Theor Appl Genet (1993) 85:541–544

Identification of RFLP markers closely linked to the *H1* gene conferring resistance to *Globodera rostochiensis* in potato

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Received August 30, 1992; Accepted September 18, 1992
Communicated by G. Wenzel

Summary. Resistance to the root cyst nematode *Globodera rostochiensis* is an agronomic trait that is at present incorporated into most new potato varieties. Major dominant genes are available that originate from wild and cultivated *Solanum* species closely related to the cultivated European potato (*Solanum tuberosum* ssp. *tuberosum*). One of those genes, *H1*, from *S. tuberosum* ssp. *andigena,* was mapped to a distal position on potato chromosome V using restriction fragment length polymorphism (RFLP) markers. The *H1* locus segregates independently from *Gro1,* a second dominant gene presumably from *S. spegazzinii* that confers resistance to *G. rostochiensis* and which has been mapped to chromosome VII. One marker, *CP113,* was linked without recombination to the *H1* locus.

Key words: Potato – *G. rostochiensis* – RFLP – Marker

Introduction

In potato the gene *H1* confers complete resistance to the root cyst nematode *Globodera rostochiensis* (Woll.) Behrens, pathotypes Ro1 and Ro4 (Kort et al. 1977). The gene was discovered in the Commonwealth Potato Collection (CPC) in 5 accessions (out of about 1300 tested) of *Solanum tuberosum* ssp. *andigena* (Ellenby 1954). Genetic analysis using selfed seeds of accession CPC 1673 revealed the presence of a single dominant gene named *H1* (Toxopeus and Huijsman 1953; Huijsman 1955). The germ plasm of CPC 1673 was subsequently incorporated into *S. tuberosum* ssp. *tuberosum* breeding lines and into many European varieties, among those cvs 'Granola', 'Maris Piper' and others widely cultivated (Ross 1986). The availability of *H1*, together with a few other genes originating from *S. vernei* and *S. spegazzinii*, was sufficient to solve the problem of infection caused by pathotype Ro1 of *G. rostochiensis* (Ross 1986).

The genetic relationship among the available genes conferring resistance to *G. rostochiensis* are to a large extent unknown. Moreover, the identity of resistance genes fom different sources is often unclear, as identification is based on their activity against different pathotypes of the nematode, and these pathotypes are themselves difficult to define and subject to change. Recently, we have mapped a major dominant gene conferring resistance to *G. rostochiensis* to chromosome VII of potato (Barone et al. 1990). This locus was named *Gro1* and, based on pedigree information and resistance tests with different *G. rostochiensis* pathotypes, should correspond to the *Fb* gene of *S. spegazzinii* first described by Ross (1962). In the present article we report the genomic position of *H1* as mapped in a genetic background where *H1* can be traced back to the founder clone CPC 1673.

Materials and methods

The *H1* gene was mapped in the progeny of a cross between the dihaploid line Amaryl H5 carrying *H1* and a 2n *Solanum phureja* line (L85DS3.1) susceptible to the nematode. Amaryl H5 was obtained at the INRA Station de Recherches sur la Pomme de Terre et les Plantes à Bulbe, INRA Kéraiber, Ploudaniel 29260 Lesneven, France, from the 4n variety 'Amaryl' via parthenogenesis after pollination with *S. phureja* (Hermsen and Verdenius 1973). 'Amaryl' had been selected from the cross 'Saskia' × (CPC 1673-20 × Furore) (Joosten 1988). CPC 1673-20 was a resistant plant (genotype *HHhh*) originating from the selfing of *S. tuberosum* ssp. *andigena* accession CPC 1673 (Toxopeus and Huijsman 1953).

Fifty-three tetraploid varieties were obtained as described in Görg et al. (1992). Twenty varieties were selected either for their

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resistance to pathotypes Ro1 and Ro4 of *G. rostochiensis* (beschreibende Sortenliste Kartoffeln 1989) or for containing the *H1* gene (Ross 1986). These varieties were: 'Accent', 'Arnika', 'Barbara', 'Certo', 'Clarissa', 'Darwina', 'Granola', 'Ilse', 'Indira', 'Katja', 'Margit', 'Monza', 'Nora', 'Optima', 'Pamir', 'Panda', 'Pirola', 'Ponto', 'Producent', and 'Quarta'. Thirty-three susceptible varieties were also selected: 'Achat', 'Astrid', 'Atica', 'Carola', 'Cosima', 'Clivia', 'Datura', 'Desirée', 'Erna', 'Erntestolz', 'Fausta', 'Forelle', 'Grandifolia', 'Grata', 'Hansa', 'Hela', 'Ilona', 'Irmgard', 'Isna', 'Isola', 'Linda', 'Luna', 'Mentor', 'Moni', 'Ostara', 'Palma', 'Prima', 'Saphir', 'Saskia', 'Selma', 'Sieglinde', 'Sommerstärke' and 'Ulla'.

Resistance to the *G. rostochiensis* Ro1 population Ecosse was tested in a petri dish according to the method of Mugniéry and Person (1976). Cuttings from plants of the first tuber generation (Mugniéry and Balandras 1986) were inoculated with five juveniles per root. A root system showing the presence of nematode females was rated as susceptible. Resistant genotypes showing the absence of cysts were transferred into pots with infested soil and examined after 3 months to confirm their resistance rating.

Between 0.3 and 0.5 g freeze-dried leaf material per genotype was used for extracting total genomic DNA. DNA extraction, restriction digests, electrophoresis, blotting and hybridization methods have been described previously (Gebhardt et al. 1989). Linkage analysis was performed as in previous investigations (Ritter et al. 1990; Barone et al. 1990). RFLP markers selected to cover all potato chromosomes (Gebhardt et al. 1991; unpublished results from this laboratory) were hybridized to filters with *Taq*I- or *Rsa*I-restricted DNA of the parents and F_1 progeny.

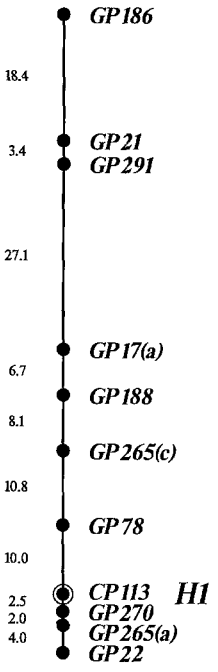

Fig. 1. Linkage map of potato chromosome V as deduced from recombination frequencies in Amaryl H5 carrying the *H1* gene. Map distances are given in centiMorgans (Kosambi 1944). *GP* markers are derived from genomic DNA; *CP* markers from the cDNA of potato. *Small letters in parenthesis* indicate that more than one locus was mapped with the same marker probe. The cDNA marker CP113 is linked with zero percent recombination to the *H1* locus

Results

One hundred and eleven hybrid lines of the cross Amaryl H5 × *S. phureja* were tested for resistance to *G. rostochiensis*, pathotype Ro1. Amaryl H5 was totally resistant to Ro1, while the *S. phureja* parent was susceptible. Among the seedlings, 58 were found to be resistant and 53 susceptible which supports the hypothesis that the *H1* gene is heterozygous in Amaryl H5 and segregates in the cross in a 1:1 ratio ($P > 0.5$).

Out of the 111 hybrid lines scored for resistance, for practical reasons only 91 lines, 44 resistant and 47 susceptible, were screened for RFLP alleles that cosegregated with the resistance trait. Linkage was detected with six out of ten informative markers of potato chromosome V: *GP22, GP265, GP270, CP113, GP78* and *GP188*. One marker, *CP113*, was linked with zero percent recombination to the *H1* locus. The linkage map of potato chromosome V, including the position of the *H1* locus as deduced from recombination values measured in the resistant parent Amaryl H5, is shown in Fig. 1. No distortion of segregation ratios was observed for chromosome V in the cross studied. The length of the linkage map of chromosome V of Amaryl H5 was 93 centiMorgans, which is a 20% increase over its length of 78 centiMorgans reported in the RFLP map of Gebhardt et al. (1991).

Fifty-three tetraploid German potato varieties were surveyed for the presence of the RFLP allele that had been found to be totally linked in coupling to *H1* in Amaryl H5. Their names are listed in the Materials and methods. Twenty varieties are described as being resistant to pathotypes Ro1 and Ro4 of *G. rostochiensis* in the Beschreibende Sortenliste Kartoffeln (1989) and in Ross (1986). The RFLP allele indicative of the *H1* gene in Amaryl H5 was, however, not present in the germ plasm surveyed (data not shown). There was also no alternative RFLP allele detectable (with the same restriction enzyme and probe) by which resistant and susceptible varieties could be separated.

Discussion

The potential of RFLPs for marker-based selection was recognized 10 years ago (Beckmann and Soller 1983; Tanksley 1983). A prerequisite for using RFLP markers is that ones have to be identified that are closely linked to agronomically relevant genes. Resistance to the root cyst nematodes *G. rostochiensis* and *G. pallida* are considered to be important traits in breeding programs. In this article we report a mapping experiment that identifies four RFLP markers linked, within 10 centiMorgans, to the *H1* gene conferring resistance to *G. rostochiensis*. One marker, *CP113*, was shown to be linked to the *H1* locus without recombination, based on an F_1 population of 91 genotypes. The *H1* locus maps at the end of potato

chromosome V and, therefore, segregates independently from the nematode resistance locus *Gro1*, which has been mapped onto chromosome VII (Barone et al. 1990). *H1* is also genetically unrelated to the *Mi* locus that induces resistance to the root knot nematode *Meloidogyne incognita*; this locus is located on chromosome VI of the homeologous tomato genome (Klein-Lankhorst et al. 1991; Messeguer et al. 1991). Two other resistance loci have also been allocated to chromosome V: the *Rx2* locus conferring extreme resistance to potato virus X (Ritter et al. 1991) and *R1* conferring race-specific resistance to *Phytophthora infestans* (Leonards-Schippers et al. 1992). Both of these latter loci map close to the marker locus *GP21* and, therefore, are located in a segment of chromosome V that is 63 centiMorgans away from *CP113* (Fig. 1). If we also take *Pto*, a *Pseudomonas* resistance gene identified on tomato chromosome V (Martin et al. 1991), into consideration and if we assume homeology between the potato and tomato genome for resistance loci, chromosome V appears to be particularly rich in disease resistance loci.

There may be several reasons for the absence of the RFLP allele linked to *H1* in Amaryl H5 in a series of resistant potato varieties having reported pathotype specificity of *H1*. First, and this seems the most likely explanation, the source of resistance in the varieties tested was not the same as in Amaryl H5. Genetic evidence, for example, indicated that resistant germ plasm derived from *S. vernei* in some cases contains the same genetic locus as that present in *S. tuberosum* ssp. *andigena* CPC 1673 (Scurrah et al. 1973). *S. vernei*, in addition to CPC 1673, has also been widely used in potato breeding for introducing resistance to *G. rostochiensis*. Second, in the *S. tuberosum* ssp. *andigena* germ plasm from which the *H1* gene originated, more than one *H1* allele was present in a polymorphic genetic background. Third, during extended backcross breeding the linkage between *H1* and closely linked marker sequences have been broken. To be able to use maker *CP113* for selecting *H1* genotypes, it is therefore necessary – as in human genetics – to determine the informative allele or the marker phase in the germ plasm employed in crosses.

A positive and important outcome of this study is that the selection of *Gro1, H1* genotypes is now feasible based on the RFLP markers flanking both loci. This should result in phenotypes having highly durable resistance to most, if not all pathotypes of *G. rostochiensis*.

Acknowledgements. The authors thank H. Thelen for technical assistance and M. Pasemann for preparing the manuscript. The project was supported by the Bundesministerium für Forschung und Technologie (BMFT) under Project no. 1.10.

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