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DNA Markers Linked to Fusarium Wilt Race 1 Resistance in Pea

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Abstract. **Dry pea (*Pisum sativum* L.) production in many areas of the world may be severely diminished by soil inhabiting pathogens such as *Fusarium oxysporum* f. sp. *pisi* race 1, the causal organism of fusarium wilt race 1. Our objective was to identify closely linked marker(s) to the fusarium wilt race 1 resistance gene (*Fw*) that could be used for marker assisted selection in applied pea breeding programs. Eighty recombinant inbred lines (RILs) from the cross of Green Arrow (resistant) and PI 179449 (susceptible) were developed through single-seed descent, and screened for disease reaction in race 1 infested field soil and the greenhouse using single-isolate inoculum. The RILs segregated 38 resistant and 42 susceptible fitting the expected 1:1 segregation ratio for a single dominant gene (χ^2 = 0.200). Bulk segregant analysis (BSA) was used to screen 64 amplified fragment length polymorphism (AFLP) primer pairs and previously mapped random amplified polymorphic DNA (RAPD) primers to identify candidate markers. Eight AFLP primer pairs and 15 RAPD primers were used to screen the RIL mapping population and generate a linkage map. One AFLP marker, ACG:CAT_222, was within 1.4 cM of the *Fw* gene. Two other markers, AFLP marker ACC:CTG_159 at 2.6 cM linked to the susceptible allele, and RAPD marker Y15_1050 at 4.6 cM linked to the resistant allele, were also identified. The probability of correctly identifying resistant lines to fusarium wilt race 1, with DNA marker ACG:CAT_222, is 96% percent. These markers will be useful for marker assisted breeding in applied pea breeding programs.**

Pea (*Pisum sativum*) is an important agricultural crop worldwide with major production centers in Europe, Asia, Canada, and the United States (FAO, 1994). Dry pea accounts for 22% of the world production of all pulses (Kelley, et al., 2000). Major growing areas for pea production in the U.S. include Wisconsin, Minnesota, New York, Washington, North Dakota, South Dakota, Montana, and northeastern Oregon (Gritton, 1986). Dry pea is grown in the Palouse region of eastern Washington and northern Idaho under dryland conditions and fulfills a vital role in this cereal-based agriculture system as a rotational crop due to its ability to break disease cycles, improve soil fertility by returning nitrogen to the soil, and enhance weed control (Muehlbauer and Tullu, 1997).

Fusarium oxysporum is distributed worldwide, however, the distribution of individual *formae specialis* is limited due to selective host preference. Dissemination occurs by wind, rain and mechanical means through movement of infested soil or seed. Symptoms of fusarium wilt include a downward turning of the leaves and stipules, with older leaves of an infected pea plant eventually becoming dry and brittle (Haglund and Kraft, 2001). The basal portion of an infected plant becomes brown and discolored before the apical portion due to the upward progression of the fungus in the vascular tissue (Haglund and Kraft, 2001). The fungus invades the root system of both susceptible and resistant cultivars, but the invasion into resistant cultivars is restricted through production of vascular plugs (CharChar and Kraft, 1989).

Four races of *Fusarium oxysporum* Schlecht. f. sp. *pisi* (*Fop*) (C.J.J. Hall) Snyder and Hansen (Brayford, 1996) are recognized based on differential pathogenicity on pea cultivars. Fusarium wilt race 1 was first encountered in Wisconsin in 1924 and later in other pea growing regions (Jones and Linford, 1925). Race 1 is now known to occur in pea production regions throughout the world (Haglund and Pepin, 1987; McPhee et al., 1999).

The fusarium wilt race 1 resistance locus to in pea is designated as *Fw* and has been mapped to linkage group (LG) III (Muehlbauer, personal communication; Weeden et al. 1998). Dirlewanger et al. (1994) reported an RFLP marker, p254, 6 (±1.9) cM from the *Fw* resistance locus, which is too far removed from the actual locus to be used effectively in marker assisted selection (MAS) (Stuber, 1992). AFLP markers are useful tools for mapping in pea, as was demonstrated by Tiwari et al. (1999) who identified a marker for the powdery mildew resistance gene, *er*-2. Bulk segregant analysis (BSA) has proven successful in quickly identifying candidate

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markers linked to a trait of interest (Michelmore et al., 1991).

In this paper we report two AFLP markers and one RAPD marker linked to *Fw* locus, the location of these markers and several others on LG III, and the potential use of these markers. The objective of this research was to combine AFLP and BSA technology to rapidly identify a marker(s) closely linked to *Fw* resistance for use in MAS.

Materials and Methods

Plant and soil materials. Eighty F_8-derived recombinant inbred lines (RILs) from the cross PI 179449 x Green Arrow (PI 614141) were developed using single-seed descent and used in all experiments except for field planting, summer 1999. All field evaluations and greenhouse experiments included seven standard pea wilt differentials. Little Marvel (W6 17515) is susceptible to races 1 and 2 (Haglund and Kraft, 2001). Darkskin Perfection (W6 17516) and WSU 23 (W6 17519) are resistant to race 1 and susceptible to race 2 (Haglund and Kraft, 2001). The remaining differentials, New Era (W6 17517), New Season (W6 17518), WSU 28 (W6 17520), and WSU 31 (W6 17521) are resistant to races 1 and 2 (Haglund and Kraft, 2001). All pea differentials are maintained by the USDA–ARS Western Regional Plant Introduction Station, Pullman, WA. The standard differentials were included in all experiments as controls to verify the sole presence of race 1 in the soil (Haglund and Kraft, 2001). Twelve inbred lines were obtained from a private breeding company. Palouse silt loam soil infested with the fungus, was obtained from the fusarium wilt nursery located at the Washington State University–Spillman Research Farm, Pullman, Wash., and used to assess disease reactions of the mapping population to *Fop* race 1.

Field evaluation. Field evaluations were done in the fusarium wilt nursery in 1999 and 2000 in order to select the RIL population segregating for *Fw* and verify the disease reactions of the selected RIL population in the greenhouse tests. The trial was established using an eight-row planter fitted with disk openers set at a depth of 5 cm. Each plot consisted of one row, 150 cm long, spaced 30 cm apart. The first and eighth rows were sown to a susceptible control, WA 788, to verify the presence and uniformity of the pathogen. The seed of nine parental lines of six RIL populations were sown on 20 Apr. 1999. The seed of Green Arrow, PI 179449, 80 RILs, and the seven pea wilt differentials were sown on 15 Apr. 2000. Fifty seeds were planted per entry for each evaluation; all seed was treated with Captan (Micro Flo Company LLC, Memphis, Tenn.) at a rate of 0.01 g/seed and sown as a single row in the nursery. The plots were scored 10 weeks later by determining whether plants were alive (resistant), dead (susceptible), or whether both live and dead plants were present (mixed).

Greenhouse experiment. Fifteen seed of Green Arrow and PI 179449 and the seven pea wilt differentials were used to verify the parental disease reaction and the presence of only race 1 in the soil before testing the entire RIL population. Scarified seed were placed on germination paper after soaking 10 min in a 1 bleach : 10 distilled water (by volume) solution followed by a 1-min rinse in distilled water. Treated seed was placed in a germination chamber without light at 20 ºC. After 4 d, the seedlings were planted in the greenhouse in 15-cm-diameter pots, and filled with soil from the fusarium wilt nursery. Each entry had three pots with five seedlings per pot; the pots were arranged in a completely randomized design with three replications. The air temperature and supplemental lighting were maintained at 22 to 24 ºC and 16 h, respectively. Starting two weeks after sowing, disease reactions (alive, dead or mixed reaction) were recorded for 5 weeks.

Twenty seeds each of the entire RIL population, the parents, and the pea differentials were then germinated as above. Fifteen of the most vigorous seedlings were planted and scored for disease reaction. The whole experiment was repeated a second time.

Single isolate inoculation. Six seed of each RIL, parent, and differential line were planted in trays with #2 vermiculite (Thermo-O-Rock, Chandler, Ariz.) at a depth of 4 cm and grown for three weeks in the greenhouse with a range of temperatures between 22 to 24 °C. Isolate F80 *Fop* race 1 was obtained from Dr. John Kraft, Prosser, Wash., and used to inoculate the RIL population. The inoculum was incubated at 22 °C in Kerr's liquid medium (Kerr, 1963) for 6 d on a rotary shaker at 70 rpm. Inoculum was adjusted to 1.1×10^5 spore/mL. The roots of 3-week-old pea seedlings were dipped into the spore suspension immediately after cutting off about one-third of the root mass with scissors. Inoculated seedlings were transplanted into individual 10-cm-diameter pots containing fresh vermiculite. Three of the six plants were inoculated with the spore suspension while the other three were inoculated with water and used as controls. The experiment was arranged in a randomized complete block design with three replications. Wilt reactions were recorded after 4 weeks as alive, dead, or mixed at the time Little Marvel bloomed (Inglis, 1996).

DNA extraction, AFLP and RAPD analyses. DNA was extracted from 1 g of young leaf material from each of the parents, RILs and 68 selected pea cultivars and advanced pea breeding lines (ABLs) using a modification of the Murray and Thompson (1980) DNA extraction method (Simon and Muehlbauer, 1997). DNA for bulk segregant analysis was prepared by bulking DNA samples from five wilt-susceptible RILs and five wilt-resistant RILs by combining equal DNA aliquots (Michelmore et al., 1991). DNA from five recombinant inbred lines, 7-08, 7-14, 7-22, 7-31, and 7-75, showing 100% survival, was combined to form the resistant bulk. Similarly, DNA from five susceptible RILs, 7-05, 7-27, 7-37, 7-49, and 7-62, showing 100% susceptibility, formed the susceptible bulk.

Amplified fragment length polymorphism procedures were conducted according to Vos et al. (1995) using preselective amplification with *Eco*RI primer (5'-GAC TGC GTA CCA ATT C-3') +A and *Mse*I primer (5'-GAT GAG TCC TGA GTA A-3') +C from AFLP Core Reagent and AFLP Starter kits (GibcoBRL, Gaithersburg, Md.). *Eco*RI infrared-labeled primers were visualized by the automated sequencer system (LI-COR, Lincoln, Neb.). Eight +3 primers from *Eco*RI: +AAC, +AAG, +ACA, +ACC, +ACG, +ACT, +AGC, +AGG; and, eight +3 primers from *Mse*I: +CAA, +CAC, +CAG, +CAT, +CTA, +CTC, +CTG, +CTT, were used. The resulting 64 AFLP primer pairs were used to screen the wilt-resistant and wilt-susceptible bulks in order to detect candidate markers. Only primer pairs showing consistent amplification across three RIL mapping populations were used for the RIL mapping (Coyne et al., 2000). The candidate markers were then used to collect AFLP genotypes for the entire mapping population.

Fifteen random amplified polymorphic DNA (RAPD) primers previously mapped to LG III in pea were used (Laucou et al., 1998; Pilet-Nayel et al., 2001). RAPD primers function by creating polymorphisms amplified by single primers of arbitrary nucleotide sequences (Williams et al., 1990). The RAPD primer sequences were obtained from Operon Technologies (California) and the University of British Columbia (Canada).

Table 1. Segregation of 80 RILs derived from Green Arrow x PI 179449 based on reaction to *Fop* race1; R = resistant, S = susceptible.

Experiment	R	S	Mixed	χ^2	*P*
Greenhouse experiment[z]	37, 38	42, 42	1, 0	0.316, 0.200	0.574, 0.655
Field evaluation[y]	40	40	0	0.000	1.00
Single isolate inoculation[x]	37	42	0	0.316	0.574

[z]Results from two tests are presented.
[y]Two RILs previously phenotyped as wilt-susceptible were wilt-resistant.
[x]One RIL did not germinate.

Marker designation. The AFLP markers were specified according to the three distinctive nucleotides used from *Eco*RI and *Mse*I primers and their base pair size. For example, the primer combination of *Eco*RI +3 ACG and *Mse*I +3 CAT produced a 222 base pair amplicon. The name of the marker was designated as ACG:CAT_222. RAPD markers from Operon Technologies were based on the name of the primer and the size of the fragment, Y15_1050. RAPD markers from the University of British Columbia were designated by the abbreviation UBC, serial number, and fragment size, UBC29_450, for example.

Statistical analysis and map construction. Chi-square analysis was done on the RIL mapping population using PROC FREQ of the SAS 6.12 program (SAS, 1996). The linkage map was constructed using Mapmaker/EXP3.0 commands Group, Order, Compare, and Try (Lincoln et al., 1992). Mapping parameters used were a LOD score of 4.00 and maximum recombination of 0.40 as the lower limit for accepting linkage between two markers. Recombination frequencies were converted to map distances in centimorgans (cM) using the mapping function of Kosambi (1944). Bayes Theorem of conditional probability was used to calculate the probability that DNA marker, ACG:CAT_222, would correctly identify the 68 advanced breeding lines and cultivars as resistant (Lowry, 2002). The conditional probability was calculated as $P(T|M) = [P(M|T)P(T)]/[P(M)$, where $P(M) = P(M|T)P(T) + P(M| - T)P(-T)$ where *P* is the probability, M is the marker, and T is the trait (Lowry, 2002).

Results

Disease evaluations. The field evaluations in 1999 and 2000 indicated that the Green Arrow and PI 179449 RIL population segregated for fusarium wilt race 1 resistance. Little Marvel and PI 179449 were highly wilt-susceptible while Green Arrow and the other six pea differentials were wilt-resistant. Tests of the field soil indicated that only race 1 of *Fop* was present and contained sufficient inoculum. PI 179449 and Little Marvel were susceptible, and Green Arrow, Darkskin Perfection, New Era, New Season, WSU 23, WSU 28, and WSU 31 were resistant in the field confirming that soils were infective for race 1. Survival of Darkskin Perfection and WSU 28 indicated the absence of *Fop* race 2 in the field evaluations and greenhouse experiments. In the field, the RILs segregated with 40 wilt-resistant and 40 RILs wilt-susceptible (Table 1). Two RILs, 7-11 and 7-70, previously characterized in the greenhouse experiments as wilt-susceptible were wilt-resistant.

The first greenhouse experiment indicated 37 RILs as wilt-resistant, 42 RILs as wilt-susceptible and one RIL as undetermined. Recombinant inbred line 7-59, had a mixed wilt-reaction in the first greenhouse test, but was wilt-susceptible in all other experiments. The segregation was not significantly different from the expected 1:1 for a single gene ($\chi^2 = 0.316$, $P = 0.57$; Table 1). Results from the second greenhouse experiment indicated that 38 RILs were resistant and 42 susceptible with a chi-square value of 0.200 ($P = 0.65$) also indicating a single gene. The single-isolate inoculation in the greenhouse verified the disease reaction scores with 37 wilt-resistant and 42 wilt-susceptible RILs. Recombinant inbred line 7-16 (wilt-resistant in previous experiments) did not germinate. Segregation fit a 1:1 ratio ($\chi^2 = 0.316$, $P = 0.57$).

AFLP and RAPD markers linked to *Fw*. In total, 64 AFLP primer pair combinations detected 45 candidate markers using BSA; 22 were associated with resistance and 23 were associated with susceptibility. The RIL mapping population was screened with eight AFLP primer pairs, generating 37 polymorphic AFLP markers. Eleven AFLP markers mapped near *Fw* on LG III; six present in the wilt-resistant parent, Green Arrow, and five present in the wilt-susceptible parent, PI 179449. Fifteen RAPD primers were screened on the RIL mapping population including six RAPD primers previously mapped to LG III (Table 2). Eight RAPD markers and eleven AFLP markers mapped to the linkage group with *Fw* as shown in Fig. 1. Six RAPD markers previously mapped to linkage group III in pea anchored the *Fw* linkage group also shown in Fig. 1.

The RIL mapping population screened with DNA marker ACG:CAT_222 was present in 37 wilt-resistant RILs. One wilt-resistant RIL, did not generate this marker. Forty-two wilt-susceptible RILs lacked this marker including one recombinant RIL that did generate this marker and one missing data. The AFLP marker ACG:CAT_222 was linked to *Fw* at 1.4 cM. Fig. 2 illustrates a partial profile of fragment ACG:CAT_222. Another AFLP marker, present in wilt-susceptible PI 179449, ACC:CTG_159 flanked the other side of *Fw* at 2.6 cM as shown in Fig. 1. This flanking AFLP marker, identified 38 wilt-resistant, 40 wilt-susceptible, and two RILs with missing data. The DNA

Table 2. RAPD primers previously mapped to LG III in pea.

RAPD Primer	Sequence	Fragment bp	Reference
UBC 29	CCG GCC TTA C	450	Pilet-Nayel et al., 2001
UBC 30	CCG GCC TTA G	900	Pilet-Nayel et al., 2001
E16	GGT GAC TGT G	550	Laucou et al., 1998
J12	GTC CCG TGG T	1280	Laucou et al., 1998
R12	ACA GGT GCG T	320	Laucou et al., 1998
Y15	AGT CGC CCT T	1050	Laucou et al., 1998

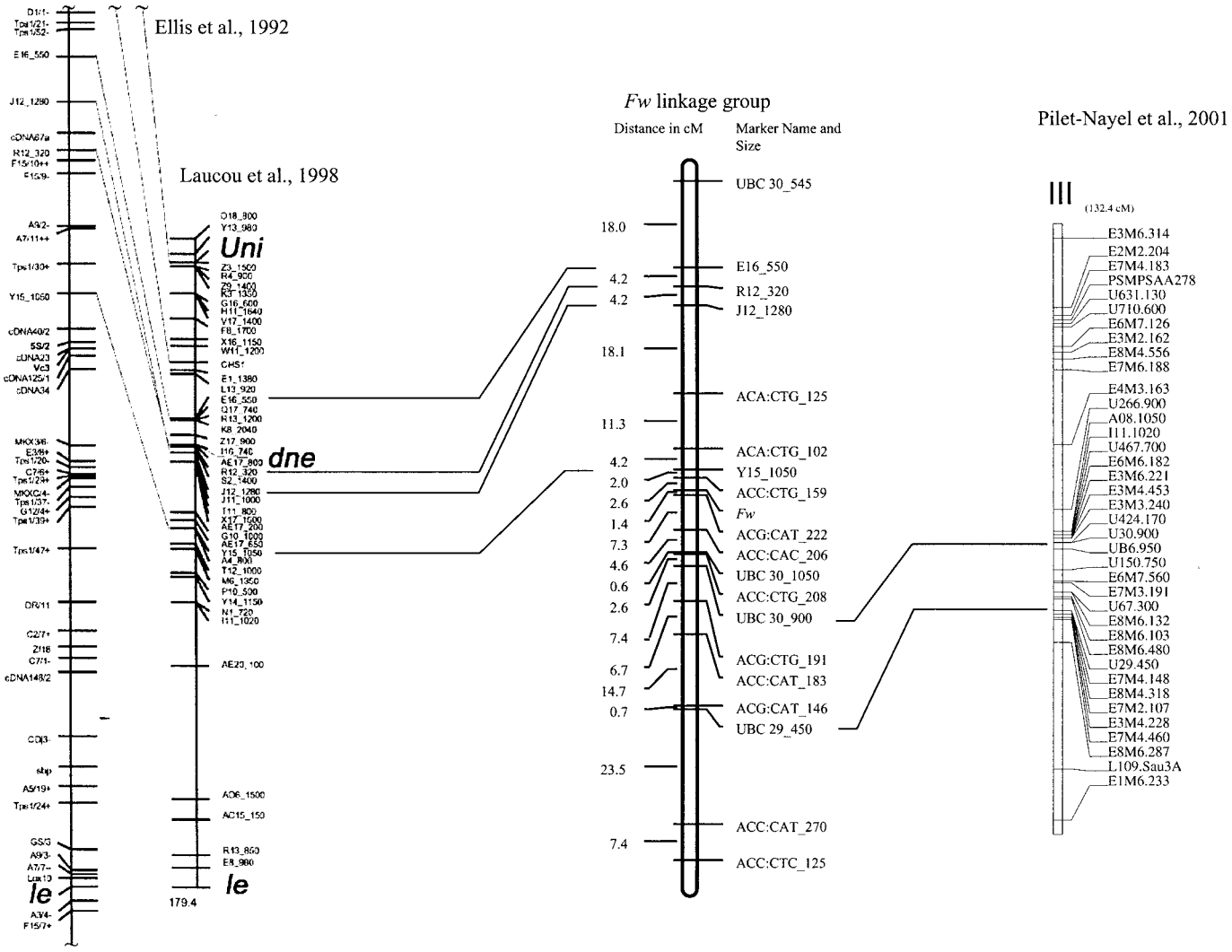

Fig. 1 *Fw* race 1 linkage group (middle) compared with the Ellis et al., 1992 (top left), Laucou et al., 1998 (bottom left) and Pilet-Nayel et al., 2001 (right) maps of linkage group III of pea. Note in the linkage map of AFLP and RAPD markers linked to the gene for race 1 resistance (*Fw*) in pea (middle), the name and size of the markers and distance is included. AFLP marker ACG:CAT_222 is 1.4 cM from *Fw*. AFLP marker ACC:CTG_159 at 2.6 cM and RAPD marker Y15_1050 at 4.6 cM both flank the other side of *Fw*.

marker was absent in one of the resistant RILs but present in three of the susceptible RILs. A RAPD marker, Y15_1050, linked to the resistant allele Green Arrow, also flanked the *Fw* gene at a distance of 4.6 cM (Fig. 3). This marker correctly identified 34 wilt-resistant RILs, 36 wilt-susceptible RILs, 7 recombinant RILs and 3 RILs with missing data. Twelve breeding lines from a private company were tested and correctly identified as resistant or susceptible using Y15_1050 (data not shown).

Of 68 ABLs and cultivars, 87% were accurately identified with AFLP marker ACG:CAT_222 (Table 3) as either resistant or susceptible. Of the wilt-resistant lines, PS710048, PS810489, PS810694, 'Joel', 'Scuba', 'Shawnee' and 'Umatilla' lacked the

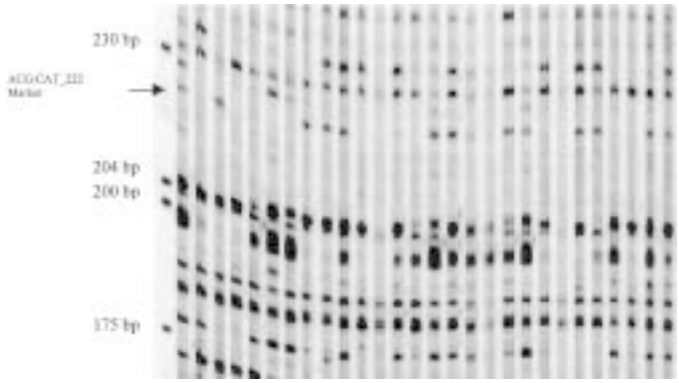

Fig. 3 Agarose gel of RAPD DNA marker Y15_1050 (1050 b0 marker) linked to LG III in pea at 4.6 cM from *Fw*, and the corresponding phenotype. The first lane (far left) is the size standard, the second lane is Green Arrow, and the third lane is PI 179449. The rest of the lanes (left to right) are RILs 7-01 to 7-21. [z]Green Arrow, [y]PI 179449, [x]R = resistant and S = susceptible to *Fusarium oxysporum* f.sp. *pisi* race 1.

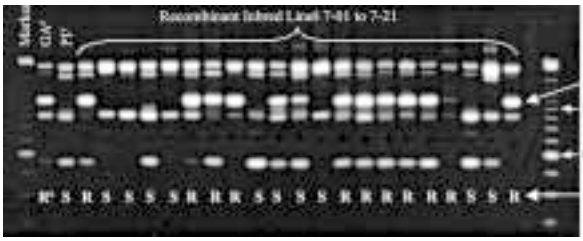

Fig. 2 Polyacrylamide gel showing the segregation of AFLP DNA marker ACG:CAT_222. The first lane (far left) is the size standard, the second lane is Green Arrow, and the third lane is PI 179449. The rest of the lanes (left to right) are RILs 7-01 to 7-22.

Table 3. Disease reaction and corresponding genotype for AFLP marker, ACG:CAT_222, for 68 advanced breeding lines and cultivars of pea grown in the fusarium wilt nursery, Spillman Farm, Pullman, WA.

Entry[z]	2000 Field evaluation	Marker	Cultivar	2000 Field evaluation	Marker
Alaska-81	Resistant	+	PS7101126	Susceptible	–
Astina	Resistant	+	PS710137	Resistant	+
Atomic	Susceptible	–	PS710255	Resistant	+
Big Daddy	Resistant	+	PS710263	Resistant	+
Carneval	Susceptible	–	PS710308	Resistant	+
CEB1170	Resistant	+	PS710416	Resistant	+
CEB1477	Resistant	+	PS710975	Resistant	+
Columbian	Resistant	+	PS810060	Resistant	+
Conso	Susceptible	–	PS810062	Resistant	+
Eiffel	Susceptible	–	PS810162	Resistant	+
Espace	Resistant	+	PS810191	Resistant	+
Fallon	Resistant	+	PS810208	Susceptible	–
Guido	Resistant	+	PS810240	Resistant	+
Joel	Resistant	–	PS810323	Resistant	+
Karita	Susceptible	+	PS810831	Resistant	+
Majoret	Susceptible	–	PS810416	Resistant	+
Maro	Resistant	+	PS810446	Resistant	+
Neuga-af	Susceptible	–	PS810466	Resistant	+
Phantom	Resistant	+	PS810489	Resistant	–
Rambo	Resistant	+	PS810513	Resistant	+
Scuba	Resistant	–	PS810514	Resistant	+
Shawnee	Resistant	–	PS810639	Resistant	+
Supra	Resistant	+	PS810694	Resistant	–
Toledo	Resistant	+	PS810765	Resistant	+
Umatilla	Resistant	–	PS810774	Susceptible	–
PS510718	Resistant	+	PS810799	Resistant	+
PS510737	Resistant	+	PS810816	Resistant	+
PS610150	Resistant	+	PS810823	Resistant	+
PS610152	Resistant	+	PS810838	Resistant	+
PS610324	Resistant	+	PS9101365	Resistant	+
PS710048	Resistant	–	PS9101368	Resistant	+
PS7101044	Resistant	+	PS9101380	Resistant	+
PS7101047	Resistant	+	PS9101921	Susceptible	+
PS7101123	Resistant	+	PS9102067	Resistant	+

[z]Advanced breeding lines and cultivars.

expected DNA band. 'Karita' and PS9101921, wilt-susceptible lines, had the DNA band present. The probability that DNA marker ACG:CAT_222 would correctly identify resistant lines was calculated as 96%.

Discussion

Greenhouse and field experiments are time consuming. In temperate environments, the opportunity to screen for disease reaction in the field occurs once per year, and may yield confounding results due to environmental influences. Field evaluations also require ≈10 weeks before the phenotype is determined. Field evaluations sometimes result in confounded data due to variable inoculum density, pathogen virulence, and the presence of other pathogens. For example, in this study two RILs consistently susceptible in the repeated greenhouse studies were scored resistant in the field study. A gradient of inoculum density across the field site may explain the contrasting phenotypes (data not shown).

The greenhouse experiments were conducted under more controlled conditions than the field evaluations in that the air and soil temperature and moisture were controlled. However, in the soil obtained from the fusarium wilt nursery used in the first greenhouse experiment, other soil pathogens and microorganisms were also present (data not shown). The results from the single isolate inoculation under greenhouse conditions were definitive and precise, but by using only one isolate of the pathogen virulence may have been compromised. Greenhouse disease evaluations take seven weeks and can be conducted year-round. However, greenhouse space is usually a limited resource and an adequate supply of naturally and uniformly infested field soil and/or a virulent isolate of *Fop* race 1 is necessary. Additionally, *Fusarium oxysporum* isolates stored in vitro lose virulence over time, and must be constantly maintained (Nelson et al., 1983). Marker assisted selection replaces lengthy (7 to 10 weeks) field or greenhouse experiments with a 2-d laboratory experiment.

Three DNA markers (2 AFLP, 1 RAPD) identified in this study were linked to fusarium wilt race 1 resistant gene, *Fw*. Two markers were linked to the resistant allele from Green Arrow, and the third marker was linked to the susceptible allele from PI 179449. Bulk segregant analysis of the AFLP primer pairs was an efficient procedure to rapidly identify candidate markers for screening the RIL mapping population. Previous mapping of AFLP markers in other mapping populations of pea were consistent in our mapping population for race 1 (Coyne et al., 2000;

Pilet-Nayel et al., 2001). The closest markers to *Fw* were AFLP marker ACG:CAT_222 and flanking AFLP marker ACC:CTG_159. These markers, while identified using a wild-type accession, were useful in identifying resistant inbred lines in our applied breeding program. The third marker, RAPD Y15_1050, previously mapped to LG III in pea (Laucou et al., 1998), anchored this marker to the pea consensus map of Weeden et al. (1998). This marker, while farther from *Fw* than the AFLP markers, was also useful in correctly identifying resistant fusarium wilt race 1 breeding lines.

Marker assisted selection improves the speed and accuracy of selecting resistant lines to fusarium wilt race 1 compared to phenotype expression experiments. The availability of markers linked to resistance to *Fw* reduces the laborious processes of traditional field or greenhouse disease screening for evaluating advanced breeding lines of pea. Genotypes can be detected easily and early in a single-day experiment using MAS, instead of several weeks required for a phenotypic assessment. Genotypes can be identified early, in the seedling stage, at 1 to 2 weeks of growth. Additionally, MAS can be conducted year round eliminating inferior genotypes from the next cycle of selection, thereby increasing efficiency.

Marker assisted selection reduces the confounding effects of environmental influences and thereby increases breeding efficiency (Lee, 1995). However, the markers identified in this study are limited to inbred lines of crosses that have the markers present in the resistant parent, and to breeding programs that have laboratories with AFLP technology. The usefulness of these race 1 markers will increase if they can be converted to codominant sequenced-characterized amplified regions (SCARs) and combined in MAS with other markers linked to economic traits of interest in pea. The conversion of the two AFLP markers and the RAPD marker reported here to SCARs would increase their utility during the early segregating generations of a breeding program and probably across a broader range of crosses (Paran and Michelmore, 1992).

Marker assisted selection for a single gene trait in a short-cycle annual crop such as pea may not be justified in terms of resource allocation in a breeding program (Lee, 1995). However, the combination of a race 1 marker with the numerous markers for agronomically important traits that have been published for pea, may justify the application of MAS. Many other DNA markers are available for MAS in pea breeding programs. A few examples of published markers linked to disease resistant traits in pea include pea seedborne mosaic virus (Timmerman et al. 1993), powdery mildew (Timmerman et al. 1994; Tiwari et al. 1999), pea common mosaic virus and *Ascochyta pisi* race C (Dirlewanger et al. 1994), pea enation mosaic virus (Yu et al. 1995), and Aphanomyces root rot (Pilet-Nayel et al. 2001).

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