

Segregation of Astringency in F_1 Progenies Derived from Crosses between Pollination-constant, Nonstringent Persimmon Cultivars

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Abstract. Japanese persimmon (*Diospyros kaki* Thunb.) cultivars are classified into four types depending on the nature of astringency loss of the fruit. The pollination-constant, non-astringent (PCNA) persimmons lose their astringency on the tree as the fruits develop. This PCNA trait is qualitatively inherited and recessive to the other three types, pollination-constant, astringent (PCA), pollination-variant, nonstringent (PVNA), and pollination-variant, astringent (PVA). In fact, crosses among Japanese PCNA cultivars yield only PCNA type in F_1 generation as shown in recent breeding programs at the National Institute of Fruit Tree Science. Despite these previous results, we demonstrated here that non-PCNA (PVNA, PVA, and PCA) type offspring were derived at relatively high rates in the F_1 generation from a cross between ‘Luo Tian Tian Shi’, a PCNA accession from China, and the Japanese PCNA cultivar, ‘Taishu’, despite the fact that ‘Luo Tian Tian Shi’ was confirmed to be a true PCNA type by measuring tannin cell size, a principal morphological characteristic to distinguish PCNA cultivars from non-PCNA ones. When segregations of tannin cell size and tannin content in three progenies of the breeding populations derived from Chinese PCNA ‘Luo Tian Tian Shi’ x Japanese PCNA ‘Taishu’, Japanese PCNA ‘Shinshu’ x Japanese PCNA ‘Taishu’, and Japanese PVNA (non-PCNA) ‘Kurokuma’ x Japanese PCNA ‘Taishu’ were investigated, all offspring between Japanese PCNA cultivars contained only small tannin cells and were PCNA types, and those between Japanese PVNA x PCNA cultivars contained only large tannin cells and were non-PCNA types. However, hybrids between ‘Luo Tian Tian Shi’ and ‘Taishu’ segregated into populations of small and large tannin cells, indicating that ‘Luo Tian Tian Shi’ is likely heterozygous for astringency. Therefore, Chinese PCNA ‘Luo Tian Tian Shi’ should be different from Japanese PCNA cultivars in genetic makeup.

Japanese persimmons are classified as astringent or nonstringent based on whether the fruits lose their astringency naturally as they mature on the tree. These astringent and nonstringent types are further classified into pollination-variant or pollination-constant, depending on whether the flesh becomes dark in the presence of the seeds. Thus, Hume (1913, 1914) and Kajijura (1946) classified Japanese persimmons into the following four types: 1) pollination-constant, nonstringent (PCNA), 2) pollination-variant, nonstringent (PVNA), 3) pollination-variant, astringent (PVA), and 4) pollination-constant, astringent (PCA).

There are qualitative differences between PCNA and non-PCNA types (PVNA, PVA, and PCA) (Yonemori et al., 2000). Recent results of persimmon breeding projects at the National Institute of Fruit Tree Science (NIFTS) show that crosses between PCNA types and non-PCNA ones yield only non-PCNA offspring in the F_1 generation (Yamada and Sato, 2002). Only when non-PCNA F_1 progeny derived from PCNA type x non-PCNA were backcrossed to a PCNA cultivar were PCNA-type offspring obtainable with a ratio of $\approx 15\%$ (Ikeda et al., 1985; Yamada and Sato, 2002). As persimmon is a hexaploid and the PCNA is recessive, two or three allele pairs regulate the inheritance of the PCNA trait (Yonemori et al., 2000).

A principal difference between PCNA and non-PCNA cultivars is the developmental pattern of tannin cells (Yonemori and Matsushima, 1985). Tannin cells in PCNA fruits stop enlarging at an early stage of fruit growth, whereas in non-PCNA fruits they continue to grow until the fruits mature. Thus, the slow size increase of small tannin cells and their dilution by fruit enlargement in PCNA-type fruit account for the loss of astringency on the tree.

Among more than 1000 persimmon cultivars in a nationwide survey in Japan (Agricultural Research Station, 1912), only six PCNA types were found, and these were developed in Japan uniquely (Yonemori et al., 2000). Recently, however, a PCNA cultivar, ‘Luo Tian Tian Shi’, was found in Luo Tian Prefecture of China (Wang, 1982; Wang et al., 1997). Despite having characteristics of Japanese PCNA cultivars [fruits have low tannin content at maturity (Yamada et al., 1993)], some F_1 offspring of Chinese PCNA ‘Luo Tian Tian Shi’ x Japanese PCNA ‘Taishu’ produced astringent fruits at the fruit mature stage. Crosses among Japanese PCNA cultivars yield only PCNA-type offspring in F_1 generations, when a few exceptions caused by experimental errors were eliminated (Ikeda et al., 1985). Therefore, in this paper, we investigated whether: 1) Chinese ‘Luo Tian Tian Shi’ is a true PCNA type with respect to tannin cell size, and 2) the cross between ‘Luo Tian Tian Shi’ and a Japanese PCNA cultivar yields non-PCNA-type offspring in F_1 generation.

Materials and Methods

Tannin content and tannin cell size. Tannin content and tannin cell sizes in mature fruits from 42 cultivars consisting of 15 PCNA, including ‘Luo Tian Tian Shi’, 7 PVNA, 6 PVA, and 14 PCA (a total of 27 non-PCNA, Table 1) were measured.

Four to five fruits sampled from each cultivar were divided into two groups. The mesocarp of one group was diced and 5-g samples were prepared in triplicate. Each sample was homogenized with 80% methanol, and then the homogenate was centrifuged at 3600 g_n for 5 min and the supernatant decanted. After the pellet was washed with fresh 80% methanol and centrifuged, the supernatants were combined and made up to 100 mL with the same solvent. An aliquot of the extract was analyzed for soluble tannin with the Folin-Ciocalteu method according to Oshida et al. (1996). The soluble tannin content is expressed as (+)-catechin equivalents.

To determine tannin cell size, small blocks of tissue from the equatorial region of the mesocarp of the other group were fixed with 2.5% glutaraldehyde, containing 0.2% tannic acid. After the blocks were washed with water, they were macerated in a 0.05 M EDTA solution (pH 10.0) at 45 °C for 5 h by oscillating at 90 rpm according to Letham (1960). A droplet of tannin cells that separated from the parenchyma cells by decanting several times was placed on a glass slide and images of the tannin cells taken with a digital camera attached to a light microscope (Olympus DP-50, Tokyo) were recorded on a computer. The area of each 100 tannin cells was measured with Scion Image, public domain software (Scion Corp., Maryland).

Changes in soluble tannin content and development patterns of tannin and parenchyma cells were determined on developing fruits of three PCNA ‘Fuyu’, ‘Hanagosho’, and ‘Luo Tian Tian Shi’; PCA ‘Kuramitsu’; and PVA ‘Monpei’. Samples were collected monthly from mid-June until the fruits matured in late

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Table 1. Four types of persimmon cultivars used for determination of tannin cell size and tannin content at mature stages.

PCNA type cultivar	Non-PCNA-type cultivar		
	PVNA type	PVA type	PCA type
1. Fujiwara-gosho	16. Amahyakume	23. Emon	29. Atago
2. Fuyu	17. Anzai	24. Hiratanenashi	30. Hagakushi
3. Hanagosho	18. Chokenji	25. Kikuhira	31. Jisha
4. Hazegosho	19. Johren	26. Koshu-hyakume	32. Kakiyamagaki
5. Jiro	20. Kurokuma	27. Monpei	33. Kuramitsu
6. Luo-tian-tian-shi	21. Ohniwa	28. Yamato-hyakume	34. Miyazaki-tanenashi
7. Maekawa-jiro	22. Shogatsu		35. Nagara
8. Mikado			36. Pung-gi-go-si
9. Okugosho			37. Shakokushi
10. Oh-gosho			38. Takura
11. Shinshu			39. Tamopan
12. Suruga			40. Watarizawa
13. Taishu			41. Yokono
14. Tenjin-ghosho			42. Yotsuya-saijo
15. Yamato-gosho			

October, depending on the cultivar. Soluble tannin content and tannin cell size were determined as above. Parenchyma cell size was determined by the same method as tannin cell size, using the macerated solution before tannin cells were collected by decantation.

Segregation pattern of tannin cell size in the F_1 population. Five to seven mature fruits from three populations: Japanese PCNA 'Shinshu' x Japanese PCNA 'Taishu' (25 offspring), Chinese PCNA 'Luo Tian Tian Shi' x Japanese PCNA 'Taishu' (34 offspring), and Japanese PVNA 'Kurokuma' x Japanese PCNA 'Taishu' (18 offspring) were analyzed for tannin content and tannin cell size as above, except that the images were recorded on a videotape through a CCD camera equipped with an inverted light microscope (Olympus IMT-2, Tokyo). The area of each 100 tannin cells was measured with a Power Macintosh 7500/100 using a public domain NIH Image v. 1.61 software.

Results and Discussion

Tannin content and tannin cell size. Astringency in PCNA cultivars, including 'Luo Tian Tian Shi', almost disappeared on the tree at maturity, whereas it showed high levels in the PVA and PCA cultivars (Fig. 1). Soluble tannin content of the PVNA cultivars varied because of the lack of seeds in some fruits. Our data revealed that tannin cell size distinguishes PCNA fruits from non-PCNA fruits. The area of tannin cells was $<33 \times 10^3 \mu\text{m}^2$ in the former and ranged from $55 \times 10^3 \mu\text{m}^2$ to $140 \times 10^3 \mu\text{m}^2$ in the latter (Fig. 1).

The developmental pattern of tannin cells in five cultivars differed between PCNA and non-PCNA cultivars (Fig. 2A). In PCNA cultivars, including 'Luo Tian Tian Shi', tannin cells gradually ceased enlarging from the end of June and stopped completely by the end of July. The area of tannin cells in non-PCNA cultivars was more than 2.5 times larger than those in the PCNA cultivars. However, there was no clear difference between PCNA and non-PCNA cultivars in the developmental patterns of parenchyma cells (Fig. 2B), despite the fact that Chinese PCNA 'Luo Tian Tian Shi' and PCA 'Kuramitsu' showed a slow enlarge-

ment. The pattern of decreasing soluble tannins differed between the Japanese PCNA cultivars and 'Luo Tian Tian Shi'. The decreasing trend in 'Luo Tian Tian Shi' was intermediate between the two PCNA types and non-PCNA ones. However, its final concentration was almost equal to all PCNA cultivars (Fig. 2C).

The dilution of tannins during fruit development correlates with the loss of astringency in PCNA fruits (Yonemori and Mastushima, 1985) and was supported in this study. Furthermore, tannin cell size in mature PCNA fruits, including 'Luo Tian Tian Shi' is much smaller than in non-PCNA fruits and the difference in the developmental pattern diverged as the season progressed. Hence, it was demonstrated that Chinese 'Luo Tian Tian Shi' is a true PCNA-type with respect to tannin content and tannin cell size.

Segregation patterns of tannin cell size in the F_1 populations. All offspring in the

F_1 population derived from Japanese PCNA 'Shinshu' x Japanese PCNA 'Taishu' had tannin cells $<35 \times 10^3 \mu\text{m}^2$ in area (Fig. 3A), and contained soluble tannins $<0.3\%$ of fresh weight (Fig. 3A), indicating that they were all PCNA types. All F_1 offspring from PVNA 'Kurokuma' x Japanese PCNA 'Taishu' had large tannin cells ranging from $70 \times 10^3 \mu\text{m}^2$ to $130 \times 10^3 \mu\text{m}^2$ (Fig. 3B), and they were two to four times larger than those of the PCNA types. Some individuals had little soluble tannin (Fig. 3B) because they inherited the PVNA trait from 'Kurokuma', i.e., the ability to produce ethanol from the seeds (Sugiura and Tomana, 1983). Nevertheless, no offspring derived from this cross were PCNA. These results confirm that the PCNA trait is recessive in Japanese cultivars as reported previously (Ikeda et al., 1985; Yamada and Sato, 2002).

The F_1 population of Chinese 'Luo Tian Tian Shi' x Japanese PCNA 'Taishu' segregated into population with either small or large tannin cells (Fig. 3C). Small tannin cells and low tannin content in progenies were similar to those derived from 'Shinshu' x 'Taishu', indicating that these offspring were PCNA. Contrarily, individuals having large tannin cells and very high soluble tannin contents in the F_1 population derived from 'Luo Tian Tian Shi' x 'Taishu' were similar to the non-PCNA offsprings of 'Kurokuma' x 'Taishu', indicating that these offspring were non-PCNA. The segregation ratio of the offspring having small tannin cells (PCNA type) to large tannin cells (non-PCNA type) was 22:12 in the present study. These results suggested that 'Luo Tian Tian Shi' is likely heterozygous for astringency.

Japanese PCNA cultivars probably originated as a mutation from a non-PCNA type because they all seem to have the same mutant allele. However, our data indicated that origin

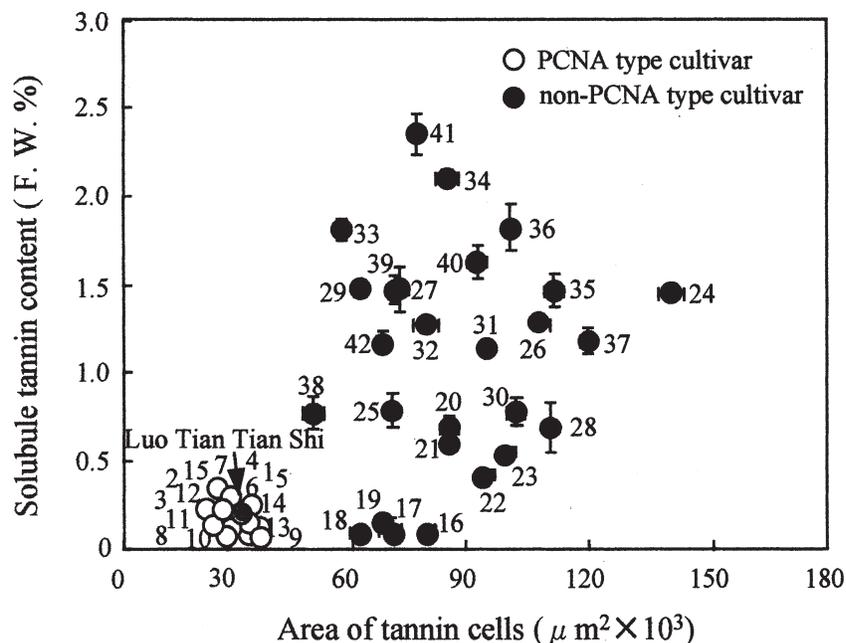


Fig. 1. Relationship between tannin cell size and soluble tannin content in persimmon cultivars. PCNA type (1–15) and non-PCNA type (PVNA type, 16–22; PVA type, 23–28; PCA type, 29–42). Cultivar number corresponds to those in Table 1. Vertical and horizontal bars represent \pm SE ($n = 100$ for tannin cell size and $n = 3$ for soluble tannin content).

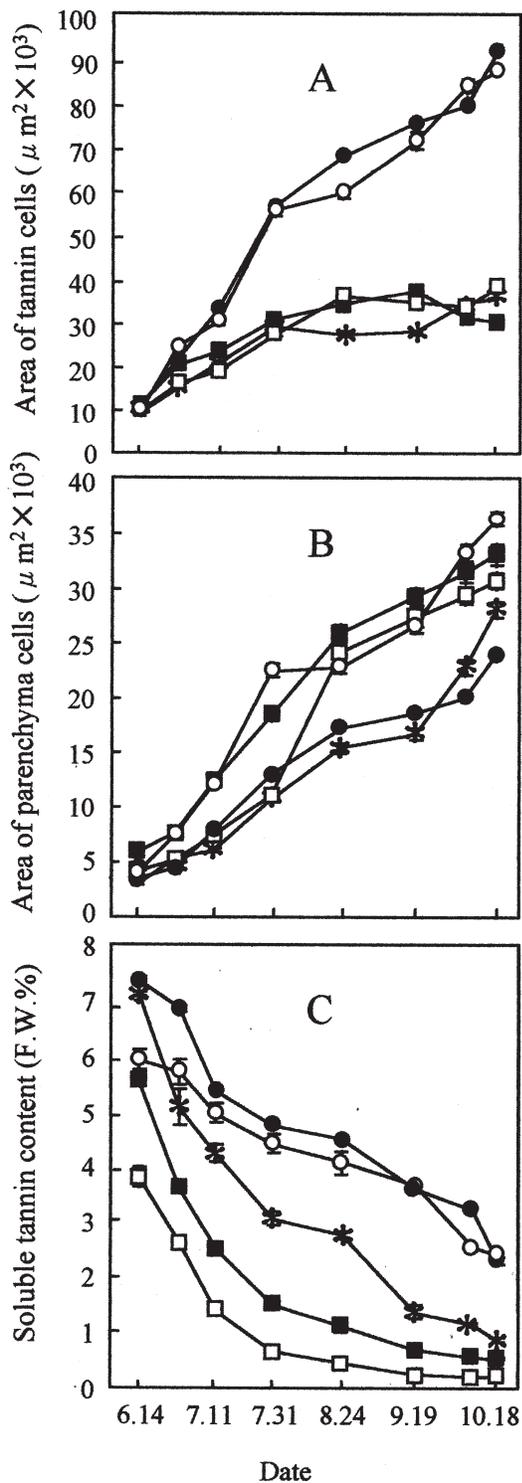


Fig. 2. Developmental patterns of tannin cell (A), parenchyma cell (B) and seasonal changes in soluble tannin content (C) in three PCNA 'Fuyu' (■), 'Hanagoso' (□), 'Luo Tian Tian Shi' (□), and two non-PCNA 'Kuramitsu' (●) and 'Monpei' (□). Vertical bars represent \pm SE ($n = 100$ in A and B; $n = 3$ in C).

of 'Luo Tian Tian Shi' differs from Japanese PCNA cultivars. In the phylogenetic study using AFLP analysis, Kanzaki et al. (2000a) also found that 'Luo Tian Tian Shi' was distantly related to Japanese PCNA cultivars. Furthermore, RFLP analysis for molecular markers, which can distinguish Japanese PCNA cultivars from non-PCNA cultivars, demonstrated that 'Luo Tian Tian Shi' has the same RFLP markers as

non-PCNA cultivars, not as PCNA cultivars (Kanzaki et al., 2000b). These results strongly suggest that genetic make up of 'Luo Tian Tian Shi' is different from Japanese PCNA cultivars and that different genes are responsible for the PCNA trait in 'Luo Tian Tian Shi'.

Currently, a serious problem for breeding new PCNA cultivars is inbreeding depression caused by narrow genetic pools among Japa-

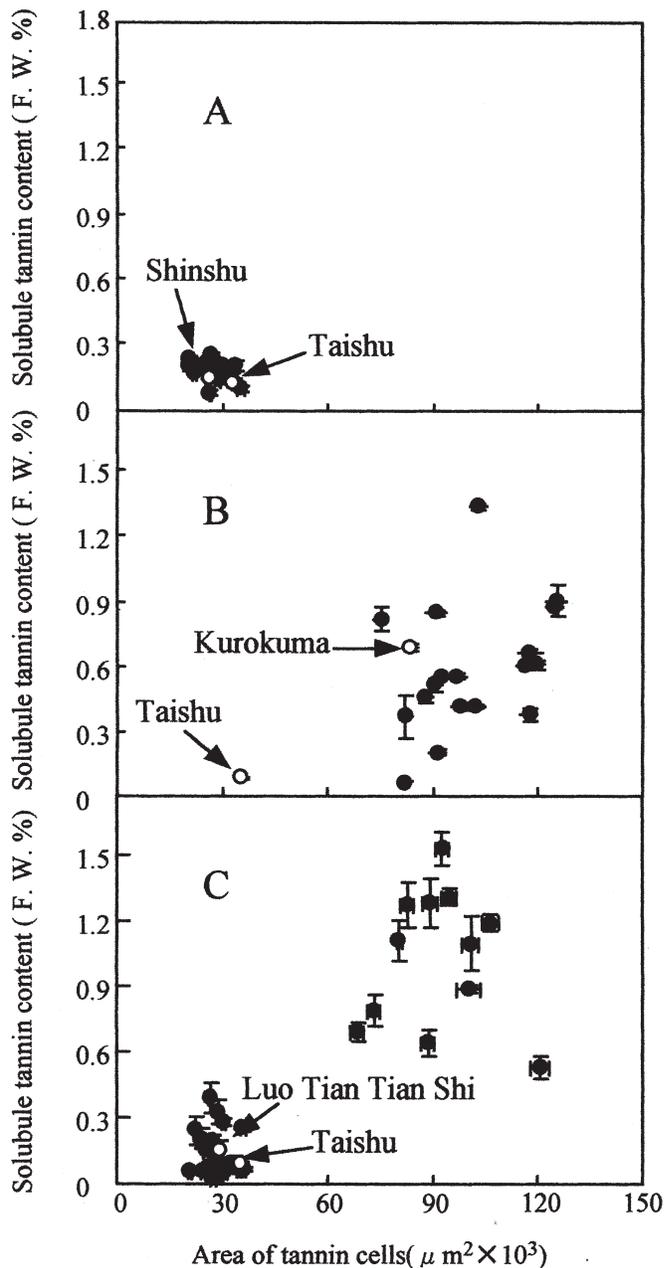


Fig. 3. Relationship between the tannin cell size and the soluble tannin content in F_1 progenies derived from PCNA 'Shinshu' x PCNA 'Taishu' (A), PVNA 'Kurokuma' x PCNA 'Taishu' (B), and PCNA 'Luo Tian Tian Shi' x PCNA 'Taishu' (C). Each dot indicates offspring in the progeny and two circles indicate the parents. Vertical and horizontal bars represent \pm SE ($n = 100$ for tannin cell size and $n = 3$ for soluble tannin content).

nese PCNA cultivars (Yamada, 1993). 'Luo Tian Tian Shi' could be used as a parent for PCNA cultivar breeding projects to overcome inbreeding depression in progenies of PCNA breeding populations, since 'Luo Tian Tian Shi' should have a different genetic background to be PCNA type. Furthermore, the cross between 'Luo Tian Tian Shi' and a non-PCNA cultivar may segregate some PCNA type offspring in

the F₁ generation, due to the involvement of different genes for PCNA trait in 'Luo Tian Tian Shi'. If this is the case, the use of 'Luo Tian Tian Shi' as a parent will open up a new strategy for PCNA cultivar breeding projects.

Literature Cited

- Agricultural Research Station. 1912. Investigation on persimmon cultivars (in Japanese). Agr. Res. Sta. (extra), 28:1–46.
- Hume, H. 1913. Effect of pollination on the fruit of *Diospyros kaki*. Proc. Amer. Soc. Hort. Sci. 10:88–93.
- Hume, H. 1914. A kaki classification. J. Hered. 5: 400–406.
- Ikeda, I., M. Yamada, A. Kurihara, T. Nishida. 1985. Inheritance of astringency in Japanese persimmon (in Japanese with English summary). J. Jpn. Soc. Hort. Sci. 54:39–45.
- Kajiura, M. 1946. Persimmon cultivars and their improvement 2 (in Japanese). Breed. Hort. 1: 175–182.
- Kanzaki, S., K. Yonemori, A. Sato, M. Yamada, and A. Sugiura. 2000a. Analysis of the genetic relationships among pollination-constant and non-astringent (PCNA) cultivars of persimmon (*Diospyros kaki* Thunb.) from Japan and China using amplified fragment length polymorphism (AFLP). J. Jpn. Soc. Hort. Sci. 69:665–670.
- Kanzaki, S., K. Yonemori, A. Sato, M. Yamada, and A. Sugiura. 2000b. Evaluation of RFLP analysis for discriminating PCNA genotype in some persimmon cultivars. J. Jpn. Soc. Hort. Sci. 69:702–704.
- Latham, D.S. 1960. The separation of plant cells with ethylenediaminetetraacetic acid. Expt. Cell. Res. 21:353–360.
- Oshida, M., K. Yonemori, and A. Sugiura. 1996. On the nature of coagulated tannins in astringent-type persimmon fruit after an artificial treatment of astringency removal. Posharvest Biol. Technol. 8:317–327.
- Sugiura, A. and T. Tomana. 1983. Relationships of ethanol production by seeds of different types of Japanese persimmons and their tannin content. HortScience 18:319–321.
- Wang, R. 1982. The origin of 'Luo Tian Tian Shi' (in Chinese). Chinese Fruit Tree 2:16–19.
- Wang, R., Y. Yang, and G. Li. 1997. Chinese persimmon germplasm resources. Acta Hort. 436:43–50.
- Yamada, M. 1993. Persimmon breeding in Japan. Jpn. Agr. Res. Quart. 27:33–37.
- Yamada, M., A. Sato, H. Yakushiji, K. Yoshinaga, H. Yamane and M. Endo. 1993. Characteristics of 'Luo Tian Tian Shi', a non-astringent cultivar of oriental persimmon (*Diospyros kaki* Thunb.) of Chinese origin in relation to non-astringent cultivars of Japanese origin (in Japanese with English summary). Bul. Fruit Tree Res. Sta. 25:19–32.
- Yamada, M. and A. Sato. 2002. Segregation for fruit astringency type in progenies derived from crosses of 'Nishimurawase' × pollination constant non-astringent genotypes in oriental persimmon (*Diospyros kaki* Thunb.). Scientia Hort. 92:107–111.
- Yonemori, K. and J. Matsushima, 1985. Property of development of the tannin cells from non-astringent and astringent type fruits of Japanese persimmon (*Diospyros kaki*) and its relationship to natural astringency (in Japanese with English summary). J. Jpn. Soc. Hort. Sci. 54:201–208.
- Yonemori, K., A. Sugiura, and M. Yamada. 2000. Persimmon genetics and breeding. Plant Breeding Rev. 19:191–225.