A novel RAPD marker linked to the Fusarium wilt race 5 resistance gene (*Fwf*) in *Pisum sativum*

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Introduction

Fusarium wilt (Fw) is a significant problem in pea-growing regions throughout the world. The causal agent, *F. oxysporum* Schlecht. emend. f. sp. *pisi* (van Hall) Snyd. & Hans., is a soil-borne fungus that enters the host vascular system at root tips or through wounds, causing progressive chlorosis of the leaves and stems, wilting, and collapse of the root system (4, 6). Fusarium wilt race 5 (Fwf) is well-adapted to environmental conditions in the northwestern United States. It has caused serious economic losses to the pea industry in the Pacific Northwest since the late 1960s (7) and has posed problems in the pea-growing regions of British Columbia (6). Fw race 5 is distinguished from races 1, 2 and 6 on the basis of host genotype interactions (10). These races also differ in vegetative compatibility (4) and molecular fingerprint profiles (9). New variants of the pathogen continue to emerge (2, 8), indicating that *F. oxysporum* f. sp. *pisi* is a dynamically evolving pathogen.

Breeding for resistance is an effective means of controlling Fw. Several mechanisms of Fw resistance have been proposed (9) including the formation of physical barriers in the xylem of resistant cultivars (3). The *Fwf* resistance gene confers complete and specific resistance to the race 5 isolate. *Fwf* segregates as a single, dominant trait (3) and has been assigned to Linkage Group II following an initial mapping of three morphological markers and four polymorphic isozymes (5). Here, we examine sixty random amplified polymorphic DNA (RAPD) primers for polymorphisms linked to *Fwf*. One of these, U693a, delineates a 5.6 cM interval adjacent to *Fwf*. Such a marker can be used to develop locus-specific PCR primers that will facilitate marker assisted selection.

Materials and Methods

Fifty-three F_7-derived RILs obtained from a cross of 74SN3B (*Fwf*) x A83-22-4(e)-A, resistance phenotyping, and initial mapping of *Fwf* were described previously (5). Bulk segregant analysis with pooled resistant and susceptible individuals (13) was used to identify RAPD polymorphic markers linked to *Fwf*. PCR amplifications were carried out according to Williams et al. (19). To generate locus-specific primers for U693a, the 450 bp U693a RAPD product was amplified from genomic DNA of 74SN3B, partitioned on an agarose gel, and purified using the Concert Rapid Gel Extraction System (Gibco/Invitrogen, Carlsbad, CA). The fragment was cloned into pCR4-TOPO vector (Invitrogen, Carlsbad, CA) and mobilized into chemically competent TOP10 *E. coli* cells (17). Plasmid DNA was purified using a QIAprep Spin Miniprep kit (Qiagen, Inc., Valencia, CA).

Sequencing of four cloned 450 bp inserts was performed by the Molecular Biology Core Sequencing Facility, School of Molecular Biosciences, Washington State University. Sequence characterized amplified region (SCAR) primers (14) were derived from the 5' and 3' ends of a consensus nucleotide sequence (GenBank accession no. pending) using PrimerSelect 5.0 (DNAStar, Inc., Madison, WI). The SCAR primers were: U693aSCF1, GACGGGGTTCAAGGTAGTG; U693aSCR1, GCCATAACCATTCTTCACG; U693aSCF2, GTTCAAGGTAGTGATAAGTGG; and U693aSCR2, GTAGTGCCATAACCATTCTTC. U693aSCF1 and U693aSCR1 were designed to amplify a 431 bp portion of the U693a fragment (nucleotide positions #6 to #437); primers U693aSCF2 and U693aSCR2 were nested within the first set (nucleotide positions #11 to #432) to generate a 421 bp product. SCAR PCR was performed for 35 cycles according to published protocols (14), using annealing temperatures of 55°C and 57°C for F1/R1, and 51°C and 54°C for F2/R2.

Linkage analysis was done using MAPMAKER/EXP 3.0, Whitehead Institute, Cambridge, MA (12).

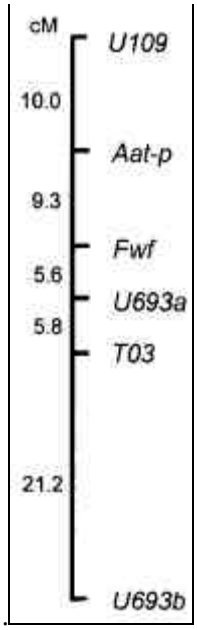

Fig. 1. Genetic map of the Fwf *region in* Pisum sativum.

Results and Discussion

A total of fourteen markers (5 morphological, 1 isozyme, and 9 RAPD) co-segregated with *Fwf* within a 123 cM interval (data not shown). The five most tightly linked (LOD = 4.0, maximum distance = 30.0) are shown in Fig. 1 and their sequences are given in Table 1. *Fwf* fell within a 14.9 cM interval between a locus for aspartate aminotransferase (*Aatp*; 5) and RAPD marker U693a, a 450 bp PCR product that segregated with *Fwf* (Fig. 2). At present, marker U693a is the most tightly linked to *Fwf*, at a map distance of 5.6 cM and about 8.5 cM closer than a previously identified marker (5). *Aatp* remains the nearest marker on the distal side of *Fwf*, at ~9 cM.

Table 1. PCR primers used to generate the *Fwf* linkage map.

Locus	Description	Primer	Product (bp)	Source
U109	**UBC RAPD**	**TGTACGTGAC**	**800**	**University of British Columbia**
U693a	**UBC RAPD**	**GACGAGACGG**	**450**	**University of British Columbia**
U693b	**UBC RAPD**	**GACGAGACGG**	**400**	**University of British Columbia**
T3_650	**RAPD**	**TCCACTCCTG**	**650**	**Operon, Inc., Alameda, CA**
V20_1100	**RAPD**	**CAGCATGGTC**	**1100**	**Operon, Inc., Alameda, CA**

To relate our linkage map to that of other *P. sativum* maps (16), we examined the segregation of two additional RAPD loci, T3_650 and V20_1100. The former marker was polymorphic in two of six populations reported by Rameau et al.; the latter marker segregated in five populations and was linked to *rms3*, a *ramosus* locus for apical dominance that is adjacent to *Aatp* in line K564. In our parental lines T3_650 was polymorphic and mapped 5.8 cM from U693a, distal to *Fwf* (Fig. 1). V20_1100 was not polymorphic in our mapping population (data not shown).

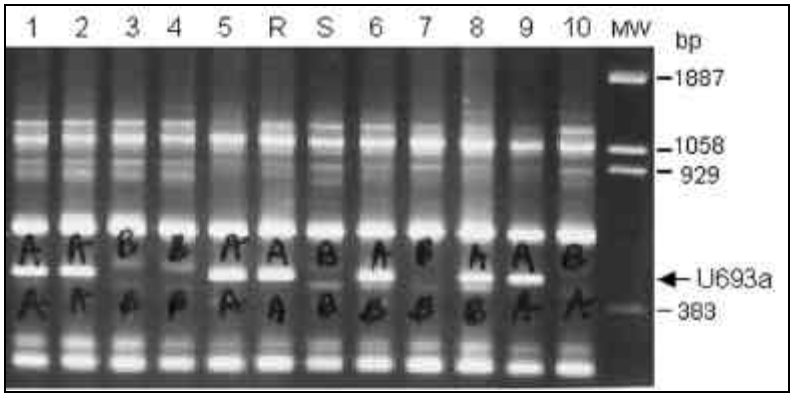

Fig. 2. Segregation of RAPD U693a (arrow) in resistant (R) 74SN3B and susceptible (S) A83-22-4(e)-A parental lines and a subset of recombinant inbred lines (1-10) from the mapping population.

The nucleotide sequence of the U693a RAPD clone encodes a partial 148-amino acid open reading frame having similarity (E value = $5e^{-28}$; 1) to polyproteins of *copia*-like retrotransposable elements. *Copia*-like elements are ubiquitous and occur in the genomes of legumes, including *Pisum* (15), *Cicer* (18) and *Cajanus cajan* L. (11). Specific sequences within these elements have been used to examine genetic diversity at the species level. It is not known whether the putative U693a *pol* sequence in 74SN3B represents a functional retrotransposon.

Both sets of U693a SCAR primers generated a ~400 bp fragment and conferred specific amplification of the U693a locus. However, the SCAR fragment was amplified from DNA of all the lines, including the

susceptible parent, at various annealing temperatures. It is unlikely that the primers were derived from a contaminating PCR product, because line 74SN3B used to generate the U693a clone showed no additional fragments within the ~400 bp size range (Fig. 2). We conclude that the polymorphism obtained with the RAPD-based method reflects the competition by which the decameric primers anneal to and amplify high-complexity target DNA.

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