PAPAYA VARIETY DEVELOPMENT IN FLORIDA

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Abstract. A papaya (Carica papaya L.) variety development program was initiated using breeding lines with transgenic resistance to papaya ringspot virus. Selected PRSV-resistant transgenic lines (R1) were all female and were crossed with six papaya genotypes to produce the R2 generation. The R2 generation was evaluated in the field in 2001-2002, and the R3 generation, derived from self-pollinated R2 selections, was evaluated in the field in 2002-2003. In the R3 generation, a total of 1263 fruits were harvested from 150 hermaphroditic trees selected from the 1196 trees planted. Mean fruit weight was 1.2 kg for those lines with ‘Solo Sunrise’ papaya as the original pollen parent and was 2.4 kg for those lines with ‘Red Lady’ as the original pollen parents. The mean weights for the other pollen parents, ‘Puerto Rico 6-65,’ ‘Experimental No. 15,’ ‘Tainung No. 5’ and ‘Solo 40’ were between these values. Those lines with ‘Solo Sunrise’ as an original pollen parent also had the highest mean concentration of soluble sugars in fruits. The concentration of soluble sugars declined in January and February when the temperatures were cool. Of the 1196 trees evaluated, 24 trees representing four transgenic lines and the six original pollen parents were selected for production of the next breeding generation.

Papaya ringspot virus (PRSV) causes one of the most economically important diseases of papaya and is a major limiting factor in papaya production in Florida (Conover, 1964). Efforts to overcome PRSV in Florida by Conover et al. (1986) resulted in the development of ‘Cariflora,’ a PRSV-tolerant variety. ‘Cariflora’ has served as a source of PRSV resistance in different papaya breeding programs around the world, resulting in currently popular PRSV-tolerant varieties such as ‘Red Lady.’ However, the PRSV-tolerance in ‘Cariflora’ is difficult to transfer by breeding and the level of tolerance is often not adequate economically. Presently, the most effective means to manage PRSV is through transgenically derived PRSV resistance (Gonsalves, 1998). Transgenes for PRSV resistance have been incorporated into the ‘Rainbow’ and ‘Sun-up’ varieties presently being commercially grown in Hawaii (Tennant et al., 2001).

Because of licensing restrictions that prohibit growing the Hawaiian PRSV-resistant transgenic varieties outside of Hawaii and the possibility the Hawaiian transgene might provide inadequate protection against strains of the virus in Florida, we have embarked on a program to develop transgenic PRSV-resistant papaya varieties for Florida. Transgenes for resistance derived from the coat protein of a Florida isolate of PRSV were transferred the ‘F65’ variety of papaya (Davis and Ying, unpublished). All transgenic lines were female, and 21 selected, PRSV-resistant lines were crossed with six papaya genotypes to initiate the papaya variety development program. The first generation (R1) from 54 crosses was installed in the field in Mar. 2001, and the second generation, derived from 18 self-pollinated hermaphroditic selections from the first generation, was installed in the field in Mar. 2002. In both plantings, the frequency of natural infection by PRSV in the transgenic lines was considerably less than for non-transgenic plants in the same fields, indicating the transgenically derived PRSV resistance can be used to manage the disease.

The present paper describes some of the horticultural characteristics of selected transgenic lines of the R3 generation of transgenic PRSV-resistant papaya in the papaya variety development program at the Tropical Research and Education Center in Homestead, Florida.

Materials and Methods

Seeds derived from self-pollinated hermaphroditic breeding lines were sown during 20-25 Feb. 2002. For germination, seeds were soaked for 3 h in a 1 g·L⁻¹ solution of Miracle-Gro fertilizer (Scotts Miracle-Gro Products, Inc., Port Washington, N.Y.) and then planted in 98-well seedling trays containing Pro Mix BX soil mix (Premier Horticulture, Ltd., Dorval, Que.) amended with 14-14-14 Osmocote (Scotts-Sierra Horticultural Products Co., Maryville, Ohio) at 6.7 kg·m⁻³ of soil mix. Seeds were germinated and seedlings grown in a greenhouse at 28-34 °C. When seedlings were 3-4 weeks old, they were sprayed to run-off with kanamycin sulfate (Agri-Bio, Miami, Fla.) at 2 mg·mL⁻² in water with 0.02% SilWet L77 (Setre Chemical Co., Memphis, Tenn.) to detect the nptII gene that conferred resistance to kanamycin and was used as a selectable marker to identify non-transgenic plants. Seedlings were sprayed using a high-pressure spray gun at 80 psi at a distance of 10-20 cm from the plants. Each tray of seedlings was immediately put into a black garbage bag and kept out of direct sunlight for 12-14 h overnight to allow the kanamycin solution to be absorbed by the plants. The trays were removed from the bags and placed back on the greenhouse bench. Chlorosis of foliage of seedlings without the nptII gene was seen after approximately 7 d. Chlorotic seedlings were discarded, and non-chlorotic seedlings were transplanted to 24-well seedling trays.

Seedlings were transplanted to the field on 28 Mar. 2002. Seedlings were planted 8 feet apart within rows, and rows were ≅3 m apart. A randomized block design was used. Each plot consisted of 30 seedlings representing the R2 generation from the original cross of one transgenic line with one of the six original male parents, and plots were replicated at least once in two blocks. A total of 1196 seedlings (4 missing in one plot) representing 18 crosses were transplanted. Prior to planting, the rows were covered with plastic mulch and had

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been fumigated with methyl bromide. Plants were irrigated and watered through drip lines. Fruit of selected breeding lines were harvested at maturity, weighed, and the total soluble solids measured with a refractometer.

**Results and Discussion**

Because the papaya plants were transgenic, all seed, except that required for breeding purposes, had to be destroyed to guard against uncontrolled release of the materials. Therefore after evaluation, we removed trees as soon as possible to limit the amount of fruit that had to be handled in the process of destroying seed. Five months after planting in late March, 329 female plants were identified and removed. Soon thereafter, bagging of flowers on hermaphroditic plants was commenced to promote self-pollination, and the collection of seed from mature, self-pollinated fruit followed until late March 2003. By mid November, 723 hermaphroditic and nine more female plants had been removed. Selection of plants for removal was based primarily on plant vigor compared to other plants of the particular breeding line. Thus, 122 hermaphroditic plants and 13 female plants were retained for further selection. The D6 and X17-2 transgenic lines had single transgene copies and represented the majority of the selections (Table 1). The other transgenic lines originally had two or more transgene copies that might or might not have been maintained as multiple copies in subsequent generations.

A total of 1263 fruits were harvested from the 150 hermaphroditic plants remaining after initial selections. The sum of the number of fruit harvested and the fruit remaining on the trees as of 5 Feb. 2003, was used to calculate the total number of fruit set for individual trees. The total number of fruit and the mean weight of the mature fruit harvested can be used to evaluate yield (Fig. 1). However, total yield calculated as the product of these two values might be misleading because it does not take into account whether or not the fruit would be marketable. For example, the fruit was so crowded on the column of some trees that they were misshapen and not marketable, and the very large fruit of some other trees would not have been marketable because of size. Fruit weight varied significantly among the breeding lines derived originally from different pollen parents (Fig. 2) but not with respect to different transgenic lines (data not shown). Those lines with ‘Solo Sunrise’ as one original parent produced the smallest fruit averaging 1.2 kg; whereas, those with ‘Tainung No. 5’ or ‘Red Lady’ produced fruit weighing over 2 kg on the average.

The brix measurements indicative of soluble sugar in the fruit also varied depending on the breeding line (Fig. 3). Those lines with ‘Solo Sunrise’ as the original pollen parent had the highest total soluble solids averaging 11.1%. Those lines with ‘Experimental No. 15’ as an original pollen parent had the lowest total soluble solids averaging 10.0%. Interestingly, ‘Experimental No. 15’ is grown locally for the “green”

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**Table 1. Number of plants of different papaya breeding lines of the R^2 generation after initial selections in the field.**

<table>
<thead>
<tr>
<th>Transgenic line</th>
<th>Experimental No. 15</th>
<th>Puerto Rico 665</th>
<th>Red Lady</th>
<th>Solo 40</th>
<th>Solo Sunrise</th>
<th>Tainung No. 5</th>
<th>Grand total</th>
</tr>
</thead>
<tbody>
<tr>
<td>D6</td>
<td>9</td>
<td>10</td>
<td>14</td>
<td>12</td>
<td>9</td>
<td>2</td>
<td>54</td>
</tr>
<tr>
<td>D75</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>D95</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>X17-2</td>
<td>19</td>
<td>7</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>X26</td>
<td>3</td>
<td>1</td>
<td>13</td>
<td>6</td>
<td>10</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Grand Total</td>
<td>25</td>
<td>17</td>
<td>14</td>
<td>19</td>
<td>24</td>
<td>23</td>
<td>122</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Number of fruit set per tree 11 months after planting seed and mean weight of mature fruit harvested per tree for 150 selected hermaphroditic plants including the 24 final selections (●) of the R^2 generation.

**Fig. 2.** Mean fruit weight for R_2 generation trees derived from original crosses of a non-transgenic pollen parents with transgenic lines of ‘F65’ papaya.
papaya market where sweetness of the fruit is not an important factor. Overall, the brix measurements declined with the onset of cooler temperatures in January and February (Fig. 4).

Although resistance to PRSV was a major selection factor in the previous R1 generation where 24% of the plants had become naturally infected by the virus in the field resulting in the elimination of some breeding lines, only 2 of the 1196 plants of the R2 generation developed PRSV symptoms. In both generations, some plants sustained substantial damage due to infestation by mites and were eliminated from further consideration. Other factors, such as uneven fruit set at different times of the year, fruit shape, and general vigor of the tree were considerations taken into account during the selection process. Altogether, 24 individual trees were selected for further variety development efforts. These selections represent four of the 17 original transgenic lines and all six of the original pollen parents.

**Literature Cited**


