Characteristics of a somatic hybrid between *Solanum melongena* L. and *Solanum sanituwongsei* Craib.

Hiroshi Asao¹, Sigeru Arai¹, Takanori Sato² and Masashi Hirai²

¹Nara Agricultural Experiment Station, Kashihara, Nara 634, Japan
²National Research Institute of Vegetables, Ornamental plants and Tea, Ano, Mie 514-23, Japan

Summary
In order to transfer the growth habit and the resistance to *Pseudomonas solanacearum* from *S. sanituwongsei* to *S. melongena*, electrosfusion was carried out. The fused protoplasts were cultured in KM(8p) medium supplemented with 1,000ppm wilt-inducing product secreted by a virulent strain of *P. solanacearum*. The regenerated plants were further screened on the soil contaminated with *P. solanacearum*. Only one plant survived. Several morphological traits of the surviving plant were intermediate between those of the parents, and the chromosome number was 48. The pollen viability averaged 82.3%, and fruits were set. The hybridity was confirmed using random amplified polymorphic DNA (RAPD) markers. The somatic hybrid exhibited a resistance to *P. solanacearum* comparable to that of *S. sanituwongsei*. The juvenile growth of the offspring (S1 plants) obtained by selfing of the somatic hybrid was superior to that of *S. sanituwongsei*. The S1 plants were also as resistant to *P. solanacearum* as *S. sanituwongsei*. When the S1 plants were used as rootstocks for eggplants, the fruit yields were as high as those of eggplants grafted on *S. sanituwongsei*. It was considered that the S1 plants could be used as rootstocks for eggplant production.

Key Words: *Solanum melongena*, *S. sanituwongsei*, electrosfusion, somatic hybrid, rootstock, resistance to *Pseudomonas solanacearum*.

Introduction

Eggplant (*Solanum melongena* L.) is an economically important non-tuberous *solanaceous* crop. Eggplant production is sometimes severely curtailed due to infection with *Pseudomonas solanacearum* in Japan. To overcome this problem, several rootstocks have been used. *S. sanituwongsei* shows a high level of resistance to *P. solanacearum*, although the growth of a young plant on a rootstock of this species is slow. Therefore, this stock has not been used widely. Somatic hybridization by protoplast fusion is expected to provide a new possibility of increasing genetic variability. Prerequisite protocols for regenerating plants from protoplast cultures in both eggplant and *S. sanituwongsei* have already been developed in our group (Asao et al. 1989). The method of the cell selection using a wilt-inducing product secreted by a virulent strain of *P. solanacearum* has also been described (Asao et al. 1992). Somatic hybrids of *S. melongena* have been produced by protoplast fusion with *S. sisymbriifolium* (Gledde et al. 1986), *S. khasianum* (Sihachakr et al. 1988), *S. torvum* (Guri and Sink 1988 a; Sihachakr et al. 1989), *S. nigrum* (Guri and Sink 1988 b), *S. integrifolium* (Kamey et al. 1990) and *S. aesthojii* (Daunay et al. 1993). However, most of the somatic hybrids were sterile and could not be used for eggplant breeding nor as rootstocks for eggplant production.

In the present report a fertile somatic hybrid between *S. melongena* and *S. sanituwongsei* obtained through electrosfusion was described. In order to evaluate the agricultural potential of the somatic hybrid and its offsprings, the plants obtained in this study were tested for their field resistance to *P. solanacearum*, their morphological characteristics, pollen viability and ability as rootstocks.

Materials and Methods

Protoplast isolation

Seeds of *Solanum melongena* L. (cv. Senryou II) and *S. sanituwongsei* Crib (cv. Karehen) were aseptically sown on agar-solidified (8g/l) hormone-free 1/2 MS medium containing 15g/l sucrose. The protoplasts were derived from the cotyledons taken from 2 to 3-week-old plants. The lamina strips were incubated overnight at 25°C, in the dark, in an enzyme solution containing salt CPW (Xu et al. 1981), 0.3% Cellulase R-10 (YAKULT CO., LTD., Japan), 0.06% Macerozyme R-10 (YAKULT CO., LTD., Japan), 0.5M mannitol and 10mM 2-(N-morpholino) ethanesulfonic acid (MES) at pH 5.8. The protoplasts were passed through a nylon mesh of 100μm and then mixed with the W5 solution (Menczel et al. 1981) and centrifuged (100×g, 3min). The pellet was further washed twice with the W5 solution by centrifugation (100×g, 3min). Prior to fusion, the protoplasts were washed once in a 0.45M glucose solution supplemented with 0.2M CaCl₂:2H₂O, and then they were suspended in the same solution at a density of 5×10⁴ protoplasts per milliliter.

Protoplast fusion and culture

Cotyledon protoplasts of *S. melongena* and *S. sanituwongsei* were mixed in a ratio of 5 : 1, and then 1ml aliquots of the mixture were pipetted into a 15×60mm Petri dish. Thereafter, an immersible electrode (Shimadzu FTC33 D5) was placed in the protoplast suspen-
sion. In order to align the protoplasts, an AC-field at 200V/cm and 1MHz was applied for 30sec; subsequently, two D. C. square pulses developing 0.75KV/cm for 20μS each (Shimadzu SSH–2) were applied to achieve protoplast fusion.

Immediately after fusion, 1ml culture medium was slowly added to the protoplast mixture. The culture medium consisted of KM(8p) (Kao and Michayluk 1975) supplemented with 0.5mg/l 2,4-dichlorophenoxy-acetic acid (2,4-D), 1mg/l kinetin, 1mg/l α-naphthaleneacetic acid (NAA), 1,000ppm wilt–inducing product (WIP) secreted by a virulent strain of P. solanacearum (Taniyama et al. 1991), 0.45M glucose as an osmoticum and 10 mM MES at pH 5.8. The protoplasts were initially cultured in the dark at 25°C for 7 days. Afterwards, they were exposed to fluorescent light for 16hrs/day at 500lux. Every week, the cultures were diluted twice with the same medium except for the osmoticum and the auxin. About 4 weeks after the fusion, when the protoplast-derived colonies reached approximately 1.0mm, the culture medium was replaced with C medium (Shepard and Totten 1977). The green calli on the C medium were transferred to the shoot regeneration medium that contained MS salts and vitamins (Murashige and Skoog 1962), 3mg/l zeatin, 0.2mg/l 3-indoleacetic acid (IAA), 30g/l sucrose and 2g/l gellan gum. Shoots were excised from the calli and rooted on hormone-free MS medium under illumination of 16hrs/day at 3,000lux and 25°C. RAPD assay

DNA was extracted from the leaves according to the method of Edwards et al. (1991). The reaction mixture (10μl) for the polymerase chain reaction (PCR) was composed of 10mM Tris–HCl (pH 8.9), 80mM KCl, 1.5mM MgCl2, 0.1% sodium cholate, 0.1% Triton X–100, 0.2mM dATP, 0.2mM dCTP, 0.2mM dGTP, 0.2mM dTTP, 0.2μM primer, 10 to 30ng template DNA, and 0.5unit Tth DNA Polymerase (Toyobo, Osaka). The primer RA 12–17 (5’-CGTCCGGGAGAA-3’) was used. Amplification was carried out in a Program Temp Control System PC–700 (Astec, Shime) with preheating at 94°C for 30sec, 45 cycles at 94°C for 30sec, at 60°C for 2min, at 72°C for 3min, and postheating at 72°C for 7min. After all the cycles were completed, 10μl of the products were analyzed by agarose gel electrophoresis. Chromosome counting and pollen viability

Root tips were pretreated with a saturated solution of 8–quinolinol for 2hrs at room temperature, fixed in ethanol acetic acid (3:1, v/v) at 25°C for 1hr, and then incubated in an enzyme solution consisting of 4% Cellulase RS (YAKULT CO., LTD., Japan), 0.1% Pectolyase Y23 (SEISHIN CO., LTD., Japan), and 75mM Na2–EDTA (pH 5.5) at 37°C for 30min. After being washed with distilled water three times, the chromosomes were stained with 1% orcein. Pollen viability was determined by staining mature pollen grains in 2% acetocarmine.

Characterization of offsprings (S1 plants) from self-pollination of the hybrid

The seeds obtained from selfing of the hybrid were immersed in 100ppm gibberellin for one day, and then they were sown in a growth chamber (5,000lux) at 20°C and at 25°C. The germination rate, the diameter of the hypocotyl and plant height were scored. The fruit production of S. melongena grafted on S1 hybrid plants was compared with that of the plants grafted on S. sanctum and on S. integrifolium Poir.

Test of resistance to P. solanacearum

The somatic hybrid was propagated by cutting, and used as rootstock of S. melongena. The grafted plants were cultivated in a field infested with P. solanacearum. S. melongena plants grafted on S. sanctum and on S. integrifolium were also grown in the same field as control.

Results

Protoplast fusion and culture

Mesophyll protoplasts from S. melongena and S. sanctum were electrofused (Fig. 1, A). First cell division occurred after about 4 days, and some of the cells divided and developed further into colonies (Fig. 1, B). After about 6 weeks of culture, the small calli formed were transferred to the regeneration medium. About 2 to 3 weeks later, green shoots were regenerated from the callus (Fig. 1, C). Based on the plant morphology, we selected a total of 8 putative somatic hybrid plants, which accounted for an overall average of 6.7% of the 120 regenerated plants obtained in the fusion treatment. These plants were then cultivated in a field contaminated with P. solanacearum for the assessment of the field resistance to P. solanacearum, and only one out of 8 putative somatic hybrid plants was survived.

Characterization of the somatic hybrid and S1 hybrid plants

Morphological observation of the parents and the somatic hybrid is summarized in Table 1. The somatic hybrid showed intermediate characteristics between those of the parents in terms of gross morphology (Fig. 2, A), leaf shape (Fig. 2, B), flower size and color (Fig. 2, C), and fruit shape and color (Fig. 2, D). Especially, the immature fruit of the somatic hybrid was black as in eggplant, while that of S. sanctum was green. The mature fruit turned orange as in the case of S. sanctum, while that of eggplant turned yellow. The pollen viability of the parents exceeded 90%, while that of the hybrid was 82.3% and this plant set fruits and seeds. Cytological analysis revealed that the chromosome number of the hybrid was 2n=4x=48, i.e. the sum of those of the parents (Fig. 3). The same chromosome number was also found in S1 plants. The band patterns of the hybrid revealed by the RAPD assay were different from those of both parents but bands from both parents were included, thus providing evidence of hybridity (Fig. 4).

In the field test on contaminated soil, 81.3% of the eggplants grafted on S. integrifolium died within 45days
after transplanting. However, the appearance of the symptoms on eggplants grafted on the somatic hybrids was delayed and only 12.5% of the eggplants died due to the disease. Similar results were also obtained in the case of the plants grafted on the S1 plants and S. sanitwongsei (Table 2).

The germination rate of S. sanitwongsei was lower than that of the other species, especially at 20°C, while the germination rate of the S1 seeds was higher than that of S. sanitwongsei regardless of temperature (Table 3). The hypocotyl of the S1 plants was about 1.5 times thicker than that of S. sanitwongsei and as thick as that of S. melongena and S. integrifolium. The S1 plants were also taller than S. sanitwongsei.

Fruit yields were investigated from July to October in 1993. The yield of eggplants grafted on S1 plants in July was higher than that of eggplants grafted on S. integrifolium and S. sanitwongsei, while the total fruit yield was similar to that of the other species (Fig. 5).

**Discussion**

In this study, 1,000ppm of wilt-inducing product (WIP) was added to the protoplast culture medium in order to select effectively the hybrids resistant to *P. solanacearum*. As the protoplasts of S. melongena seldom divided in the medium supplemented with 1,000 ppm WIP (Asao et al. 1992), it was likely that the regenerated plants were somatic hybrids or S. sanitwongsei plants. Since only 8 plants were regenerated in the present study, WIP applied to the fused protoplasts may have killed most of the unfused eggplant protoplasts. However, we analyzed only one of them. Effectiveness of the present strategy requires further studies. As the plating efficiency of S. sanitwongsei was higher than that of S. melongena, the protoplasts of S. sanitwongsei and S. melongena were mixed in the ratio of 1 : 5, respectively prior to fusion. A similar experiment was reported by Guri and Sink (1988a, 1988b). Several papers on protoplast fusion using the three methods have already been published in eggplants, including electrofusion (Sihachkr et al. 1988, Sihachkr et al. 1989, Daunay et al. 1993), polyethylene glycol treatment (Gledie et al. 1985, Gledie et al. 1986, Guri and Sink 1988a, Guri and Sink 1988b) and dextran treatment (Kameya et al. 1990).

Although it is generally recognized that somatic hybridization enables to overcome the sexual barrier between eggplant and other wild species, the utilization of the

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**Table 1.** Chromosome number and morphological characteristics of *S. melongena*, *S. sanitwongsei* and the somatic hybrid

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>S. melongena</th>
<th>Somatic hybrid</th>
<th>S. sanitwongsei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome number</td>
<td>24</td>
<td>48</td>
<td>24</td>
</tr>
<tr>
<td>Leaf shape</td>
<td>Lobed</td>
<td>Slightly divided</td>
<td>Deeply divided</td>
</tr>
<tr>
<td>Anthocyanin on stem</td>
<td>Present</td>
<td>Minimal amount</td>
<td>absent</td>
</tr>
<tr>
<td>Flowering</td>
<td>Solitary flower</td>
<td>Cyme (4-7 flowers)</td>
<td>Cyme (4-7 flowers)</td>
</tr>
<tr>
<td>Flower diameter (cm)</td>
<td>5.0</td>
<td>4.2</td>
<td>2.8</td>
</tr>
<tr>
<td>Fruit size (height×width, cm)</td>
<td>13.0×5.0</td>
<td>2.9×2.7</td>
<td>1.0×1.1</td>
</tr>
<tr>
<td>Fruit color (immaturity : maturity)</td>
<td>Black : Yellow</td>
<td>Black : Orange</td>
<td>Green : Orange</td>
</tr>
<tr>
<td>Seed number per fruit</td>
<td>300 &lt;</td>
<td>133</td>
<td>53</td>
</tr>
<tr>
<td>Pollen viability (%)</td>
<td>98.0</td>
<td>82.3</td>
<td>93.7</td>
</tr>
</tbody>
</table>

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Fig. 1. The electrofusion and culture of protoplasts.
A: Electrofusion of protoplasts.
B: Colony formation after 2 weeks of culture.
C: Shoots regeneration after 8 weeks of culture.
Fig. 2. Characteristics of plant morphology of *S. melongena* (left), the somatic hybrid (center) and *S. santoungseii* (right). A: Plant morphology. B: Leaf shape. C: Flower shape. D: Shape of immature fruit (upper) and mature fruit (lower).

Table 2. Resistance of eggplants on the soil contaminated with *Pseudomonas solanacearum*

<table>
<thead>
<tr>
<th>Plant</th>
<th>No. of plants examined</th>
<th>No. of dead plants</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rootstock</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>somatic hybrid</td>
<td>8</td>
<td>1</td>
<td>12.5</td>
</tr>
<tr>
<td>S1 plants</td>
<td>80</td>
<td>12</td>
<td>15.0</td>
</tr>
<tr>
<td><em>S. santoungseii</em></td>
<td>16</td>
<td>2</td>
<td>12.5</td>
</tr>
<tr>
<td><em>S. integrifolium</em></td>
<td>16</td>
<td>13</td>
<td>81.3</td>
</tr>
<tr>
<td>Control plant</td>
<td>16</td>
<td>16</td>
<td>100.0</td>
</tr>
</tbody>
</table>
| 1: *S. melongena* was grafted on each rootstock.  
2: Offspring from the self-pollinated fertile hybrid plant.  
3: Seedlings without grafting.  
4: Mortality was scored at 70 days after transplanting.

Table 3. Effect of temperature on germination (%)

<table>
<thead>
<tr>
<th>Plant</th>
<th>10 days&lt;sup&gt;1&lt;/sup&gt;</th>
<th>13 days&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 °C</td>
<td>25 °C</td>
<td>20 °C</td>
</tr>
<tr>
<td>S1 plants</td>
<td>29.7</td>
<td>68.8</td>
</tr>
<tr>
<td><em>S. santoungseii</em></td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td><em>S. melongena</em></td>
<td>62.5</td>
<td>96.9</td>
</tr>
<tr>
<td><em>S. integrifolium</em></td>
<td>98.4</td>
<td>100.0</td>
</tr>
</tbody>
</table>

1: Days after seeding.  
2: See Table 2.
hybrid plants in breeding programs of eggplant has been limited by their sterility. Up to the present, Kameya et al. (1990) and Daunay et al. (1993) have described fertile somatic hybrids between S. melongena and S. integrifolium, S. melongena and S. aethiopicum, respectively.

Few seeds were obtained from the crosses between S. melongena and S. saniculifera, and all of them were empty seeds (unpublished data). The hybrid can not be used in the breeding program of a rootstock for eggplant. On the contrary, this study showed that a fertile hybrid of S. melongena and S. saniculifera could be produced by protoplast electofusion. The somatic hybrid had 48 chromosomes, corresponding to the sum of the chromosomes of the parents. The same chromosome number was also observed in the S1 plants. The fertility of the present hybrid may be attributed to the presence of two sets of genome in a single cell. These findings suggest that fertility can be maintained in the progeny of this hybrid. Therefore, the present somatic hybrid could be used in breeding programs for the development of resistance to P. solanacearum.

We previously released a line of S. saniculifera, designated as ‘Kareben’, as a rootstock resistant to bacterial wilt of eggplant. However, germination and seedling growth of this species are slow and the resulting seedlings display slender hypocotyls. Therefore, the time from seedling to grafting is prolonged. Moreover, the scion showed a slow growth even after the grafting, which resulted in a low productivity of the rootstock in the early stage. Somatic hybridization with S. melongena modified considerably the growth habit of the rootstock. The rapid germination and growth of the present hybrid and S1 plants may be inherited from S. melongena. The current study also showed the superiority of the S1 plants as rootstocks for eggplant production.

Literature Cited


