THE EUROPEAN APPLE MAP

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Abstract

In the European Apple Genome Mapping project a linkage map of apple was generated in a 'Prima' x 'Fiesta' progeny. Some 155 F_1 seedlings were genotyped, using 208 markers – RFLPs, RAPDs, isoenzymes and microsatellites. Markers heterozygous in 'Prima' could be assigned to 17 linkage groups, markers heterozygous in 'Fiesta' to 19 linkage groups. Markers heterozygous in both parents allowed the identification of 17 homologous pairs. Skewed segregation was observed for 14 marker loci mainly at two regions of the genome. Recombination frequencies were usually higher in 'Fiesta' than in 'Prima'. RFLP results indicated a large number of duplicate loci in the apple genome and provided evidence for at least three duplicated regions. Map positions of the *Vf* gene for scab resistance, the Sd_1 gene for resistance to rosy leaf curling aphid and the *S* locus for self-incompatibility were determined in this cross.

1. Introduction

Breeding of apple (*Malus pumila*) is seriously hampered by the long juvenile period and the high costs involved in maintaining progenies in the field. Many traits have to be assessed over several years or, as in the case of fruit traits, can only be observed when the adult phase is reached. Genetic analysis in apple is further complicated by its outbred character, the high chromosome number (2n=34), and the absence of unselected progenies in breeding programmes. Still, a large number of genes has already been identified in the *Malus* genus (Brown, 1992) and simple monogenic inheritance has been observed for many of these.

In many crops molecular markers have proved to be good tools for the detection of genes and for indirect selection of phenotypic traits. Since they provide a possibility of early selection independent of environmental conditions for the trait of interest, molecular

markers may be very useful in apple breeding. They also provide possibilities to differentiate between different alleles of a gene, combine genes contributing to a single or multiple traits and to control selection of homozygous or heterozygous individuals. These considerations led to the establishment of the European Apple Genome Mapping Project (King *et al.*, 1991).

Isoenzyme markers were the first molecular markers used for genetic analysis in apple. Isoenzyme studies reported linkage of GOT-1 (= AAT-1) to the self-incompatibility locus *S* (Manganaris and Alston, 1987), ACP-1 to pale green lethal gene *l* (Manganaris and Alston, 1988), LAP-2 to the mildew resistance gene present in 'White Angel' (Manganaris and Alston, 1992) and PGM-1 to *Vf* scab resistance (Manganaris *et al.*, 1994). Since then, RAPD markers too have been used to detect linkage to apple genes (Yang and Krüger, 1994; Koller *et al.*, 1995; Tartarini, 1996; Markussen *et al.*, 1995) and more reliable SCAR and CAPS markers have been developed from RAPDs linked to *Vf* (Gianfranceschi *et al.*, 1996; Yang and Korban, 1996) and the mildew resistance gene Pl_1 from *M. robusta* (Markussen *et al.*, 1995).

The first linkage map for apple was based on a progeny of 56 trees from a 'Rome Beauty' x 'White Angel' cross and combined isoenzyme, RAPD and some RFLP markers (Hemmat *et al.*, 1994). In the present study, RAPD markers and isoenzyme markers have been used to establish the genetic maps of the separate parents, while RFLPs and microsatellite markers heterozygous in both parents provide allelic bridges and allow the identification and alignment of the homologous linkage groups.

2. Materials and methods

2.1. Plant material

In 1988, a 'Prima' x 'Fiesta' cross was produced at CPRO-DLO, The Netherlands. 'Prima' is a scab resistant variety, carrying the *Vf* gene originating from *M. floribunda* clone 821. 'Fiesta', selected at East Malling from a cross between 'Cox's Orange Pippin' and 'Idared', is susceptible to scab and carries the Sd_1 gene, conferring resistance to two biotypes of rosy leaf curling aphid (*Dysaphis devecta*) (Alston and Briggs, 1977).

A seedling population was established in the field at Elst, The Netherlands, and replicate sets of 161 bud-grafted trees on M27 rootstocks were distributed to HRI East Malling (UK), HRI Wellesbourne (UK), INRA Angers (FR), IZZ Ahrensburg (GE, half the population) PIN Naoussa (GR) and DCA Bologna (IT, half the population) in 1993. A set of bud-grafted trees was also planted at the CPRO-DLO orchard in Elst (NL) in addition to the original seedlings. These trees were evaluated for a number of traits at all sites and provided material for molecular marker analysis and propagation (e.g. for greenhouse testing). Molecular markers indicated five outcross plants and a triploid tree. These six trees were excluded from linkage analysis.

2.2. Resistance evaluation

Scab infection was evaluated at six different sites (Ahrensburg, Angers, Bologna, East Malling, Elst, Naoussa) in field and greenhouse tests in 1994 and 1995. A consensus score of the resistance was developed based on scores which were in agreement between sites and years and taking into account the possible presence of scab race 6 in Ahrensburg and the lower infection pressure in Naoussa and Bologna. Also during 1994 and 1995, resistance to rosy leaf curling aphid (*Dysaphis devecta*) was evaluated on potted bench-grafted trees on M9 rootstocks in greenhouse tests at HRI-East Malling.

2.3. Molecular markers

A total number of 208 isoenzyme, microsatellite, RAPD and RFLP markers (Table 1) were used in linkage analysis. Markers scored on a subset of less than 90 trees, were

excluded. Also, dominant markers heterozygous in both parents were not used, since this marker type, segregating 3:1 in the progeny, is not very informative and likely to cause difficulties in establishing the linkage phase and marker order. Since RAPD markers are typically dominant markers, these were used only if they were heterozygous in one of the parents. Both genomic and cDNA probes were used for RFLP analysis. RFLP markers were selected specifically to provide codominant markers heterozygous in both parents. Therefore, most RFLP markers were first assessed in a subset containing the parents and 10 progeny plants, using six restriction enzymes. If a segregation pattern with any of these enzymes indicated possible heterozygozity of both parents, the plants in the progeny were screened as well.

Table 1 – Markers used in linkage analysis of 'Prima' x 'Fiesta' progeny

Marker type	Number of loci
Isoenzyme	14 (from 10 enzymes)
RFLP	91 (from 71 probes)
RAPD	97
Microsatellite	6
Total	208

Fourteen markers from ten isoenzyme systems segregated in the 'Prima' x 'Fiesta' progeny. Five microsatellite markers, developed at Hort+Research, New Zealand, and one microsatellite marker kindly obtained from Dr N. Weeden, Cornell University, USA, were also analysed in the progeny.

Three *S* allele specific primers indicating the presence of S_2, S_3 and S_5 (Broothaerts *et al.*, 1995; Janssens *et al.*, 1995) were used to determine the genotype at the *S* locus of the parents and the progeny plants.

Segregation in the progeny was scored for all markers and marker genotypes were collected in the central AppleStore database (Hyne, 1995). Linkage analysis was performed with JoinMap version 2.0 (Stam, 1993; Stam and Van Ooijen, 1995), a version which can handle all common segregation types occurring in outbred progenies. A LOD score of 4.0 was used to divide markers into linkage groups. The Kosambi map function was used to convert recombination frequencies into map distances.

3. Results and discussion

3.1. The linkage map

Table 2 shows the segregation types of the molecular markers used in the 'Prima' x 'Fiesta' cross. Markers heterozygous in 'Prima' were arranged into 17 linkage groups covering 753 cM, markers heterozygous in 'Fiesta' into 19 linkage groups covering 820 cM. The markers heterozygous in both parents allowed the identification of 17 homologous pairs. The two extra linkage groups of 'Fiesta' for which no 'Prima' counterpart was available consisted of a single marker pair each. Three markers could not be assigned to any linkage group. Marker pairs heterozygous in both parents allowed the comparison of the recombination frequencies in 'Prima' and 'Fiesta'. The recombination frequency was usually higher in 'Fiesta' than in 'Prima' (Figure 1). In a few cases a large difference was observed e.g. between the isoenzyme marker AAT-1 and the *S* locus with a recombination frequency estimate of 0.01 for the 'Fiesta' alleles and 0.24 for the 'Prima' alleles. Strongly skewed segregation was observed for the 'Prima' S_2 allele. Skewed segregation also occurred for 14 other marker loci ($P < 0.01$), mainly in two regions of the genome. In 11 cases the segregation distortion concerned alleles segregating from 'Fiesta', the male parent.

Table 2 – Marker segregations in 'Prima' x 'Fiesta' progeny

Segregation ratio	Number of loci
1:1 ('Prima' heterozygous)	88
1:1 ('Fiesta' heterozygous)	71
1:2:1	14
1:1:1	35
Total	208

3.2. Duplicate loci

The RFLP data provided evidence for the presence of a large number of duplicate loci. Of a total number of 133 RFLP probes that have been tested at CPRO-DLO on a subset of ten seedlings using six restriction enzymes, 75 corresponded to two loci. For 45 probes there was one segregating locus plus an extra non-segregating band (which may indicate a second locus for which one of the parents was homozygous). For eight probes, one segregating locus was observed without any indications of a second locus. For only five probes no polymorphic bands were found with any of the restriction enzymes.

In at least three cases, pairs (one triple) of duplicate loci were linked on different linkage groups, e.g. MC064a was linked to MC029b on one linkage group, while their counterparts MC064b and MC029a were linked on another, with similar recombination frequencies. This may indicate that larger regions of the apple genome are duplicated.

3.3. Mapped genes

Vf could be mapped to a linkage group comprising four RFLP markers, 12 RAPD markers and the isoenzyme marker PGM-1. In general, the order of the gene and the markers was in agreement with Gianfranceschi *et al.* (1996), Tartarini *et al.* (1996) and with the *Vf* region in a Gala x A679-2 cross of Gardiner *et al.* (1996). Tight linkage of OPM-18-0900, OPAL-07-0580, OPAM-19-2200 and OPU-01-0400 to *Vf* was confirmed. No recombinants were observed between the consensus resistance score and OPM-18-0900. Also with the CAPS marker M18, produced from OPM-18-0900 by Gianfranceschi *et al.* (1996), used as an RFLP probe, no recombinants were detected. Isoenzyme marker TPI-5 was mapped to the 'Fiesta' homologue of the *Vf* linkage group.

The gene Sd_1, conferring resistance to biotypes 1 and 2 of the aphid, is present in 'Fiesta'. The gene could be mapped to a linkage group together with three RFLP and four RAPD markers (Roche *et al.*, 1997). All three RFLP markers were closely linked, within a distance of 2 cM from the gene.

The *S* locus could be assigned to a small linkage group also comprising AAT-1, one RAPD and two RFLP markers.

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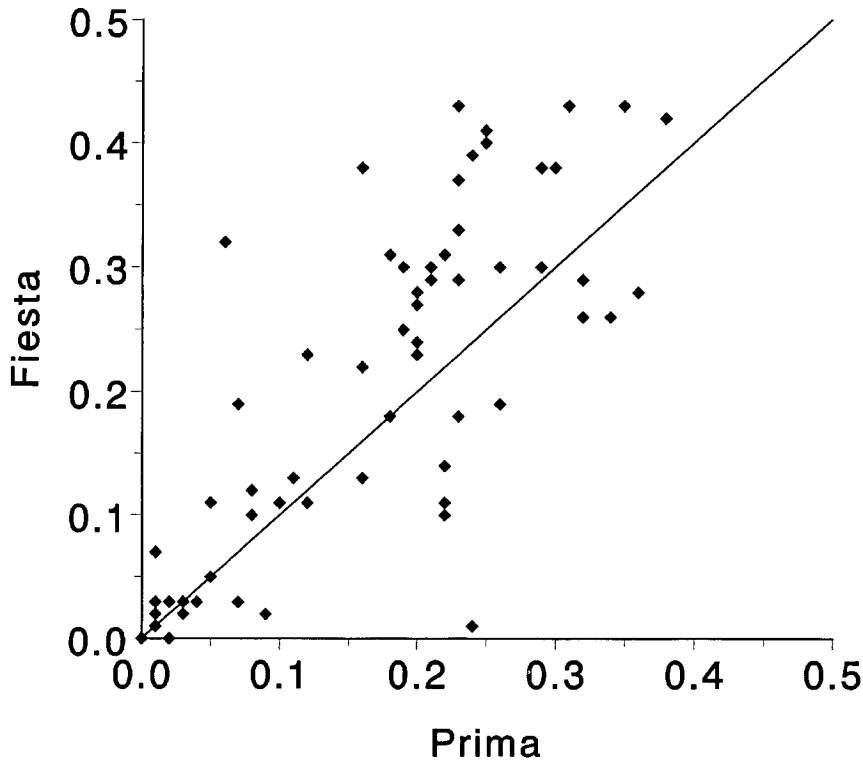

Figure 1 - Recombination frequencies in 'Prima' (female parent) and 'Fiesta' (male parent)