Transgenic Plant Journal ©2007 Global Science Books

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 carryies 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

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, axially buds, callus, protoplast, leaf disk, 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.

Received: 13 March, 2007. Accepted: 4 May, 2007.

Invited Mini-Review

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), protoplast 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 protoplants from interspecific somatic hybrids between *D. glandulosa* and *D. kaki* is reported (Tamura *et al*. 1998). Although regeneration of whole plants have 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 *in vitro* shoots of Fuyu', 'Jiro' and 'Nishimurawase'. Although plants were regenerated from tumor derived callus, which amplification of the expected size of DNA fragments had been confirmed by PCR analysis, a few regenerated plants from these calluses showed no amplification of expected size of DNA fragment. They suggested that this might be well known phenomenon caused by cross-feeding of phytohormones from transformed cells to untransformed cells. They also reported that dwarfness and decreased rooting ability were observed in the transformants. Tamura (1997) transformed the Japanese persimmon 'Jiro' using three wild-type strains of *A. rhizogenes*, M123, 1724, and A13, and reported that callus induced from M123-inoculated shoot stems developed into whole plants possessing mikimopine-synthesizing ability, which is often used as an index of the classification of Ri plasmids of *A. rhizogenes*. However, no amplifications of inserted core T-DNA genes including *rolC* and *rolD* were observed in all shoots lines obtained from the infection of *A. rhizogenesis* 1724 and A13. Tao *et al*. (1995) determined the relative virulence of several wild-type strains of *A. tumefacients* by inoculating stems of axenic shoots of 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 *cry*IA (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, delicate irrigation management is required. Moreover, recent environmental concerns,

Table 1 Examples of regeneration systems of Japanese persimmon

Source of regeneration	References
Shoot tip	Sugiura *et al*. 1986; Fukui et *al*. 1988a, 1992; Kagami 1999
Dormant buds	Sugiura *et al*. 1986; Murayama *et al*. 1989
Axially bud	Sarathchandra and Burch 1991
Leaf segment of *in vitro* shoots	Nishimura and Yamada 1992; Choi *et al*. 2001
Roots of *in vitro* plant	Tetsumura and Yukinaga 1996
Endosperm	Tao *et al*. 1997
Protoplast (derived from callus)	Tao *et al*. 1991; Tamura *et al*. 1993, 1996
Protoplast (electrofused)	Tamura *et al*. 1995, 1998
Callus	Yokoyama and Takeuchi 1976; Tao *et al*. 1988; Tamura *et al*. 1992
Hypocotyl segments	Nakamura *et al*. 1998

Table 2 Studies conducted on transformation of Japanese persimmon.

Introduced foreign gene	Characteristics vested to transformant	Reference
*cry*IA(c)	Insect resistance	Tao *et al*. 1997
codA	Salt tolerance	Gao *et al*. 2000
S6PDH	Salt tolerance	Gao *et al*. 2001
rolC	Dwarfness	Koshita *et al*. 2002
PGIP	Disease resistance	Tamura *et al*. 2004

reduction of agricultural chemicals in the process of protection from diseases or insects is important. Therefore, characteristics such as insect and disease resistance are the important improvements envisioned for cultivars, because they might be able to reduce the use of agricultural chemicals. Attempts to overcome these deficiencies have been made by the introduction of foreign genes. Examples of the transformation of Japanese persimmon are shown in **Table 2**. This indicates that genetic transformation to improve the characteristics of the native persimmon cultivars is one of the best ways to transfer desired characteristics because all of the experiments presented in **Table 2** led to improvements in the transformants.

To increase resistance against insects

The *cry*IA(c) gene of *Bacillus thuringiensis*, a soil bacterium, produces insecticidal crystal proteins. Although sporecrystal suspensions have been used as biological insecticides, their instability or inactivation under field conditions has often limited their practical use. Tao *et al.* (1997) introduced the *cry*IA(c) gene of *B. thuringiensis* into the genome of the Japanese persimmon cv. 'Jiro,' which is one of the most popular and economically important PCNA typed cultivars in Japan. In this experiment, they obtained 10 lines of transgenic persimmon from 720 infected leaf disc and these transgenic plants showed insect resistance.

To increase salt tolerance

Environmental damage to the plant is a limiting factor of fruit production. Especially, the productivity of fruit trees is severely affected by soil salinity. To elucidate this problem, the *codA* gene of *Arthrobacter globiformis* and a cDNA encoding sorbitol-6-phosphate dehydrogenase (S6PDH) were introduced into the Japanese persimmon cv. 'Jiro' by Gao *et al.* (2000, 2001) to increase the environmental stress tolerance. The *codA* gene of *A. globiformis* encodes choline oxidase, which catalyzes the oxidation of choline to glycinebetaine. Glycinebetaine, which is commonly found in higher plants, is known to protect cells from salt stress (reviewed in Öktem *et al.* 2006), and many reports have indicated that exogenously applied glycinebetaine improved the growth of stressed plants (Ashraf and Foolad 2007). Since the *codA* gene had been cloned and introduced to *Arabidopsis thaliana* and tolerance to environmental stress was successfully vested (Hayashi *et al.* 1997), introduction of this gene into the plant is an effective way to vest environmental stress tolerance. Gao *et al.* (2000) introduced this gene into the genome of the Japanese persimmon cv. 'Jiro' for the genetic engineering of salt tolerance of this species. They assayed the *codA* DNA by Southern blot analysis, and the insertion of this gene into the genome was confirmed. Furthermore, immunoblot analysis of choline oxidase revealed that all of the tested transgenic plant lines produced the protein of choline oxidase protein. In this experiment, at least 16 lines of coda gene-introduced transformants were obtained from 100 leaf disks infection. Gao *et al.* (2001) also introduced another foreign gene, apple cDNA encoding NADP-dependent sorbitol-6-phosphate dehydrogenase (S6PDH), to vest environmental tolerance. S6PDH is a key enzyme in the sorbitol biosynthesis. The enzyme was purified, and its cDNA was isolated (Kanayama *et al.* 1992; Kanayama and Yamaki 1993). Sorbitol, a sugar alcohol, is believed to allow plants to tolerate environmental factors, such as drought or salt. When S6PDH was introduced into persimmon, persimmon synthesized sorbitol successfully and the transformants showed salt tolerance (Gao *et al.* 2001). They obtained 7 transgenic lines from 200 infected leaf discs.

For dwarf culture

Since the Japanese persimmon is one of the tallest fruit trees, there is demand for a dwarf culture of this species. Tao *et al.* (1994) first reported about dwarf transformants produced by genetic transformation of Japanese persimmon. They inoculated *A. rhizogenes* wild-type strain A4 into the stems of micropropagated cvs. 'Fuyu', 'Jiro' and 'Nishimurawase'. Although crown gall tumors were formed on all of the tested cultivars, not only transformants but also non-transformants were regenerated from the tumor-derived calli and decreased rooting ability was observed in most transgenic lines. Tamura (1997) also inoculated the wild-type strains of *A. rhizogenes* wild-type strains M123, 1724 and A13 to Japanese persimmon cv. 'Jiro' and reported that crown galls formed in all tested strains. In this experiment, not all shoots from tumor-derived calli synthesized mikimopine. This phenomenon indicate that the tumor-derived calli were chimera of transformed cells and non-transformed cells. Most of the transformant which synthesized mikimopine showed abnormal growth such as dwarfness and wrinkled leaves. One transgenic line showed abnormal growth without synthesizing mikimopine. 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

Albersheim P, Anderson AJ (1971) Proteins from plant cell walls inhibit polygalacturonase secreted by plant pathogens. *Proceedings of the National Academy of Science USA* **68**, 1815-1819

Ashraf M, Foodlad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany* **59**, 206-216

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* Tuunb.). *In Vitro Cellular and Developmental Biology – Plant* **37**, 274-279

Fukui H, Nishimura K, Murase K, Nakamura M (1988a) Effect of rooting induction in medium on in vitro rooting of Japanese persimmon (*Diospyros kaki* Thunb.). *Research Bulletin of the Faculty of Agriculture, Gifu University* **53**, 133-137 (in Japanese with English summary)

Fukui H, Nishimoto K, Murase I, Nakamura M (1988b) Somatic embryogenesis from the leaf tissue of continuously subcultured shoots in Japanese persimmon (*Diospyros kaki* Thunb.) *Japanese Journal of Breeding* **38**, 465-469

Fukui H, Nishimoto K, Nakamura M (1992) Varietal differences in rooting of *in vitro* subcultured Japanese persimmon shoots. *Journal of the Japanese Society for Horticultural Science* **60**, 821-825

Gao M, Sakamoto A, Miura K, Murata N, Sugiura A, Tao R (2000) Transformation of Japanese persimmon (*Dipspyros kaki* Thunb.) with a bacterial gene for choline oxidase. *Molecular Breeding* **6**, 501-510

Gao M, Tao R, Miura K, Dandekar AM, Sugiura A (2001) Transformation of Japanese persimmon (*Diospyros kaki* Thunb.) with apple cDNA encoding NADP-dependent sorbitol-6-phosphate dehydrogenase. *Plant Science* **160**, 837-845

Gomathi V, Gayathri S, Anupama B, Teixeira da Silva JA, Gnanamanickam SS (2006) Molecular aspects of polygalacturonase-inhibiting proteins (PGIPs) in plant defence. In Teixeira da Silva JA (Ed) *Floriculture, Ornamental and Plant Biotechnology: Advances and Tropical Issues* (1st Edn, Vol III), Global Science Books, London, pp 373-379

Hayashi H, Alia ML, Deshnium P, Ida M, Murata N (1997) Transformation of *Arabidopsis thaliana* with the *codA* gene for coline oxidase; accumuration of glycinebetaine and enhanced tolerance to salt and cold stress. *Plant Journal* **12**, 133-142

Kagami H (1999) Effect of sugars on rooting of shoots of Japanese persimmon propagated *in vitro*. *Plant Biotechnology* **16**, 371-374

Kanayama Y, Mori H, Imaseki H, Yamaki S (1992) Nucreotide sequence of a cDNA encoding NADP-sorbitol-6-phosphate dehydrogenase from apple. *Plant Physiology* **100**, 1607-1608

Kanayama Y, Yamaki S (1993) Purification of properties of NADP-dependent sorbitol-6-phosphate dehydrogenase from apple seedling. *Plant and Cell Physiology* **34**, 819-823

Koshita Y, Nakamura Y., Kobayashi S, Morinaga K (2002) Introduction of *rolC* gene into the genome of the Japanese persimmon causes dwarfism. *Journal of Japanese Society for Horticultural Science* **71**, 529-531

Murayama H, Tao R, Tanaka T, Sugiura A (1989) *In vitro* shoot proliferation and rooting of several Japanese persimmon cultivars. *Journal of the Japanese Society for Horticultural Science* **58**, 55-61 (in Japanese with English summary)

Nakamura Y, Kobayashi S, Nakajima I (1998) *Agrobacterium*-mediated transformation and plant regeneration from hypocotyl segments of Japanese persimmon (*Diospyros kaki* Thunb). *Plant Cell Reports* **17**, 435-440

Nishimura K, Yamada K (1992) Plantlet regeneration of Japanese persimmon from leaf segment through adventitious bud formation. *Bulletin of the Shimane Agricultural Experiment Station* **26**, 106-113 (in Japanese with English summary)

Öktem HA, Eyidoğan F, Selçuk F, Teixeira da Silva JA, Yücel M (2006) Osmotic stress tolerance in plants: Transgenic strategies. In: Teixeira da Silva JA (Ed) *Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues* (1st Edn, Vol III), Global Science Books, London, pp 194-208

Sarathchandra SU, Burch G (1991) Micropropagation of Japanese persimmon (*Diospyros kaki* Thunb.) cv. 'Hiratanenashi'. *New Zealand Journal of Crop and Horticultural Science* **19**, 113-120

Sugiura A, Tao R, Murayama H, Tomana T (1986) *In vitro* propagation of Japanese persimmon. *HortScience* **21**, 1205-1207

Tao R, Murayama H, Moriguchi K, Sugiura A (1988) Plant regeneration from callus cultures derived from primordial leaves of adult Japanese persimmon. *HortScience* **23**, 1055-1056

Tao R, Tamura M, Yonemori K, Sugiura A (1991) Plant regeneration from callus protoplast of adult Japanese persimmon (*Diospyros kaki* L.). *Plant Science* **79**, 119-125

Tao R, Handa T, Tamyra M, Sugiura A (1994) Genetic transformation of Japanese persimmon (*Diospyros kaki* L.) by *Agrobacterium rhizogenes* wild type strain A4. *Journal of the Japanese Society for Horticultural Science* **63**, 283-289

Tao R, Uratsu SL, Dandekar AM (1995) Relative virulene of *Agrobacterium* strains on persimmon (*Diospyros kaki* L.). *Acta Horticulturae* **392**, 171-178

Tao R, Dandekar AM, Uratsu SL, Vali PV, Tebbets JS (1997a) Enginerring genetic resistance against insects in Japanese persimmon Using the *cry*IA(c) gene of *Bacillus thuringiensis*. *Journal of the American Society for Horticultural Science* **122**, 764-771

Tao R, Ozawa K, Tamura M, Sugiura A (1997b) Dodecaploid plant regeneration from endosperm culture of persimmon (*Diospyros kaki* L.). *Acta Horticulture* **436**, 116-128

Tamura M, Tao R, Sugiura A (1992) Highly stable regeneration from long-term cultures of Japanese persimmon callus. *HortScience* **27**, 1048-1048

Tamura M, Tao R, Sugiura A (1993) Improved protoplast culture and plant regeneration of Japanese persimmon (*Diospyros kaki* L.). *Japanese Journal of Breeding* **43**, 239-245

Tamura M, Tao R, Sugiura A (1995) Regeneration of somatic hybrids from electrofused protoplasts of Japanese persimmon (*Diospyros* kaki L.). *Plant Science* **108**, 101-107

Tamura M, Tao R, Sugiyra A (1996) Production of dodecaploid plants of Japanese persimmon (*Diospyros kaki* L.) by colchicine treatment of protoplasts. *Plant Cell Reports* **15**, 470-73

Tamura M (1997) Transformation of persimmon by wild type *Agrobacterium rhizogenes*. *Memories of the School of B.O.S.T. of Kinki University* **1**, 53-38

Tamura M, Tao R, Suguira A (1998) Production of somatic hybrids between *Diospyros gladulosa* and *D. kaki* by protoplast fusion. *Plant Cell, Tissue and Organ Culture* **54**, 85-91

Tamura M, Gao M, Tao R, Labavitch JM, Dandekar AM (2004) Transformation of persimmon with a pear fruit polygalacturonase inhibiting protein (PGIP) gene. *Scientia Horticulturae* **103**, 19-30

Tetsumura T, Yukinaga H (1996) High-frequency shoot regeneration from roots of Japanese persimmon. *HortScience* **31**, 463-464

Yamada M (2005) Persimmon genetic resources and breeding in Japan. *Acta Horticulture* **685**, 51-64

Yamane Y, Yamada M, Yoshinaga K (1990) New Japanese persimmon cultivars, 'Shinshuu' and 'Youhou'. *Journal of the Japanese Society for Horticultural Science* **59 (Suppl. 2)**, 218-219 (in Japanese)

Yamane Y, Yamada M, Kurihara A, Yoshinaga K, Hirakawa N, Sato A, Matsumoto R, Iwanami H (1995) New Japanese persimmon cultivar 'Taishuu'. *Journal of the Japanese Society for Horticultural Science* **64 (Suppl. 2)**, 184-185 (in Japanese)

Yokoyama T, Takeuchi M (1976) Organ and plantlet formation from callus in Japanese persimmon (*Diospyros kaki*). *Phytomorphology* **26**, 273-275