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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Report:	Annual report, September 2013
Previous report:	None
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Date project completed (or expected completion date):	1 March 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.]

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

GROWER SUMMARY

Headline

Phenotypic 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 high fruit quality. These traits rely on good plant architecture and high levels of pest and disease resistance. To remain competitive and financially viable, it is essential that growers extend the cropping season and adapt their production systems to the particular growing environment.

The objective of this project is to provide both phenotyping and genetic techniques to improve high-throughput trait identification in industry-funded breeding programmes. Very little is currently 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 the 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, a 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. Two different mapping populations were raised and used/will be used for phenotyping different traits. A total of 22 different traits were recorded of ‘Redgauntlet’ × ‘Hapil’ population. The preliminary data analysis suggests that there are both expected and unexpected correlations between traits. This information can be used for the identification and phenotyping only of the most important traits leading to significant cost and time savings.

The parental genotypes (‘Redgauntlet’ and ‘Hapil’) were screened for root architecture. Clear differences between genotypes have already been observed, but it is not known if this trait is correlated to Verticillium wilt resistance and/or drought tolerance. Further analysis will be carried out in 2014 to evaluate this hypothesis.

‘Sonata’ × ‘SDBL123’ mapping population will mainly be used for fruit quality phenotyping, QTL identification and the development of novel molecular markers that can be later used in MAS.

Financial benefits

For this annual 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.

SCIENCE SECTION

Introduction

The cultivated strawberry (*Fragaria × ananassa*) is an important fruit crop in temperate regions of the world and has a long history of artificial selection for improved cultivars (Hummer et al. 2011). During the last decade, an enormous amount of research has been conducted towards the development of marker-assisted selection (MAS) in plant breeding. The use of MAS in plant breeding programmes can improve the pre-selection process by significantly reducing the number of seedlings at a very early stage of breeding, thus enhancing breeding efficiency (Jannink et al. 2010; Whitaker, 2011). However, despite advances in molecular biology, such as next-generation sequencing technologies, and due to cultivated strawberry's complex octoploid genetics, MAS strategies have had limited success in improving the pre-selection process in this crop.

Markers for fruit quality and disease resistance traits are developed by a process known as QTL mapping. The process involves associating phenotypic variation, in this case resistance to wilt and key fruit quality traits with genetic markers, using the progeny from a biparental cross. However, it is well known that 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 level of resistance (Sargent et al. 2012). It is only recently that series of molecular markers have been developed for fruit quality traits in *Fragaria*. These include genes linked to seasonal flowering (Sugimoto et al. 2005), fruit flavour, size, firmness, shape and metabolites (Lerceteau-Köhler et al. 2012).

Understanding the association between phenotype and genotype is essential for plant breeders when selecting for optimal cultivars. In general, QTL mapping approaches consider only a trait at a time (or a handful of traits) and often there is little information about how different traits are correlated at the phenotypic and genotypic level. This is important to understand, as it could be that there are tradeoffs between traits either caused by the same QTL (i.e. the QTL is affecting multiple traits) or by linkage drag, (the desirable QTL is coupled with some other genetically linked undesirable traits). This might limit the effectiveness of MAS in breeding, depending upon the nature of the marker-trait combinations being utilized.

In this study, work has been carried out to understand how complex traits are correlated in the octoploid strawberry (*Fragaria × ananassa*) at the phenotypic and genotypic level, by developing novel phenotyping approaches to study this and to investigate how this affects the deployment of markers in breeding. The discovery of the best and easiest traits to phenotype and developing simple ways of capturing phenotypic data in an automated fashion will speed up the development of novel breeding approaches, first through MAS and then genomic selection (GS). GS is a technique that estimates the breeding value (specified based on the breeder's criteria) for a given seedling based purely upon its genotype. To be successful the GS model requires excellent phenotypic data for a full range of traits to be gathered on a training population.

A particular focus of current research is phenotyping two suites of traits, disease resistance and fruit quality. An octoploid strawberry mapping population containing a total of 122 seedlings and parental genotypes ('Redgauntlet' and 'Hapil') was used for phenotyping flower-related traits, root architecture and plant characteristics. The population segregates for a number of disease resistance, such as *Verticillium* wilt and powdery mildew and work is underway to characterize the nature of *Verticillium* wilt disease resistance and to uncover and map molecular markers linked to it. In addition, a new mapping progeny was raised ('Sonata' × 'SDBL123') to investigate correlations within fruit quality traits in octoploid strawberry. The population will be phenotyped for key fruit quality traits such as firmness, sugar levels, fruit size and shape in 2014 summer for the first time. Data will be used for molecular marker development for fruit quality traits in cultivated strawberry using QTL mapping.

A summary of 22 traits phenotyped within 'Redgauntlet' × 'Hapil' mapping population are provided for 2013. Preliminary data suggests that there are both expected and unexpected correlations between different physical traits in a strawberry mapping population. This might mean that there are trade-offs present, which may inform the deployment of markers in strawberry breeding programmes. The existing SSR-based linkage map ('Redgauntlet' × 'Hapil') will be used for locating genes of interest for MAS and to identify QTLs associated with phenotyped traits.

Materials and methods

Plant material

An F_1 mapping population was raised from the cross between two octoploid strawberry (*Fragaria* × *ananassa*) cultivars 'Redgauntlet' × 'Hapil'. A total of 188 seedlings were raised from the cross and of those 122 seedlings were randomly selected and further propagated by pinning down the runners. Ten replications of the 122 seedlings and parental genotypes ('Redgauntlet' and 'Hapil') were produced, thus 1,240 plants (including parents) were planted in the open field at East Malling Research late September 2012. The segregating population and parental genotypes were grown under tunnels from late spring until September 2013 (Figure 1).

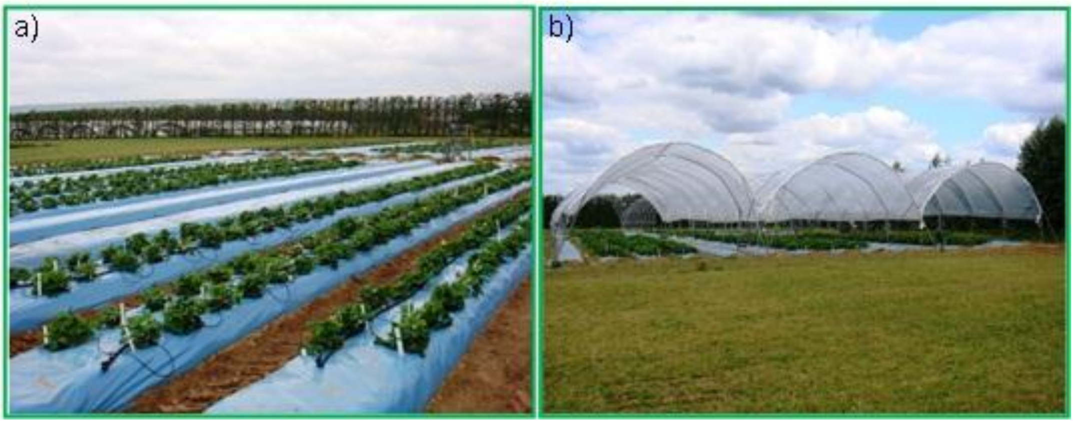

Figure 1. Seedlings of 'Redgauntlet' × 'Hapil' mapping population and parental genotypes planted in September 2012; a) Seedlings without cover before phenotyping, photograph was taken on 17.05.2013; b) seedlings under cover while recording the phenotypic data, photograph was taken on 25.06.2013

A new F_1 mapping population of 211 individuals was obtained from the cross between the octoploid strawberry variety 'Sonata' and the genotype 'SDBL123' for phenotyping fruit quality traits (Figure 2). Seedlings are growing under polytunnel conditions at East Malling Research.

Figure 2. ‘Sonata’ × ‘SDBL123’ mapping population in the polytunnel. Seedlings were propagated in August 2013. Photographs were taken on 07.08.2013 and 16.09.2013

Phenotyping

A total of 22 flower related, plant characteristics, fruit and root related traits were phenotyped on ‘Redgauntlet’ × ‘Hapil’ mapping population and parental genotypes for 2013. The summary of traits measured is presented in Table 1. Due to a shortage of tunnel coverage, six replications of each seedling and parental genotypes were phenotyped (Figure 1 b).

Data analysis

For the two parents and the progeny, the mean and standard deviation of some of the traits were calculated using measurements across replications. Pairwise comparisons among parental genotypes were done for all recorded traits to identify possible segregating traits. Spearman rank correlation coefficients were calculated for the different trait values using R Statistical analysis software (R Development Core Team (2011), Austria).

Results

Phenotypic analysis of parental genotypes

The mean of the trait values of the two parental genotypes ‘Redgauntlet’ and ‘Hapil’ were calculated combining all data between replications for the 22 traits analysed (Table 1). Under tunnel conditions, the two parental genotypes exhibited different behaviours throughout the phenotyping season from late-April to the end of August. Although a number of traits phenotyped were similar in both genotypes, some traits expressed clear differences. The most significant differences between parental genotypes were observed for flower diameter, the number of flowers per plant, the length of the longest runner, the number of runners per plant (Table 1), flowering time (Figure 3) and root architecture (Figure 4). Compared to ‘Hapil’, ‘Redgauntlet’ started to flower 17 days earlier and had more than twice the number of flowers. It also had longer runners than those of ‘Hapil’. However, ‘Hapil’ had larger diameter flowers, longer stamens and stronger trusses than ‘Redgauntlet’.

It is worth noticing that truss length, truss width and number of crowns per plant were recorded twice; once when the first flower opened and again when all flowers were opened on each plant. The data shows that the length and width of trusses increased as expected. The length of truss increased nearly three and two times in ‘Redgauntlet’ and ‘Hapil’ respectively, whereas width of truss increased by 0.7 mm and 1.2 mm. The number of crowns increased in both cultivars.

When root architecture was compared between parental genotypes, clear differences were observed between two cultivars (Figure 4). ‘Redgauntlet’ produced many wider and longer lateral roots (branching) and less main roots, whereas ‘Hapil’ showed many primary roots (main roots) with less branched laterals.

Table 1. The summary of recorded traits for mapping population and mean values for ‘Redgauntlet’ and ‘Hapil’ parents

Trait	Unit	Genotype	
		'Redgauntlet'	'Hapil'
Flower related traits			
Date of first opened flower		27 April	13 May
Date of 25% opened flowers		13-15 May	29-31 May
Date of 50% opened flowers		22-24 May	31 May - 3 June
Date of 75% opened flowers		31 May - 3 June	5-7 June
Date of 100% opened flowers		19 June	19 June
Total number of flowers per plant		85.7	41.5
Flower diameter	mm	25.3	32.8
Number of petals per flower		6.2	6.1
Plant characteristics traits			
Plant vigour		4-5	2-5
Plant height	cm	19-33	7-30
Plant width	cm	90-132	34-132
Length of longest stamen	mm	88.6	95.6
Number of leaflets per leaf		3-4	4
Truss length	mm	49.1[a]	84.5[a]
	mm	145[b]	115.7[b]

Truss width	mm	3.1[a]	3.9[a]
	mm	3.8[b]	5.1[b]
Total number of trusses per plant		8.2	4.8
Date of first runner		21 May	21 May
Length of the longest runner	mm	55.1	47.9
Total number of runners per plant		14	10.3
Number of crowns per plant		1.5[a] 1.7[b]	1.3[a] 1.5[b]
Fruit related traits			
Date of first ripped fruit		21-24 June	24 June
Root related traits[c]			
Root architecture		More branching	More main root

[a] Values were recorded when first flower opened per plant

[b] Values were recorded when all flowers were opened per plant

[c] Phenotyped only parental genotypes

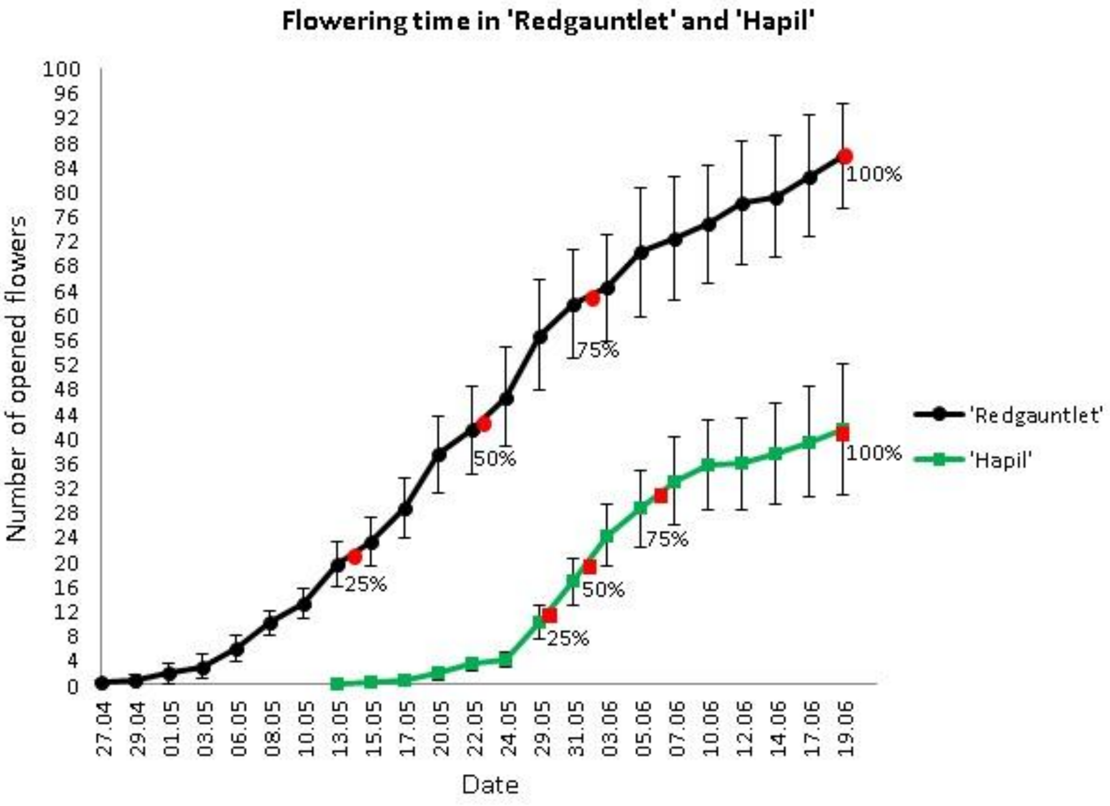

Figure 3. Flowering behaviours of the parental genotypes. Red dots indicate the percentage of open flowers

Figure 4. The differing root structure of parental genotypes (‘Hapil’ and ‘Redgauntlet’ respectively). Plants were grown in ‘mini’-rhizotrons from small runners for 7 weeks. Photo was taken on 23.07.2013

The flowering time related traits were measured by counting opened flowers per seedling and the parental genotypes three times a week (Monday, Wednesday and Friday). Three flowers per plant were selected randomly to measure flower diameter using manual fruit size calibration (Figure 5). Strawberry plant height and plant width were recorder once a week (Wednesday) using a 50 cm long rule. The length of the three longest runners was measured once using the 50 cm rule, whereas the length of stamen, length and width of truss were recorded using calipers twice (Figure 5).

Figure 5. Trait phenotyping using different instruments of the ‘Redgauntlet’ × ‘Hapil’ mapping population. Photographs were taken between May – July 2013

Phenotypic analysis of mapping population

Six replications of 122 seedlings were phenotyped for 22 different traits presented in Table 1. Although phenotypic data analysis of mapping population is still an on-going process, preliminary results suggest that there are both expected and unexpected correlations between different physical traits.

Here we present correlations between 10 different traits phenotyped within ‘Redgauntlet’ × ‘Hapil’ mapping population (Figure 6).

The 10 traits showed continuous variation in the progeny and close to normal distributions were observed for almost all traits (Figure 6). Progeny showed high variations in most trait values, with the exception of number of petals and flower diameter, as expected.

Phenotypic correlations among 10 traits were calculated using R statistical software. Correlation values between traits are shown along the diagonal in Figure 6.

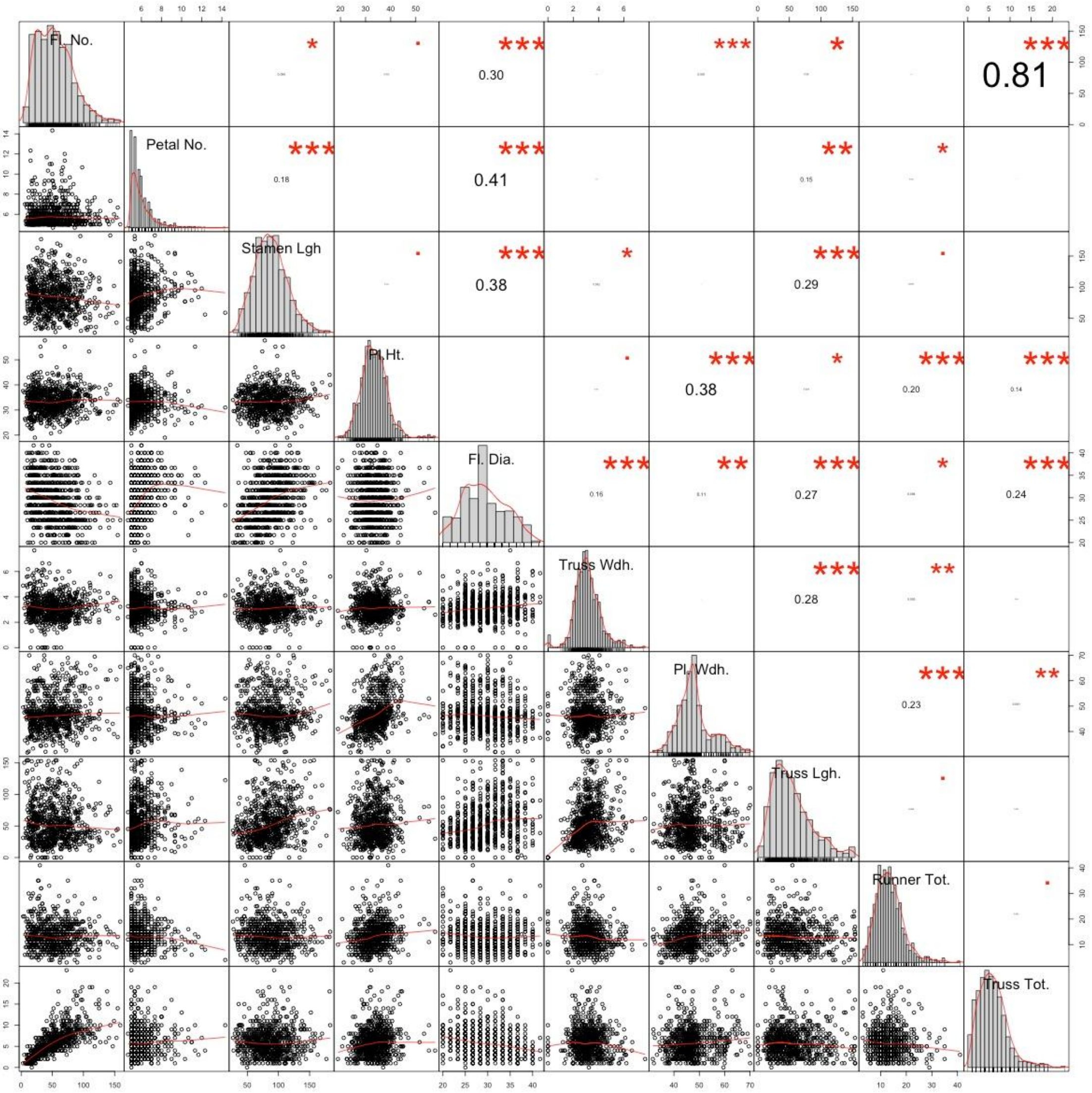

Figure 6. Scatterplot matrix of 10 plant architecture traits phenotyped within 'Redgauntlet' × 'Hapil' mapping population. Histograms, kernel density overlays, Spearman rank correlations and significance asterisks are presented (***=p<0.001, **p<0.01, *=p<0.05). Traits are the following: number of flowers, Fl. No.; number of petals, Petal No.; length of stamen, Stamen Lgh.; plant height, Pl. Ht.; flower diameter, Fl. Dia.; width of truss, Truss Wdh.; width of plant, Pl. Wdh.; length of truss, Truss Lgh.; total number of runners, Runner Tot.; total number of truss, Truss Tot.

Out of 45 possible correlations 20 showed no correlations, six correlations were significant at (*=p<0.05), four correlations showed a higher level of significance (**p<0.01) and the remaining 15 correlations were highly significant (***=p<0.001). The number of flowers (Fl. No.) showed a strong positive correlation with a total number of trusses (0.81). Correlations between the number of petals (Petal No.) and flower diameter (Fl. Dia.), the length of stamen (Stamen Lgh.) and flower diameter, and plant height (Pl. Ht.) and width of plant (Pl.

Wdh.) were also highly significant (***=p<0.001). In contrast, the number of flowers did not show correlation with plant height and total number of runners (Runner Tot.). Interestingly, flower diameter was correlated (p<0.001 - p<0.05) with eight other measured traits, with the exception of plant height. Thus, bigger plants have a higher number of longer trusses and produce longer stamens and larger fruits. However, data shows a negative correlation between the number of flowers and flower diameter, thus on plants that have more flowers the size of the flowers is smaller.

Discussion

The main aim of this study was to investigate any possible phenotypic correlations between traits of the octoploid strawberry (*Fragaria* × *ananassa*) and better understand the associations between phenotype and genotype. The data collected and analyzed here will help to identify the most important traits to be phenotyped in 2014, thus phenotypic time will be significantly reduced.

The parental genotypes (‘Redgauntlet’ and ‘Hapil’) studied here showed highly significant differences in a number of traits phenotyped, such as flowering time, number of flowers, flower diameter and root architecture, suggesting that although ‘Redgauntlet’ is an early highly cropping cultivar, ‘Hapil’ produces larger fruits that are easier to pick. Interestingly, highly significant differences in root architecture suggested that ‘Redgauntlet’ has more branching with less main roots, which is very helpful for wider absorption of minerals and water from the soil. Whereas the root structure of ‘Hapil’ appears to be completely opposite to that of ‘Redgauntlet’, with more main roots and less branching. This may explain the differences in drought tolerance between cultivars. In addition, it is unknown at present whether this phenotype is correlated with the differential response to *Verticillium dahliae* between these two parents. Phenotyping for root architecture of mapping population will be evaluated in summer 2014.

Comparison of the trait correlation values (Table 1, Figure 6) obtained for the population studied indicated expected and unexpected correlations between traits documented in this study. For example, the number of flowers is positively correlated with the total number of trusses (***=p<0.001). In contrast, unexpected correlation was observed between the number of petals and flower diameter, suggesting that larger flowers have more petals.

Fruit quality traits were phenotyped on the ‘Redgauntlet’ × ‘Hapil’ mapping population and data was kindly provided by A. Llorente. However, data analysis is still an on-going process and will be reported in the next annual report.

The objectives to be achieved in year 2 will be carried out as follows:

- The development of a genetic linkage map of ‘Sonata’ × ‘SDBL123’ population. This linkage map will be used for the identification of QTLs linked to fruit quality traits and the development and mapping of novel molecular markers;
- Screening of root traits in rhizoboxes;
- The investigation of the nature of *Verticillium* wilt disease resistance by analysis of the genome sequence data of six different wilt strains;
- Phenotyping the most important traits of the ‘Redgauntlet’ × ‘Hapil’ mapping population and QTL analysis. Novel marker development linked to disease resistance;
- Phenotyping key fruit quality traits of the ‘Sonata’ × ‘SDBL123’ population. Correlations between fruit quality traits will be investigated.

Conclusions

- A mapping population (‘Redgauntlet’ × ‘Hapil’) containing a total of 122 seedlings and parental genotypes was used for phenotyping flowering time-related, plant characteristics, root-related and fruit-related traits in the field.
- Correlations between phenotypes have been investigated. However this is still an on-going process. Preliminary phenotypic data analysis showed expected and unexpected correlations between the traits recorded.

Knowledge and Technology Transfer

9-10 September 2013 - HDC annual conference - A poster was presented entitled ‘Phenotyping fruit quality disease resistance traits in octoploid strawberry (*Fragaria × ananassa*)’.

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