Theor Appl Genet (1999) 98 : 509–520 © Springer-Verlag 1999

D. Leister · J. Kurth · D. A. Laurie · M. Yano
T. Sasaki · A. Graner · P. Schulze-Lefert

# RFLP- and physical mapping of resistance gene homologues in rice (*O. sativa*) and Barley (*H. vulgare*)

Received: 24 July 1998 / Accepted: 14 August 1998

**Abstract** The deduced peptide sequences of 25 gene fragments of NBS-LRR resistance (*R*) gene homologues from rice and barley and of characterized *R* genes were compared, revealing a string of six conserved motifs. Mapping of the *R*-gene candidates in rice showed linkage to genes conferring race-specific resistance to rice blast (*Pi-k*, *Pi-f* and *Pi-1*) and bacterial blight disease (*Xa-1*, *Xa-3* and *Xa-4*), in barley to powdery mildew (*Mla*) and the rust fungus (*Rpg1*). In rice four mixed clusters were detected, each harboring at least two highly dissimilar NBS-LRR genes. A YAC-contig was established for one of these mixed clusters. YAC fragmentation experiments revealed the presence of at least five NBS-LRR genes within 200 kb in head-to-tail orientation.

**Key words** Resistance gene · RFLP · YAC contig · Fragmentation · Gene cluster

Communicated by F. Salamini

D. Leister[1] (✉) · J. Kurth · P. Schulze-Lefert
The Sainsbury Laboratory, John Innes Centre, Colney Lane, NR4 7UH Norwich, UK

D. A. Laurie
The John Innes Centre, Colney Lane, NR4 7UH Norwich, UK

M. Yano · T. Sasaki
Rice Genome Research Program/STAFF Institute, 446-1, Ippaizuka, Tsukuba, Ibaraki 305, Japan

A. Graner
Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Corrensstr. 3, D-06466 Germany

*Present address:*
[1]Max-Planck-Institute for Plant Breeding, Carl von Linne Weg 10, D-50829 Cologne, Germany
Fax: +49-221-5062-413
E-mail: leister@mpiz-koeln.mpg.de

DL and JK contributed equally to this work

## Introduction

The arrest of attempted pathogen attack is mediated in almost every characterized plant pathogen interaction by cognate genes in the host (resistance gene; *R* gene) and pathogen (avirulence gene; *Avr* gene; Flor 1971). These gene-for-gene relationships explain disease resistance to all classes of plant pathogens. The specificity of these interactions has been used as an argument that a host resistance reaction is triggered by a recognition event in which *R* and *Avr* genes are either directly or indirectly involved. Several *R* genes corresponding to race-specific interactions have been isolated by transposon tagging or map-based cloning approaches (reviewed in Baker et al. 1997). Although these genes were isolated from distantly related species (tomato, tobacco, *Arabidopsis*, flax and rice) and although they control resistance to viral, bacterial or fungal isolates, their deduced gene products reveal structural similarities. A comparison of the shared motifs has led to the definition of at least two *R*-gene classes (Hammond-Kosack and Jones 1997; Jones and Jones 1997). The major class comprises genes containing both a 5′ terminal nucleotide binding site (NBS; Traut 1994) and 3′ terminal leucine-rich repeats (LRR) of various length. This NBS-LRR class consists of *Rps2*, *Rpm1*, *N*, *L6*, *M*, *Rpp5* and *Xa-1* (Bent et al. 1994; Mindrinos et al. 1994; Whitham et al. 1994; Grant et al. 1995; Lawrence et al. 1995; Anderson et al. 1997; Parker et al. 1997; Yoshimura et al. 1998).

Although the overall sequence homology among members of the NBS-LRR class is low and insufficient to be detected by cross-hybridization, short stretches of peptide sequences are well-conserved (Staskawicz et al. 1995; Dangl 1995; Hammond-Kosack and Jones 1997; Jones and Jones 1997). The conserved motifs, adjacent to the NBS, enabled a novel polymerase chain reaction (PCR)-based approach that used degenerate primers to amplify resistance gene homologous sequences from

plant genomes. This approach was successfully applied to isolate NBS-LRR genes from several monocot and dicot species (Leister et al. 1996, 1998; Kanazin et al. 1996; Yu et al. 1996). The possible functions of the homologue sequences were evaluated by testing their linkage to characterized resistance specificities by means of restriction fragment length polymorphism (RFLP) mapping. Twelve NBS-LRR genes representing 7 different classes in potato were isolated and subsequently, revealed either linkage or cosegregation with a fungal and nematode resistance locus (Leister et al. 1996). Similar results were obtained independently by Kanazin et al. (1996) and Yu et al. (1996) who isolated 9 and 11 different classes of NBS-LRR genes in soybean, respectively, and detected correlations with characterized resistance loci.

A common feature of many but not all *R* genes is their apparent complex physical organization (Hulbert and Michelmore 1985; Hulbert and Bennetzen 1991; Ellis et al. 1995; Holub and Beynon 1996). This was shown for both NBS-LRR and LRR/Kinase-type *R* genes. The *R* genes *N* from tobacco, *Pto* and the *Cf* genes in tomato, *Xa-21* in rice and *M* in flax were found to detect several cross-hybridizing homologues, often at the same genetic loci, forming small gene families (Whitham et al. 1994; Martin et al. 1993; Jones et al. 1994; Song et al. 1995, 1997; Anderson et al. 1997). A characterization of the complex organization of *Pto* revealed the presence of several *Pto* homologues in a region of approximately 70 kb (Jia et al. 1997), for *M* approximately 15 related genes clustered in less than 1,000 kb (Anderson et al. 1997) and for the *Cf-9* family in *L. pimpinellifolium* 5 copies clustered within 50 kb (Parniske et al. 1997). This complex structure may suggest mechanisms of *R*-gene evolution, but it complicates map-based cloning approaches since it increases the number of *R*-gene candidates in the region of interest.

Comparative mapping of rice and barley NBS-LRR homologues in rice, barley and foxtail millet frequently revealed a lack of co-linearity (synteny) among these closely related species. This indicates a faster gene diversification for NBS-LRR genes than for the rest of monocot genomes (Leister et al. 1998). In this study we describe the analysis of NBS-LRR homologue sequence diversity and conservation and their linkage to genetically characterized *R* genes in rice and barley. We also provide a detailed analysis of the physical organization of a detected *R*-gene homologue cluster in rice.

## Materials and methods

### RFLP and Southern analysis

RFLP and Southern analysis with rice and barley genomic DNA was performed as described earlier (Leister et al. 1998). For Southern analysis of YACs total yeast DNA (100 ng) was digested with *Hin*dIII, submitted to electrophoresis on a 0.6 % (w/v) agarose gel and transferred to nylon membranes using standard protocols (Sambrook et al. 1989). Hybridization experiments with yeast and rice DNA were performed using the ECL system (Amersham) and in barley by use of [$^{32}$P]-labeled probes.

### Sequence analysis

Sequence data were analysed with the Genetics Computer Group, Wisconsin Programme, version 8 (GCG; Devereux et al. 1984). Display of aligned deduced amino acid sequences was carried out by using the programme "BOXSHADE".

### Construction of the rice YAC contig

DNA sequences of the five RFLP markers R1506, Y6854L, L1044, Y2668L and G181, previously mapped in the telomeric region of rice chromosome 11 (Kurata et al. 1994), were obtained from the *GenBank* database, and primers were derived for the generation of PCR markers (Table 1). Sequences of YAC termini were obtained according to Leister et al. (1997) and converted into PCR-based markers (Table 1). The insert size of the identified rice YACs was evaluated by the preparation of high-molecular-weight (HMW) DNA according to Kleine et al. (1993). Subsequent pulsed field gel electrophoresis (PFGE) analysis was carried out by using the CHEF-DR III System (Biorad) using the following parameters: 28-h run time, 120°, switch time of 15–100 s, 1% (w/v) agarose, 5.5 V/cm. Southern blots of PFGE-separated yeast HMW DNA were hybridized by probing with *Hin*dIII-cut pYAC4 vector DNA.

**Table 1** PCR primer sequences

| Primer | Sequences (5′ → 3′) |
|---|---|
| R2s | TCTTCGTAATATGGGAATTCGA |
| R2as | TCTCTTGGTTGATGTTCATGC |
| R10s | GTTCTTAGTAATATGAAAATTCATT |
| R10as | CCTGTGTCCCGTAGATTTTTC |
| 2668Ls | GAATTCACCGGAATGGATTTTC |
| 2668Las | CACTATAGGGAGACTGTGGA |
| 6854Ls | CGGCCGCCTCAGCCTCGC |
| 6854Las | GAATTCGGATATCCTTTTTCAC |
| L1044s | GATCCTAAAATTATCCTCCATG |
| L1044as | CCAGGGATGCCACCAACAGC |
| G181s | CTGCAGGGAAGGGAAGTTCA |
| G181as | TGAAGAACAATAGACCTCATGG |
| R1506s | CTGGTGATTTGGTGAAAGTCT |
| R1506as | ACTCTCCTGACCACCAAAGC |
| 2977Ls | GAATTCCAAACTTTAGAAGC |
| 2977Las | GATATCTATTTTAAAAGTTATGG |
| 0043Ls | GAATTCCAGTTCGATCTACGG |
| 0043Las | AAGCTTTGCTTTTAAAAGAAATGTC |
| 2977Rs | GAATTCATCCTTTTTCCTTTTGAA |
| 2977Ras | GTTGCAGATTTGGGGGAGTGA |
| 5131Ls | GAATTCTTCAGGTTTTGCTGGA |
| 5131Las | AGCGGATTCATCCTGAAAGTAGT |
| 6416Ls | GATGCGGCACGGAGATGCTA |
| 6416Las | GGAGATACCATGAGGTATTATAAG |
| 2668Rs | CATAGACTGCACCTGATCTATG |
| 2668Ras | GCGACCAGAGGTATCTCCAA |
| 2668Ls | GAATTCACCGGAATGGATTTTC |
| 2668Las | TCCCCGGAATAAGGGAAGACAT |
| 5131Rs | GAATTCTACAGCTACATATTTTTTG |
| 5131Ras | GTCGACTGAAATAAGCATGATAA |
| 6416Rs | TACGGCCGGATGCAACGTGC |
| 6416Ras | GAAACTGACATGTGGGTCCC |

| | (I) P-Loop/Kinase-1a | (II) Kinase-2 | (III) | (IV) "GLPLAL" |
|---|---|---|---|---|
| *Rps2* | rgIIgVyGpGGVGKTTLmqsInne | rqKRfLLLLDDVWee | ckVMfTTRsi | cgGLPLALitLGgaMah |
| *Rpm1* | riVVaVvGMGGsGKTTLsanIFks | qsKRyIVVLDDVWt. | srVMMTTRdm | cqGLPLAIasLGsmMst |
| *Rpp5* | arMVgIwGqsGIGKSTIgraLFsq | nhKKvLILLDDV... | srIIViTqdr | vgsLPLGLsvLGssL.. |
| *Prf* | ldVIsIvGMpGLGKTTLakkIYnd | ltKRfLILIDDVWd. | srIILTTRln | crGLPLSVvlVAgvLkq |
| *I2C1* | laVVpIvGMGGMGKTTLakaVYnd | ngKRfLVVLDDVWnd | skIIVTTRke | ckGLPLALkaLAgmLrs |
| *N* | vrIMgIwGMGGVGKTTIaraIFdt | rsKKvLIVLDDI... | srIIITTRdk | akGLPLALkvwGslL.. |
| *L6* | vtMVgLyGMGGIGKTTtakaVYnk | srfKiLVVLDDV... | srfIITSRsm | taGLPLtLkvIGslL.. |
| *M* | vtMVgLyGMGGIGKTTtakaVYnk | sksKiLVVLDDV... | trfIITSRnq | tgGLPLtLkvtGsfL.. |
| *Xa1* | itVLpIvGnGGIGKTTLaqlVckd | ksKKFLIVLDDVWei | nmIILTTRiq | lkGnPLAaktVGslLgt |
| r1 | vsVLpIvGMGGLGKTTLtqlVYnd | egKRfLLVLDDVWne | srIVVTTRnk | lkGLPLAakaIGslLct |
| r2 | vykLaIvGtGGVGKTTLaqkIFnd | anKsffLVLDDVWn. | gvILITTRdd | cgGLPLAIrvIAtvLas |
| r3 | -------LaqkIYne | rgKsvfLVLDDVWk. | shILVTSRnl | gy------ |
| r4 | -------LarfVYrd | reKRfLIILDDMWed | caVLaTTRrn | lk------ |
| r5 | -------LaqkIYnd | seKsfLLVLDDVWq. | gvILVTTRld | cy------ |
| r6 | -------LaanVYrn | mdqKyLIVLDDVWv. | srVLVTTRid | ce------ |
| r7 | -------L...Veqk | keqKyLLVIDg.... | srIVhiT..e | tg------ |
| r8 | -------LvayVYya | hgKRyVLILDDVWa. | .rfVITSRih | cs------ |
| r9 | -------LaqmVYnd | qdmKffLVLDNVWnv | gmILLTTRde | cg------ |
| r10 | -------LaqkIFnd | adKsffLVLDDVWh. | giILVTTRde | cgGLPLAIraIAkvLas |
| r11 | -------LaqlVYnd | khKmvLLVLDDLWne | cmIIVTTRse | ckGLPLAIktLAsmLcy |
| r12 | -------LaqlVYnd | qgKsfLLVLDDVWte | crIMVTTRne | cr------ |
| r13 | -------LtqlVYnd | kgKRfLLVLDDVWne | skIMVTTRne | lr------ |
| r14 | -------LailVFne | htRRvLLVLDDVqtd | smVLVTTRyh | le------ |
| r15 | ipVLgIwGMGGVGKTTLlklIhne | wnKnfLLLLDDLWek | hkVVLaTRse | ckGLPLALvsVGrtMsi |
| r16 | -------LarlVYnd | kgKKiLLVfDDVWne | shMIITTRne | sd------ |
| b1 | -------Llhvfnnd | arKRfVILLDDVrkk | skLILTSRyq | cgGLPLALnvIGtaVag |
| b2 | -------LaryVYhd | rnKRfLLVLDDMWed | cmVLaTTRtk | lk------ |
| b3 | -------LvhhVYka | qgsKyVLILDDVWn. | | ------ |
| b4 | lsILpIvGMGGLGKTTLtqlVYnd | kgKRfLLVLDDVWne | srIVVTTRnk | lk------ |
| b5 | lnVLpVvGIGGVGnTTLarfVckd | rnKRfLLVLDDMWed | cmILaTTRmd | lkGcPLAaqsVGalLnt |
| b6 | srVItVsGMGGLGKTTLvknVYdr | qdRKcLIVLDDVWd. | chVMITTRke | cr------ |
| b7 | -------LareVYrk | qdKRyLIVIDDIWs. | nrIIaTTRvv | cg------ |
| b8 | -------LakeLYrr | qdKRyLIVIDDVWa. | sgILMTTevd | cdGLPLAVvtVAglLvn |
| b9 | -------LakmVYnd | gkKRfMLVLDDVWde | svIVVTTRsq | crGLPLALktMGglLss |
| *Consensus* | XX.X.GXGGXGKTTL...XX | KR.XXVLDDVW | XXXTTR | GLPLAX..XX..L |

| | (V) | (VI) |
|---|---|---|
| *Rps2* | alLkfSYdnLes | vkMHNVVrsfAl. |
| *Rpm1* | siMfLSFndL.p | fkMHDVIweIAl |
| *Rpp5* | etLrVgYdrLnk | ieMHNLLekLGre |
| *Prf* | .iIgfSYknL.p | crIHDLLhkfcm. |
| *N* | dkLkISYdgLep | vqMHDLIqdMGky |
| *I2C1* | paLmLSYndL.p | flMHDLVndLAq. |
| *L6* | drLkISYdaLnp | fkMHDqLrdMGre |
| *M* | drLkISYdaLka | leMHDqLrdMGre |
| *Xa1* | qaLkLSYdhL.s | fvMHDLMhdLAqk |
| r1 | paLrLSYnhL.p | yvMHDaMhdLAq |
| r2 | gaLyLSYevL.p | |
| r10 | gaLyLSYevL.p | ckMHDLLrqLAs |
| r11 | psLeLSYknM.p | |
| r15 | atLkLTYdnLss | |
| b1 | gqLkySYdsLtp | vkMHhVIrqwGfg |
| b5 | pvLkLSYdyL.p | yvMHDLMheLAg |
| b8 | qvLnLSYnnL.p | |
| b9 | siLkLSYihL.s | |
| *Consensus* | L.XSY..L | MHDXX..XX |

**Fig. 1** Alignment and consensus sequences between characterized *R* genes and deduced amino acid sequences of rice and barley *R* gene candidates. Consensus sequences of aligned rice (*r*) and barley (*b*) *R* gene candidates and the *R* genes *N*, *Rps2*, *Rpm1*, *Rpp5*, *Prf*, *12C1*, *L6*, *M* and *Xa-1* are indicated. Regions of homology are highlighted in *black* (identity), *dark gray* (highly conservative exchange) or *light gray* (conservative exchange). Conserved motifs are designated *I–VI*

A
5
0.0 TEL1
88.1 C624
C1402
98.9 Os-b8.2
99.4
102.5 Os-(r1,r13)/b4
103.5 C1018
123.7 C1230
4
0.0 R2373
95.9 R2737
C600
107.9
[Xa-1] 108.0 Os-r4.1/b2.1 RHC-D
114.0 R99
129.0 C445
1
0.0 R687
11.5 Os-r3 C936
19.1 C470
114.1 3.0 Os-b9.1 C122
3
0.0 R1468A
73.4 C1677
87.3 Os-r9 C269B
97.4 R19
168.5
9
0.0 C152
9.6 P107
25.2 P104
27.9 Os-r15 Os-r16 RHC-C
36.4 C1454
42.3 R79
43.2 Os-r14
46.7 C397
100.5 L984
2
0.0 C1357
50.5 G243 Os-b9.2
51.3
54.7 C196 -3.0
156.1 C1470
8
0.0 P122
42.2 C1121
53.7 G104
[Pi-11(t)] 54.4 Os-r6.3
56.7 C411
63.6 C623
116.5 Os-b9.3
124.8 C83
10
0.0 L769
8.4 R1933
10.9 Os-r8
11.7 C913A
20.3 Os-r6.1 G1084
22.3 P133
85.6 C223
7
0.0 R1744
68.5 L1073
71.1 Os-r7
76.1
128.7 C794B
11
0.0 TEL2B
36.4 G320
37.2 Os-b6
59.9 G202
66.2 Os-r4.2/b2.2
66.5 Os-r11 RHC-B
67.7 Os-r12
70.5 G257
[Pi-7(t)]
87.1 P32
88.1 Os-r6.2
89.1 C189
[Xa-10] 95.3 G4001
102.1 Os-r5
102.6 G1465
[Pi-1(t)] G181
[Xa-4][Xa-3] 116.0 Os-r(2,10)
117.8 Os-r6.4 RHC-A
Os-b8.1
[Pi-k][Pi-f] 123.3 TEL3
B
1H
(I x F) (I x T) (B x K)
0 Hor2 0 0 Hor2
Mla-6 4 Hv-r6 4 1 Hor1
[Ml-k] 6 Hv-b6.1 Hv-b6.2
9 Hv-b7.1 12 Hv-b6.1 3 PSR805a
10 5
[Ml-nn] 20 MWG896 20 Hor1
30 ABG500a
33 Hv-r1
38 cMWG758
132 ABG55 130 BCD304 154 PSR305a
2H
(I x F) (I x T)
0 MWG844a 0 PSB31
80 MWG874
91 MWG865
97 MWG2240 96 PSB141
105 Hv-b2.1
109 MWG866 112 Hv-b2.2
117 PSB130
124 MWG882
162 WG645
204 MWG2225
3H
(I x F) (C x Hs) (I x T)
0 cMWG691 0 C30 0 Hv-b6.3
1 MWG584
Hv-b6.3
MWG595
43 MWG584 22 P19M81-3-1
53 MWG595
101 R1545
108 Cxp1
112 C742 112 Cxp1
125 C742
147 MWG2149
150 Hv-b3.1 144 PSB77a
166 C112 150 C112
167 MWG85
4H
(I x T) (B x K)
0 WG622 0 WG622
50 PSR305
56 Hv-b3.2
58 MWG58 58 PSR604b
[Ym-11] 63 PSR305
70 WG181
124 bAmy1
155 bAmy1
5H
(I x F) (I x T)
0 MWG502 0 MWG502
79 ABC168
83 84 BCD351e
BCD351e
95 MWG604
102 PSR120
111 MWG914
114 Hv-b8 119 BCD221c
120 MWG550 120 Hv-b8
121 PSR145
131 PSB301
152 CDO506
240 MWG891
6H
(I x T)
0 PSR899
112 PSR154
[Rph-11] 122 Hv-b7.2
130 Hv-b2.3
137 PSB56
7H
(I x F) (I x T) (B x K) (C x Hs)
0 ABG312 0 Hv-b9 0 PSR392 0 PSR634b
[Rpg-1] 3 Hv-b9 PSB95 Hv-b9 4 Hv-b9
5 MWG851a BCD129 BCD129
wx 13 PSR835
CDO475(S)
22 MWG530
29 wx
15 PSR965
16 PSR148
201 MWG2062

Fragmentation

The YAC clone Y5131 was transferred from its original genetic background (strain AB1380: *his5*) into the strain YLBW3 (*his3-Δ200*; Hamer et al. 1995) by the *kar1*-transfer procedure (Spencer et al. 1994) to enable the use of a fragmentation vector carrying *HIS3*. The resulting YAC clone Y5131(W) was analysed by PFGE and PCR (with markers for the loci: *Y5131L*, *Y0043L*, *Y6854L*, *Y2668R*, *Y6854L′*, *Os-r2.2*, *Y2977R*, *Y6416R*, *Os-r2.1*, *Os-r10* and *Y5131R*) for integrity. Six different constructs were used for fragmentation: the rice *R*-gene homologues r2, r6 and r10 were introduced in both directions into vector pBP103 (Pavan et al. 1991) by directional cloning (by ligating either an *Apa*I/*Not*I or a *Spe*I/*Sac*II restriction fragment from pGEM-T into pBP103). *LacZ*-positive clones were verified by PCR. Transformation with *Sph*I-linearized constructs was performed using the Alkali-Cation yeast transformation system (Bio101). Transformants were recovered in 100 μl SOS medium and spread onto SD lacking tryptophan and histidine (SD/-Trp-His). Fast-growing clones were picked out and transferred onto SD/-Trp-His plates and onto AHC plates lacking uracil and tryptophan. Clones growing on SD/-Trp-His, but not growing on AHC, exhibited the desired phenotype (HIS +, URA –) and were analysed for the presence of the above-mentioned PCR markers and by PFGE of yeast HMW-DNA.

## Results

### Conserved protein motifs in barley and rice NBS-LRR homologues

The isolation of NBS-LRR homologues from rice and barley via PCR from genomic DNA and subtracted DNA or cDNA, respectively, has been described before (Leister et al. 1998). A total of 16 different NBS-LRR genes from rice (designated r1–r16) and 9 different NBS-LRR homologues from barley (designated b1–b9) were isolated. The application of primers matching consensus sequences GG(V/I)GKTT and GLPL(A/T)L, derived from characterized dicot *R* genes, did not provide authentic sequence information of these two domains in the isolated monocot NBS-LRR homologues. To obtain sequence information of these and other possibly shared motifs between dicot and monocot NBS-LRR genes, we had to extend the barley and rice PCR products by rapid amplification of cDNA ends (RACE).

Analysis of the extension products with previously characterized *R* genes revealed at least six conserved peptide motifs (Fig. 1) of which two, the P-loop/Kinase-1a (motif I) and "GLPLAL" (motif IV), represent the targets of the employed consensus primers. Motifs II and III, located between the annealing sites of the consensus primers, represent parts of the NBS together with motif I. At least two additional conserved motifs are located in 3′ orientation from the GLPLAL domain, designated motif V (consensus L..SY..L) and VI (consensus MHD). The presence of the latter two motifs and the "GLPLAL" consensus discriminates the isolated gene fragments from genes containing merely a NBS. The presence and order of motifs I–VI appear to be a shared feature of dicot NBS-LRR genes and the monocot *R*-gene homologues described here. Further downstream of motif V and VI leucine-rich repeats of the consensus (L)xxLxxLx(L) were found (data not shown). Sequences 5′ from the P-loop are more diverse. No conserved motif was identified, although individual *R*-gene homologues share weak similarities among each other and with characterized *R* genes.

### Linkage analysis of NBS-LRR homologues in rice and barley

Rice and barley *R*-gene homologues were used as probes in Southern analysis to detect corresponding loci in segregants of the rice *indica* × *japonica* crosses 'Kasalath' × 'Nipponbare' (Kurata et al. 1994) and 'IR20' × '63-83' (Quarrie et al. 1997), and in the barley crosses 'Igri × Franka' (Graner et al. 1991), 'Igri' × 'Triumph' (Laurie et al. 1995), 'Blenheim' × 'Kym' (Bezant et al. 1996) and 'Captain' × *Hordeum spontaneum* (Devos et al. 1993; Dunford et al. 1995) as described earlier (Leister et al. 1998). Since this previous study focused on the syntenic relationships of NBS-LRR homologue map positions, we provide here a comprehensive analysis of all detected map positions in rice and barley. In rice all 16 rice NBS-LRR homologues and five out of nine barley homologue probes could be mapped, detecting a total of 24 different loci on 10 of

**Fig. 2A, B** Map positions of rice (*r*) and barley (*b*) NBS-LRR genes. **A** Map positions of rice (*r*) NBS-LRR genes in progeny of the crosses 'Kasalath' × 'Nipponbare' and 'IR20' × '63-83'. The orientation of linkage groups 1, 2, 3, 4, 8, 11 and 12 is reversed compared to the original orientation according to the centromere mapping by Singh et al. (1996). Positions of resistance traits are given on the *left* of each chromosome. *Os*-loci were identified by using the NBS-LRR genes as marker probes for RFLP mapping, with the exception of *Os-r6.4* which was identified by physical mapping on YAC clones (see Fig. 3). Loci for resistance traits were anchored by flanking molecular or morphological markers. *R* genes to bacterial blight are: *Xa-1* (Yoshimura et al. 1996), *Xa-3* (Yoshimura et al. 1992), *Xa-4* (Yoshimura et al. 1992) and *Xa-10* (Xinghua et al. 1996; Ronald et al. 1992); rice blast resistance genes: *Pi-1(t)* (Xiao et al. 1992; Abenes et al. 1994), *Pi-7 (t)* (Wang et al. 1994), *Pi-11 (t)* (Causse et al. 1994), *Pi-k* (Khush and Kinoshita 1991) and *Pi-f* (Abenes et al. 1994). *R* gene homologue clusters (RHC) harboring at least two dissimilar NBS-LRR genes are located on rice chromosomes 4, 9, and 11. Allelic bridges are symbolized by *dotted lines*. **B** Map positions of barley (*b*) NBS-LRR genes derived from progeny analysis of the crosses 'Igri' × 'Franka' (I × F), 'Igri' × 'Triumph' (I × T), 'Captain' × '*Hordeum spontaneum*' (C × Hs), and 'Blenheim' × 'Kym' (B × K). The powdery mildew resistance gene *Mla-6* was previously mapped using I × F progeny (Graner et al. 1991). Loci for other resistance traits were anchored by flanking molecular or morphological markers: *Ml-k* and *Ml-nn* (resistance to powdery mildew; Saghai Maroof et al. 1994), *Rpg-1* (resistance to stem rust; Kilian et al. 1995), *ym-11* (resistance to yellow mosaic virus; A. Graner, personal communication) and *Rph-11* (resistance to leaf rust; Feuerstein et al. 1990). Allelic bridges are symbolized by dotted lines

the 12 linkage groups (Fig. 2A). Most probes detected apparently single loci with variable copy numbers whereas probes r4, r6, b2 and b9 detect 2 or more different loci. Interestingly, some probes map to the same genetic locus, although they do not cross-hybridize to each other, identifying *R*-gene homologue clusters (RHC) RHC-A (*Os-b8.1*, *Os-r6.4*, *Os-r2*, *Os-r10*), RHC-B (*Os-r12*, *Os-r11*, *Os-r4.2*) and RHC-C (*Os-r15*, *Os-r16*). In barley, most NBS-LRR probes also detected more than one locus and showed differences in copy numbers between different cultivars. Seven probes were mapped detecting 14 different loci (Fig. 2B). Two rice NBS-LRR probes, r1 and r6, detected clear cross-hybridizing fragments which could be located on barley chromosome 1HS (*Hv-r1* and *Hv-r6*) whereas other rice NBS-LRR probes revealed only weak signal intensities or no polymorphism.

The apparent linkage of NBS-LRR genes with map positions of previously described resistance loci is shown in Fig. 2. We tested in an exemplary manner in rice the linkage of *Os-r4.1*/*Xa-1* and in barley the linkage *Hv-b6*/*Mla-6*. Both *r4* and *Xa-1* represent members of NBS-LRR genes, and probes of each gene were found to cross-hybridize with the same chromosome 4-derived YAC Y5212 from cv 'Nipponbare' containing the single copy *Xa-1* homologue (Yoshimura et al. 1998). However, a comparison of the *r4*-gene fragment and the corresponding *Xa-1* domain reveals only 58.6% sequence identity at the nucleotide level (39% sequence identity at the protein level). Because YAC Y5212 physically links these two highly divergent NBS-LRR genes, we have designated the corresponding region RHC-D (Fig. 2A).

In barley, *Hv-b6.1* was found to cosegregate with the map position of powdery mildew resistance *Mla-6* in a population of 72 dihaploid progeny derived from the cross 'Igri' × 'Franka' (Graner et al. 1991). Next we tested this linkage on a high-resolution mapping population involving 1,800 individuals segregating for *Mla-6* (Descenzo et al. 1994). Eleven recombinants were identified between *Hv-b6.1* and *Mla-6*, positioning the *R*-gene homologue at a distance of 0.6 cM distal to *Mla-6*.

## Physical analysis of the RHC-A cluster in rice

The genetic clustering of highly divergent NBS-LRR genes prompted us to analyse their physical organization in more detail for the RHC-A cluster on rice chromosome 11. We identified seven rice YACs (Umehara et al. 1995) in colony hybridization experiments with the cross-hybridizing rice r2 and r10 probes which detected multiple and genetically linked copies on genomic Southerns. Southern analysis of the seven YACs with probe r2 showed a variable number of different cross-hybridizing copies (Fig. 3C). The genetically tight linkage of *Os-b8.1* (1.3 cM distal from *Os-r2/r10*) motivated us to test for the presence of b8 cross-hybridizing copies in the YACs positive for r2. Only YAC Y2977 DNA revealed the presence of multiple b8 cross-hybridizing copies (Fig. 3A). Unexpectedly, we discovered also one copy of r6 (*Os-r6.4)* in four of the YACs positive for probe r2, a locus that we were unable to locate on the basis of RFLPs (Fig. 3B). Finally, the presence of r10 could be distinguished from r2 copies in six of the seven YACs by specific primers derived from the r10 sequence (Fig. 3D and Materials and methods).

Insert sizes of each of the seven YACs were determined by PFGE separations, and YAC termini were isolated and converted into PCR-based markers (see Materials and methods). By integrating YAC end probes, RFLP markers known to be located in this genomic interval and PCR markers derived from the NBS-LRR homologues r2 and r10, we were able to deduce a physical contig of the seven rice YACs covering more than 950 kb (Fig. 3D). Two YACs, Y6854 and Y0043, were found to be chimaeric. The availability of a PCR-based marker for r2 in each of the YACs together with the Southern analysis data using the r2 hybridization probe led us to address the question of whether r2 copies could be differentiated in the contig. Restriction enzyme digestion of r2 amplification products with *Alu*I enabled us to discriminate at least two r2 copies (*Os-r2.1* and *Os-r2.2*), characterized by the presence or absence of the restriction site. Since YAC Y5131 was the only non-chimaeric clone in the contig containing all four rice NBS-LRR gene copies (*Os-r2.1*, *Os-r2.2*, *Os-r10* and *Os-r6.4*), the maximal physical region covered by these genes could be reduced to the corresponding YAC insert size of 420 kb.

To increase the resolution of the physical mapping, we carried out terminal deletions of Y5131 by selecting for homologous recombination events in the NBS-LRR genes (Pavan et al. 1990). Since homologous recombination can only occur if both sequences are in parallel orientation, fragmentation provides direct evidence for the orientation of the target sequence in the YAC. Acentric fragmentation of YAC Y5131 was performed by using a fragmentation vector which contains no centromere to delete inserts adjacent to the right (centromere-less) vector arm and in which either of the NBS-LRR homologues r2, r10 or r6 were inserted in both orientations. For each of the six constructs 200 clones exhibiting the expected auxotrophy were inspected for absence of the right YAC-end by PCR. Positive clones were characterized for presence or absence of PCR markers distributed within the insert of Y5131 and assembled into different groups accordingly (Fig. 4A).

For r2, three different fragmentation products (F-[r2.1] to F-[r2.3])were obtained, indicating the presence of an additional copy of this NBS-LRR homologue on Y5131 (designated *Os-r2.3*; see below). For r10 and r6 only one fragmentation product was

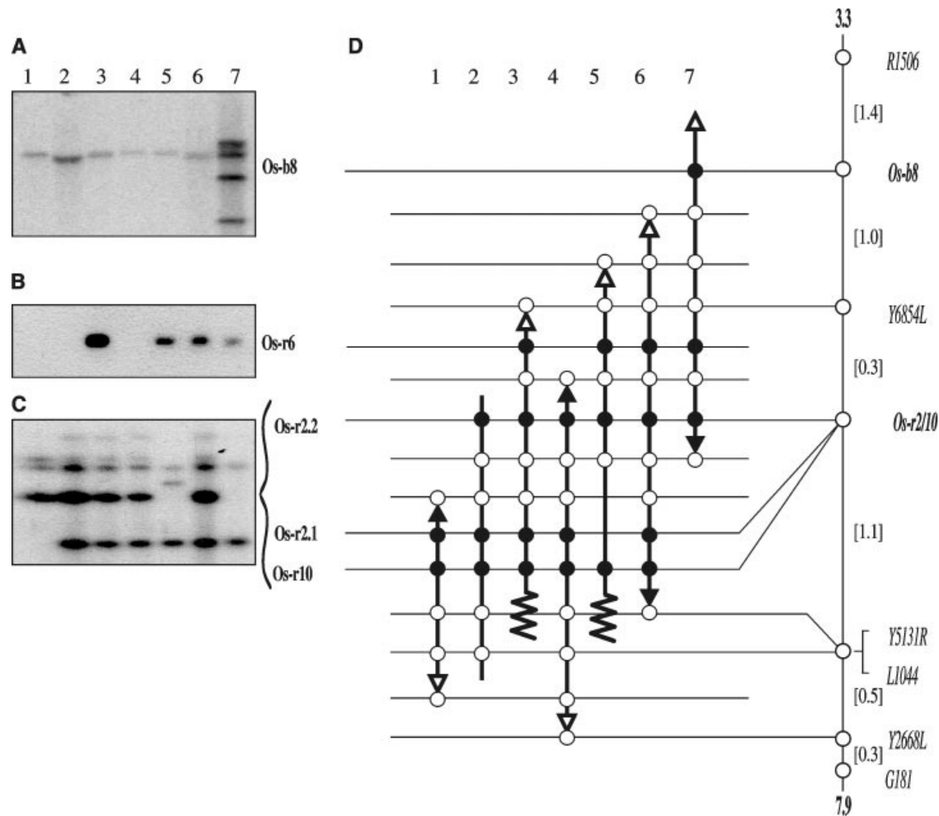


**Fig. 3A–D** A YAC contig of RHC-A on rice chromosome 11. **A–C** YAC-Southern analysis of Y6416 (*lane 1*), Y4894 (*2*), Y6854 (*3*), Y2668 (*4*), Y0043 (*5*), Y5131 (*6*) and Y2977 (*7*). DNA filters were probed with the barley probe b8 **A**, the rice probe r6 **B** and rice probe r2 **C**. Total yeast DNA was digested with *Hin*dIII prior to size separation by agarose gel electrophoresis. **D** Combined genetic and physical map at RHC-A. The relative position of relevant genetic markers and their distance in centiMorgans on rice chromosome 11 is shown on the *right* between the *square brackets*. The deduced overlap of rice YACs originally isolated by rice probe r2 is shown on the *left*. Insert sizes of the YACS were estimated after PFGE size separation (Y6416, 300 kb; Y4894, 400 kb; Y6854, 550 kb; Y2668, 520 kb; Y0043, >650 kb; Y5131, 420 kb; Y2977, 650 kb). *Black arrows* indicate right YAC termini, and *white arrows* indicate left YAC ends. *Bold-line circles* with *no filling* symbolize markers anchored in the RGP map (Kurata et al. 1994). *Zigzag lines* indicate chimaeric parts of YACs. *Small circles* with *no filling* indicate positions of PCR markers derived from YAC ends, and *black solid circles* indicate positions of NBS-LRR homologues that were identifiable as PCR markers with the exception of *Os-r6.4*, *Os-r2.1* and *Os-r2.2*, which were discriminated from each other by restriction enzyme digestion of r2 amplification products with *Alu*I and are characterized by the presence or absence of the restriction site, respectively. The only non-contiguous region is found in Y0043 between the marker Y2977R and *Os-r10* and may be due to an internal deletion

found. Although r2 and r10 cross-hybridize to each other (87% identity at nucleotide level) and generate an identical RFLP pattern on rice genomic DNA, distinct fragmentation products were obtained. Complementary information was obtained by probing the DNA of 2 independently isolated clones of each fragmentation product shown in Fig. 4A with r6 or r2 after Southern blotting of PFGE separations (Fig. 4B, C). Expectedly, the strong r6 hybridization signal obtained with DNA from the 90-kb fragmentation product F[Os-r6.4] showed only background signals if probed with r2. Interestingly, all fragmentation products obtained were derived from homologous recombination with fragmentation constructs that contained the NBS-LRR homologues r2, r6 and r10 in the same orientation, indicating that all detected NBS-LRR homologues on YAC Y5131 have a head-to-tail orientation.

## Discussion

We have shown the feasibility of isolating *R*-gene candidates of the NBS-LRR class in two monocot genomes, rice and barley, by using degenerate PCR primers matching conserved motifs of characterized dicot *R* genes. The efficiency of the approach appears to be related to genome size (in barley $5.3 \times 10^9$ bp/haploid genome equivalent; in rice $0.45 \times 10^9$ bp/haploid genome equivalent; Bennett and Smith 1991). In contrast to rice genomic DNA, barley genomic template DNA yielded no candidate *R*-gene fragment unless subtractive procedures or RT-PCR were applied to reduce the complexity of the target. Amplification

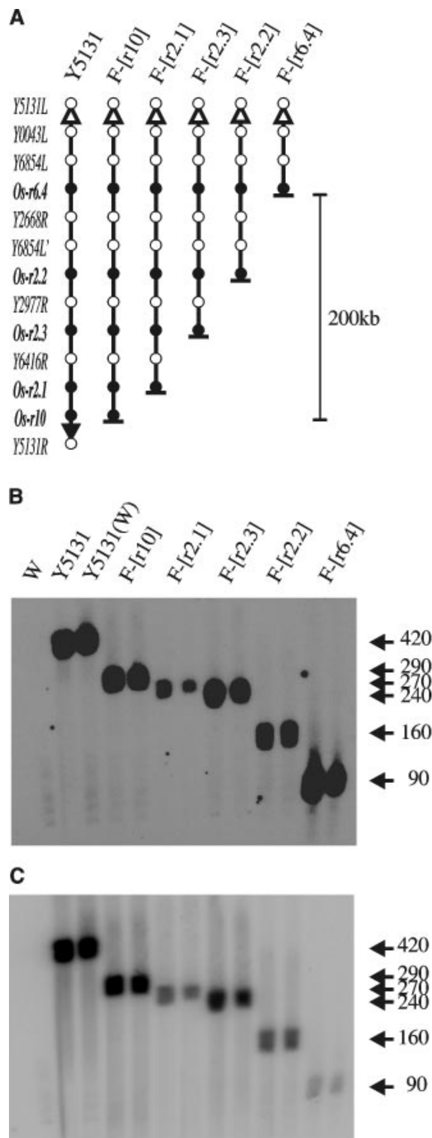


**Fig. 4A–E** Fragmentation analysis of rice YAC Y5131. **A** Analysis of YAC fragmentation products for the presence or absence of PCR markers as described in Fig. 3D. **B**, **C** PFGE-Southern analysis of the window strain (*W*), the native YAC Y5131 (in AB1380), Y5131 after transfer into the window strain [Y5131(W)] and five different fragmentation products of Y5131 (2 independent clones each) probed with r6 (**B**) and r2 (**C**). The transfer from AB1380 into the window strain shows no change in the insert size of Y5131 indicating stable propagation of the YAC. PFGE conditions were: 5–50 s, 26 h, 12°C, 5.5 V/cm, 1% (w/v) agarose. Fragmentation products at *Os-r2.1*, *Os-r2.2* and *Os-r2.3* were obtained with the NBS-LRR homologue r2 as fragmentation probe. Fragmentation products at *Os-r10* and *Os-r6.4* were obtained with r10 and r6, respectively, as fragmentation probes. Fragmentation constructs with r2, r6 and r10 lead only to fragmentation events when cloned in antiparallel orientation to the introduced telomere, indicating a head-to-tail orientation of *Os-r2.1*, *Os-r2.2*, *Os-r2.3*, *Os-r10* and *Os-r6.4*. **C** Note that probe r2 reveals only background signals in F[Os-r6.4]

products from non-subtracted barley genomic template revealed mainly retrotransposon sequences. Retrotransposons of the *BARE-1* family are known to be present in large numbers in barley (*Apa2*, a copia-like retrotransposon, constitutes approximately 2.4% of the barley genome; Kleine et al. 1993), and it has been shown that retrotransposons constitute at least 50% of the maize genome (SanMiguel et al. 1996). Their high copy number in barley is likely to have interfered with the generation of specific amplification products. The reduction in template complexity by subtractive approaches should also be applicable to other complex monocot genomes such as wheat and maize.

## Structure of candidate *R* genes

Structurally related genes of the NBS-LRR class have been shown to encode race-specific resistance to bacterial, viral and fungal pathogens in dicot species (Dangl 1995; Staskawicz et al. 1995). This finding implies that it is impossible to predict the role of a *R*-gene candidate to a particular class of pathogen from deduced amino acid sequences. We were unable to identify any signatures in the deduced monocot NBS-LRR protein sequences which would discriminate them from those in dicot plants (Fig. 1). The finding of at least six conserved sequence motifs amongst monocot and dicot plants is relevant for the following reasons. Each of these motifs and their order has been identified in the *ced-4* gene of *Caenorhabditis elegans* and its human homologue, *Apaf-1* (van der Biezen and Jones 1998). These two genes serve as adaptor proteins during programmed cell death in animal development (Hengartner and Horvitz 1994; Zou et al. 1997). Whether the shared motif string between mammalian cell death regulators and NBS-LRR genes indicates overlapping mechanisms with the hypersensitive host cell death response observed in plant race-specific resistance remains to be shown. Our findings indicate that plant NBS-LRR genes existed before the separation of monocot and dicot plant species from each other 120–200 million years ago. The lack of a monocot signature indicates a similar selection pressure towards sequence conservation for each detected motif after these two major classes of flowering plants have diverged from each other.

For 3 homologues (r1, r10, b1) for which carboxy terminal extensions 3′ of motif VI were isolated, we found the characteristic spacing of leucine residues reported for cytoplasmatic LRRs but no other conserved motifs (Jones and Jones 1997). It has been shown that a subclass of NBS-LRR genes (*N*, *L6*, and *Rpp5*) contains an aminoterminal domain which exhibits a low similarity to the TOLL/Interleukin-1R receptors (TIR domain; Parker et al. 1997), whereas the *R* genes *Rps2* and *Rpm1* contain a putative leucine zipper in this region (Bent et al. 1994; Mindrinos et al. 1994; Grant et al. 1995). We found no similarity to these domains in the six monocot homologues for which sequences 5′ from the P-loop domain are available,

but this could be explained by a lack of full length clones.

## How many NBS-LRR genes do plants have?

This study and related work in potato (Leister et al. 1996) and soybean (Kanazin et al. 1996; Yu et al. 1996) have shown an unexpected degree of amino acid variability among the homologues within each species even in those segments connecting the conserved subdomains in the NBS domain. This raises the question of how many distinct classes of NBS-LRR genes exist in a single plant species. The PCR assay isolated multiple clones of 13 rice and 9 barley classes, suggesting that we have reached saturation with the tested degenerate primer combinations.

Interestingly, comparable numbers of distinct NBS-LRR gene classes have been found in potato and soybean (6 and 9, respectively; Leister et al. 1996; Kanazin et al. 1996; Yu et al. 1996). It could be argued that this is due to a limited set of tested degenerate primer combinations in each species. This assumption is testable since the NBS-LRR candidates isolated from soybean (Kanazin et al. 1996; Yu et al. 1996) represent an independent sample isolated by using different priming sites (P-loop/kinase 3a and P-loop/GLPLAL motifs) and different methods to create degeneracy (inosine or presence of N = A/G/C/T). A comparison of the NBS-LRR classes in soybean reveals that only 3 out of 19 were isolated by both of these methods, favouring the idea that we have uncovered only a fraction of the actual number in the rice and barley genome. Similarly, when we compared 4 rice and 3 barley NBS-LRR homologues isolated either with a different set of degenerate primer combinations (Collins et al. 1998) or by homology to the wheat *Cre3* nematode *R*-gene family (Lagudah et al. 1997; Robertson et al. 1997; Seah et al. 1997) with those isolated with our technique, we found that they identify 2 additional novel classes of rice NBS-LRR homologues and 3 novel classes of barley NBS-LRR homologues. Taken together, the current data suggest that NBS-LRR genes represent a major diverse gene family in plant genomes and that this family shows unusual sequence variability also in domains flanking the LRR region. This becomes evident by a comparison of sequences isolated by RACE. There is a consensus of [G(M/T/I)GG(V/L)G(K/N)TT] for the P-loop and [G(L/C)PL(T/A)(L/V/I/A)] for the internal hydrophobic domain, revealing both a high sequence variability and an unexpected annealing flexibility of the degenerate primers that were originally designed for binding only to the consensus among *Rps2*, *N* and *L6*, respectively ([GG(I/V)GKTT] and [GLPL(T/A)L]).

The existence of a conserved string of six motifs between monocot and dicot NBS-LRR provides a basis to isolate other NBS-LRR homologues since the motifs expand the number of potential target sites which can be utilized to design degenerate primers. For example, motifs V and VI are likely to be useful targets in combination with either of the three NBS domains. Thus, the combinatorial use of primer combinations matching the conserved six motifs might open a route towards a comprehensive PCR-based isolation of NBS-LRR genes in any monocot or dicot species.

## NBS-LRR gene clusters

Many race-specific *R* genes are known to occur in clusters both in monocot and dicot plant species (Hulbert and Bennetzen 1991; Jones et al. 1993; Ellis et al. 1995; Holub and Beynon 1996; Anderson et al. 1997; Parniske et al. 1997). Clustering has been observed both for NBS-LRR as well as *Cf*-like *R* genes. Noteworthy is the observation that *R* genes to different pathogens can also be genetically tightly linked, as observed in potato, wheat and rice (Leonard-Schippers et al. 1992; DeJong et al. 1997; McIntosh 1992; Singh 1992; Song et al. 1995). This may provide clues as to how *R* genes evolve; evidence has been provided that unequal crossing-over and/or gene conversion events between related sequences contribute to the generation of new specificities (Sudupak et al. 1993; Ellis et al. 1995; Parniske et al. 1997). The repeated structure of LRRs may also promote DNA rearrangements within a *R* gene itself. The allelic series of flax rust specificities at the *L* locus (Lawrence et al. 1995) and the observation of a perfect 270-bp intragenic duplication event in a partially defective *Rpp5* allele (Parker et al. 1997) support this suggestion. In this study we have observed clusters of candidate *R* genes in rice (Figs. 2–4). The detailed physical analysis of the *RHC-A* cluster *via* YAC fragmentation enabled us to delimit the locus to 200 kb containing at least five head-to-tail copies of NBS-LRR genes. Particularly interesting is the observation that *RHC-A* is composed of a family consisting of 4 closely related NBS-LRR gene copies (*r2.1-3* and *r10*) and 1 unrelated (non-cross-hybridizing) NBS-LRR, *r6.4*. Another family of unrelated NBS-LRR homologues (*Osb8.1*) is physically linked to this cluster. All NBS-LRR homologues in the RHC-A cluster are arranged in a head-to-tail orientation. The same head-to-tail orientation has also been observed for a different class of plant *R* genes, the *Cf-9* gene family in tomato (Parniske et al. 1997). Direct repetitions of sequence-related genes are generally thought to increase the rate of novel sequence variants by presenting substrates for mechanisms like unequal crossing-over and gene conversion events between non-allelic homologues, rendering the evolutionary rate of an individual copy dependent on interactions between family members in a cluster (concerted evolution; Arnheim 1983; Dover 1993). Thus, tandem repeat organization of *R*-gene loci

may be a general mechanism to increase the rate of novel resistance specificities.

## Candidate resistances

If the isolated rice and barley NBS-LRR genes represent candidates of functional *R* genes, it would be expected that several exhibit genetic linkage to characterized resistance loci. Here we have shown that genetic and physical clustering of NBS-LRR homologues is frequently observed in rice and barley. This phenomenon and the abundance of NBS-LRR genes in plant genomes complicate the utilization of *R*-gene homologues as a tool to isolate biologically characterized resistance specificities. Thus, the availability of high-resolution genetic maps for a particular resistance specificity and of mutant alleles will be crucial complementary tools. One possibility would be to link the PCR-based approach for the isolation of NBS-LRR homologues with map-based isolation techniques, e.g. by a directed PCR-based isolation of homologues from large-insert genomic clones. The string of shared conserved motifs among monocot and dicot NBS-LRR genes, described above, may provide a rationale towards a comprehensive homologue isolation from physically delimited target intervals.

**Acknowledgements** This work was supported by a long-term EMBO post-doctoral fellowship to D.L., an EC biotechnology research grant to J.K., and a Gatsby Foundation grant to P.S.-L. The experiments comply with the current laws of the countries in which the experiments were performed.

## References

Abenes MLP, Tabien RE, McCouch SR, Ikeda R, Ronald P, Khush GS, Huang N (1994) Orientation and integration of the classical and molecular genetic map of chromosome 11 in rice. Euphytica 76: 81–87

Anderson PA, Lawrence GJ, Morrish BC, Ayliffe MA, Finnegan EJ, Ellis JG (1997) Inactivation of the flax rust resistance gene *M* associated with loss of a repeated unit within the leucine-rich repeat coding region. Plant Cell 9: 641–651

Arnheim N (1983) Concerted evolution of multigene families. In: Nei M, Koehn RK (eds) Evolution of genes and proteins. Sinauer Assoc, Boston, pp 38–61

Baker B, Zambryski P, Staskawicz B, Dinesh-Kumar SP (1997) Signaling in plant-microbe interactions. Science 276: 726–733

Bennett MD, Smith JB (1991) Nuclear DNA amounts in angiosperms. Philos Trans R Soc London 334: 309–345

Bent AF, Kunkel BN, Dahlbeck D, Brown KL, Schmidt R, Giraudat J, Leung J, Staskawicz BJ (1994) *RPS2* of *Arabidopsis thaliana*: a leucine-rich repeat class of plant disease resistance genes. Science 265: 1856–1860

Bezant JH, Laurie DA, Pratchett N, Chojecki J, Kearsey MJ (1996) Marker regression mapping of QTL controlling flowering time and plant height in a spring barley (*Hordeum vulgare* L.) cross. Heredity 77: 64–73

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Wu Z, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley S (1994) Saturated molecular map of the rice genome based on a interspecific backcross population. Genetics 138: 1251–1274

Collins NC, Webb CA, Seah S, Ellis JG, Hulbert SH, Pryor AJ (1998) The isolation and mapping of disease resistance gene analogs in maize. Mol Plant Microbe Interact 11: 968–978

Dangl JF (1995) Piece de resistance: novel classes of plant disease resistance genes. Cell 80: 363–366

DeJong W, Forsyth A, Leister D, Gebhardt C, Baulcombe D (1997) A potato hypersensitive resistance gene against potato virus X maps to a resistance gene cluster on chromosome 5. Theor Appl Genet 95: 246–252

Descenzo RA, Wise RP, Mahadevappa M (1994) High-resolution mapping of the Hor1-Mla-Hor2 region on chromosome 5S in barley. Mol Plant Microbe Interact 7: 657–665

Devereux J, Haeberli P, Smithies O (1984) A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res 12: 387–395

Devos KM, Gale MD (1993) Extended genetic maps of the homoeologous group 3 chromosomes of wheat, rye and barley. Theor Appl Genet 85: 649–652

Dover GA (1993) Evolution of genetic redundancy for advanced players. Curr Opin Genet Dev 3: 902–910

Dunford RP, Kurata N, Laurie DA, Money TA, Minobe Y, Moore G (1995) Conservation of fine-scale DNA marker order in the genomes of rice and the *Triticeae*. Nucleic Acids Res 23: 2724–2728

Ellis JG, Lawrence GJ, Finnegan EJ, Anderson PA (1995) Contrasting complexity of two rust resistance loci in flax. Proc Natl Acad Sci USA 92: 4185–4188

Feuerstein U, Brown AHD, Burdon JJ (1990) Linkage of rust resistance genes from wild barley hordeum-spontaneum with isozyme markers. Plant Breed 104: 318–324

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

Graner A, Jahoor A, Scholdelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of barley. Theor Appl Genet 83: 250–256

Grant MR, Godiard I, 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

Hamer L, Johnston M, Green ED (1995) Isolation of yeast artificial chromosomes free of endogenous yeast chromosomes: construction of alternate hosts with defined karyotypic alterations. Proc Natl Acad Sci USA 92: 11706–11710

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

Hengartner MO, Horvitz HR (1994) Programmed cell death in *Caenorhabditis elegans*. Curr Opin Genet Dev 4: 581–586

Holub EB, Beynon, JL (1997) Symbiology of mouse-ear cress (*Arabidopsis thaliana*) and oomycetes. Adv Bot Res 24: 228–273

Hulbert SH, Bennetzen JL (1991) Recombination at the *Rp1* locus of maize. Mol Gen Genet 226: 377–382

Hulbert SH, Michelmore, RW (1985) Linkage analysis of genes for resistance to downy mildew (*Bremia lactucae*) in lettuce (*Lactuca sativae*). Theor Appl Genet 70: 520–528

Jia Y, Loh YT, Zhou J, Martin GB (1997) Alleles of *Pto* and *Fen* occur in bacterial speck-susceptible and fenthion-insensitive tomato cultivars and encode active protein kinases. Plant Cell 9: 61–73

Jones DA, Jones IDG (1997) The roles of leucine-rich repeats in plant defences. Adv Bot Res Adv Planat Pathol 24: 89–167

Jones DA, Dickinson MJ, Balint-Kurti P, Dixon M, Jones JDG (1993) Two complex resistance loci revealed in tomato by classical and RFLP mapping of the *Cf-2*, *Cf-4*, *Cf-5* and *Cf-9* genes for resistance to *Cladosporium fulvum*. Mol Plant Microbe Interact 6: 348–357

Jones DA, Thomas CM, Hammond-Kosack KE, Balint-Kurti PJ, Jones JDG (1994) Isolation of the tomato *Cf-9* gene for resistance

to *Cladosporium fulvum* by transposon tagging. Science 266: 789–793

Kanazin V, Marek LF, Shoemaker RC (1996) Resistance gene analogs are conserved and clustered in soybean. Proc Natl Acad Sci USA 93: 11746–11750

Kilian A, Kudrna DA, Kleinhofs A, Yano M, Kurata N, Steffenson B, Sasaki T (1995) Rice-barley synteny and its application to saturation mapping of the barley *Rpg1* region. Nucleic Acids Res 23: 2729–2733

Kleine M, Michalek W, Graner A, Herrman RG, Jung C (1993) Construction of a barley (*Hordeum vulgare* L.) YAC library and isolation of a *Hor1*-specific clone. Mol Gen Genet 240: 265–272

Khush GS, Kinoshita T (1991) Rice karyotype, marker genes, and linkage groups. In: Khush GS, Toenissen GH (eds) Rice biotechnology. CAB Int and IRRI, Wallingford, Oxon, UK and Manila, Philippines, pp 83–108

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shomura A, Shimizu T, Lin SY, Inoue T, Fukada A, Shimano T, Kuboki Y, Toyama T, Miyamoto Y, Kirihara T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang ZX, Momma T, Umehara Y, Yano M, Sasaki T, Minobe Y (1994) A 300-kb kilobase interval genetic map of rice including 883 expressed sequences. Nat Genet 8: 365–372

Lagudah ES, Moullet O, Appels R (1997) Map based cloning of a gene sequence encoding a nucleotide binding domain and a leucine-rich region at the *Cre3* nematode resistance locus of wheat. Genome 40: 659–665

Laurie DA, Pratchett N, Bezant JH, Snape JW (1995) RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter × spring barley (*Hordeum vulgare* L.) cross. Genome 38: 575–585

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 reistance gene *N*. Plant Cell 7: 1195–1206

Leister D, Ballvora A, Salamini F, Gebhardt C (1996) A PCR based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. Nat Genet 14: 421–429

Leister D, Thompson R, Gebhardt C (1997) Production of long inverse PCR products facilitates the isolation of YAC insert termini. Trends Genet 13: 122 (Technical Tips Online: http://www.elsevier.com/locate/tto T40066)

Leister D, Kurth J, Laurie DA, Yano M, Sasaki T, Devos K, Graner A, Schulze Lefert P (1998) Rapid reorganization of resistance gene homologues in cereal genomes. Proc Natl Acad Sci USA 95: 370–375

Leonards-Schippers C, Gieffers W, Salamini F, Gebhardt C (1992) The *R1* gene conferring race-specific resistance to *Phytophtora infestans* in potato is located on potato chromosome V. Mol Gen Genet 233: 278–283

Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spivey R, Wu T, Earle, ED, Tanksley S (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262: 1432–1436

McIntosh RA (1992) Close genetic linkage of genes conferring adult-plant resistance to leaf rust and stripe rust in wheat. Plant Pathol 41: 523–527

Mindrinos M, Katagiri F, Yu,GL, Ausubel FM (1994) The *A. thaliana* disease resistance gene *RPS*2 encodes a protein containing a nucleotide-binding site and leucine-rich repeats. Cell 78: 1089–1099

Parker JE, Coleman MJ, Szabo V, Frost LN, Schmidt R, Moores T, Dean C, Daniels MJ, Jones JDG (1997) *Rpp5*, a gene in Arabidopsis specifying resistance to the oomycete pathogen *Peronospora parasitica*, is highly related to the tobacco viral resistance gene *N* and the flax rust resistance gene *L6*. Plant Cell 9: 879–894

Parniske M, Hammond-Kosack KE, Golstein C, Thomas CM, Jones DA, Harrison K, Wulff BB, 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

Pavan WJ, Hieter P, Reeves RH (1990) Generation of deletion derivatives by targeted transformation of human-derived yeast artificial chromosomes. Proc Natl Acad Sci USA 87: 1300–1304

Pavan WJ, Hieter P, Sears D, Burkhoff A, Reeves RH (1991) High-efficiency yeast artificial chromosome fragmentation vectors. Gene 106: 125–127

Quarrie SA, Laurie DA, Zhu J, Lebreton C, Semikhodski A, Steed A, Witsenboer H, Calestani C (1997) QTL analysis to study the association between leaf size and abscisic acid accumulation in droughted rice leaves and comparisons across cereals. Plant Mol Biol 35: 155–165

Robertson M, Moullet O, Lagudah E (1997) Cloning of a barley disease resistance gene analog by homology to the wheat *Cre3* nematode resistance gene (abstract). In: Plant Anim Genome V. p 94

Ronald PC, Albano B, Tabien R, Abenes L, Wu KS, McCouch S, Tanksley SD (1992) Genetic and physical analysis of the rice bacterial blight disease resistance locus *Xa-21*. Mol Gen Genet 236: 113–120

Saghai Maroof MA, Zhang Q, Biyashev RM (1994) Molecular marker analyses of powdery mildew resistance in barley. Theor Appl Genet 88: 733–740

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning, a laboratory manual. Cold Spring Harbor Press, New York

SanMiguel P, Tikhonov A, Jin YK, Motchoulskaia N, Zakharov D, Melake-Berhan A, Springer PS, Edwards KJ, Lee M, Avramova Z, Bennetzen JL (1996) Nested retrotransposons in the intergenic regions of the maize genome. Science 274: 765–768

Seah S, Sivasithamparam K, Lagudah ES (1997) Isolation and mapping of disease resistance gene sequences from wheat and barley (abstract). In: Plant Anim Genome V. p 81

Singh RP (1992) Genetic association of leaf rust resistance gene *Lr34* with adult plant resistance to stripe rust in bread wheat. Phytopathology 82: 835–838

Singh K, Ishii T, Parco A, Huang N, Brar DS, Khush GS (1996) Centromere mapping and orientation of the molecular linkage map of rice (*Oryza sativa* L.). Proc Natl Acad Sci USA 93: 6163–6168

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald P (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. Science 270: 1804–1806

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

Spencer F, Hugerat Y, Simchen G, Hurko O, Connelly C Hieter P (1994) Yeast *kar1* mutants provide an effective method for YAC transfer to new hosts. Genomics 22: 118–126

Staskawicz BJ, Ausubel FM, Baker BJ, Ellis JG, Jones JDG (1995) Molecular genetics of plant disease resistance. Science 268: 661–667

Sudupak MA, Bennetzen JL, Hulbert SH (1993) Unequal exchange and meiotic instability of disease-resistance genes in the *Rp1* region of maize. Genetics 133: 119–125

Traut TW (1994) The functions and consensus motifs of nine types of peptide segments that form different types of nucleotide-binding sites. Eur J Biochem 222: 9–19

Umehara Y, Akiko I, Hiroshi T, Yuhji Y, Yoshiaki N, Shoko S, Yoshiaki O, Tatsuhito F, Nori K, Yuzo M (1995) Construction and characterization of a rice YAC library for physical mapping. Mol Breed 1: 79–89

van der Biezen EA, Jones JDG (1998) The NB-ARC domain: a novel signalling motif shared by plant resistance gene products and regulators of cell death in animals. Curr Biol 8: R226-R227

Wang GL, Mackill DJ, Bonman JM, McCouch SR, Champoux MC, Nelson RJ (1994) RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics 136: 1421–1434

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

Xiao J, Fulton T, McCouch S, Tanksley S, Kishimoto N, Ohsawa R, UkaiY, Saito A (1992) Progress in integration of the molecular maps of rice: Rice Genet Newsl 9 : 124–128

Xinghua L, Duanpin Z, Yuefeng X, Qifa Z, Heping G (1996) Mapping a new gene for resistance to bacterial blight using RFLP markers. Int Rice Res Newsl 21 : 30

Yoshimura S, Nelson R, Yoshimura A, Mew TW, Iwata N (1992) RFLP mapping of the bacterial blight resistance genes *Xa-3* and *Xa-4*. Rice Genet Newsl 9 : 136–138

Yoshimura S, Umehara Y, Kurata N, Nagamura Y, Sasaki T, Minobe Y, Iwata N (1996) Identification of a YAC clone carrying the *Xa-1* allele, a bacterial blight resistance gene in rice. Theor Appl Genet 93 : 117–122

Yoshimura S, Yamanouchi U, Katayose Y, Toki S, Wang ZX, Kono I, Kurata N, Yano M, Iwata N, Sasaki T (1998) Expression of *Xa-1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. Proc Natl Acad Sci USA 95 : 1663–1668

Yu YG, Buss GR, Saghai Maroof MA (1996) Isolation of a superfamily of candidate disease-resistance genes in soybean based on a conserved nucleotide-binding site. Proc Natl Acad Sci USA 93 : 11571–11756

Zou H, Henzel WJ, Liu X, Lutschg A, Wang X (1997) Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell 90 : 405–413