Host responses elicited by a satellite virus-associated synergism in multiple Brachypodium distachyon accessions

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ABSTRACT

Panicum mosaic virus (PMV) and its satellite virus (SPMV) infect several species of grasses (Poaceae). SPMV is an 824-nucleotide single-stranded RNA virus that is dependent upon PMV, its helper virus, for replication and movement in host plants. The 17-kDa capsid protein of SPMV (SPCP) is the main effector of a distinct disease synergism with its helper virus, causing pronounced disease symptoms. Our previous studies in millets and Brachypodium, a model grass species in the family Poaceae, have shown that SPCP localizes to the nucleus/nucleolus and plasmodesmata. The PMV+SPMV co-infection mis-regulates the expression of several genes involved with metabolism, photosynthesis, and pathogen defense. We performed a screen of 189 geographically diverse inbred accessions of Brachypodium by co-infecting with PMV+SPMV. Plants were scored for disease symptoms at 7, 14, and 21 days post inoculation (dpi) and subsequently analyzed for accumulation of the 26-kDa PMV capsid protein and SPCP, as markers for mixed virus infections, by immunoblot analyses. At 21 dpi, analysis of virus accumulation on upper-non-inoculated leaves of the 189 accessions revealed that only 24 accessions consistently supported SPMV, characterized by the typical severe disease symptoms of PMV+SPMV-infected plants, while the others did not. Plants that did not support SPMV had milder symptoms. The future goal of this study is to identify genetic factors that contribute to the PMV and SPMV-synergism, thus facilitating the engineering of new strategies for host plant resistance.

RESULTS AND DISCUSSION

Figure 1. Phenotypic and molecular characterization of PMV and PMV+SPMV infection in Brachypodium. Brachypodium plants infected with PMV and PMV+SPMV displays chlorosis and necrosis of shoots and are stunted (A, B). Inset picture in Figure 1A shows the deep chlorosis symptoms typical associated with the co-infection of PMV+SPMV compared to PMV alone infection. Immunoblotting using PMV and SPMV anti-serum consistently detected PMV and SPMV coat protein (CP) in the infected plants (C). Coomassie- and Ponceau S-stained gel and membrane, respectively, are shown to demonstrate approximately equal loading in the lanes. The asterisk (*) represents lower molecular-weight CPs, possibly produced from leaky scanning or post-translational modifications.

Table 1. Microarray analysis of PMV and PMV+SPMV infection.

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>PMV</th>
<th>PMV+SPMV</th>
<th>Description</th>
</tr>
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<tr>
<td>Bradi2g09850</td>
<td>1.11</td>
<td>1.58</td>
<td>Pre-mRNA splicing factor PRP21 like protein, splicing factor 3A subunit 1</td>
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<tr>
<td>Bradi5g07850</td>
<td>1.11</td>
<td>2.09</td>
<td>Splicing factor 1/branch point binding protein (RRM superfamily), Zinc knuckle domain</td>
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<td>Bradi3g06560</td>
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<td>1.55</td>
<td>AP2 domain</td>
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<tr>
<td>Bradi4g34700</td>
<td>1.02</td>
<td>1.63</td>
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</tbody>
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Figure 2. Genetic screen of 189 Brachypodium accessions following inoculation with PMV+SPMV. Brachypodium accessions (A) were collected from different geographical locations in Turkey. A representative map of the few accessions are indicated in Figure 2C. Accessions were categorized based on the presence or absence of PMV and SPMV upon infection, as determined by immunoblotting using PMV and SPMV anti-serum and disease symptoms.

CONCLUSIONS AND FUTURE EXPERIMENTS

• Brachypodium is a supercalifragilisticexpialidocious (good) genetic model for studying host-virus interactions and synergism between PMV and SPMV.

• Genetic diversity among Brachypodium accessions will allow further molecular characterization of the PMV and SPMV synergism.

• SPMV specifically affects expression of transcription and splicing factors.

• Expression analysis of SPMV-specific nuclear factors among the Brachypodium accessions using quantitative RT-PCR will allow us to determine if their regulation is conserved among different Brachypodium accessions that support SPMV.

REFERENCES