Somaclonal Variation: a Tool for the Improvement of Cavendish Banana Cultivars

Ching-Yan Tang
Taiwan Banana Research Institute, P.O. Box 18, Chiuju, Pingtung
Taiwan

Abstract

An improvement program for disease resistance and horticultural traits in Cavendish banana cultivars was established using somaclonal variants as a source of variation. The improvement procedure included single plant selection, clonal evaluation and experimental variety trials. Resistance to Fusarium wilt (race 4) is the most important breeding objective of the program. Resistant clones were selected by the pot screening technique using the fungal pathogen as the inoculum. A large quantity of young plants derived from tissue culture were subjected to the challenge of the disease. Putative resistant plants were identified and were multiplied by tissue culture for further evaluation. Through this procedure, several promising clones showing a high level of resistance to Fusarium wilt were selected and subjected to further evaluation.

Somaclonal variation is also an effective tool in the improvement of horticultural traits of banana. A semi-dwarf resistant clone, TC1-229, which is about 70 cm shorter, was identified. It was derived from 'Tai-Chiao No. 1' which is tall and susceptible to wind damage. This clone was registered as 'Tai-Chiao No. 3' in 2000 and released for commercial planting in 2001. Further selection from TC1-229 resulted in the identification of clones with improved bunch size and shape, and these are now undergoing further evaluation. Triploid Cavendish bananas are highly sterile and hence very difficult to improve by conventional hybridization method. Selection among somaclonal variants has proved to be an effective alternative in the improvement of Cavendish bananas.

INTRODUCTION

Plant tissue culture has been used as a tool for crop improvement in many different ways. Somaclonal variation (variation in plants regenerated from tissue culture) has occurred in many different crops, including bananas (Hwang, 1986; Reuveni and Israeli, 1990; Vuylsteke et al., 1991; Daniells and Smith, 1993). Somaclonal variation was claimed to be a source of variation for crop improvement, especially for crops reproduced vegetatively (Larkin and Scowcroft, 1981). In 1984, Taiwan Banana Research Institute started a selection program for resistance to Fusarium wilt in Cavendish bananas using somaclonal variation (Hwang and Ko, 1987). In 1992, a resistant cultivar, 'Tai Chiao No. 1', was successfully selected and released to banana growers in Taiwan (Hwang et al., 1992). In the last ten years, somaclonal variation has also been used to improve the horticultural traits of banana cultivars, such as plant stature, bunch size and the length of growing cycle etc. (Hwang and Tang, 1999). This paper reports on recent progress in cultivar development of Cavendish banana using somaclonal variation as a tool for improvement.

PROCEDURES OF SELECTION AND EVALUATION

Single Plant Selection

1. Selection for Resistance to Fusarium Wilt. In the early phase of selection, field evaluation was made by planting tissue-culture plantlets in a disease nursery. After 3-5 months, the rhizomes of surviving plants were unearthed, cut and examined for the browning of the vascular tissue. In recent years, pot evaluation has been made in the screen-house using artificial inoculation of the pathogen to the roots of 1-month-old plantlets propagated by tissue culture (Tang et al., 2002). After 6-10 weeks, the plants...
were removed from the pot and examined for internal symptoms of infection. Plants without symptoms or only slightly infected were considered as putative resistant plants and selected for further evaluation.

2. Selection for Improved Horticultural Traits. In order to minimize the environmental effect, uniform orchards were established with tissue-culture plants for selection of horticultural traits in banana. By the time of flowering and harvesting, every plant in the orchard was checked for superior characteristics. Plants with superior traits were tagged and the suckers were dug and used for multiplication by tissue culture. Selection criteria were early flowering, short stature and large bunch size. In addition, normal leaf shape and better plant habit were considered for improvement in a semi-dwarf resistant clone, TC1-229, registered as 'Tai Chaio No. 3'.

Clonal Evaluation of Selected Traits
Fifty to one hundred plants were multiplied from each selected plant by tissue culture. These clones were subjected to evaluation in the banana orchard. For selection of Fusarium wilt resistance, 40-50 plants from each clone were planted in a single-row plot in the disease nursery in two replications. Both susceptible and resistant check cultivars were included in the experiment. After 4-5 months, both external and internal symptoms of Fusarium wilt were examined. Percentages of diseased plants in each clone were calculated and used as a criterion for selection.

For the improvement of horticultural traits, 50-100 plants derived from the selected plants were planted in a two-row plot as an observation trial. The heritability and stability of the selected traits in the somaclones were critically evaluated for two generations.

Field Evaluation of Selected Clones
Superior clones with stable expression of the selected traits were evaluated in designed plot experiments and in the farmer’s field. These experiments included preliminary and advanced experimental variety trials, regional adaptation trials, on-farm and agronomic experiments, etc.

RECENT PROGRESS ON THE SELECTION AND EVALUATION OF SOMACLONES

Selection for Resistance to Fusarium Wilt
'Pei Chiao' and 'Tai Chiao No. 2' are two Cavendish cultivars grown in Taiwan. Both are susceptible to Fusarium wilt (race 4). A total of 422 somaclones derived from the single plant selection as described in the previous section were evaluated for Fusarium wilt resistance under field conditions. The percentages of somaclones showing resistance, moderate resistance, moderate susceptibility and susceptibility were 0.9, 1.2, 6.4 and 91.5, respectively (Table 1). Clones that were resistant and superior in horticultural traits, or were similar to the parental cultivar, were selected for further evaluation. 'GC-1089' was a recently selected somaclone that was highly resistant to Fusarium wilt. In a two-year study, the average occurrence of diseased plants was 0% for both the clone ‘GC-1089’ and the resistant check cultivar (Tai Chiao No. 3), and 17.05% for the susceptible cultivar (Tai Chiao No. 2), as evaluated by external symptoms (Table 2). For internal symptoms, the results were 3.35, 11.15 and 77.55%, respectively (Table 2). Therefore, ‘GC-1089’ was selected for further evaluation in the advanced trials.

In Taiwan, over two million banana tissue-culture plantlets are multiplied and distributed to banana growers every year. With the cooperation of the farmers, superior somaclones are often discovered from the large number of tissue-culture plants. One of these superior clones is GCTCV-218, which is derived from 'Pei Chiao'. It was found to be resistant to Fusarium wilt by a farmer in Southern Taiwan. Besides the resistance, it is also a high yielding cultivar. In an advanced variety trial conducted in 2000-01, the bunch weight was 33.2 kg, which was about 22.2% higher than the local cultivar, 'Pei Chiao'
This somaclone was registered as 'Formosana' and was released to farmers in 2001 (TBRI, 2001). However, the growing cycle of ‘Formosana’ is about one month longer than ‘Pei Chiao’ under growing conditions in Taiwan.

**Improvement of Horticultural Traits**

Four Cavendish cultivars have been grown in Taiwan in the last decade. Each cultivar has its own merits and weaknesses that need improvement. Selection criteria included early flowering, dwarfism, leaf habit and bunch size. Since 1992, a total of 463 somaclones derived from single plant selection of the above cultivars have been evaluated. Seventy-two clones were stable in the traits selected. Among them, ten were superior in horticultural traits, such as shorter plant stature, larger bunch size, shorter growing cycle etc. A semi-dwarf resistant clone, TC1-229, was identified from a tall resistant cultivar, 'Tai Chiao No. 1' (Tang and Hwang, 1998; Tang et al., 2000). It has the advantages of ease in field management and wind tolerance. In 2000, it was registered as 'Tai Chiao No. 3'. Since the yield of 'Tai Chiao No. 3' was low, especially in orchards with poor soil, further improvement was needed. In 73 somclones selected from 'Tai Chiao No. 3', TC3-600 and TC3-1035 (Table 4) were superior in plant habit and yield potential. TC3-600 was higher in yield, while TC3-1035 was shorter in growing cycle than their parental cultivar, ‘Tai Chiao No. 3’. Selection of these two clones demonstrated the possibility of improving a cultivar step by step through a series of selection.

**DISCUSSION**

As in other breeding procedures, use of somaclonal variation for crop improvement has its advantages and disadvantages. The disadvantages include the fact that somaclonal variation is a random process and thus it is non-directional. The kind of variation and the frequency of occurrence are unpredictable and not controllable. Secondly, the occurrence of a useful variant is very low. It was estimated that the occurrence of a particular trait in mutation breeding was about one in a million of cells treated with mutagen (Micke et al., 1987). In bananas, the frequency of occurrence of different traits in somaclonal variation were variable. Dwarfism was the most common type of variant with a frequency of up to 75% of off-types derived from tissue culture (Israeli et al., 1995). In our study, the occurrence of a variant resistant to Fusarium wilt was about one in thirty thousand plants used in the screening process. Unfortunately, as in other single gene mutation, a useful variant was always associated with inferior characteristics, such as longer growing cycle, smaller bunch size and change in the fruit quality etc. (Hwang and Tang, 1999). Therefore, the chance of getting a useful variant is even lower. Another disadvantage is the occurrence of epigenetic variation. A typical example of transient expression of somaclonal variation in banana was observed in mosaic-like off-type (Cote et al., 1993). In our study, it was found that the multiple-bunch (Tang et al., 1994) and uneven degreening of banana (Tang, 1998) were transient in the expression of genetic factor(s).

On the other hand, the procedure used for selection of useful somaclonal variants is simple and straight-forward. This is especially true for vegetatively propagated crops such as banana. When a stable variant is found, it can be 'fixed' and transmitted to its 'offspring' for further evaluation. In our study, it was demonstrated that somaclonal variation was a useful source of variation for the selection of both disease resistance and improved horticultural traits, including earliness, dwarfism and yield potential etc. In order to make use of somaclonal variation for crop improvement, several requirements are needed:

1. The breeding objective should be simple enough for improvement one at a time. In order to achieve several objectives, stepwise improvement must be possible.
2. An efficient micropropagation system to produce large quantities of tissue culture plants is a basic requirement.
3. A simple and efficient screening technique to select for a particular trait is needed. Molecular markers and in vitro selection should be helpful in the identification of useful variants.

4. A comprehensive evaluation program, including field trials and establishment of nursery stock to maintain breeding stocks are important in an improvement program using somaclonal variation.

ACKNOWLEDGEMENTS

This research was financed by the Council of Agriculture (90AST-1.1.1-FAD-Z5-7), Executive Yuen, R.O.C.

Literature Cited


Tables

Table 1. Performance of putative selected clones for resistance to Fusarium wilt.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>No. of clones tested</th>
<th>Resistant (%)</th>
<th>Moderately resistant (%)</th>
<th>Moderately susceptible (%)</th>
<th>Susceptible (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pei Chiao</td>
<td>233*</td>
<td>2(0.85)**</td>
<td>3(1.28)</td>
<td>14(6.01)</td>
<td>214(91.8)</td>
</tr>
<tr>
<td>Tai Chiao #2</td>
<td>189</td>
<td>2(1.06)</td>
<td>2(1.06)</td>
<td>13(6.88)</td>
<td>172(91.0)</td>
</tr>
<tr>
<td>Total/means</td>
<td>422</td>
<td>4(0.95)</td>
<td>5(1.18)</td>
<td>27(6.40)</td>
<td>386(91.5)</td>
</tr>
</tbody>
</table>

*40-50 plants from each clone were evaluated in the disease nursery.
**Figures in ( ) are percentages.

Table 2. Mean percentages of diseased plants of a new resistant clone (GC-1089) and check cultivars in the disease nursery.

<table>
<thead>
<tr>
<th>Clone/cultivar</th>
<th>External sym.</th>
<th>Diseases (%)</th>
<th>Internal sym.</th>
<th>Diseases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of plants observed</td>
<td></td>
<td>No. of plants observed</td>
<td></td>
</tr>
<tr>
<td>GC-1089</td>
<td>46</td>
<td>0</td>
<td>25</td>
<td>3.35</td>
</tr>
<tr>
<td>TC3*(resistant check)</td>
<td>37</td>
<td>0</td>
<td>30</td>
<td>11.15</td>
</tr>
<tr>
<td>TC2 (susceptible check)</td>
<td>144</td>
<td>17.05</td>
<td>139</td>
<td>77.55</td>
</tr>
</tbody>
</table>

* TC2 is Tai Chiao #2; TC3 is Tai Chiao #3.

Table 3. Characteristics of three cultivars in an advanced experimental variety trial.

<table>
<thead>
<tr>
<th>Clone/cultivar</th>
<th>Parental cultivar</th>
<th>Days (plant to harvest)</th>
<th>Plant height (cm)</th>
<th>Girth (cm)</th>
<th>No. of hands</th>
<th>Bunch weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCTCV-218</td>
<td></td>
<td>426a*</td>
<td>266b</td>
<td>69.4c</td>
<td>8.5b</td>
<td>25.3a</td>
</tr>
<tr>
<td>Pei Chiao**</td>
<td></td>
<td>383c</td>
<td>268c</td>
<td>69.9c</td>
<td>8.5b</td>
<td>27.0b</td>
</tr>
<tr>
<td>Tai Chiao #1</td>
<td></td>
<td>410b</td>
<td>297a</td>
<td>71.5b</td>
<td>8.1c</td>
<td>27.1b</td>
</tr>
</tbody>
</table>

*Values are means of 4 replications, 20 plants in each replication. Means with different letters in the same column differ significantly (P<0.05).
**Pei Chiao is the susceptible check and Tai Chiao 1 is the resistant check.

Table 4. Performance of four cultivars in an advanced experimental variety trial.

<table>
<thead>
<tr>
<th>Clone/ Cultivar</th>
<th>Parental cultivar</th>
<th>Days (plant to harvest)</th>
<th>Plant height (cm)</th>
<th>Girth (cm)</th>
<th>No. of hands</th>
<th>Bunch weight (kg)</th>
<th>% diseased plants***</th>
<th>Leaf habit</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC3-1035</td>
<td>TC3</td>
<td>372c**</td>
<td>266b</td>
<td>69.4c</td>
<td>8.5b</td>
<td>25.3a</td>
<td>16.7</td>
<td>Normal</td>
</tr>
<tr>
<td>TC3-600</td>
<td>TC3</td>
<td>431a</td>
<td>259b</td>
<td>79.8a</td>
<td>10.6a</td>
<td>24.7a</td>
<td>5.3</td>
<td>Split</td>
</tr>
<tr>
<td>TC3(ck2)*</td>
<td>TC1</td>
<td>423a</td>
<td>245c</td>
<td>76.3b</td>
<td>10.1a</td>
<td>23.8a</td>
<td>6.7</td>
<td>Split</td>
</tr>
<tr>
<td>TC1(ck1) Pei Chiao</td>
<td>404b</td>
<td>288a</td>
<td>70.2c</td>
<td>9.9a</td>
<td>23.5a</td>
<td>7.5</td>
<td>Split</td>
<td></td>
</tr>
</tbody>
</table>

*TC1 is Tai Chiao #1; TC3 is Tai Chiao #3.
** Values are means of four replications, 20 plants in each replication. Means with different letters in the same column differ significantly (P<0.05)
***Disease scores are means from 3 different locations in farmers’ fields.