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Identification of an AFLP molecular marker for oat stem rust resistance in the diploid *Avena strigosa*

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An AFLP marker associated with resistance to NA67 was identified

Introduction

Oat stem rust (*Puccinia graminis* Pers. f. sp. *avenae* Eriks. and E. Henn) is an important disease (Figure 1) causing significant losses in Canada (Martens, 1978). The disease can be effectively controlled with host genetic resistance. Gold Steinberg et al. (2005) identified resistance in the diploid species *Avena strigosa* Schreb. to race TJJ (NA67), the highly virulent and prevalent oat stem rust race in western Canada (Fetch, 2005). Molecular markers associated with resistance alleles can be identified by several DNA fingerprinting techniques. Amplified Fragment Length Polymorphism (AFLP), which is based on polymerase chain reaction (PCR) amplification of restriction fragments utilizing genomic DNA (Vos et al. 1995) is the technique used in this study. The objective of this study was to identify molecular marker(s) associated with the resistance in *A. strigosa* to race TJJ (NA67) using AFLP.

Figure 1. *Avena strigosa* accession (CN56979) susceptible to stem rust race TJJ (NA67)

Materials and methods

Two *A. strigosa* accessions (CN22000 and CN57130) resistant to race TJJ (NA67) were crossed to a susceptible accession (CN56979). F_2 populations were produced (151 and 152 plants, respectively) from two F_1 plants in each cross. F_3 families were produced for each cross. F_1, F_2 and F_3 seedlings were tested with race TJJ (NA67) at the first leaf stage and disease reactions were rated 14 days after inoculation using a 0–4 rating scale (Stakman et al. 1962). Leaf tissue samples were collected from F_2 plants and genomic DNA was extracted using Qiagen DNeasy 96 plant Kit (Qiagen Inc., Mississauga, Ontario, Canada). The AFLP technique, described by Vos et al. (1995), was used to identify molecular markers.

Screening for candidate markers was performed by bulked segregant analysis (BSA) (Michelmore et al. 1991), with 256 primer combinations using 10 resistant and 10 susceptible F_2 plants. Mapping F_2 populations (24 resistant, 46 segregating, and 24 susceptible, based on reactions to race TJJ [NA67] at F_3) for each cross, including the parents, were tested with candidate markers. PCR products were resolved by capillary electrophoresis using an ABI 3100 (3130 Genetic Analyser, AB Applied Biosystems - Hitachi) and Genographer software (version 1.6). A genetic map was produced using JoinMap (version 3.0).

Results

Polymorphism was observed between the resistant and susceptible parents as well as between the resistant and susceptible bulks. For the CN56979/CN57130 population, BSA analysis showed the presence of a band at 370-bp for the resistant parent (CN57130), the resistant bulk, and for nine of the ten resistant F_2 individuals (Figure 2). The susceptible parent (CN56979), the susceptible bulk, and nine of the ten susceptible F_2 individuals were missing the band. The genetic map produced for CN56979/CN57130 F_2 population displayed 13 AFLP markers flanking the resistance gene (*Sr_57130*). The AFLP markers *P-acg/M-cga370* and *P-aag/M-ctc140* flanked *Sr_57130* at 9cM and 25 cM, respectively (Figure 3).

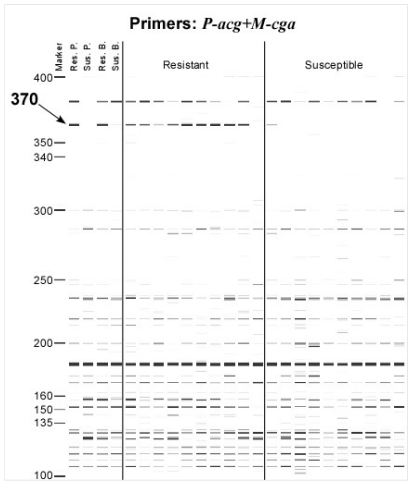

Figure 2. ABI gel analysis for resistant and susceptible F_2 individuals from CN56979/CN57130 tested with primers *P-acg+M-cga*. Lane one is the ladder, lane 2 - resistant parent, lane 3 - susceptible parent, lane 4 - resistant bulk, lane 5 - susceptible bulk, lanes 6 to 15 - 10 resistant F_2 individuals, and lanes 16 to 25 - 10 susceptible F_2 individuals.

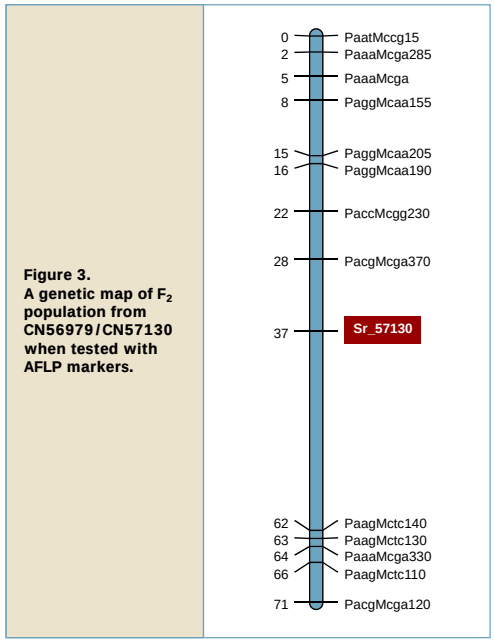

Figure 3. A genetic map of F_2 population from CN56979/CN57130 when tested with AFLP markers.

Conclusion

The result of this study shows that the AFLP marker (*P-acg/M-cga370*) is associated with the resistance allele in CN56797/CN57130 population. However, since there are gaps between the markers and also there is a lack of closely associated markers flanking the resistance gene, the genetic map needs to be more saturated to identify additional closely linked markers. No useful marker was identified for CN56979/CN22000 population.

References

Fetch, T. 2005. Can J. Plant Pathol. 27:572-580.
Gold Steinberg, et al. 2005. Plant Disease. 89:521-525.
Martens, J. W. 1978. Can. Plant Dis. Surv. 58: 51-52.
Michelmore, et al. 1991. Poc. Natl. Acad. Sci. USA. 88:9828-9832.
Stakman, et al. 1962. USDA-ARS publ. E617.
Vos, et al. 1995. Nucleic Acids Res. 23(21):4407-4414.

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