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Plant Gene Register

Molecular Characterization of Disease-Resistance Response Gene *DRR206-d* from *Pisum sativum* (L.)[1]

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The nonhost DRR in plants is associated with the rapid production of a number of proteins, many of which are likely to be involved in preventing a successful pathogen attack. We have previously described the isolation of a number of cDNA clones representing DRR transcripts that rapidly accumulate after inoculation of pea (*Pisum sativum* [L.]) pod tissue with spores of the bean pathogen *Fusarium solani* f. sp. *phaseoli* (Riggelman et al., 1985). Expression of one of these clones, pI206 (DRR206-a), was shown by northern analysis to be induced to high, sustained levels very early in the successful resistance response to *F. solani* f. sp. *phaseoli*, but expressed only transiently to low levels in the unsuccessful resistance response to the pea pathogen *Fusarium solani* f. sp. *pisi*. A preliminary western analysis with rabbit polyclonal antibodies directed against the DRR206 protein indicates that a protein with an apparent molecular mass of approximately 23,000 D is induced in pea pod tissue in response to challenge with *F. solani* f. sp. *phaseoli*.

The nucleotide sequence of the genomic clone DRR206-d reported here is identical to the pI206 (DRR206-a) cDNA clone sequence (Fristensky et al., 1988) with the exception of a single conservative change in the coding region (base position 1836) and five base differences in the 3′ untranslated region. These differences are most likely due to allelic variation resulting from the use of different pea cultivars to isolate the cDNA and genomic clones (Dot versus Alcan, respectively).

Southern hybridization analysis of pea DNA using the DRR206-d coding region as a probe indicated that it is a member of a multigene family consisting of at least five loci. The DRR206 sequence appears to be evolutionarily conserved, as indicated by hybridization of the coding region probe to genomic sequences in bean (*Phaseolus vulgaris*), potato (*Solanum tuberosum*), tomato (*Lycopersicon esculentum*), and corn (*Zea mays*). Primer extension analysis indicated that transcription is initiated at base position 1433, 56 bases 5′ of the ATG start codon. Two potential TATA boxes are present at positions −31 and −79 relative to the transcription initiation site. Several regions that may be involved in regulation of the *DRR206-d* gene are present in the 5′ sequence. Two identical 10-bp sequences at positions −166 and −417 show homology with the TCA motif present in the promoters of a number of elicitor/stress-induced genes (Goldsbrough et al., 1993). Tandem 23-bp perfect direct repeats, separated by 2 bases, are present from position −333 to −286. From position −962 to −810 there is a region consisting of 28 bases of perfect AT repeats (14 repeat units) followed by a region of potentially strong

Table I. *Characteristics of the DRR206-d gene from pea*

Organism:	*Pisum sativum* L. cv Alcan.
Source:	Unamplified λ-Dash (Stratagene) library constructed from a partial *Sau*3A digest of pea (cv Alcan) genomic DNA.
Techniques:	A 15.2-kb positive clone, λg206–13, was obtained by screening the pea genomic library with a 600-bp coding region probe from the cDNA clone pI206 (DRR206-a) (Fristensky et al., 1988). A 4.5-kb *Bam*HI subclone (DRR206-d) containing the coding region, approximately 3.7 kb of 5′ sequence, and 260 bp of 3′ sequence was generated in Bluescript KS+ (Stratagene). Overlapping sequence of both strands was obtained for the 3′ 2.3 kb of the *Bam*HI subclone using the dideoxy chain-termination method in conjunction with primer walking. The transcription initiation site was identified by primer extension with RNA obtained from fungus-induced pod tissue and the polyadenylation site was determined by comparison with the sequence of the pI206 cDNA clone.
Confirmation:	Sequence similarity with the inducible cDNA clone pI206 (DRR206-a).
Characteristics of the Nucleotide Sequence:	2298 nucleotides containing 1432 bases of 5′ region, 189 bases of 3′ sequence, and a single exon of 677 bases containing a 56-base 5′ untranslated region, a coding region of 552 bases, and 69 bases of 3′ untranslated region.
Expression Characteristics:	A transcript of approximately 700 bp induced to high levels in pea pod tissue in response to fungal challenge or the elicitor chitosan.
Features of the Deduced Protein:	Deduced amino acid sequence is 184 amino acids with a molecular mass of 20,371 D and a calculated pI of 9.66.

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Abbreviation: DRR, disease-resistance response.

secondary structure (change in Gibbs free energy = −60 kcal). Promoter fusion studies are being performed to determine the significance of these regions in the regulation of the DRR206-d gene.

Computer analysis of the deduced amino acid sequence of DRR206-d indicates a molecular mass of 20,371 D, a calculated pI of 9.66, and a short stretch of predominantly hydrophobic amino acids extending for approximately 20 residues from the amino terminus. A search of the GenBank, SwissProt, and PROSITE data bases using the TFASTA, FASTA, and MOTIFS programs from the Genetics Computer Group (Madison, WI) package did not reveal any proteins or protein domains with significant similarity to the deduced protein sequence of DRR206-d. Transgenic studies are currently underway to investigate the role of the DRR206-d protein in the DRR.

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