Chromosomal location of *Fwf*, the Fusarium wilt race 5 resistance gene in *Pisum sativum*

Coyne, C.J., USDA-ARS Plant Introduction, Pullman, WA
Inglis, D.A., Research and Extension, Washington State University, Mount Vernon, WA
Whitehead, S.J., Elsevier Science, London, UK
McClendon, M.T. and Dept. of Horticulture, Washington State University, Pullman, WA
Muehlbauer, F.J. USDA-ARS, Washington State University, Pullman, WA

Introduction

Fusarium wilt, a serious and widespread vascular wilt disease of pea (*Pisum sativum* L.) which is caused by the fungus *Fusarium oxysporum* f.sp. *pisi* (C.J.J. Hall) Synder & Hansen (1). Fusarium wilt race 5 was identified only as a disease problem in pea in the in high rainfall regions west of the Cascade mountains of Washington, Oregon and British Columbia (6). Resistance to race 5 is conferred by a single dominant gene, *Fwf* (5). F_7:derived recombinant inbred lines (RILs) from a cross of the lines A83-22-4(e) and 74SN3 were used to map *Fwf*. A83-22-4(e) is a genetic stock line originally selected by Dr. N. F. Weeden to have the 'slow' allozyme in a number of isozyme systems. It is susceptible to race 5. 74SN3 (PI 608036) is a germplasm release line which is resistant to race 5 (8). The RILs were screened for disease reaction in the greenhouse by inoculating the plants with pure culture isolates of *Fusarium oxysporum* f.sp. *pisi* race 5. The plants were scored as resistant (i.e. alive) or susceptible (i.e. dead). The mapping population was also scored for five morphological traits and four polymorphic isozymes. The locus coding the plastid isozyme of aspartate aminotransferase (*Aat*p) was found to be linked to *Fwf* , at a distance of 9.1 cM. *Aat*p was previously mapped to pea linkage group II (15), recently assigned to pea chromosome 6 based on translocation breakpoints (7).

Materials and Methods

Fifty-eight RILs from the cross between 74SN3 (PI 608036)(resistant to race 5) and A83-22(e) (susceptible to race 5) were used as a mapping population. The parents 74SN3 and A83-22(e), the F_7-derived RILs, and seven differential cultivars (Little Marvel, Dark Skin Perfection, New Era, New Season, WSU 23, WSU 28, WSU 31) (5) were planted in the greenhouse. The differential cultivars were used to verify the isolate used in the inoculation was race 5 (5). Five plants were grown in 3" pots containing vermiculite in each of three replicate sets for each RIL and cultivar. The plants were inoculated when they reached 4 nodes with pure cultures of race 5 isolates from the Mount Vernon Research and Extension Unit, Mount Vernon, WA using the procedure of Wells et al. (19). The conidia concentration was adjusted to 1×10^6 cells per ml. An uninoculated plant from each replicate was included in the test to serve as a water-only healthy controls. The plants were scored as alive or dead 21 days after inoculation.

Data relating to three morphological traits were collected from all the RILs, the presence of anthocyanin production (*A*), round cotyledon (*R*). and yellow pods (*gp*). Young leaves were collected before flowering and ground in extraction buffer to score for the allozymes using starch gel electrophoresis (20). Polymorphism was found for four isozymes: aspartate aminotransferase (AAT) (EC 2.6.1.1), phosphoglucomutase (PGM) (EC 5.4.2.2), 6-phosphogluconate dehydrogenase (6PGD) (EC 1.1.1.44) and shikimate dehydrogenase (SKDH) (EC 1.1.1.25).

Linkage between morphological and isozyme markers and the disease score were calculated using MAPMAKER/EXP3.0 (9), with LOD score of 4.0, maximum recombination 3.0, $P<0.0001$, expressed in Kosambi mapping units.

Results

The resistance/susceptible segregation ratio of this mapping population did not significantly differ from 1:1 (28 resistant lines, 27 susceptible, 3 mixed reaction, $\chi^2=0.171$). Thus, we suggest that a single gene is responsible for resistance to race 5, as reported by Hagedorn (5). Data collected for two morphological traits and six polymorphic allozymes fit the expected 1:1 segregation indicating a normal segregation in this mapping population (Table 1). However, *Pgdc* is on the statistical edge of rejecting the hypothesis of a 1:1 ratio (Table 1).

Table 1. Observed values for F_7-derived recombinant inbred lines for morphological markers and polymorphic isozymes from the cross of 74SN3 × A83-22(e).

Locus	Linkage group	Fast allele	Slow allele	Hetero-zygous	χ^2	*P* value
***Aat*p**	**II**	**28**	**25**	**0**	**0.169**	**0.75 >*P*> 0.50**
***Aat*m**	**VII**	**22**	**26**	**0**	**0.333**	**0.50 >*P*> 0.25**
***Pgm*c**	**VII**	**24**	**21**	**2**	**0.200**	**0.75 >*P*> 0.50**
***Pgd*p**	**VII**	**25**	**28**	**0**	**0.169**	**0.75 >*P*> 0.50**
***Pgd*c**	**V**	**20**	**33**	**0**	**3.186**	**0.10 >*P*> 0.05**
Skdh	**VII**	**26**	**23**	**1**	**0.169**	**0.75 >*P*> 0.50**
		Dominant allele	**Recessive allele**			
A	**II**	**33**	**25**	**0**	**1.100**	
Gp	**V**	**24**	**34**	**0**	**1.100**	

[1] **Nomenclature from earlier *Pisum sativum* isozyme research publications (16, 17, 18).**

RIL polymorphisms in four isozymes (20) were used in the linkage analysis and for anchoring resistance markers to the consensus linkage map of pea (15). The typical polymorphism for AAT-2 (initially reported by Weeden and Marx [17]) was observed to segregate in the RILs (Fig. 1). Joint segregation analysis gave clear linkage between *Aatp* and *Fwf* (45 lines with parental genotypes, 8 lines with recombinent genotypes). MAPMAKER calculations gave a map distance between the two loci of 9.1 cM (Fig. 2). *Fwf* mapped 9.1 cM from *Aat*p (Fig. 2).

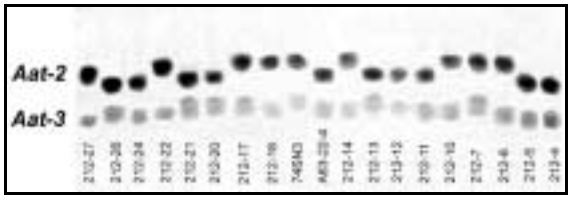

Fig. 1. Isozyme analysis on a starch gel of pea plastid isozyme aspartate aminotransferase of the RILs segregating for the fast and slow AAT-2 allozymes.

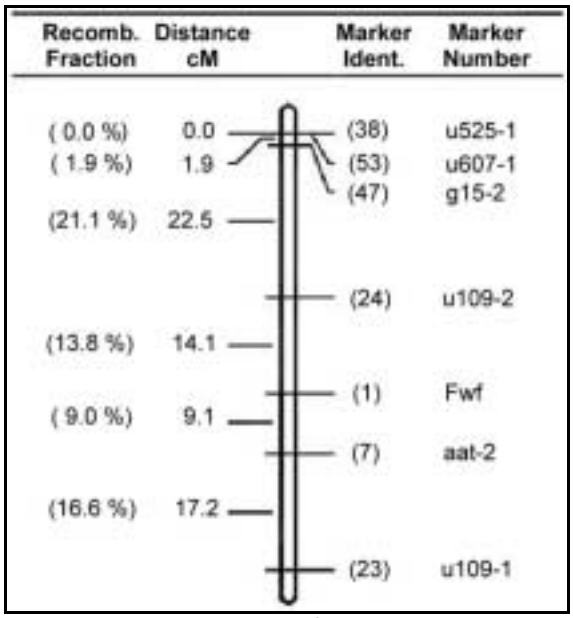

Fig. 2. Relative positions of isozyme locus Aatp *and* Fwf, *the locus responsible for resistance to Fusarium wilt race 5.*

Discussion

The morphologic and isozyme data facilitated localization the Fusarium wilt race 5 gene (*Fwf*) to pea linkage group II. A marker line A83-22-4(e) from the Dr. Gerald A. Marx genetic stock collection was successfully used to anchor *Fwf* to the current consensus map of pea (15). Localizing useful genes to a linkage group should speed the discovery of closely linked markers for use in marker assisted selection in pea breeding programs. Recently several increasingly marker-dense maps have been published which will be of great utility in the fine mapping of useful disease resistance alleles (3, 7, 10, 13, 14, 15).

Unlike some other monogenic plant resistance genes (11), *Fwf* is not clustered with the other mapped Fusarium wilt resistance gene, race 1 (*Fw*). *Fw* has been mapped to pea linkage group III in several crosses (2, 4, 12). Preliminary results of Shultz et al (12), indicated that Fusarium near wilt resistance gene (*Fnw*, race 2) is also not clustered with *Fwf*. The linkage relationship, if any, between *Fw* and *Fnw* is not yet known. Grajal-Martin and Muehlbauer (4) reported that the genes were inherited independently in three crosses.

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