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Identification of a RAPD marker linked to the oat stem rust gene *Pg3*

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Summary. The feasibility of identifying molecular markers linked to disease resistance genes in oats was investigated utilizing random primers in conjunction with polymerase chain reaction technology. A pair of near-isogenic oat lines were screened for polymorphic DNA fragments linked to the stem rust resistance gene *Pg3*. Two primers were identified which amplified DNA fragments that were polymorphic between the lines analyzed. One primer (ACOpR-2) was shown to be completely linked to the *Pg3* locus; the other primer was not linked to either the ACOpR-2 or the *Pg3* loci. This type of analysis, combined with rapid leaf disc DNA extraction techniques, offers an effective means of identifying useful molecular markers and of applying them to plant breeding selection strategies.

Key words: Polymerase chain reaction – Disease resistance loci – Oat DNA – RAPD primers – Rapid DNA extraction

Introduction

Identification of sources of both stem rust (*Puccinia graminis* Pers. f. *avenae* Eriks and E. Henn.) and crown rust (*Puccinia coronata* Cda f. sp. *avenae* Eriks.) resistance genes in oats has been a major challenge, and one well met by a number of researchers over the past several decades (Harder et al. 1977; Martens et al. 1980). The introduction of more than one novel resistance gene into new cultivars will enhance the durability of each single gene, but the time constraints imposed by phenotypic scoring of individual rust race reactions on breeding lines and the pressure of producer demand for resistant cultivars have resulted in most new cultivars possessing only single novel resistance genes. The phenotypic screening of rust reactions can be further confounded by the cross-reactivity of single resistance genes to several rust races. In some cases differentials of rust races simply do not exist that would allow the differentiation of resistance genes. This can be a significant problem for plant breeders attempting to introgress two or more resistance genes into one cultivar.

Molecular markers which exhibit a linkage to a rust resistance gene would offer a solution to this problem. By exploiting a linkage between a molecular marker (which may have no functional significance on its own) and a rust resistance allele, we would be able to predict the presence or absence of the allele without having to rely on the expressed product of the allele by way of disease testing. Several storage protein loci have already been utilized in this manner in wheat (Dyck et al. 1987; Poperelya and Babayants 1978; Howes 1986; Dhaliwal et al. 1988) and in oats (Howes et al. 1992). Storage protein and isozyme loci are, however, limited to a few sites within the oat genome, thereby limiting their application to the subset of rust resistance genes that reside in their immediate neighborhood. The essentially unlimited number of restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers which can be generated allow an assay of the entire genome. RFLP analysis of near-isogenic lines of tomato has led to the identification of molecular markers linked to disease resistance genes (Young et al. 1988; Sarfatti et al. 1989). However, the application of RFLP

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marker technology is time consuming, labor intensive and expensive both to develop and to apply.

Williams et al. (1990) have developed a novel analytical approach that utilizes the polymerase chain reaction (PCR) and random primers to amplify random genomic sequences (RAPD analysis). Recently, Martin et al. (1991) and Paran et al. (1991) have demonstrated that RAPD analysis of near-isogenic tomato and lettuce lines, respectively, provides a rapid alternative to RFLP analysis as a means of identifying markers for disease resistance loci. The purpose of the study presented here was to determine whether RAPD analysis could be successfully extended to the development of molecular markers for a disease resistance locus in a cereal species such as oats where the genome is much larger and the proportion of repetitive DNA much higher than in the tomato genome. One marker, very closely linked to the oat stem rust resistance *Pg3* locus, was identified in this study.

Materials and methods

A pair of near-isogenic oat lines, Rodney O (Rodney*[5]/Exeter) and Rodney *Pg3* (Rodney O//Rodney*[6]/Jostrain) (referred to hereafter as RO and *Pg3*, respectively), which differed by the absence or presence of the stem rust resistance allele *Pg3*, were screened for random amplified polymorphic (RAPD) fragments. The *Pg3* allele has already been shown to be tightly linked (in coupling) to an unidentified crown rust resistance gene (McKenzie et al. 1968). The segregation of polymorphisms was analyzed on an F_2 population of 93 individuals derived from a cross between the two near-isogenic lines with Rodney O as the female parent. Genomic DNA was extracted from leaf discs by the rapid extraction method of Edwards et al. (1991) and resuspended in 100 µl TE. The F_2 plants were then raised to maturity for progeny (F_3) testing for stem and crown rust resistance.

All RAPD primers were obtained from Dr. J. Carlson (University of British Columbia, Vancouver, Canada). All primers were 10 mers of random sequence with no palindromes and a G/C content of between 50% and 80%. The 204 primers used in this study had all been previously identified as successfully amplifying discrete DNA fragments in different oat cultivars (data not published). PCR reactions were performed in a manner similar to that of Williams et al. (1990). All reaction volumes were 25 µl overlayered with 12.5 µl of light mineral oil (Fisher). Each reaction consisted of 1 × Promega Biotech Taq activity buffer, 0.625 U Taq polymerase (Promega Biotech), 200 µ*M* total deoxyribonucleoside-5′-triphosphates (dNTPs) (50 µ*M* each), 200 µ*M* primer (1 µl from a 5 p*M*/µl stock solution), and 1 µl genomic DNA. Primer concentration was calculated on the basis of base composition. Only one primer and one genomic DNA sample were added to any single reaction.

A total of 45 PCR cycles were performed, with each cycle consisting of a 1-min 94 °C denaturation segment, a 1-min 34 °C annealing segment, and a 2-min 72 °C extension segment. Following the final cycle, all transcripts were completed with a 10-min 72 °C segment followed by storage at 4 °C. If the samples could not be electrophoresed within 12 h of the run completion they were stored at − 20 °C. Electrophoresis was performed in 1.3% agarose with a TRIS/acetate/EDTA (TAE) buffer for 3 h at 70 V (constant voltage). Ethidium bromide-stained gels were visualized on an ultraviolet light transmitting transilluminator.

To determine the genetic association of polymorphic DNA fragments with rust resistance, 80 F_3 families (described above) were tested for segregation to stem rust and crown rust resistance. Approximately 25 seedlings from each F_3 family (i.e., 80 × 25 plants) were inoculated with stem rust race NA3 at the one leaf-stage, and 6 days later (two-leaf stage) with crown rust isolate (CR181) (Agriculture Canada, Winnipeg, accession number). Infection types were scored 14 days after each inoculation. Plants homozygous for the presence of the gene *Pg3* gave a resistant infection type 1–2 +, whereas heterozygous (RO/Pg3) plants displayed a resistant, mesothetic × infection type NA3. All of the resistant plants, including the heterozygous ones, gave a resistant; or; 1 type reaction to CR181. Homozygous susceptible (RO/RO) plants gave an infection type 4 response to both rust isolates. Linkage evaluation was based on the 78 datapoints in common between the two analyses (rust scoring and RAPD priming).

Results

We tested 204 random primers on the pair of near-isogenic lines Rodney O and Rodney Pg3 (RO and Pg3). Of these primers 161 amplified DNA in both

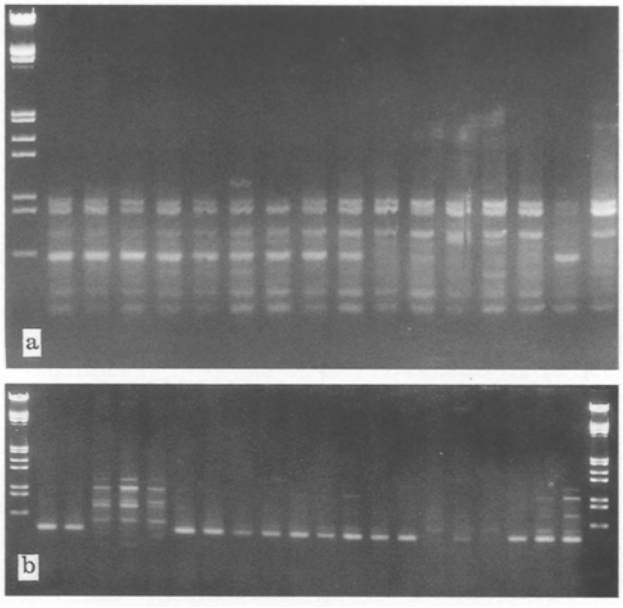

Fig. 1a, b. Amplification of genomic DNA from the near-isogenic lines Rodney O and Rodney Pg3, and several segregants with random primers ACOpR-1 and ACOpR-2. **a** ACOpR-1-primed products. *Lane 1* molecular weight markers [*Hin*dIII- and *Eco*RI-digested DNA (in order of decreasing mobility: 564 bp, 831 bp, 947 bp, 1375 bp, 1584 bp, 1904 bp, 2027 bp, 3530 bp)], *lanes 2–14* different Rodney O × Rodney Pg3 F_2 segregants, *lane 15* Rodney O, *lane 16* Rodney Pg3. **b** ACOpR-2 primed product. *Lanes 1* and *22* molecular weight markers (as above), *lanes 2* and *3* separate DNA extractions from Rodney O, *lanes 4* and *5* separate DNA extractions from Rodney Pg3, *lanes 6–21 different Rodney O* × Rodney Pg3 F_2 segregants

Table 1. Segregation of random amplified polymorphisms on F_2 segregants from the cross Rodney O × Rodney Pg3

Primer	Number of F_2 plants			χ^2
	RO/RO	RO/Pg3	Pg3/Pg3	
ACOpR-1	29	40	23	2.348[a]
ACOpR-2	77	–	15	3.710[b]

[a] Based on expected ratio of 1:2:1 ($P > 0.30$, < 0.50)
[b] Based on expected ratio of 3:1 ($P > 0.5$, < 0.10)

Table 2. Co-segregation of rust reaction to NA3 and CR181 of F_3 families derived from the cross Rodney O × Rodney Pg3, with polymorphisms exhibited by F_2 individuals with the RAPD primer ACOpR-2

DNA pattern	Number of F_3 families		
	Homozygous resistant	Heterozygous resistant	Homozygous susceptible
Pg3/Pg3	15	0	0
RO/-	0	43	20
No data	0	1	1
Total[a]	15	44	21

[a] χ^2 (1:2:1) = 1.7 ($P > 0.3$, < 0.5)

plant lines. Of these, two primers, designated ACOpR-1 and ACOpR-2, amplified DNA fragments that differed between the two near-isogenic lines (Fig. 1a, b). These primers were then used to amplify DNA derived from F_2 segregants resulting from a cross between the two near-isogenic lines. The primer ACOpR-1 yielded a pattern that was the additive result of the two parental patterns on some segregants (Fig. 1a, lanes 2–10, and 12), while the primer ACOpR-2 did not (Fig. 1b). The higher molecular weight bands evident in lanes 4, 5, 6, 13, 20, and 21 were not consistently reproducible; moreover, they were also amplified from the parent RO genomic DNA in other replications. The 437-bp fragment evident in lanes 2 and 3 was consistently amplified when RO genomic DNA was used as a template and was consistenly not amplified when Pg3 genomic DNA was used as a template. The lack of a discernible additive pattern was interpreted to mean that the amplification of the 437-bp fragment was expressed in a dominant fashion for the RO genotype; that is, both RO/RO and RO/Pg3 genotypes yielded RO patterns, while Pg3/Pg3 genotypes yielded a Pg3 pattern. Both of the amplified polymorphisms described simple single locus segregation ratios (Table 1). Linkage analysis conclusively demonstrated that these two polymorphisms were not linked to each other.

Rust reaction data on F_3 families derived from the F_2 plants analyzed with the RAPD primers also exhibited a good fit to a single locus Mendialian segregation pattern ($\chi^2 = 1.7$; Table 2). In addition, there was an absolute correlation between the segregation pattern exhibited by primer ACOpR-2 and the rust race reaction data (Table 2).

Discussion

The linkage reported herein is, to our knowledge, the first reported linkage between an agronomically useful trait and a RAPD marker in *Avena*. On average, the primers analyzed in this study amplified approximately four fragments each. In total, we analyzed approximately 644 different amplified fragments. The detection of only two polymorphic fragments represents a variation of only 0.3% between these two near-isogenic lines. Based on theoretical calculations we would have expected the two lines to move to within 98.5% of total homozygosity. In other studies, we have demonstrated RAPD detectable polymorphisms of approximately 30% between *Avena sativa* cultivars. This means that we should have expected 0.45% polymorphism between these two near-isogenic lines (1.5% residual heterozygosity × 30% polymorphism frequency). Therefore, the low level of observed polymorphism is well within theoretical expectations.

We have shown that RAPD analysis coupled with rapid DNA extraction technology can be effectively utilized to generate molecular markers for traits in a crop species with a very large genome and a high percentage content of repetitive DNA. In contrast, RFLP analysis in such crops is technically more difficult to perform consistently and is relatively costly. The ability, with RAPD analysis, to rapidly and cost effectively screen hundreds of seedlings moves molecular marker technology into a plant breeder's arsenal of feasible selection tools.

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