Cloning of Molecular Markers for Disease Resistance in Sunflower *Helianthus annuus* L.

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Summary

A candidate gene approach to analyse the resistance of plants to phytopathogenic fungi is presented. Resistance of sunflower (*Helianthus annuus* L.) to downy mildew (*Plasmopara halstedii*) shows gene-for-gene interactions (monogenic resistance). By homology cloning, probes were obtained, homologous to some plant resistance genes (Nucleotide Binding Site-like genes or NBS genes). These clones were used as probes for linkage mapping of the corresponding genes. It was demonstrated that at least three NBS-like loci are located on linkage group 1, in the region where downy mildew resistance loci have been described.

Introduction

Resistance of sunflower to downy mildew (*Plasmopara halstedii*) is controlled by single dominant genes designated *Pl* (Vranceanu 1970) and has been found for all known races of the pathogen. It has been demonstrated recently that at least some *Pl* genes are clustered (Mouzeyar et al. 1995; Vear et al 1997). A number of plant resistance genes have been already cloned (for review see Bent 1996) These resistance genes fall in two different classes (Staskawicz et al. 1995): Leucine Rich Repeats (LRR) genes with or without Nucleotide Binding Site (NBS), and genes with a serine/threonine protein kinase domain such as *Pto* in tomato (Martin et al. 1993). The *Xa21* gene in rice (Song et al. 1995) is a gene containing the two motifs, *i.e* a LRR domain and a kinase domain. A possible hypothesis concerning the *Pl* downy mildew resistance genes of sunflower is that they could be coded either by a LRR or NBS-like gene or a PK-like gene.

Materials and methods

Homology cloning and probes

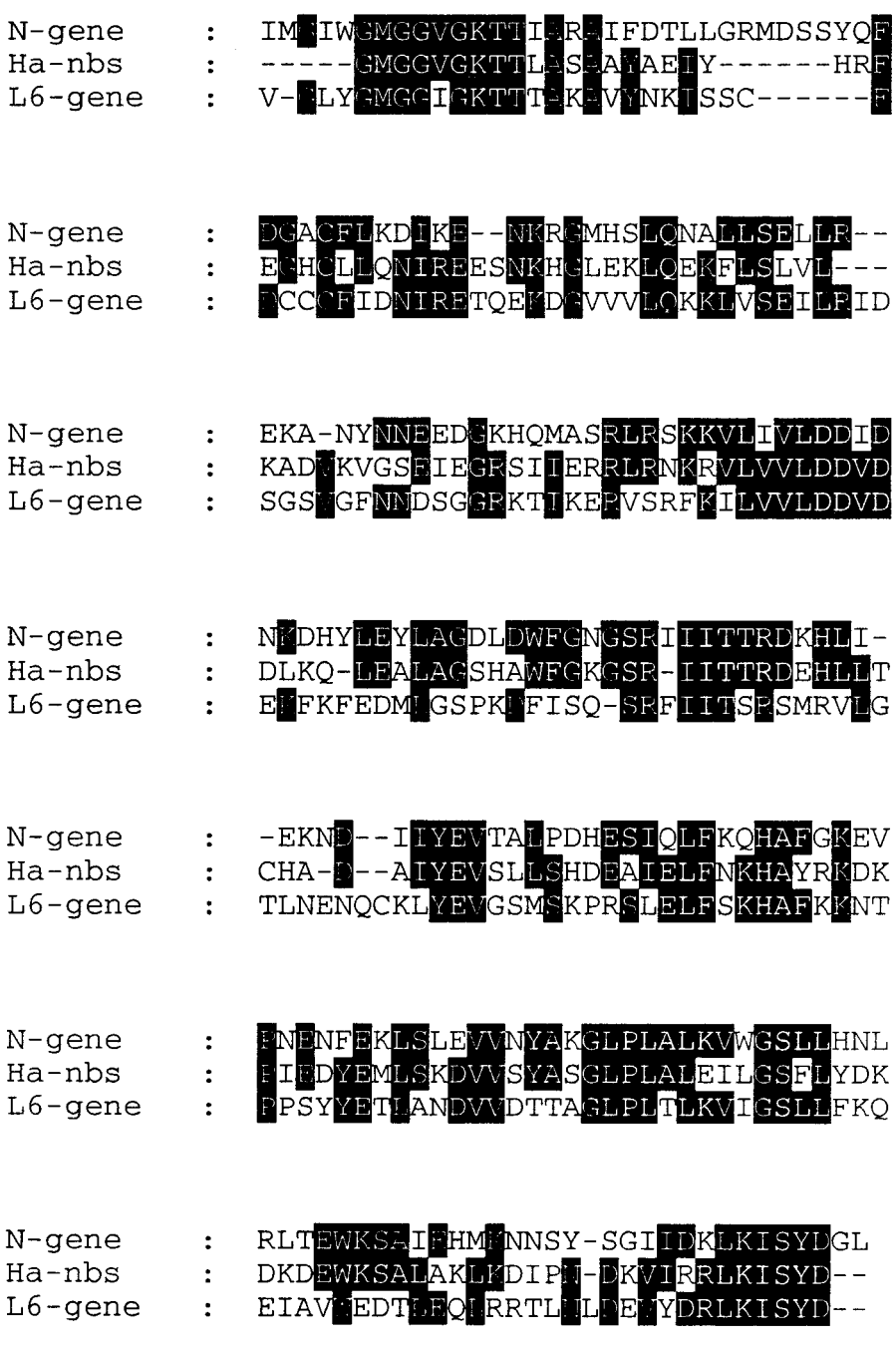
For the homology cloning of the NBS-like probe, the *N* gene sequence (accession A54810) and the *L6* gene sequence (accession U27081) were aligned with the CLUSTAL program (Higgins et al. 1992). Two degenerate primers were then generated : forward primer : (5') GGA ATG GGK GGA GTY GGY AAR AC (3') and reverse primer: (5') ATC ATA ACT TAT TTT KAG (3'). These primers were synthetized by OLIGO-EXPRESS (Paris; France).

Amplification was made on genomic DNA of a downy mildew resistant line (RHA266) with the following PCR conditions : 94°C (5 min) for denaturation; then 40 cycles of 94°C (30 sec) for denaturation; 42°C (30 sec) for annealing; 72°C (1min 30sec) for extension; then a final extension of 72°C (10 minutes). PCR products were checked on standard agarose gels.

Cloning was carried out in a T/A vector (pCR2.1, InVitrogen, Netherlands) following the manufacturer's instructions. After sequencing, the resulting sequence of the probe was analysed for homology with data banks using the BLAST program (Altschul et al. 1990).

Plant material and disease evaluations

Resistance to downy mildew analyses involved the GH X PAC2 cross and were previously described by Vear et al. (1997). The 111 F_2 progenies from this cross were assessed for resistance both to race 1 and race D of *Plasmopara halstedii*.


N-gene : IMSIWGMGGVGKTTIARAIFDTLLGRMDSSYQF
Ha-nbs : -----GMGGVGKTTLASAAYAEIY------HRF
L6-gene : V-RLYGMGGIGKTTTAKEVYNKISSC------F
N-gene : DGACFLKDIKE--NKRGMHSLQNALLSELLR--
Ha-nbs : EGHCLLQNIREESNKHGLEKLQEKFLSLVL---
L6-gene : DCCCFIDNIRETQEKDGVVVLQKKLVSEILFID
N-gene : EKA-NYNNEEDGKHQMASRLRSKKVLIVLDDID
Ha-nbs : KADVKVGSFIEGRSIIERRLRNKRVLVVLDDVD
L6-gene : SGSTGFNNDSGGPKTIKEPVSRFKILVVLDDVD
N-gene : NKDHYLEYLAGDLDWFGNGSRIIITTRDKHLI-
Ha-nbs : DLKQ-LEALAGSHAWFGKGSR-IITTRDEHLLT
L6-gene : EIFKFEDMIGSPKTFISQ-SRFIITSPSMRVLG
N-gene : -EKND--IIYEVTALPDHESIQLFKQHAFGKEV
Ha-nbs : CHA-D--AIYEVSLLSHDEAIELFNKHAYRKDK
L6-gene : TLNENQCKLYEVGSMSKPRSLELFSKHAFKKNT
N-gene : PNENFEKLSLEVVNYAKGLPLALKVWGSLLHNL
Ha-nbs : HIEDYEMLSKDVVSYASGLPLALEILGSFLYDK
L6-gene : PPSYYETLANDVVDTTAGLPLTLKVIGSLLFKQ
N-gene : RLTEWKSAIFHMLNNSY-SGIIDKLKISYDGL
Ha-nbs : DKDEWKSALAKLKDIPN-DKVIRRLKISYD--
L6-gene : EIAVLEDTLFQLRRTLLLDEYYDRLKISYD--

Linkage mapping

DNA preparation, Southern blotting and hybridization were carried out by standard techniques. The MAPMAKER/EXP program (Lander et al. 1987) was used to compute the linkage map of the cross (minimum LOD score 3.0, maximum recombinant fraction 0.40). The overall method for linkage mapping, including nomenclature of the loci, follows that of Gentzbittel et al. (1995)

Results

Homology cloning and mapping.

By restricting the multiple sequence alignments to the *N* gene (Whitham et al 1994) and *L6* gene (Lawrence et al. 1995), it was possible to design degenerate primers to two conserved domains of these genes. After PCR reaction on genomic DNA (T = 42°C) of a resistant line (RHA266), the product obtained was about 650 bp long. Cloning and sequencing of the NBS-R3 product (GenBank accession U96642) revealed a significant homology with the *N* (Whitham et al. 1994) and *L6* (Lawrence et al. 1995) genes at the deduced amino-acid level (Figure 1).

Mapping of the NBS-R3 probe was carried. Autoradiographs revealed a rather complex pattern, suggesting a multigenic family. Each NBS-R3 locus (NBSR3-E1, NBSR3-E5_1 and NBSR3-E5_2) co-segregated with loci assigned to linkage group 1 (Figure 2).

Discussion

Co-localisation of the NBS-like probe and Pl loci.

The hypothesis on which this study was based was that LRR-NBS-like genes could be involved in the expression of resistance of sunflower to

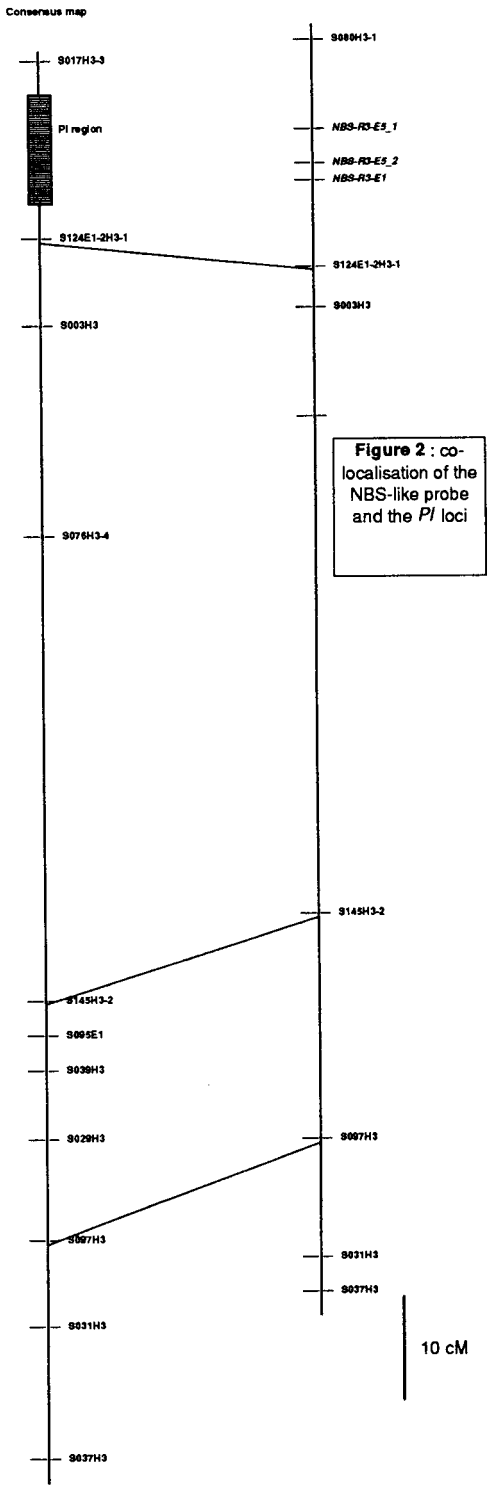


Figure 2 : co-localisation of the NBS-like probe and the *PI* loci

downy mildew. It may be suggested that the NBS-R3 probe is homologous to the *N* and *L6* genes. Autoradiographs suggest that the NBS-R3 probe is a repeated sequence, of which all the polymorphic bands are located on linkage group 1, in the region where the *Pl* loci have been assigned (Mouzeyar et al. 1995; Vear et al. 1997). Thus, the NBS-R3 gene could be a candidate for resistance to downy mildew in sunflower. The same approach was used in recent studies to map candidate disease-resistance genes in soybean (Kanazin et al. 1996; Yu et al. 1996) and in potato (Leister et al. 1996). Further studies are in progress to define the genetics of the *Pl* locus in sunflower, in order to determine whether each *Pl* gene (specific to a given race of downy mildew) corresponds to one NBS-R3 locus.

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