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Identification of quantitative trait loci contributing to Fusarium wilt resistance on an AFLP linkage map of flax (*Linum usitatissimum*)

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Abstract An AFLP genetic linkage map of flax (*Linum usitatissimum*) was used to identify two quantitative trait loci (QTLs) on independent linkage groups with a major effect on resistance to Fusarium wilt, a serious disease caused by the soil pathogen *Fusarium oxysporum* (lini). The linkage map was constructed using a mapping population from doubled-haploid (DH) lines. The DH lines were derived from the haploid component of F_2 haploid-diploid twin seed originating from a cross between a polyembryonic, low-linolenic-acid genotype (CRZY8/RA91) and the Australian cultivar 'Glenelg'. The AFLP technique was employed to generate 213 marker loci covering approximately 1400 cM of the flax genome (n = 15) with an average spacing of 10 cM and comprising 18 linkage groups. Sixty AFLP markers (28%) deviated significantly ($P < 0.05$) from the expected segregation ratio. The map incorporated RFLP markers tightly linked to flax rust (*Melamspora lini*) resistance genes and markers detected by disease resistance gene-like sequences. The study illustrates the potential of the AFLP technique as a robust and rapid method to generate moderately saturated linkage maps, thereby allowing the molecular analysis of traits, such as resistance to Fusarium wilt, that show oligogenic patterns of inheritance.

Key words Flax · *Linum usitatissimum* · AFLP · Genetic mapping

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Introduction

Flax (*L. usitatissimum*) is a model organism used in classical genetics to elucidate the basis for the specific recognition events involved in host-pathogen interactions between the flax host plant and the rust pathogen *Melamspora lini* (Flor 1956). More recently this system was used in molecular genetics to isolate genes conferring resistance to flax rust (Lawrence et al. 1995; Anderson et al. 1997). However, crop improvement of this species relies on an understanding of the genetic control of other important agronomic traits such as yield potential, resistance to lodging and the soil fungal-disease Fusarium wilt (*Fusarium oxysporum* lini). The inheritance of Fusarium wilt resistance was studied previously within a recombinant doubled-haploid (DH) population from a cross between parental lines that differ widely in their resistance status to wilt (Spielmeyer et al. 1998). Most of the phenotypic variation was attributable to the segregation of two independent genes with additive effects. Minor resistance genes may have also contributed by modifying the resistance response. In the present study, the application of molecular-marker technology, through the construction of genetic linkage maps, allowed the dissection of Fusarium wilt resistance into its genetic components at the molecular level.

Genetic linkage maps of plant species constructed thus far using molecular markers have been based primarily on the RFLP technique (Helentjaris et al. 1986; McCouch et al. 1988; Gebhardt et al. 1991; Graner et al. 1991; Tanksley et al. 1992; Ferreira et al. 1994; Langridge et al. 1995). The co-dominant mode of inheritance, reliability and locus specificity, are still the principal reasons behind the continued use of this class of marker in mapping studies. The advent of PCR-based marker systems provides new opportunities to identify a large number of DNA polymorphisms within genomes of autogamous species such as *L. usitatissimum*. In particular, the recent introduction of the

AFLP technique allows the identification of a sufficient number of markers to construct moderately saturated linkage maps by one researcher in less than 6 months (Vos et al. 1995). The high-stringency PCR conditions used to generate AFLP markers allow good reproducibility of amplification products. The AFLP technique has been successfully employed as a mapping tool in barley (Becker et al. 1995), potato (van Eck et al. 1995), soybean (Lin et al. 1996) and rice (Mackill et al. 1996).

The estimated genome size (7.0×10^8 bp/1C) of *L. usitatissimum*, which is considered an ancient tetraploid (2n = 30), is relatively small (Cullis 1981). Preliminary linkage mapping within *L. usitatissimum* previously identified 19 RFLP and 69 RAPD markers covering 15 linkage groups (Cullis et al. 1995). To identify molecular markers linked to Fusarium wilt resistance, the current study has generated a more comprehensive linkage map of this species by characterising AFLP and RFLP markers employing a subset of the DH lines previously used in the inheritance study of wilt resistance (Spielmeyer et al. 1998). The linkage map formed the basis for quantitative trait analysis to identify genomic regions that contribute to wilt resistance. In addition, the utility of a bulked-segregant approach was assessed to target quantitative trait loci (QTLs) involved in the resistance response to wilt (Michelmore et al. 1991; Wang and Paterson 1994).

Materials and methods

Plant material

A population of homozygous recombinant lines was developed through chromosome doubling of the haploid component of haploid-diploid twin F_2 seedlings derived from a biparental cross designed to segregate for wilt resistance, twinning, and various other traits. One parent of the cross was the highly-inbred, wilt-susceptible, non-twinning Australian linseed cultivar Glenelg. The other parent (CRZY8/RA91) was a wilt-resistant, high-twinning, Linola™ oil-quality (low linolenic form of flax, Green 1986) doubled-haploid (DH) plant derived from a cross between a wilt-resistant, non-twinning Linola line (CRZY8) and the high-twinning linseed DH genotype RA91 developed by Green and Salisbury (1983). The wilt resistance characteristic of the CRZY8 line was contributed by the resistant cultivar Croxton, which was a later selection from the same crossbred population that gave rise to Glenelg. The parental lines in the final cross are thus related and are expected to have a degree of genetic identity. The recombinant DH lines were produced by germinating F_2 twin seeds, identifying the haploid component on the basis of sterility at flowering, and subsequent colchicine treatment of decapitated main stems using the methods of Rajhathy (1976). A total of 143 DH lines were scored for resistance to Fusarium wilt in the glasshouse and field nursery as described by Spielmeyer et al. (1998).

AFLP template

The method for AFLP template preparation was based on the protocol published by Vos et al. (1995) with only minor modifications. The amount of genomic DNA used in the restriction digest was increased from approximately 0.5 μg to 2 μg/sample.

Restriction enzymes, adapters and oligonucleotide primers

Two restriction-enzyme combinations were used to generate separate DNA fragment-pools for analysis. Mapping of marker loci was initially performed using amplification products derived from *Eco*RI/*Mse*I-digested genomic DNA, and these were later supplemented by markers derived from a *Pst*I/*Mse*I enzyme combination. Adapter and oligonucleotide primer sequences were designed according to Vos et al. (1995) and synthesised at CSIRO Plant Industry, Australia.

Amplification of ligated DNA fragments

A two-step amplification method was used as described by Vos et al. (1995). During the pre-amplification step oligonucleotide primers were employed with one selective nucleotide attached to the 3′ terminus. The PCR temperature profile was identical to the conditions described by Vos et al. (1995). The pre-amplified DNA was diluted 1:10 with double-distilled water before being amplified a second time using oligonucleotide primers with three selective nucleotides attached. Before the second round of amplification the *Eco*RI or *Pst*I primers were [^{33}P]ATP end-labelled. The 'touchdown' PCR conditions consisted of a total of 36 cycles with a constant denaturation step (30 s, 94°C) and extension step (60 s, 72°C). The duration of all annealing steps was kept constant at 60 s. The annealing temperature for the first cycle was 65°C which was reduced to 64°C for cycles 2 and 3, to 63°C for cycle 4, to 62°C for cycles 5 and 6, to 61°C for cycle 7, to 60°C for cycles 8 and 9, to 59°C for cycle 10, to 58°C for cycles 11 and 12, and to 57°C for the remaining 24 cycles. The PCR was performed on a Corbett FTS-960 thermocycler.

Gel analysis

The PCR products were separated on 5% denaturing polyacrylamide gels run in 1 × TBE buffer pre-heated to 65°C at 25 W for 2.5 h. The gels were fixed in 10% acetic acid/20% methanol for 30 min, dried at 80°C for 3 h and exposed to X-ray film (Kodak MR) for 1–3 days.

DNA pooling

AFLP templates from individual DH lines were prepared as described above. Following the ligation of adapter sequences, DNA from ten resistant and ten susceptible DH lines was bulked into separate pools. Resistant and susceptible DNA bulks in conjunction with AFLP templates from parental lines were subjected to a two-step PCR amplification employing the same primer combinations as used for the construction of the linkage map.

RFLP analysis

DNA hybridisation was carried out as described by Lagudah et al. (1991). Two DNA fragments (Lu-1 and X-22) linked to the flax rust resistance loci *L* and *M* respectively were obtained from J. G. Ellis (personal communication). The probe Lu-1 was derived from the promoter region of *L6*, whilst X-22 detects an RFLP marker located two map units from the *M* locus (Ellis et al. 1995). An additional clone from flax (Ltt) was isolated by heterologous probing of a flax

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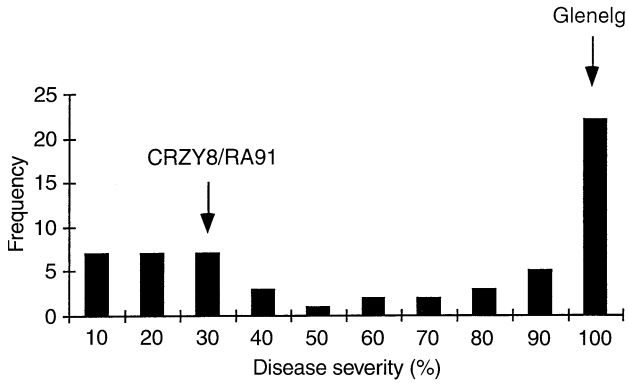


Fig. 1 Frequency distribution of Fusarium wilt severity (%) for 59 recombinant doubled-haploid lines used as a mapping population. The average level of resistance displayed by the resistant parental line CRZY8/RA91 and the susceptible cv Glenelg is indicated

genomic library using a putative cereal cyst nematode (CCN) resistance gene from *Triticum tauschii* (Lagudah et al. 1997). Using primers for conserved motifs that are characteristic for a class of disease resistance genes containing nucleotide-binding domains as well as leucine-rich repeat regions, it had been confirmed that the Ltt clone (4.8 kb) most likely contains a resistance gene-like sequence. When used as a probe Ltt detected RFLP loci *pr-1*, pr-*3* and *pr-4*.

Map construction and data analysis

From a total of 143 DH lines a mapping population of 59 DH lines was selected on the basis of lines displaying either a resistant (29 lines) or susceptible (30 lines) phenotype in response to infection by Fusarium wilt (Fig. 1). This selection of phenotypes was carried out to increase the efficiency in determining quantitative trait loci (QTLs) that contribute to wilt resistance (Tanksley 1993). Linkage analysis was performed using 'MapMaker/EXP 3.0' computer software (Lander et al. 1987). Markers were grouped according to two-point analysis (LOD score > 4.0) and framework orders were established in most cases with a LOD score > 3.0, except in linkage groups which were comprised entirely of markers deviating significantly from the expected 1 : 1 ratio (LOD score > 2.5). Additional markers were fitted into the reference frame with LOD scores > 2.0. Recombination values were converted into map distances (centi-Morgan, cM) by applying the Kosambi function. The numbers denoting individual linkage groups were assigned arbitrarily.

The linkage map was used for interval mapping using MAPMAKER/QTL software (Lander and Botstein 1989). A QTL was declared for chromosomal regions which showed LOD scores greater than 2.5 indicating a significant association between marker genotype and trait data. Significant associations between marker genotypes and resistance was tested for markers located within the confidence interval of QTLs by 2×2 contingency tests. Interactions between loci were also tested by using contingency tables for a significant association with resistance.

Results

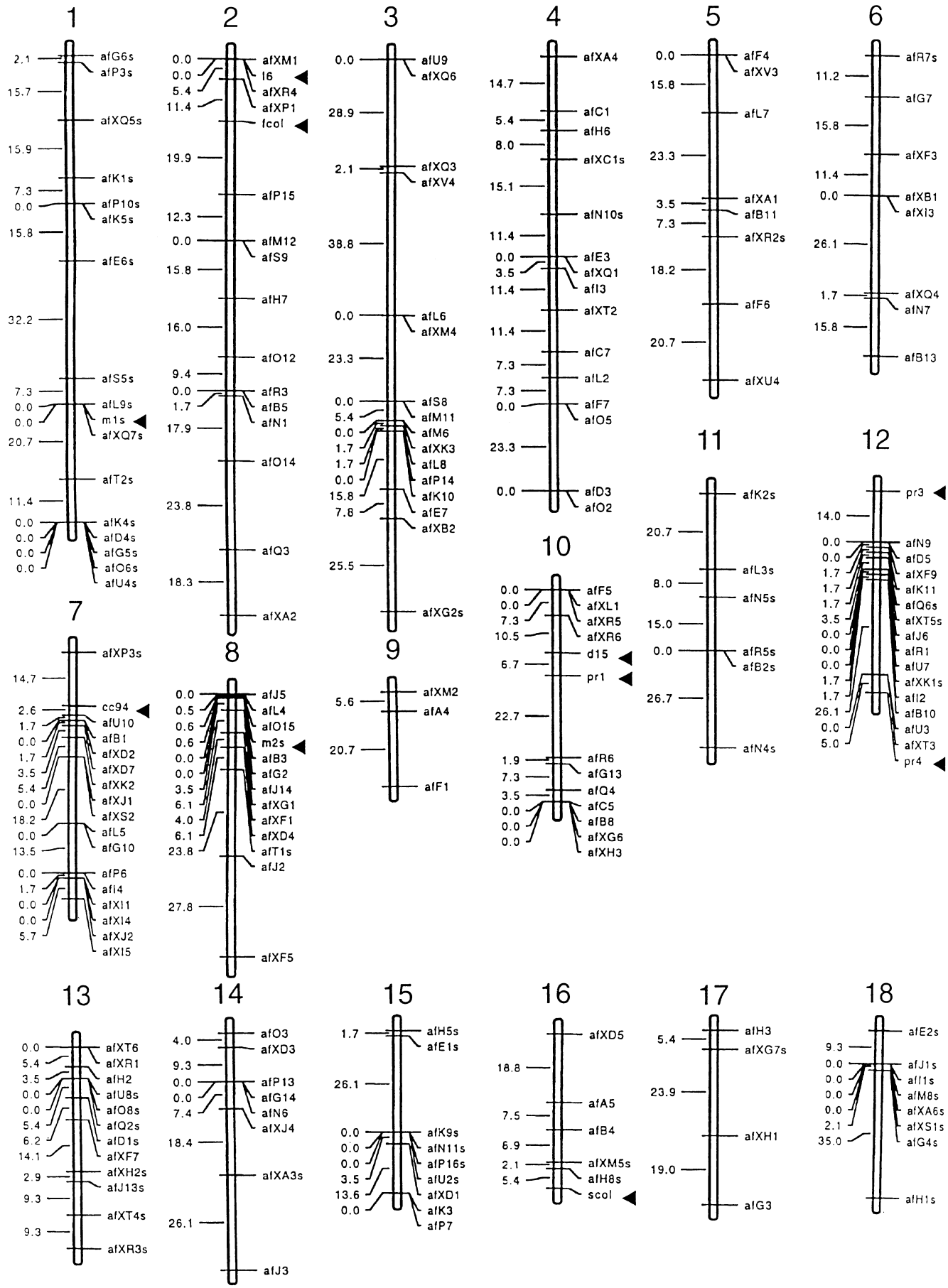
Genetic linkage map

The two parents, 'CRZY8/RA91' and 'Glenelg', were screened for polymorphisms using 160 primer combinations. A subset of 21 *Eco*RI/*Mse*I and 24 *Pst*I/*Mse*I primer combinations was chosen on the basis of the high level of polymorphic bands identified between parents. Each primer combination generated up to 80 bands of which on average 5.7 were polymorphic. Marker loci derived from *Eco*RI/*Mse*I and *Pst*I/*Mse*I fragments mapped with a similar frequency across most linkage groups.

The linkage relationships of two morphological (seed and flower colour), eight RFLP and 213 AFLP marker loci were determined (Fig. 2). Approximately 1400 cM of the flax genome was covered with an average spacing of 10 cM between loci comprising 18 linkage groups of three or more loci. Outside the 18 linkage groups, nine pairs of loci showed pairwise linkage or co-segregation. A further eight marker loci did not show any linkage to other loci. A considerable number of AFLP marker loci (35%) co-segregated with other AFLP or RFLP markers, therefore not improving the overall map resolution. The segregation of 60 AFLP markers (28%) deviated significantly ($P < 0.05$) from the expected 1 : 1 ratio. Most of these marker loci clustered in five linkage groups, three of which (groups 1, 11 and 18) were composed entirely of marker loci showing significant deviations (Table 1). The loci on linkage groups 1 and 18 are skewed towards the 'Glenelg' parental alleles whereas linkage groups 11, 13 and 15 have a prevalence of the 'CRZY8/RA91' alleles. The degree of distortion of marker loci varied notably within linkage groups 1 and 11 (Fig. 3). Marker loci positioned at the ends of linkage groups deviated considerably less than more centrally located markers, perhaps indicating the presence of disturbance factors in the central regions of linkage groups. Marker loci located furthest away from any hypothesised disturbance factors are expected to show less segregation distortion due to more opportunity for recombination.

AFLP amplification products segregated as dominant markers resulting in either the presence or absence of polymorphic products. However, 42 individual segregating bands (19%) were classified as putative co-dominant alleles, because two polymorphic amplification products originating from either parent and displaying only minor differences in mobility segregated as alternatives to each other in expected 1 : 1 ratios (see marker 5, Fig. 4). These putative allelic markers mapped randomly across linkage groups.

RFLP markers associated with putative disease resistance loci mapped independently to five linkage groups (Fig. 2). The genomic clone Lu-1 detected the RFLP marker *l6* that mapped to linkage group 2. Lu-1 was previously shown to be tightly linked to the *L6* rust resistance gene (Ellis et al. 1995). The clone X-22 linked to the complex rust resistance locus *M* detected RFLPs *m1* and *m2* which mapped to linkage groups 1 and 8 respectively. The probe Ltt detected additional RFLP markers (*pr-1*, *pr-3* and *pr-4*) on linkage groups 10 and 12.


1
2.1 afG6s
afP3s
15.7 afXQ5s
15.9 afK1s
7.3 afP10s
0.0 afK5s
15.8 afE6s
32.2 afS5s
7.3 afL9s
0.0 m1s
0.0 afXQ7s
20.7 afT2s
11.4 afK4s
0.0 afD4s
0.0 afG5s
0.0 afO6s
afU4s
2
0.0 afXM1
0.0 I6
5.4 afXR4
11.4 afXP1
fcol
19.9 afP15
12.3 afM12
0.0 afS9
15.8 afH7
16.0 afO12
9.4 afR3
0.0 afB5
1.7 afN1
17.9 afO14
23.8 afQ3
18.3 afXA2
3
0.0 afU9
afXQ6
28.9 afXQ3
2.1 afXV4
38.8 afL6
0.0 afXM4
23.3 afS8
0.0 afM11
5.4 afM6
0.0 afXK3
1.7 afL8
1.7 afP14
0.0 afK10
15.8 afE7
7.8 afXB2
25.5 afXG2s
4
afXA4
14.7 afC1
5.4 afH6
8.0 afXC1s
15.1 afN10s
11.4 afE3
0.0 afXQ1
3.5 afI3
11.4 afXT2
11.4 afC7
7.3 afL2
7.3 afF7
0.0 afO5
23.3 afD3
0.0 afO2
5
0.0 afF4
afXV3
15.8 afL7
23.3 afXA1
3.5 afB11
7.3 afXR2s
18.2 afF6
20.7 afXU4
6
afR7s
11.2 afG7
15.8 afXF3
11.4 afXB1
0.0 afXI3
26.1 afXQ4
1.7 afN7
15.8 afB13
7
afXP3s
14.7 cc94
2.6 afU10
1.7 afB1
0.0 afXD2
1.7 afXD7
3.5 afXK2
5.4 afXJ1
0.0 afXS2
18.2 afL5
0.0 afG10
13.5 afP6
0.0 afI4
1.7 afXI1
0.0 afXI4
0.0 afXJ2
5.7 afXI5
8
0.0 afJ5
0.5 afL4
0.6 afO15
0.6 m2s
0.0 afB3
0.0 afG2
3.5 afJ14
6.1 afXG1
4.0 afXF1
6.1 afXD4
23.8 afT1s
afJ2
27.8 afXF5
9
5.6 afXM2
afA4
20.7 afF1
10
0.0 afF5
0.0 afXL1
7.3 afXR5
10.5 afXR6
6.7 d15
pr1
22.7 afR6
1.9 afG13
7.3 afQ4
3.5 afC5
0.0 afB8
0.0 afXG6
0.0 afXH3
11
afK2s
20.7 afL3s
8.0 afN5s
15.0 afR5s
0.0 afB2s
26.7 afN4s
12
pr3
14.0 afN9
0.0 afD5
0.0 afXF9
1.7 afK11
1.7 afQ6s
1.7 afXT5s
3.5 afJ6
0.0 afR1
0.0 afU7
0.0 afXK1s
1.7 afI2
1.7 afB10
26.1 afU3
0.0 afXT3
5.0 pr4
13
0.0 afXT6
5.4 afXR1
3.5 afH2
0.0 afU8s
0.0 afO8s
5.4 afQ2s
6.2 afD1s
14.1 afXF7
2.9 afXH2s
9.3 afJ13s
afXT4s
9.3 afXR3s
14
4.0 afO3
afXD3
9.3 afP13
0.0 afG14
0.0 afN6
7.4 afXJ4
18.4 afXA3s
26.1 afJ3
15
1.7 afH5s
afE1s
26.1 afK9s
0.0 afN11s
0.0 afP16s
0.0 afU2s
3.5 afXD1
13.6 afK3
0.0 afP7
16
afXD5
18.8 afA5
7.5 afB4
6.9 afXM5s
2.1 afH8s
5.4 scol
17
5.4 afH3
afXG7s
23.9 afXH1
19.0 afG3
18
afE2s
9.3 afJ1s
0.0 afI1s
0.0 afM8s
0.0 afXA6s
2.1 afXS1s
35.0 afG4s
afH1s

QTL mapping

Interval analysis using the AFLP linkage map of flax identified two QTLs for resistance to Fusarium wilt located on linkage groups 6 (LOD 7.3) and 10 (LOD 3.4) that contribute 38% and 26% respectively to the phenotypic variation (Fig. 5). At both QTLs the alleles inherited from the resistant parent CRZY8/RA91 at markers located within the confidence intervals on linkage group 6 (*afB13*) and linkage group 10 (*afXR6*) contributed significantly to wilt resistance (Table 2). Using contingency tests the loci *afB13* and *afXR6*, showed a significant association, indicating that both loci interact by having an additive effect on resistance (Table 2).

Bulked-segregant analysis to target QTLs

DNA pools from resistant and susceptible DH lines together with both parental lines were screened by evaluating 160 AFLP primer combinations that detected approximately 10 000 marker bands. Twelve marker bands originating from one of the two parents were polymorphic between the two DNA bulks. These polymorphisms were analysed on 59 DH lines of either resistant or susceptible phenotypes. The majority of polymorphic markers between DNA pools were the result of segregation distortion at these loci and hence were not associated with resistance. However, the amplification product of *afB13* was polymorphic between parental lines and DNA pools, and segregated according to the expected 1:1 ratio when screened against the 59 DH lines (Fig. 4). Subsequent interval mapping placed this marker into a chromosomal region containing a putative QTL for resistance to Fusarium wilt (Fig. 5).

Discussion

Genetic linkage map

In this study, AFLP and RFLP markers were generated to construct a genetic linkage map of *L. usitatissimum*. The expected number of 15 linkage groups for a comprehensive linkage map of flax (2n = 30) is exceeded by three linkage groups and nine paired markers, which could not be placed into any of the larger linkage groups with LOD values > 2.0. Because some linkage groups are relatively small, the apparent excess of linkage groups is probably the result of incomplete coverage of the genome with marker loci. Alternatively, the common ancestry of the parental lines used to derive the mapping lines may have contributed genetically identical chromosome sections, for which no polymorphic markers were detected. If these identical regions are sufficient in length, they may effectively separate linkage groups that are located on the same chromosome.

Several linkage groups showed considerable clustering and co-segregation of AFLP marker loci (Fig. 2). This was particularly common with markers derived from *Eco*RI/*Mse*I fragments. A different enzyme combination, *Pst*I/*Mse*I, was used to generate additional informative marker loci taking advantage of the DNA methylation sensitivity of the *Pst*I restriction enzyme. By employing this restriction enzyme, which cuts less frequently within methylated DNA, a greater proportion of DNA fragments is expected to be generated from chromosomal regions potentially containing transcribed genes. This strategy enabled the identification of new marker loci by filling in 'gaps' and extending linkage groups. Despite this approach 18 regions remain where distances between pairs of adjacent markers exceed 20 cM. Some of these regions devoid of marker loci may represent identical regions on homologous chromosomes due to the common ancestry of parental lines.

A substantial percentage of AFLP markers (28%) deviated significantly from the expected Mendelian ratio of 1:1. Segregation distortion has frequently been observed in mapping studies and was reported at similar frequency amongst RFLP markers in a DH mapping population of rapeseed (Ferreira et al. 1994). Significant proportions of RFLP markers showing distorted segregation were observed in DH progeny of barley (Graner et al. 1991) and in F_2 mapping populations of sunflower (Berry et al. 1995), rice (McCouch et al. 1988), lettuce (Landry et al. 1987) and rapeseed (Landry et al. 1991). One cause for the disturbed segregation shown by some markers in this study could derive from the fact that the 59 individuals in the mapping family all originate from F_2 twins. If twinning is influenced by genotype there would be a strong selection for those alleles that promote twinning. Early studies by Kappert (1933) showed that the genotype of the plant on which the seed develops, and not the genotype of the gamete, determines twinning frequency. Since all the F_2 haploids used to generate DH lines originated from the same F_1 plant, it is unlikely that the DH lines are genetically biased towards genes linked to polyembryony.

Fig. 2 Genetic linkage map of *L. usitatissimum* consisting of AFLP, RFLP and morphological marker loci. AFLP marker loci were assigned the prefix '*af*', a *capital letter* encoding the specific primer combination ('*X*' denotes loci generated from *Pst*1/*Mse*1 DNA fragments) followed by a *number* describing the polymorphic marker band scored. The lower case letter '*s*' as a suffix denotes that loci were not segregating according to the expected ratio ($P < 0.05$). RFLP markers are denoted by *arrowheads* and phenotypic markers '*scol*' and '*fcol*' are loci controlling seed colour and flower colour respectively. *Numbers* on the left hand side of linkage groups denote genetic distances in centiMorgans

Table 1 Summary data for AFLP, RFLP and morphological markers distributed across 18 linkage groups of three or more marker loci showing expected and distorted segregation patterns ($P < 0.05$) in DH mapping lines

Linkage group	cM	Total markers	Skewed markers	Individ. loci	AFLP *Eco*RI/ *Mse*I	AFLP *Pst*I/ *Mse*I	RFLP markers	Morph. markers
1	128	17	17	10	14	2	1	–
2	151	16	–	12	10	4	1	1
3	151	16	–	11	9	7	–	–
4	118	15	2	12	11	4	–	–
5	88	8	1	7	4	4	–	–
6	82	8	1	7	4	4	–	–
7	68	17	1	11	6	10	1	–
8	73	13	2	7	8	4	1	–
9	26	3	–	3	2	1	–	–
10	59	13	–	8	6	5	2	–
11	70	6	6	5	6	–	–	–
12	57	16	3	10	10	4	2	–
13	56	12	8	9	6	6	–	–
14	65	8	1	6	5	3	–	–
15	44	9	6	5	8	1	–	–
16	42	6	3	6	3	2	–	1
17	48	4	1	4	2	2	–	–
18	46	8	8	4	6	2	–	–
Paired markers		18						
Total	1372	213	60	137	120	83	8	2

Mapping of putative disease resistance loci

Quantitative trait loci were identified on two independent linkage groups that show association with resistance to Fusarium wilt (Fig. 5). Interval regression analysis determined that one QTL on linkage group 6 and one on linkage group 10 had major effects on wilt resistance, explaining 38% and 26% of the observed phenotypic variation respectively. While the efficiency of detecting QTLs increases using a map constructed with individuals from distributional extremes, the accuracy of estimating QTL effects may be reduced (Tanksley 1993). Selection within mapping populations may also result in incorrect linkage estimates between independently inherited loci and compromise the accuracy of locating QTLs if false linkage relationships between markers are used as a framework in QTL mapping (Martinez 1996). However, in the present study the segregation analysis of over 200 marker loci showed that the majority of loci possess a ratio compatible with the expected 1:1 ratio for this DH progeny. Although distorted segregation of markers was observed within several linkage groups, the two putative QTLs with major effects on wilt resistance are not associated with genomic regions showing segregation distortion. Furthermore, the identification of two QTLs associated with wilt resistance is in accordance with earlier inheritance studies that concluded that two independent loci accounted for most of the phenotypic variation observed for wilt resistance in this DH population (Spielmeyer et al. 1998).

Bulked-segregant analysis of DNA pools constructed on the basis of extreme phenotypes predicted an association between marker locus *afB13* (linkage group 6) and Fusarium wilt resistance. Interval mapping confirmed this association by showing that this marker is linked to a putative resistance locus on linkage group 6. It was therefore possible to tag a QTL with major effect using a pooling strategy similar to the one proposed for

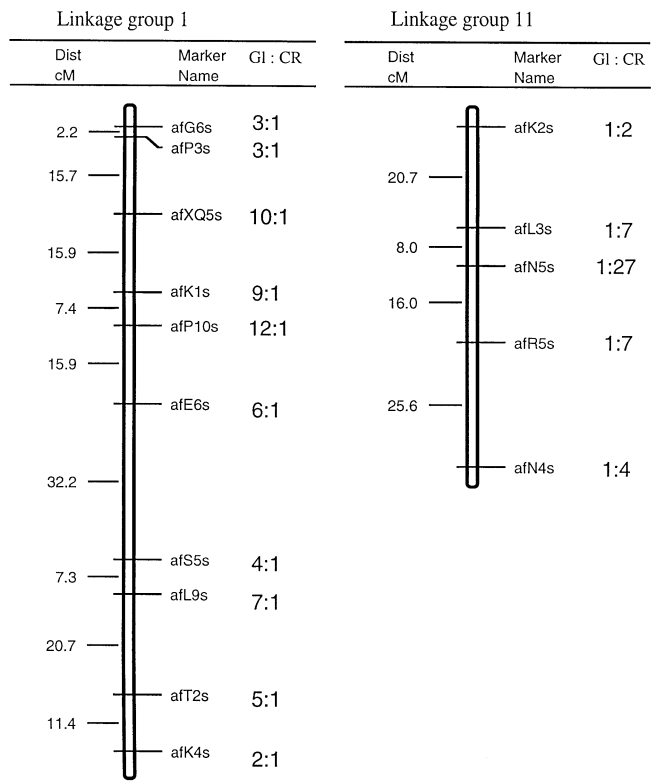


Fig. 3 Two linkage groups which consist entirely of marker loci showing distorted segregation ($P < 0.05$). Loci on linkage group 1 are skewed towards the 'Glenelg' (*Gl*) parental alleles and loci on linkage group 11 are skewed towards the 'CRZY8/RA91' (*CR*) alleles

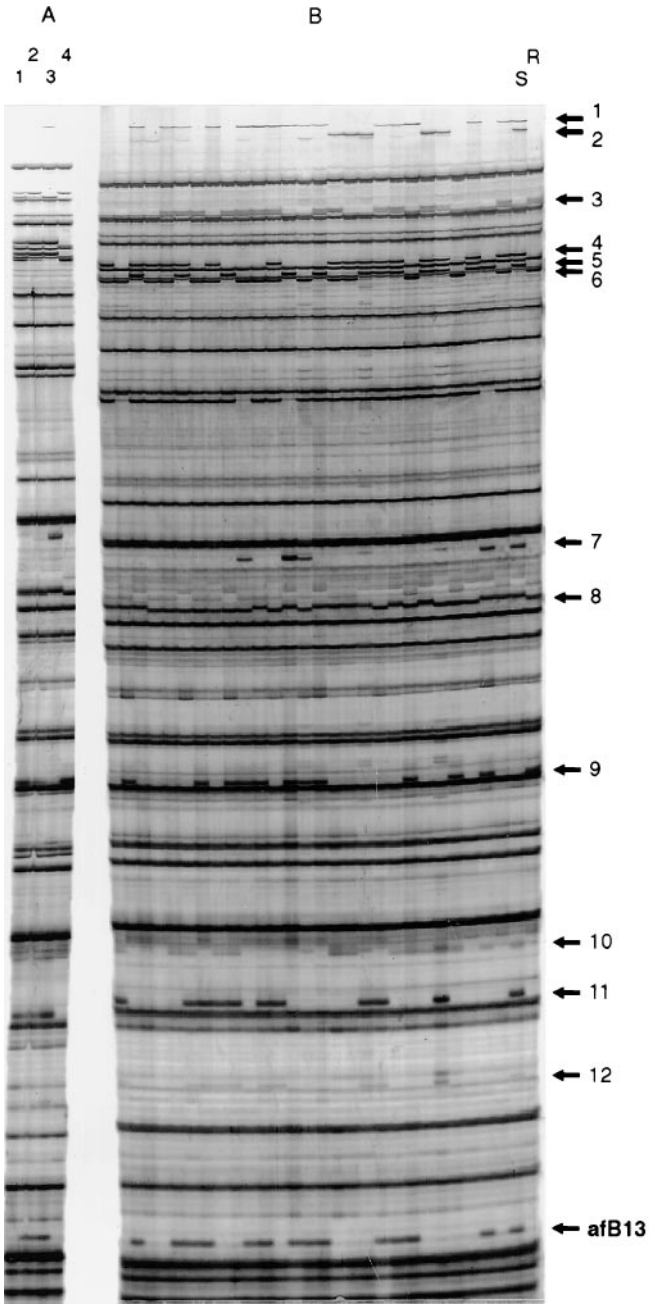


Fig. 4A, B AFLP profile using primer combination *Mse*I.GAG/*Eco*RI.AGT. **A** *Lane 1* = pool of DNA from ten resistant DH lines, *lane 2* = pool of DNA from ten susceptible lines, *lane 3* = susceptible parent, *lane 4* = resistant parent. Product 'afB13', amplified from the susceptible parent, is polymorphic between DNA pools. **B** *Lane S* = susceptible parent, *lane R* = resistant parent. Mendelian segregation of amplification product 'afB13' (approximately 80–100 bp) in doubled-haploid lines. The size of amplification products range from approximately 100 to 800 base pairs

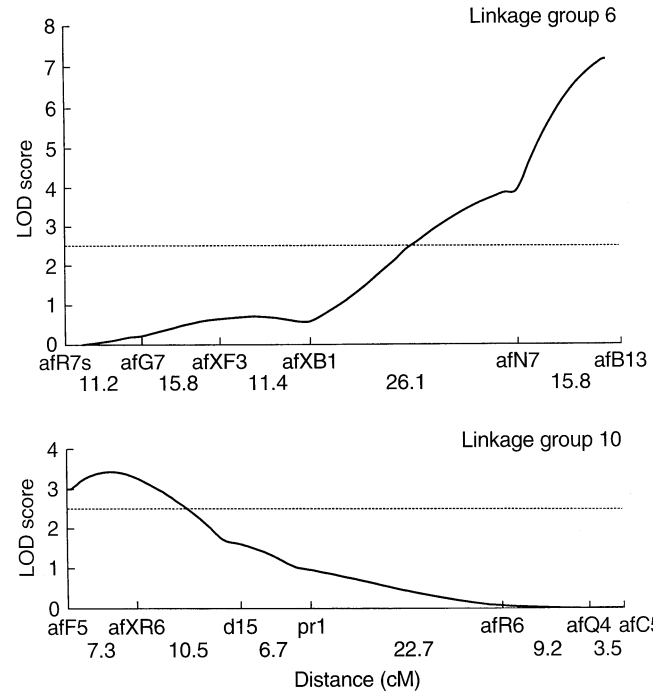


Fig. 5 QTL likelihood profiles of linkage groups 6 (LOD 7.3) and 10 (LOD 3.4) contributing to Fusarium wilt resistance. The AFLP marker (afB13) linked to resistance on linkage group 10 has been amplified using the primer combination *Mse*I.GAG/*Eco*RI.AGT. The markers linked to resistance on linkage group 6 have been amplified using the primer combinations *Mse*I.GTT/*Pst*I.ACA (afXR6) and *Mse*I.GCT/*Eco*RI.AGA (afF5). The LOD significance threshold of 2.5 is marked as a *dotted line*

targeting single loci (Michelmore et al. 1991; Wang and Paterson 1994).

Additional putative disease resistance loci were mapped in this DH population using RFLP probes linked to known resistance genes. The rust resistance locus *L* was mapped to linkage group 2 with probe Lu-1, a clone that was derived from the promoter region of the L6 rust resistance gene (Lawrence et al. 1995) and which detected a single fragment as RFLP marker locus *l6*. Probe X-22, which had previously been shown to be linked (2 cM) to the *M* resistance cluster (Ellis et al. 1995), detected a strongly hybridising fragment and a minor fragment associated with RFLP loci *m*1 and *m2* respectively. The RFLP marker *m1* on linkage group 1 is likely to detect the homologous sequence linked to the rust resistance cluster *M*. RFLP loci *l6* and *m1* map on independent linkage groups consistent with previous reports from classical linkage analysis of both loci (Lawrence et al. 1994).

The construction of genetic linkage maps within minor crop species such as flax, for which comprehensive RFLP linkage maps do not exist, is set to benefit greatly from the application of the AFLP technique. This technique enabled the rapid generation of a reference linkage map, thereby enabling the identification of genomic regions contributing to a significant proportion of the resistance to Fusarium wilt. Future work will concentrate on identifying additional AFLP markers in an attempt to delineate QTLs in the chromosomal regions more accurately. AFLP markers

Table 2 Contingency tests of markers within confidence intervals of putative QTLs for their association and two-way interaction to Fusarium wilt resistance in recombinant DH lines

FW[a]	Marker effect tested										
	af B13			af XR6			af B13*af XR6				
	CR[b]	GL[c]	Total	CR	GL	Total	CR/CR	CR/GL	GL/CR	GL/GL	Total
R	21	4	25	17	8	25	14	3	7	1	25
S	6	25	31	10	21	31	4	6	2	19	31
Total	27	29	56	27	29	56	18	9	9	20	56
	$\chi^2 = 23$**			$\chi^2 = 7$*			$\chi^2 = 25$**				

[a] FW: response to Fusarium wilt, either resistant R or susceptible S classes
[b] CR: CRZY8/RA91 (resistant allele)
[c] GL: Glenelg (susceptible allele)
* Significant association at $p < 0.01$
** Significant association at $p < 0.001$

flanking genomic regions of interest will be screened against a wide range of flax germplasm with the view to develop a robust PCR assay for marker-assisted selection of wilt resistance.

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