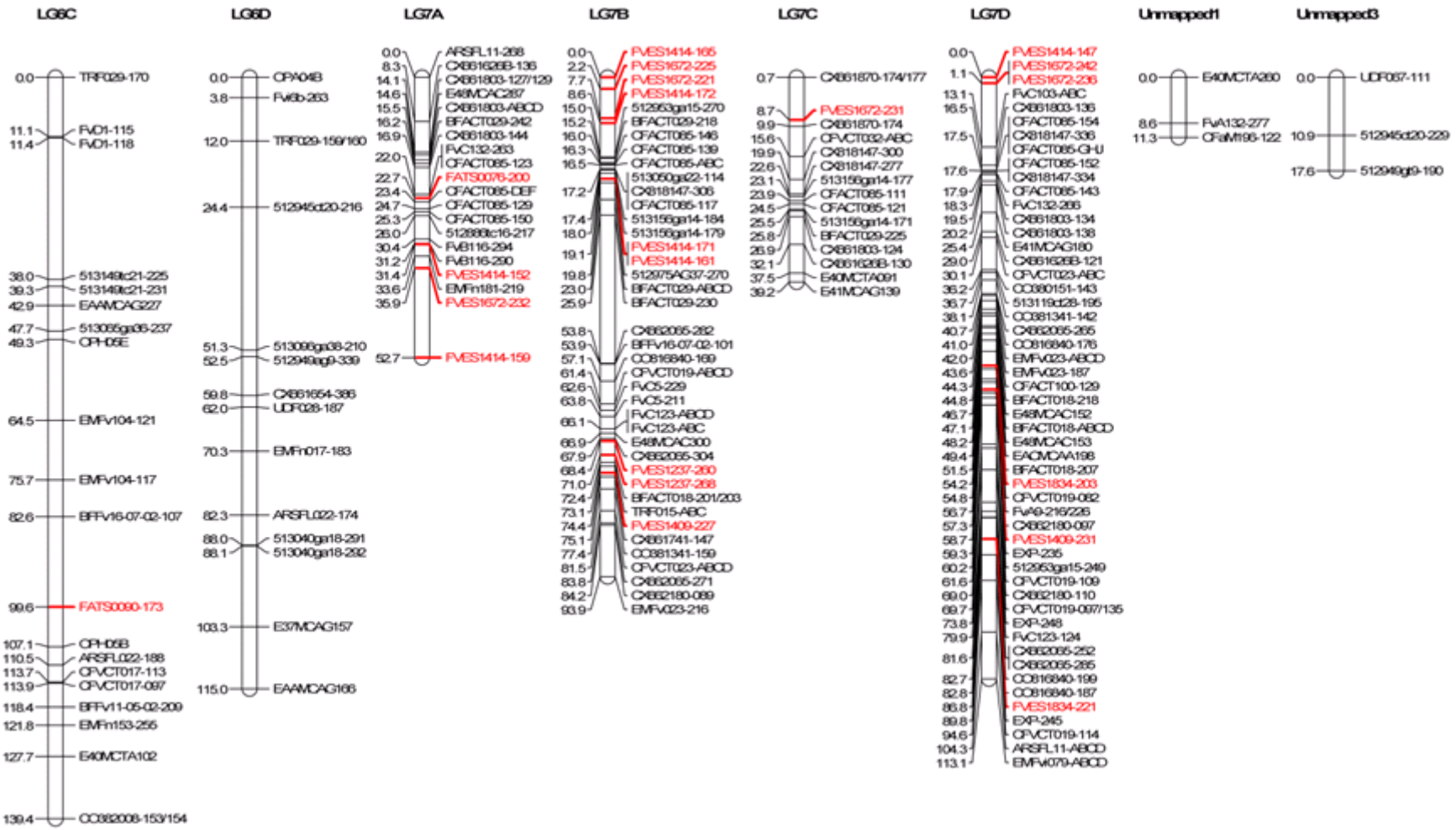

Figure 4. continued

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The linkage groups to which novel markers were mapped in this study were compared to their original locations reported by Isobe et al. 2012. Some discrepancies were observed between linkage groups between the two maps. Twenty SSR markers were mapped to the same linkage groups between the two maps as expected. However the linkage groups of six SSRs representing 11 loci were inconsistent between the the maps (Table 3, highlighted in bold). Most noticeable discrepancies were found for FVES1409 marker previously mapped to LG4D. In this study, this marker amplified five loci that mapped to different linkage groups (LG1D, LG6A, LG6Ba, LG7B and LG7D).

Due to our recent findings of errors in the genotypic data of 'Redgauntlet' × 'Hapil' progeny which was used for map construction in previous studies, the result highlighted above was not surprising. The result supports the theory that the existing SSR-based 'Redgauntlet' × 'Hapil' linkage map was constructed using erroneous genotypic data. To improve the quality and density of the existing linkage map, a 90K strawberry SNP array will be genotyped in the 'Redgauntlet' × 'Hapil' population in Year 3 of this study. This will allow map re-construction, which will be used for QTL detection.

Phenotypic data analysis and relationship between traits

Phenotypic data collected for 25 different flowering-related, fruit-related and plant characteristic-related traits was analysed using statistical software. Means were calculated across six replications, and histograms representing distribution of progeny mean values were plotted (Figure 5). Most of the traits showed continuous variation in the progeny and close to normal distributions were observed for almost all traits. With the exception of petal number, leaflet number and crown number, the progeny means for given traits were not normaly distributed as expected, due to the small scale used for evaluation of these traits. Progeny showed high variation in most trait values, as was previously reported in the Year 1 report.

Correlations among the 25 traits were calculated for data collected in 2013. Similar correlation matrices were produced, showing the relationship between different traits to the matrices reported last year (Figure 6). However the correlation matrix produced this year was plotted using mean values across the six replications. In total, 122 data points were plotted in this study. In contrast, scatterplot matrices were plotted using all data, thus 732 data points were plotted and reported last year.

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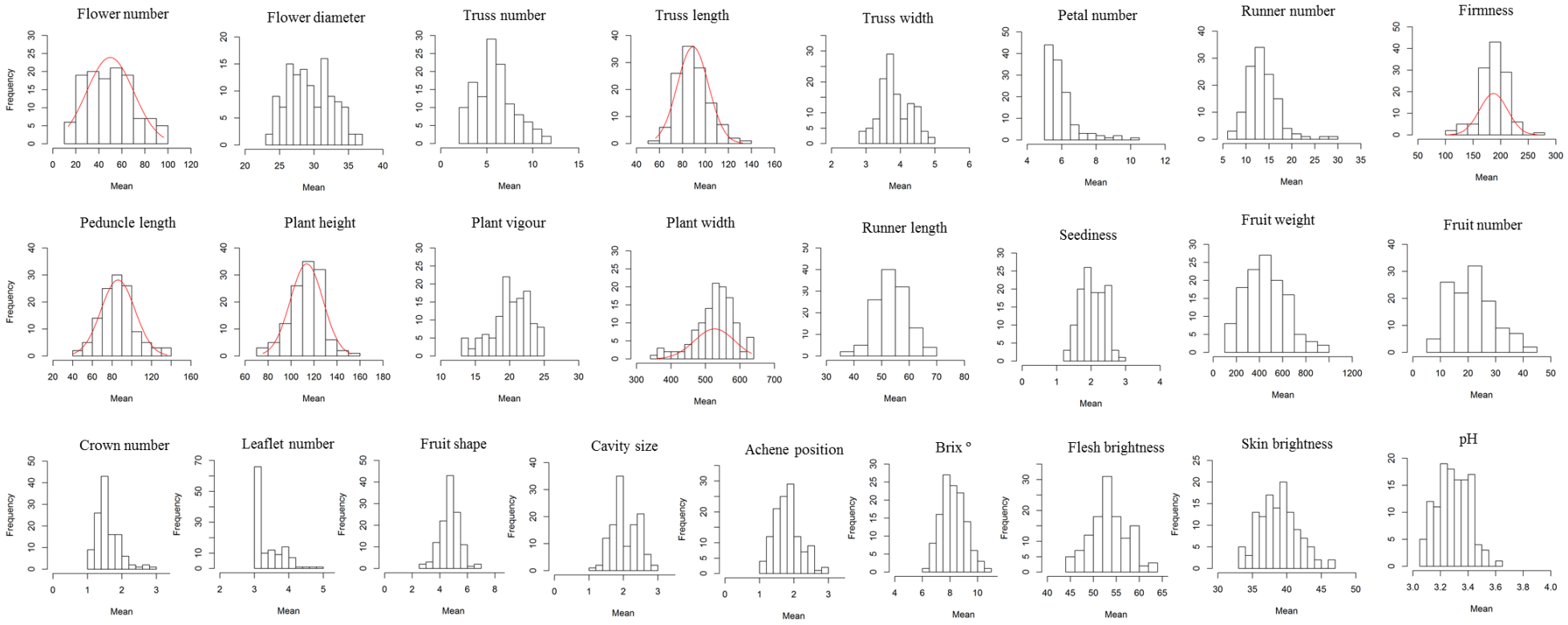

Figure 5. Distribution of the progeny mean values for 25 different traits. Mean values are the means of the six replications for 2013.

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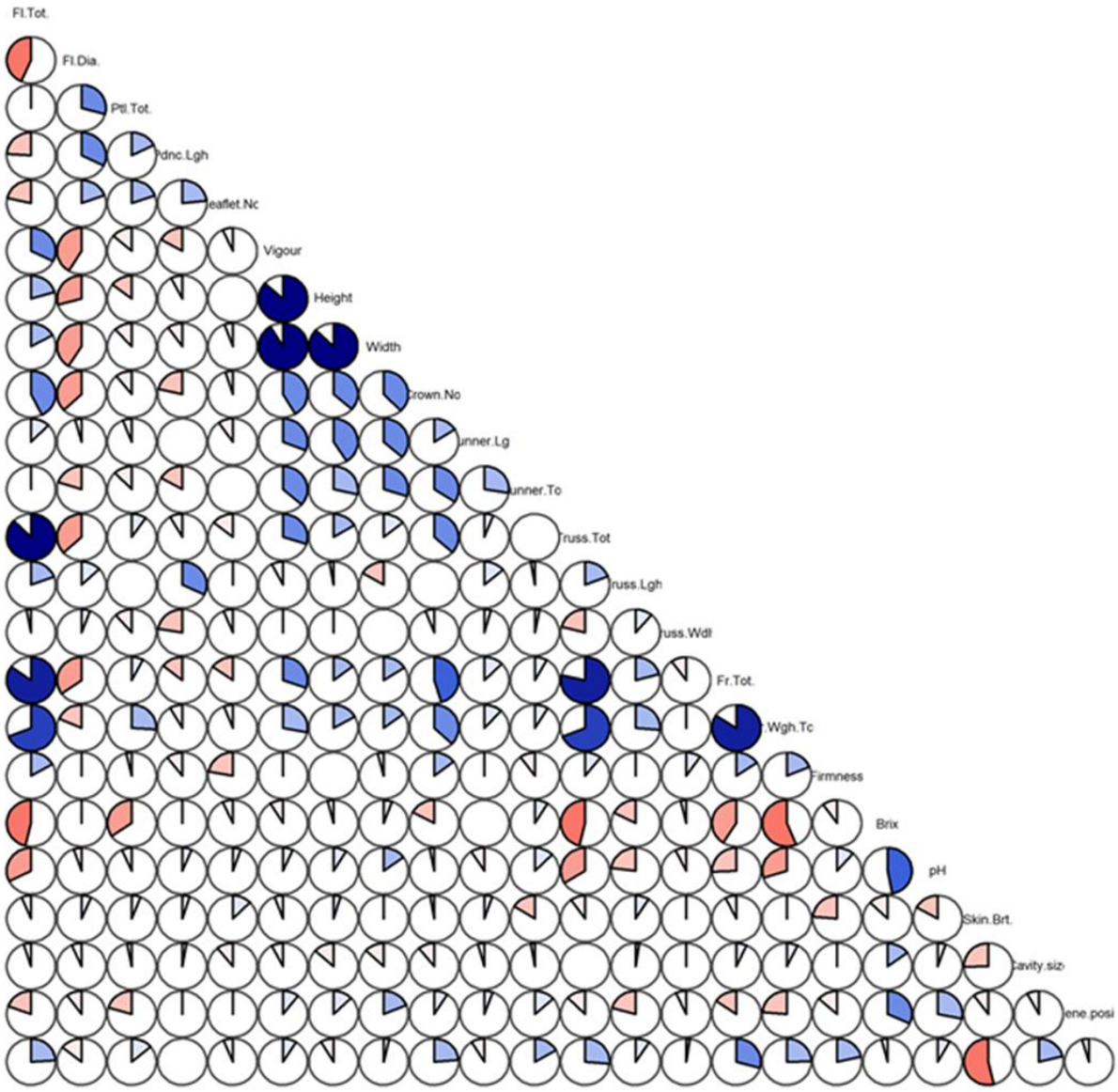

Figure 6. Pair-wise correlation matrix of 22 different plant characteristic and fruit quality traits phenotyped within 'Redgauntlet' × 'Hapil' mapping population. Positive correlations are highlighted in blue, negative correlations are highlighted in red colour, whereas white pies represents non-correlated traits. Different blue and red shades represents the level of correlation coefficient. * The last circle on the bottom right represents seediness.

Similar results reported in Year 1 were observed when correlations between different traits were investigated. The most correlated traits were flower number and diameter, plant vigour, height and width, runner and truss number, seediness, firmness, Brix º and pH. The least correlated traits were peduncle length, crown number, fruit shape, cavity size and flesh and skin brightness.

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Preliminary QTL analysis

Genotype means of Year 1 (2013) phenotypic data for the mapping population were used for QTL analysis. The Kruskal-Wallis analysis was performed for detection of QTL using the consensus 'Redgauntlet' × 'Hapil' linkage map. A total of 33 QTL were detected, 21 associated with plant characteristics-related traits and 12 associated with fruit-related traits (Table 4). All QTL locations on the linkage map detected in this study are presented in Figure 8.

Table 4. The most significant potential QTL detected using the non-parametric Kruskal-Wallis test. Linkage groups (LG) highlighted in red represent QTL which are present in 'Redgauntlet' strawberry cultivar. Linkage groups highlighted in blue indicate QTL which are present in 'Hapil', whereas linkage groups in pink represent the QTL which is present in both parental genotypes.

Traits	No. of QTLs	Location of QTLs
Plant characteristic traits		
Flower number	1	LG3D
Flower diameter	3	LG2C, LG4A, LG7B
Petal number	1	LG1A
Peduncle length	1	LG3A
Leaflet number	2	LG3C, LG5D
Vigour	1	LG7A
Height	2	LG7A, LG7D
Width	1	LG1C
Crown number	2	LG2A, LG3D
Runner length	2	LG6Bb, LG7A
Runner number	1	LG3D
Truss number	1	LG3D
Truss length	2	LG3A, LG7D
Truss width	1	LG3D
Total	**21**	

Traits	No. of QTLs	Location of QTLs
Fruit quality traits		
Fruit number	1	LG3D
Fruit weight	1	LG3D
Firmness	2	LG1A, LG7B
Brix	1	LG5B
pH	1	LG5C
Skin brightness	1	LG3D
Flesh brightness	1	LG4A
Cavity size	2	LG3C, LG5D
Achne position	1	LG5B
Seediness	1	LG3D
Total	**12**	

A total of 12 QTL were derived from 'Redgauntlet', 20 were derived from 'Hapil' and the remaining one was derived from both parents of the cross. The majority of plant characteristics traits, such as leaflet number and flower diameter, were associated with QTL derived from 'Redgauntlet'. In contrast, fruit-related trait QTL, including fruit number, fruit weight, seediness and pH, were derived mainly from 'Hapil'. Plant width QTL identified on LG1C was derived from both parental genotypes.

Interestingly, QTL associated with traits which were closely correlated from correlation analysis (Figure 6), were detected on the same linkage groups and nearly the same

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regions. For example, flower number correlated with truss number and both QTL were mapped on LG3D. Similarly, plant height and vigour showed closely related correlation and QTL associated with these two traits were identified within nearly the same region on LG7A.

Out of 33 QTL identified, 16 mapped to a single location on the linkage map, 7 mapped to two different linkage groups and one QTL associated with flower diameter mapped to three different linkage groups (Table 4, Figure 8). Linkage group 3D had the most QTL mapped (associated with nine different traits) and the majority of them are overlapping in the chromosome region (Figure 7). This may suggest that the same gene(s) are controlling different traits. Similar findings were reported in apple, where QTL associated with major categories of fruit quality, such as flavour, firmness, fruit size and nutritional composition, were allocated on the same linkage group 16.

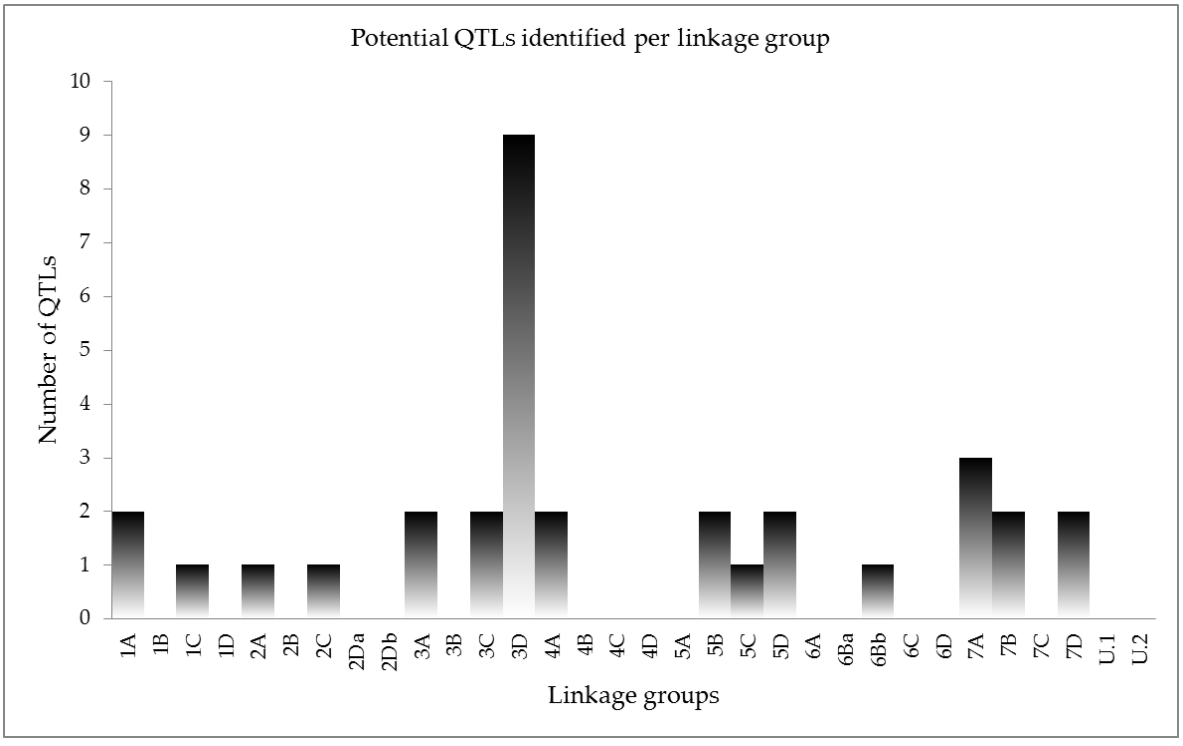

Figure 7. Number of QTL identified per linkage group of the 'Redgauntlet' × 'Hapil' linkage map.

QTL detected to date are preliminary. Further analyses, such as permutation test and interval mapping, will be performed later in the research in order to estimate QTL locations more precisely. Moreover, QTL analysis will be based on 2 to 3 years of phenotypic data (2013, 2014 and 2015) to investigate the environmental effect.

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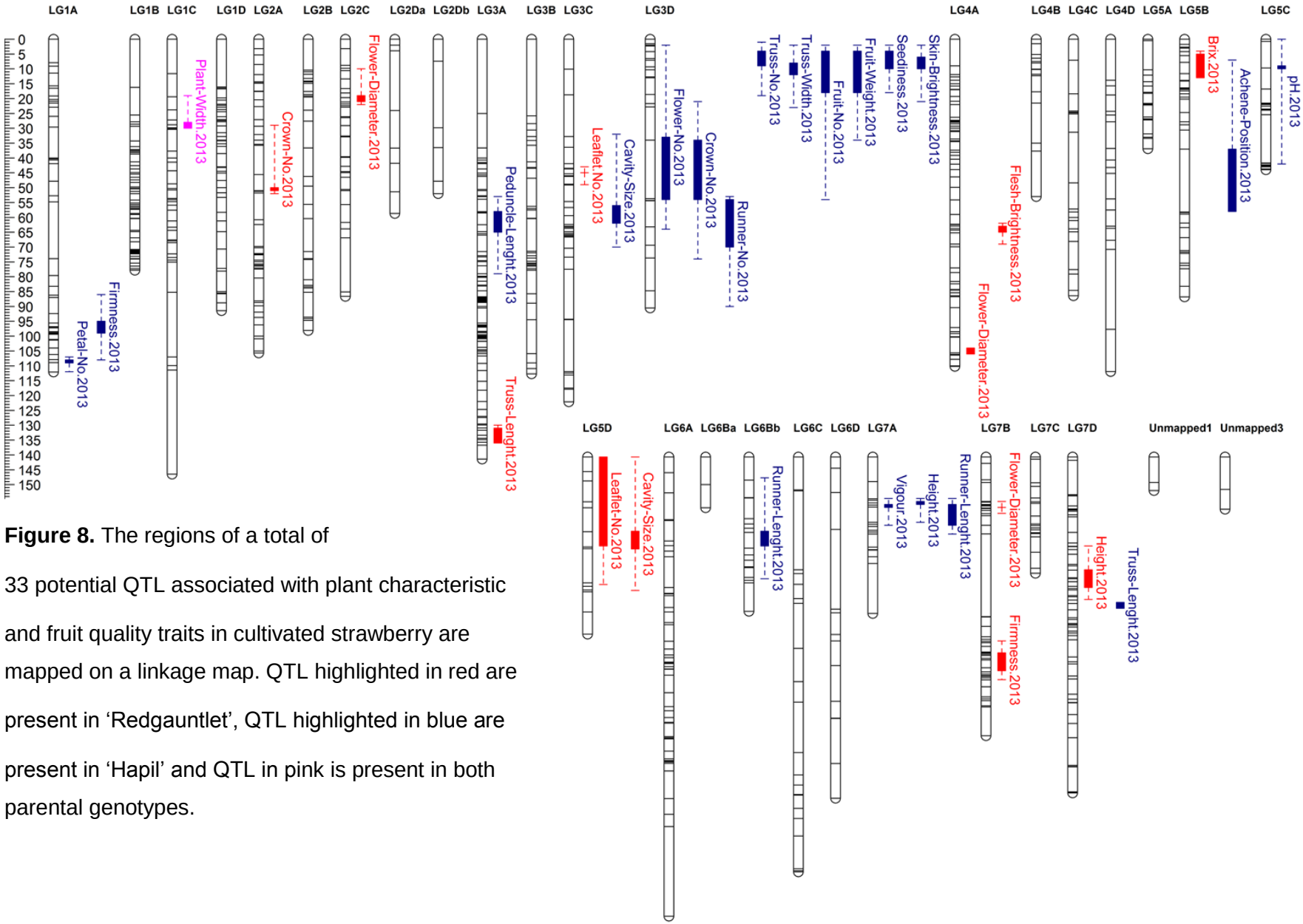

Figure 8. The regions of a total of 33 potential QTL associated with plant characteristic and fruit quality traits in cultivated strawberry are mapped on a linkage map. QTL highlighted in red are present in 'Redgauntlet', QTL highlighted in blue are present in 'Hapil' and QTL in pink is present in both parental genotypes.

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The objectives to be achieved in Year 3

- The saturation of genetic linkage map of 'Redgauntlet' × 'Hapil' population using 20K SNP array.
- Year 3 (2015) phenotyping of the most important fruit quality traits of 'Redgauntlet' × 'Hapil' mapping population.
- QTL detection across three years (2013, 2014 and 2015) phenotypic data.
- Candidate gene identification linked to fruit quality and development and mapping novel molecular markers.
- Testing novel markers in different octoploid mapping population ('Sonata' × 'SDBL123') for their applicability and potential usage in MAS.

Conclusions

- An existing SSR-based 'Redgauntlet' × 'Hapil' linkage map was used for saturation of gaps. A total of 63 new loci were successfully mapped.
- Phenotypic data from Year 1 (2013) and genotypic data was used for preliminary QTL identification. Thirty-three potential QTL were detected linked to fruit quality and plant characteristics. A "hotspot" of 9 QTL mapped to the same linkage group 9 suggests that the same gene(s) may control the majority of fruit quality and plant characteristics traits.
- Phenotypic data for Year 2 (2014) was collected of 'Redgauntlet' × 'Hapil' population. Data analysis is still on-going.

Knowledge and Technology Transfer

- 24-26 June – 7th International Rosaceae Genomics conference. A poster was presented entitled 'QTL identification and phenotyping of fruit quality and disease resistance traits in octoploid strawberry (*Fragaria* × *ananassa*)'.
- 16-17 September – HDC Studentship Conference. A poster was presented entitled 'QTL identification and phenotyping of fruit quality and disease resistance traits in octoploid strawberry (*Fragaria* × *ananassa*)'.

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