A detailed linkage map around an apple scab resistance gene demonstrates that two disease resistance classes both carry the Vf gene

Abstract A detailed genetic map has been constructed in apple (Malus × domestica Borkh.) in the region of the Vf gene. This gene confers resistance to the apple scab fungus Venturia inaequalis (Cooke) Wint.. Linkage data on four RAPD (random amplified polymorphic DNA) markers and the isoenzyme marker PGM-1, previously reported to be linked to the Vf gene, are integrated using two populations segregating for resistance to apple scab. Two new RAPD markers linked to Vf (identified by bulked segregant analysis) and a third marker previously reported as being present in several cultivars containing Vf are also placed on the map. The map around Vf now contains eight genetic markers spread over approximately 28 cM, with markers on both sides of the resistance gene. The study indicates that RAPD markers in the region of crab apple DNA introgressed with resistance are often transportable between apple clones carrying resistance from the same source. Analysis of co-segregation of the resistance classes 3A (weakly resistant) and 3B (weakly susceptible) with the linked set of genetic markers demonstrates that progeny of both classes carry the resistance gene.

Key words Malus × domestica · Venturia inaequalis · Malus floribunda · Genetic map · Disease resistance

Introduction

Combining disease resistance with the valuable characteristics of a commercially successful cultivar is a prime objective of most plant breeding programmes. In the domesticated apple (Malus × domestica) there has been a concerted effort to introgress genes that are derived from wild crab apples and that confer resistance to the apple scab fungus Venturia inaequalis (Crosby et al. 1992). This was achieved by modified backcrossing programmes which entailed repeated crossing of resistant progeny with commercial cultivars of Malus × domestica. In 1914, Crandall initiated a cross between cv Rome Beauty and the crab apple Malus floribunda (Sieb. ex Van Houtte) clone 821 that possesses the disease resistance gene Vf (Crandall 1926). The first commercial cultivar resulting from this cross was ‘Prima’, a fourth generation descendent of Malus floribunda 821 (introduced in 1970). This example demonstrates how slow traditional methods for introgressing genes from related species can be in woody plants with long generation times.

The advent of molecular markers has made the prospect of producing genetic maps which can be used to accelerate plant breeding a reality. In apple, a genetic map containing molecular markers has recently been published (Hemmat et al. 1994) and other maps are being constructed (Gardiner et al. 1994; King 1994). The ability to map the position of agronomically important apple genes has several benefits. Selecting for molecular genetic markers linked to an important gene(s) can be used to accelerate traditional methods of breeding, a technique known as marker assisted selection or MAS (Lande and Thompson 1990; Gianfrancheschi et al. 1994). The confidence that progeny identified by marker assisted selection actually contain the gene of interest can be increased by molecular markers on both sides of the gene (Koller et al. 1994). The same molecular markers can also be used to initiate the construction of a fine structure map around the gene, which is a pre-requisite for map-based positional cloning strategies (Tanksley et al. 1995).
The first successful map-based cloning projects involving plant disease resistance genes were published recently (Martin et al. 1993; Mindrinos et al. 1994). Notwithstanding the difficulties associated with somaclonal variation, gene expression, and new problems such as sense suppression (Napoli et al. 1990), it should be possible to transform such cloned genes into commercial cultivars and thereby confer natural resistance on these cultivars while retaining their unique blend of characteristics. This has not been possible using traditional breeding approaches. The importance of being able to clone disease resistance genes for direct transfer into cultivars is underlined by the recent appearance of races of *V. inaequalis* that can overcome *Vf* resistance (Parisi et al. 1993; Roberts and Crute 1994).

When combined with the need to continually bring out improved cultivars, the traditional route of introgressing resistance factors is too slow relative to the ability of the fungus to overcome resistance based on single genes. Disease management strategies involving strategic planting of unrelated cultivars in orchards that are designed to slow down the spread of resistant races have been proposed (Gessler and Blaise 1994). However, cloning resistance factors offers one of the few practical strategies that can circumvent the almost inevitable breakdown of single gene resistance. In addition it offers the alternative of pyramid-ing a series of cloned resistance genes into cultivars to reduce the chance of new isolates being able to overcome a single resistance gene in isolation. Molecular markers can also improve the efficiency of incorporating quantitatively inherited (multi-component) disease resistance systems that are often described as being more durable than single resistance genes (Dayton et al. 1983). However, the latter strategy still involves several generations of backcrosses to select the rare recombinants containing the multiple resistance factors and all of the vital fruit and plant characteristics required in a modern cultivar. Cultivars with quantitatively inherited resistance are not favoured in traditional breeding strategies since the effective resistance is difficult to transmit to progeny.

The scab resistance in the crab apple *M. floribunda* 821 is thought to consist of a single major effect gene (*Vf*) and a number of modifying genes (Dayton et al. 1983). The resistance evident in this crab apple produces a classic pin-point or hypersensitive reaction in response to inoculation with *V. inaequalis* races unable to overcome the resistance. When the *Vf* resistance is passed on to progeny, a range of reactions from hypersensitive responses (HR) to weakly susceptible reactions are evident (Hough et al. 1953). Chevalier et al. (1991) carried out detailed scanning electron microscopy and histological studies and defined five classes of reactions. These were: class 1 – a typical HR pin-point reaction, resistant; class 2 – chlorotic lesions with slight necrosis, resistant; class 3A – necrotic lesions with occasional slight sporulation, weakly resistant; class 3B – clearly sporulating necrotic and chlorotic lesions, weakly susceptible; class 4 – abundant sporulation with no chlorotic or necrotic response, susceptible. Classes 1, 2 and 3A are generally accepted as being resistant. However, there has been a degree of doubt that the weakly susceptible progeny in class 3B actually carry the *Vf* resistance gene and whether 3B progeny should be classed as susceptible.

In this manuscript we present a detailed linkage map around the *Vf* resistance gene using RAPD and isoenzyme markers. We integrate the position of several previously published markers which were reported to be linked to *Vf* (Koller et al. 1994; Manganaris et al. 1994; Tartarini 1994; Yang and Kruger 1994) into a single map around this gene and have identified several additional linked RAPD markers by bulked segregant analysis (Michelmore et al. 1991). By analyzing the segregation of the markers linked to *Vf* in class-3A and -3B progeny, we have also been able to address the question of whether both of these classes contain the resistance allele of this gene.

### Materials and methods

#### Segregating families

Two sets of progeny segregating for scab resistance were used in this study. One set of 98 progeny were derived from a cross between cv ‘Granny Smith’ (susceptible) and A679-2 (a *Vf* heterozygote derived from a cross between cv ‘Worcester’ and A363-38). This progeny family is referred to as family 1. A set of 160 progeny (referred to as family 2) were chosen from amongst 658 seedlings of a cross between cv ‘Royal Gala’ (susceptible) and A172-2 (a *Vf* heterozygote derived from a cross between cv ‘James Grieve’ and OR33T90). Approximately equal numbers of progeny were selected from each of the three main classes of plant/fungal interactions segregating in this family (3A, 3B and 4) in order to determine if the *Vf* gene segregates with both classes 3A and 3B. The three groups selected consisted of 49 weakly resistant (class 3A), 55 weakly susceptible (class 3B), and 56 susceptible (class 4) progeny.

#### DNA isolation

DNA was isolated from young apple leaves less than 1.5 cm long. Leaves were frozen and stored at -70°C before extraction. Two leaves were placed in a plastic bag and 2 ml of DNA extraction buffer (140 mM Sorbitol, 220 mM Tris-HCl pH 7.5, 20 mM EDTA, 0.8 M NaCl, 0.8% w/v Cetyl trimethylammonium bromide, 1% w/v N-Lauroylsarcosine, 1% w/v Polyvinyl-pyrrolidone 4000) was added and the bag was heat-sealed. The leaves were ground to a homogeneous pulp by applying a Teflon roller to the sealed bag placed on a flat surface. A 1.6-ml sample of the pulp was extracted with 400 μl of chloroform:octanol (24:1 v/v) for 30 min at 65°C and centrifuged in a 2-ml Eppendorf tube at 15000 rpm for 10 min. The aqueous layer was collected and DNA was precipitated by adding 1.0 ml of ice-cold isopropanol. DNA was recovered by centrifugation at 12000 rpm for 5 min, the pellet washed twice with 70% ethanol, dried under vacuum and re-suspended in sterile distilled water.

#### PCR amplification conditions and RAPD Primers

PCR amplifications were performed in a 1.25-μl reaction mix containing 10 mM Tris-HCl pH 8.8, 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin, 1.5% formamide, 0.1 mM dNTP, 0.2 μM of each primer, 1 ng of apple DNA and 1.25 U of Taq polymerase (Stratagene, La Jolla, Calif., USA, or Life Technologies, Gaithersburg, Md., USA). The amplification reactions were overlaid with 14 μl of paraffin. DNA was amplified in a Perkin Elmer Cetus DNA Thermal cycler with the following programme: 4 min at 94°C, 40 cycles of 1 min at 94°C, 1 min at 40°C and 2 min at 72°C, a final extension cycle of 10 min.