Stylonychia and what are the structure and function of the NPY receptors in *Stylonychia*.

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Isolation of candidate *R* disease resistance genes from rice

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Abstract Using a polymerase chain reaction (PCR) based method six distinct candidate disease resistant gene (*R*) homologs from rice have been isolated. The rice sequences are organized into two phylogenetic groups with contrasting genomic organization patterns. The first group, represented by a single sequence, *Osh*359-1, is more similar to non-rice *R* sequences than to rice ones and has a simple genomic organization. The second group, represented by *Osh*359-3, contains the remaining five rice sequences. *Osh*359-3 consists of a multi-gene family. The members of *Osh*359-3 family are further found to be clustered together in the genome.

Keywords: **polymerase chain reaction**, **disease resistance** (R) **genes**, ***Oryza sativa***.

PLANTS display a wide array of mechanisms to fend off pathogen attacks. One of the most studied mechanisms is the so-called "gene-for-gene" resistance which is characterized by the presence of a resistance gene, *R*, in a host plant and an avirulence gene, *Avr*, in a pathogen. Recognition of *Avr* by *R* leads to resistance to pathogen infection, often accompanied by a hypersensitive response (HR), a localized cell-death around infected sites[1]. Molecular and biochemical characterization of this interaction will provide invaluable insights into pathogenesis and disease resistance and possibly lead to generation of novel disease resistant crops.

Recently, the first *R* genes have been isolated from several species using a positional cloning or a transposon-tagging strategy[2—4]. With one exception (tomato *Pto*) most of the isolated *R* genes are found remarkably similar to each other and are characterized by leucine-rich repeat (LRR) domains, thought to be involved in protein-protein interaction. Within this LRR class of *R* genes two subclasses could be identified, a nucleotide binding site (NBS)-containing subclass including Arabidopsis *Rps*2 and *Rpm*1, tobacco N, flax L6 and tomato *Prf* and a *Cf*-2/*Cf*-9 subclass. More importantly, several well-conserved domains were identified in the NBS-containing regions[2]. These conservations provide a basis to design degenerate primers for PCR isolation of sequences homologous to *R* genes, in particular, from

species where positional cloning or transposon-tagging is yet to be developed or difficult to perform. We used such a PCR strategy to isolate candidate *R* from a number of species including Antirrhinum, rice, wheat, maize, barley, rye and pearl millet. Furthermore, we isolated six distinct candidate *R* genes from rice and showed that they are organized as a cluster in the genome. Our results show that the PCR strategy for isolating *R* homologs described here has possible wide applications in other crop species.

1 Materials and methods

DNA from different species were obtained through the following sources: Antirrhinum (*majus* X *hispanicum*) strains P330[29] and P329[67] (see ref. [5]), rice (*O. sativa* cv. H359, Acc. 8558, Gui 630, and Taigen) and an RI population (100 individuals) from a cross between *O. sativa* cv. H359 and Acc. 8558 (Weiming Li *et al.*, unpublished), wheat (*T. æstivum* cv. Shannong 7859, Shinong 3251, Beijng 837 and Neixiang 182) (provided by Hui Zhang, The Chinese Academy of Agricultural Sciences, Beijing), barley (*Hordeum vulgare*), pearl millet (*Pennisetum typhoides*), maize (*Zea mays*), rye (*Secale cereale*) (provided by Long Mao, John Innes Centre, UK).

Fifty nanograms of genomic DNA was used as template for PCR amplification using two degenerate primers (Y16, 5′ GGX (C/A)(C/T)X GGX GGX (A/G)TX GGX AA(A/G) ACX AC 3′; LP1, 5′ AG XG(T/C) XAG XGG XAG XCC 3′ where X is G, A, T or C) corresponding to conserved domains of the NBS-containing regions of *R* genes[2]. PCR was performed according to the following conditions: 94℃, 1 min, 42℃, 1 min, 72℃, 1 min for 35 X with a final extension of 10 min at 72℃. The PCR products were purified by a Wizard PCR Preps kit from Promega before being cloned in pGEM-T vector (Promega). Insert sizes were determined by colony PCR analysis using forward and reverse primers.

DNA gel blot analysis was carried out as previously described[5]. DNA sequence and amino acid analyses were performed using the Genetics Computer Group (Madison, WI) package. DNA sequences were submitted to the EMBL data base under the following accession numbers: *Osh*359-1 (Y09807), *Osh*359-2 (Y09808), *Osh*359-3 (Y09809), *Osh*359-5 (Y09810), *Os*8558-3 (Y09812) and *Os*8558-12 (Y09811).

2 Results and discussion

PCR was performed on genomic DNA isolated from several plant species including Antirrhinum, rice, wheat, rye, barley, maize and pearl millet. The PCR result indicated that patterns of PCR products are species-specific and similar profiles are observed among different cultivars of the same species (data not shown). Their sizes vary between 300 bp and over 1 kbp and differently sized major amplified products are obtained between species. The predominately amplified product in rice is around 500 bp, similar to the predicted size of the corresponding region of *R* genes between the two primers used, but the major PCR products in wheat is about 1.3 kbp. In this report we will focus on rice PCR product analysis.

To further analyse the identity of PCR fragments from rice they were cloned and sequenced (see sec. 1). A large number of recombinants were obtained (data not shown). Eight clones each were randomly selected from the recombinants obtained from the PCR products of *O. sativa* cv. H359 and Acc. 8558, respectively, for DNA sequencing analysis. Database searches identified six distinct clones, *Osh*359-1, *Osh*359-2, *Osh*359-3, *Osh*359-5, *Os*8558-3 (*Os*8558-8) and *Os*8558-12, showing significant homology to known *R* genes and the rest clones did not show homology to *R* gene sequences and their origins are presently unknown (data not shown). The predicted polypeptide sequences of the six different rice *R* homologous sequences are shown in fig. 1 together with *R* proteins from Arabidopsis and tobacco. They generally showed identities over 35%.

To determine their relationships a phylogenetic analysis was performed on the *R* and candidate *R* proteins. Two groups of rice sequences were detected (data not shown). The first group consists of five of the six classes of rice sequences. The second group contains only one sequence, *Osh*359-1, which is more similar to Arabidopsis Rpm1 than to the other rice candidate *R* analysed.

To reveal how candidate *R* homologs are organized in rice genome DNA gel blot hybridization analysis was carried out using two representative sequences from the two phylogenetic groups of rice sequences, *Osh*359-1 and *Osh*359-3 (fig. 2). A simple hybridization pattern was detected using *Osh*359-1 as a probe (fig. 2(a)). *Osh*359-1 hybridized to a single fragment of BglII-digested genomic DNA of *O.*

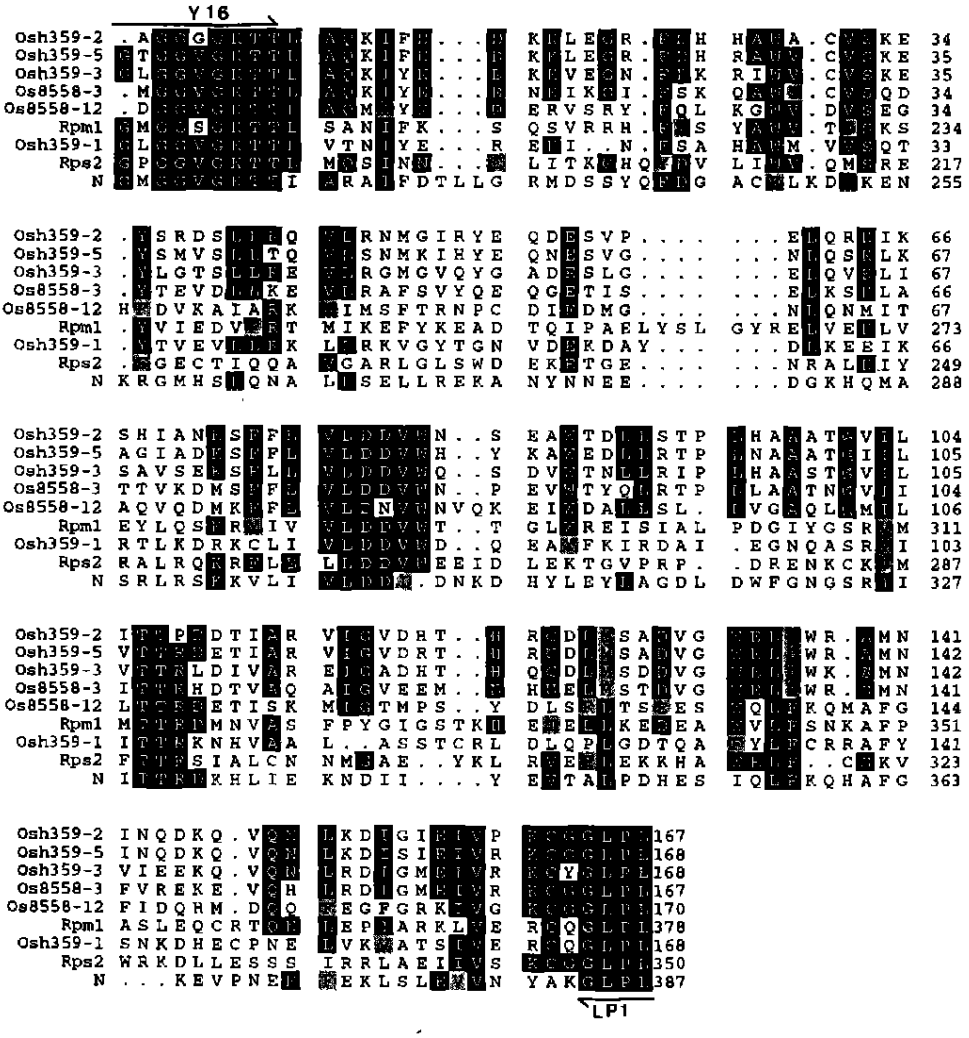

Fig. 1. Amino acid sequence alignment of rice candidate *R* with other *R* sequences. The alignment was generated using the Genetics Computer Group (Madison, WI) LOCALPILEUP and PRETTY programs. Conserved residues are indicated by black or grey boxes. Black dots are gaps introduced to maximize the alignment. *Osh*359-1, -2, -3, and -5 and *Os*8558-3 and -12 are candidate rice *R* proteins. *Rpm*1 and *Rps*2 are from Arabidopsis and *N* from tobacco. Two regions corresponding to primers Y16 and LP1 are indicated.

sawita cv. H359. In *O. sativa* Acc. 8558, two more weakly hybridizing fragments were detected in addition to the strong BglII fragment. Because no BglII restriction site was found within *Osh*359-1, these weakly hybridizing fragments might represent homologous sequences to *Osh*359-1. The three fragments appear to be linked because they are found to segregate together in a recombinant inbreed (RI) population (fig. 2(a)). In contrast, *Osh*359-3 hybridized to at least 6—8 fragments in addition to a strongly hybridizing BglII fragment (fig. 2(b)). Since no internal BglII site was detected in *Osh*359-3, these fragments could also potentially represent homologous sequences to *Osh*359-3. Furthermore, these fragments appear to segregate together in the RI population (fig. 2(b)), suggesting that these candidate rice *R* homologs are probably clustered in rice genome. The finding is consistent with the result from the phylogenetic analysis showing that the *Osh*359-3 group had five sequences. Unfortunately, none of the hybridizing fragments to *Osh*359-1 and *Osh*359-3 was found to segregate with a gene potentially resistant to *Xanthomonas campestris* pv. *oryzicola* (Dingzhong Tang, unpublished), which causes bacterial leaf streak on rice.

We have isolated candidate *R* sequences from rice and other crop species using a PCR strategy. Six distinct putative rice *R* sequences were characterized by DNA sequencing and hybridization analyses and could be divided into two phylogenetic groups, represented by *Osh*359-1 and *Osh*359-3, respectively. They belonged to the subclass of NBS-containing disease resistance genes including Arabidopsis *Rps*2 and *Rpm*1, tobacco *N*, flax *L*6 and tomato *Prf*. Similar class of the *R* homologs has also been isolated from soybean and potato[6—8]. Rice *R* homologous sequences showed a contrast pattern of genomic organization. *Osh*359-1 is possibly a single or low copy number genes, but *Osh*359-3 consists of a gene family with at least 6—8 members, of which all are linked. Such a close linkage of disease resistance genes seems a common feature of several characterized *R* genes including recently isolated candidate soybean *R* genes[3,6—8]. Recently, the first *R* gene from rice (*Xa*21) conferring resistance to *Xanthomonas campestris* pv *oryzae* has been isolated using a positional cloning strategy and found to be different from NBS-containing *R* genes[9]. Therefore, the 6 candidate *R* homologs isolated here are different from *Xa*21. Whether rice candidate *R* homologs or candidate *R* from other species encode functional disease resistance genes needs further confirmation.

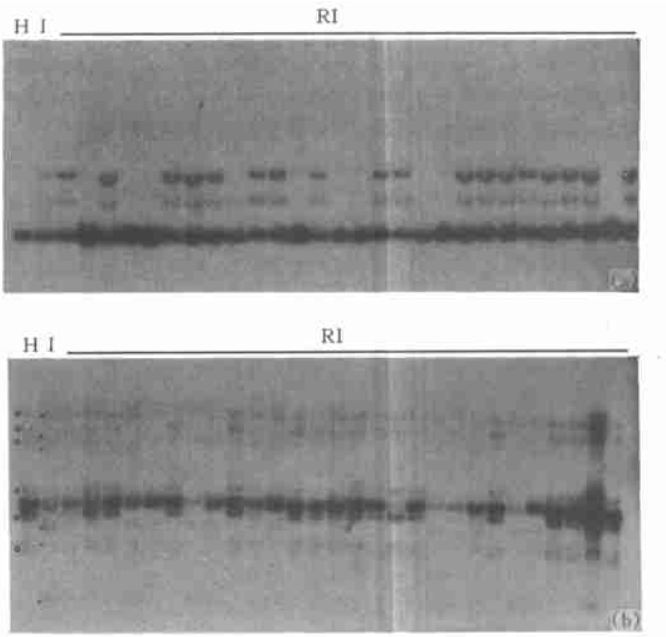

Fig. 2. Genomic organizations of rice candidate R genes. Five micrograms of genomic DNA were digested by BglII and probed with *Osh*359-1 (A) and *Osh*359-3 (B). DNAs were from *O. sativa* cv H359 (H), Acc. 8558 (I), an RI population from a cross between *O. sativa* cv. H359 and Acc. 8558 (RI). Co-segregating fragments are indicated by black circles or stars.

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