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Segregations of Astringent Progenies in the F_1 Populations Derived from Crosses between a Chinese Pollination-constant Nonastringent (PCNA) 'Luo Tian Tian Shi', and Japanese PCNA and Pollination-constant Astringent (PCA) Cultivars of Japanese Origin

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Abstract. **Pollination-constant and nonastringent (PCNA) is one of the most desirable traits in persimmons as this type of cultivar loses its astringency while still on the tree before harvest. Among Japanese PCNA cultivars, the trait is qualitatively inherited and recessive to pollination-constant, astringent (PCA), pollination-variant, nonastringent (PVNA), and pollination-variant, astringent (PVA) types. However, in a previous trial, both astringent and nonastringent types segregated in the F_1 population that resulted from a cross between a Chinese PCNA 'Luo Tian Tian Shi' and a Japanese PCNA cultivar. Because of the unusual segregation, in this study, we crossed another Japanese PCNA 'Okugosho' with 'Luo Tian Tian Shi' to confirm the segregation of astringent types by measuring the tannin cell size and tannin concentration at harvest. Previously, we found that astringent types have larger tannin cells than PCNA-type. The F_1 hybrid progenies from the cross segregated into both PCNA and astringent-type individuals in approximately 1:1 ratio. Likewise, the F_1 population from the astringent-type 'Yotsumizo' and 'Iwasedo' × 'Luo Tian Tian Shi' were ascertained to contain both PCNA and astringent types, which indicates that the PCNA trait of 'Luo Tian Tian Shi' was dominant. Thus, this Chinese cultivar has the potential to become an important parental material for future breeding of PCNA persimmons.**

Persimmon (*Diosypyros kaki* Thunb.; 2n = 6x = 90) cultivars are distinguished into four types (PCNA, PCA, PVNA, PVA), according whether they are astringent (A) or nonastringent (NA), and if they are pollination-variant (PV) or pollination-constant (PC). In the PV type, the dark flesh is associated with the presence of the seeds (Hume, 1913, 1914). Among the different types, the PCNA persimmons are the most desirable for fresh consumption because the developing fruit loses its astringency while on the tree. Therefore, one of the main objectives of the persimmon breeding is to obtain PCNA-type that are large, early ripening, having high fruit quality, free of physiological disorders (Yamada, 1993, 2005). Persimmon fruits accumulate a large quantity of high molecular weight tannins in specific cells called tannin cells, but in the PCNA type, the enlargement of these cells terminates at an early stage of fruit development (Yonemori and Matsushima, 1985, 1987). However, PCNA trait is qualitatively inherited and it is recessive to non-PCNA (PCA, PVNA, and PVA)-type (Ikeda et al., 1985). Japanese PCNA trait resides in a single, recessive locus. The crosses between PCNA- and non-PCNA-type yield only non-PCNA F_1 progenies (Ikeda et al., 1985; Yamada and Sato, 2002). PCNA-type offspring are obtainable at about 15% only when a non-PCNA F_1 individual derived from a cross between PCNA-type and non-PCNA-type was backcrossed to a PCNA cultivar or selection (Ikeda et al., 1985; Yamada and Sato, 2002). Zhuang et al. (1990) suggested that *D. kaki* might be an allohexaploid rather than autohexaploid based on the meiotic behavior and relatively high fertility of *D. kaki*. However, Choi et al. (2003) suggested that *D. kaki* might be an autoallohexaploid based on the physical mapping of 45S rDNA by fluorescent in situ hybridization in *D. kaki* and its wild diploid or tetraploid relatives. Additionally, by the segregation of the RFLP markers for the trait of astringency-loss, Kanzaki et al. (2001) believe that *D. kaki* is not allohexaploid, but an autohexaploid or autoallohexaploid, with two allele pairs that may regulate the inheritance of the PCNA trait.

PCNA-type persimmons were thought to have originated uniquely in Japan during a recent era; and there is little diversity among this type (Kanzaki et al., 2000a; Yamada et al., 1993, 1994; Yonemori et al., 2000). Current PCNA breeding programs face serious inbreeding depression problems caused by restricted gene pools for parents, because a few limited number of male cultivars or selections of PCNA-type were repeatedly used as parents (Yamada, 1993; 2005). However, recently, a PCNA cultivar, 'Luo Tian Tian Shi' was found in Luo Tian county of China (Wang, 1983; Wang et al., 1997). Despite having the same characteristics of the Japanese PCNA cultivars (Yamada et al. 1993), the cross between 'Luo Tian Tian Shi' and a Japanese PCNA 'Taishu' segregated non-PCNA-type and PCNA-type individuals in F_1 population (Ikegami et al., 2004), indicating that the Chinese PCNA trait is dominant unlike the Japanese PCNA trait. Hence we hypothesize that PCNA offspring are possible to obtain in the F_1 population derived from cross between 'Luo Tian Tian Shi' and non-PCNA cultivars. In this paper, we tested the hypothesis by crossing 'Luo Tian Tian Shi' with 1) a Japanese PCNA 'Okugosho' to confirm the segregation of both PCNA- and non-PCNA-type individuals, and with 2) the astringent 'Yotsumizo' and 'Iwasedo'. We discussed our hypothesis that the Chinese PCNA is dominant based on our results.

Materials and Methods

The F_1 individuals of Chinese PCNA 'Luo Tian Tian Shi' × Japanese PCNA 'Okugosho' (62 offspring), 'Luo Tian Tian Shi' × Japanese PCA 'Yotsumizo' (21 offspring), and 'Luo Tian Tian Shi' × Japanese PCA 'Iwasedo' (4 offspring), were analyzed for tannin content and tannin cell size. Scions of each seedling were top-grafted on 'Fuyu' or 'Hiratanenashi' trees to promote early bearing, and fruits from each top-grafted F_1 tree were sampled from mid-October to late November in 2004. The mesocarp of one group was diced and the 5-g samples prepared in triplicate were homogenized with 80% methanol and the homogenate centrifuged at 3600 g for 5 min. The supernatant was decanted and pellet was washed with fresh 80% methanol and recentrifuged. The supernatants were made up to 100 mL with 80% methanol. An aliquot was analyzed for tannin concentration with Folin-Ciocalteau 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% gultaraldehyde, containing 0.2% tannic acid. After the blocks were washed with water, they were macerated in a 0.05 M EDTA solution adjusted to pH 10 at 45 °C for 5 h by oscillating at 90 rpm, according to Letham (1960).

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A droplet of tannin cells that separated from the parenchyma cells by decanting several times was placed on a glass slide; images of the tannin cells were taken with a digital camera attached to a light microscope DP-50 (Olympus Corporation, Tokyo, Japan) and recorded on a computer. The area of each 100 tannin cells was measured with Scion Image, public domain software (Scion Corporation, Frederick, Md.).

Results and Discussion

Offspring in the F_1 generation derived from 'Luo Tian Tian Shi' × Japanese PCNA 'Okugosho' segregated into two types with small and large tannin cells, respectively (Fig. 1). Individuals that have small tannin cells and low tannin content were PCNA-type, however, those having large tannin cells and very high tannin concentration were non-PCNA-type. The segregation ratio of PCNA-type to non-PCNA-type was 29:33 in this cross. Our previous report (Ikegami et al., 2004) showed that the segregation of PCNA-type to non-PCNA-type in the F_1 population from the cross between 'Luo Tian Tian Shi' and Japanese PCNA 'Taishu' was 22:12.

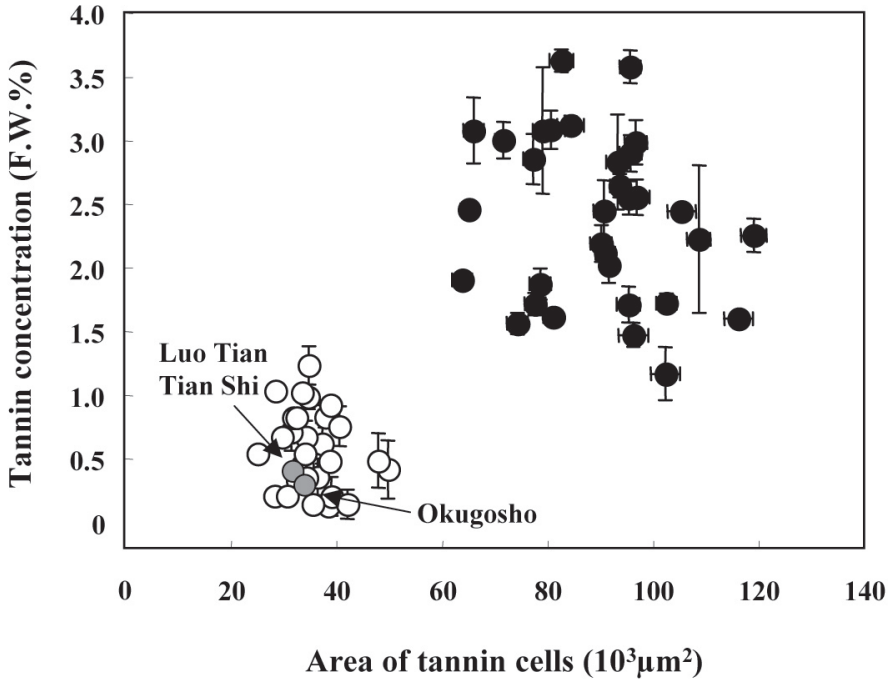

Fig. 1. Distribution plot showing the relationship between the tannin cell size and the tannin concentration in F_1 offspring derived from PCNA 'Luo Tian Tian Shi' × PCNA 'Okugosho'. Each circle indicates F_1 individuals in the progeny and two grey circles indicate the parents. White and black circles indicate PCNA and non-PCNA individuals, respectively. Vertical and horizontal bars represent ±SE (n = 100 for tannin cell size and n = 3 for soluble tannin content).

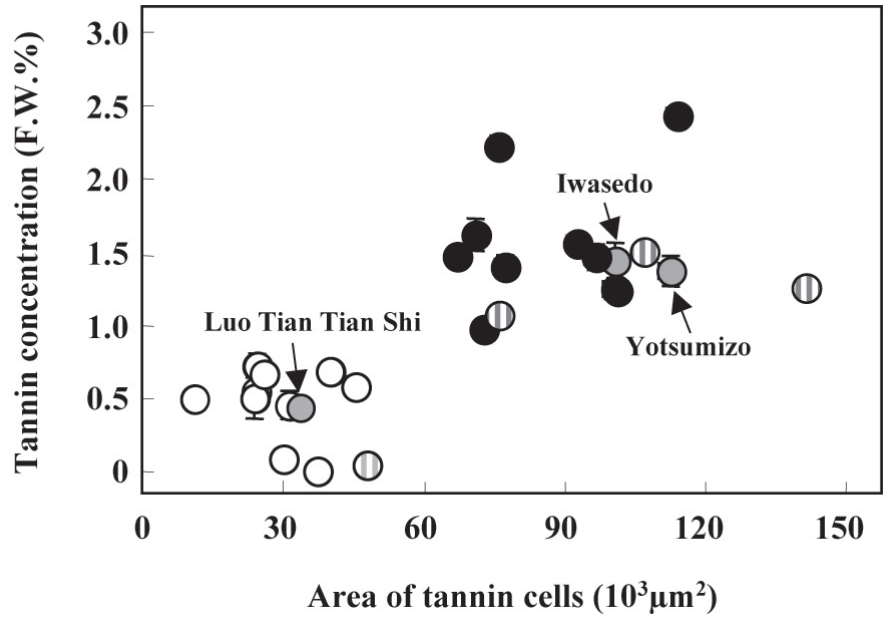

Fig. 2. Distribution plot showing the relationship between the tannin cell size and the tannin concentration in F_1 offspring derived from PCNA 'Luo Tian Tian Shi' × PCA 'Yotsumizo' or 'Iwasedo'. Each circle indicates F_1 individuals in the progeny and three grey circles indicate the parents. White and black circles indicate PCNA and non-PCNA individuals derived from 'Luo Tian Tian Shi' and 'Yotsumizo', respectively. The four striped circles indicate individuals derived from 'Luo Tian Tian Shi' × 'Iwasedo'. Vertical and horizontal bars represent ±SE (n = 100 for tannin cell size and n = 3 for soluble tannin content).

PCNA-type individuals obtained from the crosses between 'Luo Tian Tian Shi' × Japanese PCA 'Yotsumizo' segregated into two populations with either small or large tannin cells (Fig. 2). The segregation ratio of the offspring having small tannin cells (PCNA-type) to large tannin cells (non-PCNA-type) was 11:10. Among the four progenies resulting between 'Luo Tian Tian Shi' × Japanese PCNA 'Iwasedo', one was PCNA-type whereas three were non-PCNA-type. However, we believe the population is too small to determine the segregation ratio.

The recessive Japanese PCNA trait is believed to be the result of a mutation in a non-PCNA-type, so that the PCNA-type persimmon is homozygous for all alleles by conferring the nonastringent trait to the fruit. By contrast, genetic background of 'Luo Tian Tian Shi' seems to be completely different from Japanese PCNA cultivars, whereas the morphological characteristic of tannin cells for termination of the enlargement at an early stage of fruit development is the same (Ikegami et al., 2004). The RFLP analysis of 'Luo Tian Tian Shi' by using molecular markers, which can distinguish Japanese PCNA cultivars from non-PCNA cultivars, demonstrated that 'Luo Tian Tian Shi' has the same RFLP pattern as non-PCNA cultivars, and not as PCNA cultivars (Kanzaki et al., 2000b), which indicates that 'Luo Tian Tian Shi' does not have any mutated alleles linked to the Japanese PCNA trait. Therefore, the mutated locus of Chinese PCNA is different from that of Japanese PCNA.

In our study, the segregation of both PCNA- and non-PCNA-type individuals in F_1, derived from Chinese PCNA 'Luo Tian Tian Shi' × Japanese PCA 'Yotsumizo' indicates that alleles for the PCNA trait are heterozygous in either 'Luo Tian Tian Shi' or 'Yotsumizo'. When the trait of Chinese PCNA is considered dominant, 'Luo Tian Tian Shi' should be heterozygous for this trait. The segregation ratios of PCNA to non-PCNA offspring obtained in our studies were 22:12 in the population of 'Luo Tian Tian Shi' × 'Taishu'; 29:33 in the population of 'Luo Tian Tian Shi' × 'Okugosho'; and 11:10 in the F_1 of 'Luo Tian Tian Shi' × 'Yotsumizo'. Therefore, the most reasonable segregation ratio is 1:1 according to the Chi-square test (Table 1). With the result of 'Luo Tian Tian Shi' × 'Taishu', the most probable segregation ratio was 3:1 due to Chi-square test ($P = 0.165$). The segregation ratio in this cross may deviate from expected result because of the small number of individuals. On the other hand, if 'Luo Tian Tian Shi' is assumed homozygous and mutated allele for the PCNA

Table 1. Expected segregation ratio and Chi-square test in F_1 of 'Luo Tian Tian Shi' × Japanese PCNA or PCA cultivars.

Crosses (segregation ratio)	Expected segregation ratio (PCNA to non-PCNA)	χ^2 value	*P*
'Luo Tian Tian Shi' × 'Taishu'			
(PCNA to non-PCNA, 22:12)	1:1	2.94	0.086
	3:1	1.92	0.165
'Luo Tian Tian Shi' × 'Okugosho'			
(PCNA to non-PCNA, 29:33)	1:1	0.26	0.611
	3:1	18.34	2.86E-07
'Luo Tian Tian Shi' × 'Yotsumizo'			
(PCNA to non-PCNA, 11:10)	1:1	0.05	0.827
	3:1	5.73	0.017

Table 2. Expected segregation ratio and their genotypes by assuming that 'Luo Tian Tian Shi' was heterozygous and the Japanese cultivar was homozygous for the mutated alleles.

Types of polyploidy	Expected genotypes	Expected segregation ratios (PCNA to non-PCNA)
Allohexaploid	Aa nn nn × aa nn nn[z]	1:1
	Aa aa nn × aa aa nn	1:1
	Aa Aa nn × aa aa nn	3:1
	Aa aa aa × aa aa aa	1:1
	Aa Aa aa × aa aa aa	3:1
	Aa Aa Aa × aa aa aa	7:1
Autoallohexaploid	Aa nnnn × aa nnnn	1:1
	Aaaa nn × aaaa nn	1:1
	AAaa nn × aaaa nn	5:1
	Aa aaaa × aa aaaa	1:1
	Aaaa aa × aaaa aa	1:1
	Aaaa Aa × aaaa aa	3:1
	AAaa Aa × aaaa aa	11:1
Autohexaploid	Aaaaaa × aaaaaa	1:1
	AAaaaa × aaaaaa	4:1
	AAAaaa × aaaaaa	19:1

[z]n = a null allele.

trait is recessive, both Japanese PCNA and non-PCNA cultivars should be heterozygous. However, in this case, a certain PCNA offspring must be yielded when a Japanese PCNA cultivar is crossed with a non-PCNA cultivar or when a non-PCNA cultivar is crossed with a non-PCNA cultivar. According to the previous reports (Ikeda et al., 1985; Yamada and Sato 2002), only a few PCNA offspring could obtain through mistakes by crossings Japanese PCNA with non-PCNA cultivars; no PCNA progeny has been obtained in a cross between Japanese PCNA 'Taishu' and PVA 'Kurokuma' in our study (Ikegami et al., 2004). Moreover, no PCNA offspring were derived from crosses between PCNA cultivars and PCA 'Yotsumizo' (unpublished data). Therefore, the hypothesis that the trait of Chinese PCNA is recessive, and that Japanese PCNA or non-PCNA cultivars is heterozygous, should be rejected. The trait of Chinese PCNA should be dominant and 'Luo Tian Tian Shi' should be heterozygous for the trait. We showed the expected segregation ratios with possible polyploidy types when the trait of Chinese PCNA is dominant and the mutated alleles are heterozygous in 'Luo Tian Tian Shi'(Table 2). Choi et al. (2003) has suggested that *D. kaki* might be an autoallohexaploid, whereas Kanzaki et al. (2001) believed that *D. kaki* is likewise, an autoallohexaploid or autohexaploid. 'Luo Tian Tian Shi' should have one dominant allele for the trait and so that the most probable genotype of 'Luo Tian Tian Shi' is ''Aaaa nn'' in the case of autoallohexaploid.

In conclusion, 'Luo Tian Tian Shi' can be a good gene resource for breeding new PCNA cultivar because half of the hybrids resulting from a cross between 'Luo Tian Tian Shi' and non-PCNA cultivars will segregate into a PCNA-type. The use of this Chinese PCNA will overcome inbreeding depression by introducing many useful, wide-ranged traits of a non-PCNA-type cultivar.

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