Theor Appl Genet (2005) 110: 895–905
DOI 10.1007/s00122-004-1908-1

ORIGINAL PAPER

**Elodie Ferrier-Cana · Catherine Macadré
Mireille Sévignac · Perrine David · Thierry Langin
Valérie Geffroy**

Distinct post-transcriptional modifications result into seven alternative transcripts of the CC–NBS–LRR gene *JA1tr* of *Phaseolus vulgaris*

Received: 31 August 2004 / Accepted: 9 December 2004 / Published online: 20 January 2005

Abstract The generation of splice variants has been reported for various plant resistance (R) genes, suggesting that these variants play an important role in disease resistance. Most of the time these R genes belong to the Toll and mammalian IL-1 receptor–nucleotide-binding site–leucine-rich repeat (TIR–NBS–LRR) class of R genes. In *Phaseolus vulgaris*, a resistance gene cluster (referred to as the *B4* R-gene cluster) has been identified at the end of linkage group B4. At this complex resistance cluster, three R specificities (*Co-9*, *Co-y* and *Co-z*) and two R QTLs effective against the fungal pathogen *Colletotrichum lindemuthianum*, the causal agent of anthracnose, have been identified. At the molecular level, four resistance gene candidates encoding putative full-length, coiled-coil (CC)–NBS–LRR R-like proteins, with LRR numbers ranging from 18 to 20, have been previously characterized. In the present study, seven cDNA corresponding to truncated R-like transcripts, belonging to the CC–NBS–LRR class of plant disease R genes, have been identified. These seven transcripts correspond to a single gene named *JA1tr*, which encodes, at most, only five LRRs. The seven *JA1tr* transcript variants result from distinct post-transcriptional modifications of *JA1tr*, corresponding to alternative splicing events of two introns, exon skipping and multiple 'aberrant splicing' events in the open reading frame (ORF). *JA1tr* was mapped at the *B4* R-gene cluster identified in common bean. These post-transcriptional modifications of the single gene *JA1tr* could constitute an efficient source of diversity. The present results provide one of the few reports of transcript variants with truncated ORFs resulting from a CC–NBS–LRR gene.

Communicated by B. Keller

Equal contributions were made by T. Langin and V. Geffroy, who should be considered joint last authors.

E. Ferrier-Cana · C. Macadré · M. Sévignac · P. David
T. Langin · V. Geffroy (✉)
Institut de Biotechnologie des Plantes, UMR-CNRS 8618,
Université Paris-Sud, LPPM, bât. 630, 91405 Orsay, France
E-mail: geffroy@ibp.u-psud.fr
Tel.: +33-1-69153370
Fax: +33-1-69153424

Introduction

Host–parasite interaction is a struggle for survival between two organisms. A variety of pathogens, including viruses, bacteria, nematodes and fungi, are able to attack plants and in turn, plants have evolved sophisticated systems to perceive and defend themselves against such attacks (McDowell and Dangl 2000). Genetic control of plant disease resistance often relies on the simultaneous occurrence of a resistance (R) gene in the plant genome and a specific corresponding avirulence (Avr) gene in the pathogen genome (Flor 1971). During this 'gene-for-gene' interaction, R genes supposedly possess two functions: molecular recognition of pathogen-derived ligands and subsequent activation of plant defence. Classical genetic studies have revealed that R genes are often located at complex loci displaying either a multiallelic structure and/or a cluster of linked genes responsible for different specificities (Parniske et al. 1997; Song et al. 1997; Meyers et al. 1998; Dodds et al. 2001; Sun et al. 2001). The majority of the R genes cloned to date in plants encode R proteins composed of an N-terminal nucleotide-binding site (NBS) region and a C-terminal leucine-rich repeat (LRR) region, suggesting that R genes are members of an ancient gene family (Meyers et al. 1999; Ellis et al. 2000; Dangl and Jones 2001; Jones 2001). In the NBS–LRR proteins, two distinct N-terminal domains have been mainly described: either a domain sharing homology with the cytoplasmic domain of *Drosophila* Toll and mammalian IL-1 receptor (TIR) or a putative coiled-coil (CC) (Nimchuk et al. 2003). Analyses revealed that the LRR region contributes to the specificity in pathogen recognition, while signal transduction is mediated through the amino

terminal region (Hammond-Kosack and Jones 1997; Dangl and Jones 2001; Hammond-Kosack and Parker 2003; Nimchuk et al. 2003). Direct physical interaction between NBS–LRR R proteins and Avr proteins have been demonstrated only twice (Jia et al. 2000; Deslandes et al. 2003). The few examples of direct interaction between R and Avr proteins and the involvement of several Avr genes in virulence in susceptible context (Kjemtrup et al. 2000) have led to the emergence of new models, such as the 'guard hypothesis' (Dangl and Jones 2001).

One feature of TIR–NBS–LRR R genes is their ability to produce alternative transcripts with truncated open reading frames (ORFs). The tobacco *N* (Whitham et al. 1994), the flax *L6* (Lawrence et al. 1995; Ayliffe et al. 1999), the *Arabidopsis thaliana RPP5* (Parker et al. 1997) and *RPS4* (Gassmann et al. 1999) and the tomato *Bs4* (Schornack et al. 2004) R genes encode either full-length or truncated R transcripts. Distinct molecular mechanisms are involved in the generation of these truncated R transcripts. The N-truncated R transcripts originate from alternative splicing of a 70-bp exon within intron 3 (Whitham et al. 1994). For *Bs4*, *L6* and *RPS4*, alternative transcripts are derived from alternative splicing of intron sequences (Lawrence et al. 1995; Ayliffe et al. 1999; Gassmann et al. 1999). Finally, for the *Arabidopsis RPP5*, the putative truncated protein is not the result of post-transcriptional modifications of the *RPP5* R gene itself, but is encoded by a closely linked gene ($RPP5^{tr}$) that encodes a putative protein with a TIR–NBS amino acid sequence identical to that of RPP5 but lacking LRRs (Parker et al. 1997). Because of their structural organisation, these truncated R transcripts are hypothesized to be involved in disease resistance. While the role of the truncated L6 (Ayliffe et al. 1999), $RPP5^{tr}$ (Parker et al. 1997) and Bs4 (Schornack et al. 2004) R transcripts have not yet been established in disease resistance, a possible involvement of the truncated N transcript (Dinesh-Kumar and Baker 2000) and the truncated RPS4 transcript (Zhang and Gassmann 2003) in resistance has been described. Indeed, variation in the ratio of the truncated and full-length products of the *N* gene seems to be regulated by *Tobacco mosaic virus* (TMV)-induced signals and involved in the disease resistance response (Dinesh-Kumar and Baker 2000). Concerning RPS4, both regular and alternative RPS4 transcripts are necessary for resistance (Zhang and Gassmann 2003). Whereas the alternative splicing of transcripts encoding TIR–NBS–LRR proteins leading to truncated forms begins to be well documented (Jordan et al. 2002), no such example have been described for the CC–NBS–LRR sub-class of plant disease R genes.

At the genomic level, truncated forms of TIR–NBS–LRR-encoding genes have been identified through a comprehensive bioinformatic analysis of the NBS–LRR-encoding genes of the *Arabidopsis* genome: the TIR–X family, which lacks both the NBS and the LRR regions, and the TIR–NBS family, which contains most of the NBS region and lacks the LRR region (Meyers et al. 2002, 2003). These truncated TIR–NBS–LRR-encoding genes are important in the context of the present paper, since they structurally reconstitute alternatively spliced TIR–NBS–LRR-encoding transcripts (Jordan et al. 2002). The functionality of these R-like genes has not been established; however, their diversity, their number and the detectable expression observed for the majority of these genes suggest that these genes encode functional proteins and are not a result of degradation or deletion events of full-length TIR–NBS–LRR R genes (Meyers et al. 2002, 2003).

In *Phaseolus vulgaris*, we have identified a complex R-gene cluster, localized at the end of linkage group B4, and referred to as the *B4* R-gene cluster. This cluster comprises three R specificities (*Co-9*, *Co-y* and *Co-z*) and two R QTLs effective against the fungal pathogen *Colletotrichum lindemuthianum*, the causal agent of anthracnose (Geffroy et al. 1999, 2000). Four expressed R-gene candidates (RGCs) that mapped at the *B4* R-gene cluster have been characterized. These RGCs encode full-length CC–NBS–LRR R-like proteins with 18 to 20 LRRs (Ferrier Cana et al. 2003). In the present paper, we describe the identification of seven additional cDNA encoded by a single gene, referred to as *JA1tr*, that belongs to the *B4* R-gene cluster and that encodes at the most 5 LRRs. These seven transcripts result from distinct post-transcriptional modifications of *JA1tr*, corresponding to the alternative splicing events of two introns, exon skipping and multiple 'aberrant splicing' events in the open reading frame. These modifications result in a single RGC encoding for seven distinct truncated R-like transcripts belonging to the CC–NBS–LRR class. To our knowledge, this constitutes one of the only reports of truncated CC–NBS–LRR R-like transcripts, resulting from alternative splicing, leading to truncated putative protein.

Materials and methods

Genomic and cDNA libraries screening

The JaloEEP558 cDNA library (Ferrier Cana et al. 2003) was screened with the *PRLJ1* probe (Geffroy et al. 1999) under the following conditions: overnight hybridization at 65°C in 5× SSC (3 M NaCl and 0.3 M sodium citrate), 5% SDS and 5× Denhardt's (0.1% Ficoll, 0.1% PVP, 0.1% BSA), followed by three washing steps of 15 min each in 0.5× SSC and 0.5% SDS at 65°C. Seven positive clones were obtained from the JaloEEP558 cDNA library of which the insert display sizes ranged from 754 bp to 2,190 bp. In order to find the genomic clone(s) corresponding to these cDNA, the JaloEEP558 genomic library (Ferrier Cana et al. 2003) was screened with the *cJA88* cDNA as representative probe. A single genomic clone, *JA1tr*, was recovered.

Sequencing and analysis of the cDNA and the genomic clones

Genomic and cDNA clones sequencing was performed using an automated 373A DNA sequencer and the Big Dye Dideoxy Chain Terminator Cycle sequencing kit (PerkinElmer, Applied Biosystems, Roissy, France). Sequence analyses and multiple alignments were performed with the MacMolly program (version 3.5.1, Softgene) and the Gap and Overlap programs of the Genetics Computer Group package (Madison, Wis., USA). Sequence similarities were established using the BLAST algorithm (Altschul et al. 1997). Exon–intron boundaries were determined by comparison between the cDNA sequences and genomic sequence. COILs analyses (Lupas et al. 1991) were performed with the Macstripe, version 2.0b1, software, using a window of 28 and the MTK matrix. The GenBank accession number is AY237123.

Genetic mapping of the *JA1tr* genomic clone

Seventy-seven F_9 recombinant inbred lines developed at the University of California, Davis, Calif., USA, and derived from a cross between the Mesoamerican BAT93 genotype and the Andean JaloEEP558 genotype, were used to map the *JA1tr* RGC on the integrated linkage map of common bean (Freyre et al. 1998). A PCR approach, using specific oligonucleotide primers j46 (5′-GGTACAAAAGTGAGAAAG-3′) and j47 (5′-CAAAGTGTGGGTGATCAA-3′), was utilized. PCR reactions were completed in a volume of 25 µl containing 50 ng DNA, 1× PCR reaction buffer, 3 pmol each primer, 50 µM each dNTP and 0.5 U Red *Taq* Goldstar polymerase (Eurogentec, Seraing, Belgium). Amplifications were performed in a GeneAmp PCR system 9600 (PerkinElmer, Norwalk, Conn., USA). The MAPMAKER software, version 3.0, was used to map the genomic clone on the integrated linkage map (Lander et al. 1987). Linkage groups were established with an LOD threshold of 3.0 and a recombination fraction of 0.3. Marker order was estimated with an LOD threshold of 2.0, based on multipoint *compare*, *order* and *ripple* analyses.

Reverse transcriptase-PCR analysis

One-week-old JaloEEP558 plants were infected with the *C. lindemuthianum* M38 avirulent strain, and cotyledonary leaves were harvested before inoculation and 24, 48 and 72 h after inoculation. Total RNA was extracted from both the non-inoculated and inoculated bean cotyledonary leaves as already described (Ferrier Cana et al. 2003). RNA was treated twice with the DNA-free DNase (Ambion, Tex., USA).

The two primers *j13* (5′-GATAACAGTGATATGGAAC-3′) and *j29* (5′-GTCTGAAGTGCATCTTC-3′), located at the 5′ and 3′ boundaries of the aberrant spliced coding region (Fig. 1a) have been used in reverse transcriptase (RT)-PCR experiments. RT-PCRs were performed with the Enhanced Avian RT-PCR Kit (Sigma, St, Louis, Mo., USA) according to manufacturer recommendations. A control experiment without reverse transcriptase was included to make sure that the products are not genomic DNA contamination.

Results

Characterization of seven cDNA showing similarities with R genes of the CC–NBS–LRR class

In a previous study, four full-length cDNA that mapped to the *B4* R-gene cluster and that might correspond to known R specificities effective against the fungal pathogen *C. lindemuthianum* were identified (Ferrier Cana et al. 2003). These cDNA (*cJA71* and *cJA78* isolated from the JaloEEP558 genotype, *cBA8* and *cBA11* isolated from the BAT93 genotype) encode putative R proteins containing an NBS domain and an LRR domain consisting of 18 to 20 repeats.

The screening of the JaloEEP558 cDNA library led to the isolation of seven additional cDNA presenting high similarities with R genes encoding NBS–LRR R proteins (Ellis et al. 2000). These cDNA are predicted to encode shorter proteins compared to the previous RGCs isolated at the *B4* R-gene cluster. Indeed, the coding sequences (ORFs) of these seven cDNA, *cJA68*, *cJA73*, *cJA74*, *cJA80*, *cJA88*, *cJA76* and *cJA102*, display variable lengths ranging from 754 bp to 2,190 bp (Fig. 1a). Nucleotide sequence comparisons of the seven cDNA reveal that they are 100% identical in their overlapping regions (Fig. 1a) and that ORF length variability has two origins. The four cDNA (*cJA73*, *cJA68*, *cJA76* and *cJA80*) are incomplete in their 5′ end due to the cDNA library construction protocol. More interestingly, detailed nucleotide sequence comparisons indicated that two distinct internal deletion events have occurred in the coding region: *cJA68*, *cJA76* and *cJA80* cDNA present a deletion of 689 bp, whereas the *cJA73* cDNA presents a deletion of 437 bp (Fig. 1a). The 689-bp and 437-bp deleted regions display two features characteristic of intron sequences. First, upon comparison with the longest sequence of the *cJA74* cDNA, we observed that the 689-bp and 437-bp deleted regions both display GT and AG nucleotides at their 5′ and 3′ extremities, respectively, as expected for an intron sequence. Second, the 689-bp and 437-bp deleted sequences are flanked by nucleotides A and G at −2 and −1 positions and nucleotides G and T at +1 and +2 positions, which correspond to the most frequent flanking nucleotides observed for introns in dicot plant genes (Simpson and Filipowicz 1996). However, the AT content of the 689-bp and 437-bp deleted regions is not higher than the AT content observed for the rest of the

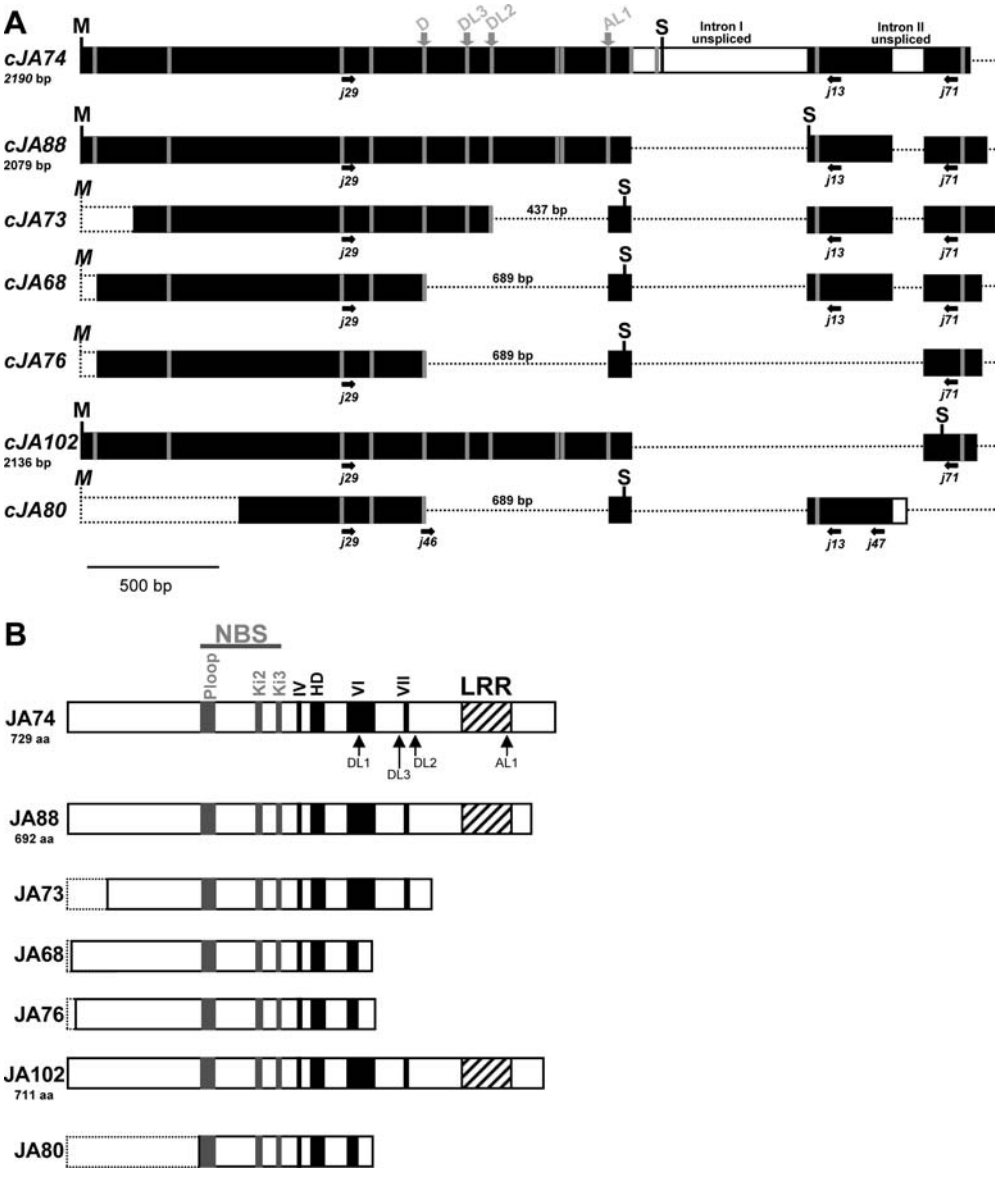

Fig. 1 **a** Schematic structural representation of the seven cDNA sequences. *M* and *S* correspond to the start and the stop codon for translation, respectively. The incomplete 5′ end sequences of the *cJA73*, *cJA68*, *cJA76* and *cJA80* cDNA, due to the cDNA library building protocol, are indicated by *dotted open boxes*. The lengths of *cJA74*, *cJA88* and *cJA102* cDNA open reading frames are indicated *under the cDNA names*. The positions of the 14 AGGT motifs, corresponding to potential donor-like (DL) sites and/or acceptor-like (AL) sites in the cDNA sequences, are indicated by *grey vertical traits*. The positions of the detected three DL sites DL1, DL2, DL3 and the AL site AL1 are indicated by *grey arrows above the cJA74 sequence*. Deleted regions in the different cDNA are indicated by *dotted lines*, and the lengths of the deletions are indicated *above the dotted lines*. Unspliced introns are symbolized by *open boxes in the sequences of the cJA74 and cJ80 cDNA*. The positions of the *j13*, *j29*, *j46* and *j47* primers are indicated by *horizontal black arrows under the sequences*. **b** Structural organisation of the seven truncated resistance (R)-like proteins deduced from the cDNA sequences. The conserved domains of the nucleotide-binding site (*NBS*) region: the P loop, kinase 2 (*Ki2*) and kinase 3 (*Ki3*) domains are symbolized by *grey boxes*. Conserved domains IV, hydrophobic domain (*HD*), VI and VII are indicated by *black boxes*. The leucine-rich repeat (*LRR*) region is denoted with *black, hatched boxes*. The incomplete N-terminal sequences of JA73, JA68, JA76 and JA80 R-like proteins, due to the cDNA library building protocol, are indicated by *dotted open boxes*. The lengths of JA74, JA88 and JA102 R-like proteins are indicated *under the protein names*

ORF (approximately 65%) as expected for an intron sequence of a dicot plant gene. Usually, in these intron sequences the percentage of AT nucleotides is above 70% (Simpson and Filipowicz 1996). At the 5′ border, the two donor-like (DL) splice sites have been designated DL1 and DL2 (Fig. 1a). At the 3′ border, only one acceptor-like (AL) splice site has been observed, and was designated AL1 (Fig. 1a). Moreover, it is remarkable that the deletion of the coding sequence involved only 4 of 14 distinct potential DL splice and/or AL splice sites present in the *cJA74* cDNA sequence (Fig. 1a).

The 3′ untranslated regions (3′ UTRs) of the seven cDNA also display variable lengths (Fig. 1a): *cJA102* (156 bp), *cJA76* (244 bp), *cJA80* (396 bp), *cJA68* (553 bp), *cJA88* (556 bp), *cJA73* (604 bp) and *cJA74* (1160 bp). Comparison of these 3′ UTR sequences reveals that distinct deletions have occurred in the 3′ UTR sequences. Like the coding sequences, the remaining nucleotide sequences shared by the seven cDNA are 100% identical (Fig. 1a).

A COILS analysis (Lupas et al. 1991) revealed that the region between amino acids 132 and 143 of the predicted cJA74 protein forms a CC structure, with a probability higher than 98%.

The seven cDNA encode distinct putative truncated CC–NBS–LRR R-like proteins

These seven cDNA are predicted to encode seven distinct proteins presenting high similarities with the JA71 and JA78 NBS–LRR R-like proteins encoded by the two *JA71* and *JA78* RGCs previously identified at the *B4* R-gene cluster (Fig. 2; Ferrier Cana et al. 2003). For example, the JA88 R-like protein exhibits at least 81.1% and 85.5% of amino acid identity and similarity, respectively, with the JA71 and JA78 R-like proteins. Moreover, the position of both the N-terminus conserved domains (from the domain P loop to the domain VII) and the beginning of the LRR region are conserved (Fig. 2).

However, in contrast to the JA71 and JA78 R-like proteins that comprise the seven conserved N-terminus domains and 18 and 20 LRRs, respectively, the putative proteins deduced from the seven cDNA isolated in the present study, are partially truncated. Because of the distinct deletion events, the corresponding seven R-like proteins are truncated in their C-terminus region, and three distinct configurations have been discovered (Fig. 1b). Splice forms JA74, JA102 and JA88 harbour the seven conserved domains (NBS conserved domain plus the four additional IV to VII conserved domains) and five LRRs. The JA73 R-like protein contains only the seven conserved domains (I to VII). Finally, the JA68, JA76 and JA80 R-like proteins harbour only the five first conserved domains (I to V) and part of the conserved domain VI.

The seven cDNA originate from various splicing events following transcription of a unique gene referred to as *JA1tr*

As the seven cDNA share a common nucleic sequence with distinct deleted parts, we studied whether these cDNA corresponded to transcription products of different truncated R genes, or whether these cDNA originated from post-transcriptional modifications of a single gene. To address this question, we screened a lambda genomic library from the JaloEEP558 genotype (12 genome equivalent) with the representative *cJA88* cDNA as a probe. Four phages corresponding to a unique genomic clone, displaying the nucleic sequence corresponding to all cDNA, were recovered. Upon comparison of the genomic sequence with the *cJA88* cDNA clone sequence, the presence of two introns was detected: intron I of 662 bp and intron II of 126 bp (Fig.1a). Both displayed a characteristic high AT content of 71.6% and 73.8%, respectively, as expected for intron sequences of a dicot plant gene (Simpson and Filipowicz 1996).

Comparisons of the seven cDNA sequences with the sequence of the recovered genomic clone revealed that the length variations observed in the 3′ UTR sequences of the cDNA are due to alternative splicing events of the two introns and four distinct situations are observed (Fig. 1a). For the *cJA88*, *cJA73* and *cJA68* cDNA, the two introns are correctly spliced, and the length variations of the 3′ UTRs are due to length variation of the 3′ end sequences located after the intron II. For the *cJA74* cDNA, the two introns are not spliced, which implies the expansion of the ORF to a stop codon reached within the sequence of intron I (Fig. 1a). For the *cJA76* and the *cJA102* cDNA, the sequence located between the donor splice site of intron I and the acceptor splice site of intron II is completely removed and corresponds to an example of exon skipping. Finally, a fourth situation of aberrant splicing is observed for the *cJA80* cDNA. In this case intron I is correctly removed, whereas the end of the 3′UTR corresponds to the first unspliced 55 bp sequence of intron II (Fig. 1a).

Serial PCR experiments were completed to confirm that only a single genomic sequence corresponding to the seven identified cDNA exists in the JaloEEP558 bean genome. Primers *j13* and *j29* were used (Fig. 1a). These primers frame the 689-bp and 437-bp sequences that are deleted in the *cJA68*, *cJA76*, *cJA80* cDNA and in the *cJA73* cDNA, respectively. If distinct genes correspond to the different cDNA, five amplification products of 0.491 kb (*cJA68* and *cJA80*), 0.744 kb (*cJA73*), 1.177 kb (*cJA88*) and 1.8 kb (*cJA74*), corresponding to each event of deletion, would be expected. A single 1.8-kb band was PCR-amplified from the JaloEEP558 genomic DNA with the *j13*/*j29* primers. This 1.8 kb size corresponds to the product expected for the longest cDNA, *cJA74* (Fig. 3). This result indicates that all cDNA derive from a single gene, of which the messenger is subjected to multiple, distinct post-transcriptional events. An additional PCR experiment performed with primers *j29* and *j71*, allowing the amplification of the *cJA76* and *cJA102* cDNA, led to the same conclusion (data not shown). This single RGC will be further referred to as *JA1tr* for first (*1*) truncated (*tr*) resistance gene candidate isolated from the JaloEEP558 genotype (*JA*). To our knowledge, these R-like transcripts constitute the first report of truncated non-TIR–NBS–LRR R-like transcripts resulting from alternative splicing.

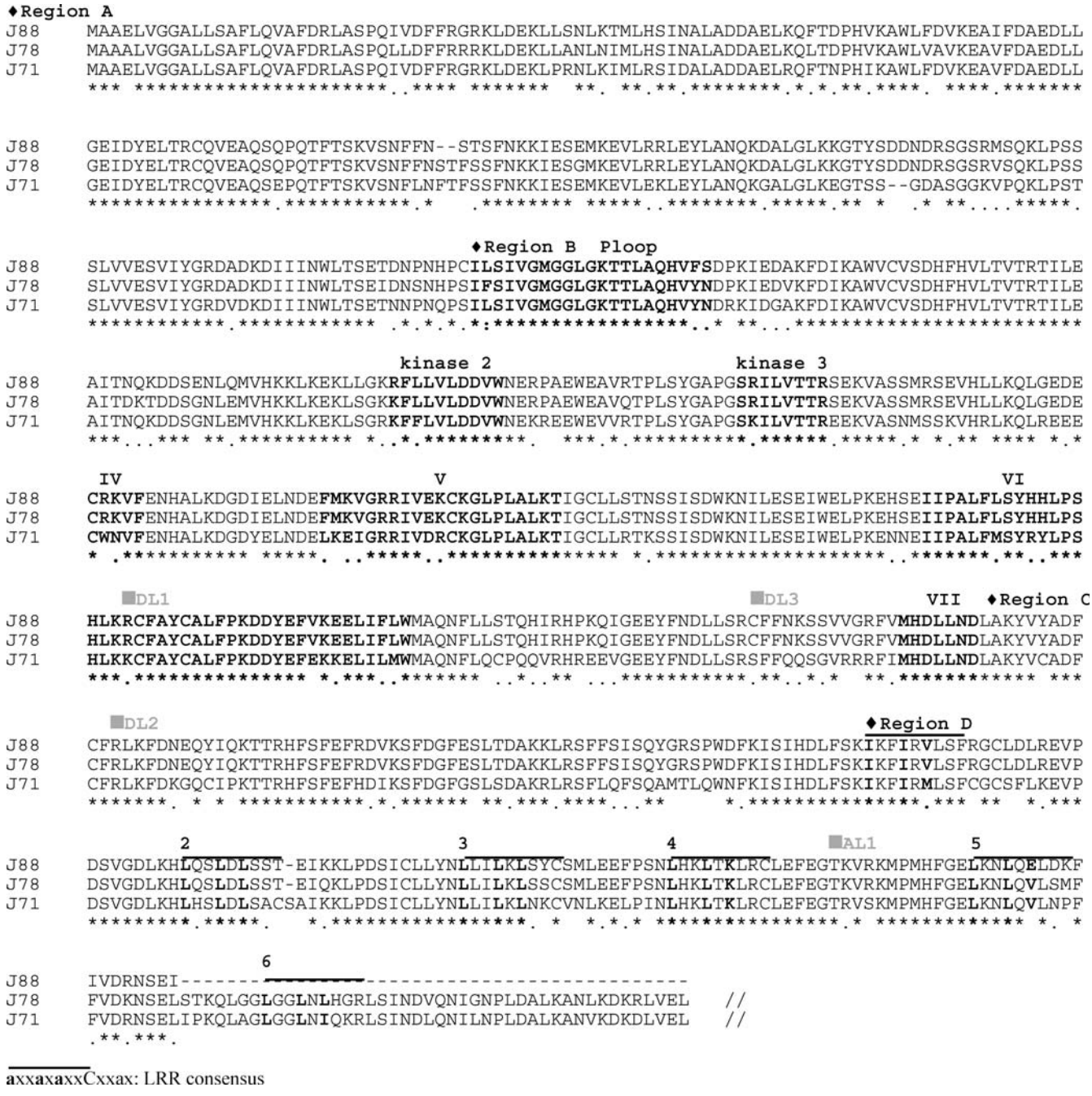

Fig. 2 Comparison of the JA88 truncated R-like protein with the corresponding region of the full-length JA71 and JA78 R-like proteins. The amino acid sequences of the four proteins are divided into four regions: the N terminus (*Region A*), the NBS (*Region B*), the spacer (*Region C*) and the LRR (Region D). *Filled diamonds* above the three sequences indicate the beginning of each region. The conserved domains of the NBS region, as well as domains IV to VII that are conserved among the NBS–LRR R-like sequences, are indicated in *boldface characters*. The number of each LRR is indicated *above the sequence*. The LRR consensus sequence is indicated at the *bottom of the figure*: *a* corresponds to an aliphatic residue (I, F, L, M or V), and *x* corresponds to any residue. The conserved aliphatic residues of the *β*-turn–*β*-sheet domains (*xxaxaxx*) are shown by *horizontal lines* and aliphatic amino acids are in *boldface*. *Asterisks* and *dots* indicate identical and similar amino acids in the consensus sequence, respectively. *Grey squares placed above the amino acid sequences* indicate the position of DL1, DL2, DL3 and AL1 splicing sites. The JA71 and JA78 R-like proteins sequences have been represented until their fifth LRR

Mapping of the *JA1tr* R gene candidate

A PCR approach, using specific primers *j46* and *j47* (Fig. 1a), was used to map the *JA1tr* RGC on the integrated linkage map of common bean (Freyre et al. 1998). The *JA1tr* RGC maps at the end of linkage group B4, on the *B4* R-gene cluster, and is co-localized with the previous characterized *JA78* RGC (Ferrier Cana et al.

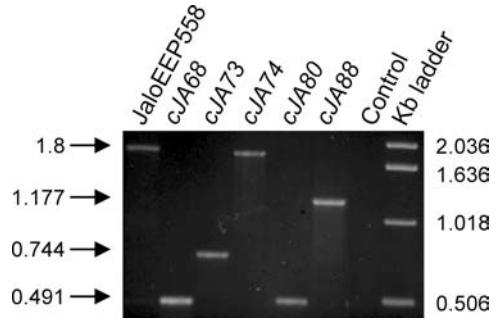

Fig. 3 Occurrence of a single genomic sequence corresponding to the different transcripts. PCR experiment was performed on genomic DNA from the JaloEEP558 genotype (*lane JaloEEP558*) and on five cDNA clones (*cJA68*, *cJA73*, *cJA74*, *cJA80* and *cJA88*), with the *j29* and *j13* primers, which flanked the coding sequence that is subject to aberrant splicing (Fig. 1a). The lengths (in kilobases) of the amplified products on the distinct cDNA are indicated on the *left*. *Control*: PCR test reaction without DNA. *Kb ladder*: Nucleic acid molecular size standard (1 kb, GIBCO-BRL, Cergy Pontoise, France) of which the size of the fragments are indicated on the *right* (in kilobases). A unique amplification product of 1.8 kb is obtained with the JaloEEP558 genomic DNA similar to the product obtained from the *cJ74* cDNA

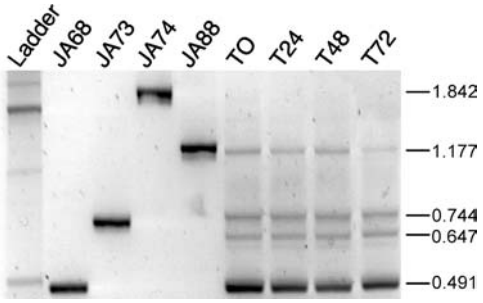

Fig. 4 Identification of additional splice variants of JA1tr through RT-PCR experiments. A reverse transcriptase (RT)-PCR experiment was done on total RNAs isolated from bean cotyledonary leaves before inoculation (*T0*) with the M38 avirulent strain of *Colletotrichum lindemuthianum* 24, 48 and 72 h after inoculation (*T24*, *T48* and *T72*, respectively), with the *j29* and *j13* primers that flank the coding region of the *JA1tr* R-gene candidate subjects to aberrant splicing (Fig. 1a). Control amplifications were also done on DNA corresponding to *cJA68*, *cJA73*, *cJA74* and *cJA88* cDNA (which give amplification product with primers *j29*/*j13*, which is not the case of cJA76, cJA102; see Fig. 1a). Lengths (in kilo bases) of the amplified products are indicated on the *right*. For each sample, control experiments without reverse transcriptase showed that the observed products corresponded to *JA1tr* transcripts and not genomic DNA contamination (data not shown). *Ladder*: Nucleic acid molecular size standard (1 kb). Note that: (1) all RT-PCR patterns are identical regardless of the time point; (2) no amplification is observed that corresponds to the size of the *cJA74* transcript form; and (3) a new amplification product of 647 bp is observed that does not correspond to any of the recovered cDNA and therefore indicates the occurrence of a new donor like splicing site, designated DL3 (see Figs. 1, 2)

2003), the *Co-y*/*Co-9* R-specificities and one R QTL effective against *C. lindemuthianum* (Geffroy et al. 1999, 2000).

Identification of an additional *JA1tr* alternative transcript through RT-PCR experiments

In order to test (1) whether all the alternative transcripts are expressed during infection to a detectable level and (2) whether additional transcripts resulting from post-transcriptional events are expressed in vivo via alternative splicing, RT-PCR experiments were performed using primers *j29* and *j13* (Fig. 1a). *j29*/*j13* gave amplification products on cJA74, cJA88, cJA73, cJA68/cJA80 and no product on cJA76 and cJA102 (Figs. 1a, 4). Three amplification products were observed in both non-inoculated and inoculated plants corresponding to the expected product for the *JA68*/*JA80*, the *JA73* and the *JA88* transcripts (Fig. 4). One additional amplification product of 647 bp has also been recovered that does not correspond to any of the seven cDNA previously identified. Cloning and sequencing of this amplification product allowed the identification of a third DL splice site (DL3, Fig. 1a). This latter product corresponds to a new class of transcripts with correct splicing of intron I but lacking a 533-bp region between the DL splice site DL3 and the AL splice site AL1. Moreover, the transcripts are observed in both inoculated and non-inoculated plants, and their level of occurrence is similar in all tested conditions: inoculated/non-inoculated, regardless of the time point. Therefore, it appears that the post-transcriptional modifications, as we describe here, are not dependent on the triggering of the defence mechanism and/or resistance response in bean upon inoculation with the fungal pathogen *C. lindemuthianum*. The level of expression of the distinct transcripts is clearly different, even if RT-PCR is not a quantitative technique (Fig. 4). Indeed, the *JA74* transcript is not detectable in this condition, in contrast to the other transcripts. Consequently, the non-splicing of intron I is likely to be a rare event.

Discussion

In common bean, the complex *B4* R-gene cluster contains a multi-allelic family of NBS resistance gene analogues, three R specificities and two R QTLs that are effective against the fungal pathogen *C. lindemuthianum* (Geffroy et al. 1999, 2000). Recently, we reported the characterization of four expressed RGCs predicted to encode CC–NBS–LRR R-like proteins (Ferrier Cana et al. 2003) that are candidates corresponding to the genetically defined *Co-9*/*Co-y* R-specificities effective against *C. lindemuthianum* (Geffroy et al. 1999). In this study, we report the characterization of an additional expressed CC–NBS–LRR RGC, *JA1tr*, that is predicted to produce at least eight distinct R-like transcripts via multiple post-transcriptional modifications. In contrast to *JA71* and *JA78* RGCs that correspond to full-length R-like proteins harbouring the seven conserved domains P loop to VII and at least 18 LRRs in the C-terminus

region, *JA1tr* encodes putative R-like proteins truncated in the C-terminus region and harbour either zero or five LRRs.

Until now, alternative transcripts have been mainly described in the TIR–NBS–LRR sub-class of disease R genes (Whitham et al. 1994; Lawrence et al. 1995; Ayliffe et al. 1999; Gassmann et al. 1999; Dinesh-Kumar and Baker 2000), and very few examples have been described so far for the CC–NBS–LRR sub-class of disease R genes. For the CC–NBS–LRR powdery mildew *Mla13* R genes, alternative splicing events have been reported. However, since these splicing events occur in the transcript leader region, they do not lead to alternative transcripts with truncated ORFs (Halterman et al. 2003). For *Mla-6*, another barley powdery mildew CC–NBS–LRR resistance gene (Halterman et al. 2001), another situation is observed. A truncated version of the barley *Mla-6* resistance gene, referred to as *Mla6-2*, has been identified. The *Mla6-2* gene is a paralogue of *Mla6* and encodes a truncated version (232 amino acids) of *Mla-6* (956 amino acids), with only four divergent bases (Halterman et al. 2001, 2003). Therefore, our study constitutes one of the only reports of truncated CC–NBS–LRR transcripts derived from alternative splicing. The rarity of truncated CC–NBS–LRR putative R proteins was already identified in a comprehensive analysis of the number of NBS–LRR sequences present in a single plant. In fact, 149 genes encoding putative full length NBS–LRR R proteins and 57 genes encoding putative truncated R proteins, i.e. without LRR domains, were identified (Initiative 2000; Meyers et al. 2002, 2003; Richly et al. 2002). These truncated NBS–LRR-encoding genes structurally reconstitute alternatively spliced NBS–LRR-encoding transcripts (Jordan et al. 2002). Of this latter class of 57, only six genes encode truncated R proteins belonging to the CC–NBS–LRR sub-class. These results suggest that, in the *Arabidopsis* genome, the number of R genes encoding truncated TIR–NBS–LRR R proteins is much more important than the number of R genes encoding truncated CC–NBS–LRR R proteins. However, whether these genes are subjected to alternative splicing like in the present study is not known. The rarity in the *Arabidopsis* genome of R-like genes encoding putative CC-NBSs with no LRR coupled with the rarity of the alternative transcripts with truncated ORFs derived from CC–NBS–LRR R genes could suggest that the events leading to such putative proteins are counter-selected. Alternatively, the genes encoding truncated TIR–NBS–LRR proteins might have different function than plant disease resistance. In the rice genome, a different situation is observed, since a large group of 50 of these CC-NBSs with no LRR or non-TIR-NBSs with no LRR have been described (Bai et al. 2002). This unique class of cereal genes probably reflect the independent evolution of the resistance gene repertoire since the divergence of grasses and dicots (Bai et al. 2002).

Several features distinguish the *JA1tr* gene from previously reported data concerning the truncated TIR–NBS–LRR R proteins. In contrast to alternative transcripts of flax *L6* (Lawrence et al. 1995; Ayliffe et al. 1999), tobacco *N* (Whitham et al. 1994; Dinesh-Kumar and Baker 2000) and *A. thaliana RPS4* (Gassmann et al. 1999) R genes, which derive from a single R gene encoding both full-length and truncated transcripts, the bean *JA1tr* RGC encodes only truncated R-like proteins when compared to related *JA71* and *JA78* RGCs previously characterized at the complex *B4* R-gene cluster (Ferrier Cana et al. 2003). The larger transcript *JA74* corresponds to a R-like protein containing an NBS, the four conserved domains IV, HD, VI and VII (Ellis et al. 2000) and five LRRs, whereas the JA71 and JA78 related R-like proteins present 18 and 20 LRRs, respectively. Moreover, unlike the RPP5 and the RPP5^{tr} R proteins that are encoded by two distinct but related genes (Parker et al. 1997), no related gene encoding an identical and full-length transcript corresponding to the *JA1tr* RGC has been recovered. Therefore, the *JA1tr* gene is 'truncated' at the genomic level since, at most, five LRRs are encoded by this gene, and various post-transcriptional events give rise to at least eight transcripts with truncated ORFs for some of them.

Two distinct post-transcriptional modifications occur on the *JA1tr* RGC primary transcript. The first consists of an alternative splicing of intron sequences, which is associated with an exon skipping as observed for the *JA76* and *JA102* transcripts. This mechanism has been previously described during the formation of the *L6*, *N* and *RPS4* splice variants (Ayliffe et al. 1999; Gassmann et al. 1999; Dinesh-Kumar and Baker 2000). The second mechanism has not yet been reported for plant R genes and corresponds to an 'aberrant' splicing of the coding region that might be explained as a malfunction of the splicing machinery. The 689-bp and 437-bp deleted regions are flanked by a DL splice site and an AL splice site and might be considered as cryptic intron sequences, despite the fact that the AT content of the deleted sequences is not as high as expected for an intron sequence (Simpson and Filipowicz 1996). Alternative splicing seems to occur at extremely different rates in human (40–60%) and *Arabidopsis* (1.5%) (Lorkovic et al. 2000; Modrek and Lee 2002; Zhu et al. 2003). Among the *Arabidopsis* genes, 327 cases of alternative splicing have been reported and categorized in five categories, alternative donor sites, alternative acceptor sites, exon skipping, alternative introns that are shifted in position at both sites and composite alternative splicing. The situation observed in the present study corresponds to this later category of composite alternative splicing. In *Arabidopsis*, only 11 cases of composite alternative splicing have been reported, which pointed out the singularity of such an event (Zhu et al. 2003).

Alternative splicing can be regulated by many biotic or abiotic stresses (König et al. 1998; Simpson and Filipowicz 1996). However, the alternative splicing of the *JA1tr* RGC transcript is not regulated by the infection of common bean with the *C. lindemuthianum* strain tested, since all the alternative transcripts are equally

observed in the non-inoculated plants as well as at all time points of the inoculated plants.

Interestingly, no post-transcriptional modifications have been observed for the four other RGCs (*JA71*, *JA78*, *BA8* and *BA11*) previously isolated at the *B4* R-gene cluster (Ferrier Cana et al. 2003), despite the fact that they present several AL/DL splicing sites and introns in their sequences. We hypothesize that the genomic context of the *JA1tr* RGC affects the outcome of pre-mRNA processing. It remains to be determined whether these truncated forms of R genes are unpurged fossils of past evolution or if, as proposed by Dangl and Jones (2001) and Meyers et al. (2002), they encode adaptor molecules that are important in signalling, as MyD88 contributes to TIR signalling in animals (Medzhitov et al. 1998). Until now, whether or not the predicted products of truncated L6 and RPP5 transcripts are indeed produced and have any involvement in resistance has not yet been established (Parker et al. 1997; Ayliffe et al. 1999). In tobacco, variation in the ratio of the full-length and truncated products of the *N* R gene has been observed during the resistance response to TMV, and both the full-length and the truncated N proteins are necessary to observe complete resistance to TMV (Dinesh-Kumar and Baker 2000; Marathe et al. 2002), although the existence of these predicted proteins has not been demonstrated. For *RPS4*, the combined presence of regular and alternative transcripts is necessary for function (Zhang and Gassmann 2003).

A total of 149 NBS–LRR R genes have been identified in *Arabidopsis* Col-0 (Meyers et al. 2002, 2003). This seems to be a surprisingly small number of genes with regard to all the possible pathogen-encoded ligands. Several models can explain this phenomenon. First, one R protein can recognize more than one Avr protein. This case has been identified several times. For example, the *Arabidopsis RPM1* gene product recognizes two distinct Avr gene products (Bisgrove et al. 1994; Grant et al. 1995); the tomato *Mi* gene is effective against nematodes, aphids and whitefly (Rossi et al. 1998; Nombela et al. 2003) and different alleles of the *RPP8*/*HRT*/*RCY1* gene confer resistance against an oomycete and two separate viruses (McDowell et al. 1998; Cooley et al. 2000; Takahashi et al. 2002). Second, post-transcriptional modifications of a single gene can lead to a remarkable number of alternative transcripts. In the present study, eight distinct transcripts corresponding to a single gene, *JA1tr*, have been characterized. This potentially constitutes an efficient source of diversity for R products that could act as a very efficient system for generating a large sample of R specificities. One limitation of this putative model is that most of the JA1tr transcript variants do not present LRR and that, on the other hand, it is now well documented that the LRR domain of different plant R proteins contribute to specificity in pathogen recognition (Dangl and Jones 2001). However, examples of regions outside of the LRR domain involved in specificity determination have been reported for alleles of the *L* locus of flax (Luck et al. 2000). Another hypothesis could be that the truncated forms of JA1tr are working in interaction with another full length NBS-LRR, such as JA78 or JA71 previously identified at the B4 R-gene cluster (Ferrier Cana et al. 2003), to specify resistance. This kind of situation was observed in *Arabidopsis*, where two closely linked genes RPP2A (TIR-NBS-LRR with short LRR domain) and RPP2B (complete TIR-NBS-LRR) are both essential determinants for isolate specific recognition of *Peronospora parasitica* isolate Cala2 (Sinapidou et al. 2004).

In any case, our results seem to suggest that alternative splicing in plants may have an under-appreciated role in expanding proteome diversity, as mentioned by Jordan et al. (2002).

Acknowledgements We thank Peter Moffet and Richard Laugé for helpful discussions and critical reading of the manuscript. E.F.C. was supported by a grant from the Ministère de l'Education Nationale. The research was supported by INRA, the European community (INCO-CT980317 project), CNRS and Ministère de la recherche.

References

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acid Res 25:3389–3402

Axtell MJ, Staskawicz BJ (2003) Initiation of RPS2-specified disease resistance in Arabidopsis is coupled to the AvrRpt2-directed elimination of RIN4. Cell 112:369–377

Ayliffe MA, Frost DV, Finnegan EJ, Lawrence GJ, Anderson PA, Ellis JG (1999) Analysis of alternative transcripts of the flax *L6* rust resistance gene. Plant J 17:287–292

Bai J, Pennill LA, Ning J, Lee SW, Ramalingam J, Webb CA, Zhao B, Sun Q, Nelson JC, Leach JE, Hulbert SH (2002) Diversity in nucleotide binding site–leucine-rich repeat genes in cereals. Genome Res 12:1871–1884

Bisgrove SR, Simonich MT, Smith NM, Sattler A, Innes RW (1994) A disease resistance gene in *Arabidopsis* with specificity for two different pathogen avirulence genes. Plant Cell 7:927–933

Cooley MB, Pathirana S, Wu HJ, Kachroo P, Klessig DF (2000) Members of the Arabidopsis *HRT*/*RPP8* family of resistance genes confer resistance to both viral and oomycete pathogens. Plant Cell 5:663–676

Dangl JL, Jones JDG (2001) Plant pathogens and integrated defence responses to infection. Nature 411:826–833

Deslandes L, Olivier J, Peeters N, Feng DX, Khounlotham M, Boucher C, Somssich I, Genin S, Marco Y (2003) Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. Proc Natl Acad Sci USA 100:8024–8029

Dinesh-Kumar SP, Baker BJ (2000) Alternatively spliced N resistance gene transcripts: their possible role in tobacco mosaic virus resistance. Proc Natl Acad Sci USA 97:1908–1913

Dodds PN, Lawrence GJ, Ellis JG (2001) Six amino-acid changes confined to the leucine-rich repeat b-strand/b-turn motif determine the difference between the P and P2 rust resistance specificities in flax. Plant Cell 13:163–178

Ellis J, Dodds P, Pryor T (2000) Structure, function and evolution of plant disease resistance genes. Curr Opin Plant Biol 3:278–284

Ferrier Cana E, Geffroy V, Macadré C, Creusot F, Imbert-Bolloré P, Sévignac M, Langin T (2003) Characterization of expressed NBS–LRR resistance gene candidates from common bean. Theor Appl Genet 106:251–261

Flor HH (1971) Current status of the gene-for-gene concept. Annu Rev Phytopathol 9:275–296

Freyre R, Skroch P, Geffroy V, Adam-Blondon A-F, Shirmohamadali A, Johnson WC, Llca V, Nodari RO, Pereira PA, Tsai SM, Tohme J, Dron M, Nienhuis J, Vallejos CE, Gepts P (1998) Towards an integrated linkage map of common bean. 4. Development of a core linkage map and alignment of RFLP maps. Theor Appl Genet 97:847–856

Gassmann W, Hinsch ME, Staskawicz BJ (1999) The *Arabidopsis RPS4* bacterial-resistance gene is a member of the TIR–NBS–LRR family of disease-resistance genes. Plant J 20:265–277

Geffroy V, Sicard D, De Oliveira JC, Sévignac M, Cohen S, Gepts P, Neema C, Langin T, Dron M (1999) Identification of an ancestral resistance gene cluster involved in the coevolution process between *Phaseolus vulgaris* and its fungal pathogen *Colletotrichum lindemuthianum*. Mol Plant–Microbe Interact 12:774–784

Geffroy V, Sévignac M, De Oliveira JCF, Fouilloux G, Skroch P, Toquet P, Gepts P, Langin T, Dron M (2000) Inheritance of partial resistance against *Colletotrichum lindemuthianum* in *Phaseolus vulgaris* and co-localization of QTL with genes involved in specific resistance. Mol Plant–Microbe Interact 13:287–296

Grant MR, Godiard L, Straube E, Ashfield T, Lewald J, Sattler A, Innes RW, Dangl JL (1995) Structure of the *Arabidopsis Rpm1* gene enabling dual specificity disease resistance. Science 269:843–846

Halterman D, Zhou F, Wei F, Wise RP, Schulze-Lefert P (2001) The MLA6 coiled-coil, NBS–LRR protein confers *AvrMla6*-dependent resistance specificity to *Blumeria graminis* f. sp. *hordei* in barley and wheat. Plant J 25:335–348

Halterman DA, Wei FS, Wise RP (2003) Powdery mildew-induced Mla mRNAs are alternatively spliced and contain multiple upstream open reading frames. Plant Physiol 131:558–567

Hammond-Kosack KE, Jones JDG (1997) Plant disease resistance genes. Annu Rev Plant Physiol Plant Mol Biol 48:575–607

Hammond-Kosack KE, Parker JE (2003) Deciphering plant–pathogen communication: fresh perspectives for molecular resistance breeding. Curr Opin Biotechnol 14:177–193

Initiative AG (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408:796–815

Jia Y, McAdams SA, Bryan GT, Hershey HP, Valent B (2000) Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. EMBO J 19:4004–4014

Jones JDG (2001) Putting knowledge of plant disease resistance genes to work. Curr Opin Plant Biol 4:281–287

Jordan T, Schomack S, Lahaye T (2002) Alternative splicing of transcripts encoding Toll-like plant resistance proteins—what's the functional relevance to innate immunity. Trends Plant Sci 7:392–398

Kjemtrup S, Nimchuk Z, Dangl JL (2000) Effector proteins of phytopathogenic bacteria: bifunctional signals in virulence and host recognition. Curr Opin Microbiol 3:73–78

König H, Ponta H, Herrlich P (1998) Coupling of signal transduction to alternative pre-mRNA splicing by a composite splice regulator. EMBO J 17:2904–2913

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lawrence GJ, Finnegan EJ, Ayliffe MA, Ellis JG (1995) The *L6* gene for flax rust resistance is related to the *Arabidopsis* bacterial resistance gene *RPS2* and the tobacco viral resistance gene *N*. Plant Cell 7:1195–1206

Lorkovic ZJ, Wieczorek Kirk DA, Lambermon MH, Filipowicz W (2000) Pre-mRNA splicing in higher plants. Trends Plant Sci 5:160–167

Luck JE, Lawrence GJ, Dodds PN, Shepherd KW, Ellis JG (2000) Regions outside of the leucine-rich repeats of flax rust resistance proteins play a role in specificity determination. Plant Cell 12:1367–1377

Lupas A, Van Dyke M, Stock J (1991) Predicting coiled coils from protein structures. Science 252:1162–1164

Mackey D, Belkhadir Y, Alonso JM, Ecker JR, Dangl JL (2003) Arabidopsis RIN4 is a target of the type III virulence effector AvrRpt2 and modulates RPS2-mediated resistance. Cell 112:379–389

Marathe R, Anandalakshmi R, Liu Y, Dinesh-Kumar SP (2002) The tobacco mosaic virus resistance gene, *N*. Mol Plant Pathol 3:167–172

McDowell JM, Dangl JL (2000) Signal transduction in the plant immune response. Trends Biochem Sci 25:79–82

McDowell JM, Dhandaydham M, Long TA, Aarts MGM, Goff S, Holub EB, Dangl JL (1998) Intragenic recombination and diversifying selection contribute to the evolution of downy mildew resistance at the *RPP8* locus of *Arabidopsis*. Plant Cell 10:1861–1874

Medzhitov R, Preston-Hurlburt P, Kopp E, Stadlen A, Chen C, Ghosh S, Janeway CAJ (1998) MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. Mol Cell 2:253–258

Meyers BC, Chin DB, Shen KA, Sivaramakrishnan S, Lavelle DO, Zhang Z, Michelmore RW (1998) The major resistance gene cluster in lettuce is highly duplicated and spans several megabases. Plant Cell 10:1817–1832

Meyers BC, Dickerman AW, Michelmore RW, Sivaramakrihnan S, Sobral BW, Young ND (1999) Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. Plant Cell 20:317–332

Meyers BC, Morgante M, Michelmore RW (2002) TIR-X and TIR-NBS proteins: two new families related to disease resistance TIR–NBS–LRR proteins encoded in Arabidopsis and other plant genomes. Plant J 32:77–92

Meyers BC, Kozik A, Griego A, Kuang H, Michelmore RW (2003) Genome-wide analysis of NBS–LRR-encoding genes in Arabidopsis. Plant Cell 13:809–834

Modrek B, Lee C (2002) A genomic view of alternative splicing. Nat Genet 30:13–19

Nimchuk Z, Eulgem T, Holt BE, Dangl JL (2003) Recognition and response in the plant immune system. Ann Rev Genet 37:579–609

Nombela G, Williamson VM, Muniz M (2003) The root-knot nematode resistance gene Mi-1.2 of tomato is responsible for resistance against the whitefly *Bemisia tabaci*. Mol Plant–Microbe Interact 16:645–649

Parker JE, Coleman MJ, Szabo V, Frost LN, Schmidt R, van der Biezen EA, Moores T, Dean C, Daniels MJ, Jones JDG (1997) The Arabidopsis downy mildew resistance gene *RPP5* shares similarity to the Toll and Interleukin-1 receptors with *N* and *L6*. Plant Cell 9:879–894

Parniske M, Hammond-Kosack KE, Golstein C, Thomas CM, Jones DA, Harrison K, Wulff BBH, Jones JDG (1997) Novel disease resistance specificities result from sequence exchange between tandemly repeated genes at the *Cf-4*/*9* locus of tomato. Cell 91:821–832

Richly E, Kurth J, Leister D (2002) Mode of amplification and reorganisation of resistance genes during recent *Arabidopsis thaliana* evolution. Mol Biol Evol 19:76–84

Rossi M, Goggin FL, Milligan SB, Kaloshian I, Ullman DE, Williamson VM (1998) The nematode resistance gene Mi of tomato confers resistance against the potato aphid. Proc Natl Acad Sci USA 17:9750–9754

Schornack S, Ballvora A, Gurlebeck D, Peart J, Ganal M, Baker B, Bonas U, Lahaye T (2004) The tomato resistance protein Bs4 is a predicted non-nuclear TIR-NB-LRR protein that mediates defense responses to severely truncated derivatives of AvrBs4 and overexpressed AvrBs3. Plant J 37:46–60

Shao F, Golstein C, Ade J, Stoutemyer M, Dixon JE, Innes RW (2003) Cleavage of Arabidopsis PBS1 by a bacterial type III effector. Science 301:1230–1233

Simpson GG, Filipowicz W (1996) Splicing of precursors to mRNA in higher plants: mechanism, regulation and sub-nuclear organisation of the spliceosomal machinery. Plant Mol Biol 32:1–41

Sinapidou E, Williams K, Nott L, Bahkt S, Tor M, Crute I, Bittner Eddy P, Beynon J (2004) Two TIR : NB : LRR genes are required to specify resistance to Peronospora parasitica isolate Cala2 in Arabidopsis. Plant J 38:898–909

Song WY, Pi LY, Wang GL, Gardner J, Holsten T, Ronald PC (1997) Evolution of the rice *Xa21* disease resistance gene family. Plant Cell 9:1279–1287

Sun Q, Collins NC, Ayliffe M, Smith SM, Drake J, Pryor T, Hulbert SH (2001) Recombination between paralogues at the *Rp1* rust resistance locus in maize. Genetics 158:423–438

Takahashi H, Miller J, Nozaki Y, Takeda M, Shah J, Hase S, Ikegami M, Ehara Y, Dinesh-Kumar SP (2002) RCY1, an Arabidopsis thaliana RPP8/HRT family resistance gene, conferring resistance to cucumber mosaic virus requires salicylic acid, ethylene and a novel signal transduction mechanism. Plant J 32:655–667

Whitham S, Dinesh-Kumar SP, Choi D, Hehl R, Corr C, Baker B (1994) The product of the tobacco mosaic virus resistance gene N: similarity to Toll and the Interleukin-1 receptor. Cell 78:1101–1115

Zhang XC, Gassmann W (2003) RPS4-Mediated disease resistance requires the combined presence of RPS4 transcripts with full-length and truncated open reading frames. Plant Cell 15:2333–2342

Zhu W, Schlueter SD, Brendel V (2003) Refined annotation of the Arabidopsis genome by complete expressed sequence tag mapping. Plant Physiol 132:469–484