Registration of the OhVRS-1 Maize Synthetic Population
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ABSTRACT
Virus diseases cause significant losses in maize worldwide. A virus-resistant synthetic maize (Zea mays L.) population, OhVRS-1 (Reg. No. GP-583, PI 665687), was developed by the USDA Corn and Soybean Research Unit and released as a germplasm resource by Ohio State University, the Ohio Agriculture Research and Development Center, and the USDA. OhVRS-1 is a synthetic population made up of tropical and Corn Belt germplasm selected for high levels of resistance to viral diseases, and B73-selected for agronomic traits. The population has undergone one complete cycle of S, recurrent selection for resistance to Maize dwarf mosaic virus, Sugarcane mosaic virus (SCMV), and Maize chlorotic dwarf virus. The OhVRS-1 cycle-1 was significantly more resistant to SCMV and had a reduction in days to mid-silk when compared with cycle-0.

Virus diseases cause significant economic losses in most food crops, estimated to be more than $60 billion annually (Hsu, 2002), and the potential losses from virus diseases were estimated to be 3% for maize (Zea mays L.) (Oerke and Dehne, 2004). At least nine different virus species cause significant disease problems in maize worldwide (Redinbaugh et al., 2004; Redinbaugh and Pratt, 2008), and emerging and reemerging virus diseases pose continual problems for maize breeders. Virus disease problems are likely to increase as biofuel grass acreage increases. For example, a recent survey revealed Sugarcane mosaic virus (SCMV) infection of Miscanthus × giganteus, energycane (Saccharum spp.), and sweet corn. In addition, a virus similar to Maize rayado fino virus, which causes disease in Central and South America, was found in switchgrass (Panicum virgatum L.) grown in Illinois (Agindotan and Perry, 2007). A recent survey of maize and johnsongrass (Sorghum halepense L.) in Ohio indicated the continued presence of Maize dwarf mosaic virus (MDMV) and Maize chlorotic dwarf virus (MCDV) (L. Stewart, personal communication, 2012).

Virus diseases in maize are most effectively controlled by using virus-resistant hybrids, removing the perennial virus reservoirs, such as johnsongrass, and using earlier or later planting dates to avoid vector populations. Although viruses such as MDMV and SCMV are currently well controlled in the U.S. Corn Belt, the studies above indicate that potentially destructive maize-infecting viruses continue to be present. Deploying virus-resistant germplasm is considered the most environmentally and economically sustainable approach for controlling virus diseases, but identifying both the causal agent of the virus disease and suitably adapted virus-resistant germplasm are not always simple for maize breeders.

The potyviruses MDMV and SCMV are found worldwide. Resistance to these viruses has been identified in germplasm from the U.S. Corn Belt, tropics, Europe, and Asia (Redinbaugh and Pratt, 2008), but the strongest resistance is frequently found in tropical materials (Jones et al., 2007). MCDV is found in the United States from the
Gulf of Mexico north to the Ohio River and west through Texas. Lines from Mississippi and Ohio with moderate MCDV resistance were identified (Pratt et al., 1994), but the only strong resistance to MCDV has been identified in tropical germplasm, including Oh1VI (Louie et al., 2002) and the inbred lines DR and Cuba (Jones, unpublished data, 1995; Louie, personal communication, 1995). In all three of these inbred lines, virus resistance is quantitative, with major quantitative trait loci for MCDV resistance identified in Oh1VI on chromosomes 3 and 10 (Jones et al., 2004). Some of these lines have more recently been identified as resistant to emerging viruses, including Wheat mosaic virus (WMoV, aka High Plains virus), Maize necrotic streak virus, Maize fine streak virus, and MRFV (Marçon et al., 1997; Redinbaugh, unpublished data, 1999; Louie et al., 2000; Redinbaugh et al., 2002).

Virus resistant germplasm identified in these studies is often very poorly adapted to Corn Belt growing conditions and has delayed flowering, high ear placement, and other deleterious traits. For example, the inbred line Oh1VI is highly resistant to several viruses, but it is also highly susceptible to Ustilago maydis (DC.) Corda (Louie et al., 2002). To provide virus-resistant germplasm with improved agronomic traits, we developed the OhVRS-1 population (Reg. No. GP-583, PI 665687) to combine resistance to multiple viruses into a genetic background that has improved adaptation to Corn Belt environments.

Materials and Methods

Population Development and Evaluation Material

Germplasm development was performed in the field at Wooster, OH. The 10 inbred lines used as parents (Table 1) were intercrossed to make 22 F1 lines in 2001, and the F1 plants were selfed in 2002. In 2003, 22 F12 families were intercrossed using a paired-row bulk-entry method. Brieﬂy, each F12 family was paired with a row containing one seed each from the other 21 F12 families, and all possible crosses were made between each pair of rows using each plant as a male only once. A 5000-seed balanced composite was made from 178 intercrossed ears that were harvested. In 2004, the population was random mated by making two 1000-seed plantings of the composite on two dates 10 d apart. Plants were randomly mated using plants from both plantings as needed and each plant as a male only once. A balanced composite of 5000 seeds from 412 ears harvested from the random mating cycle was made to form OhVRS-1 cycle 0.

In 2005, 500 OhVRS-1 cycle-0 seeds were planted, and the plants were self-pollinated to create 213 S1 lines. In 2006, 100 of the OhVRS-1 cycle 0 S1 lines were evaluated for their responses to MDMV, SCMV, and MCDV inoculation as outlined below. MDMV and SCMV incidence and MCDV severity were ranked (Jones et al., 2004, 2007). Twenty lines were selected for intermating using a rank and replace method (Jones et al., 1993). In 2007, 15 lines that developed no symptoms after MDMV and SCMV inoculation, and 5 lines with a mean disease severity rating of 1 (i.e., no symptoms developed) after MCDV inoculation were selected for intermating via the paired bulk-entry method outlined above, and a 5000-seed balanced composite was made from the 254 ears harvested. In 2008, 500 seed of the composite were planted, and plants were randomly intermated using plants as males only once. A balanced composite of 5000 seeds was made from the 370 harvested ears to form OhVRS-1 cycle 1. In 2009, 500 seeds of OhVRS-1 cycle 1 were planted and self-pollinated to form 216 S1 lines. In 2010 and 2011, ear height, days to mid-silk, and the responses to MDMV, SCMV and Wheat streak mosaic virus (WSMV) inoculation were evaluated on 30 randomly selected S1 lines from OhVRS-1 cycle 0 and 30 from OhVRS-1 cycle 1.

Evaluation of Responses to Virus Inoculation

Single-row plots (6 m long and 0.76 m apart) of 25 seeds for each S1 line were evaluated for their responses to virus inoculation. Two replicates were evaluated in randomized blocks. Plots were inoculated with individual viruses four times at 2-d intervals starting at the V2 stage as previously described (Louie et al., 1983). Ohio isolates of MDMV, SCMV (Louie, 1986), and WSMV (McMullen and Louie, 1991) were maintained by serial inoculation of the susceptible maize inbred line Oh28. Plants were scored for the appearance of general and limited mosaic symptoms beginning 2 wk after the first inoculation and continuing at weekly intervals until no further increase in symptom development

### Table 1. Germplasm used for development of OhVRS-1.

<table>
<thead>
<tr>
<th>Inbred line</th>
<th>Type</th>
<th>Origin†</th>
<th>Virus resistance‡</th>
<th>No. crosses§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pa405</td>
<td>Dent</td>
<td>PSU</td>
<td>MDMV, SCMV, WSMV, WMoV, MFSV</td>
<td>5</td>
</tr>
<tr>
<td>Oh1EP</td>
<td>Dent</td>
<td>USDA/OSU</td>
<td>MDMV, SCMV, WSMV, MCDV</td>
<td>6</td>
</tr>
<tr>
<td>Pa11</td>
<td>Dent</td>
<td>PSU</td>
<td>MDMV, SCMV, WSMV</td>
<td>6</td>
</tr>
<tr>
<td>Oh1VI</td>
<td>Flint</td>
<td>Virgin Islands</td>
<td>MDMV, SCMV, MCDV, WMoV, MFSV, MNeSV, MRFV</td>
<td>6</td>
</tr>
<tr>
<td>Cuba Pl</td>
<td>Flint</td>
<td>Cuba 551</td>
<td>MDMV, SCMV, MCDV</td>
<td>6</td>
</tr>
<tr>
<td>DR PI</td>
<td>Flint</td>
<td>Dominican Republic</td>
<td>MDMV, SCMV, MCDV</td>
<td>5</td>
</tr>
<tr>
<td>Mp705</td>
<td>Dent</td>
<td>Mississippi</td>
<td>MDMV, SCMV, MCDV</td>
<td>6</td>
</tr>
<tr>
<td>Kp291</td>
<td>Dent</td>
<td>Australia</td>
<td>MDMV, SCMV, WSMV</td>
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</tr>
<tr>
<td>HIX4231</td>
<td>Dent</td>
<td>Hawaii</td>
<td>MDMV, SCMV, WSMV, MMV</td>
<td>1</td>
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<tr>
<td>B73</td>
<td>Dent</td>
<td>Iowa</td>
<td>Agronomic traits</td>
<td>2</td>
</tr>
</tbody>
</table>

†Origin of inbred line; PSU, Penn State University; OSU, Ohio State University.

‡Virus resistance was previously identified; MDMV, Maize dwarf mosaic virus; SCMV, Sugarcane mosaic virus; WSMV, Wheat streak mosaic virus; WMoV, Wheat mosaic virus (also known as High Plains virus); MFSV, Maize fine streak virus; MCDV, Maize chlorotic dwarf virus; MNeSV, Maize necrotic streak virus; MRFV, Maize rayado fino virus; MMV, Maize mosaic virus.

§Inbred lines were crossed and the resulting F1 lines were selfed, and the resulting F12 families were used in the paired bulk-entry intercross to form cycle 0.

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was seen, as previously described (Jones et al., 2011). Mean days to mid-silk and ear height were also scored. The general linear model of the Statistical Analysis System 9.1 (SAS Institute) was used for ANOVA.

**Evaluation of Responses to MCDV Inoculation**

In 2006, 10 plants for each of 100 OhVRS-1 cycle 0 S<sub>i</sub> lines were evaluated for their responses to inoculation with MCDV-S (Gingery and Nault, 1990) using three inoculations at 2-d intervals with viruliferous leafhoppers [Grainella nigrifrons (Forbes)] (Louie and Anderson, 1993). After inoculation, the plants were fumigated to remove the leafhoppers and transferred to a greenhouse for symptom development. Plants were scored for the vein banding that is diagnostic of MCDV infection using a 1–5 scale (where 1 = no symptoms, 5 = severe vein banding) for 3 wk beginning 1 wk after the last inoculation (Jones et al., 2004). Mean scores for each line were used for resistance evaluation. Susceptible Oh28 and resistant Oh1VI controls were included in each cage.

### Characteristics

**Responses of OhVRS-1 Cycle-0 S<sub>i</sub> Lines to Inoculation with MDMV, SCMV, and MCDV**

In the 2006 field trial, 100% of (virus-susceptible) Oh28 plants became symptomatic after inoculation with MDMV or SCMV, indicating that the virus inoculation protocol was successful. Of the 100 S<sub>i</sub> lines inoculated with MDMV, 32 did not develop symptoms. In the remaining 68 lines, infection ranged from 3 to 76%, with a mean infection of 11.9%. Similarly, 30 of the 100 S<sub>i</sub> lines did not develop symptoms after inoculation with SCMV. Of the remaining lines, infection ranged from 3 to 73%, with a mean of 17.3%. The S<sub>i</sub> lines were all apparently more resistant to MDMV and SCMV than Oh28, because none of the former became 100% infected.

In tests for resistance to MCDV, Oh28 plants developed clear symptoms after multiple inoculations with viruliferous leafhoppers, with a mean disease severity score of 4.79 at 14 d after the last inoculation, indicating successful virus inoculation. The mean disease severity score was 4.23 for all S<sub>i</sub> lines, suggesting a modest increase in resistance relative to Oh28. However, 9 of the 100 S<sub>i</sub> lines tested did not develop symptoms after MCDV inoculation, similar to the highly resistant inbred line, Oh1VI.

**Comparison of OhVRS-1 Cycle-0 and OhVRS-1 Cycle-1 Responses to Inoculation with MDMV, SCMV, and WSMV**

In the 2010 field test, all Oh28 plants became symptomatic within 14 d after the final inoculation with MDMV, SCMV or WSMV, indicating that there was effective virus transmission (data not shown). Less than 5% of plants from the selected S<sub>i</sub> lines developed symptoms after MDMV inoculation, and infection was not significantly different for lines derived from OhVRS-1 cycle-0 and OhVRS-1 cycle-1 plants (Table 2). In contrast, significantly fewer OhVRS-1 cycle-1-derived S<sub>i</sub> plants (6.6%) developed symptoms than OhVRS-1 cycle-0-derived S<sub>i</sub> plants (29.4%) after SCMV inoculation, indicating that there was an increase in population resistance to this virus. No plants showed symptoms after WSMV inoculation, which was as expected since three independent genes control WSMV resistance (McMullen et al., 1994).

The relative rates of infection with SCMV, MDMV, and WSMV were consistent with previous studies indicating that the Ohio SCMV isolate infects more lines than the Ohio MDMV isolate, and that WSMV can infect only a few maize lines (Jones et al., 2007, 2011). Increased resistance to WSMV and MDMV could not be detected due to the relatively high levels of resistance to these viruses in the OhVRS-1 cycle-0 S<sub>i</sub> lines. However, SCMV resistance was improved in the OhVRS-1 cycle-1 population relative to the cycle-0 population, indicating that favorable allelic combinations of resistance genes resulting from recurrent selection can improve SCMV resistance. The Ohio SCMV is more aggressively than the MDMV or WSMV isolates, and near-isogenic lines carrying one or two of three loci from the inbred line Pa405 develop symptoms after SCMV inoculation (Jones et al., 2011). Similar improvements in virus resistance through recurrent selection have been previously reported (Findley et al., 1977; Jones et al., 1993).

There was a 3-d reduction in the mean number of days to mid-silk between cycle 0 and cycle 1 (Table 2). It is possible that unintentional bias against late flowering plants was introduced by the hand pollinations in the intermating and random mating steps and may have contributed to the reduction in mean days to mid-silk. Ear heights were not significantly different between cycle-0 and cycle-1 plants. These data indicate that the recurrent selection process resulted in a population with increased resistance to SCMV and fewer days to mid-silk. Inbred lines developed from this population have the potential to combine high levels of resistance to multiple virus diseases with earlier flowering, an improved trait for Corn Belt environments.

### Availability

OhVRS-1, a maize population with resistance to MDMV, SCMV, WSMV, and MCDV, was released from Ohio State University Crop Variety Release and Distribution Committee in 2011. Seed will be available in 500-seed lots on request to the authors at the USDA Corn and Soybean Unit, 1680 Madison Ave., Wooster, OH 44691. Seed of this germplasm has been deposited at the National Plant Germplasm System.
in Ft. Collins, CO for curation. Appropriate recognition should be made if this population contributes to the development of new breeding material.

References


