Fishing Genes from germplasm: Cloning, testing and use of gene-like fragments associated with root-knot nematode resistance in tuber-bearing Solanum genetic resources

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Watanabe, J. A.,1,2) M. Ikegawa1,3), T. Yamada1) & K. N. Watanabe1,2)

1. Dept. Biotech. Sci., Kinki University, Uchita, Wakayama, Japan
2. Gene Research Center, Univ. of Tsukuba, Ibaraki, Japan
3. Nara Advanced Inst. for Sci. and Technol., Ikoma, Nara, Japan
Root-knot nematodes infest over 2000 plant species

Mi is a known plant resistance gene to some Meloidogyne incognita
Root-knot nematode resistance genes in Potato

RKN is an important pest in warm and tropical zones including Southern part of Japan such as Nagasaki

1) Original genetics lines developed (Watanabe et al. 1994)
2) Genetics and physiology of the trait analyzed well
   -> Quantitative, additive, temperature insensitive in phenotype.
   -> Broad range of reactions to different taxa of the nematode.
3) Diverse sources of resistances in various wild and cultivated species
   (Watanabe and Watanabe 2000)
4) Base work and patent filed in potatoes on markers(PCT/JP02/12392)
Specific focuses

• Cloning gene-like fragments associated with root-knot nematode (*Meloidogyne* spp) resistance genes in potatoes
• Testing transgenic plants using the fragments
• Use of the gene information for diversity study and molecular breeding
Structure of Plant R genes
(Science 292 #5525, Nature 411 #6839)

Common Features in Plant R genes

- LZ: Leucine Zipper (Coiled coil)
- NBS: Nucleotide Binding Site
- LRR: Leucine-Rich Repeat
- TIR: Drosophila Toll and mammalian interleukin-1 receptor (IL-1R) homologous region
- PK: Protein Kinase

Common Defense Mechanisms among Plants

Common features in plant resistance genes

<table>
<thead>
<tr>
<th>LZ(CC)/ TIR</th>
<th>NBS</th>
<th>T/S Kinases</th>
<th>LRR</th>
</tr>
</thead>
</table>

LRR: leucine rich repeats  
LZ: Leucine zipper/ CC: Coiled coil  
NBS: Nucleotide binding site  
TIR: Homologous region with Toll/IL-1R
Categories of primary products from Plant disease resistance genes
(Simplified from Dangl & Jones 2001, Science 411:826-827)
RKN resistance gene in Sweetpotato

- Similar structure to known plant R genes but not identical
- Variation occurs in hexasomic hexaploid genome
- Diversity among 6x genotypes
- Possibility of SNPs for identification of R/S
- Weak expression
- Need elaboration of transgenic manipulation I
- Base knowledge filed as patent (JP 2001-223606)
Materials

• Diploid potato breeding lines with high levels of resistance to RKN (Watanabe et al. 1996, 1999)
• Tetraploid potato cultivars with known S for comparison
Methods

- **PCR:** Primers designed from common motifs in PDR genes especially from *Mi* and sweetpotato information
- **Cloning:** Random cloning of PCR products
- **Sequence comparison:** Difference between fragments from R and S genotypes
- **Amino acid identity:** with known PDR genes
  Construction of binary vector: pBI121 or pBE2113 Not
- **Transformation:** *Agrobacterium tumefaciens* LBA4404
- **Resistance evaluation:** regenerants on *N. benthamiana* and Desiree
## NBS region comparison

<table>
<thead>
<tr>
<th>Proteins</th>
<th>85.37.38</th>
<th>Beniazuma (SP)</th>
<th>Mi-1.1</th>
<th>Mi-1.2</th>
<th>PRF</th>
<th>I2C-1</th>
<th>I2C-2</th>
<th>RPM1</th>
<th>N</th>
<th>RPP5</th>
<th>RPP2</th>
<th>ADG2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GMPGSGKTTLAYRYYNDKSV</td>
<td>GMGGIGKTTLAERVYNDKAV</td>
<td>GMPGSGKTTLAYKVYNDKSV</td>
<td>GMPGSGKTTLAYKVYNDKSV</td>
<td>GMPGGLKKTTLAKKICYNDPEV</td>
<td>GMPGGMGKTTLAKAVYNDERV</td>
<td>GMPGGMGKTTLAKAVYNDERV</td>
<td>GMPGGSKTTLSANVFKSKQS</td>
<td>GMPGGVGGKTTLIARALFDTL</td>
<td>GQSIGKSTLGRAF</td>
<td>GPGGGKTTLLMSI</td>
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</tr>
<tr>
<td>Kinase 1a</td>
<td>ISSYFDLHAWCVT</td>
<td>ISSYFDLRAWTTISSQQHNL</td>
<td>SSRFDLRAWCVTVDQGCD</td>
<td>SSRFDLRAWCVTVDQGYDK</td>
<td>TSRFDVHAWCVTVDQLYS</td>
<td>QKHFGLTAWFCSVEAYDAF</td>
<td>KNHFDLKAWFCSVSEAYNADF</td>
<td>RRFESYAHWVTISKSYYVE</td>
<td>RMD</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Beniazuma (SP)**

**Mi-1.1**

**Mi-1.2**

**PRF**

**I2C-1**

**I2C-2**

**RPM1**

**N**

**RPP5**

**RPP2**

**ADG2**
Deduced amino acid identity with known PDR genes

<table>
<thead>
<tr>
<th>Species</th>
<th>Protein Description</th>
<th>Identity Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solanum acaule</td>
<td>NBS-LRR protein</td>
<td>607/867 (70%)</td>
</tr>
<tr>
<td>Solanum tuberosum</td>
<td>RGC1 protein, 607/867</td>
<td>(70%)</td>
</tr>
<tr>
<td>Solanum tuberosum</td>
<td>NBS-LRR protein, 611/867</td>
<td>(70.5%)</td>
</tr>
<tr>
<td>Solanum tuberosum</td>
<td>Disease resistance protein Gpa2, 611/867</td>
<td>(70.5%)</td>
</tr>
<tr>
<td>Solanum tuberosum</td>
<td>Rx protein</td>
<td>604/867 (70%)</td>
</tr>
<tr>
<td>Capsicum chacoense</td>
<td>Disease resistance protein BS2, 482/870</td>
<td>(55.4%)</td>
</tr>
<tr>
<td>Lycopersicon esculentum</td>
<td>PRF protein, 403/742</td>
<td>(54%)</td>
</tr>
<tr>
<td>Lycopersicon pimpinellifolium</td>
<td>PRF protein, 391/726</td>
<td>(54%)</td>
</tr>
<tr>
<td>Lycopersicon esculentum</td>
<td>Root-knot nematode resistance protein, 410/816</td>
<td>(50%)</td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
<td>RPP13 protein, 338/682</td>
<td>(50%)</td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
<td>rpp8 protein, 399/822</td>
<td>(49%)</td>
</tr>
<tr>
<td>Arabidopsis lyrata</td>
<td>NBS/LRR disease resistance protein RPM1, 352/675</td>
<td>(52%)</td>
</tr>
</tbody>
</table>
Summary results of cloning and sequence study

- 2613 bps fragment containing NBS and LRR regions with incomplete CC
- Conserved in NBS and LRR, but not identical to known genes
- Mapped at Chrom VI of potato around *Mi* of tomato
- R & S alleles: probably stop codons at introns in S
- Variation in S alleles
RT-PCR using LRR region specific to the potato RKN gene
1. Marker, 2. *N.bensamiana* control, 3. And 4 transgenic plants,
5. *S. tuberosum* Susceptible control, 6 and 7 *S. tuberosum* donor,
8. And 9. Negative control
Resistance evaluation in *N. benthamiana*

- Root-knot nematodes (RKN) were maintained in soil pots with susceptible tomato genotypes.
- Minimum of five cuttings per line were used for the evaluation. Cuttings were made three weeks before transplanting to the RKN infested soil to let rooting enough.
- General infection method Iwanaga et al. (1989) except the incubation temperature of the plants were conducted between 30-35 degree Celsius at day temperature for 16 hours and 25-30 degree Celcius at night temperature for 8 hours at the controlled growth chambers.
Resistance evaluation

• Galls were scored in roots.
• Clones with only no galls were subjected to microscopic observation using erioglaucine (0.05% aqueous solution for 10 min, Yaghoobi et al. 1995) for egg observation.
• Further microscopic observation was conducted on the presence of adults.
Resistance scoring

• HR: No gall and no egg in root
• R: No gall and presence of some egg in root mass
• MR: Some galls @ specific root region and presence of eggs in root mass
• MS: Many galls @ specific root region
• S: Many galls over root system