Introduction of the rolC gene into the Genome of the Japanese Persimmon Causes Dwarfism

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Summary

The rolC gene from Agrobacterium rhizogenes was introduced into a seedling of the Japanese persimmon (Diospyros kaki L.) ‘Saijo’ genome by Agrobacterium-mediated transformation. Integration of the rolC gene into the genome was confirmed by Southern blot analysis. Northern blot analysis revealed that the transgene was transcribed in the transgenic persimmon. The transformed plant (PC1) had shorter internodes and smaller leaves and produced more branches than the regenerated plants from the open-pollinated ‘Saijo’ seedlings. These results show the potential use of the rolC gene to produce dwarf Japanese persimmon plants.

Key Words: dwarfism, persimmon, rolC gene, transformation.

Introduction

Persimmon, one of the most important fruit-tree species in Japan, grows to more than 4 m in height, which has created considerable demands for dwarfing persimmon rootstocks to reduce labor costs in the orchard. Although Diospyros rhombifolia, a bushy dwarf persimmon, has been reported to reduce the growth of the scion when it is used as a rootstock (Yamada et al., 1997) and cutting technique for propagation had developed (Tetsumura et al., 2000), it is less than ideal because possible graft-incompatibility between ‘Fuyu’ persimmon and D. rhombifolia was suggested.

The introduction of a transgene that causes dwarfism of the plant is assumed to be effective for the production of dwarf plants. Several experiments have shown that the introduction of rol genes from A. rhizogenes induces dwarfism. For example, transformed trifoliate orange with the rolC gene has a shorter internode and better propagability from cuttings than the control plant (Kaneyoshi and Kobayashi, 1999). The introduction of the rolC gene is considered to have a potential for producing dwarf trees with superior horticultural characteristics that include easier propagation.

In Japan, seedling of the ‘Saijo’ is a popular persimmon rootstock. However, a scion grafted on a ‘Saijo’ seedling grows into a large tree. Hence, the introduction of the rolC gene into ‘Saijo’ seedlings to reduce their vigor and size and to produce dwarfing rootstock for Japanese persimmon were the objectives of this study.

Materials and Methods

Plant material and transformation

The transformed plants were produced according to Nakamura et al. (1998). The strain of Agrobacterium tumefaciens used for inoculation was EHA101 (Hood et al., 1986) harboring the binary vector pSMAK251-rolC, which originated in pSMAK251 (Yamashita et al., 1995). The plasmid pSMAK251-rolC contains the rolC gene, linked to the califlower mosaic virus (CaMV) 35S promoter, and the NPT II gene, linked to the Nos promoter. The rooted plants were transferred to sterilized vermiculite and moved to a growth chamber for four months. Conditions in the growth chamber were 30 °C, 80 % humidity, and 600 ppm CO₂ under a 16hr photoperiod. The photosynthetic photon-flux density was 110 μmol·m⁻²·sec⁻¹. The plants were then potted in soil and grown in a greenhouse. To compare their morphological features, untransformed plants were derived from ‘Saijo’ seedlings and grown as described above.

Southern blot analysis

Genomic DNA was extracted from in vitro grown shoots according to Nakamura et al. (1998). DNA (5 μg) was double-digested with Hind III followed by EcoRI, separated by electrophoresis in an 0.8% agarose gel, and then transferred to a nylon membrane. The rolC probe was generated by PCR using a PCR DIG Probe Synthesis Kit (Roche Diagnostics). The primers were designed from the coding region of the rolC gene (Slightom et al., 1986); the sequences were 5’ GAA
GAC GAC CTG TGT TCT C 3' and 5' TTA GCC GAT TGC AAA CTT GC 3'. Hybridization was carried out at 42℃, using a hybridization buffer, Dig Easy Hyb (Roche Diagnostics). After hybridization, the membrane was washed twice with 2 × standard saline citrate (SSC), 0.1% sodium dodecyl sulfate (SDS) at room temperature for 5 min each, then washed twice with 0.1 × SSC and 0.1% SDS at 68℃ for 15 min each. The hybridization signal was visualized by the chemiluminescent detection of DIG-labeled nucleic acids (Roche Diagnostics).

**Northern blot analysis**

Total RNA was extracted from young leaves of the potted plants, according to Levi et al. (1992). To recognize the expression of the introduced gene, 20 μg of total RNA was separated by electrophoresis in a 1.2% agarose gel, containing 0.66 M formaldehyde, then transferred to a nylon membrane. The RNA was hybridized with the same probe as that used for Southern blot analysis in a DIG Easy Hyb buffer for 16 hr at 50℃. Washing the membrane and detection of target RNA were carried out under the same conditions as for Southern blot analysis.

**Growth characteristics of transformed plants**

After growing for 2 months in the greenhouse, the height, internodal length, and number of internodes on 3 plants / clone were measured.

**Comparison of rooting of control and transformed plants**

The rooting rates of 10 to 12 in vitro shoots / clone cultured for 30 days were compared with that of the control plant by counting the number of rooted shoots.

![Image](image_url)

**Fig. 1.** Southern and Northern blot analysis of the control and the PC1 plant. (A) Southern blot analysis probed with a 537bp internal fragment of the rolC gene of the control (lane 1) and PC1 (lane 2) plant following restriction digest with Hind III. PC1 produced a few more bands than the control plant. Southern analysis of the control (lane 3) and PC1 (lane 4) plant following restriction digest with EcoRI and Hind III produced the expected 1.8kb rolC fragment in lane 4. (B) Northern blot analysis probed with a 536bp internal fragment of the rolC gene of the control and PC1 plant. The rolC transcript was estimated to be about 1.0kb.

**Results and Discussion**

Southern blot analysis revealed that at least one copy of the rolC gene was integrated into the genome of the PC1 plant (Fig. 1A). The expected 1.8 kb fragment was detected when the DNA of the PC1 plant was digested with Hind III–EcoRI (lane 4). Furthermore, Northern blot analysis showed that the integrated rolC gene was transcribed in the PC1 plant (Fig. 1B). No signal was detected in the untransformed plant. The morphological features of the PC1 plant (Fig. 2, Table 1) reveal that the height and length of the internodes of the PC1 plant were shorter than those of the untransformed plant. Because there was a significant difference at the 5% level by t-test between three PC1 plants and five untransformed plants regenerated from different ‘Saijo’ seedlings, we concluded that this difference in the height was not caused by the differences observed in individual ‘Saijo’ seedlings. The PC1 plant produced more branches with smaller, wrinkled leaves than did the untransformed plant (Fig. 2A, B). These data indicate that the rolC gene was being expressed in the transformed persimmon and causing dwarfism of the plant, although we could not deny the possibility that the rolC gene-inserted position was affecting the normal growth of the Japanese persimmon. The rooting rate of the PC1 plant was higher than that of the untransformed plant (Table 1).

The introduction of the rol genes into the plant alters morphological characteristics (van der Salm et al.,

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**Fig. 2.** (A) Growth of untransformed and PC1 plant after six months: untransformed plants (left) and PC1 (right). (B) Leaf morphology of the untransformed (left) and PC1 (right) plant.
Table 1. Mean height, number of nodes, average internode length of six-month-old plants, and rooting rate of the control and PC1 plants.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Height (cm)</th>
<th>Number of internodes</th>
<th>Internode length (cm)</th>
<th>Rooting rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>60.0 ± 11.6(^t)</td>
<td>23.3 ± 9.0</td>
<td>3.0 ± 0.2</td>
<td>50</td>
</tr>
<tr>
<td>PC1</td>
<td>35.0 ± 3.2</td>
<td>38.0 ± 2.4</td>
<td>2.4 ± 0.1</td>
<td>80</td>
</tr>
</tbody>
</table>

\(^t\)Mean ± SE (n=3)

1997), particularly, severe dwarfism (Kaneyoshi and Kobayashi, 1999; Bell et al., 1999). As in our transformed persimmon with rolC, leaves of a transgenic pear with rolC were smaller than those in an untransformed plant as Bell et al. (1999) also found. Tao et al. (1994) produced the transformed Japanese persimmon with A. rhizogenes wild-type strain A4. The Ri TL-DNA transformed in vitro shoots showed dwarfism along with a decreased rooting rate. Although the improved rooting rate of the PC1 plant is a horticulturally preferred characteristic, whether the introduction of the rolC gene generally improves the rooting rate of Japanese persimmon is unclear because only one transgenic line was investigated in this study.

Plant rootstocks containing the rol gene have recently been shown to alter the growth of the scion. For instance, the rolA-, -B-, and C- introduced rose rootstock increased bud release axially (van der Salm et al., 1998). This result suggests that rol gene introduced rootstock might possibly change the shape of the scion. Since the PC1 has a superior rooting rate, it may be an ideal rootstock if the growth of the scion can be reduced.

Literature Cited


rolC遺伝子導入による‘西条’実生わい性 形質転換体の作出

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摘 要

Agrobacterium rhizogenes由来のrolC遺伝子をカキ‘西条’の実生に導入した。サザンプロット解析により本遺伝子の導入が、ノーザンプロット解析により発現が確認された。形質転換体(PC1)は他の‘西条’実生由来の植物と比較し、節間が短く葉が小さく、分枝が多かった。これらの結果よりrolC遺伝子は‘西条’実生のわい化を引き起こすことが示された。