Mapping quantitative physiological traits in apple (*Malus × domestica* Borkh.)

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Abstract

Efficient breeding and selection of high-quality apple cultivars requires knowledge and understanding of the underlying genetics. The availability of genetic linkage maps constructed with molecular markers enables the detection and analysis of major genes and quantitative trait loci contributing to the quality traits of a genotype. A segregating population of the cross between the apple varieties 'Fiesta' (syn. 'Red Pippin') and 'Discovery' has been observed over three years at three different sites in Switzerland and data on growth habit, blooming behaviour, juvenile period and fruit quality has been recorded. QTL analyses were performed, based on a genetic linkage map consisting of 804 molecular markers and covering all 17 apple chromosomes. With the maximum likelihood based interval mapping method, the investigated complex traits could be dissected into a number of QTLs affecting the observed characters. Genomic regions participating in the genetic control of stem diameter, plant height increment, leaf size, blooming time, blooming intensity, juvenile phase length, time of fruit maturity, number of fruit, fruit size and weight, fruit flesh firmness, sugar content and fruit acidity were identified and compared with previously mapped QTLs in apple. Although 'Discovery' fruit displayed a higher acid content, both acidity QTLs were attributed to the sweeter parent 'Fiesta'. This indicated homozygosity at the acidity loci in 'Discovery' preventing their detection in the progeny due to the lack of segregation.

Introduction

Breeding apple varieties of high quality, meeting grower demands and satisfying consumer requests is a time-consuming and challenging task. Strong self-incompatibility, slow growth and a long juvenile phase of the plants hamper the efficient crossing and the fast selection of desired genotypes. Between 20 and 25 years elapse between the actual crossing and the release of a new variety (Kellerhals and Meyer, 1994). Further complicating is the fact that a large number of characters has to be taken into consideration such as resistance against a variety of diseases and pests, growth habit and tree vigour, fruit characters like flesh firmness, flavour, sugar and acid balance, let alone numerous generative traits such as juvenile phase length, blooming habits, and alternate fruit bearing.

The long generation cycles and the impossibility of back crossing make apple breeding a very promising field for marker-assisted selection and breeding (MAS and MAB). This method applies molecular markers, linked to the genes of desired traits, for selection instead of selecting the plants according to their phenotypic behaviour (Kellerhals *et al.*, 2000).

The availability of molecular markers and genetic linkage maps both allows the localisation of major genes with bulk segregant analysis (BSA) as well as the dissection of complex, polygenic traits into a number of quantitative trait loci (QTLs) (Lander and Botstein, 1989; Haley and Knott, 1994; van Ooijen

and Maliepaard, 1996). With this focus, the genetic determinants of a number of physiological traits, such as growth, blooming, and fruit qualities have been investigated.

Vegetative growth of apple trees is largely determined by environmental conditions and can be influenced by means of light, temperature, and nutrition. Growth characters and tree form of apple trees however, are mostly controlled through pruning and the selection of rootstocks on which the varieties are grafted.

Few studies have been carried out on the underlying genetics of growth habit. Lawson *et al.* (1995), Seglias (1997) and Conner *et al.* (1998) reported a number of genomic region-carrying QTLs involved in the control of growth characters. Lawson and Seglias reported only few responsible factors, one for branching habit and two for plant size, whereas Conner described over 10 chromosomal regions influencing growth characters, indicating a very complex trait involving a large number of QTLs, each contributing only little to total variability. This makes it difficult to detect and identify influential genes. Moreover, these properties are easily affected by environmental factors, which additionally complicate their detection.

Nevertheless, the knowledge of the genetic factors and the understanding of their mode of action will simplify their application in breeding and the production of new varieties including growth-governing rootstocks will be put on a larger genetic basis and potentially be accelerated (Fischer, 1994a, b).

Blooming behaviour and fruit quality traits are good candidates for a successful application of marker assisted selection, since the characters concerned are expressed after only several years. This represents a great potential of saving time, thus accelerating breeding and selection progress.

Most important with respect to blooming is the length of the juvenile period, determined by the appearance of the first inflorescences, which can last up to 12 years (Fischer, 1994a). A short juvenile phase is desired to quickly reach productivity or to have the plants available for further crosses. Various methods have been described to shorten the time until first flowering, including growing under ideal conditions in the greenhouse, and/or grafting on the dwarfing rootstock M9 (Visser, 1964; Aldwinckle, 1975; Fischer, 1994a). However, dwarfing rootstock M27 was reported to have very little effect on juvenile phase length (Verhaegh *et al.*, 1988). Accelerated growing in the greenhouse, although resulting in first flowering as early as 16 months after sowing, is impractical since it results in tree lengths of 2 to 5 m (Zimmermann, 1971; Aldwinckle, 1975). Several crab apples have been described to normally have short juvenile periods (Zimmermann, 1972), so time to first flowering can be shortened not only by cultural methods but also with targeted breeding for this quality (Visser, 1965).

Visser (1970) disclosed the relation between seedling vigour and juvenile phase length and demonstrated them to correlate inversely (Visser, 1970; Verhaegh, 1988). Independent of whether they are grown on their own roots or grafted, seedlings with thicker stems flowered earlier and were more productive. Therefore, seedling vigour, expressed as stem diameter, could be used as a marker for precocity and productivity. It is expected to find the genetic determinants of these characters close to each other or at least on the same chromosome.

Apple cultivars are known to vary in flowering date by up to more than 30 days. Since apple trees are most sensitive to frost when they are in full bloom, late-flowering selections often avoid freezing injury (Mehlenbacher and Voordeckers, 1991). For growers in regions with a high risk of spring frost, planting of late-flowering varieties might be a welcome alternative to the investment in wind machines and heaters. On the other hand, late blooming increases the risk of fire blight infection (Spotts *et al.*, 1976). Work has been devoted to establishing methods for the early selection of late-blooming genotypes by correlating late blossoming with time of leaf bud break (Tydeman, 1958), but Lawson *et al.* (1995) showed that the two characters are not correlated and are inherited independently. Among European cider apples, there are many late-flowering genotypes (Murawski, 1967) which represent valuable germplasm for the development of late-flowering breeding selections. Knowledge of the genetic factors involved can considerably simplify and accelerate their introgression in commercially grown cultivars.

Fruit qualities such as appearance, texture and flavour are key factors for the success of a cultivar on the market. Texture as a major component of consumer preference has been analysed in detail by King *et al.* (2001). Mechanical as well as sensory evaluations were performed and several QTLs have been detected for a variety of wedge fracture and compression measurements and qualities like crispness and juiciness (King *et al.*, 2001). Fruit appearance, form, size, colour and flavour are the other determinants of consumer preference. Flavour, which consists of

odour and taste, is most complex to analyse. Over 350 volatiles have been detected in apple (Maarse, 1991), 11 organic acids were identified in apple pulp with an additional 5 in whole fruit (Hulme and Rhodes, 1971).

In apple, the predominant factor of variation in flavour is the balance between sugars and acids. Malic acid, being the main substrate for respiration in apples, also represents the principal acid in apple fruit (Hulme, 1963). Content of malic acid in fruit has been proposed by several authors to be controlled by a single gene (Nybom, 1959; Knight, 1963; Visser, 1968; Visser and Verhaegh, 1978) although not always in unison (Visser *et al.*, 1968). The exact position of the *Ma* gene was determined by Maliepaard *et al.* (1998) by means of a genetic linkage map of the cross 'Prima' × 'Fiesta'. Since most varieties are of the heterozygous *Ma/ma* genotype (Brown and Harvey, 1971), 25% of the progeny of a cross will be homozygous for *ma/ma*, expressing an unacceptable low-acid phenotype and 25% will be homozygous for *Ma/Ma*, expressing an unpleasantly sour phenotype. Early pH indicators like leaf acidity as proposed by Visser and Verhaegh (1978) have not been widely applied due to their unreliability and their impracticability. Therefore, the knowledge of major genes and QTLs governing fruit acidity and the development of linked molecular markers enables an early selection and prevents the waste of resources by maintaining plants which will never express an acceptable phenotype.

For sugar content a polygenic, quantitative inheritance, independent of acidity, was proposed (Visser *et al.*, 1968, Brown and Harvey, 1971). Wide variation between cultivars was observed in total sugar content as well as in the proportions of the main sugars present, namely fructose, sucrose and glucose, whereas within cultivars contents and compositions were fairly constant from tree to tree and from year to year (Brown and Harvey, 1971). From these results a rather robust genetic determination of sugar content, which is not overly influenced by the environment, can be expected.

In our study, several physiological traits were investigated with the aim to determine the number and the location of effective major genes and QTLs governing these traits. The detection of such genes and genomic regions and the development of markers linked to genes of interest are important steps towards a successful MAS and MAB strategy. With the availability of closely linked markers, the presence of genes of interest can already be verified in the parents, enabling the breeders to select the right parents for the aimed

cross, which is not always unambiguously possible otherwise (C. Gessler, unpublished).

Here we report the analysis of several quantitative traits in an apple progeny of the cross 'Fiesta' × 'Discovery'. Growth characters, blooming traits and fruit quality QTLs were investigated at three different locations of clearly distinct climatic conditions in Switzerland over several years. QTLs for the respective characters are presented and their potential applicability in accelerated fruit tree breeding is discussed.

Materials and methods

Plant material and orchard locations

A cross between the apple varieties 'Fiesta' (syn. 'Red Pippin') and 'Discovery' was carried out in 1995 at Plant Research International, Wageningen, Netherlands. 'Discovery' is an open-pollinated descendant of 'Worcester Pearmain' and, supposedly, 'Beauty of Bath', discovered in 1949 and named in 1962. With its low to medium vigour, 'Discovery' reaches productivity rather late. The early-ripening summer variety produces fruit with excellent flavour, tightly connected to the tree, so there is almost no fruit fall at maturity (Silbereisen *et al.*, 1996). 'Fiesta' was introduced in 1985 as a cross of 'Cox Orange' × 'Idared' at HRI East Malling. It is a slightly later-ripening variety than 'Discovery', more vigorous and reaches a steady productivity early (Aeppli *et al.*, 1989).

Seed of this cross were purchased by the Swiss Federal Institute of Technology, Zurich, sown in 1996 and grown on their own roots for three years at the Swiss Federal Research Station for fruit growing, viticulture and horticulture (FAW), Wädenswil, Switzerland. The entire progeny of about 330 plants was tripled in summer 1998 by bud-grafting on M27 rootstocks and planted in winter 1998/1999 at three different locations in Switzerland, Wädenswil (Zurich) at 47° 13′ 20″ N, 8° 40′ 05″ E, 455 m altitude, Conthey (Wallis) at 46° 12′ 30″ N, 7° 18′ 15″ E, 478 m altitude, and Cadenazzo (Ticino) at 46° 09′ 35″ N, 8° 56′ 00″ E, 203 m altitude. The trees were grown in rows 3.5 m apart and with tree-to-tree distances of 50 cm in Wädenswil and Conthey and of 125 cm in Cadenazzo. Orchard and plant maintenance included mulching between the rows, in-row herbicide treatment and sprays against aphids at all sites and sprinkler watering in Conthey. No fungicides were applied. Climatic conditions of the three locations are characterized with the

average temperature in °C, rainfall in mm and hours of sunshine in the growing period between March and October of the years 1999 to 2001 (Table 1).

Field data collection and harvest

Growth data were collected in 1997 and 1998 on the seedlings in Wädenswil and from 1999 to 2001 on the replicates in all three locations. On the seedlings, length and width of mature leafs 6, 7 and 8, counted from the base of the one-year old shoot, were measured in the autumn of 1997. Leaf area was calculated by considering the leaves to be elliptic. Stem diameter was determined 30 cm above the ground in the summer of 1998 with a Vernier calliper. On the replicates, stem diameter was measured 30 cm above grafting point at the end of each growing season (late autumn 1999 to 2001). Height increment was evaluated in 1999 at all three locations.

Blooming data were collected from 1999 to 2001. In 1999, only blooming versus not blooming was recorded. A more accurate observation was performed in the springs of 2000 and 2001. At the estimated time of full bloom of the population, the percentage of open flowers and the number of flower bunches were determined per tree. The percentage of open flowers was recorded as a measure of flowering time. Early-blooming genotypes have a considerable part of open or already withered flowers at the time of evaluation, whereas late-flowering trees still carry unopened flower buds and just a few open flowers. This evaluation allowed scorings greater than 100% when the observation took place after full bloom and most flowers on a tree were withered. As an indication of juvenile phase length, the year of first bloom was recorded. Plants flowering for the first time in 1999 were assigned a 0, those first flowering in 2000 a 1, those first flowering in 2001 a 1, and 3 those not yet flowering in 2001 a 3. Although the recorded values are discrete, the underlying trait is continuous albeit observed with a rather low resolution.

Apples were harvested up to twice a week as they reached maturity in 2000 and 2001. All fruit from one tree were harvested at once and all collected apples were assayed. Harvested apples were immediately analysed or stored for no more than 10 days at 4 °C before analysis. Since parental trees were not planted before the autumn of 2000, fruit of these varieties were harvested and analysed only in 2001 in Wädenswil. Of each cultivar, 10 apples were collected and analysed as described below.

Fruit trait assessment

Harvest and fruit data consist of date of harvest, number of fruit harvested per tree, single-fruit weight, fruit flesh firmness, sugar content and acidity. Date of harvest and number of fruit were recorded in both years (2000 and 2001) at all locations. Fruit weight, sugar content and fruit flesh firmness were evaluated in Conthey and Cadenazzo in both years and in Wädenswil only in 2000, and, additionally, fruit acidity was measured.

Analyses of the fruit harvested from Conthey and Cadenazzo were conducted at the Swiss Federal Research Station for plant production in Changins (RAC), Centre les Fougères, Conthey, on a fully automated fruit analysis device, Pimprenelle (Setop Giraud Technologie, 84300 Cavaillon, France). Fruit from Wädenswil were analysed at the Swiss Federal Research Station for fruit growing, viticulture and horticulture (FAW), Wädenswil. Fruit flesh firmness was determined with an automated Magness-Taylor penetrometer (Stevens CR Analyser, C. Stevens & Son, St. Albans UK). Small areas of skin were removed with a peeler either two at opposite sides of the fruit or four at angles of 90° along the fruit equator. Fruit were then placed on a cork stand and penetrated by the mechanized probe. Penetrometer settings were identical in both systems (Pimprenelle, CR Analyser). Penetration speed was 4.0 mm/s and penetration depth 8.0 mm, both penetrometers were equipped with a 11 mm probe, and the readings were recorded as maximum force in g/cm^2 of skinned cortex tissue.

Penetrated fruit were pressed with a hand operated squeezer and the juice collected. Sugar content was determined with a digital refractometer (model PR-1, Atago, Tokyo Japan) by adding a few drops of apple juice onto the lens of the measuring device. Refractometry results were recorded in °Brix which equals to grams sugar per 100 ml juice at 20 °C (1 °Brix corresponds to ca. 18 g/l sugar).

Acidity measurements were executed immediately after harvest, or collected juice was stored at −20 °C for up to 2 months. Fruit acidity was determined as titratable acid and results were recorded in grams malic acid per kg juice. For these acidity tests a Mettler-Toledo autotitrator DL 67 in combination with a sample changer ST20A (20 positions) and a connected analysis scale model PM 4600 was utilized.

Table 1. Climatic characterization of the three orchard locations with average temperature, rainfall and hours of sunshine during the growing season (1 March to 31 October). Standard values are based on climatic data of the period 1960 to 1990.

	Year	Average temp. (°C)	Deviation in °C from standard	Total rainfall (mm)	Rainfall in % of standard	Hours of sunshine	Sunshine in % of standard
Wädenswil	1999	13.6	1.2	1186	118.6	1314	103.4
	2000	13.9	1.5	1045	105.6	1414	108.5
	2001	13.6	1.2	1296	140.4	1384	107.9
Conthey	1999	14.7	1.4	475	131.8	1580	95.7
	2000	14.9	1.6	371	100.2	1686	101.9
	2001	14.6	1.3	505	136.5	1649	98.9
Cadenazzo	1999	16.2	1.5	2179	155.7	1347	88.0
	2000	16.3	1.6	1789	128.3	1473	95.7
	2001	15.8	1.1	1493	113.7	1585	103.7

Data analysis

Plants that had not been genotyped previously (31 plants) (Liebhard *et al.*, in press) and outcrossed individuals (15 plants) were excluded from the analyses or analysed separately. A set of 251 phenotyped and genotyped individuals were available for trait analyses.

The phenotypic effect was estimated for each genotype by means of a linear model with genotype, location, year or group (i.e. location-year combinations) as explanatory factors. The corresponding ANOVAs were performed with S-Plus 6.0 (Insightful Corp.). Fruit harvest data were available with several measures (fruit) per individual and year and location or even several measures (penetrometer readings) per fruit and individual and year and location. Such data were averaged over fruit and over tree, and the ANOVA was performed as described above with the average values. The amount of total variability explained by those factors was estimated from the 'type III sum of squares'.

QTL mapping

QTL analyses of the ANOVA estimates of the traits for each genotype as well as of the year and location specific data sets were performed with MapQTL 3.0 (van Ooijen and Maliepaard, 1996). Both single parent genetic linkage maps as well as the integrated linkage map of 'Fiesta' × 'Discovery' (Liebhard *et al.*, in press) were used to determine the parental chromosome, carrying the effective allele, the positions and

the effects of the QTL with the Kruskal-Wallis single-locus analysis and the maximum-likelihood-based interval mapping approach. Since the integrated map consists of markers with different segregation types, the 'all marker mapping approach' (Knott and Haley, 1992) was applied. This method employs not only the flanking markers but also markers from neighbouring intervals to calculate the probabilities of a QTL. For the interval mapping approach, five neighbouring intervals and a step size of 1 cm was used. Significance thresholds for the presence of a QTL were determined according to van Ooijen (1999) and fixed at a LOD score of 2.5 for single parent maps and 3.0 for the integrated map.

Results of the QTL analysis consisted of information about the parental chromosome on which the more effective QTL allele/s is/are located, i.e. F+, F−, D+ or D−, the genetic position of the QTL on the parental map (Liebhard *et al.*, in press), the LOD score for the presence of a QTL at this position, and the percentage of phenotypic variability attributed to the genotype explained. Since the QTL analysis detects phenotypic differences associated with the two involved parental alleles, the results presented include information on the specific expression of the trait connected with the indicated allele.

Results

Phenotypic data

Growth

Stem diameters of two-year old seedlings ranged from 0.7 mm to 32.9 mm with a population average of 17.1 mm. Leaf size obtained from one-year old seedlings ranged from 0.2 cm^2 to 60.1 cm^2 with an average of 22.7 cm^2. The correlation coefficient between stem diameter data and leaf size data was 0.31, and the measures are considered not to correlate so that the traits must be inherited independently. The largest values for all growth traits were obtained from trees grown in Cadenazzo, followed by plants grown in Conthey and Wädenswil. Taking the climatic conditions into account (Table 1) these results become plausible since Cadenazzo clearly provides the most favourable growing conditions, i.e. highest temperatures combined with sufficient rainfall and hours of sunshine.

Weak correlations were obtained between stem diameter and height increment. R values of 0.41, 0.39, and 0.34 were calculated for Wädenswil, Conthey and Cadenazzo, respectively.

For stem diameter measurements, the variability proportion contributed by the genotype amounted steadily to about 50% whereas the proportion contributed by the environment increased from 20% in 1999 to 27% in 2001. For height increment, the variance proportions amounted to 32% and 25% for genotype and environment, respectively (Table 2).

Blooming

More flower bunches were observed and more trees were blooming in Cadenazzo in 2001 than in the two other locations, indicating an advanced development of these trees (Table 3). This observation corresponds with the growth measurement, showing the same ranking of the three locations. Since the number of flower bunches is strongly influenced by treatments like pruning in the previous year and parental trees were planted only in the autumn of 2000, no data was recorded on these varieties.

Since percentage of open flowers observed is highly dependent on the time of evaluation, population means as well as minimum and maximum scores have no significance on their own, except as an indication of the time of evaluation. These records are valuable for the ranking of the individuals with respect to their blooming time or for a comparison with the parental varieties. However, blooming time showed transgressive segregation in the progeny both towards earlier and later flowering, i.e. earlier than Discovery and later than Fiesta.

Variability proportions contributed by the genotype and the environment were 27% and 39% for number of flower bunches, and 52% and 14% for juvenile phase length, respectively (Table 3).

Fruit harvest

A comparison of the population means showed that the trees grown in Cadenazzo ranked at the top end of the scale, bearing earlier ripening, heavier and softer fruit with higher sugar content, followed by the plants in Conthey and Wädenswil. In 2000, the largest number of fruit-bearing trees was found in Cadenazzo, although more trees were flowering in Conthey. In 2001, trees from Conthey yielded most fruit although most trees were flowering in Cadenazzo (Table 4).

In all fruit quality traits, the variability contributed by the genotype ranged from 40% to 68% and the contribution of the environment was less than 24% (Table 4A).

The results of the fruit analysis of the parental varieties showed clear differences between the two varieties in harvest time, fruit weight, sugar content and acidity, whereas fruit flesh firmness was about the same (Table 4B).

QTL analysis

Growth traits (Table 5)

With the analysis of seedling data, five QTLs for growth characters were identified, three associated with stem diameter and two associated with leaf size (Table 5). None of these seedling QTLs could be detected with data obtained on the replicated (grafted) plants. Nine genomic regions associated with stem diameter were identified in the replicates and even though three are positioned on the same chromosomes as QTLs for seedling stem diameters, they could be clearly distinguished by their location. Six genomic regions were found to be associated with height increment. Five of them are located on the same chromosomes as QTLs for stem diameter and four are actually coinciding with stem diameter QTLs (Table 5). The QTL for stem diameter on linkage group F1 showed to be more effective in 1999 than in later years, whereas the QTLs for the same trait on linkage groups D2 and F15 could not be detected or only weak in 1999 and grew increasingly stronger from 1999 to 2001. Since

Table 2. Stem diameter and leaf size of 251 'Fiesta' × 'Discovery' seedlings and height increment and stem diameter of bud-grafted triplicates at three different sites with population minima, maxima, means and standard deviations (SD). Variability proportions contributed to each trait by genotype and environment are indicated.

Measurements on seedlings		Leaf size 1997 in cm^2	Stem diam. 1998 in mm		
Min		0.2	0.7		
Mean		22.7	17.1		
Max		60.1	32.9		
SD		10.4	5.1		

Measurements on replicated plants		Height increment 1999 in cm	Stem diameter 1999 in mm	Stem diameter 2000 in mm	Stem diameter 2001 in mm
Wädenswil	Min	0.0	4.0	10.0	12.0
	Mean	24.7	11.9	16.3	21.6
	Max	102.0	18.0	30.0	32.0
	SD	17.0	1.7	2.9	3.7
Conthey	Min	0.0	5.0	5.0	6.0
	Mean	34.5	12.0	16.8	20.8
	Max	105.0	20.0	27.0	36.0
	SD	21.3	2.1	3.4	4.8
Cadenazzo	Min	0.0	4.0	2.0	5.0
	Mean	50.5	14.1	21.0	26.8
	Max	101.0	22.0	31.0	47.0
	SD	19.3	2.9	5.0	5.9
Variability proportions					
Variability contributed by the genotype		32%	52%	50%	51%
Variability contributed by the environment (= site)		25%	20%	26%	27%
Variability contributed by interactions genotype × environment (within-genotype variability)		43%	28%	24%	23%

effects like growth of continuously expressed traits are accumulated and summed over the years, QTL results presented on stem diameter are, unless otherwise stated, based on the measurements of 2001.

The presence of the alleles indicated for growth traits are inducing thicker trunk, bigger leaves and larger height increment than their alternative alleles.

Blooming traits (Table 6)
Five QTLs were detected for the investigated blooming characters. Three were associated with blooming time and were located on linkage groups 7, 10 and 17. Two were associated with the number of flower bunches and were located on linkage groups 8 and 15. A further two were found to be associated with juvenile phase length and were located on linkage groups 3 and 15 (Table 6). The QTLs for number of flower bunches and for juvenile phase length on linkage group 15 were found to be very close to each other but on the alternative chromosomes F− and F+.

The presence of the alleles observed for blooming traits induces earlier flowering, a larger number of flower bunches and shorter juvenile phase compared to the alternative alleles.

Table 3. Blooming data of 251 'Fiesta' × 'Discovery' progeny plants in 2000 and 2001 at three sites with population minima, maxima, means, and standard deviations (SD). '% open flowers' represents blooming stage of the trees at time of evaluation (before full bloom < 80%, at full bloom = 80%, after full bloom > 80%), as an indication of flowering time. 'Time of first flowering' represents the end of juvenile phase by the appearance of first inflorescences. Variability proportions contributed by the genotype and the environment to each trait are indicated.

	Spring 2000	Spring 2001	
Wädenswil	68 of 251 trees flowering	161 of 251 trees flowering	
	number of flower bunches	number of flower bunches	% open flowers
Minimum value observed	1.0	1.0	0.0
Population mean	4.2	10.0	11.2
Maximum value observed	10.0	53.0	80.0
Standard deviation	3.1	9.1	19.1
Fiesta	n.a.	n.a.	3.0
Discovery	n.a.	n.a.	36.0
Conthey	146 of 251 trees flowering	189 of 251 trees flowering	
	number of flower bunches	number of flower bunches	% open flowers
Minimum value observed	n.a.	1.0	0.0
Population mean	n.a.	11.8	8.2
Maximum value observed	n.a.	71.0	80.0
Standard deviation	n.a.	11.9	17.9
Fiesta	n.a.	n.a.	4.0
Discovery	n.a.	n.a.	33.0
Cadenazzo	135 of 251 trees flowering	227 of 251 trees flowering	
	number of flower bunches	number of flower bunches	% open flowers
Minimum value observed	1.0	1.0	0.0
Population mean	8.9	29.9	39.4
Maximum value observed	43.0	97.0	80.0
Standard deviation	7.9	18.5	26.2
Fiesta	n.a.	n.a.	17.0
Discovery	n.a.	n.a.	61.0
Variability proportions	number of flower bunches		Time of first flowering
Variability contributed by the genotype	27%		52%
Variability contributed by the environment (= year × location or location)	39%		14%
Variability contributed by interactions genotype × environment (within genotype variability	37%		37%

Fruit traits (Table 7)

One strong QTL was found for the trait 'harvest date' on linkage group 3, originating from the early ripening parent Discovery and explaining 16% of the phenotypic variability. The trait 'number of fruit' could be attributed to three genomic regions on linkage groups 5, 15, and 16, whereas for 'fruit weight' not less than 8 QTLs were identified.

Four QTLs were found for fruit flesh firmness with the one on linkage group 3 accounting for 27% of phenotypic variability followed by the ones on LGs 6, 11, 12, and 14, with 16%, 11,%, 8% and 6%, respec-

Table 4A. Fruit harvest data of 'Fiesta' × 'Discovery' progeny genotypes in 2000 and 2001 at three sites with population minima, maxima, means, and standard deviations (SD) of harvest date ([Julian] Day of harvest), number of fruit, single fruit weight in gram, fruit flesh firmness (FFF) in g/cm^2 penetration resistance, sugar content in °Brix, and fruit acidity in grams titratable acid per kg juice as well as variability proportions contributed by the genotype and the environment to the traits evaluated more than once.

	Harvest 2000						Harvest 2001					
	day of harvest	number of fruit	fruit weight	FFF	sugar content	fruit acidity	day of harvest	number of fruit	fruit weight	FFF	sugar content	fruit acidity
Wädenswil	54 of 251 fruit-bearing trees						110 of 251 fruit-bearing trees					
Min	206	1.0	33	5173	9.5	3.3	221	1.0	n.a.	n.a.	n.a.	n.a.
Mean	219.3	6.5	99	10905	12.3	8.9	235.0	7.3	n.a.	n.a.	n.a.	n.a.
Max	232	30.0	199	17936	16.4	18.7	250	36.0	n.a.	n.a.	n.a.	n.a.
SD	7.0	5.7	35	2301	1.1	3.7	7.5	6.3	n.a.	n.a.	n.a.	n.a.
Conthey	69 of 251 fruit-bearing trees						165 of 251 fruit-bearing trees					
Min	207	1.0	53	5213	10.9	n.a.	210	1.0	55.5	4540	11	n.a.
Mean	216.2	5.8	100	9688	13.9	n.a.	219.4	9.7	105.4	9728	14.1	n.a.
Max	267	14.0	195	14066	19.6	n.a.	236	43.0	251	14124	17.7	n.a.
SD	16.7	3.2	28	2159	1.7	n.a.	11.0	7.9	31.4	1994	1.3	n.a.
Cadenazzo	84 of 251 fruit bearing trees						158 of 251 fruit bearing trees					
Min	204	1.0	52	1180	7.3	n.a.	205	1.0	52.2	3280	10.8	n.a.
Mean	212.6	4.4	159	9520	14.6	n.a.	214.4	8.2	119	9379	15.3	n.a.
Max	218	18.0	171	14875	17.3	n.a.	229	32.0	232.1	14790	19.5	n.a.
SD	6.8	3.3	29	2572	1.4	n.a.	10.5	5.9	39.7	2045	1.5	n.a.

Variability proportions	Day of harvest	No. of fruit	Fruit weight	FFF	Sugar content
Variability contributed by the genotype	45%	40%	52%	68%	47%
Variability contributed by the environment (= year × location)	15%	10%	20%	4%	24%
Variability contributed by interactions genotype × environment (within-genotype variability	40%	50%	28%	28%	29%

Table 4B. Fruit harvest data of the parental varieties harvested in Wädenswil.

Parental varieties	Day of harvest	Number of fruit	Fruit weight	FFF	Sugar content	Fruit acidity
Fiesta	252	10	190	7195	10.7	7.3
Discovery	210	10	160	7109	13.3	8.1

tively. Sugar content of juice could be attributed to five genomic regions on linkage groups 3, 6, 8, 9 and 14.

Variability contributed by the genotype in the trait 'fruit acidity' was almost completely explained by two QTLs on linkage groups 8 and 16 accounting for 46% and 42%, respectively. Linkage group 16 is known to carry the *Ma* gene for malic acid (Maliepaard *et al.*,

1997). Two markers were identified as being tightly linked with the respective genes for acidity. On F8 the AFLP marker E31M38-0193 with the *aa* allele and on F16 the SSR marker CH05e04z with the 167 bp fragment. Fruit from trees carrying both acidity alleles showed an average of 11.2 g titratable acid per kg of juice, fruit from trees carrying only one of the alleles

Table 5. Growth QTLs detected in the segregating population of 251 progeny genotypes of the cross 'Fiesta' × 'Discovery' for leaf size and stem diameter in seedlings and for height increment and stem diameter in budgrafted plants at three different sites. For each QTL, the parental chromosomes from which it originates are indicated (chr), the position with the maximal likelihood on the parental map (Liebhard *et al.*, in press) (pos), the LOD score at this position (LOD), and the percentage of explained variability for this parent (expl). For weak QTLs or very small contributions, only the parental chromosome and possibly the position is indicated or the line is left bank. For the integrated 'FxD' map (integr), information is provided (in bold) on the LOD score (top line) and the variability explained (bottom line).

Linkage group	Leaf size, seedlings 1997					Stem diameter, seedlings 1998					Height increment, replicates 1999					Stem diameter, replicates 2001				
	chr.	pos	LOD	expl	integr	chr	pos	LOD	expl	integr	chr	pos	LOD	exp	integr	chr	pos	LOD	expl	integr
1																F-[a]				**6.1**[a]
																D-	25	5.3	11%	**13%**
2						F-	57	2	4%	**3.1**										**3.5**[b]
						D-				**6%**						D-	36	2.5	5%	**6%**
3															**2.4**					**2.6**
											D-	83	2	4%	**5%**	D+	91			**7%**
5															**3.2**					
											D+	85			**7%**					
8											F−				**4.0**					**2.9**
											D−	46	3.3	6%	**7%**	D−	33	2.2	4%	**5%**
9			**3.0**																	
	D−	54	2.9	5%	**6%**															
11															**3.3**					**3.5**
											D−	84	1.8	4%	**6%**	D−	15			**6%**
13															**3.7**					**1.7**
											D+	30	3.3	6%	**10%**	D+	30	1.5	3%	**4%**
14																F+	2	2.0	4%	**2.5**
																D+	9	2.3	4%	**5%**
15						F−	60	2.2	7%	**3.5**						F+[b]	0	2.0	4%	**2.0**
						D+	75	2.9	9%	**10%**										**5%**
16																				
17	F+	10	3.9	7%	**4.2**	F+	15	3.2	6%	**4.8**	F+	15	4.2	8%	**6.2**					**6.5**
					8%					**10%**	D+	6	2.1	4%	**11%**	D+	3	2.1	4%	**13%**

[a]QTL is more effective in 1998 and 1999 then in the later years.
[b]QTL undetectable or weak in 1998, grew increasingly stronger from 1999 to 2001.

averaged at 7.9 g and 7.4 g/kg titratable acid for the F8 and the F16 alleles, respectively, and trees which inherited none of the two Fiesta alleles produced fruit with an average of 5.8 g/kg titratable acid.

The presence of the alleles detected confer earlier ripening, more fruit, heavier fruit, harder fruit, sweeter fruit and more acidic fruit relative to the alternative alleles at the same loci.

All of the detected QTLs, except for seedling traits and fruit acidity, were found with several, if not with all, independent data sets of the specific trait evaluations per year or per location. This fact justifies the presentation of also weak QTLs like the ones for stem diameter on LG 8 or for height increment on LG 3. Although explaining only 5% of the variability, the QTLs were detected in all years and all locations and are therefore regarded as real QTLs and not artefacts, even if they might not reach a significance level of 0.05.

Discussion

All investigated traits, which could be compared with parental phenotypes, showed transgressive segregation. Since apple is an outbred, self-incompatible species it is rather expected to find heterozygous than homozygous loci. Therefore, a combination of the superior parental alleles is likely to result in a phenotype exceeding the parental value.

A direct comparison with parental trees was not always possible. However, a dissection of the complex traits into their responsible factors, as conducted here,

Table 6. Bloom QTLs found in the segregating population of 251 progeny genotypes of the cross 'Fiesta' × 'Discovery' for flowering time, expressed as '% open flowers' at the time of evaluation, number of flower bunches, and juvenile phase length, expressed as the year when the trees bloom for the first time. For each QTL, the parental chromosomes from which it originates are indicated (chr), the position with the maximal likelihood on the parental map (Liebhard *et al.*, in press) (pos), the LOD score at this position (LOD), and the percentage of explained variability for this parent (expl). For weak QTLs or very small contributions, only the parental chromosome and possibly the position is indicated or the line is left blank. For the integrated map (integr), information is provided (in bold) on the LOD score (top line) and the proportion explained (bottom line).

Linkage group	Flowering time					Number of flower bunches					Juvenile phase length				
	chr	pos	LOD	expl	integr	chr	pos	LOD	expl	integr	chr	pos	LOD	expl	integr
3											F+	62	3.2	7%	**4.0**
											D+				**8%**
7	F−	26	3.1	6%	**3.6**										
	D+	35	2.8	7%	**7%**										
8						F−				**3.6**					
						D−	54	2.9	6%	**7%**					
10	F−	2			**3.5**										
	D+	5			**13%**										
15						F−	18	4.2	13%	**5.1**	F+	17	3.0	6%	**3.2**
										10%					**6%**
17	F+	7	1.8	4%	**2.5**										
					5%										

is also possible without information on parental phenotypes, since the contribution of the parents can be assigned by means of the genetic linkage maps.

Growth

Yearly stem diameter increments recorded were believed to be too small and not robust enough to be used for QTL analysis. Therefore stem diameter records were considered, representing the sum of all increments in the past. As the phenotypic effects accumulate, most significant results were obtained with the latest records of 2001. Likewise, an accumulation of the environmental influence can be observed. Over the years, the differences between the locations were increasing, expressed by the increasing variability proportion contributed by the site. While at the same time, the within genotype variability, possibly originating from the nursery, levels out (decreases). Furthermore as trees grew thicker, measurement inaccuracies due to not completely round trunks and small unevenness in the bark like buds and knots proportionally decreased.

Based on data obtained from 7–8-year old seedlings, Visser (1970) reported a correlation between seedling vigour and precocity, which enables an early selection. Such results include a huge proportion of root growth and nutrient uptake influence, which is not given in the case of our observations on replicates on genetically identical rootstocks. However, a similar correlation, albeit weaker, was described for grafted plants (Visser, 1970). All differences observed on our test plants originate from the above surface part of the plants and the scion/rootstock interaction. Since seedling measurements are largely influenced by root growth genes, it is not surprising that the correlation analysis of growth parameters between seedling measurements and measurements on the grafted plants did not reveal any correlation. A correlation analysis of growth and blooming data of the replicates revealed only very poor correlations between stem diameter and juvenile phase length with R values below 0.3 and could therefore not confirm the findings of Visser.

Since plants behave differently depending on their age, it can be assumed that during the different stages in the life of an apple tree, different sets of genes are expressed for certain traits. Stem diameter records of consecutive years showed correlations with r values of up to 0.86, but grew continuously weaker with larger time differences between the measurements, reaching a low r value of 0.29 after 3 years. This result indicates that different genotypes reach top scores across evaluations, and furthermore, that possibly different genes govern one trait over time. In fact, the QTLs found to be responsible for seedling stem diameter were not the same detected in the replicates and even in the

Table 7. Fruit quality QTLs found in the segregating population of 251 progeny genotypes of the cross 'Fiesta' × 'Discovery' for the characters indicated. For each QTL, the parental chromosomes from which it originates are indicated (chr), the position with the maximal likelihood on the parental map (Liebhard *et al.*, in press) (pos), the LOD score at this position (pos), and the percentage of explained variability for this parent (expl). For weak QTLs or very small contributions, only the parental chromosome and possibly the position is indicated or the line is left blank. For the integrated map (integr), information is provided (bold) on the LOD score (top line) and the variability proportion explained (bottom line).

Linkage group	Harvest date chr	pos	LOD	expl	integr	Number of fruit chr	pos	LOD	expl	integr	Fruit weight chr	pos	LOD	expl	integr	Fruit flesh firmness chr	pos	LOD	expl	integr	Sugar content chr	pos	LOD	expl	integr	Fruit acidity chr	pos	LOD	expl	integr
1											F−	71	2.6	6%	**3.5** / **19%**															
3	D−	75	5.7	13%	**4.7** / **16%**						F− / D+	88			**3.3** / **8%**	F− / D+	84	16.8	34%	**12.3** / **27%**	F−	44	2.0	5%	**3.1** / **7%**					
4																														
5						F+ / D−	45 / 70			**3.2**ᵃ / **9%**																				
6											F+	51	4.0	11%	**3.9** / **12%**	F− / D+	48 / 24	2.8 / 2.9	16% / 6%	**6.5** / **16%**	F− / D−	44 / 62	4.9 / 4.2	17% / 15%	**5.1** / **10%**					
8											F−	13	2.4	5%	**3.6** / **9%**						D+	62	1.9	4%	**3.6%** / **11%**	F+	32	4.8	33%	**4.7** / **46%**
9																					D+	5	3.3	7%	**4.8** / **11%**					
10											F−	70	2.1	5%	**3.3** / **7%**															
11																F− / D−	38	4.8	1%	**4.7** / **11%**										
12											D+	50	2.7	6%	**3.8** / **8%**	F+ / D−	50	3	7%	**3.7** / **8%**										
13																														
14																F+	47	2.3	5%	**3.6** / **6%**	F− / D+	3 / 7	4.2 / 3.3	11% / 7%	**4.5** / **12%**					
15						F−	19	3.6	13%	**3.8** / **8%**	F+ / D+	41 / 75	2.5 / 2.3	13% / 5%	**2.5** / **15%**															
16						D−				**4.5** / **10%**	F− / D+	18 / 17	4.8 / 6.1	10% / 13%	**17.0** / **31%**											F−	12	4.9	36%	**6.2** / **42%**

ᵃdetected only in 2001.

Table 8. Comparison of QTLs detected in 'Fiesta' × 'Discovery' with other crosses. Linkage groups (LG) and traits are indicated.

Fiesta × Discovery	Prima × Fiesta	Iduna × A679-2	Wijcik McIntosh × NY75441-58 and -67	Rome Beauty × White Angel
Liebhard *et al.*, in press	Maliepaard *et al.*, 1997; King *et al.*, 2000, 2001	Seglias and Gessler, 1997	Conner *et al.*, 1997, 1998	Hemmat et al., 1994 Lawson et al., 1995
LG1: stem diameter		LG A 5: size		
LG3: FFF	LG3: FFF			
LG6: FFF fruit weight	LG6: compression stiffness fruit weight			
LG8: size			LG WM 7: size	
LG12: FFF	LG12: FFF			
LG14: stem diameter			LG NY58 2: stem diameter	
LG16: acidity fruit weight	LG16: malic acid gene fruit weight			
LG17: blooming time size			LG NY58 1: size	LG WA 1: blooming time

replicates different QTLs were identified to be most effective at different times during the three years of observation.

Although no strong correlation could be detected between stem diameter and height increment, QTLs detected for both traits on linkage group (LG) 8 possibly and on LG 13 and 17 certainly coincide.

Again the fact that the QTLs are not expressed at the same time as well as the influence of the other QTLs might obscure such a correlation. Furthermore it is known that trees change their growth rate when entering the reproductive stage (Verhaegh *et al*., 1988). A detailed analysis of stem diameter QTL on LG 14 showed that in 'Discovery' the trait is more pronounced in plants not yet bearing fruit, whereas in 'Fiesta' the contrary was the case.

Blooming

To avoid frost injuries during full bloom in spring, flowering time can be an interesting trait to breed for. To meet these specific demands, it is sufficient to incorporate the characters in certain varieties whereas a short juvenile phase should be globally introduced in breeding selection to accelerate breeding progress.

The trait 'number of flower bunches' is much more difficult to exploit since an optimum can hardly be defined. The right number of flowers depends on the size of the tree, on the time of blooming and the risk of frost, and on the fruit fall in June. Furthermore, the number of flower bunches is influenced by thinning and yield in the previous year. Investigations over a longer period of time are necessary to record more relevant aspects of the blooming physiology including alternate fruit bearing.

Fruit traits

Fruit quality traits are among the most promising for early and marker-assisted selection because they have the greatest potential of resulting in a significant reduction of costs since unfavourable genotypes can be discarded long before the insufficient fruit qualities become evident.

For obvious reasons, blooming traits and fruit traits are not completely independent. Intensively flowering trees will have better chances to achieve a high yield. One of the QTLs detected for the number of flower bunches, located on LG 15, actually coincides with a QTL detected for number of fruit. However, no correlation could be revealed between the two phenotypic traits ($r = 0.41$). The poor correlation may be due to the influence of the number of other QTLs affecting the same traits or due to the fact that still not all trees have been flowering and bearing fruit. It is possible that blooming behaviour as well as fruit traits change when trees reach their full productivity. Since plants are indeed still very young, the traits observed might

not be identical with the expression in later years. Therefore it is important to keep the trees under investigation to conclude whether such early records are representative and useful for selection.

For acidity a monogenic inheritance was proposed (Nybom, 1959; Knight, 1963; Visser, 1968; Visser and Verhaegh, 1978) with homozygous individuals being either too sweet (flat) or too sour. The result obtained here revealed two genetic locations, explaining 98% of the phenotypic variability attributed to the genotype. Surprisingly, both originate from Fiesta although Discovery is the more acidic parent. The QTLs determined map on LG 8 and 16, and the position on LG 16 correspond with the *Ma* locus determined by Maliepaard *et al.* (1998). The fact that no QTLs could be determined in Discovery indicates that no differences result when a progeny individual inherits one or the other Discovery allele. This inevitably leads to the conclusion that Discovery must be homozygous at all loci involved in fruit acidity or that the effects of the two alleles per locus are close to identical.

It is important to recognize that a QTL analysis can only detect differences between the inherited, parental alleles and not absolute effects. As the acidity situation shows, Discovery, due to its homozygousity, cannot inherit different alleles, so no QTLs can be detected. But this indicates by no means that Discovery does not inherit alleles affecting fruit acidity.

The same considerations justify the presentation of weak QTLs, which do not reach the predefined significance level. It is not the small effect of the QTL but the small difference between the involved alleles leading to low LOD scores. Furthermore the QTLs were detected in all years and/or all location and therefore regarded to be reproducible results and not artefacts.

To determine the genetic status of Discovery with respect to fruit acidity, further crosses will be necessary between 'Discovery' descendants with low acid content and, ideally, a known very low acid genotype. In such an F_2 cross, the measured difference between the two grand parental alleles, carried by the Discovery low-acid descendant, will be maximized and will be close to the absolute effect of the effective acidity allele from Discovery.

The detection of Discovery's homozygosity at the *Ma* locus further shows that other flavour and taste compounds are able to counterbalance a 'sour' genotype (*Ma/Ma*) and achieve a favourable phenotype. A situation which is rather unlikely to appear with a homozygous sweet genotype (*ma/ma*).

The plants investigated were very young, both with respect to 'genetic age', i.e. age since germination, and 'clonal age', i.e. age since grafting. It is known that plant age has an influence on fruit characters in that first harvests might not reach the best possible quality. However, ranking of the genotypes will not be affected by such changes and, furthermore, age effects are considered negligible compared to the environmental effects from the three different locations.

Comparison with mapped QTLs

QTLs for growth traits, blooming time and fruit qualities have been investigated and mapped by several groups (Lawson *et al.*, 1995; Seglias, 1997; Conner *et al.*, 1998; King *et al.*, 2000, 2001). Due to the lack of co-dominant microsatellite markers, full alignment is not feasible except in the case of 'Prima' × 'Fiesta' (Maliepaard *et al.*, 1998; King *et al.*, 2000, 2001). Therefore, only a limited comparison of the mapped QTLs is possible, which considers chromosomes but no map positions. Disclosed correspondences are listed in Table 8. Fruit flesh firmness and fruit weight were also evaluated and mapped by King *et al.* (2000, 2001) and the methods are directly comparable. For penetrometer readings, 12 and 5 QTLs were detected in 'Prima' × 'Fiesta' and in 'Fiesta' × 'Discovery', respectively, with only 3 on corresponding chromosomes. A similar situation appeared with fruit weight, with 8 and 3 QTLs in the 'Prima' × 'Fiesta' and the 'Fiesta' × 'Discovery' progeny, respectively, of which 2 were located on homologous chromosomes.

The detection of different QTLs for the same traits in half sib families at different locations as it is the case with the 'Fiesta' × 'Discovery' and the 'Prima' × 'Fiesta' progeny investigated here and by King *et al.* (2000, 2001), respectively, may have several explanations. If the alleles responsible for the phenotypic difference originate from the common parent ('Fiesta'), the genetic background might be responsible by influencing their expression differently. Also the environmental conditions might possibly be responsible for the expression only at particular locations. If the responsible alleles originate from the parent not in common, the same considerations are true, but additionally not both parents might carry alleles inducing phenotypic differences in the progeny.

For breeding purposes such comparisons are of great importance and value since they provide information on other alleles of the same QTL, reveal more

QTLs affecting the same trait and allow an estimation of the effectiveness of the QTL alleles in other genetic environments.

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