

MOLECULAR MAPPING AND CONSTRUCTION OF SCAR MARKERS OF THE STRAWBERRY *Rpfl* RESISTANCE GENE TO *PHYTOPHTHORA FRAGARIAE* AND THEIR USE IN BREEDING PROGRAMMES

K.M. Haymes, W.E. van de Weg, P. Arens, B. Vosman and A.P.M. den Nijs

Centre for Plant Breeding and Reproduction Research (CPRO-DLO),
PO Box 16, 6700 AA Wageningen, The Netherlands

Additional keywords: *Fragaria x ananassa*, red stele, PCR, RAPD, SCAR, marker-assisted selection, disease resistance

Summary

The commercial strawberry (*Fragaria x ananassa*) resistance gene *Rpfl* conferring resistance to various isolates of *Phytophthora fragariae*, was mapped using 7 RAPD markers. A DNA fragment representing a RAPD marker linked to susceptibility was cloned, sequenced and converted into a sequence characterized amplified region (SCAR) marker. Next, SCAR primers to the resistant allele (SCAR-R) were developed based upon a deletion region between susceptible and resistant plants. The SCAR-R primers were tested on a F₁ progeny of 60 plants in which *Rpfl* segregated in a 1:1 ratio. SCAR-R primers amplified DNA from resistant plants only.

The SCAR-R primers and three RAPD primers linked to and surrounding the *Rpfl* gene were used to screen numerous European and North American strawberry genotypes. The molecular data supported the currently known plant resistance testing and indicates that the resistance gene region has been conserved in most crosses. The linkage to *Rpfl* with these markers was lost in some genotypes due to crossing-over, for example the subsequent generations of 'Stelemaster'. The accuracy of these molecular markers for the detection of the gene demonstrates that they can be used in a marker-assisted strawberry breeding programme.

1. Introduction

The commercial strawberry (*Fragaria x ananassa*) is susceptible to the fungus *Phytophthora fragariae*, causal agent of red stele root rot. Symptoms of the disease are reddening of the stele and rotting away of the infected roots, dwarfism, wilting and finally plant death. Due to growing concern and pressure from environmental groups towards chemical control of this pathogen, the use of resistant cultivars will play a more substantial role in future cultivation.

Williams *et al.* (1990) and Welsh and McClelland (1990) demonstrated that short 10 bp length single primers of arbitrary nucleotide sequence could be used to amplify segments of genomic DNA from a variety of species using PCR technology. Polymorphisms among the amplification products are typically detected as DNA segments which amplify from one parent but not the other and can be used to construct genetic maps. The dominant

nature of RAPD markers does not allow the complete determination of genotype in the segregating population. RAPD-PCR has been used to a limited extent in the commercial strawberry for cultivar and genotype identification (Parent and Pagé, 1995; Levi *et al.*, 1994; Gidoni *et al.*, 1994) and in the diploid strawberry for mapping (Haymes, 1993).

Classical breeding methods already allowed the incorporation of resistances to various fungal and viral diseases, but the pyramiding of resistance genes is still problematic in the commercial strawberry (Maas *et al.*, 1989). The gene-for-gene interaction model by Van de Weg (in press) includes five resistance genes to red stele root rot. One of the major methods of detecting these useful genes is by Bulk Segregant Analysis (BSA) described by Michelmore *et al.* (1991). RAPD markers in combination with BSA have previously been used to tag various disease resistance genes in many crops, but not yet in the strawberry. In this report, such mapping of one gene for red stele root rot resistance, *Rpfl*, was mapped using BSA in conjunction with RAPD markers (Haymes *et al.*, 1997). Next, specific primers (SCARs) to the gene were developed and assessed in European and North American genotypes.

2. Materials and methods

2.1. Plant material

From the cross of two strawberry genotypes, MD683 (*Rpfl*; resistant) and 'Senga Sengana' (*rpfl*; susceptible), 60 F₁ progeny plants were obtained. They were grown and runner-propagated in a greenhouse and previously tested against the virulent race 2.3.4 isolate NS2-25 (Nickerson and Murray, 1993) of *P. fragariae* by Van de Weg *et al.* (submitted).

2.2. DNA isolation/Bulk Segregant Analysis

DNA from the mapping population was isolated using a modification of the method of Torres *et al.* (1993) or by a mini-prep DNA isolation method for the North American and European genotypes (Haymes, 1996). Nine resistant and ten susceptible plants, previously identified as by a disease index were utilized in the BSA. A total of 576 RAPD primers were tested on the bulks. Each primer that produced polymorphisms between bulk samples were repeated two times and then tested on all the individuals constituting the bulks. Putatively linked markers were then tested on the entire population. Those markers which still appeared to be linked were tested twice more at the population level. RAPD-PCR was done according to the method of Haymes *et al.* (1997). Amplification products were resolved by electrophoresis in a 2.0% TBE agarose gel. This part of the research was done in conjunction with T. Davis (University of New Hampshire, Durham, NH, USA).

2.3. Linkage map of the *Rpfl* region

Map positions of the RAPD markers and the *Rpfl* gene were calculated with JoinMap 2.0[®] (Stam, 1993; Stam and Van Ooijen, 1995) with a minimum 3 LOD using the Kosambi function.

2.4. Cloning and sequencing of RAPD Marker (M6)

DNA from RAPD marker M6(450) was cloned into the *Hinc* II site of plasmid pBluescript SK+, (Stratagene) and transformed to *E. coli* DH5 α according to the method of Sambrook *et al.* (1989). Recombinant clones were screened for appropriately sized inserts.

The DNA inserts were sequenced from both sides on an Applied Biosystems Inc. (ABI) 373 Automated Sequencer using a *Taq* DyeDeoxy™ Terminator Cycle Sequencing kit (ABI). Template DNA preparations and sequence reaction mixtures were done according to the ABI kits recommended procedure. SCAR-S primers were designed that amplified DNA in both susceptible (S) and resistant (R) plants (Haymes *et al.*, submitted). The amplified band fragments from both S and R plants were sequenced and then using a deletion region in R plants, dominant SCAR primers were designed called SCAR-R.

3. Results and discussion

We are the first to identify molecular markers linked with a commercially interesting trait in the strawberry. We could do so due to the disomic behavior of the *Rpfl* region and the reliable classification into resistance and susceptibility of the individuals of the strawberry progeny. Moreover, the seven markers for *Rpfl* all had distinct presence or absence polymorphisms between the paired bulks. These markers linked to *Rpfl* were produced from four primers. The 7 markers were mapped to within 1.7 - 13.9 cM of the gene (*Rpfl*) (Figure 1).

MD683 and its descendants have been used as progenitors of resistance in various breeding programmes. RAPD markers are difficult to reproduce, therefore they preferentially should be converted into sequence characterized amplified region (SCAR) markers (Paran and Michelmore, 1993). An advantage of SCAR markers (when dominant) is their potential for quick and robust assessment.

RAPD marker M6, located 3.0 cM from *Rpfl* (Figure 1), produced a bright band and therefore was chosen for cloning even though it was linked to the susceptibility allele of the *Rpfl* gene. This marker was then used for the construction of SCAR markers. The markers, amplified by the same RAPD primer, linked in coupling phase to the gene are more difficult to clone due to the faint band intensity (M4 and M5; Figure 1).

SCAR-S primers designed from the M6 sequence, amplified band fragments in both S and R plants from the mapping population. The S plants either had a single band or a doublet while R plants had a doublet or a triplet band pattern amplified from the SCAR-S primers. The amplified fragments were sequenced and three primer sets (SCAR-R) were developed from a deletion region between the M6 sequence and the R sequences. The SCAR-R markers mapped to the same location as M4-M6.

These SCAR-R and the closest flanking RAPD markers were assessed in European and North America genotypes. The RAPDs and SCARs are good indicators of crossing-over events occurring in the genotypes (Figure 2). Out of the 115 genotypes assessed, about 5% non-conforming genotypes were observed using the SCAR-R primers. This percentage is in agreement with the markers distance from the gene (3.0 cM). This error is comparable to what is observed in plant screening tests, considering the number of escapes. Most of the non-conforming genotypes could be traced back to a crossing-over

event in one of the earlier genotypes. Such an event occurred in 'Stelemaster' between the SCAR-R marker and *Rpfl* thereby affecting progenies like 'Scott' and 'Sunrise'. However, when RAPD M3 was assessed in conjunction with the SCAR-R, then only about 4% genotypes could not be properly classified (results not shown).

The linked markers to *Rpfl* allowed us to trace this resistance gene by pedigree analysis (Figure 2). Many important breeding stock materials could accurately be assessed with these markers. These markers can therefore be integrated into a marker-assisted breeding programme facilitating the pyramiding and screening for resistance genes.

4. Acknowledgments

The authors would like to thank the North American Strawberry Growers Association and the Dutch strawberry growers and nurseries for partial financial support of this research.

5. References

- Gidoni, D., M. Rom, T. Kunik, M. Zur, E. Izsak, S. Izhar and N. Firon, 1994. Strawberry-cultivar identification using random amplified polymorphic DNA (RAPD) markers. *Plant Breeding* 113:339-342.
- Haymes, K.M., 1993. Genome mapping and *Agrobacterium*-mediated transformation and regeneration of the diploid strawberry. MSc Thesis, University of New Hampshire, USA.
- Haymes, K.M., 1996. A DNA mini-prep method suitable for a plant breeding program. *Plant Molecular Biology Reporter* 14(3):280-284.
- Haymes, K.M., B. Henken, T.M. Davis and W.E. van de Weg, 1997. Identification of RAPD markers linked to a *Phytophthora fragariae* resistance gene (*Rpfl*) in the cultivated strawberry. *Theoretical and Applied Genetics* (in press).
- Haymes, K.M., P. Arens and B. Vosman. Construction of primers highly specific for *Phytophthora fragariae* resistance gene *Rpfl* in the commercial strawberry. (submitted).
- Levi, A., L.J. Rowland, G.J. Galletta, G. Martelli and I. Greco, 1994. Identification of strawberry genotypes and evaluation of their genetic relationships using random amplified polymorphic DNA (RAPD) analysis. *Advances in Strawberry Research* 13:36-39.
- Maas, J.L., G.J. Galletta and A.D. Draper, 1989. Resistance in strawberry to races of *Phytophthora fragariae* and to isolates of *Verticillium* from North America. *Acta Horticulturae* 265:521-526.
- Michelmore, R.W., I. Paran and R.V. Kesselli, 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. *Proceedings of the National Academy of Science USA* 88:9828-9832.
- Nickerson, N.L. and R.A. Murray, 1993. Races of the red stele root rot fungus, *Phytophthora fragariae*, in Nova Scotia. *Advances in Strawberry Research* 12:12-15.

- Paran, I. and R.W. Michelmore, 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. *Theoretical and Applied Genetics* 85:985-993.
- Parent, J.G. and D. Pagé, 1995. Authentification des 13 cultivars de fraiser du programme de certification du Québec par l'analyse d'ADN polymorphe amplifié au hasard (RAPD). *Canadian Journal of Plant Science* 75:221-224.
- Sambrook, L., E.F. Fritsch and T. Maniatis, 1989. *Molecular cloning: 2nd edition a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA.
- Stam, P., 1993. Construction of integrated linkage maps by means of a new computer package: JoinMap®. *The Plant Journal* 3:739-744.
- Stam, P. and J.W. van Ooijen, 1995. JoinMap (tm) version 2.0: Software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen, The Netherlands.
- Torres, A.M., N.F. Weeden and A. Martin, 1993. Linkage among isozyme, RFLP and RAPD markers in *Vicia faba*. *Theoretical and Applied Genetics* 85:937-945.
- Weg, W.E. van de, 1997. A gene-for-gene model to explain interactions between cultivars of strawberry and races of *Phytophthora fragariae* var. *fragariae*. *Theoretical and Applied genetics* (in press).
- Weg, W.E. van de, H.J. Schouten and B. Henken. Resistance to *Phytophthora fragariae* var. *Fragariae* in strawberry: the *Rpfl*-gene. (submitted).
- Welsh, J. and M. McClelland, 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* 18:7213-7218.
- Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18:6531-6535.

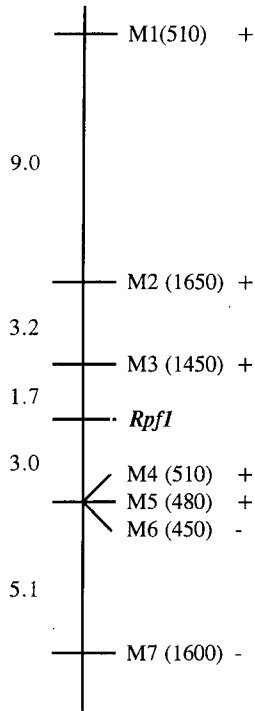


Figure 1. Linkage map of the region surrounding the *Rpf1* resistance gene and 7 RAPD DNA markers, in the F_1 of the cross MD683 x 'Senga Sengana' of the cultivated strawberry. Map distances, written on the left, are in centiMorgans. The order and distances of the loci were derived from multiple linkage analysis using the JoinMap 2.0[®] software programme with a threshold of 3 LOD for determining linkage and using the Kosambi function (Stam, 1993; Stam and Van Ooijen, 1995). The fragment size of the marker, in bp, is given in parentheses. The phase of each marker is indicated by either a (+), coupling phase, or by a (-), repulsion phase.

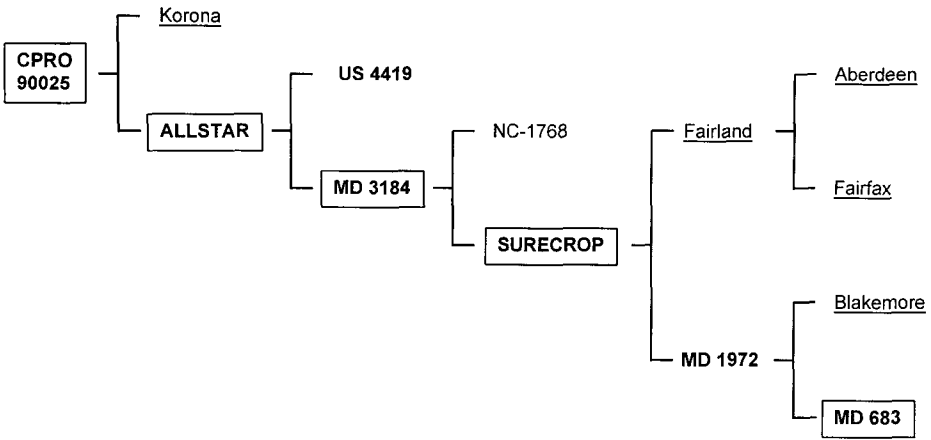


Figure 2 . Pedigree of genotype CPRO 90025. Cultivars possessing *Rpf1* are in bold while susceptible are normal text. Genotypes that were tested positive for the presence of RAPD and SCAR markers are indicated by a box, while genotypes that were absent for the markers are underlined.