New Sources of Resistance in Sorghum (Sorghum bicolor) Germplasm Are Effective Against a Diverse Array of Potyvirus spp.

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Abstract


Sorghum is a host to numerous Potyvirus spp. and its germplasm encompasses a wide range of infection responses to these viruses. We determined how 183 mini-core-collection sorghum germplasm accessions responded to mechanical inoculation with Maize dwarf mosaic virus (MDMV) in growth regimes in which they were maintained at 30°C followed by 16°C for 5 days. Accessions that appeared immune to MDMV in this initial screening were evaluated for their response in a similar temperature maintenance regime to mechanical inoculation with MDMV, Sugarcane mosaic virus strain MDB (SCMV-MDB), Sorghum mosaic virus (SrMV), Zea mosaic virus (ZeMV), and Kansas, Nigerian, and Australian isolates of Johnsongrass mosaic virus (JGMV-KS, -N, and -Aus, respectively). In both experiments, MDMV systemically infected all accessions except international sorghum accession number (IS) 7679 and IS 20740. These accessions also proved resistant to MDMV, SCMV-MDB, SrMV, and JGMV-N but both were susceptible to the JGMV-KS and JGMV-Aus isolates. IS 7679 but not IS 20740 was resistant to infection with ZeMV. These observations suggest that IS 7679 and IS 20740 might serve as new sources of resistance to several Potyvirus spp. that systemically infect sorghum.

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braska source of SCMV-MDB (formerly MDMV-B) was obtained from Stan Jensen (University of Nebraska, Lincoln), and the ZeMV isolate was the original used in 2000 to describe the virus (18). Three isolates of JGMV were used: a Kansas isolate (JGMV-KS) (17); the Nigerian isolate of JGMV (JGMV-N), which was used to describe the virus (17); and the Australian isolate (JGMV-Aus, obtained from Dennis Persley, Indooroopilly, Australia) that infects sorghum with the Krish gene for resistance (11,15). The PV 51 isolate of SrMV was obtained from the American Type Culture Collection (2). All isolates were increased in ‘GA TE 76’ sorghum in growth chambers (Percival Model PGC-15 WC) set at 30°C with a 12-h photoperiod of fluorescent light (250 µE s⁻¹ m⁻²). Symptomatic GA TE 76 sorghum plants separately infected with each virus were harvested at intervals of 21 days post inoculation (DPI) and frozen at 80°C just prior to the start of these studies. All inoculations of the seedlings in the experiments were conducted using the 21 DPI frozen virus sources. GA TE 76 sorghum was used because it is susceptible to systemic infection with all the Potyvirus spp. examined in this study (D. L. Seifers, unpublished).

Indirect enzyme-linked immunosorbent assay and sources of antisera. The third leaves from the base of individual plants were sampled separately at 14 DPI. Each leaf sample was ground in a 1.5-ml microcentrifuge tube with a wood applicator stick (Fisher Scientific) at a 1:30 (wt/vol) dilution in 0.05 M carbonate buffer, pH 9.6 (6). Extracts (200 µl) were pipetted into wells of enzyme-linked immunosorbent assay (ELISA) plates (Immulon 1; Fisher Scientific) for 1 h at 37°C. Following a rinse step, the wells were incubated for 1 h at 37°C with the appropriate antivirus antibody (200 µl) in dilution buffer (6). The wells were rinsed and blocked for 1 h in blocking buffer (5% nonfat dry milk, 0.01% antifoam A, and 0.02% sodium azide in phosphate-buffered saline, pH 7.4) at 37°C. After blocking, 200 µl of anti-rabbit antibody/alkaline phosphatase conjugate (Southern Biotechnology Associates) in dilution buffer (1:3,000 vol/vol) was added to each well. The plates were held at 37°C for 1 h. The wells were rinsed, and 200 µl of p-nitrophenyl phosphate substrate (0.714 mg/ml), in substrate buffer (6), was added to each well. The plates were held at 37°C for 1 h. The wells were rinsed and blocked for 1 h in blocking buffer (5% nonfat dry milk, 0.01% antifoam A, and 0.02% sodium azide in phosphate-buffered saline, pH 7.4) at 37°C. After blocking, 200 µl of anti-rabbit antibody/alkaline phosphatase conjugate (Southern Biotechnology Associates) in dilution buffer (1:3,000 vol/vol) was added to each well. The plates were held at 37°C for 1 h. The wells were rinsed, and 200 µl of anti-rabbit antibody/alkaline phosphatase conjugate (Southern Biotechnology Associates) in dilution buffer (1:3,000 vol/vol) was added to each well. The plates were held at 37°C for 1 h. The wells were rinsed, and 200 µl of p-nitrophenyl phosphate substrate (0.714 mg/ml), in substrate buffer (6), was added to each well. The plates were then held at 20 to 22°C for 30 min. Absorbance at 405 nm was measured using an iMark plate reader (Bio-Rad Laboratories). Absorbance values were arbitrarily considered positive if they were at least twice those of the equivalent mock-inoculated control value. Plants below this arbitrary threshold were considered negative in ELISA. The terms “positive” and “negative” are used for the purposes of brevity; rather than repeatedly stating that extracts from plants reacted with a given antibody set at a two times or greater than the healthy control, we state that such a plant or plants were positive and a plant or plants below the arbitrary threshold are negative.

The MDMV antiserum (PVAS 55) was obtained from the American Type Culture Collection and the ZeMV antiserum was obtained as previously described (18). A single antiserum prepared to a Kansas isolate as previously described was used for analyses of all JGMV isolates (17). The SCMV-MDB antiserum was from Ray Louie (United States Department of Agriculture–Agricultural Research Service, Wooster, OH) and the SrMV antiserum was obtained as previously described (18). Three isolates of JGMV were used: a Kansas isolate (JGMV-KS) (17); the Nigerian isolate of JGMV (JGMV-N), which was used to describe the virus (17); and the Australian isolate (JGMV-Aus, obtained from Dennis Persley, Indooroopilly, Australia) that infects sorghum with the Krish gene for resistance (11,15).

### Table 1. Sorghum accessions that developed only a mosaic symptoms 19 days after mechanical inoculation with Maize dwarf mosaic virus in a growth chamber

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*IS = International sorghum accession numbers are as given by Upadhyaya et al. (23).*

*Country of origin of the sorghum is as given by Upadhyaya et al. (23).*

*Phenotype: M1 = faint mosaic, M2 = moderate mosaic, M3 = severe mosaic, N1= necrotic spots and dashes (1 to 10% of the leaf), N2 = necrotic streaks and stripes (11 to 40% of the leaf), N3 = whole leaf necrosis (41 to 100% of the leaf), and NS = no symptoms. Following inoculation, the plants were held at 30°C for 14 days followed by 5 days at 16°C.*
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Table 2. Sorghum accessions that developed necrosis 19 days after mechanical inoculation with Maize dwarf mosaic virus in a growth chamber*

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a IS = International sorghum accession numbers are as given by Upadhyaya et al. (23).
b Country of origin of the sorghum is as given by Upadhyaya et al. (23).
c Phenotype: M1 = faint mosaic, M2 = moderate mosaic, M3 = severe mosaic, N1 = necrotic spots and dashes (1 to 10% of the leaf), N2 = necrotic streaks and stripes (11 to 40% of the leaf), N3 = whole leaf necrosis (41 to 100% of the leaf), and NS = no symptoms. Following inoculation, the plants were held at 30°C for 14 days followed by 5 days at 16°C. Plants of these lines developed necrosis after exposure to 16°C.

Responses of IS 7679 and IS 20740 to inoculation with a diverse array of Potyvirus spp. Plants of the accessions inoculated with the seven viruses were differentially infected depending upon isolate (Table 3). All GA TE 76 plants were systemically infected when inoculated with each virus isolate. Plants of accession IS 7679 were systemically infected with JGMV-KS and JGMV-Aus; those of IS 20740 with ZeMV, JGMV-KS, and JGMV-Aus; and those of ICI 5616 with only the JGMV-Aus isolate. In the three experiments, only symptomatic plants reacted with their respective homologous antibody, indicating that immunity, rather than latent infection, accounted for the absence of symptoms.

Discussion

The mini-core collection of sorghum accessions represents 1% of a core collection of 2,247 sorghum accessions that are representative of the species diversity in sorghum (23). In the initial experiment in which we analyzed the responses of 183 mini-core-collection accessions to inoculation with MDMV, the diverse array of host responses (Tables 1, 2, and 3) encompassed a subset of accessions (8%) which developed some level of necrosis and a much larger subset (79%), which developed only a mosaic symptom in a 16°C maintenance regime. If we had failed to discover the resistance expressed by accessions IS 7679 and IS 20740, convention would have led us to advance the numerous accessions that responded with mosaic symptoms, because the mosaic reaction has been considered the resistant, tolerant, or preferred reaction to infection (1,3,5,10,22). This preference, however, has lessened since sorghum with higher levels of resistance such as Krish, QL20, and Wiru (IS 8789) have been identified (10,13,15). We do not know why plants in 19 of the accessions had a variable response, with some individuals developing only mosaic symptoms while others developed mosaic combined with necrosis (Table 3). This variation in phenotype may be because the accessions were not genetically homogenous.

The accessions IS 7679 and IS 20740 distinguished themselves from all others by consistently failing to develop symptoms in both experiments in which the response to MDMV was used to identify candidates for resistant host responses to other Potyvirus spp. The question of the sources or sources of the apparent resistance needs to be addressed. We considered the possibility that IS 7679 and IS 20740, like Krish, are photoperiod sensitive (R. Perumal, personal communication) and might have derived their resistance from that source by crossing. This uncertainty prompted us to conduct an extended analysis of the IS 7679 and IS 20740 accessions at 30°C by separately inoculating with MDMV, SCMV-MDB, SrMV, ZeMV, and JGMV-KS, JGMV-N, and JGMV-Aus.

Table 3. Sorghum accessions with mixed disease phenotypes 19 days after mechanical inoculation with Maize dwarf mosaic virus in a growth chamber*

<table>
<thead>
<tr>
<th>IS number</th>
<th>Countryb</th>
<th>Phenotypec</th>
</tr>
</thead>
<tbody>
<tr>
<td>7305</td>
<td>Nigeria</td>
<td>M3 &amp; M3N1</td>
</tr>
<tr>
<td>8774</td>
<td>South Africa</td>
<td>M2 &amp; M2N1</td>
</tr>
<tr>
<td>12697</td>
<td>Australia</td>
<td>M3 &amp; M3N1</td>
</tr>
<tr>
<td>12945</td>
<td>Nicaragua</td>
<td>M3 &amp; M3N2</td>
</tr>
<tr>
<td>12965</td>
<td>Cuba</td>
<td>M2 &amp; M2N2</td>
</tr>
<tr>
<td>15170</td>
<td>Cameroon</td>
<td>M2 &amp; M2N2</td>
</tr>
<tr>
<td>19389</td>
<td>Bangladesh</td>
<td>M3 &amp; M3N1</td>
</tr>
<tr>
<td>20632</td>
<td>United States</td>
<td>M3 &amp; M3N1</td>
</tr>
<tr>
<td>21863</td>
<td>Syrian Arab Republic</td>
<td>M3 &amp; M3N1</td>
</tr>
<tr>
<td>24453</td>
<td>South Africa</td>
<td>M3 &amp; M3N1</td>
</tr>
<tr>
<td>24492</td>
<td>South Africa</td>
<td>M3 &amp; M3N2</td>
</tr>
<tr>
<td>26737</td>
<td>South Africa</td>
<td>M3 &amp; M3N3</td>
</tr>
<tr>
<td>29239</td>
<td>Swaziland</td>
<td>M3 &amp; M3N3</td>
</tr>
<tr>
<td>29236</td>
<td>Swaziland</td>
<td>M3 &amp; M3N1</td>
</tr>
<tr>
<td>29392</td>
<td>Lesotho</td>
<td>M2 &amp; M2N2</td>
</tr>
<tr>
<td>30450</td>
<td>China</td>
<td>M2 &amp; M2N2</td>
</tr>
<tr>
<td>30466</td>
<td>China</td>
<td>M2 &amp; M2N1</td>
</tr>
<tr>
<td>30838</td>
<td>Cameroon</td>
<td>M2 &amp; M2N1</td>
</tr>
<tr>
<td>31043</td>
<td>Uganda</td>
<td>M3 &amp; M3N1</td>
</tr>
</tbody>
</table>

a IS = International sorghum accession numbers are as given by Upadhyaya et al. (23).
b Country of origin of the sorghum is as given by Upadhyaya et al. (23).
c Phenotype: M1 = faint mosaic, M2 = moderate mosaic, M3 = severe mosaic, N1 = necrotic spots and dashes (1 to 10% of the leaf), N2 = necrotic streaks and stripes (11 to 40% of the leaf), N3 = whole leaf necrosis (41 to 100% of the leaf), and NS = no symptoms. Following inoculation, the plants were held at 30°C for 14 days followed by 5 days at 16°C.

In addition to providing sources of resistance to MDMV, the IS 7679 and IS 20470 accessions may well serve as effective new sources of resistance to other Potyvirus spp., including SCMV-MDB and SrMV. For IS 7679, resistance extends to ZeMV as well. These two sources do not confer resistance to all the isolates of JGMV we tested, and variant isolates of other viruses yet to be tested might also attenuate the case we make here for their versatil-
Table 4. Enzyme-linked immunosorbent assay (ELISA) values and numbers of infected sorghum plants mechanically inoculated with different *Potyvirus* spp. after being held in a growth chamber at 30°C for 14 days<sup>a</sup>

<table>
<thead>
<tr>
<th>Virus&lt;sup&gt;b&lt;/sup&gt;</th>
<th>N</th>
<th>IS 7679</th>
<th>N</th>
<th>IS 20740</th>
<th>N</th>
<th>ICI 5616</th>
<th>N</th>
<th>GA TE 76</th>
<th>N</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDMV</td>
<td>0/15</td>
<td>0.005 (±0.001)</td>
<td>0/22</td>
<td>0.004 (±0.000)</td>
<td>0/22</td>
<td>0.004 (±0.000)</td>
<td>3/3</td>
<td>(±0.068)</td>
<td>0/3</td>
<td>(±0.001)</td>
</tr>
<tr>
<td>SCMV-MDB</td>
<td>0/19</td>
<td>0.005 (±0.000)</td>
<td>0/22</td>
<td>0.004 (±0.001)</td>
<td>0/25</td>
<td>0.004 (±0.001)</td>
<td>3/3</td>
<td>(±0.043)</td>
<td>0/3</td>
<td>(±0.004)</td>
</tr>
<tr>
<td>SrMV</td>
<td>0/14</td>
<td>0.003 (±0.001)</td>
<td>0/22</td>
<td>0.003 (±0.001)</td>
<td>0/28</td>
<td>0.003 (±0.001)</td>
<td>3/3</td>
<td>(±0.091)</td>
<td>0/3</td>
<td>(±0.003)</td>
</tr>
<tr>
<td>ZeMV</td>
<td>0/14</td>
<td>0.032 (±0.013)</td>
<td>0/23</td>
<td>0.771 (±0.034)</td>
<td>0/25</td>
<td>0.076 (±0.009)</td>
<td>3/3</td>
<td>(±0.067)</td>
<td>0/3</td>
<td>(±0.024)</td>
</tr>
<tr>
<td>JGMV-KS</td>
<td>13/13</td>
<td>0.641 (±0.090)</td>
<td>18/18</td>
<td>0.560 (±0.018)</td>
<td>0/23</td>
<td>0.031 (±0.002)</td>
<td>3/3</td>
<td>(±0.015)</td>
<td>0/3</td>
<td>(±0.003)</td>
</tr>
<tr>
<td>JGMV-N</td>
<td>17/17</td>
<td>0.012 (±0.003)</td>
<td>0/23</td>
<td>0.018 (±0.003)</td>
<td>0/27</td>
<td>0.016 (±0.003)</td>
<td>3/3</td>
<td>(±0.093)</td>
<td>0/3</td>
<td>(±0.003)</td>
</tr>
<tr>
<td>JGMV-Aus</td>
<td>13/13</td>
<td>0.408 (±0.031)</td>
<td>22/22</td>
<td>0.309 (±0.043)</td>
<td>22/22</td>
<td>0.372 (±0.031)</td>
<td>3/3</td>
<td>(±0.028)</td>
<td>0/3</td>
<td>(±0.001)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values (405 nm) are the mean from three experiments followed by ± the standard error.

<sup>b</sup> IS 7679 and IS 20740 are sorghum accessions and ‘GA TE 76’ and ‘ICI 5616’ are cultivars. Healthy = mock-inoculated GA TE 76 sorghum. For each value N, the numerator represents the number of sorghum plants with symptoms and positive in ELISA for a respective antiserum and the denominator the total number of plants inoculated with a respective virus in the three experiments. For the GA TE 76 positive controls, a single infected plant was sampled from a set of 6 to 10 symptomatic control plants for a given virus treatment in each experiment, so that three plants were analyzed. ELISA values in bold are considered positive, indicating that virus-specific antibodies reacted with extracts from plants above the arbitrary positive threshold of twice the healthy control value. Only symptomatic plants were positive.

<sup>c</sup> MDMV = *Maize dwarf mosaic virus*, SCMV-MDB = *Sugarcane mosaic virus* strain maize dwarf mosaic B, SrMV = *Sorghum mosaic virus*, ZeMV = *Zea mays mosaic virus*, JGMV = *Johnsongrass mosaic virus* (KS = Kansas, N = Nigerian, and Aus = Australian isolates).

Identification of the resistance conditioned by these accessions is inherited, and how effectively they protect yield of elite sorghum germplasm when under pressure from infection with a range of *Potyvirus* spp. Fresh crosses were made using IS 7679 and IS 20420 separately as donor parents with ‘RTX 430’, a universal adaptable sorghum susceptible to these *Potyvirus* spp. Evaluation of the F<sub>1</sub> to study the gene action and subsequent segregating generations will be evaluated to develop recombinant inbred lines for marker-assisted selection to MDMV and other *Potyvirus* spp. resistance.

Acknowledgments

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Literature Cited