Identification of a CAPS marker in an eIF4E gene linked to Zucchini yellow mosaic virus resistance in watermelon

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Abstract

Genes that encode eukaryotic initiation factors (eIF) 4E and iso(4E) have been associated with the recessively inherited resistance to potyviruses in a number of plant species. Using previously developed degenerate primers, partial eIF4E and eIF(iso)4E gene sequence regions were obtained through polymerase chain reaction of the genomic DNA templates prepared from the Zucchini yellow mosaic virus (ZYMV)-resistant U.S. Plant Introduction (PI) 595203, and the ZYMV-susceptible ‘New Hampshire Midget’ (NHM). A single nucleotide polymorphism (SNP) was identified in the intron II region of the eIF4E sequence between the resistant and susceptible parental genotypes. F2 and BC1 populations were developed using PI 595203 and NHM and each plant was phenotyped for resistance to ZYMV and resistance was found to be conferred by a single recessive gene. A codominant Cleaved Amplified Polymorphic Sequence (CAPS) marker was created using the identified SNP in the eIF4E gene. This marker was linked to the ZYMV resistance locus (zym) at a linkage distance of 7 cM as estimated with the F2 and BC1 populations. A marker in the eIF(iso)4E gene was created and was not linked to the ZYMV resistance locus.

INTRODUCTION

Zucchini yellow mosaic virus (ZYMV), Watermelon mosaic virus (WMV), Papaya ringspot virus (PRSV), and Cucumber mosaic virus (CMV) are considered major viruses of cucurbits. Provvidenti et al. (1984) identified two major ZYMV strains ZYMV-CT and ZYMV-FL in the United States. ZYMV-FL is the most prevalent strain in cucurbit crops and ZYMV-CT is limited to the Northeastern U.S. In screening U.S. watermelon germplasm, Provvidenti (1991) identified four watermelon (Citrullus lanatus) landraces (PI 482322, PI 482299, PI 482261 and PI 482308) with potential resistance to ZYMV. Inheritance studies indicated that a single recessive gene (zym) is conferring ZYMV resistance in PI 482261. Boyhan et al. (1992) was the first to identify ‘Egun’ (apparently PI 595203) with resistance to ZYMV. Lecoq et al. (1998) also identified other PI accessions with resistance to

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ZYMV. Guner (2004) re-evaluated a large PI collection of watermelon for ZYMV-resistance and identified a few more PIs (including PI 595203). Xu et al. (2004) examined ZYMV-China Strain (ZYMV-CH) and WMV resistance in watermelon (PI 595203) and determined that the resistance to ZYMV-CH is also conferred by a single recessive gene (zym-CH).

The Potyvirus is the largest genus of plant viruses and recessive Potyvirus resistance genes were shown to correspond to eIF4E gene family members (Robaglia and Caranta 2006). eIF4E binds the 5’ cap of a mRNA that is to be translated and eIF4E associates with eIF4G, which serves as a scaffold, to form the eIF4F complex (Zhang et al. 2006). Many examples exist that link potyvirus resistant genes to eIF4E. For example, the pepper pvr2 resistance gene against Potato virus Y (PVY) and Tobacco etch virus (TEV), the sbm1 gene for pea resistance to Pea seed-borne mosaic virus (PSbMV), the pot1 gene for tomato resistance to PVY and TEV, and the mol gene for lettuce resistance to Lettuce mosaic virus were shown to be associated with mutations in eIF4E homologs (Robaglia and Caranta 2006). The objective of this study was to identify a molecular marker linked to ZYMV resistant locus in watermelon. Genes eIF4E and eIF(iso)4E were chosen as candidate genes to generate markers linked to the zym locus in watermelon.

MATERIALS AND METHODS
Host plant and genetic materials
F1, F2, and reciprocal BC1 populations, derived from a cross between PI 595203 (ZYMV-resistant) and ‘New Hampshire Midget’ (ZYMV-susceptible), were developed at North Carolina State University (Guner 2004). Seeds were germinated in an insect-free greenhouse with a temperature at 18-30°C for 14-16 hours of natural lighting at the U.S. Vegetable Laboratory in Charleston, SC. The two youngest leaves were collected from young seedlings (4-5 leaf stage) for DNA isolation. Plants were then mechanically inoculated with the ZYMV-FL culture.

Virus isolate and inoculation
The ZYMV-FL culture (Provvidenti et al. 1984) was propagated and maintained on Gray zucchini squash. Virus inoculum was prepared by macerating virus-infected leaves (1:5 w/v) in 0.01 M phosphate buffered saline, pH 7.4 with a mortar and pestle. Seedlings were inoculated by lightly dusting the leaves with carborundum, then mechanical rubbing the leaf with a cotton Q-tip soaked in the virus inoculum. Excess carborundum was rinsed with water and the inoculated seedlings were placed in shade for a few hours to minimize direct sunlight damage to the newly inoculated leaves. Three weeks after inoculation, plants were evaluated for virus symptoms. Virus disease severity was rated as: 0 - no symptoms, 1- slight mosaic on leaves, 2 - mosaic patches and/or necrotic spots on leaves, 3 - leaves near apical meristem are slightly deformed, with yellow color and reduced leaf size, 4 - apical meristem has deformed shape and with mosaic appearance; 5 - extensive mosaic appearance and severe leaf deformation, or plant is dead (Xu et al. 2004).

ELISA
Enzyme linked immunosorbent assay (ELISA) was performed according to the manufacturer’s instructions (BioReba, Switzerland). Microtiter plates were first
coated with 1 µg/ml of ZYMV antibody, and virus particles were trapped after incubating the tissue extract on the coated plates. Leaf extract was prepared by processing the collected leaf tissue with a tissue homogenizer in tissue extraction buffer (1:20 w/v). The alkaline phosphatase conjugated antibody to ZYMV was then added. Yellow color developed upon substrate addition was measured with an ELISA reader. A sample with absorbance value of at least twice that of the mean healthy control (OD_{405nm}) was regarded as positive.

**Identification of SNPs in eIF4E gene and development of a codominant CAPS marker linked to ZYMV resistance in watermelon**

Genomic amplicons of eIF4E and eIF(iso)4E from ‘New Hampshire Midget’ and PI 595203 were produced with PCR using degenerate primers as described (Nieto et al. 2006). Two amplicons were produced, 1.9 kb for eIF4E and 500 bp for eIF(iso)4E. These PCR products were cloned into TA cloning vector (Invitrogen, Carlsbad, CA). Clones with inserts of the expected size were identified and sequenced. Single nucleotide polymorphism (SNP) markers were identified through multiple sequence alignments. The identified SNP marker was converted to a Cleaved Amplified Polymorphic Sequence (CAPS) marker to allow genotyping of lines from the F₂ and BC₁ populations. JoinMap 3.0 was used to calculate the genetic distance of the CAPS marker to the zym locus in watermelon.

**RESULTS AND DISCUSSION**

**Inheritance of resistance**

All 19 F₁ plants produced from the cross between NHM and PI 595203 were susceptible, indicating that ZYMV resistance in PI 595203 is recessively inherited. The F₂, BC₁R and BC₁S segregation data were tested against the expected ratio for a single recessive gene confirming the observations by Provvidenti (1991) and Xu et al. (2004). The F₂ segregation data support the expected 3:1 and BC₁R population in 1:1 (susceptible: resistance) ratio (Tab. 1 and 2).

**Table 1. Distribution of genotypes using a codominant CAPS marker in an intron of the eIF4E gene in a F₂ population derived from ‘New Hampshire Midget’ and PI 595203.**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>No. of plants</th>
<th>P/P</th>
<th>P/N</th>
<th>N/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant</td>
<td></td>
<td>19</td>
<td>15</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Susceptible</td>
<td></td>
<td>62</td>
<td>4</td>
<td>37</td>
<td>21</td>
</tr>
</tbody>
</table>

²P: PI 595203, N: ‘New Hampshire Midget’
³R:S expected ratio 1:3; χ² = 0.10, P = 74.8
⁴P:P:N:N expected ratio 1:2:1, χ² = 0.13, P = 93.6
Table 2. Distribution of genotypes using a codominant CAPS marker in an intron of the eIF4E gene in a BC₁ population derived from ‘New Hampshire Midget’ and PI 595203.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>No. of plants</th>
<th>P/P</th>
<th>P/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant</td>
<td></td>
<td>53</td>
<td>47</td>
<td>6</td>
</tr>
<tr>
<td>Susceptible</td>
<td></td>
<td>61</td>
<td>2</td>
<td>59</td>
</tr>
</tbody>
</table>

z P: PI 595203, N: ‘New Hampshire Midget’

² R:S expected ratio 1:1 ; $\chi^2 = 0.56$ P = 45.4

³ P:P:P/N expected ratio 1:1; $\chi^2 = 2.24$ P= 13.4

eIF4E gene is linked to ZYMV resistance in watermelon

With the link between eIF4E and resistance to potyviruses, eIF4E and eIF(iso)4E was amplified in watermelon using degenerate primers (Nieto et al. 2006). A 1.9 kb amplicon was generated for eIF4E and a 500 bp amplicon for eIF(iso)4E. These amplicons were cloned and sequenced and a SNP was identified between NHM and PI 595203 in the eIF4E and eIF(iso)4E sequence, respectively. Amplicons that contained these SNPs were amplified in 86 plants of BC₁ population and sequenced. The eIF4E SNP, but not eIF(iso)4E SNP, was linked to the ZYMV resistance locus. The eIF4E SNP was converted to a codominant CAPS marker and this marker was used to screen 81 F₂ plants (Tab. 1, Fig. 1) and 114 BC₁ plants (Tab. 2, Fig. 2). The genetic distance was calculated and the CAPS marker was 7.3 cM in the F₂ population and 7.0 cM from the ZYMV resistance locus in the BC₁ population.

Although eIF4E does not appear to be the gene for watermelon resistance to ZYMV, clustering of resistance loci in many plant genomes is well documented (Hulbert et al. 2001; Meyers et al. 2003; Kuang et al. 2004). Thus use of the CAPS marker in the eIF4E gene not only selects for those plants with resistance to ZYMV but also has the potential of selecting for resistance to other pathogens.

CONCLUSION

Here we show that a single recessive gene confers resistance to ZYMV in PI 595203. Furthermore, a CAPS marker within the eIF4E gene is linked to the resistance gene against ZYMV. This marker can be used in marker-assisted selection to develop watermelon cultivars with resistance to ZYMV.

ACKNOWLEDGEMENTS

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Figure 1. Amplicons were generated from DNA isolated from plants in a F$_2$ population (‘New Hampshire Midget’ x PI 595203) using the eIF4E CAPS marker and were cleaved using $MseI$. M: 1 kb plus DNA ladder; P: PI 595203; N: ‘New Hampshire Midget’

Figure 2. Amplicons were generated from DNA isolated from plants in a BC$_1$ population [F$_1$ (‘New Hampshire Midget’ x PI 595203) x PI 595203] using the eIF4E CAPS marker and were cleaved using $MseI$. M:1kb plus DNA ladder.
Literature Cited