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X. Li · H. J. van Eck · J. N. A. M. Rouppe van der Voort
D.-J. Huigen · P. Stam · E. Jacobsen

# Autotetraploids and genetic mapping using common AFLP markers: the *R2* allele conferring resistance to *Phytophthora infestans* mapped on potato chromosome *4*

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**Abstract** Due to the complexity of tetrasomic inheritance, mapping studies in potato (*Solanum tuberosum* L.) are generally conducted at the diploid level. In the present study we tested the feasibility of Bulked Segregant Analysis (BSA) using a tetraploid offspring for the identification of AFLP markers linked to the *R2* allele, which confers race-specific resistance to *Phytophthora infestans*. Eleven bulk-specific AFLP markers, detected in fingerprints of 205 AFLP primer combinations, could be mapped in a linkage group encompassing the *R2* locus. The efficiency of BSA at the tetraploid level, determined by the frequency of single-dose restriction fragments (SDRF), was much higher than expected on the basis of overall genetic dissimilarity between the parental clones. The fortuitous detection of AFLPs with linkage to the *R2* allele is explained on the basis of specific genetic dissimilarity between cultivated potato and the chromosomal segment introgressed from *S. demissum* carrying the resistant *R2* allele. AFLP markers common to those with linkage to *R2* were visually recognized by their electrophoretic mobility in the AFLP fingerprint in a parental clone of a reference mapping population. Using these common AFLP markers we anchored the linkage group comprising the *R2* allele to potato chromosome 4.

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X. Li[1] · H. J. van Eck (✉) · D.-J. Huigen · P. Stam · E. Jacobsen
The Graduate School of Experimental Plant Sciences, Wageningen Agricultural University, Department of Plant Breeding, P.O. Box 386, 6700 AJ Wageningen, The Netherlands
Fax: + 31 317 483457
E-mail: herman.vaneck@users.pv.wau.nl

J. N. A. M. Rouppe van der Voort
Department of Nematology, Wageningen Agricultural University, P.O. Box 386, 6700 AJ Wageningen, The Netherlands

*Present address:*
[1]Chinese Academy of Agricultural Sciences, Institute of Crop Breeding and Cultivation, Beijing 100081, P. R. China

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## Introduction

Mapping studies at higher ploidy levels require simplex-inherited marker alleles [single-dose restriction fragments (SDRF); Wu et al. 1992] for the detection of linkage in coupling phase with simplex trait alleles. Linkage analysis between loci with higher allele dosage is hardly informative. In this paper we examine the feasibility and evaluate the efficiency of the identification of AFLP markers linked to the *R2* locus in a tetraploid potato offspring using Bulked Segregant Analysis (BSA) (Michelmore et al. 1991). BSA is a fast and technically simple approach to identify markers linked to monogenic traits. Elegant strategies for the composition of the bulks have been applied for diploid mapping populations from non-inbred parents (Meksem et al. 1995; Ballvora et al. 1995). However, the cultivated potato is a highly heterozygous autotetraploid species (2n = 4x = 48). All of the mapping studies conducted so far have been at the diploid level to avoid the complexities of tetrasomic inheritance (Bonierbale et al. 1988; Gebhardt et al. 1991; Jacobs et al. 1995; Van Eck et al. 1995) at the costs of the laborious development of diploid populations (Hermsen and Verdenius 1973; Veilleux et al. 1995). A disadvantage of primary dihaploids (2n = 2x = 24) is their often reduced fertility and vigour due to inbreeding depression as well as skewed segregations.

Mapping studies at the tetraploid level could be an alternative, if the inconvenience of tetrasomic inheritance and the at least fourfold reduction in the efficiency of BSA could be overcome by a more efficient marker technology. The polymerase chain reaction (PCR)-based marker technique AFLP

(Vos et al. 1995) offers the advantage that large numbers of locus-specific markers (Rouppe van der Voort et al. 1997) can be studied simultaneously. However, a priori information on allele dosage and the resulting utility of AFLP markers in tetraploid potato is lacking.

Late blight, caused by the fungus *Phytophthora infestans* (Mont.) de Bary, is one of the major diseases in potato (*S. tuberosum* L.). Eleven *R* genes conferring race-specific resistance, all originating from the hexaploid *S. demissum* (Black et al. 1953; Malcolmson and Black 1966), have been successfully introgressed into the cultivated potato to control this fungal disease. Up till now 4 *R* genes have been localized using diploid mapping populations: *R1* on chromosome *5* (Leonards-Schippers et al. 1992; El-Kharbotly et al. 1994) and *R3*, *R6* and *R7* on chromosome *11* (El-Kharbotly et al. 1994, 1996). The latter 3 mapped to the same region of chromosome *11*, suggesting a clustering of *R* genes similar to that of resistance genes in maize, lettuce and soybean (Saxena and Hooker 1968; Hulbert and Michelmore 1985; Ashfield et al. 1995).

In the study presented here we demonstrated (1) the application of BSA in an autotetraploid offspring, and (2) the efficient use of common AFLP markers to map *R2*. The basic idea behind common AFLP markers is that AFLP bands with equal electrophoretic mobility, visually recognized in AFLP fingerprints of different genotypes, represent a unique allele at a given locus. Once AFLP markers have been mapped in a (diploid) mapping population, they can be used to assign map positions to other markers with which they cosegregate in another mapping population.

## Materials and methods

### Plant material

The tetraploid mapping population, EJ96-4601, comprising 86 offspring genotypes, descended from BET95-4200-3 ($R_2rrr$) and DJ93-6707-10 (*rrrr*). The female resistant parent BET95-4200-3 was derived from a cross between Cebeco44158-4 and the highly fertile and susceptible genotype J90-6001-3. Clone Cebeco44158-4 was originally used for the characterization of *R*-gene specificity (Black et al. 1953; kindly provided by L. Turkensteen, IPO-DLO, Wageningen) and originated from the *S. demissum* accession CPC2127 (synonymous to BGRC10030; PI205625). *S. demissum* germplasm was represented by the accessions BGRC9990, BGRC10022 and BGRC10030 (kindly provided by Jouke Kardolus, Department Plant Taxonomy, WAU), and *S. tuberosum* germplasm by the tetraploid clones 6701-4 and Am66-42 and the cultivars 'Bintje', 'Katahdin', 'Kondor', 'Desiree', 'Adora' and 'Frieslander'; all susceptible to *P. infestans* race 2 (Joosten 1991). Five reference clones which have been used in previous AFLP mapping studies were USW5337.3 and 77.2102.37 (Jacobs et al. 1995; Van Eck et al. 1995) and 3778-16 (encoded AM), SH82-93-488 and RH89-039-16 (Rouppe van der Voort et al. 1997).

### Evaluation of resistance to *P. infestans*

The resistance of the plant material was evaluated using the detached leaf test (El-Kharbotly et al. 1994) with *P. infestans* race 0 to recognize segregation of any *R* allele. The identity of the *R* allele in this material was confirmed by re-testing the resistant plants with race 2 (the races of *P. infestans* were kindly provided by Francien Govers, Department of Phytopathology, WAU, NL).

### DNA isolation, AFLP procedure and bulk composition

Total genomic DNA was extracted from the plant material as described by Van der Beek et al. (1992).

The AFLP technique was performed according to Vos et al. (1995), including a 20-fold dilution of the ligation mixture prior to a selective pre-amplification, with primers that were extended with a single 3′ nucleotide (**E+A** and **M+C**). PCR employed a stringent touch-down temperature profile. In the subsequent PCR amplification a radioactively labeled ($^{33}$P) **E+3** primer was used in combination with a **M+3** primer. [$^{33}$P]-labeled amplification products were loaded, after a 15 min pre-run, on a 5% denaturing polyacrylamide sequence gel system (BioRad, Richmond, USA) buffered with 1 × TBE. The anodal 1 × TBE buffer was supplemented with 0.5 *M* sodium acetate to establish a salt gradient in order to slow down the migration of the smallest fragments. The gels were dried on Whatman 3MM paper and exposed to X-ray film (Konica, Japan) for 2–6 days to visualize AFLP fragments on autoradiograms.

The resistant and susceptible bulks ($B_R$ and $B_S$) were composed from equal aliquots of secondary AFLP template from 8 randomly chosen resistant or susceptible plants, respectively. AFLP template DNA of a *S. demissum* accession was obtained from a mixture of leaf tissue from 5 seedlings.

### Nomenclature of AFLP markers

AFLP markers were designated according to the letters of the three selective nucleotide extention at the 3′ end of the *Eco*RI + 3 primer, followed by a slash, then the three letters of *Mse*I + 3 primers and the size of the amplified product. The approximate size of each marker was expressed in nucleotides as estimated in comparison with the mobility of the bands of the SequaMark 10 base ladder (Research Genetics, Huntsville, Ala.) by linear interpolation. Amplification products with a size below 100 nucleotides were omitted from further analysis.

### Linkage analysis

Marker order and map distances were estimated using JOINMAP version 2.0 (Stam 1993).

## Results

### Inheritance of the resistance

Detached leaf tests with *P. infestans* race 0 unambiguously revealed the resistance genotype of the individuals of the mapping population EJ96-4601. The observed segregation of 44 resistant and 40 susceptible plants fitted a 1 : 1 ratio, indicating a simplex inheritance of the resistance allele (two missing observations).

## Levels of polymorphism

To identify AFLP marker alleles with putative linkage to the *R2* allele we used the greater part of the 16 **E+ANN** × 16 **M+CNN** (= 256) primer combinations to generate AFLP fingerprints from four samples: the resistant and susceptible parents ($P_R$ and $P_S$) and two DNA pools ($B_R$ and $B_S$) comprising 8 resistant and susceptible offspring genotypes, respectively, as identified in the detached leaf test described above. Fifty-one primer combinations were omitted from further analysis because of the low quality of the fingerprint (too dense or too weak) or when PCR amplification failed in one (or more) of the four samples. The fingerprints obtained with the remaining 205 primer combinations were used to evaluate the frequency of polymorphisms per lane.

The total number of unique AFLP bands per primer combination, observed in the two parents, varied between 90 and 36 (mean = 54.2 bands). Across all primer combinations 12.4% and 12.8% of the amplification products were unique to the resistant and susceptible parent, respectively. The remaining 74.8% of the amplification products were present in both parents.

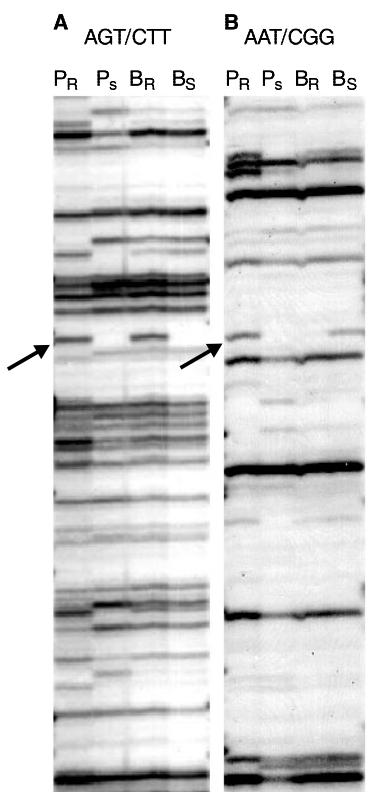


**Fig. 1A, B** AFLP fingerprints (sections) generated with primer combinations ACT/CTT (**A**) and AAT/CGG (**B**) showing bulk-specific amplification products. The bulk specific products are indicated by *arrows*. *Lanes* $P_R$ and $P_S$ represent resistant and susceptible parents, $B_R$ and $B_S$ represent resistant and susceptible bulks. **A** The specific amplification of the AFLP marker in a + - + - pattern suggests linkage to the *R2* locus in coupling phase. **B** The AFLP marker specific to the resistant parent and susceptible bulk suggests linkage to the *R2* locus in repulsion phase

By means of BSA, 12 AFLP markers were detected that were present in $P_R$ and $B_R$ and absent in $P_S$ and $B_S$. These were candidate markers for linkage to the *R2* resistance gene in coupling phase (Fig. 1A). Ten AFLP markers specific to $P_R$ were found to be specific for the susceptible pool ($B_S$) and for the resistant parent, suggesting linkage in repulsion to the *R2* allele (Fig. 1B). These AFLP markers were omitted from further analysis because the size of the mapping population was too small to allow reliable detection of linkage and accurate estimation of recombination values between linked markers in repulsion phase.

## Identification of linked markers

The entire offspring population (86) was used to confirm linkage of these 12 AFLP markers with the *R2* allele and to estimate recombination frequencies (Fig. 2). Analysis of the AFLP fingerprints of the offspring generated using these 12 primer combinations showed that on average 37% of the amplification products of the fingerprint segregated as presence/absence polymorphism. This rate of 37% includes AFLP markers heterozygously present in both parents (besides the 12.4% and 12.8% possibly segregating parental polymorphisms mentioned above).

Linkage between 11 of the 12 bulk-specific AFLP markers and the *R2* allele was confirmed (Fig. 2) with highly significant LOD values (between 7 and 24). No recombination was detected in this population between the *R2* resistance allele and markers ACC/CAT-535, ACT/CAC-189 and AGC/CCA-369. The order of the markers around the resistance locus was calculated using JOINMAP and is shown in Fig. 3. One candidate AFLP marker segregated in a 5 present: 1 absent ratio, suggesting a duplex parental genotype and, consequently, linkage to *R2* locus was not significant.

## Assignment of the R2 linkage group

To identify the genomic position of the linkage group comprising the AFLP markers and the *R2* allele on previously published maps of the potato genome, we made use of the locus-specific character of AFLP markers. Locus specificity – a unique AFLP amplification product corresponding to a unique genetic locus – was substantiated by Rouppe van der Voort et al. (1997). They showed that comigrating AFLP markers generated by the same primer combination in different parental genotypes map to indistinguishable positions on the genetic maps that correspond to these different genotypes and exhibit the same nucleotide sequence.

Five parental potato genotypes which had been used extensively in previous mapping studies (Jacobs et al.

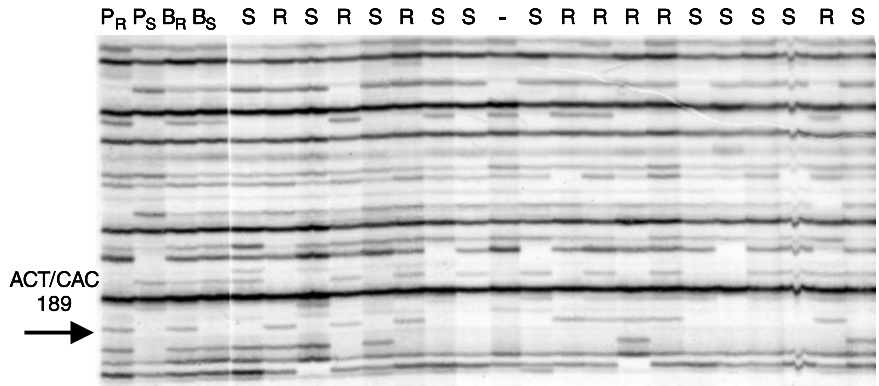


**Fig. 2** Part of an AFLP fingerprint showing the cosegregation of marker ACT/CAC-189 with the phenotype of *P. infestans* resistance (only 20 genotypes shown). *Lanes 1*, *2* $P_R$ and $P_S$ represent resistant and susceptible parents, *lanes 3*, *4* $B_R$ and $B_S$ represent resistant and susceptible bulks, *lanes 5–25* offspring genotypes showing resistant (*R*) or susceptible (*S*) phenotype

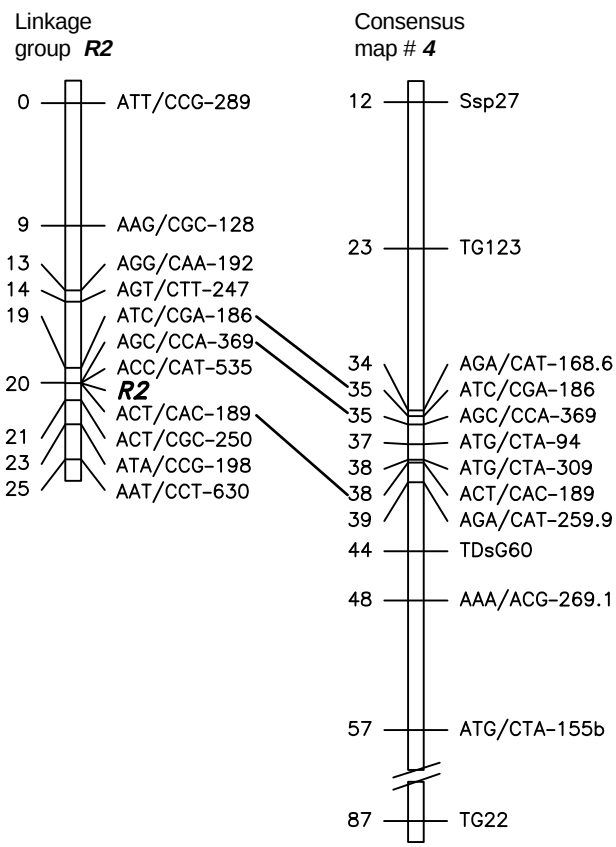


**Fig. 3** Linkage map showing the position of the *R2* locus and AFLP markers (distances in cM)

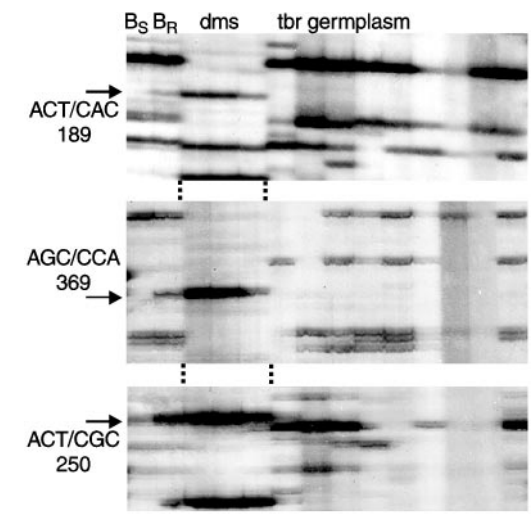


**Fig. 4** Three AFLP markers, ACT/CAC-189, AGC/CCA-369, ACT/CGC-250, with linkage to the *R2* locus are specifically amplified in the resistant bulk and three accessions of *S. demissum* but are absent in other lanes. *Lanes 1*, *2* $B_S$ susceptible bulk and $B_R$ resistant bulk, *lanes 3–5 S. demissum* accessions BGRC9990, BGRC10022 and BGRC10030, *lanes 6–14 S. tuberusom* germplasm represented by 'Bintje', 'Katahdin', Am66-40, 'Desiree', 'Frieslander', 'Adora', USW5337.3, 77.2102.37, 'Kondor', respectively

1995; Van Eck et al. 1995; Rouppe van der Voort et al. 1997) were analysed for the presence of these 11 AFLP markers. Only in the fingerprints of the reference clone "AM" were amplification products visually recognized with an electrophoretic mobility corresponding to that of 3 AFLP markers with linkage to *R2*. In the diploid reference mapping population "$F_1$AM × RH" (Rouppe van der Voort et al. 1997) the 3 AFLP alleles ACT/CAC-189, AGC/CCA-369 and ATC/CGA-186 again showed tight mutual linkage, as well as linkage to other potato chromosome *4* markers. The assignment of the linkage group comprising the *R2* locus on the consensus map of potato chromosome *4* was inferred on the basis of common AFLP marker alleles (Fig. 3).

In a wider search for these 11 AFLP alleles in *S. tuberosum* germplasm using the cultivars 'Bintje', 'Katahdin', 'Desiree', 'Kondor', 'Frieslander' and 'Adora' as well as progenitor lines Am66-42 and 6701-4 only occasionally (three times) an AFLP marker was observed with the same mobility as the AFLP alleles linked to *R2*. However, these 11 common AFLP markers were always identified in the fingerprints of three accessions of *S. demissum* (Fig. 4). These results suggest that AFLP alleles with linkage to the *R2* allele are of *S. demissum* origin and located on an introgression segment surrounding the *R2* locus.

## Discussion

### Efficiency of BSA at the tetraploid level (emperical)

In this study the feasibility of BSA was tested in a non-inbred polysomic polyploid. In diploid non-inbred potato Meksem et al. (1995) and Ballvora et al. (1995) demonstrated the efficient use of BSA in combination with AFLP markers. In Bulked Segregant Analysis AFLP can be used as a dominant marker only, hence the contrast between the DNA pools should be a presence/absence polymorphism. Such polymorphisms are expected to be bulk-specific only if at the marker locus the parental combination represents a simplex and nulliplex genotypes, with the AFLP allele in simplex condition linked in coupling phase with the 'simplex' target allele. Therefore, the efficiency of BSA in polysomic polyploids depends on (1) the linkage phase and (2) the fraction of the loci in simplex/nulliplex condition. Wu et al. (1992) arrived at the same conclusion for codominant restriction fragment length polymorphic (RFLP) markers and proposed the selection of one highly heterozygous and one highly inbred parent to increase the frequency of Single Dose Restriction Fragments (SDRFs $\approx$ simplex/nulliplex condition).

In this study 205 primer combinations were evaluated, and 11 AFLP markers were identified with linkage to the *R2* allele, whereas Meksem et al. (1995) detected 29 AFLP loci with linkage to *R1* using 108 primer combinations in a diploid offspring. Comparing this result at the diploid level with BSA at the tetraploid level our result is surprisingly good, taking into account the expected eightfold reduction in efficiency in our genetic material. A fourfold efficiency reduction results from tetrasomic inheritance where marker alleles with linkage in repulsion cannot be used (Wu et al. 1992).

Another twofold reduction in efficiency is caused by the construction of the bulks. In this study no prior information was available regarding the design of the bulks other than the disease resistance phenotype, while Meksem et al. (1995) could group progeny into four bulks according to the genotypic classes present in full-sib offspring of non-inbred diploids, as revealed with multi-allelic RFLP markers linked to the target locus ($A_1A_2 \times A_3A_4 \rightarrow A_1A_3$, $A_1A_4$, $A_2A_3$, $A_2A_4$). Such a bulk composition also allows the identification of marker alleles present on both homologous chromosomes from the susceptible parent.

### Efficiency of BSA at the tetraploid level (theoretical)

The proportion of loci with the simplex/nulliplex condition in two parents is a function of the frequency of AFLP marker alleles in the population from which the parents were sampled. An estimate of the average allele frequency of an arbitrary AFLP amplification product can be obtained from the similarity between the AFLP fingerprints of two random individuals (Van Eck et al., in preparation). In the Appendix we derive the relation between mean allele frequency (over loci) and the probability of an observable polymorphism between two parents in a tetraploid random mating population.

Across 205 primer combinations 25.2% of the AFLP amplification products were polymorphic between the two tetraploid parents of this study, indicating a value for the mean allele frequency of AFLPs of $\hat{p} = 0.38$ using the formula $\hat{p} = 1 - (A)/(2 - A))^{1/4}$, where A is the fraction of monomorphic bands (See Appendix). Consequently, the expected proportion of markers with the simplex/nulliplex condition is $4p(1 - p)^3(1 - p)^4 = 0.054$.

We assume that at any given locus we have detected at most one observable allele, which means that every AFLP marker corresponds to a unique genetic locus. Assuming further that AFLP markers unique to the resistant parent are in simplex condition, we estimated that $205 \times 54.2 \times 0.124 = 1378$ polymorphic loci were tested for bulk specificity. A correction for duplex, triplex and quadruplex conditions results in a more conservative estimate for the number of loci tested: $205 \times 54.2 \times 0.054 = 599$. Correcting next for the fourfold reduction in efficiency resulting from markers linked in repulsion to the *R2* allele, we arrive at an estimated number of loci tested for bulk specificity between 345 and 150. Given a 1000-cM maplength and assuming a homogeneous distribution of AFLP markers along the genome, this number is less than 1 marker per 3 cM, yet sufficient to ascertain feasibility of BSA in tetraploid potato using AFLP. This holds true even without the advantage of specific genetic disimilarity between wild species' introgression segments and the homologous potato chromosome segment.

### Identification of linkage drag from *S. demissum* by AFLP

Both the emperical consideration and the estimation above indicated that the number of AFLP alleles linked to *R2*, as identified with BSA at the tetraploid level, exceeded the expected number, suggesting a higher proportion of loci in simplex/nulliplex condition flanking the *R2* allele. This suggests the presence of linkage drag flanking the *R2* locus carrying AFLP alleles which have a low allele frequency in *S. tuberosum* germplasm. The AFLP alleles with linkage to *R2* were generally absent in germplasm representing cultivated potato, but in germplasm representing *S. demissum*, the donor species of the resistance, these AFLP alleles were consistently present, thereby confirming their *S. demissum* origin.

These results also imply that BSA at the tetraploid level using AFLP efficiently selects those AFLP markers which discriminate between alleles from wild donor species and cultivated potato. Exploitation of the contrast between alleles from wild species and those from cultivars was described earlier by Van der Beek et al. (1992). They demonstrated that most of the polymorphic loci in the tomato genome correspond to introgressed chromosome segments from wild species carrying resistance genes. However, the high level of heterozygosity of the potato genome (Bonierbale et al. 1993) is incomparable with the narrow genetic basis of cultivated tomato. Nevertheless and despite the higher ploidy level our results indicate that AFLP markers can recognize introgression segments surrounding resistance genes from wild species origin, and this enhances the efficiency of mapping studies.

The unequivocal recognition of marker alleles is a basic requirement for marker-assisted breeding. Earlier attempts to specifically identify certain marker alleles with Allele Specific Oligos (ASO) derived from RFLP alleles failed (Niewöhner et al. 1994). Their ASOs linked to the *Gro1* and *H1* nematode resistance locus were not correlated with the presence of resistance in a wider collection of potato cultivars.

## Visual recognition of common AFLP markers

A novel strategy was used to identify the genomic position of the linkage group comprising the *R2* allele. This strategy consists of the identification of common AFLP markers and their map position in reference material. Common AFLP markers are visually recognized as co-migrating bands in fingerprints generated from unrelated genotypes and result from the highly selective and reproducible conditions under which AFLP fingerprints are generated. Validation of locus specificity and the sequence homology of co-migrating bands was recently described by Rouppe van der Voort et al. (1997).

In AFLP fingerprints from 5 reference genotypes 3 of the 11 AFLP alleles which are linked to *R2* were recognized visually on the basis of their electrophoretic mobility. These 3 common AFLP markers also showed mutual linkage in the reference mapping population and were located on potato chromsome *4* in the reference map. Hence, the linkage group comprising *R2* could be aligned to a potato consensus map without cloning or sequencing of DNA. Currently a catalogue is available at URL http://www.spg.wau/pv/aflp/catalog.html describing the mobility names and chromosome specificity of all segregating AFLP alleles from these 5 reference clones using a wide array of commonly used primer combinations (Rouppe van der Voort et al. 1998). Markers recognized in downloaded images can help others to infer the genomic position of their AFLP markers.

When the highly selective and reproducible AFLP fingerprints allow recognition of homologous stretches of DNA in other potato germplasm, a similar approach can be used to identify these sequences in YAC or BAC clones. By recognition of the plant genomic alleles in the fingerprint of a YAC or BAC clone and the corresponding allele in reference material, the YAC or BAC clones directly map to the genetic map of potato without cloning or sequencing steps. Conversely, the AFLP markers without recombination to *R2* can be used to identify a corresponding YAC or BAC clone. We conclude that visual recognition of comigrating AFLP markers is a straightforward strategy both for mapping studies and for gene cloning using the concept of chromosome landing as outlined by Tanksley et al. (1995).

## Gene mapping in potato

Genetic analysis in potato has been carried out at the diploid level when efficient methods could be used for reducing the ploidy level (Hermsen and Verdenius 1973; Veilleux et al. 1995). Efforts to develop segregating populations at the diploid level were compensated for by the genetic resolution offered at the diploid level, although crossing and selection could take years (El-Kharbotly et al. 1994 and 1996). The highly efficient AFLP technology, both in number and specificity of the markers, reopened interest for genetic analysis at the tetraploid level (Meyer et al. 1996, Brignetti et al. 1997).

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## Appendix

Let the population frequency of an allele at a given locus (detected as a PCR product with AFLP primers: a band) be p. Let q be the joint frequencies of the alternative alleles at this locus; these are not detected as a band ($p + q = 1$). We assume that the genotypes in the population occur in frequencies corrsponding to the random mating equilibrium (Hardy-Weinberg).

The probabilities for the presence or absence of the band in a tetraploid genotype are:

$$\text{Prob. (band)} = 1 - q^4 \quad \text{and} \quad \text{Prob. (no band)} = q^4$$

When comparing the fingerprints of two tetraploid parental clones, the following applies:

$$\text{Prob. (both have band)} = (1 - q^4)^2$$

$$\text{Prob. (polymorphism)} = 1 - (1 - q^4)^2 - (q^4)^2$$

$$\text{Prob. (both without a band)} = (q^4)^2$$

$$\sum = 1$$

Since the joint absence of a band with a probability of $(q^4)^2$ cannot be visualized, the following applies to the visible part $(1 - (q^4)^2)$ of the fingerprint:

$$\text{Prob. (both have band)} = (1 - q^4)^2/(1 - (q^4)^2)$$

$$\text{Prob. (polymorphism)} = 1 - (1 - q^4)^2 - (q^4)^2/(1 - (q^4)^2)$$

$$\sum = 1$$

The probability that both parents have a band can be rewritten:

$$\text{Prob. (both have band)} = A = (1 - q^4)^2/(1 - q^4)(1 + q^4)$$

$$= (1 - q^4)/(1 + q^4)$$

Assume for simplicity that all AFLP-detectable alleles have equal frequency, p. Then $q = 1 - p$ can be estimated from $A = (1 - q^4)/(1 + q^4)$, where A is the fraction of bands present in both parents. This leads to

$$q^4 = (1 - A)/(1 + A)$$

$$\text{or } p = 1 - ((1 - A)/(1 + A))^{1/4}$$

When p varies across loci, application of the above estimation procedure leads to underestimation of the average value of p (A is a concave function of p).

## References


Ashfield T, Keen NT, Buzzell RI, Innes RW (1995) Soybean resistance genes specific for different *Pseudomonas syringae* avirulence genes are allelic, or closely linked, at the RPG1 locus. Genetics 141:1597–1604

Ballvora A, Hesselbach J, Niewöhner J, Leister D, Salamini F, Gebhardt C (1995) Marker enrichment and high-resolution map of the segment of potato chromosome VII harbouring the nematode resistance gene *Gro1*. Mol Gen Genet 249: 82–90

Black W, Mastenbroek C, Mills WR, Peterson LC (1953) A proposal for an international nomenclature of races of *Phytophthora infestans* and of genes controlling immunity in *Solanum demissum* derivatives. Euphytica 2:173–179

Bonierbale MW, Plaisted RL, Tanksley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120: 1095–1103

Bonierbale MW, Plaisted RL, Tanksley SD (1993) A test of the maximum heterozygosity hypothesis using molecular markers in tetraploid potatoes. Theor Appl Genet 86:481–491

Brignetti G, Garcia-Mas J, Baulcombe DC (1997) Molecular mapping of the potato virus Y resistance gene $Ry_{sto}$ in potato. Theor Appl Genet 94:198–203

El-Kharbotly A, Leonards-Schippers C, Huigen DJ, Jacobsen E, Pereira A, Stiekema WJ, Salamini F, Gebhardt C (1994) Segregation analysis and RFLP mapping of the R1 and R3 alleles conferring race-specific resistance to *Phytophthora infestans* in progeny of diploid potato parents. Mol Gen Genet 242: 749–754

El-Kharbotly A, Palomino-Sanchez C, Salamini F, Jacobsen E, Gebhardt C (1996) 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. Theor Appl Genet 92:880–884

Gebhardt C, Ritter E, Barone A, Debener T, Walkemeier B, Schachtschabel U, Kaufmann H, Thompson RD, Bonierbale MW, Ganal MW, Tanksley SD, Salamini F (1991) RFLP maps of potato and their alignment with the homoeologous tomato genome. Theor Appl Genet 83:49–57

Hermsen JGT, Verdenius J (1973) Selection from *Solanum tuberosum* Group *phureja* of genotypes combining high frequency haploid induction with homozygosity for embryo-spot. Euphytica 22:244–259

Hulbert SH, Michelmore RW (1985) Linkage analysis of genes for resistance to downy mildew (*Bremia lactuca*) in lettuce (*Lactuca sativa*). Theor Appl Genet 70:520–528

Jacobs JME, van Eck HJ, Arens P, Verkerk-Bakker B, te Lintel Hekkert B, Bastiaanssen HJM, El-Kharbotly A, Pereira A, Jacobsen E, Stiekema WJ (1995) A genetic map of potato (*Solanum tuberosum*) integrating molecular markers, including transposons and classical markers. Theor Appl Genet 91: 289–300

Joosten A (1991) Geniteurslijst voor aardappelrassen. ISSN 0169–641, COA, Wageningen, The Netherlands

Leonards-Schippers C, Gieffers W, Salamini F, Gebhardt C (1992) The *R1* gene conferring race-specific resistance to *Phytophthora infestans* in potato is located on potato chromosome *V*. Mol Gen Genet 233:278–283

Malcolmson JF, Black W (1966) New R genes in *Solanum demissum* Lindl. and their complementary races of *Phytophthora infestans* (Mont.) de Bary. Euphytica 15:199–203

Meksem K, Leister D, Peleman J, Zabeau M, Salamini F, Gebhardt C (1995) A high-resolution map of the vicinity of the *R1* locus on chromosome *V* of potato based on RFLP and AFLP markers. Mol Gen Genet 249:74–81

Meyer R, Milbourne D, Collins A, Bradshaw J, Powell W, Waugh R (1996) Development and application of molecular markes for QTL analysis in tetraploid potatoes. In Abstracts conf papers, posters and demonstrations, 13th Triennial Conf Eur Assoc Potato Res. EAPR, Veldhoven, The Netherlands, pp 69–70

Malcolmson JF, Black W (1966) New *R* genes in *Solanum demissum* Lindl. and their complementary races of *Phytophthora infestans* (Mont.) de Bary. Euphytica 15:199–203

Meksem K, Leister D, Peleman J, Zabeau M, Salamini F, Gebhardt C (1995) A high resolution map of the vicinity of the *R1* locus on chromosome *V* of potato based on RFLP and AFLP markers. Mol Gen Genet 249:74–81

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Niewöhner J, Salamini F, Gebhardt C (1994). Development of PCR assays diagnostic for RFLP markers closely linked to alleles *Gro1* and *H1*, conferring resistance to the root cyst nematode *Globodera rostochiensis* in potato. Mol Breed 1:65–78

Rouppe van der Voort JNAM, Van Eck HJ, Folkertsma RT, Van Zandvoort P, Hutten RCB, Draaistra J, Gommers FJ, Jacobsen E, Helder J, Bakker J (1997) Locus specificity of AFLP markers used to align genetic maps from different potato genotypes. Mol Gen Genet 255:438–447

Rouppe van der Voort JNAM, Van Eck HJ, Draaistra J, Van Zandvoort PM, Jacobsen E, Bakker J (1998) An online catalogue of AFLP markers covering the potato genome. Mol Breed 4:73–77

Saxena KMS, Hooker AL (1968) On the structure of a gene for disease resistance in maize. Proc Natl Acad Sci USA 68: 1300–1305

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JOINMAP. Plant J 3: 739–744

Tanksley SD, Ganal MW, Martin GB (1995) Chromosome landing: a paradigm for map-based gene cloning in plants with large genomes. Trend Genet 11:63–68

Van Eck HJ, Rouppe van der Voort J, Draaistra J, Van Zandvoort P, Van Enckevort E, Segers B, Peleman J, Jacobsen E, Helder J, Bakker J (1995) The inheritance and chromosomal localization of AFLP markers in a non-inbred potato offspring. Mol Breed 1:397–410

Van der Beek JG, Verkerk R, Zabel P, Lindhout P (1992) Mapping strategy for resistance genes in tomato based on RFLPs between cultivars: *Cf9* (resistance to *Cladosporium fulvum*) on chromosome 1. Theor Appl Genet 84:106–112

Veilleux RE, Shen LY, Paz MM (1995) Analysis of the genetic composition of anther-derived potato by randomly amplified polymorphic DNA and simple sequence repeats. Genome 38:1153–1162

Vos P, Hogers R, Bleeker M, Reijans M, Van der Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new concept for DNA fingerprinting. Nucleic Acids Res 23(21):4407–4414

Wu KK, Burnquist W, Sorrells ME, Tew TL, Moore PH, Tanksley SD (1992) The detection and estimation of linkage in polyploids using single-dose restriction fragment. Theor Appl Genet 83:294–300