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Identification of AFLP and SSR markers associated with quantitative resistance to *Globodera pallida* (Stone) in tetraploid potato (*Solanum tuberosum* subsp. *tuberosum*) with a view to marker-assisted selection

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Abstract Seventy eight clones from the cross between SCRI clone 12601ab1 and cv Stirling were used to explore the possibility of genetical linkage analysis in tetraploid potato (*Solanum tuberosum* subsp. *tuberosum*). Clone 12601ab1 had quantitative resistance to *Globodera pallida* Pa2/3 derived from *S. tuberosum* subsp. *andigena*. The strategy adopted involved identifying single- (simplex) and double- (duplex) dose AFLP markers in the parents from segregation ratios that could be unambiguously identified in their offspring, detecting linkage between a marker and a putative quantitative trait locus (QTL) for resistance, and placing the QTL on the linkage map of markers. The numbers of scorable segregating markers were 162 simplex ones present only in 12601ab1, 87 present in Stirling, and 32 present in both; and 72 duplex markers present only in 12601ab1 and 45 present in Stirling. The total map length was 990.9 cM in 12601ab1 and 484.6 cM in Stirling. A QTL with a resistance allele present in double dose (QQqq) in 12601ab1 was inferred from the associations between resistance scores (square root of female counts) and two duplex markers linked in coupling, which, in turn, were linked in coupling to four simplex markers also associated with resistance, but to a lesser degree. The largest marker class difference was the one for the duplex marker P61M34 = 15. It accounted for 27.8% of the phenotypic variance in resistance scores, or approximately 30% of the genotypic variance. Subsequently, this duplex marker was found to be linked in coupling with a duplex SSR allele Stm3016 = a, whose locus was shown to be on chromosome IV in a diploid reference mapping population. The other QTLs for resistance segregating in the progeny were not identified for one or more of the following reasons: the markers did not cover the whole of the genome, there were unfavourable repulsion linkages between the QTLs and markers, or the gene effects were not large enough to be detected in an experiment of the size conducted. It is concluded that prospects appear good for detecting QTLs and using marker-assisted selection in a tetraploid potato breeding programme, provided that, in future, the population size is increased to over 250 and more SSR markers are used to complement the AFLPs; the same is likely to be true for other autotetraploid crops.

Key words Potato · *Solanum tuberosum* · Nematode resistance · *Globodera pallida* · Linkage in autotetraploids · Quantitative trait locus

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Introduction

The European cultivated potato (*Solanum tuberosum* subsp. *tuberosum*) is a tetraploid species (2n = 4*x* = 48) which displays tetrasomic inheritance. It was derived from a narrow genetic base of a few introductions of subsp. *andigena* from South America in the late 16th century, and possible further casual introductions in the 17th and 18th centuries (Bradshaw and Mackay 1994). As a consequence, it lacked genes for adequate levels of resistance to pathogens and pests such as late blight [*Phytophthora infestans* (Mont.) de Bary] and potato cyst nematodes (PCN) (*Globodera* spp.) which became problems once it had assumed its role as a staple food crop (Bradshaw and Mackay 1994).

During the 20th century, attempts have been made to remedy these deficiencies by the introgression of resistance genes into subsp. *tuberosum* from the wild and cultivated *Solanum* species of Central and South America (Hermsen 1994). However, a *Solanum demissum*-derived major gene resistance to late blight failed to give durable resistance (Malcolmson 1969) and the widespread deployment of the *H*1 gene from subsp. *andigena* CPC1673 to control pathotype Ro1 (and Ro4) of the golden potato cyst nematode [*Globodera rostochiensis* (Woll.)] has inadvertently selected for the white potato cyst nematode [*Globodera pallida* (Stone)] (Bradshaw et al. 1996). More recently, cultivars with high levels of field resistance to late blight, such as Stirling and Torridon, have been bred from *S. tuberosum* germplasm derived from *S. demissum*, but they have not been widely grown (Wastie 1991; Bradshaw et al. 1995 a). Furthermore, high levels of quantitative resistance to *G. pallida* have also been incorporated into subsp. *tuberosum* from subsp. *andigena* (for example, SCRI clone 12601ab1), and to both nematode species from *Solanum vernei*, but only partial resistance has been achieved in successful cultivars such as Santé and Nadine (Bradshaw et al. 1996). The major gene resistances to *G. pallida* found to-date are only effective against some of the populations/pathotypes found in Europe; for example the *H2* gene for resistance to pathotype Pa1 from *Solanum multidissectum* (Dunnett 1961) and *Gpa2* for resistance to pathotype Pa2 from *S. tuberosum* subsp. *andigena* CPC1673 (Rouppe van der Voort et al. 1997).

Therefore, in Europe, there is a pressing need to combine the high levels of quantitative resistance to late blight and the white potato cyst nematode, available in subsp. *tuberosum*, with the commercially acceptable yields and qualities demanded by processors and supermarkets. Hence, it seemed timely to explore the possibility of genetical linkage analysis in tetraploid potato as a prerequisite to using molecular-marker-assisted selection to achieve this objective in a tetraploid breeding programme, without the need to spend time and effort on haploidisation, diploid analysis, and polyploidisation in order to avoid the complexities of tetrasomic inheritance. The efficient introgression of new resistances from wild species is a different issue, and is not the subject of this paper.

AFLPs (Vos et al. 1995) were chosen as the primary marker system because they allow a large number of segregating markers to be followed in a single experiment. They are based on the selective PCR amplifications of small restriction fragments (80–400 bp) of genomic DNA and are normally dominant markers. They have already been used in diploid potatoes for the construction of high-density linkage maps (Van Eck et al. 1995), the saturation mapping of potato chromosome VII around the *Gro1* gene for resistance to *G. rostochiensis* (Ballvora et al. 1995) and mapping the *Gpa2* gene for resistance to *G. pallida* (Rouppe van der Voort et al. 1997), as well as by Milbourne et al. (1997) for the analysis of genetical relationships in tetraploid potato cultivars. Subsequently, some of the highly polymorphic co-dominant SSR-based markers which are currently being developed for potato (Milbourne et al. 1998) were included to aid the chromosomal identification of a linkage group containing a putative quantitative trait locus (QTL) for resistance to *G. pallida*.

The cross between clone 12601ab1 and cv Stirling was chosen because these parents were known to have both high levels of resistance to *G. pallida* and *P. infestans*, respectively, and also high general combining abilities for these resistances (Bradshaw et al. 1995 b). The PCN results are presented and discussed in this paper; the blight results and more detailed theory on the construction of the marker linkage map can be found elsewhere (Hackett et al. 1998; Meyer et al. 1998).

Materials and methods

Plant material

The cross 12601ab1 × Stirling was one of a diallel set of crosses made in 1992 (Bradshaw et al. 1995 b). The reciprocal cross was made in 1993. Seedlings were raised from seed in a glasshouse in 10-cm-square pots during the summer of 1994 and tubers were harvested in mid-September from 331 plants (262 from the cross and 69 from its reciprocal). The 331 clones were maintained by growing two plants of each in 12.5-cm-square pots in a glasshouse in 1995, the spare tubers being used to raise plants for a foliage blight test. The maintenance plants were harvested in mid-September and the tubers kept in a dark store at 8°C until required. By mid-January 1996 they were just beginning to sprout. Two tubers of each clone were again planted for maintenance, leaving the remainder for use in testing for resistance to potato cyst nematodes (PCN).

PCN tests

There were sufficient tubers of 281 clones for them to be assessed for resistance to *G. pallida* Pa2/3 (Lindley) in a closed container test with four replicates, as described by Phillips et al. (1980). The test was set up on 13 and 14 February 1996 with one tuber per 60-ml Clearopac container unless the tubers were very small, when two were used. The numbers of females visible through the transparent walls of the containers were counted from 16 to 22 April 1996. Since small tubers of clone 12601ab1 and cv Stirling were not available at the same physiological age as those of the clones, they were assessed later in the year along with 18 clones covering the range of female counts found in the first test. This second test also comprised four replicates and was set up on 17 June 1996 and scored on 8 August 1996. The variance-stabilising square-root transformation was performed on the female counts before doing more detailed statistical analyses on the resulting PCN scores.

Mapping population and DNA extraction

A subset of 94 clones was randomly chosen for the initial mapping population from the first 211 of those available from the cross 12601ab1 × Stirling. Between 5 and 10 g of young, fully expanded

leaves were harvested from the plants grown in the glasshouse and freeze-dried. Total genomic DNA was extracted from the freeze-dried leaf material using the CTAB method of Saghai-Maroof et al. (1984). The quality and quantity of DNA were evaluated after ethidium-bromide staining of 0.8% agarose gels and UV transillumination.

AFLP assays

AFLP assays were performed using a modification of the protocol of Vos et al. (1995) as described by Milbourne et al. (1997). The 6-bp cutting enzymes *Eco*RI and *Pst*I were obtained from Boehringer Mannheim and the 4-bp cutting enzyme *Mse*I from New England Biolabs. T4 DNA ligase and T4 polynucleotide kinase came from GIBCO BRL. Ampli*Taq* and Ampli*Taq* LD DNA polymerase, as well as 1 × PCR buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl pH 8.3), were purchased from Perkin Elmer and the nucleotide-triphosphates from Pharmacia. Finally, AFLP primers and adapters were obtained from Genset, Paris. Autoradiograms were scored manually and independently by two people. Each band was treated as a locus with a dominant versus a recessive allele. Intensity differences were observed in segregating bands, but visual interpretation was not considered reliable enough to assign allelic dosage to a given clone.

SSR assays

Sufficient forward primer for 100 PCRs was 5′ end-labelled with γ^{32}P-ATP in a 20-µl reaction consisting of 0.3 µmol of primer, 30 µCi of 4500 Ci/mmol γ^{32}P-ATP (ICN), 30 U of T4 Polynucleotide Kinase (Gibco) and 1 × Gibco Forward Reaction Buffer (70 mM Tris-HCl pH 7.6, 10 mM $MgC1_2$, 100 mM KCl, 1 mM 2-mercaptoethanol), incubated at 37°C for 1 h, after which the reaction was terminated by heating to 70°C for 10 min.

Ten-microliter PCRs consisted of 20 ng of genomic DNA, 1 × PCR buffer (10 mM Tris-HCl, 1.5 mM $MgC1_2$, 50 mM KCl pH 8.3), 0.3 U of *Taq* polymerase (Boehringer-Mannheim), 0.3 µM of forward and reverse primers and 200 µM of dNTPs. The cycling conditions for PCR on a PE 9600 thermocycler or MJ Research DNA Engine were as follows: 94°C for 3 min, followed by 25 cycles of 94°C for 30 s, annealing temperature for 30 s, 72°C for 30 s, followed by 72°C for 5 min. Equal volumes of electrophoresis loading buffer (95% formamide, 10 mM EDTA, 0.5 mg/ml bromophenol blue and xylene cyanol) were added to the samples, which were then denatured at 95°C for 5 min, snap-cooled on ice, and subjected to polyacrylamide-gel electrophoresis on 6% acrylamide, 7 M Urea Easigel (Scotlab) in 1 × TBE (89 mM Tris-Borate, 2 mM EDTA). An M13 sequencing marker (prepared by performing the control reaction in the Sequenase V.2 sequencing kit from Amersham) was also run to estimate product sizes. Visualisation of results was achieved by exposure of fixed, dried gels to X-ray film.

Identification of segregation type

Markers occurring in just one parent were accepted as present in single- (simplex) or double-(duplex) dose if their observed segregation ratio was not significantly different from 1:1 or 5:1, respectively, as tested by a χ^2 goodness of fit with a 1% significance level. Markers occurring in both parents were likewise accepted as present in single-dose if their segregation ratio was not significantly different from 3:1, but this time at the 5% significance level to avoid overlap with markers giving the 11:1 segregation ratio expected from a duplex × simplex cross. These are the only segregation ratios that can be distinguished with the population size used (Bailey 1961). Although no double reduction (Fisher and Mather 1943) is assumed, its presence would not prevent the unambiguous identification of the three types of cross, Aaaa × aaaa, AAaa × aaaa and Aaaa × Aaaa, where A is the dominant marker present in either single- or double-dose. Histograms were plotted of the number of simplex and duplex AFLP bands from each parent carried by each clone in order to identify any selfs or anomalous results. As a consequence, 16 clones were eliminated from the experiment (see later), and the analyses repeated on the remaining 78 clones.

Linkage-map construction

Recombination frequencies and lod scores were calculated between pairs of markers as described by Hackett et al. (1998) and Meyer et al. (1998), using the statistical programme Genstat (Genstat 5 Committee 1993). These were then used for input to the linkage software, JoinMap 2.0 (Stam and Van Ooijen 1995) to obtain combined maps for each parent in which homologous groups were merged. The maps were printed with Drawmap (Van Ooijen 1994).

QTL analysis

For all segregating markers, a single-marker analysis was performed by determining the difference in mean PCN scores between the two marker classes, presence and absence of marker, and evaluating its significance by a *t*-test. All differences significant at the 5% level were noted, whilst recognising that some of them could have arisen by chance. Hence, a more appropriate threshold value for declaring a significant QTL effect was sought through the empirical method of Churchill and Doerge (1994). This was done for the markers segregating 1:1 and 5:1 from each parent and for those segregating 3:1, making five sets of analyses in total. In each analysis, the PCN scores for the 78 clones were shuffled, analysed for QTL effects at all markers in the set, the largest *t*-value stored, and the entire procedure repeated 1000 times. The stored *t*-values were then ordered and the 95% quantile taken as the estimated critical value at which the overall type-I error rate for the experiment was 0.05 or less. The differences between marker classes are functions of the frequency of recombination (i.e. recombinant chromatids) between the marker and the QTL and the genotypic values of the genotypes at the QTL (there could be up to eight different alleles segregating), but these can not be estimated from a simple single-marker analysis.

Results

AFLP marker analysis

Thirty nine AFLP primer combinations allowed 3173 loci to be surveyed and, of the 1066 markers which segregated in the population, 573 were considered scorable with the required accuracy. The percentage of informative loci was not significantly different between the *Eco*RI and *Pst*I templates, but the *Pst*I autoradiograms were much clearer and easier to score than the *Eco*RI gels due to a reduced number of bands per gel.

Although analysis of the parental source of markers in the 94 clones failed to detect any selfs, it did highlight a distinct group of 16 clones with an unusually low number of paternal products (Fig. 1). As there was no obvious experimental or genetical explanation for the origin of these distinct clones, they were omitted from

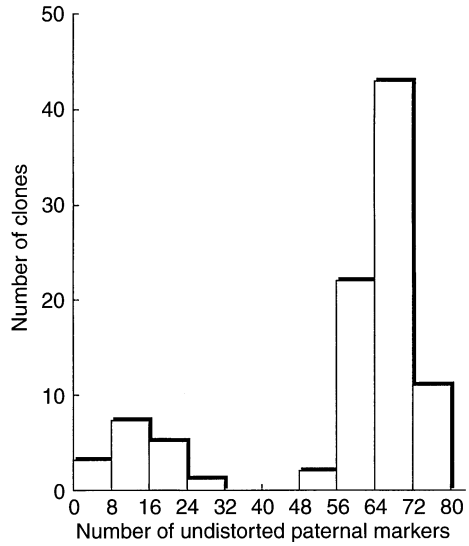

Fig. 1 Low number of paternal markers in 16 out of 94 clones from the cross 12601ab1 × Stirling

the subsequent marker, QTL, and linkage analyses which were, therefore, done on the remaining 78 clones. This left 559 scorable, segregating markers, of which 446 were present in one parent and absent in the other, while 113 were present in both parents.

Figure 2 shows the observed segregation ratios for the 446 markers which were present in one parent and absent in the other, and the theoretical distributions for 284 markers segregating 1:1 (53:25 is more likely to be a 1:1 than a 5:1 ratio) and 162 markers segregating 5:1 (54:24 is more likely to be a 5:1 than a 1:1 ratio). For a population size of 78, one would expect a clearcut separation of single- and double-dose markers, but this was not observed. Therefore, the linkage analyses were confined to markers that could be accepted as present in single- or double-dose on the criteria given earlier, whilst recognising that extreme but acceptable ratios appeared to be more numerous than expected by chance. These markers amounted to: 162 simplex markers present only in 12601ab1; 87 present in Stirling; and 32 present in both; and 72 duplex markers present only in 12601ab1 and 45 in Stirling.

AFLP linkage map

The map for 12601ab1 comprised 17 groups of four or more markers and 13 groups of just two or three markers with 35 out of 266 markers remaining unlinked (8/162 single-dose 1:1 markers, 10/72 double-dose 5:1 markers, and 17/32 single-dose 3:1 markers). The sizes of the linkage groups ranged up to 96.6 cM, with a total length of 990.9 cM.

The map for Stirling comprised nine groups of four or more markers and 17 groups of just two or three markers with 47 out of 164 markers remaining unlinked (12/87 single-dose 1:1 markers, 19/45 double-dose 5:1 markers, and 16/32 single-dose 3:1 markers). The sizes of the linkage groups ranged up to 73.0 cM, with a total length of 484.6 cM.

The parental maps contained seven bridging (3:1) markers which allowed seven maternal groups to be identified as homologous to seven paternal groups.

PCN scores

The histogram of PCN scores for the 78 clones is shown in Fig. 3. The differences between clones were statistically significant ($P < 0.001$), and an analysis of the components of variation (between clones and clones × replicates interaction) revealed that 93.7% of the observed variation was genetical. The correlation between the scores of the 18 clones in the first and in the second test was $r = 0.859$ ($P < 0.001$) and the regression equation, test 1 = 0.895 + 1.23 test 2, allowed the

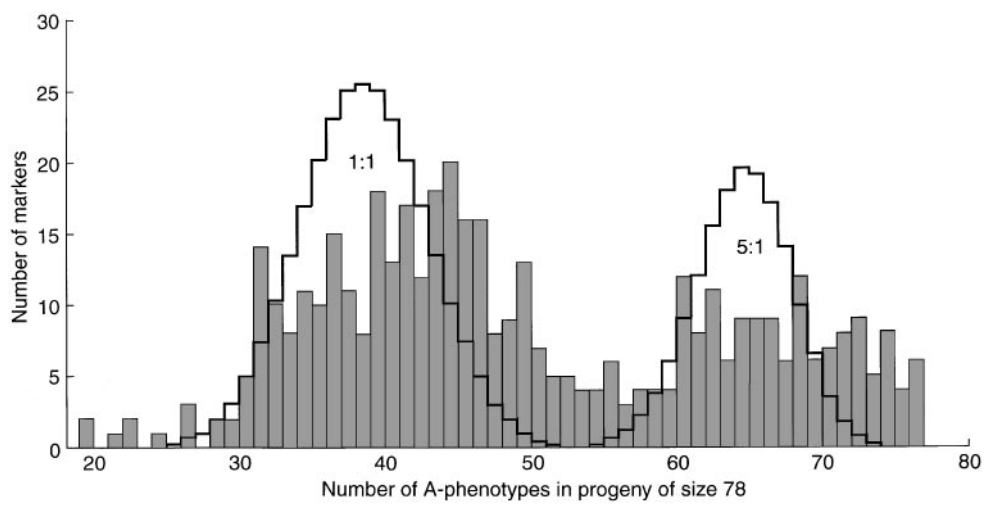

Fig. 2 Observed and expected (1:1 and 5:1) segregation ratios for 446 markers present in one parent in either single-(Aaaa) or double-dose (AAaa) and absent in the other (aaaa)

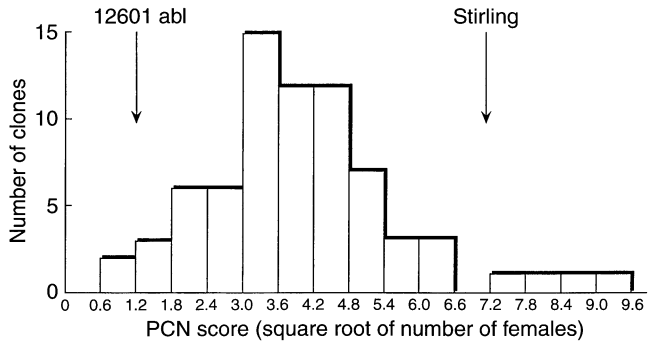

Fig. 3 PCN (*G. pallida*) scores of 78 clones from the cross 12601ab1 (resistant) × Stirling (susceptible)

parental values to be estimated as 1.203 for 12601ab1 and 7.102 for Stirling. These are also shown in the histogram.

QTL analysis

The single-marker analysis detected a number of associations between PCN scores and AFLP markers when the significance level was 5% ($t \approx 2.00$): 12 (1:1), 7 (5:1) and 1 (< 5:1) in 12601ab1, 12 (1:1), 4 (5:1) and 4 (> 5:1) in Stirling, and 3 (3:1). However, just 2 (5:1) associations in 12601ab1, together with the 1 (< 5:1) association, remained significant on using the more stringent *t*-value of 3.49 found by the method of Churchill and Doerge (1994).

Table 1 PCN resistance associated with AFLP markers and SSR marker Stm3016 on chromosome IV (b and c are homologous chromosomes)

Marker (−single dose, = double dose)	*t*-test	Percentage variance accounted for	PCN score	
			Marker present	Marker absent
IVb				
Stm3016 = a	− 4.38***	21.1	3.62	5.44
P46M37 − 5	− 2.47*	6.6	3.55	4.45
P61M34 = 15	− 5.36***	27.8	3.60	6.01
E34M61 − 27	-1.87^{NS}	3.4	3.69	4.43
E44M42 = 6	− 4.84***	23.8	3.63	5.86
E35M37 − 6	-1.90^{NS}	3.5	3.65	4.36
E39M42 − 22	− 2.11*	4.7	3.65	4.45
E38M39 − 37	-1.40^{NS}	1.3	3.71	4.24
E31M61 = 17	-0.34^{NS}	0.0	3.95	4.21
E35M121 − 2	-1.22^{NS}	0.7	3.77	4.24
E34M121 = 5	-0.15^{NS}	0.0	3.96	4.06
P40M34 − 11	− 2.28*	5.8	3.65	4.53
E46M37 = 9	-0.56^{NS}	0.0	3.94	4.27
P61M47 − 1	-1.16^{NS}	0.5	3.81	4.27
IVc				
Stm3016 = a	− 4.38***	21.1	3.62	5.44
P61M34 = 15	− 5.36***	27.8	3.60	6.01
E44M42 = 6	− 4.84***	23.8	3.63	5.86
P46M37 − 4	− 2.22*	5.2	3.55	4.37

***$P < 0.001$; **P 0.01–0.001; *P 0.05–0.01; NS, not significant

The two duplex markers associated with PCN resistance, P61M34 = 15 and E44M42 = 6, were closely linked in coupling (Table 1 and Fig. 4). Furthermore, they were also linked in coupling to four simplex (1:1)

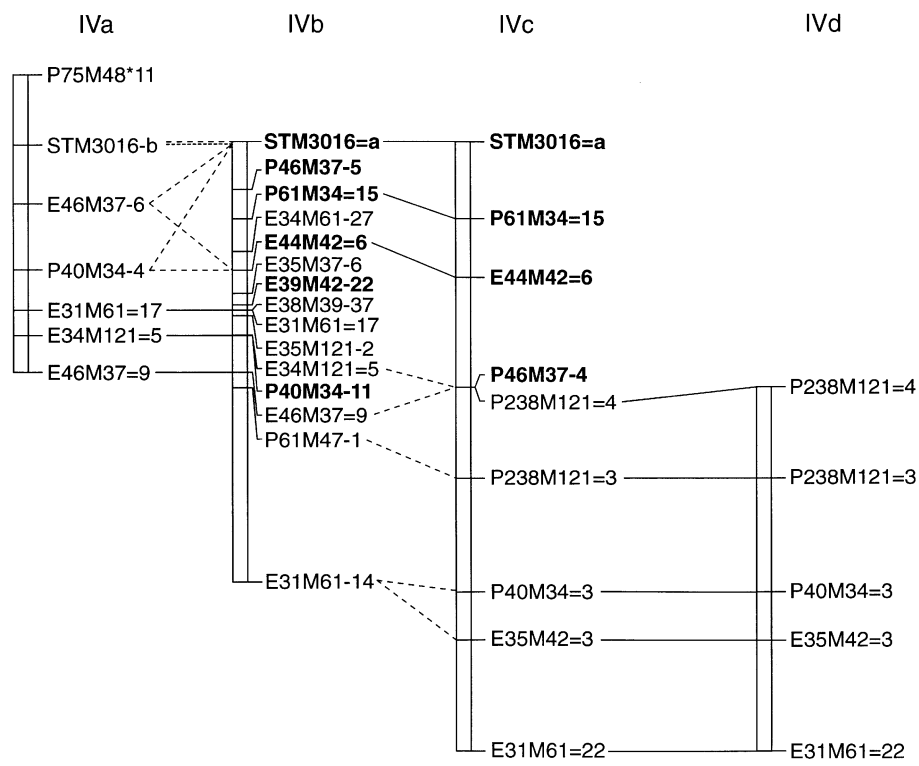

Fig. 4 AFLP markers (− simplex, = duplex, ∗ simplex also present in Stirling) and SSR locus (STM3016) on four homologues of chromosome IV (– – – repulsion linkage of simplex and duplex marker, — duplex marker, - - - alleles at SSR locus) in the female parent 12601ab1 and their association (*bold print*) with PCN resistance

markers which were associated with PCN resistance by the *t*-test. Three of these markers, P46M37–5, E39M42–22 and P40M34–11, were linked to each other in coupling, while the fourth (P46M37–4) was on a homologous chromosome. As all of these markers were associated with increased resistance, one can postulate that a QTL with an allele for PCN resistance in double-dose (QQqq) was present in the 12601ab1 parent and that the two copies of the allele were linked to the duplex and simplex markers in duplex/duplex and simplex/duplex coupling, respectively (AQ/AQ/aq/aq $\times$ aq/aq/aq/aq where A is P61M34 = 15).

It is not possible to obtain an accurate estimate of the magnitude of the effect of the resistance gene because the marker class difference ($\overline{\text{A---}} - \overline{\text{aaaa}}$) of 2.41 for marker P61M34 = 15 depends on four unknown parameters, the recombination frequency (r) and three genotypic values (μ):

$$\overline{\text{A---}} - \overline{\text{aaaa}} = \tfrac{1}{5}[(1 - 6r^2)\mu_{\text{QQqq}} + 4(1 - 3r + 3r^2)\mu_{\text{Qqqq}} - (5 - 12r + 6r^2)\mu_{\text{qqqq}}].$$

However, one can say that marker P61M34 = 15 accounted for 27.8% of the variance in PCN scores (Fig. 5).

The third marker associated with PCN resistance, E39M42 = 11, was not placed on the linkage map because it did not make the criterion for acceptance as segregating 5:1. It accounted for 14.7% of the variance in PCN scores, but one can not rule out the possibility that it may be linked to the markers just described.

The lack of associations between the PCN scores and the other markers in the linkage group (Table 1 and Fig. 4) can be attributed to unfavourable linkage relationships between the markers and the two copies of the resistance allele.

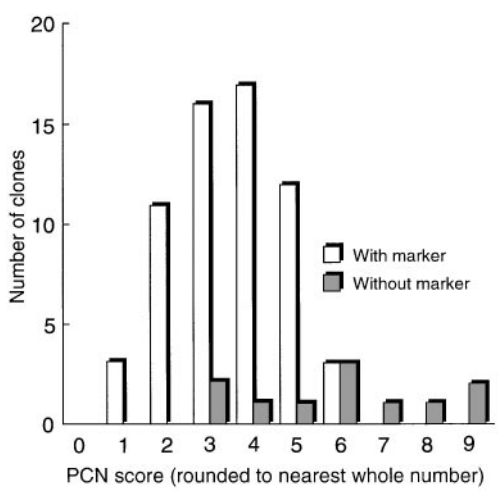

Fig. 5 PCN (*G. pallida*) scores (square root of number of females rounded to nearest whole number to give a 0 to 9 scale) of clones with and without the AFLP marker P61M34 = 15 which was present in a double dose in female parent 12601ab1 but was absent from the male parent Stirling

Chromosomal identification of the linkage group containing PCN resistance

Subsequently, newly developed SSR-based markers (Milbourne et al. 1998) were included to aid the chromosomal identification of the linkage group containing the putative QTL for resistance to *G. pallida*. Of 79 alleles from 36 loci, 34 segregated 1:1, 13 segregated 5:1, and 20 segregated 3:1. The single-marker analysis detected eight associations between PCN scores and these SSR alleles when the significance level was 5% ($t \approx 2.00$), but just one association remained significant with the more stringent *t*-value used for the AFLP markers. It involved allele Stm3016 = a which segregated 5:1 and was linked in coupling with the AFLP duplex marker P61M34 = 15 (Table 1 and Fig. 4). Furthermore, the Stm3016 locus was mapped in the diploid potato population of Gebhardt et al. (1991) and this allowed the linkage group to be identified as chromosome IV.

Discussion

The use of AFLPs allowed a large number of segregating markers to be followed in a single experiment. The occurrence of 117 markers displaying a 5:1 segregation ratio confirmed that *S. tuberosum* is a tetraploid which displays tetrasomic inheritance. The large number of markers also revealed 16 anomalous clones which would not have been detected by their phenotypes for a single trait, although they did resemble the female parent for PCN scores. More experimental work is needed to determine the origin of these clones as there is no obvious explanation in terms of selfing, contamination, or experimental error. A detailed study of meiosis in the parents and offspring is one possible line of inquiry. It was also clear from the segregation ratios of all of the markers present in one parent and absent in the other, that there were departures from the theoretical separation into two groups segregating 1:1 and 5:1. Again, further work is required to determine the causes but, assuming no misclassification of markers, three possibilities are worthy of investigation. Although potatoes have small chromosomes with few chiasmata per chromosome, double reduction is known to occur, an example being the inheritance of resistance to Potato Virus Y (Mendoza et al. 1996). Double reduction would be expected to reduce the mean number of A--- phenotypes in a progeny of size 78 (Fig. 2) from 39 towards 35.75 (simplex markers) and from 65 towards 60.67 (duplex markers), and also create a new peak at or above 74.75 (triplex markers), but one would still expect two non-overlapping distributions. The second possibility is departures from tetrasomic inheritance due to non-random pairing of homologous chromosomes, with the extreme being disomic inheritance. In

other words, a marker present in one parent in double-dose could segregate with a ratio between 5:1 (65:13) and 3:1 ($58\frac{1}{2}:19\frac{1}{2}$). This might occur in regions of the genome where genes from wild species have been introgressed into subsp. *tuberosum*. However, in order to get the apparent excess of markers between a 1:1 (39:39) and 3:1 ratio (Fig. 2), one would need linked duplicate genes (loci 1 and 2) under tetrasomic ($A_1A_2/a_1a_2/a_1a_2/a_1a_2 \times a_1a_2/a_1a_2/a_1a_2/a_1a_2$) or disomic inheritance ($A_1A_2/a_1a_2 \times a_1a_2/a_1a_2$), which is unlikely with AFLP markers. The third possibility is distorted segregation from the linkage of markers to genes affecting survival ability, a likely occurrence in an outbreeding crop like potato, which is known to suffer inbreeding depression. Distorted segregation ratios have already been reported in diploid genetical studies with potatoes (Bonierbale et al. 1988; Gebhardt et al. 1991). Clones and markers displaying anomalous results were eliminated from the further analyses in order to avoid unnecessary difficulties of interpretation.

The PCN scores displayed continuous variation (Fig. 3), as previously found by Dale and Phillips (1982) in a cross with this source of *G. pallida* resistance from subsp. *andigena* (Howard et al. 1970). One genetical component of this resistance has been identified in this present experiment, a gene in 12601ab1, in the duplex state, linked in coupling to marker P61M34 = 15, also in the duplex state, and accounting for 27.8% of the phenotypic variance in PCN scores, or approximately 30% of the genotypic variance. It is perhaps not surprising that the resistance gene was present in the duplex state as it could have been inherited from both the maternal and paternal grandparent of 12601ab1 {[*H1H3*(6) × P. Ivory] × [*H1H3*(32) × P. Ivory]}, where the *H1H3* clones were from crosses between the subsp. *andigena* source and subsp. *tuberosum*. It is also clear from Fig. 5 that this gene is necessary for high levels of resistance to *G. pallida*. Hence it might be worthwhile cloning and sequencing the AFLP fragment corresponding to the duplex marker P61M34 = 15 in order to develop an STS primer for use in marker-assisted selection and for confirming the chromosomal location of the gene on chromosome IV of the published diploid map of potato (Gebhardt et al. 1991), as already done by Meyer et al. (1998) for a blight resistance QTL. However, the SSR marker Stm3016 = a may prove even more suitable for marker-assisted selection.

As there is synteny between the 12 chromosomes of potato and tomato (Gebhardt et al. 1991; Tanksley et al. 1992; Milbourne et al. 1998), one can tentatively conclude that the *Gpa* QTL from 12601ab1 is on the same arm of chromosome IV (near GP221) as the *Hero* gene (near GP180) which gives a wide spectrum of resistance against *G. rostochiensis* in tomato (Ganal et al. 1995). Other genes for PCN resistance discovered to-date are as follows. Kreike et al. (1994) found that about 50% of the variation in quantitative resistance to *G. pallida* pathotypes Pa2/3 from *Solanum spegazzinii* could be explained by a major locus *Gpa* on chromosome V, whereas two minor loci on chromosomes IV and VII accounted for less than 10% each. The major dominant gene *Gpa2* for resistance to pathotype Pa2 of *G. pallida* has been mapped at the distal end of chromosome XII (Rouppe van der Voort et al. 1997). Dominant major genes for qualitative resistance to *G. rostochiensis* pathotype Ro1 have been mapped to chromosome V: *H1* from *S. tuberosum* ssp. *andigena* (Gebhardt et al. 1993; Pineda et al. 1993) and *GroV1* from *Solanum vernei* (Jacobs et al. 1996); and to chromosome VII: *Gro1* from *S. spegazzinii* (Barone et al. 1990). The *H1* gene was in fact present in single dose in 12601ab1 and was found to be linked in coupling to AFLP markers E40M42-1 and P238M61-1, at 21 ± 4.7 cM from the latter (data not presented). It remains to be seen if other chromosomes contain genes for resistance to potato cyst nematodes.

Although cv Stirling was not the source of PCN resistance in the population under study, clones more susceptible than Stirling are known (Bradshaw et al. 1995 b) and hence one can not rule out the possibility that some of the variation in PCN scores came from Stirling, although no associations with Stirling markers were clearly identified.

There are a number of possible reasons which alone, or in combination, could explain why the other QTLs which must have been segregating for PCN resistance were not identified in this experiment. They could have been missed because the markers did not cover the whole of the genome. The total tetraploid female map length of 990.9 cM is only about 25% of the genome, based on a known total diploid map length of 1120 cM (Jacobs et al. 1995). They could also have been missed because of unfavourable linkages between the QTLs and markers, for example, AQ/Aq/aQ/aq. Indeed, in Table 1 and Fig. 4, one can see the importance of favourable linkages for the detection of a QTL and markers suitable for marker-assisted selection in an autotetraploid. The production of dihaploids from 12601ab1 and QTL analysis in appropriate diploid crosses would make detection easier, but one would then encounter problems in deciding if any markers found linked to QTLs were suitable for marker-assisted selection in the tetraploid population because dihaploid AQ/aq could have come from the suitable linkages AQ/AQ/aq/aq and AQ/aq/aq/aq, the less suitable AQ/aQ/aq/aq, or the unsuitable AQ/aQ/Aq/aq and AQ/Aq/aq/aq. Similar problems would occur if 12601ab1 was crossed to tetraploid clones containing the marker A, but lacking PCN resistance. However, these problems may be overcome as more of the highly polymorphic co-dominant SSR-based markers which are currently being developed for potato become available (Milbourne et al. 1998). A third possibility is that gene effects were not large enough to be detected in an experiment of the size conducted. If, as found by the

permutation test, a *t*-value of 3.32 is required to declare a significant association with a marker segregating 1:1, then the smallest marker class difference that could have been detected with a population of 78 clones would be about 1.20, based on the standard error of 0.362 for the largest difference actually found of 0.98. For comparison, the difference in PCN scores between 12601ab1 and Stirling was 5.90. Doubling the population size would reduce the detectable difference from 1.20 to 0.85, and doubling it again would reduce it to 0.60. Hackett et al. (1998) concluded that a population size of at least 150 individuals should be used in future to develop a linkage map in an autotetraploid species and that a larger number, say 250, would provide a better chance of identifying homologous chromosomes. These population sizes would also seem sensible for detecting the major QTLs segregating in a tetraploid population.

Therefore, with an increased population size of 250 and SSR markers to complement AFLPs, prospects appear good for marker-assisted selection in a tetraploid breeding programme. Indeed, the necessary development work could be done as part of the second stage of the breeding strategy advocated by Bradshaw and Mackay (1994) for clonally propagated potatoes. Namely, having used progeny tests to identify the best crosses, 250 seedlings of each are raised from residual seed in a glasshouse for subsequent clonal evaluation and cultivar production. As selection is not practised in the glasshouse, such populations are ideal for phenotypic and marker evaluation with a view to marker-assisted selection in later clonal generations, in choosing parents for the next round of crossing, and in subsequent cycles of recurrent selection.

Finally, given these encouraging results, it would seem worthwhile developing the theory and practice of QTL analysis in autotetraploids by considering the effects of distorted ratios and double reduction in more detail, as well as the prospects for more sophisticated approaches to locating QTLs and determining their effects using marker means and individual observations in the marker regression and interval mapping techniques already developed in diploids (Kearsey and Pooni 1996).

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