Transgenic Japanese Persimmon (*Diospyros kaki* L.)

Yoshiko Koshita

Grape and Persimmon Research Station, National Institute of Fruit Tree Science, National Agriculture and Food Research Organization, Japan

**Correspondence:** koshita@affrc.go.jp

**ABSTRACT**

Introduction of foreign genes into the genome of Japanese persimmon (*Diospyros kaki* L.) is mainly performed using a disarmed strain of *Agrobacterium tumefaciens* that carries a binary vector. Plant regeneration systems from callus derived from leaf disc or hypocotyl segments prepared from seed had been adapted to *Agrobacterium*-mediated transformation. Several foreign genes have been introduced into the genome of Japanese persimmon by *Agrobacterium*-mediated gene transfer, and the characteristics of the transformants revealed that agriculturally important characteristics, such as insect resistance, salt tolerance, dwarfness, and disease resistance, had been successfully vested to Japanese persimmon. On the other hand, not only a disarmed strain of *A. tumefaciens* but also the wild-type of *Agrobacterium rhizogenes* has been used in the natural genetic transformation of Japanese persimmon. The transformants showed different growth from non-transformants, such as dwarfness, short internode and decreased rooting ability. These studies showed that genetic transformation of the Japanese persimmon is one of the most effective ways to improve the characteristics of this species. The procedure of genetic transformation and the characteristics of transgenic plants are discussed in this review.

**Keywords:** *Agrobacterium*-mediated transformation, regeneration, insect resistance, salt tolerance, dwarfing, disease resistance

**CONTENTS**

INTRODUCTION......................................................................................................................... 215

REGENERATION SYSTEMS OF THE JAPANESE PERSIMMON....................................................... 216

GENETIC TRANSFORMATION OF THE JAPANESE PERSIMMON................................................... 216

- Transformation with wild-type strains of *Agrobacterium*......................................................... 216
- Leaf-disc transformation............................................................................................................. 216
- Transformation with a hypocotyl segment............................................................................... 216

INTRODUCTION OF FOREIGN GENES TO VEST AGRICULTURALLY IMPORTANT CHARACTERISTICS .............................................................................................................................. 216

- Engineering genetic resistance against insects........................................................................ 217
- Engineered salt tolerance......................................................................................................... 217
- For dwarf culture...................................................................................................................... 217
- To increase disease resistance............................................................................................... 217

CONCLUSION............................................................................................................................ 218

REFERENCES............................................................................................................................... 218

**INTRODUCTION**

Japanese persimmon (*Diospyros kaki* Thunb) is one of the most popular fruit crops in Japan. According to the statistics of Ministry of Agriculture, Forestry, and Fisheries of Japan, the land devoted to growing persimmon was third, following citrus and apple, in 2006, demonstrating the importance of this species. Therefore, the breeding program of the National Institute of Fruit Tree Science (NIFTS) was established in the 1930s to improve persimmon the cultivars. The types of Japanese persimmon are divided into four classes: pollination-constant non-astringent (PCNA), pollination-valiant non-astringent (PVNA), pollination-valiant astringent (PVA) and pollination-constant astringent (PCA). Because of market needs, the main breeding objective is to develop PCNA cultivars. The breeding of Japanese persimmon by NIFTS is mainly executed by traditional cross breeding (Yamada et al. 2005). Although some excellent cultivars, such as ‘Shinshuu’ (Yamane et al. 1990) or ‘Taishuu’ (Yamane et al. 1995), had been released, it takes a long time to obtain improved cultivars in a process of selection because of the long juvenile period or genetic heterogeneity and the inbreeding depression that is often observed. Therefore, to resolve these insufficiencies of cross breeding, introducing the desired characteristics by genetic transformation is thought to be an effective way to improve the characteristics of the Japanese persimmon.

To obtain transformants of plants by introducing foreign genes into the plant genome requires an *in vitro* regeneration system. In the case of the Japanese persimmon, regeneration systems from shoot tips, dormant buds, axillary buds, callus, protoplast, leaf disc, or hypocotyl segments have been established. Therefore, it is possible to obtain transformants by selecting transformed cells from these regeneration systems. Only *Agrobacterium*-mediated transformations have been reported in the transformation of the Japanese persimmon, although many plants had been transformed using other transformation systems, such as electroporation or particle bombardment.

In this review, examples of the transformation of Japanese persimmons and characteristics of the transformants which foreign genes were introduced into the genome are described.
**REGENERATION SYSTEMS OF THE JAPANESE PERSIMMON**

The establishment of a regeneration system from explants is the first step in the production of a transgenic plant. Some regeneration systems of the Japanese persimmon have already been developed and been used to obtain whole plants. Examples of the regeneration of the Japanese persimmon are shown in Table 1. So far, regeneration starts from shoot tips (Sugiura et al. 1986; Fukui et al. 1988a, 1992; Kagami 1999), dormant buds (Sugiura et al. 1986; Murayama et al. 1989) axillary buds (Sarathchandra and Burch 1991), leaf segments of *in vitro* shoots (Nishimura and Yamada 1992; Choi et al. 2001), roots of *in vitro* plants (Tetsumura and Yukinaga 1996), endosperm (Tao et al. 1997b), protoplasts delivered from callus (Tao et al. 1991; Tamura et al. 1993, 1996) or electrically fused protoplasts (Tamura et al. 1995), callus (Yokoyama and Takeuchi 1976; Tao et al. 1988; Tamura et al. 1992), and hypocotyl segments (Nakamura et al. 1998) has been reported. Most of the species used in these regeneration systems were Diospyros kaki; however, only one example of regeneration from electrically fused protoplasts from interspecific somatic hybrids between *D. glan- dulosa* and *D. kaki* is reported (Tamura et al. 1998). Although regeneration of whole plants has not been reported, adventitious buds formation from leaf segments (Yokoyama and Takeuchi 1988) and somatic embryogenesis from leaf tissue of the *in vitro* shoots (Fukui et al. 1988b) also have been reported. These results also show the possibility of the efficient use these systems for transformation of this species.

**GENETIC TRANSFORMATION OF THE JAPANESE PERSIMMON**

**Transformation with wild-type strains of Agrobacterium**

Tao et al. (1994) reported on the genetic transformation of the Japanese persimmon by *Agrobacterium rhizogenes* wild-type strains of A4. They first induced a crown gall by inoculating this strain to *D. kaki* cv. ‘Jiro’ and ‘Nishimurawase’. Although plants were regenerated from callus (Tao et al. 1997), crown galls formed cells to untransformed cells. They also reported that dwarfness and decreased rooting ability were observed in the transformants. Tamura (1997) transformed the Japanese persimmon cv. ‘Jiro’ and concluded that the strains of A281 and C58 were the most virulent on persimmon. They suggested the possibility of practical use these disarmed strains in the transformation of the Japanese persimmon.

**Leaf-disc transformation**

A foreign gene was first successfully introduced by leaf disc transformation system with a disarmed strain of *A. tumefaciens* developed by Tao et al. (1997a). Using this system, the cry1A (c) gene of *Bacillus thuringiensis* (Tao et al. 1997a), the codA gene of *Arthrobacter globiformis* (Gao et al. 2000), apple NADP-dependent sorbitol-6-phosphate dehydrogenase cDNA (S6PDH) (Gao et al. 2001), and pear fruit polygalacturonase-inhibiting protein (PGIP) gene (Tamura et al. 2004) had been successfully introduced into Japanese persimmon. With this system, adventitious shoots were regenerated from the callus derived from cultured leaf disks. Tao et al. (1997a) reported that a callus at the intermediate stage was indispensable to obtain transformants with high efficiency. Gao et al. (2000) slightly modified this method by reducing the concentration of zeatin in the initial period of transformation, and obtained a higher transformation efficiency than that reported by Tao et al. (1997a).

**Transformation with a hypocotyl segment**

*Agrobacterium* mediated transformation and plant regeneration from hypocotyl segments of Japanese persimmon is another method for the transformation of this fruit species. This method was reported by Nakamura et al. (1998). Using this system, the rolC gene from *A. rhizogenes* was introduced into the genome of Japanese persimmon seedlings (Koshita et al. 2002). The advantage of this system are higher transformation efficiency (11.1%) and shorter time required for obtaining transformants (4-5 months) than the method of leaf disc transformation developed by Tao et al. (1997). However, the system cannot be used to introduce foreign genes into the cultivars because the hypocotyl segments using this system are of a cross-bred origin (Nakamura et al. 1998).

**INTRODUCTION OF FOREIGN GENES TO CONFER AGRICULTURALLY IMPORTANT CHARACTERISTICS**

Although Japanese persimmon is one of the most important fruit crops in Japan, this species has some horticultural disadvantages. For example, because the tree grows to heights exceeding 5 meters, pruning, flower bud thinning, and harvesting are more challenging than for small trees. Therefore, there is a considerable demand for dwarfed trees that can facilitate field practices in the orchards. From a breeding viewpoint, inbreeding depression is a serious problem when selecting PCNA cultivars. This plant is sensitive to drought, and especially for potted plants, dedicated irrigation management is required. Moreover, recent environmental concerns,

---

**Table 1 Examples of regeneration systems of Japanese persimmon**

<table>
<thead>
<tr>
<th>Source of regeneration</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot tip</td>
<td>Sugiura et al. 1986; Fukui et al. 1988a, 1992; Kagami 1999</td>
</tr>
<tr>
<td>Dormant buds</td>
<td>Sugiura et al. 1986; Murayama et al. 1989</td>
</tr>
<tr>
<td>Axillary bud</td>
<td>Sarathchandra and Burch 1991</td>
</tr>
<tr>
<td>Leaf segment of <em>in vitro</em> shoots</td>
<td>Nishimura and Yamada 1992; Choi et al. 2001</td>
</tr>
<tr>
<td>Roots of <em>in vitro</em> plant</td>
<td>Tetsumura and Yukinaga 1996</td>
</tr>
<tr>
<td>Endosperm</td>
<td>Tao et al. 1997</td>
</tr>
<tr>
<td>Protoplast (derived from callus)</td>
<td>Tao et al. 1991; Tamura et al. 1993, 1996</td>
</tr>
<tr>
<td>Protoplast (electrofused)</td>
<td>Tamura et al. 1995, 1998</td>
</tr>
<tr>
<td>Callus</td>
<td>Yokoyama and Takeuchi 1976; Tao et al. 1988; Tamura et al. 1992</td>
</tr>
<tr>
<td>Hypocotyl segments</td>
<td>Nakamura et al. 1998</td>
</tr>
</tbody>
</table>
transgenic plant lines produced the protein of choline oxidase, which catalyzes the oxidation of choline to glycine betaine. Glycine betaine, which is commonly found in higher plants, is known to protect cells from salt stress (reviewed in Öktem and Tao et al. 2007). Since the Japanese persimmon is one of the tallest fruit trees, there is demand for a dwarf culture of this species. For the purpose of dwarfing rootstock development, the rolC gene from A. rhizogenes was introduced into the seedling of cv. ‘Saijo’ persimmon (Koshita et al. 2002). The transformant also produced more branches than regenerated plants from the calli. They inoculated the rolC gene into the genome of Japanese persimmon cv. ‘Jiro,’ and reported that crown galls formed in all tested strains. In this experiment, new shoots from tumor-derived calli synthesized miki-mopine. This phenomenon indicate that the tumor-derived calli were chimera of transformed cells and non-transformed cells. Most of the transformant which synthesized miki-mopine showed abnormal growth such as dwarfness and wrinkled leaves. One transgenic line showed abnormal growth without synthesizing miki-mopine. This might indicate that the entire gene was not integrated into the plant genome. These results might be very important because these transformants obtained by inoculation of wild-type strain of A. rhizogenes have the possibility to be used for dwarfing culture of Japanese persimmon in the open field.

Some fruit trees are dwarfed using a dwarfing rootstock or interstock. In the case of the Japanese persimmon, no dwarfing rootstock has been developed, and therefore, there are considerable demands for dwarfing rootstock development of this species. For the purpose of dwarfing rootstock development, the rolC gene from A. rhizogenes was introduced into the seedling of cv. ‘Saijo’ persimmon (Koshita et al. 2002). The plant into which the rolC gene had been introduced had shorter internodes and smaller leaves and produced more branches than regenerated plants from the open-pollinated ‘Saijo’ seedlings. The transformant also showed higher rooting ability than the control. Although these transformants were dwarfed, there have been no other reports about these transformants being used for the development of a dwarf culture. It is possible that these transformants will be used as resources for elucidating a dwarfing mechanism, practical rootstock, or interstock for a dwarfing culture of Japanese persimmon.

To increase disease resistance

Recent environmental concerns and demands for reducing labors of plant protection, developing disease resistant cultivars are important, because such cultivars are expected to reduce the use of agricultural chemicals. Tamura et al. (2004) introduced the pear fruit polygalacturonase (PG) inhibiting protein (PGIP) gene into the genome of Japanese persimmon cv. ‘Jiro’ to enhance disease resistance. PGIP is presented apoplastically in many plant species (Gomathi et al. 2006), and secreted polygalacturonase from the pathogen is inhibited by PGIP (Albersheim and Anderson 1971). The crude extracts from the PGIP gene-introduced transformants successfully inhibited fungal PG activity (Tamura et al. 2004). This result demonstrated that the introduction of this gene into the genome of Japanese persimmon is one of the effective methods for increasing the fungal disease resistance of this species. In this experiment, 9 PGIP gene-introduced transgenic lines were obtained from 1191 infected leaf discs.
CONCLUSION

Since transformation of Japanese persimmon has been successful, this system contributes to the transfer of positive characteristics to this fruit species. There are some fruit-tree specific physiological phenomena, such as a juvenile phase, and persimmon-specific problem such as difficulties of propagating from cuttings, that will need to be overcome. The proposed regeneration and transformation systems should assist in overcoming these physiological phenomena.

REFERENCES

Choi JY, Kim HJ, Lee CH, Bae JM, Chung YS, Shin JS, Hyung NI (2001) Efficient and simple plant regeneration via organogenesis from leaf segment cultures of persimmon (Diospyros kaki). In Vitro Cellular and Developmental Biology – Plant 37, 274-279
Tamura M (1997) Transformation of persimmon by wild type Agrobacterium rhizogenes. Memories of the School of B.O.S.T. of Kinki University, 53-38