Mol Gen Genet (1998) 259: 150–160

ORIGINAL PAPER

R. C. Meyer · D. Milbourne · C. A. Hackett
J. E. Bradshaw · J. W. McNichol · R. Waugh

Linkage analysis in tetraploid potato and association of markers with quantitative resistance to late blight (*Phytophthora infestans*)

Received: 1 October 1997 / Accepted: 18 March 1998

Abstract We have constructed a partial linkage map in tetraploid potato which integrates simplex, duplex and double-simplex AFLP markers. The map consists of 231 maternal and 106 paternal markers with total map lengths of 990.9 cM and 484.6 cM. The longer of the two cumulative map lengths represents approximately 25% coverage of the genome. In tetraploids, much of the polymorphism between parental clones is masked by 'dosage' which significantly reduces the number of individual markers that can be scored in a population. Consequently, the major advantage of using AFLPs – their high multiplex ratio – is reduced to the point where the use of alternative multi-allelic marker types would be significantly more efficient. The segregation data and map information have been used in a QTL analysis of late blight resistance, and a multi-allelic locus at the proximal end of chromosome VIII has been identified which contributes significantly to the expression of resistance. No late blight resistance genes or QTLs have previously been mapped to this location.

Key words AFLP · Linkage map · *Solanum tuberosum*

Introduction

The European cultivated potato (*Solanum tuberosum* ssp. *tuberosum*) is a highly heterozygous autotetraploid with four homologous sets of twelve chromosomes (2n = 4x = 48). Studies using contemporary genetic approaches have focused on diploid accessions to avoid the problems of interpretation associated with working at higher ploidy levels. Using diploids, linkage maps based on RFLP, RAPD, and AFLP markers have been constructed (Bonierbale et al. 1988; Gebhardt et al. 1991; Jacobs et al. 1995), and these have been used to map major genes (Ritter et al. 1991, Van Eck et al. 1993) and the genetic components of polygenic characters (Bonierbale et al. 1994; Leonards-Schippers et al. 1994). Molecular markers have also been used to identify and select specific genotypes for genetic studies (Meyer et al. 1993), and for genotyping diploid and tetraploid clones (Gorg et al. 1992; Demecke et al. 1993).

Most traditional potato breeding takes place at the tetraploid level, where a major objective is the combination of durable resistance to *Phytophthora infestans* (Mont.) de Bary, the cause of late blight (LB), and to the white potato cyst nematode *Globodera pallida* (Stone) Behrens (PCN). These resistance 'traits' display continuous variation in segregating populations and are assumed to be controlled by several genetic loci which act collectively on the expression of resistance. The component loci cannot be recognised by simple Mendelian analysis because discrete phenotypic classes cannot be identified. Although quantitative traits are very difficult to manipulate at a practical level, the genetic loci controlling quantitative traits (QTLs) can be identified through linkage to genetic markers (Paterson et al. 1988).

Tetrasomic inheritance implies the random pairing of four homologous chromosomes at meiosis, and in a highly heterozygous outbreeding species results in a large number of possible allelic combinations at a single locus. In the most extreme case, eight different alleles could segregate independently in a population, resulting in 36 possible genotypic classes in the progeny. The implications of this for the study of quantitative traits are particularly significant, as these different allelic combinations could result in the phenotypic transformation of an essentially Mendelian character into a 'trait' which exhibits continuous variation. Approaches to the study of genetic linkage in polyploids have been

Communicated by R. Hagemann

R. C. Meyer (✉) · D. Milbourne · J. E. Bradshaw · R. Waugh
Scottish Crop Research Institute, Invergowrie,
Dundee DD2 5DA, UK
Fax: +44-1382-562426; e-mail: rmeyer@scri.sari.ac.uk

C. A. Hackett · J. W. McNichol
Biomathematics and Statistics Scotland, Invergowrie,
Dundee DD2 5DA, UK

proposed (Wu et al. 1992). In the main, these are based on the use of simplex (or single-dose) markers that are derived from only one parent and segregate in a 1:1 ratio in the progeny. These methods have been used extensively in octoploid sugarcane (Sobral and Honeycutt 1993). To identify and merge homologous co-segregation groups, duplex (or double-dose) and triplex (or triple-dose) markers have also been exploited in sugarcane (Da Silva 1993; Da Silva et al. 1995) and alfalfa (Yu and Pauls 1993). Hackett et al. (1998) have presented a simulation study on the use of simplex and duplex markers to assemble a linkage map in an autotetraploid species.

Here, we have applied molecular marker analysis to a tetraploid F_1 population developed within a potato breeding programme. Our objectives were to explore the feasibility of performing linkage analysis, and to examine the behaviour of quantitative traits, in tetraploid potatoes. Based on results from a previous study (Milbourne et al. 1997) AFLPs were chosen as the primary marker system. AFLPs are dominant and are based on the selective PCR amplification of small restriction fragments (80–400 bp) of genomic DNA. They allow a large number of segregating markers to be followed in a single experiment and have been used for the construction of high-density linkage maps (Van Eck et al. 1995) and for saturation mapping (Ballvora et al. 1995) in diploid potato. Here we describe the results of this tetraploid potato experiment and discuss the implications of these results for marker-assisted selection in a practical potato improvement programme.

Materials and methods

Plant materials and DNA extraction

A population resulting from a cross between the cultivar Stirling (male parent) and the advanced SCRI breeding line 12601ab1 (female parent) was used (Bradshaw et al. 1995). The parents express complementary quantitative resistances to late blight (Stirling) and the white potato cyst nematode (12601ab1). The mapping population consisted of a subset of 94 randomly chosen F_1 plants. The plants were grown in the greenhouse and 5–10 g of young, fully expanded leaves were harvested and freeze-dried. Total genomic DNA was extracted from freeze-dried leaf material using the cetyl trimethyl ammonium bromide (CTAB) method according to Saghai-Maroof et al. (1984). The quality and quantity of the DNA were evaluated under UV illumination after ethidium-bromide staining of 0.8% agarose gels.

A diploid reference population (Gebhardt et al. 1991) was used to assign sequence-tagged-site (STS) markers to specific chromosomal locations (Milbourne et al. 1998).

Resistance screens and QTL analysis

Two complex races of *Phytophthora infestans* were chosen for the late blight test. These were [1, 2, 3, 4, 7] and [1, 3, 4, 7, 10, 11], which are virulent on plants bearing the corresponding major resistance genes. Six plants of each parent were inoculated with each race. No difference in response was observed for the same parent inoculated with different races (data not shown). Testing proceeded using an inoculum of race [1, 2, 3, 4, 7] at 5×10^4 zoospores/ml on 219 out of 262 F_1 clones and the two parental lines, planted in two randomised blocks. There were four plants of each parent in each block and one of each F_1. For inoculation and testing the protocol of Stewart et al. (1983) was followed. Malcomson's 1–9 scale of increasing resistance (Cruickshank et al. 1982) was used to score the level of blight infection.

Associations between markers and resistance scores were tested using the non-parametric Mann-Whitney U-test (Mann and Whitney 1947). A significant difference between the median blight scores of marker classes (presence or absence of marker) was interpreted as indicating the presence of a QTL. This first round of analysis was verified by a permutation test using 1000 repeats, as described by Churchill and Doerge (1994). The significance level chosen was 5%.

AFLP assays

AFLP assays were carried out using a modification of the protocol described in Vos et al. (1995) *Eco*RI and *Pst*I were obtained from Boehringer Mannheim, *Mse*I from New England Biolabs, and T4 DNA ligase and T4 polynucleotide kinase from GIBCO BRL. AmpliTaq, AmpliTaq LD and PCR buffers were purchased from Perkin Elmer and nucleotide triphosphates from Pharmacia. AFLP primers and adapters were obtained from Genset, Paris, France. The exact protocol followed is described in Milbourne et al. (1997). We used 22 *Eco*RI/*Mse*I combinations, and 17 *Pst*I/*Mse*I combinations. The primer nomenclature of Keygene is used throughout and the primer sequences can be deduced from the marker designations. Full information on primers used is available on request from the authors. Autoradiograms were scored manually and independently by two people. Each band was treated as a locus with a dominant (presence = A) versus a recessive (absence = O) allele. Intensity differences in segregating bands were observed but visual interpretation was not considered reliable enough to assign allelic dosage to a given individual in this population.

Identification of segregation type

Markers were divided into three groups depending on their presence or absence in each parent (i.e. present in female parent only, present in male parent only or present in both parents). Markers present in just one parent are expected to segregate in a 1:1 ratio if present in single dose (simplex) or a 5:1 ratio if present in a double dose (duplex), in the absence of double reduction. Markers were accepted as 1:1 or 5:1 if their observed segregation ratio was not significantly different from that expected, as tested by a χ^2 goodness-of-fit test with a significance level of 1%. Markers present in both parents are expected to segregate with a 3:1 ratio if both parents have a simplex configuration (double-simplex); this was again tested using a χ^2 test. In this case the 5% level had to be applied to avoid overlap with the 11:1 segregation class (duplex × simplex). Markers that showed segregation ratios significantly different from those expected were omitted from the linkage analysis.

Calculation of recombination fractions (r)

Recombination fractions were estimated between all pairs of markers, assuming random pairing of homologous chromosomes, and absence of double reduction. Table 1 lists the configurations and terminology used in this study. General formulae for the recombination fractions are given for simplex markers by Wu et al. (1992). Da Silva (1993) and Da Silva et al. (1995) give phenotypic frequencies for octoploids. Hackett et al. (1998) present formulae for the standard errors of the estimates of the recombination fractions for autotetraploids. None of these authors gives estimates for double-simplex markers, which are included in the present study. As an example the estimation of recombination fractions for simplex/double-simplex coupling linkage is detailed below. In this situation, the parental configurations are assumed to be AB/OO/OO/OO × AO/OO/OO/OO. In the female parent, the AB chro-

Table 1 Marker pair configurations and terminology used in this study

Allele dosage	OOOO	AOOO	AAOO	AAAO	AAAA
Designation	Nulliplex	Simplex	Duplex	Triplex	Quadruplex
Marker pair configuration[a]			Linkage phase designation		
AB/OO/OO/OO × OO/OO/OO/OO			Simplex/simplex coupling		
AO/OB/OO/OO × OO/OO/OO/OO			Simplex/simplex repulsion		
AB/AO/OO/OO × OO/OO/OO/OO			Simplex/duplex coupling		
AO/AO/OB/OO × OO/OO/OO/OO			Simplex/duplex repulsion		
AB/AB/OO/OO × OO/OO/OO/OO			Duplex/duplex coupling		
AB/AO/OB/OO × OO/OO/OO/OO			Duplex/duplex mixed		
AO/AO/OB/OB × OO/OO/OO/OO			Duplex/duplex repulsion		
AB/OO/OO/OO × AO/OO/OO/OO			Simplex/double-simplex coupling		
AO/OB/OO/OO × AO/OO/OO/OO			Simplex/double-simplex repulsion		
AB/OO/OO/OO × AB/OO/OO/OO			Double-simplex/double-simplex coupling		
AB/OO/OO/OO × AO/OB/OO/OO			Double-simplex/double-simplex mixed		
AO/OB/OO/OO × AO/OB/OO/OO			Double-simplex/double-simplex repulsion		

[a] The marker pair configuration shows the distribution of the alleles of two loci on the four homologous chromosomes (separated by/in the Table) in a tetraploid cross. Each chromosome is represented by its configuration for the two loci. A: presence of band at locus A, B: presence of band at locus B, O: absence of band

Table 2 Expected phenotype frequencies for various configurations of pairs of simplex and double-simplex markers

Marker pair configuration	AB	AO	OB	OO
Simplex/double-simplex Coupling AB/OO/OO/OO × AO/OO/OO/OO	$\frac{2-r}{4}$	$\frac{1+r}{4}$	$\frac{r}{4}$	$\frac{1-r}{4}$
Simplex / double-simplex Repulsion AO/OB/OO/OO × AO/OO/OO/OO	$\frac{4+r}{12}$	$\frac{5-r}{12}$	$\frac{2-r}{12}$	$\frac{1+r}{12}$
Double simplex / double-simplex Coupling AB/OO/OO/OO × AB/OO/OO/OO	$\frac{3-2r+r^2}{4}$	$\frac{r(2-r)}{4}$	$\frac{r(2-r)}{4}$	$\frac{(1-r)^2}{4}$
Double simplex / double-simplex Mixed AB/OO/OO/OO × AO/OB/OO/OO	$\frac{7-r^2}{12}$	$\frac{2+r^2}{12}$	$\frac{2+r^2}{12}$	$\frac{1-r^2}{12}$
Double-simplex / double-simplex Repulsion AO/OB/OO/OO × AO/OB/OO/OO	$\frac{19+2r+r^2}{36}$	$\frac{8-2r-r^2}{36}$	$\frac{8-2r-r^2}{36}$	$\frac{1+2r+r^2}{36}$

mosome will pair with one carrying neither marker, and every gamete will carry information about whether a cross-over has occurred between A and B. The gametes will therefore have the probabilities: AB/OO: $(1-r)/2$; AO/OO: $r/2$; OB/OO: $r/2$; and OO/OO: $(1-r)/2$, where r is the recombination fraction. The male parent will be expected to produce gametes of types AO/OO and OO/OO in equal proportions. The log-likelihood can be written as

$$L = a\log((2-r)/4) + b\log((1+r)/4) + c\log(r/4) + d\log((1-r)/4)$$

The maximum likelihood equation is a cubic function of r, and so r is best estimated by maximising the log-likelihood numerically. Table 2 gives expected genotype frequencies for various configurations of pairs of double-simplex and simplex markers. The asymptotic variances of the estimates of r in each case may be calculated as $-1/E(d^2L/dr^2)$, where L is the log-likelihood and E denotes the expected value.

Figure 1 shows the asymptotic standard errors associated with various marker configurations. Linkages between double-simplex and duplex pairs had very large standard errors, even in the coupling phase, and were omitted from the analysis. The configurations with smallest standard error are simplex/simplex coupling, double-simplex/double-simplex coupling, duplex/duplex coupling, simplex/double-simplex coupling and duplex/simplex (both coupling and repulsion). As discussed by Hackett et al. (1998), the duplex/duplex repulsion and the duplex/duplex mixed configuration cannot be distinguished. These, and all configurations involving a double-simplex marker in a repulsion phase, were excluded from further analysis. Simplex/simplex repulsion linkages were included, taking into consideration the size of the standard errors.

Recombination fractions were estimated by an analytical expression where possible, or by maximising the log-likelihood numerically. LOD scores were calculated for each pair as $\log_{10}$(likelihood for $r = \hat{r}$) – $\log_{10}$(likelihood for $r = 0.5$). These calculations were carried out using the statistical package Genstat (Payne et al. 1993). Such pairwise estimates and LOD scores are suitable for input to the linkage analysis package JoinMap 2.0 (Stam and van Ooijen 1995).

STS development

Selected AFLP fragments were cloned into pGEM-T Easy (Stratagene) and transformed into *E. coli* DH5αMAX (GIBCO BRL), following the suppliers' instructions. Clones were sequenced on an ABI 377 automated sequencer using the Dye Terminator Cycle sequencing kit from Perkin Elmer. STS PCR primer pairs were designed using the PRIMER0.5 package (Lincoln et al. 1991). STS PCR comprised a denaturing step at 94° C for 5 min, five cycles of denaturation at 94° C for 30 s, annealing at 65–60° C (with a 1° C decrease each cycle) for 30 s, elongation at 72° C for 30 s, followed

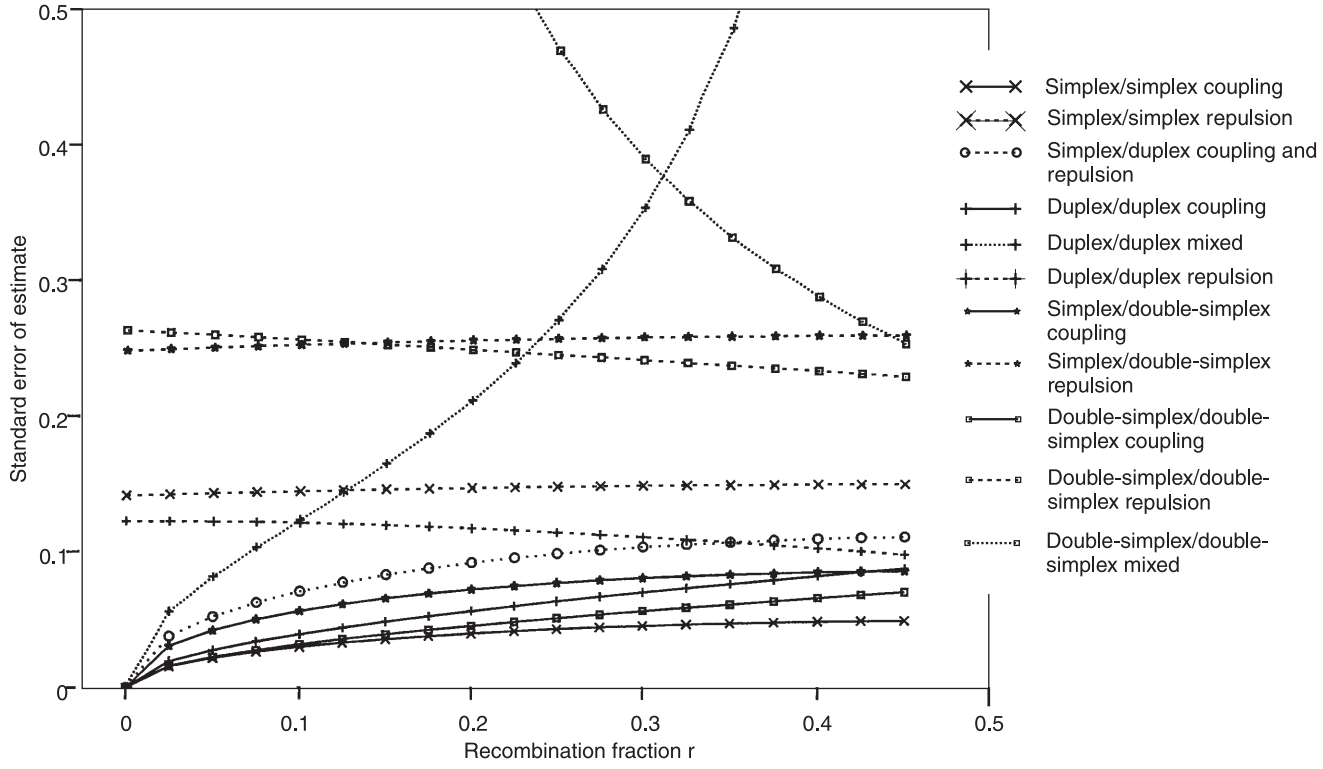


Fig. 1 Distribution of standard errors for the estimate of the recombination fraction for various linkage phases in a population of size 78

by 30 cycles of denaturation at 94° C for 30 s, annealing at 60° C for 30 s, elongation at 72° C for 30 s, and finally elongation at 72° C for 5 min. The products were separated on 1% agarose gels in 0.5 × TBE buffer (1 × TBE is 90 mM TRIS-borate pH 8.3), or in 6% denaturing polyacrylamide gels. Mapping of STS in the tetraploid population was achieved using the sequence-specific amplification polymorphisms (S-SAP) procedure described by Waugh et al. (1997) using a single STS primer.

Results

Marker analysis

Thirty-nine AFLP primer combinations were used to obtain segregation data for linkage analysis. Of the 3173 loci surveyed, 1066 markers segregated in the population, and 573 of these were considered scorable with the required accuracy. The percentages of informative loci obtained with the *Eco*RI/*Mse*I and the *Pst*I/*Mse*I templates were not significantly different. However, 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. Because a large percentage of markers did not fit clearly into an expected segregation class, all clones in the F_1 were checked for possible selfing of 12601ab1 or the use of mixed pollen. All F_1 plants were found to contain AFLP alleles derived from the paternal clone and no non-parental bands were observed in the population. Analysis of the segregating simplex and duplex markers originating from Stirling highlighted a group of 16 lines that had an unusually low number of paternal products. We have no experimental or genetical explanation for this group. The 16 anomalous genotypes were omitted from subsequent linkage analysis, leaving 78 lines. Exclusion of the 16 genotypes decreased the proportion of markers that exhibited unclassified segregation ratios from 24% to 17%, and increased the percentage of simplex and duplex markers from 39% to 45% and 19% to 22%, respectively, whereas the proportion of double-simplex markers decreased slightly from 6.6% to 5.6%.

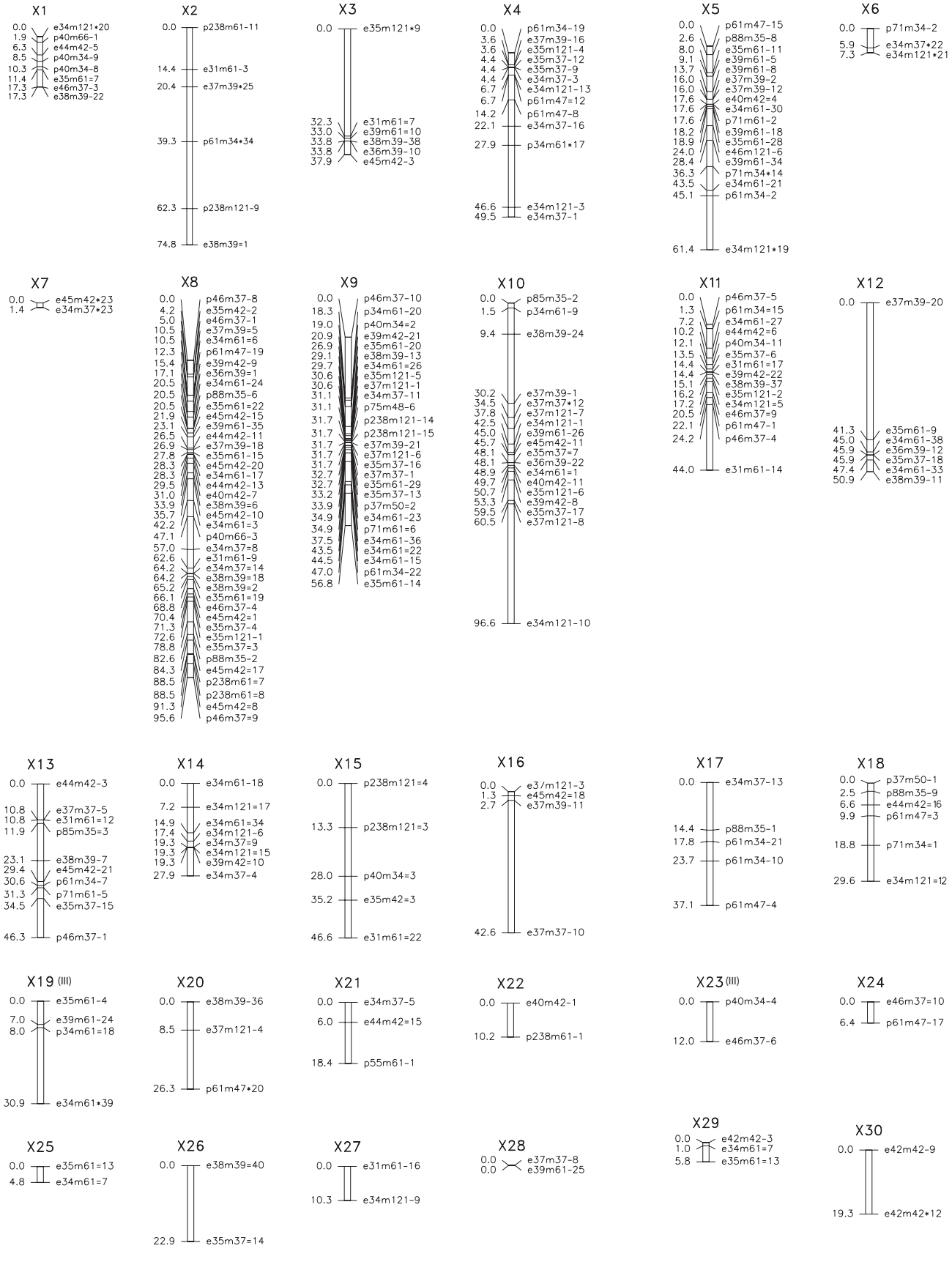
Map construction

The map was constructed by entering the pairwise distances and LOD scores of 249 simplex markers (162 maternal, 87 paternal), 32 double-simplex markers and 117 duplex markers (72 maternal, 45 paternal) into JoinMap 2.0, then using standard JoinMap procedures to obtain both parental maps (Fig. 2). Figure 3 illustrates the use of duplex markers to assemble homolo-

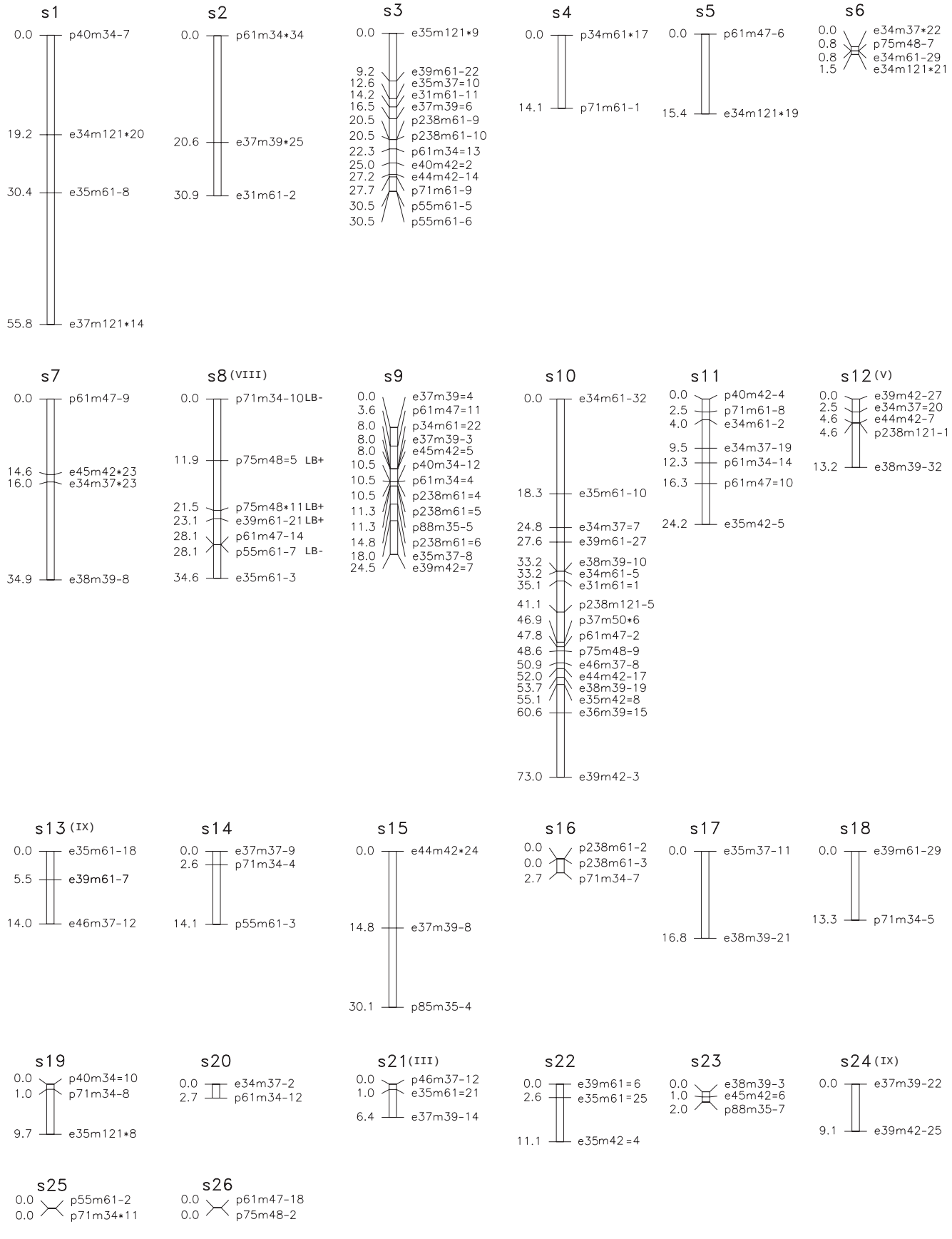
►

Fig. 2A, B Maternal 12601ab1map (**A**) and paternal Stirling map (**B**). The tetraploid potato linkage map assembles 346 markers (68 unlinked) distributed over 56 linkage groups. Numbers to the left of linkage groups are map distances in centiMorgans calculated using the Haldane function. Locus names appear to the right of linkage groups (– simplex markers, = duplex markers, * double simplex markers). X indicates the maternal map, s indicates the paternal map. Roman numerals in parentheses give the designation of the corresponding diploid chromosome, where known

A


X1
0.0 e34m121*20
1.9 p40m66-1
6.3 e44m42-5
8.5 p40m34-9
10.3 p40m34-8
11.4 e35m61=7
17.3 e46m37-3
17.3 e38m39-22
X2
0.0 p238m61-11
14.4 e31m61-3
20.4 e37m39*25
39.3 p61m34*34
62.3 p238m121-9
74.8 e38m39=1
X3
0.0 e35m121*9
32.3 e31m61=7
33.0 e39m61=10
33.8 e38m39-38
33.8 e36m39-10
37.9 e45m42-3
X4
0.0 p61m34-19
3.6 e37m39-16
3.6 e35m121-4
4.4 e35m37-12
4.4 e35m37-9
4.4 e34m37-3
6.7 e34m121-13
6.7 p61m47=12
14.2 p61m47-8
22.1 e34m37-16
27.9 p34m61*17
46.6 e34m121-3
49.5 e34m37-1
X5
0.0 p61m47-15
2.6 p88m35-8
8.0 e35m61-11
9.1 e39m61-5
13.7 e39m61-8
16.0 e37m39-2
16.0 e37m39-12
17.6 e40m42=4
17.6 e34m61-30
17.6 p71m61-2
18.2 e39m61-18
18.9 e35m61-28
24.0 e46m121-6
28.4 e39m61-34
36.3 p71m34*14
43.5 e34m61-21
45.1 p61m34-2
61.4 e34m121*19
X6
0.0 p71m34-2
5.9 e34m37*22
7.3 e34m121*21
X7
0.0 e45m42*23
1.4 e34m37*23
X8
0.0 p46m37-8
4.2 e35m42-2
5.0 e46m37-1
10.5 e37m39=5
10.5 e34m61=6
12.3 p61m47-19
15.4 e39m42-9
17.1 e36m39=1
20.5 e34m61-24
20.5 p88m35-6
20.5 e35m61=22
21.9 e45m42-15
23.1 e39m61-35
26.5 e44m42-11
26.9 e37m39-18
27.8 e35m61-15
28.3 e45m42-20
28.3 e34m61-17
29.5 e44m42-13
31.0 e40m42-7
33.9 e38m39=6
35.7 e45m42-10
42.2 e34m61=3
47.1 p40m66-3
57.0 e34m37=8
62.6 e31m61-9
64.2 e34m37-14
64.2 e38m39-18
65.2 e38m39=2
66.1 e35m61=19
68.8 e46m37-4
70.4 e45m42=1
71.3 e35m37-4
72.6 e35m121-1
78.8 e35m37=3
82.6 p88m35-2
84.3 e45m42=17
88.5 p238m61=7
88.5 p238m61=8
91.3 e45m42=8
95.6 p46m37=9
X9
0.0 p46m37-10
18.3 p34m61-20
19.0 p40m34=2
20.9 e39m42-21
26.9 e35m61-20
29.1 e38m39-13
29.7 e34m61=26
30.6 e35m121-5
30.6 e37m121-1
31.1 e34m37-11
31.1 p75m48-6
31.7 p238m121-14
31.7 p238m121-15
31.7 e37m39-21
31.7 e37m121-6
31.7 e35m37-16
32.7 e37m37-1
32.7 e35m61-29
33.2 e35m37-13
33.9 p37m50=2
34.9 e34m61-23
34.9 p71m61=6
37.5 e34m61-36
43.5 e34m61=22
44.5 e34m61-15
47.0 p61m34-22
56.8 e35m61-14
X10
0.0 p85m35-2
1.5 p34m61-9
9.4 e38m39-24
30.2 e37m39-1
34.5 e37m37*12
37.8 e37m121-7
42.5 e34m121-1
45.0 e39m61-26
45.7 e45m42-11
48.1 e35m37=7
48.1 e36m39-22
48.9 e34m61=1
49.7 e40m42-11
50.7 e35m121-6
53.3 e39m42-8
59.5 e35m37-17
60.5 e37m121-8
96.6 e34m121-10
X11
0.0 p46m37-5
1.3 p61m34=15
7.2 e34m61-27
10.2 e44m42=6
12.1 p40m34-11
13.5 e35m37-6
14.4 e31m61=17
14.4 e39m42-22
15.1 e38m39-37
16.2 e35m121-2
17.2 e34m121=5
20.5 e46m37=9
22.1 p61m47-1
24.2 p46m37-4
44.0 e31m61-14
X12
0.0 e37m39-20
41.3 e35m61-9
45.0 e34m61-38
45.9 e36m39-12
45.9 e35m37-18
47.4 e34m61-33
50.9 e38m39-11
X13
0.0 e44m42-3
10.8 e37m37-5
10.8 e31m61=12
11.9 p85m35=3
23.1 e38m39-7
29.4 e45m42-21
30.6 p61m34-7
31.3 p71m61-5
34.5 e35m37-15
46.3 p46m37-1
X14
0.0 e34m61-18
7.2 e34m121=17
14.9 e34m61=34
17.4 e34m121-6
19.3 e34m37=9
19.3 e34m121=15
19.3 e39m42=10
27.9 e34m37-4
X15
0.0 p238m121=4
13.3 p238m121=3
28.0 p40m34=3
35.2 e35m42=3
46.6 e31m61=22
X16
0.0 e3/m121-3
1.3 e45m42=18
2.7 e37m39-11
42.6 e37m37-10
X17
0.0 e34m37-13
14.4 p88m35-1
17.8 p61m34-21
23.7 p61m34-10
37.1 p61m47-4
X18
0.0 p37m50-1
2.5 p88m35-9
6.6 e44m42=16
9.9 p61m47=3
18.8 p71m34=1
29.6 e34m121=12
X19 (III)
0.0 e35m61-4
7.0 e39m61-24
8.0 p34m61=18
30.9 e34m61*39
X20
0.0 e38m39-36
8.5 e37m121-4
26.3 p61m47*20
X21
0.0 e34m37-5
6.0 e44m42=15
18.4 p55m61-1
X22
0.0 e40m42-1
10.2 p238m61-1
X23 (III)
0.0 p40m34-4
12.0 e46m37-6
X24
0.0 e46m37=10
6.4 p61m47-17
X25
0.0 e35m61=13
4.8 e34m61=7
X26
0.0 e38m39=40
22.9 e35m37=14
X27
0.0 e31m61-16
10.3 e34m121-9
X28
0.0 e37m37-8
0.0 e39m61-25
X29
0.0 e42m42-3
1.0 e34m61=7
5.8 e35m61=13
X30
0.0 e42m42-9
19.3 e42m42*12

B


s1
0.0 p40m34-7
19.2 e34m121*20
30.4 e35m61-8
55.8 e37m121*14
s2
0.0 p61m34*34
20.6 e37m39*25
30.9 e31m61-2
s3
0.0 e35m121*9
9.2 e39m61-22
12.6 e35m37=10
14.2 e31m61-11
16.5 e37m39=6
20.5 p238m61-9
20.5 p238m61-10
22.3 p61m34=13
25.0 e40m42=2
27.2 e44m42-14
27.7 p71m61-9
30.5 p55m61-5
30.5 p55m61-6
s4
0.0 p34m61*17
14.1 p71m61-1
s5
0.0 p61m47-6
15.4 e34m121*19
s6
0.0 e34m37*22
0.8 p75m48-7
0.8 e34m61-29
1.5 e34m121*21
s7
0.0 p61m47-9
14.6 e45m42*23
16.0 e34m37*23
34.9 e38m39-8
s8 (VIII)
0.0 p71m34-10 LB-
11.9 p75m48=5 LB+
21.5 p75m48*11 LB+
23.1 e39m61-21 LB+
28.1 p61m47-14
28.1 p55m61-7 LB-
34.6 e35m61-3
s9
0.0 e37m39=4
3.6 p61m47=11
8.0 p34m61=22
8.0 e37m39-3
8.0 e45m42=5
10.5 p40m34-12
10.5 p61m34=4
10.5 p238m61=4
11.3 p238m61=5
11.3 p88m35-5
14.8 p238m61=6
18.0 e35m37-8
24.5 e39m42=7
s10
0.0 e34m61-32
18.3 e35m61-10
24.8 e34m37=7
27.6 e39m61-27
33.2 e38m39-10
33.2 e34m61-5
35.1 e31m61=1
41.1 p238m121-5
46.9 p37m50*6
47.8 p61m47-2
48.6 p75m48-9
50.9 e46m37-8
52.0 e44m42-17
53.7 e38m39-19
55.1 e35m42=8
60.6 e36m39=15
73.0 e39m42-3
s11
0.0 p40m42-4
2.5 p71m61-8
4.0 e34m61-2
9.5 e34m37-19
12.3 p61m34-14
16.3 p61m47=10
24.2 e35m42-5
s12 (V)
0.0 e39m42-27
2.5 e34m37=20
4.6 e44m42-7
4.6 p238m121-1
13.2 e38m39-32
s13 (IX)
0.0 e35m61-18
5.5 e39m61-7
14.0 e46m37-12
s14
0.0 e37m37-9
2.6 p71m34-4
14.1 p55m61-3
s15
0.0 e44m42*24
14.8 e37m39-8
30.1 p85m35-4
s16
0.0 p238m61-2
0.0 p238m61-3
2.7 p71m34-7
s17
0.0 e35m37-11
16.8 e38m39-21
s18
0.0 e39m61-29
13.3 p71m34-5
s19
0.0 p40m34=10
1.0 p71m34-8
9.7 e35m121*8
s20
0.0 e34m37-2
2.7 p61m34-12
s21 (III)
0.0 p46m37-12
1.0 e35m61=21
6.4 e37m39-14
s22
0.0 e39m61=6
2.6 e35m61=25
11.1 e35m42=4
s23
0.0 e38m39-3
1.0 e45m42=6
2.0 p88m35-7
s24 (IX)
0.0 e37m39-22
9.1 e39m42-25
s25
0.0 p55m61-2
0.0 p71m34*11
s26
0.0 p61m47-18
0.0 p75m48-2

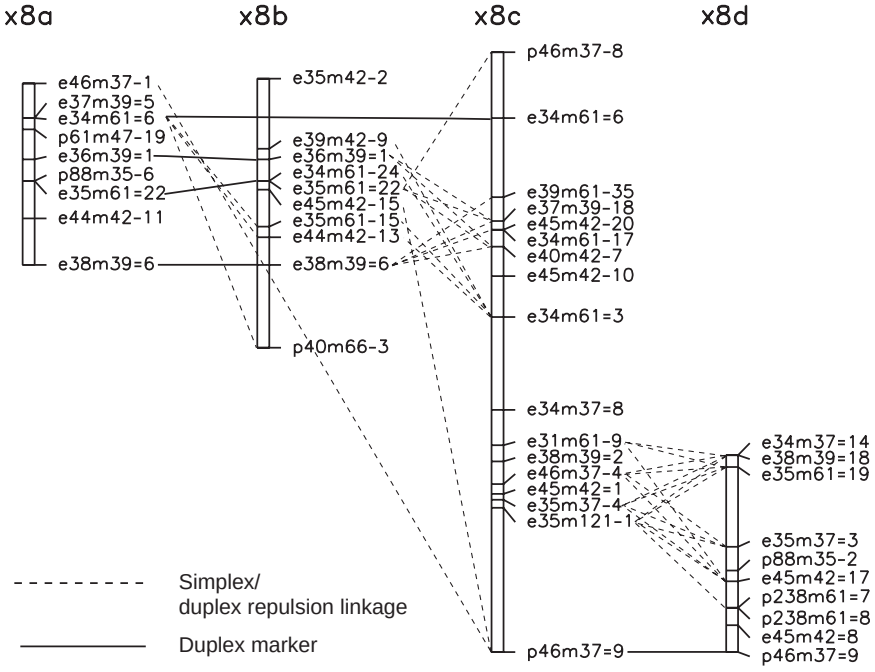


Fig. 3 Homologous co-segregation groups of 12601ab1 linkage group X8

gous co-segregation groups, identified by simplex markers in coupling, into a single linkage group by exploiting the equally informative coupling and repulsion linkages between duplex and simplex markers. The resulting linkage group X8 is shown in Figure 2A. The final map of the maternal parent 12601ab1 comprises fourteen major groups (Nos. x1, x2, x4, x5, x8, x9, x10, x11, x12, x13, x14, x15, x17, x18), and sixteen groups each containing less than five markers, with 35 (13%) of the maternal markers remaining unlinked. Overall, 154 (95%) and 62 (86%), respectively, of the simplex and duplex markers, but only 15 (47%) of the double-simplex markers, could be placed on the map. The largest linkage group is 96.6 cM long, and the cumulative length of the maternal map is 990.9 cM. The markers from the paternal parent Stirling assemble into eight major groups (Nos. s1, s3, s7, s8, s9, s10, s11, s12) and eighteen groups of less than five markers. Forty-eight (29%) of the paternal markers remain unlinked. In total, 75 (86%) of the simplex, 25 (58%) of the duplex and 16 (50%) of the double-simplex markers could be placed on the map. The paternal groups are up to 73.0 cM long, and the cumulative length is 484.6 cM. The parental maps contain seven bridging (3:1) markers, which allowed us to identify 14 linkage groups as homologous maternal and paternal chromosomes (linkage groups 1–7 in both maps in Fig. 2).

QTL analysis of the LB response

The distribution of the LB resistance scores in 219 clones of the F_1 population is shown in Fig. 4. In this experiment, Stirling was resistant, but the female parent 12601ab1 showed a higher score than the 4.0–4.5 expected from previous glasshouse and field results (H. Stewart, personal communication). As the scores do not follow a normal distribution, the non-parametric Mann-Whitney test (Mann and Whitney 1947) was chosen for analysis. A total of 21 associations between components of blight response and AFLP markers were significant at the 5% level (Table 3). The magnitude of the difference between median presence and median absence depends on the distance of the QTL from the marker, the marker segregation type, and the alleles present at the QTL. We have included the percentage of variation explained by

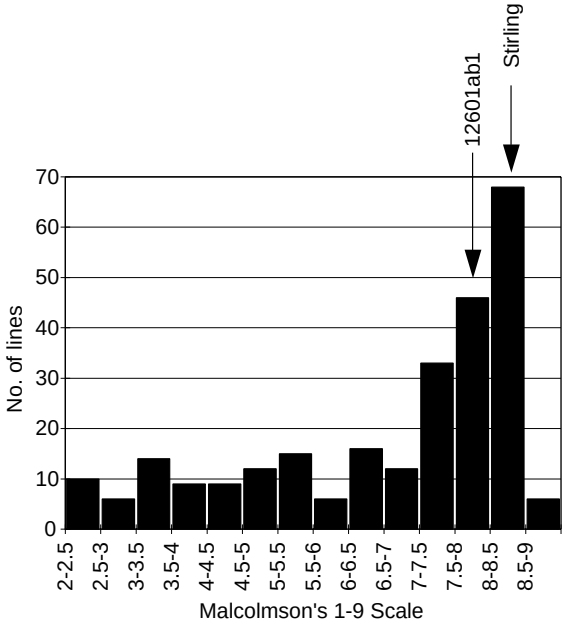


Fig. 4 Late blight resistance scores in 219 F_1 lines

Table 3 Association of markers with QTLs for late blight resistance

Marker	Median presence	Median absence	Significance level	Percent phenotypic variation explained	Sign	Group
A Association of Stirling markers with components of late blight resistance						
e35m61-8	7.0	7.5	0.03	1.4	−	s1
e34m61-29	7.0	7.5	0.03	0.9	−	s6
p75m48-7	7.0	7.5	0.04	0.9	−	s6
p75m48 = 5	7.5	4.5	0.00	31.6	+	s8
e39m61-21	7.5	7.0	0.01	7.8	+	s8
p55m61-7	7.0	7.5	0.00	10.0	−	s8
p71m34-10	7.0	7.5	0.01	3.4	−	s8
e34m61-5	7.0	7.5	0.01	9.6	−	s10
p61m47-2	7.0	7.5	0.03	4.6	−	s10
e37m39-14	7.0	7.5	0.03	1.5	−	s21
e39m42 = 14	7.5	6.3	0.02	3.3	+	–
B Association of 12601ab1 markers with components of late blight resistance						
p61m34-19	7.0	7.5	0.02	1.2	−	X4
e44m42-13	7.5	7.0	0.03	3.8	+	X8
p61m47-19	7.0	7.5	0.01	4.9	−	X8
p88m35-6	6.9	7.5	0.00	12.1	−	X8
e38m39 = 2	7.0	7.5	0.05	4.4	−	X8
e45m42 = 1	7.0	7.8	0.01	6.1	−	X8
p37m50-4	7.0	7.5	0.00	5.9	−	–
e39m42 = 6	7.5	6.3	0.01	2.9	+	–
C Association of shared markers with components of late blight resistance						
p75m48*11	7.5	6.0	0.00	9.3	+	s8
e35m121*9	7.5	6.4	0.01	2.8	+	s3/X3

linear regression at the trait on each marker as an indication of their importance for breeding, although, strictly speaking, it is not valid because the data are not normally distributed. The empirical permutation test of Churchill and Doerge (1994) makes adjustments for multiple marker testing, resulting in more stringent 5% threshold values, and only one significant association involving duplex marker p75m48 = 5 from Stirling could be declared. Regression on this marker accounts for 31.6% of the phenotypic variation. Marker p75m48 = 5 is located on Stirling linkage group s8, together with four other markers indicated as associated with LB response by the Mann-Whitney test. Figure 5 shows how these markers are distributed among the homologous co-segregation groups, and reveals that all three markers associated with resistance are linked in coupling and that these three markers are also linked in repulsion to markers associated with susceptibility.

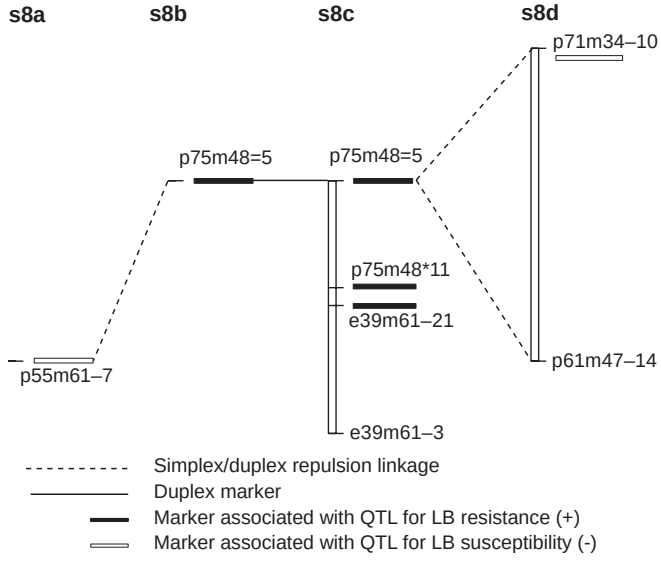


Fig. 5 Location of LB QTL alleles for LB resistance on Stirling linkage group s8

STS development

The AFLP fragment corresponding to the duplex marker p75m48 = 5 was cloned and sequenced. Two pairs of STS primers were designed to the nucleotide sequence excluding flanking AFLP adapters (StS01.1: 5′-CACATTCATATGGAATCCGTCA-3′, StS01.2: 5′-CTGAGGCCATGAGTGCAAC-3′; StS02.1: 5′-TGACATTCCGTTATTTTTGGAA-3′, StS02.2: 5′-GAGGCCATGAGTGCAACAA-3′). PCR fragments of the expected size were obtained with DNA from the plasmid containing the cloned fragment and with DNA of the resistant parent Stirling. PCRs using all combinations of these primers generated a monomorphic product in the parents and F1 individuals of the tetraploid population, and could not be used directly to confirm that they amplify the same locus as p75m48 = 5. This was however achieved by both StS02.1- and StS02.2-driven S-SAPs. As neither chromosomal designations nor polarity could

be assigned to the tetraploid linkage groups because of a lack of markers common to previously mapped populations, a diploid reference population (Gebhardt et al. 1991) was examined with StS01 and StS02. Primer pair StS01 revealed a length polymorphism between the parental clones, and StS02 a dominant/null polymorphism. The segregation of StS01 and StS02 was then followed in the entire population and the data entered alongside the existing RFLP data. This allowed us to locate StS01 and StS02 at the proximal end of the diploid reference chromosome VIII, opposite to the *Waxy* locus. No LB QTLs have previously been identified at this chromosomal location.

Discussion

The mapping population consisted of 94 randomly chosen F_1 plants from a total of 262 available lines. The decision to use 94 lines was based on practical considerations but we also determined that doubling the population size would still not enable us to distinguish unequivocally the expected segregation ratio of 11:1 for a marker in the duplex × simplex configuration from the duplex × duplex (35:1) class at the 95% confidence level (Bradshaw et al. 1998). Using these data and the linkage phase relationships with the lowest associated standard errors (Hackett et al. 1998), a partial linkage map has been constructed. The map consists of 231 maternal and 115 paternal markers with total map lengths of 990.9 cM and 484.6 cM, respectively. Given a total diploid potato map length of about 1000 cM (Jacobs et al. 1995), the cumulative length of a tetraploid map based on coupling-phase simplex markers would be expected to be four times that length (assuming similar recombination frequencies in diploid and tetraploid crosses). The longer of the two cumulative map lengths, 990.9 cM for 12601ab1, therefore represents approximately 25% coverage of the genome. It should be noted however, that the tetraploid map length determined on a population of this size is likely to be an underestimate (Hackett et al. 1998).

χ^2 analysis revealed that the proportion (17%) of markers exhibiting distorted segregation ratios was lower than the 27% observed in diploids. Gebhardt et al. (1991) attributed part of the distortion they observed to the self-incompatibility locus on chromosome I and to the presence of non-*tuberosum* genetic material in one of their diploid parents. Polyploidy disables the gametophytic self-incompatibility system of diploid potato, and so could result in a lower percentage of distorted segregation ratios. In contemporary cultivars and breeding lines, the presence of segments of non-*tuberosum* DNA is common due to the widespread use of other cultivated and wild species in modern improvement programs. Stirling and 12601ab1 have *Solanum demissum* in their pedigrees, and 12601ab1 contains *Solanum tuberosum* ssp. *andigena* (CPC2775 and CPC2802). As proposed by Gebhardt et al. (1991), the genomic regions containing the non-*tuberosum* material are likely to deviate from the expected segregation patterns.

The derived linkage map has not yet been resolved into the expected 2×12 parental linkage groups, and additional markers will be required to cover the entire genome and facilitate the merging of homologous co-segregation groups. With the population size of 78 used in this study, linkage between simplex markers in coupling with recombination frequencies of up to 25% are unlikely to be spurious (Hackett et al. 1998) and they have been used to identify the core homologous groups. Based on this grouping, incorporation of the duplex markers enabled eight sets (six maternal, two paternal) of linkage groups with two to four homologous co-segregation groups each, to be identified. The double-simplex markers identified seven associations between the two parental maps, although more bridging markers are necessary to confirm the homology of these seven pairs of parental linkage groups. More markers will be grouped as more data become available, and more loci fall within the threshold distance. However, the ordering of closely linked markers depends on the small number of individuals in the whole population which show recombination. A more representative map will require not only additional markers but – equally important – a larger mapping population (Hackett et al. 1998).

A comprehensive map cannot be constructed effectively with dominant markers alone. Although there are documented examples of the use of AFLPs as co-dominant markers based on band intensity differences (Rouppe van der Voort et al. 1997), we had insufficient confidence in our ability to assign a specific class to all individuals in our population to use this approach in tetraploids. In diploid potato six AFLP primer combinations generated a total of 264 markers and gave good overall genome coverage (Van Eck et al. 1995). In tetraploids, much of the polymorphism between parental clones is masked by marker 'dosage', which significantly reduces the number of individual AFLP alleles that can be scored in a population. Thus, only about 10% of the total markers generated in this study were submitted to linkage analysis, compared to around 50% reported for diploids (Rouppe van der Voort et al. 1997). Combined with the requirement to map all four homologous co-segregation groups in each parental clone, this represents a relative drop in efficiency to only 5% of that observed in diploids. In addition, the outcome of even a comprehensive mapping exercise would be a collection of co-segregation groups (with or without bridging loci linking homologous groups), to which neither a chromosomal designation nor polarity could be assigned. At this point, the original advantage of using AFLPs because of its high multiplex ratio (Powell et al. 1996) is so small that alternative marker types such as SSRs would be significantly more efficient. These results have prompted us to abandon any further attempts to map tetraploid potato populations with AFLPs alone in favour of the use of highly polymorphic SSR-based markers. These are currently being developed for potato

(Milbourne et al. 1998). In preliminary experiments, mapped SSRs in potato genes STIIKA (M15186), STPOACUTR (X55748) and STGLGPB (L13771) have allowed us to assign diploid chromosome designations to tetraploid linkage groups x19, x23 and s21 (III), s12 (V), and s13 and s24 (IX) (data not shown).

The marker segregation data were used to perform a QTL analysis of LB response by means of a non-parametric analysis of variance. Although some of the marker/QTL associations could have arisen by chance, as shown by the permutation test (Churchill and Doerge 1994), given the additional map information, we expect that the linkages between QTL and AFLP markers on Stirling group s8 are genuine. We believe, however, that simple tests based on presence/absence of a band are insufficiently powerful for QTL mapping in outbreeding polyploids, and that a more powerful QTL model needs to be developed. We need an identifiable marker allele which is closely linked in coupling to the QTL resistance allele for an effective MAS potato breeding program. Defining the composition of a locus in the parental material (mono- or multi-allelic, monomorphic or polymorphic) will provide valuable information about the predicted allelic configurations in the progeny, and this will in turn advance the interpretation of all the other assembled linkage data. Furthermore, the association of specific marker alleles with different QTL alleles will allow a breeder to select in the progeny for combinations of desirable alleles from both parents. Although the SSR located in the *actin* gene on chromosome V is in a region which has been previously identified as linked to late blight resistance, we could not detect a QTL in a comparable location on linkage group s12 in our study. In the absence of previously mapped markers, the AFLP product p75m48 = 5, which is strongly associated with quantitative resistance to LB from Stirling (accounting for 31.6% of the phenotypic variation), was developed into an STS marker for both the tetraploid and diploid mapping population. This allowed us to assign the Stirling linkage group s8 to chromosome VIII of the diploid reference map, identifying a multi-allelic LB locus at the proximal end. Although no LB resistance genes or QTL have been mapped to this area, it is interesting that Leister et al. (1996) mapped a single resistance gene analogue to this same region in a diploid population.

In conclusion, this study presents the first linkage map of tetraploid potato. Further work is currently under way to align this map with existing diploid linkage maps, using SSR markers. This is necessary to compare the location of QTLs for LB resistance and assess the transferability of markers and QTLs not only between populations, but also between ploidy levels.

Acknowledgements The authors thank Helen Stewart for help with the late blight assay, and Dr. Christiane Gebhardt for sharing her diploid RFLP mapping data. RCM is supported by UNDP/CIP. DM is a PMB-funded Ph.D. student. All other authors are funded by the Scottish Office (Agriculture, Environment and Fisheries Department).

References

Ballvora A, Hesselbach J, Niewohner 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

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, O. Pineda O, Tanksley SD (1994) QTL analysis of trichome-mediated insect resistance in potato. Theor Appl Genet 87: 973–987

Bradshaw JE, Stewart HE, Wastie RL, Dale MFB, Phillips MS (1995) Use of seedling progeny tests for genetical studies as part of a potato (*Solanum tuberosum* subsp. *tuberosum*) breeding programme. Theor Appl Genet 90:899–905

Bradshaw JE, Hackett CA, McNicol JW, Meyer RC, Milbourne D, Phillips MS, Waugh R (1998) Identification of AFLP markers associated with quantitative resistance to *Globodera pallida* (Stone) in tetraploid potato (*Solanum tuberosum* subsp. *tuberosum*) with a view to marker assisted selection. Theor Appl Genet, in press

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Cruickshank G, Stewart HE, Wastie RL (1982) An illustrated assessment key for foliage blight of potatoes. Potato Res 25:213–214

Da Silva J (1993) A methodology for genome mapping of autopolyploids and its application to sugarcane (*Saccharum* spp). Ph. D. dissertation, Cornell University, Ithaca, New York

Da Silva J, Sorrells ME, Burnquist WL, Tanksley SD (1993) RFLP linkage map and genome analysis of *Saccharum spontaneum* L. Genome 36:782–791

Demecke T, Kawchuk LH, Lynch DR (1993) Identification of potato cultivars and clonal variants by random amplified polymorphic DNA analysis. Amer Potato J 70:561–570

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

Gorg R, Schachtschabel U, Ritter E, Salamini F, Gebhardt C (1992) Discrimination among 136 tetraploid potato varieties by fingerprints using highly polymorphic DNA markers. Crop Sci 32:815–819

Hackett CA, Bradshaw JE, Meyer RC, McNicol JW, Milbourne D, Waugh R (1998) Linkage analysis in tetraploid potatoes: a simulation study. Genet Res, in press

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

Leister D, Ballvora A, Salamini F, Gebhardt C (1996) A PCR based approached for isolating pathogen resistance genes from potato with potential for wide application in plants. Nature Genet 14:421–429

Leonards-Schippers C, Gieffers W, Schäfer-Pregl R, Ritter E, Knapp SJ, Salamini F, Gebhardt C (1994) Quantitative resistance to Phytophthora infestans in potato: a case study for QTL mapping in an allogamous plant species. Genetics 137:67–77

Lincoln SE, Daly MJ, Lander ES (1991) PRIMER: a computer program for automatically selecting PCR primers. Whitehead Institute for Biomedical Research, Cambridge, Mass.

Mann HB, Whitney DR (1947) On a test of whether one of two random variables is stochastically larger than the other. Ann Math Stat 18:50–60

Meyer R, Salamini F, Uhrig H (1993) Isolation and characterization of potato diploid clones generating a high frequency of monohaploid or homozygous diploid androgenetic plants. Theor Appl Genet 85:905–912

Milbourne D, Meyer RC, Bradshaw JE, Baird E, Bonar N, Provan J, Powell W, Waugh R (1997) Comparison of PCR-based marker systems for the analysis of genetic relationships in cultivated potato. Mol Breed 3:127–136

Milbourne D, Meyer RC, Collins AJ, Ramsay LD, Gebhardt C, Waugh R (1998) Isolation, characterisation and mapping of simple sequence repeat loci in potato. Mol Gen Genet, in press

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Payne RW, Lane PW, Digby PGN, Harding SA, Leech PK, Morgan GW, Todd AD, Thompson R, Tunnicliffe Wilson G, Welham SJ, White RP (1993) Genstat 5 Release 3 Reference Manual. Oxford University Press, Oxford, UK.

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996) The utility of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed 2:225–238

Ritter E, Debener T, Barone A, Salamini F, Gebhardt C (1991) RFLP mapping on potato chromosomes of two genes controlling extreme resistance to potato virus X (PVX). Mol Gen Genet 227:81–85

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

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Sobral BWS, Honeycutt RJ (1993) High-output genetic mapping in polyploids using PCR-generated markers. Theor Appl Genet 86:105–112

Stam P, van Ooijen JW (1995) JoinMap version 2.0: software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen, The Netherlands

Stewart HE, Flavelle PH, McCalmont DC, Wastie RL (1983) Correlation between glasshouse and field-tests for resistance to foliage blight caused by *Phytophthora infestans*. Potato Res 26:41–48

Van Eck HJ, Jacobs JME, van Dijk J, Stiekema WJ, Jacobsen E (1993) Identification and mapping of three flower colour loci of potato (*S. tuberosum* L.) by RFLP analysis. Theor Appl Genet 86:295–300

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 Oijen JW (1994) Drawmap: a computer program for drawing genetic linkage maps. J Hered 85:66

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

Waugh R, McLean K, Flavell AJ, Pearce SR, Kumar A, Thomas WBT, Powell W (1997) Genetic distribution of *Bare-1*-like retrotransposable elements in the barley genome revealed by sequence-specific amplification polymorphisms (S-SAP). Mol Gen Genet 253:687–694

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 fragments. Theor Appl Genet 83:294–300

Yu KF, Pauls KP (1993) Segregation of random amplified polymorphic DNA markers and strategies for molecular mapping in tetraploid alfalfa. Genome 36:844–851