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Regeneration of intergeneric somatic hybrid plants between *Lycopersicon esculentum* and *Solanum muricatum*

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Summary. Mesophyll protoplasts of tomato (*Lycopersicon esculentum*) and pepino (*Solanum muricatum*) were fused by using an electrofusion method and cultured in modified MS medium supplemented with naphthaleneacetic acid and kinetin, in which only pepino and somatic hybrid protoplasts could divide. Somatic hybrid plants showing intermediate characteristics in morphology were regenerated from the calli exhibiting vigorous growth in contrast with those of pepino. The hybrid nature of these plants was confirmed by cytological observation and biochemical analyses of phosphoglucomutase isozymes and the fraction-1-protein. The regenerated somatic hybrids grew to flowering stage and set fruits.

Key words: *Lycopersicon esculentum* – *Solanum muricatum* – Intergeneric somatic hybrid – Electrofusion – Isozyme analysis

Introduction

Tomato (*Lycopersicon esculentum*) is an important vegetable crop grown all over the world. The traditional tomato improvement has been performed by sexual crossing with wild relatives belonging to *Lycopersicon* and *Solanum* genera, which have desirable genetic traits (Rick 1982). However, the genetic resources used by plant breeders have been limited due to the sexual incompatibility barrier. Somatic hybridization via protoplast fusion offers possibilities for overcoming this barrier and for increasing the amount of germ plasm available. Many researchers have successfully produced interspecific hybrids between tomato and wild species in *Lycopersicon* (O'Connell and Hanson 1985; Kinsara et al. 1986) and intergeneric hybrids between tomato and *Solanum* species (Melchers et al. 1978; Handley et al. 1986; O'Connell and Hanson 1986; Guri et al. 1988; Schweizer et al. 1988).

Pepino (*Solanum muricatum*) is a subtropical fruit originating in the Andes in South America. The fruit of pepino, which has whitish-yellow skin with clear purple stripes and strong flavor similar to a melon, has been appreciated not only as a food but also for its appearance. This species is an interesting genetic resource for the improvement of tomato fruit characteristics such as color, shape, flavor, nutrient contents, and flesh quality.

In the present paper, we report on the regeneration and characterization of intergeneric somatic hybrid plants between tomato and pepino produced by protoplast fusion.

Materials and methods

Plant materials

Tomato (*Lycopersicon esculentum* cv. 'Petit') and pepino (*Solanum muricatum*), which had been maintained in vitro as shoot culture on MS medium (Murashige and Skoog 1962) supplemented with 0.01 mg/l naphthalene acetic acid (NAA) at 25 °C under 16-h light conditions (3,000 lx), were used.

Protoplast isolation and fusion

Fully expanded leaves of tomato and pepino were cut into about 1-mm wide strips and incubated in the enzyme solution containing 1% Cellulase Onozuka RS, 0.5% Macerozyme R-10, 0.1% Pectolyase Y-23, 0.5 *M* mannitol, and 20 m*M* $CaCl_2 \cdot 2H_2O$ (pH 5.6) for 2–3 h at 28 °C on a reciprocal shaker (60 strokes/min). Protoplasts were separated from undigested tissue by filtration through a 53-μm nylon sieve and centrifuged at $80 \times g$ for 5 min. The supernatant was removed and the protoplast pellet was resuspended in 0.5 *M* sucrose. Following centrifugation at

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$100 \times g$ for 10 min, viable protoplasts floating on the surface were collected and washed twice in 0.5 *M* mannitol with 20 m*M* $CaCl_2 \cdot 2H_2O$ and finally adjusted to a density of 2×10^5 cells/ml. Protoplast suspensions of tomato and pepino were mixed in a 1:1 ratio.

Protoplasts were fused by means of electrofusion using a Somatic Hybridizer SSH-1 (Shimadzu Corp., Japan). A 1.5-ml aliquot of mixed protoplast suspension was introduced into the fusion chamber, aligned with an a.c. (alternating current) field of 2 MHz, 50 V/cm, and fused by applying a 50-μs d.c. (direct current) pulse of 1 kV/cm three times. Following fusion treatment, the protoplast suspension was transferred to a plastic petri dish (60 × 15 mm) and an equal volume of twofold strength culture medium was added.

Protoplast culture

Fusion-treated protoplasts were cultured in a half strength MS medium containing 0.5 mg/l NAA, 0.25 mg/l kinetin, 1% sucrose, and 0.5 *M* mannitol at 25 °C in the dark. After 2 weeks, an equal volume of the fresh medium supplemented with 1 mg/l NAA, 0.5 mg/l kinetin, and 3% sucrose was added. When small calli were formed after 1 month of culture, they were transferred onto MS agar medium containing 0.1 mg/l NAA and 1 mg/l kinetin. Vigorously growing calli were picked up and transferred to the regeneration medium (MS with 0.5 mg/l zeatin, 0.5 mg gibberellin, and 0.25% Gelrite). Shoots emerging from the calli were subcultured to MS medium containing 0.01 mg/l NAA and 0.25% Gelrite for rooting. Finally, rooted plantlets were transferred to sterilized soil and grown to maturity.

Chromosome counts

Chromosome numbers were determined after Feulgen staining. Root tips of regenerated plants were pretreated overnight at 0 °C, fixed in ethanol-acetic acid (3:1, v/v) for 12 h and, hydrolyzed in 1 *N* HCl at 60 °C for 10 min before staining.

Isozyme analysis

Leaf tissues (100 mg) were homogenized in 0.1 *M* HEPES buffer (pH 7.5) with 2 m*M* EDTA and 12 m*M* $MgCl_2$. The homogenates were absorbed into paper wicks and analyzed by ultrathin-layer isoelectric focusing (Radola 1980). Gels were stained for phosphoglucomutase (PGM) according to the procedure of Tanksley (1979).

Fraction-1-protein analysis

Leaf tissues (1 g) were homogenized in 0.1 *M* Tris-H_2SO_4 (pH 8.0) with 10 m*M* $MgSO_4$, 1 m*M* 2-mercaptoethanol, and 10% polyvinylpyrrolidone. Leaf extracts were obtained by squeezing the homogenate through four layers of cheesecloth, and 1% Sephadex G-25 was added to the extracts. Following centrifugation (15,000 rpm, 30 min), the supernatant was used for sucrose gradient centrifugation (7–22%, 25,000 rpm, 14 h), as described by Goldthwaite and Bogorad (1971). Peak fraction of absorption at 280 nm was analyzed by isoelectric focusing according to the procedure of Hirai (1982).

Results and discussion

Fusion frequency in the electrofusion experiment carried out under optimal conditions (50 μs d.c. pulse of 1 kV/cm, three times) was approximately 1%, but the rate of heterokaryon could not be determined. Fusion-treated protoplasts began to divide after 3–5 days of culture and formed small calli, 1–2 mm in diameter, 1 month after plating. The division frequency was around 5% after 7 days of culture. In the control culture of pepino, the frequency was about 20%, while tomato protoplasts did not divide in the same medium. Therefore, it seemed that small calli formed after fusion treatment were derived from pepino protoplasts or fusants between tomato and pepino protoplasts.

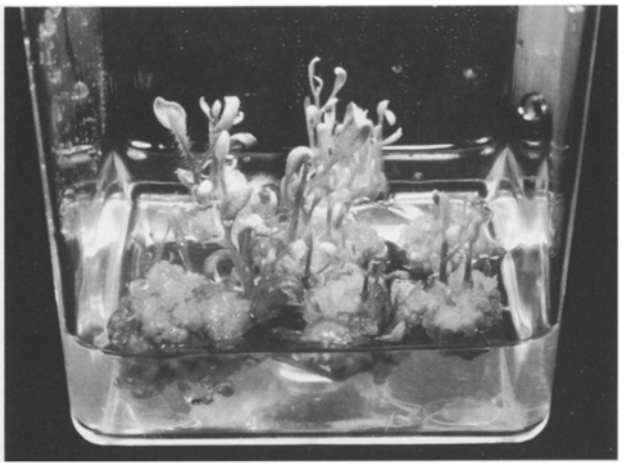

Fig. 1. Shoot regeneration from a callus assumed to have been derived from the fusion product of tomato and pepino protoplasts

After transfer of small calli to proliferating medium (MS with 0.1 mg/l NAA and 1 mg/l kinetin), a few calli showed vigorous growth compared to the other ones. These vigorously growing calli were transferred again to the regeneration medium. Out of seven calli transferred, two produced many shoots about 3 months after fusion treatment (Fig. 1). In the control culture of pepino, however, shoot formation from calli was not observed. After they grew up to 1–2 cm in height, the shoots were transferred to the rooting medium. Roots were formed within 2 weeks and whole plants were obtained.

The regenerated plants exhibited intermediate morphological characteristics between tomato and pepino (Fig. 2A). Tomato had compound leaves consisting of 9–10 leaflets with an indented edge, whereas pepino had single leaves with a smooth edge. In contrast, regenerated plants had 3–5 leaflets on each petiole with a smooth edge, which were intermediate between both parental species (Fig. 2B). The flowers of regenerated plants were also intermediate morphologically (Fig. 2C). Their shape and size resembled those of pepino, but the color was intermediate (yellowish white) between the two parents. All regenerants from two hybrid cell lines had normal leaves and flowers, and morphological abnormality was not observed.

The results on the counts of chromosome numbers of regenerants revealed that they had 2n = 48 (Fig. 3), which

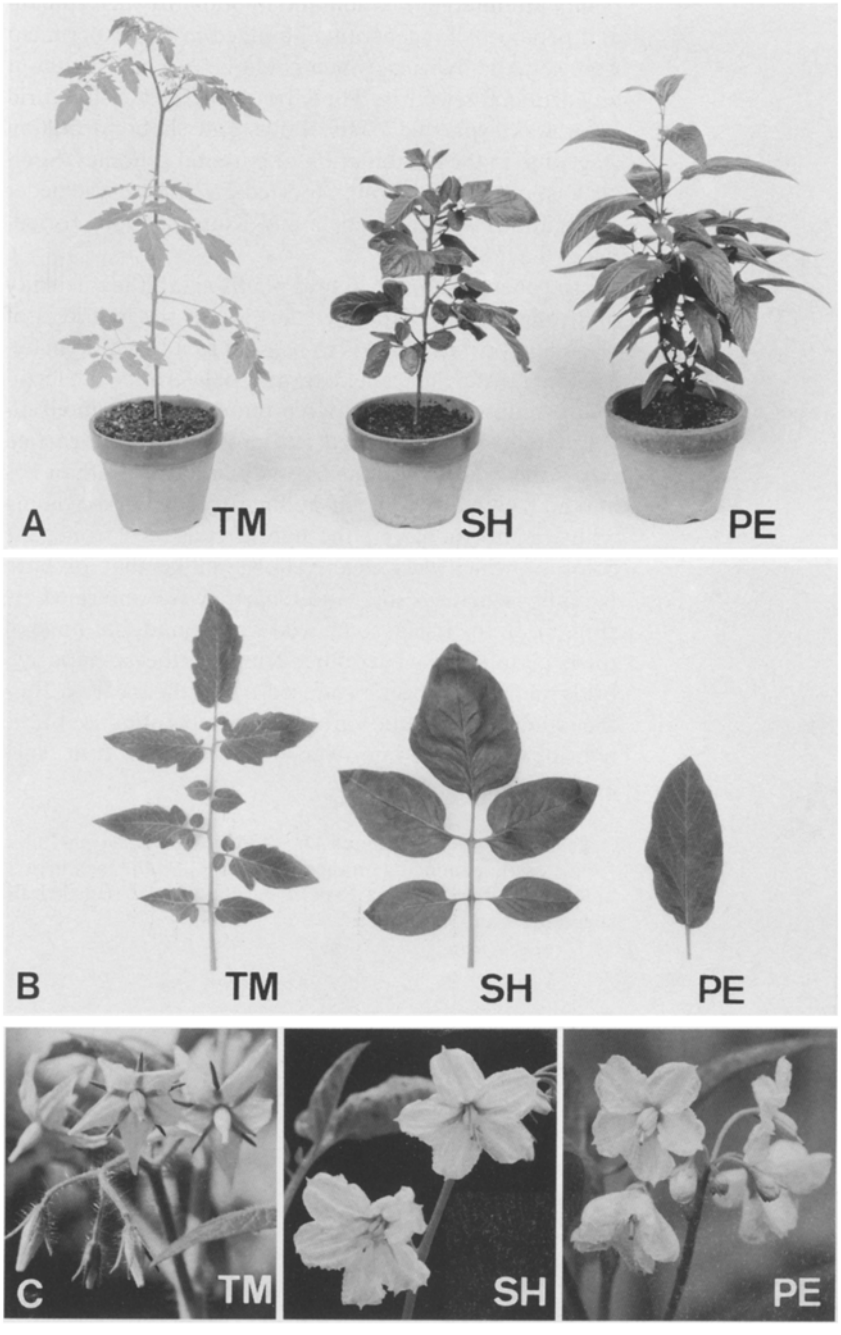

Fig. 2 A–C. Plant (**A**), leaf (**B**), and flower (**C**) of parental species and somatic hybrid. *TM:* tomato, *SH:* somatic hybrid, *PE:* pepino

was equal to the sum of tomato (2n = 24) and pepino (2n = 24). No aneuploids were observed. Therefore, it was assumed that regenerated plants were intergeneric somatic hybrids between tomato and pepino, which were amphidiploid.

To confirm the hybridity of regenerated plants, PGM isozymes and the fraction-1-protein were analyzed. Figure 4A shows PGM isozyme patterns. Regenerated plants had five bands, two of which were common to both parents, while the others were specific to each parent. Every regenerant expressed the summation of the parental bands. Isoelectric focusing of the fraction-1-protein provided additional evidence for hybridization (Fig. 4B). Tomato and pepino each had three bands that were different from one another. The regenerants had seven bands consisting of both parental bands and one additional unique band.

The presented data on morphology, chromosome number, and biochemical analyses of PGM isozymes and the fraction-1-protein indicate that the regenerated

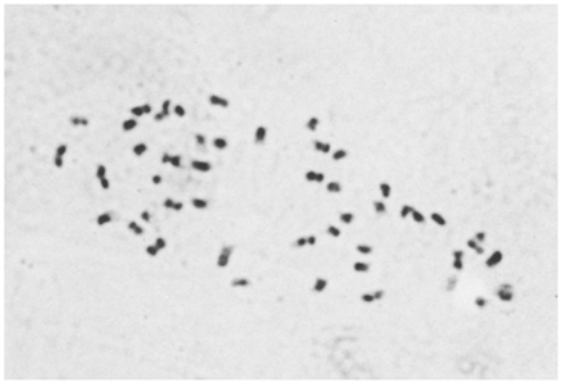

Fig. 3. Metaphase chromosomes (2n = 48) in a root-tip cell of somatic hybrid

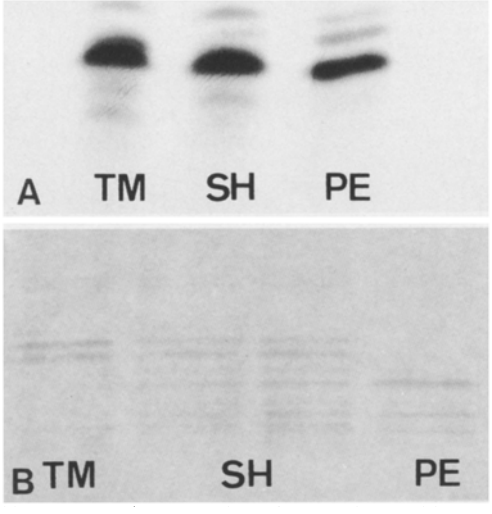

Fig. 4 A and B. Phosphoglucomutase (PGM) isozyme patterns (**A**) and fraction-1-protein isoelectric focusing patterns (**B**) for parental species and somatic hybrid. *TM:* tomato, *SH:* somatic hybrid, *PE:* pepino

plants are intergeneric somatic hybrids between tomato and pepino. All regenerants obtained in this experiment were somatic hybrids, which could be produced without any artificial selection. These results suggest that hybrid vigor was expressed in the callus and shoot formation stage due to the combination of parental genomes. Similar instances have been reported in *Datura* (Schieder 1978) and *Brassica* (Taguchi and Kameya 1986; Toriyama et al. 1987).

In general, somatic hybrid plants exhibit low fertility or strong sterility. However, fertility of the hybrid is of utmost importance if it is to be used as a breeding material for transferring genetic traits. To determine the fertility of somatic hybrids between tomato and pepino, pollen viability was examined and cross tests were carried out. Pollen viability of the hybrid plant was less than 1% and no fruit was set by self-pollination. By back-crossing with tomato, however, the hybrid produced fruits, the color of which was clear yellow, unlike that of both parents, whereas size and shape were intermediate (Fig. 5). In the fruits, some seed were found, but none of them germinated. Therefore, fruits of the somatic hybrids might have been produced by parthenocarpy. Further studies on genetic variation and potential use of the hybrids for tomato improvement and for new fruit types are in progress.

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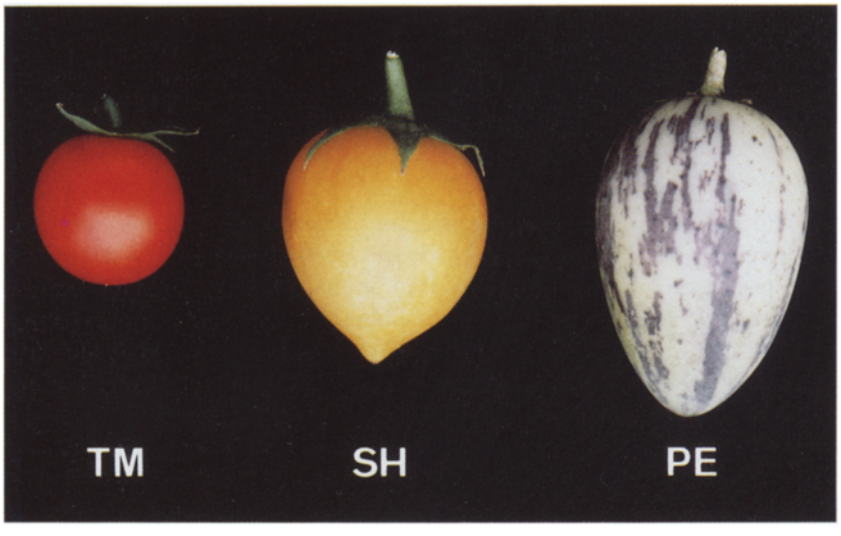

Fig. 5. Fruits of the parental species and somatic hybrid. *TM:* tomato, *SH:* somatic hybrid, *PE:* pepino

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