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A. El-Kharbotly · C. Palomino-Sánchez · F. Salamini
E. Jacobsen · C. Gebhardt

R6 and *R7* alleles of potato conferring race-specific resistance to *Phytophthora infestans* (Mont.) de Bary identified genetic loci clustering with the *R3* locus on chromosome XI

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Abstract In potato, 11 resistance alleles (*R1–R11*) are known which confer race-specific resistance to the fungus *Phytophthora infestans*. *R1* has been mapped previously to potato chromosome V and *R3* to chromosome XI. Here we report on the localization of the *R6* and *R7* alleles on the genetic map of potato. Differential resistant strains of tetraploid *Solanum tuberosum*, clones Ma*R6* and Ma*R7*, were used as parental plants for the parthenogenetic induction and selection of diploid genotypes containing the *R6* or the *R7* resistance allele to *P. infestans*. One resistant dihaploid from Ma*R7* could be used directly as a parent to produce diploid F_1 progeny suitable for phenotypic and RFLP analysis. Ma*R6* did not produce useful dihaploids directly. After crossing Ma*R6* with a tetraploid susceptible genotype, resistant F_1 clones were selected. The resistant genotypes were then used as parents for the induction of dihaploids. Six dihaploids bearing *R6* were identified that could be crossed with a diploid susceptible genotype. Two diploid F_1 populations, segregating for *R6* and *R7*, respectively, were analysed with RFLP markers known to be linked with previously identified *R* genes. Markers linked with *R3* were found also to be linked with *R6* and *R7*. The resistance alleles *R6* and *R7* mapped to a similar distal position on chromosome XI as the *R3* allele.

Key words *Solanum tuberosum* · *P. infestans* · Resistance · Dihaploid induction · RFLP mapping

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A. El-Kharbotly (✉) · E. Jacobsen · C. Palomino-Sánchez [1]
Graduate School of Experimental Plant Sciences,
Department of Plant Breeding, Agricultural University
Wageningen, P.O. Box 386, 6700 AJ Wageningen,
The Netherlands

F. Salamini · C. Gebhardt
Max-Planck-Institut für Züchtungsforschung,
Carl-von-Linné-Weg 10, D-50829 Köln, Germany

Present address:
[1] Centro de investigacion y desarollo agrario (C.I.D.A.)
Departamento de Mejora y Agronomia, Apartado 4240,
14080 Cordoba, Spain

Introduction

In potato, two types of resistance to *Phytophthora infestans* are known: one horizontal and the other vertical. The inheritance of horizontal resistance is based on polygenes (Umaerus et al. 1983; Wastie 1991; Leonards-Schippers et al. 1994),while the vertical resistance is controlled by major dominant genes (*R*-genes) (Mastenbroek 1953; Malcolmson and Black 1966). *R*-genes which confer race-specific resistance to *P. infestans* have been introduced from the wild species *Solanum demissum* into the cultivated tetraploid potato by repeated back crossing. Eleven *R*-genes are known (Malcolmson and Black 1966; Shaw 1991) and their isolation and characterization would help in understanding the mechanism of resistance to the fungus.

The mapping of *R*-genes is the first step towards molecular cloning either by transposon tagging or by map-based cloning.Two loci, named *R1* and *R3*, have recently been identified (Leonards-Schippers et al. 1992; El-Kharbotly et al. 1994) on chromosomes V and XI, respectively, using RFLP markers (Gebhardt et al. 1991; 1994). In the present paper we have determined the positions of the race-specific resistance alleles *R6* and *R7* in the potato genome by analyzing segregating populations at the diploid level. We find *R6* and *R7* to be located in the same chromosomal segment as the *R3* locus.

Materials and methods

Plant material

The tetraploid clones Ma*R6* and Ma*R7*, two differential tester clones carrying the *R6* and *R7* resistance alleles, respectively (C. Mastenbroek, unpublished), were used to develop diploid populations suitable for RFLP mapping. The clones were kindly supplied by Dr. L. J. Turkensteen (Research Institute for Plant Protection, IPO-DLO Wageningen, The Netherlands). From these clones dihaploids (2n=2x=24) were induced through prickle pollination using clones IVP 35 and 48 (Hermsen and Verdenius 1973) and IVP 101 (Hutten et al. 1994) of the diploid species *Solanum phureja*. The dihaploids obtained were tested with *P. infestans* race 0. Resistant dihaploids

were grafted onto tomato root stocks of cv Virosa for flower induction and pollination, and used as seedstock in crosses with susceptible genotypes. In these crosses the fertile diploid clones 87-1024-2 (Jacobsen et al. 1989) and the amylose-free tetraploid clone J90-6001-3 (Flipse et al. 1996), which are both susceptible to *P. infestans* (*rr*), were used as pollen donors.

Screening for resistance

The resistance genotype of Ma*R6* and Ma*R7* was confirmed using different races of *P. infestans*. The same test was carried out for all susceptible pollinators and resistant F_1 hybrids. Races of *P. infestans* were kindly supplied by Dr. L. J. Turkensteen (Research Institute for Plant Protection, IPO-DLO Wageningen, The Netherlands) and Dr. F. Govers (Department of Phytopathology, Wageningen Agricultural University, The Netherlands). The preparation of *P. infestans* inoculum (races 0, 7, 2.7, 1.3.4.7 and 1.2.3.6.7) and the inoculation procedure were carried out according to El-Kharbotly et al. (1994).

Ploidy level determination

The ploidy level of the putative dihaploid seedlings was determined by counting the number of chloroplasts in their stomatal guard cells (Frandsen 1967).

RFLP analysis and mapping

RFLP analysis was carried out as described by El-Kharbotly et al. (1994) and Gebhardt et al. (1989). The linkage between the two resistance genes *R6* and *R7* and RFLP markers of chromosome XI was established on the basis of the fraction of recombinants recorded in the mapping populations. The linear order of loci was determined by three-point analysis. Closely linked loci were ordered by rejecting all alternate linear orders involving unlikely double-crossover events.

Results

Development of plant material for the localization of *R*-genes

Genotypes carrying a *R*-gene show an incompatible interaction resulting in hyper-sensitive resistance (R) when inoculated with *P. infestans* races carrying the corresponding avirulence gene. The same genotypes show a compatible interaction, leading to sporulating lesions (S), when inoculated with races lacking the corresponding avirulence gene. Clones not expressing *R*-genes are expected to be compatible (S) with all *P. infestans* races. The reaction pattern of clones Ma*R6* and Ma*R7* when inoculated with races 0, 7, 2.7, 1.3.4.7 and 1.2.3.6.7 was as expected, with the *R6* allele present in Ma*R6* and the *R7* allele in Ma*R7*. Pollinators used in dihaploid induction and crosses were susceptible to all races tested and did not, therefore, contain detectable *R*-genes (Table 1).

Because of its short flowering period, the production of dihaploids from clone Ma*R6* was difficult. From the mock pollination using *S. phureja*, three dihaploids were, nevertheless, isolated. All were resistant to *P. infestans* race 0 (Table 2), but either they did not flower at all, or else produced flower buds aborting before or after pollination. To overcome the problem of low dihaploid induction, the tetraploid clone Ma*R6* was crossed with the tetraploid susceptible clone J90-6001-3. Forty five F_1 plants (progeny J91-6145) were classified with respect to the *R6* genotype by testing them with *Phytophthora* race 0. A 1:1 or 5:1 (resistance versus susceptibility) segregation ratio was expected depending on the simplex (one *R6* allele) or duplex (two *R6* alleles) allelic constitution of Ma*R6*. A segregation of 39 resistant to 6 susceptible genotypes was found which fits a 5:1 ratio (χ^2=0.36, P>0.5), indicating that two *R6* alleles are present in Ma*R6* (Table 1). The induction of dihaploids was much easier in the J91-6145 population because of its long flowering period and high berry set. From five selected tetraploid genotypes resistant to *Phytophthora,* 42 parthenogenetic dihaploids were isolated (Table 2), of which 29 were resistant to race 0 (Table 2). They were grown to flowering and tested for seed set after pollination with the susceptible clone 87-1024-2. Six fertile genotypes were recovered (Table 2).

The dihaploids isolated from Ma*R7* flowered and produced enough seeds in 2x×2x crosses for segregation analysis. Out of six dihaploids, two showed resistance to race 0 (Table 2).

Segregation of *R* alleles in diploid F_1 families

Few of our resistant dihaploids set seeds when pollinated with line 87-1024-2, due to the well known problem of male and female partial sterility of the primary dihaploids of potato. Seven diploid F_1 families were obtained, six segregating for *R6* and one for *R7*, when screened using race 0. Four *R6*-segregating F_1 families, J92-6589, J92-6595, J92-6601 and BE93-4035, showed a segregation ratio not

Table 1 Resistance reaction of parental potato clones to five different races of *P. infestans*

Potato clone	Genotype	Inoculation with *P. infestans* race[a]				
		0	7	2.7	1.3.4.7	1.2.3.6.7
Ma*R6*	*R6R6r6r6*	R	R	R	R	S
Ma*R7*	*R7r7r7r7*	R	S	S	S	S
S. phureja	*rr*	S	S	S	S	S
J90-6001-3	*rrrr*	S	S	S	S	S
87-1024-2	*rr*	S	S	S	S	S

[a] R = resistant; S = susceptible

Table 2 Numbers of dihaploids isolated from tetraploid genotypes with defined *R*-genes and their reaction to *P. infestans* race 0

Potato clone	Number of dihaploids			Fertile dihaploids (codes)
	Total	Inoculation with race 0		
		R	S	
Ma*R6*	3	3	0	
J91-6145-2	6	4	2	J92-6435-1
J91-6145-4	6	5	1	
J91-6145-6	5	2	3	
J91-6145-7	17	12	5	J92-6441-1, 2 J92-6442-1,8,10
J91-6145-8	8	6	2	
Total	42	29	13	
Ma*R7*	6	2	4	BE93-4053-6

Table 3 Segregation in the progeny of crosses of resistant dihaploids (*Rr*) with the susceptible clone (*rr*) 87-1024-2. The inoculation was done with *P. infestans* race 0

Dihaploid resistant parent	Progeny-code	Inoculation with race 0		χ^2 1:1
		Number of plants		
		Resistant	Susceptible	
R6				
J92-6435-1	J92-6589	19	24	0.58
J92-6441-1	J92-6590	5	14	4.26*
J92-6441-2	J92-6592	1	2	nd
J92-6442-1	J92-6595	3	9	3.00
J92-6442-8	J92-6601	35	53	3.68
J92-6442-10	BE93-4035	4	7	0.82
R7				
BE93-4053-6	BE94-4101	39	64	6.07*

nd, not determined
* Significant at P=0.05

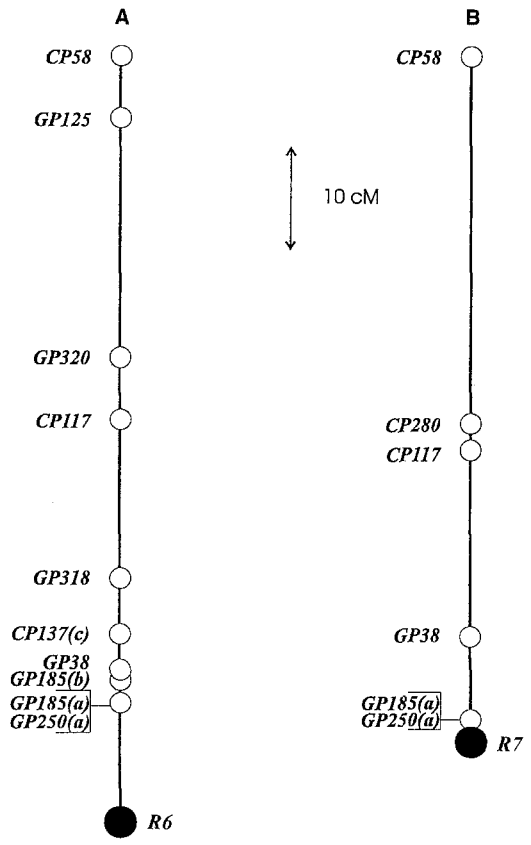

Fig. 1A, B Map position of the *R6* and *R7* resistance alleles on potato chromosome XI. **A** Linkage group derived from recombination in the *R6*-carrying parent J92-6442-8 of family J92-6601. **B** Linkage group derived from recombination in the *R7*-carrying parent BE93-4053-6 of family BE94-4101. GP markers are based on genomic DNA, CP markers on cDNA of potato. *Letters in parenthesis* indicate that more than one locus is detected by the same marker probe (Gebhardt et al. 1989)

significantly different from 1:1 at P=0.05. Family J92-6590 had an excess of susceptible genotypes (Table 3).

A 1:1 segregation ratio was expected also for the family BE94-4101 that segregated for the *R7* allele. In this population, however, a surplus of susceptible genotypes was observed (Table 3). The size of families J92-6601 and BE94-4101 was sufficient to assess the linkage between *R* genes and RFLP markers. The parental clones and the resistant genotypes of these two populations were tested with all available races of *P. infestans*. They showed the same phenotypes as the tetraploid clones *MaR6* and *MaR7* (Table 1). This test confirmed the presence of the *R6* allele in population J92-6601 and of the *R7* allele in BE94-4104.

Map positions of *R6* and *R7*

Parents and 85 plants of family J92-6601 and 96 plants of family BE94-4101 were analyzed with selected RFLP markers of known map position (Gebhardt et al. 1994). First preference was given to markers known to be linked to resistance genes in potato (Gebhardt 1994). Using RFLP marker probes GP185 and GP250, which detect closely linked RFLP loci [*GP185(a), GP185(b), GP250(a)*] on the distal end of chromosome XI in the vicinity of the *R3* locus (El-Kharbotly et al. 1994), linkage was evident to *R6* in family J92-6601 and to *R7* in family BE94-4101. Analysis with further RFLP markers of chromosome XI established the position of *R6* and *R7* distal to *GP185(a)* and *GP250(a)* (Fig. 1). *R7* was separated by one recombination event from marker loci *GP185(a)* and *GP250(a)*. The resistance phenotype of the plant with the recombinant chromosome was ambiguous and may have been miscored. If this plant is not considered, *R7* is linked without recombination to loci *GP185(a)* and *GP250(a)*. *R6* was separated from the same markers by ten recombination events. All ten recombinant plants had the *R6* allele, while the second class of recombinants bearing the susceptible *r6* allele was absent in family BE94-4101.

Discussion

The *R6* and *R7* alleles inducing resistance against *P. infestans* races have been extracted from tetraploid potato genotypes and incorporated into diploid parental lines. As previously observed (El-Kharbotly et al. 1994), the recovery of fertile diploid lines was difficult. An additional complication was the short flowering period of the tetraploid clone Ma*R6*. This was avoided by selecting long-flowering tetraploids carrying the *R6* allele from the cross of Ma*R6* with a highly fertile tetraploid clone susceptible to *Phytophthora*. The cross provided new basic material for the development of diploid clones. Although four of five diploid F_1 families in which the *R6* allele segregated did not deviate significantly from the expected 1:1 segregation ratio (R vs S), the pooled data indicated an excess of susceptible genotypes. Significant deviations from canonical segregating ratios are frequent when considering the product of sexual reproduction in this vegetatively propagated species (Gebhardt et al. 1991). In spite of this, two F_1 families of sufficient size were obtained in which *R6* and *R7*, respectively, segregated as single dominant factors. Probing these families with RFLP markers known to be linked to other *R* genes of potato (Leonards-Schippers et al. 1992; El-Kharbotly et al. 1994) revealed that both *R6* and *R7* are located on the same segment of chromosome XI distal to the RFLP loci *GP185(a)* and *GP250(a)*, in a region containing the *R3* resistance locus. *R7* was closely linked with loci *GP185(a)* and *GP250(a)*, whereas *R6* was separated from the same marker loci by 12 cM. All recombinant plants in the interval *GP185(a)-R6* had the R6 phenotype. The absence in this population of the susceptible class of recombinants indicates that the allelic combination present in those genotypes may have been gametically or zygotically lethal.

R3, *R6* and *R7* were mapped in independent F_1 families relative to the same closely linked RFLP markers. Despite the fact that the linkage values between *R3*, *R6*, *R7* and these closely linked RFLP loci differed, the possibility exists that all three are alleles of the same locus. Alternatively, *R3*, *R6* and *R7* may be alleles at closely linked loci. Both possibilities exist in other systems of plant-fungus interactions which are compatible with the gene-for-gene hypothesis. Examples are flax and flax rust, barley and powdery mildew, and maize and its rust (reviewed in Pryor 1987). The clustering of *R3*, *R6* and *R7* is the first example for a similar genetic architecture of fungal resistance genes in the potato.

The *R3*, *R6*, *R7* gene cluster of potato occupies a chromosomal position homologous to that of the *Fusarium oxysporum* resistance locus *I2* which maps to the same segment of chromosome 11 of tomato (Segal et al. 1992). The RFLP locus *TG105(a)* is closely linked to *I2* in tomato (Segal et al. 1992) and to the RFLP loci *GP185(a)* and *GP250(a)* in potato (El-Kharbotly et al. 1994). In the crosses analyzed here, the marker TG105 was not informative. It is possible, that the genes encoding *I2* in tomato and *R3*, *R6*, *R7* in potato are members of an homologous gene family. DNA-sequence analysis of resistance genes cloned from distantly related plant species, such as *Arabidopsis* and tobacco, has revealed structural similarities between genes conferring resistance to very different pathogens, such as *Pseudomonas* and tobacco mosaic virus (Mindrinos et al. 1994; Whitham et al. 1994). Based on such structural similarities we have amplified related gene fragments of potato by PCR (unpublished results from the laboratory of C. Gebhardt). One of these fragments, when used as a marker probe, identifies an RFLP locus that co-segregates with the *R7* allele with the exception of only one plant which had an ambiguous resistance phenotype and may have been mis-scored (unpublished results). This opens up the possibility that resistance genes located in this syntenic chromosome segment of potato and tomato are structurally related to the resistance genes effective against bacterial and viral pathogens in *Arabidopsis* and tobacco, respectively. The homology between different fungal resistance genes in the closely related genera *Solanum* and *Lycopersicon* based on their precise chromosomal location, as reported here, might be even more significant than in the case of resistance genes effective against more different pathogens. In the future, molecular cloning of different members of resistance gene families may, therefore, provide a versatile and adaptive instrument for engineering disease resistance in crop plants.

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