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Varietal differences in sweetness value and flesh juiciness among persimmon cultivars

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Abstract: To promote persimmon breeding project, we analyzed the sugar composition (a ratio of sucrose to hexose sugars, SH ratio) and flesh juiciness of 43 persimmon cultivars (*Diospyros kaki* Thunb.) consisting of 24 pollination-constant non-astringent (PCNA)-types and 19 non-PCNA-types, together with other fruit quality traits. The cultivar collection includes newly-released cultivars after 1990 and commercially-produced local cultivars in Japan. These cultivars were broadly classified into three types: sucrose accumulators, intermediate accumulators, hexose accumulators. Analysis of variance showed that the genotypic effect on the SH ratio and flesh juiciness is high with negligibly small environmental variance, indicating that SH ratio and flesh juiciness can be determined by a one-year trial without tree replication. Highly varietal diversity in the SH ratio and flesh juiciness was observed within and between persimmon cultivar types. Sweetness value (SSC × SH ratio) of the cultivars/selections seems to be a useful predictor of fruit sweetness. In terms of palatability, however, persimmon cultivar's improvement should be performed on the sweetness value in association with flesh juiciness.

1. Introduction

Persimmon (*Diospyros kaki* Thunb.) is believed to have originated in Eastern Asia, is produced worldwide including in Azerbaijan, Brazil, China, Iran, Israel, Italy, Japan, Korea, New Zealand, and Spain (FAOSTAT, 2017). A number of local varieties has been developed in China, Korea, and Japan during a long history of domestication (Parfitt *et al.*, 2015; Sato and Yamada, 2016; Yesiloglu *et al.*, 2018). Persimmon cultivars can be classified into four types: pollination-constant astringent (PCA); pollination-variant astringent (PVA); pollination-variant non-astringent (PVNA); pollination-constant non-astringent (PCNA), based on seed formation, change in flesh color, and nature of astringency loss (Hume, 1914; Ikeda *et al.*, 1985; Yonemori *et al.*, 2000). Among these types, fruits of PCA- and PVA-type cultivars are always astringent without postharvest treatment such as application of carbon dioxide gas or ethanol vapor. PVNA-type cultivars require pollination and seed formation to lose astringency as well as flesh

browning. Fruits of PCNA-type cultivars naturally lose their astringency because they terminate accumulating tannins at early fruit development stage. In particular, PCNA-type persimmon cultivars are highly desired for fresh consumption on a worldwide level.

Regarding eating quality of PCNA-type cultivar, the great stress is laid upon the evaluation of soluble solids content (SSC, °Brix) using refractometer because sugars generally contribute a very large proportion to the SSC in the ripening stage (reviewed by Giordani *et al*., 2011). However, Ban *et al*. (2010) and Mitani *et al.* (2015) postulated that flesh juiciness is a decisieve parameter to determine the texture of persimmon fruit, where fruits with higher firmness had a tendency to be less juicy as observed in apple (Harker *et al*., 2003). On the other hand, the percentage sucrose in flesh significantly affects the sweetness of fruit, such as East Asian pear (Kajiura *et al*., 1979), oriental melon (Zhang and Li, 2005), peach (Suzuki *et al.*, 1990; Cirilli *et al.*, 2016) and strawberry (Sone *et al*., 2000). Thus, understanding how varietal differences influence sugar composition will provide valuable information for genetic improvement of the palatability of persimmon fruit in future breeding programs. Giordani *et al*. (2011) classified worldwide persimmon cultivars such as 'Atago', 'Fuyu', 'Rojo Brillante' and 'Triumph' into three groups based on the cluster analysis of sugar composition. To date, however, there has been no comprehensive research on the sugar composition of the recent Japanese PCNA- and non-PCNA-type cultivars. Since 1990, new PCNA- and non-PCNA-type persimmon cultivar have been released in Japan by the National Institute of Fruit Tree Science (Yakushiji and Nakatsuka, 2007; Yamada *et al*., 2012 a, b). These new cultivars have large sized fruit, brilliant skin color, and are highly palatable, but their sugar composition remains largely unknown.

Asakuma and Shiraishi (2017) showed that genotypic effect of sugar composition (SH ratio) and flesh juiciness is significantly high, whereas the year, genotype × year interaction, among trees within genotype, and tree × year interaction is small or negligible. In experimental field of the current study, we have preserved a Japanese persimmon cultivar collection of both PCNA- and non-PCNA-type cultivars as breeding stocks since 1980. Although the total number of preserved cultivars is less than 50, the cultivar collection includes newly-released cultivars after 1990 and commercially-produced local cultivars in Japan. In the present study, we (1) re-confirmed small year variability in the sugar composition and flesh juiciness by the analysis of variance, and (2) discussed varietal differences in the sugar composition and flesh juiciness among persimmon cultivars in association with palatability.

2. Materials and Methods

Plant materials for yearly variation of sugar composition

Four PCNA-type cultivars of Japanese persimmon ('Akiou', 'Fuyu', 'Matsumotowase-Fuyu', 'Taishu') were used. These cultivars are grown for commercially marketable fruit production by normal cultural practices, including pruning, flower and fruit thinning, irrigation, soil and pest management (Yamada, 2006) in an open-field of the Fukuoka Agricultural and Forestry Research Center, Fukuoka, Japan (33°50′ N and 130°57′ E). According to the reference value derived from our annual survey from 2008 to 2015 (Table S1), mature representative eight fruits per tree were sampled from three trees per genotype for late-October to late-November in 2014 to 2016 seasons depending on each cultivar's optimal ripening time and skin color.

The statistical fixed-effect model (Table S2) that we adopted to express the phenotypic value (Asakuma and Shiraishi, 2017) is:

$$P_{ijkc} = m + G_i + Y_k + (GY_{ik}) + T_{ij} + (TY_{ijk}) + E_{ijkc}$$

where P_{ijkc} is the phenotypic value of the *c*th fruit of the *j*th tree of the *i*th genotype in the *k*th year; *m* is the overall mean; G_i is an effect contributed by the *i*th genotype; Y_k is an effect of the *k*th year; GY_{ik} is the interaction between the *i*th genotype and the *k*th year; T_{ij} is an effect of the *j*th tree of the *i*th genotype; TY_{ijk} is the interaction between the *j*th tree of the *i*th genotype and the *k*th year; and E_{ijkc} is an effect of the *c*th fruit of the *j*th tree of the *i*th genotype in the *k*th year. ANOVA provided the variance associated with genotype (σ_g^2), among years (σ_y^2), genotype × year interaction (σ_{gy}^2), among trees within genotypes (σ_t^2), tree × year interaction (σ_{ty}^2) and among fruits within tree (σ^2).

Plant materials for varietal difference in sugar composition

A total of 43 of Japanese persimmon cultivars consisting of 23 PCNA-types (Fig. S1), 9 PVNA-types (Fig. S2), 6 PVA-types (Fig. S3), and 5 PCA-types (Fig. S4) was analyzed in 2016. Cultural practices were con-

formed to *Plant materials for yearly variation of sugar composition*. Depending on the optimal ripening time of the cultivar, five to eight mature fruits per one-tree of each cultivar were harvested from late-September to early-December in 2016 season according to the reference values based on above-described cultivation records (Table S1). Classification of fruit ripening time was performed in accordance with the definitions by Yamada *et al.* (1995). Fruit shape index, FSI (longitudinal diameter/transverse diameter) was defined according to Maeda *et al.* (2018) with slight modifications (Fig. S5). Cracking of apex and calyx-end of fruit was examined by UPOV guideline (UPOV, 2004). Astringency removal of PCA- and PVA-type cultivars was performed by treatment with either ethanol vapor or carbon dioxide gas depending on the cultivar (Yamada, 2006). The sampled fruit was weighed, and each fruit was horizontally cut to measure fruit skin color, flesh firmness, flesh juiciness, soluble solids content (SSC, °Brix), and sugar composition.

Analysis of fruit quality traits

Fruit skin color, flesh firmness, flesh juiciness, soluble solids content, and sugar composition was measured according to Asakuma and Shiraishi (2017). Fruit skin color around fruit apex was measured using a chromameter (CR-300, Minoruta, Tokyo, Japan), and expressed as the value of color chart following formula:

$$\text{Color chart (CC)} = -9.485 \text{ Ln (hue angle)} + 44.503, R^2 = 0.9278.$$

Flesh firmness (kg) was determined by a handheld universal pressure tester with a 5.0-mm-diameter ×10.0-mm-height columnar plunger (KM-5, FUJIWARA SCIENTIFIC, Tokyo, Japan). Fifteen to 20 g of peeled flesh was weighed and wrapped in one layer of medical gauze. After hand-pressing (only one press) for 15 s, the squeezed juice (flesh juiciness) was measured using a 25-mL mess cylinder and expressed as mL g^{-1} FW. Soluble solids content (SSC) of the resulting juice samples was determined as the °Brix value using a portable calibrated electronic refractometer (PAL-1, Atago, Tokyo, Japan).

For the analysis of sugar composition, 8 to 10 g of peeled flesh was weighed and transferred to a 50-mL heat-tolerant tube and partially screw-capped. The flesh sample was immediately microwave-irradiated at 730 W for 60 s before extracting the sugars. The irradiated sample was ground in a laboratory blender with ~40 mL deionized water. The puree was centrifuged at 5000 × *g* at 25°C for 10 min. The resulting supernatant was brought to 50 mL with deionized water and filtered through a 0.45-µm filter. Sugar composition was analyzed using a HPLC (LC-10A, Shimadzu, Kyoto, Japan) consisting of a SCL-10A system controller, LC-10AD pumps, a CTO-10A column oven, and a RID-10A refractive index detector. The column (SCR-101N, 7.9 × 300 mm, Shimadzu, Kyoto, Japan) was operated at 60°C with 0.8 mL min^{-1} of water. The injection volume was 10 to 20 µL.

3. Results and Discussion

Variation in sugar composition

HPLC profiles of sugar composition were obtained from persimmon cultivars examined with three major peaks assigned as sucrose, glucose, and fructose, respectively, thereby expressing as the SH ratio, a ratio of sucrose to hexose sugars (Hirano *et al.*, 1995). As shown in figure 1, we classified persimmon cultivars into three types according to Zheng and Sugiura (1990) with slight modification; sucrose accumulators (percentage sucrose ≥55.1%, SH ratio≥ 1.23), intermediate accumulators (percentage sucrose in 45.0 to 55.0%, SH ratio in 0.82 to 1.22), hexose accumulators (percentage sucrose ≤44.9%, SH ratio ≤0.81). Previous studies have shown that the

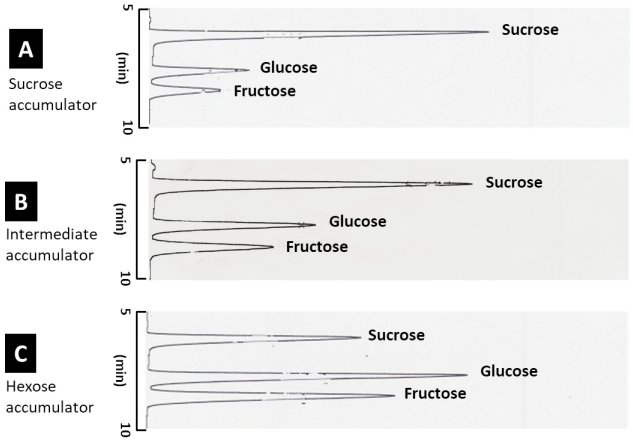

Fig. 1 - Sugar composition of persimmon cultivars from the HPLC analysis. A: Sucrose accumulators ['Fuyu (PCNA-type)', 'Akagaki (PVNA-type)', 'Atago (PCA-type)']; B: Intermediate accumulator ['Maekawajiro (PCNA-type)', 'Saefuji (PVNA-type)', 'Aizumishirazu (PVA-type)']; C: Hexose accumulators ['Soshu (PCNA-type)', 'Nishimurawase (PVNA-type)', 'Hiratanenashi (PVA-type)'] PCA: pollination-constant astringent PVA: pollination-variant astringent PVNA: pollination-variant non-astringent PCNA: pollination-constant non-astringent.

sucrose percentage in persimmon cultivar ranged between 12 and 70%, resulting in varietal difference in the sugar composition or SH ratio (Tsuji and Komiyama, 1987; Zheng and Sugiura, 1990; Hirano *et al.*, 1995; Hirai *et al.*, 2004; Suzuki *et al*., 2010; Asakuma and Shiraishi, 2017). Sugar profiles of PCNA- and non-PCNA-types from well-known cultivars such as 'Atago' (sucrose accumulator), 'Hiratanenashi' (hexose accumulator), 'Soshu' (hexose accumulator) and 'Fuyu' (sucrose accumulator) in previous reports are in agreement with those in the present study.

Table 1 shows highly varietal difference in the SH ratio among the PCNA-type cultivars, especially for Mid- to Late-Oct. ripening ones. In cultivars as sucrose accumulators, large amounts of sucrose recorded in 'Sodawase (SH ratio = 3.29)' followed by 'Okugosho (SH ratio = 2.35)', 'Hanagosho (SH ratio = 2.17)', 'Suruga (SH ratio = 2.02)', 'Fuyu (SH ratio = 1.93)', and 'Shinshu (SH ratio = 1.89)'. In the hexose accumulator, large amounts of hexose were present in 'Kishu (SH ratio = 0.21)', 'Soshu (SH ratio = 0.23)', 'Izu (SH ratio = 0.35)', followed by 'Tenjingosho (SH ratio = 0.61)', 'Taishu (SH ratio = 0.64)' and 'Taiga (SH ratio = 0.67)'. Among the remainders, sucrose and hexose seemed to be accumulated approximately equal amounts with 1.10 in SH ratio ('Maekawajiro'), 1.03 in SH ratio ('Misatogosho'), and 0.96 in SH ratio ('Reigyoku').

Table 1 - Sugar composition of pollination-constant non-astringent (PCNA) type of Japanese persimmon cultivars in 2016

Fruit ripening time/ cultivar or selection	Sugar composition (g 100 g^{-1} FW)				Sugar composition (%)			Type of sugar accumulation	SH ratio	Sweetness value	Flesh juiciness (mL g^{-1} FW)
	Sucrose	Glucose	Fructose	Total	Sucrose	Glucose	Fructose				
Early-Oct.											
Soushu	1.98	4.96	4.60	11.5	17.2	43.0	39.9	Hexose	0.21	3.1	0.27
Mid-Oct.											
Izu	3.03	4.53	4.22	11,8	25.7	38.5	35.8	Hexose	0.35	5.3	0.29
Shinshuu	9.32	2.62	2.32	14.3	65.4	18.4	16.3	Sucrose	1.89	32.3	0.22
Late-Oct.											
Sodawase	11.66	1.90	1.64	15.2	76.7	12.5	10.8	Sucrose	3.29	58.9	0.27
Reigyoku	6.44	3.59	3.12	13.2	49.0	27.3	23.7	Intermediate	0.96	16.1	0.36
Taiga	5.25	4.21	3.68	13.1	40.0	32.0	28.0	Hexose	0.67	11.1	0.32
Kanshu	5.93	3.87	3.48	13.3	44.7	29.1	26.2	Hexose	0.81	13.2	0.17
Kishu	2.66	5.90	5.56	14.1	18.8	41.8	39.4	Hexose	0.23	3.4	0.24
Taishu	5.30	4.52	3.76	13.6	39.0	33.3	27.7	Hexose	0.64	10.1	0.35
Maekawajiro	6.83	3.48	2.71	13.0	52.5	26.7	20.8	Intermediate	1.10	18.2	0.22
Tenjingosho	6.00	5.33	4.46	15.8	38.0	33.8	28.2	Hexose	0.61	10,5	0.17
Early-Nov.											
Akiou	9.14	3.40	2.68	15.2	60.1	22.3	17.6	Sucrose	1.57	27.6	0.38
Misatogosho	7.37	4.18	2.96	14.5	50.8	28.8	20.4	Intermediate	1.03	18.6	0.23
Uenishiwase	7.63	3.35	2.48	13.5	56.7	24.9	18.4	Sucrose	1.31	20.8	0.13
Matsumotowase-Fuyu	9.16	2.85	2.41	14.4	63.5	19.8	16.7	Sucrose	1.74	26.8	0.27
Mid-Nov.											
Mushirodagosho	5.53	3.77	3.57	12.9	43.0	29.3	27.7	Hexose	0.75	10.8	0.33
Youhou	5.99	4.17	4.11	14.3	42.0	29.2	28.8	Hexose	0.72	12.2	0.18
Late-Nov.											
Fuyu	9.79	2.75	2.32	14.9	65.9	18.5	15.6	Sucrose	1.93	31.7	0.27
Okitsu-20 (Ro-19)	8.64	3.61	3.06	15.3	56.4	23.6	20.0	Sucrose	1.30	25.5	0.31
Taiho	7.92	3.50	2.88	14.3	55.4	24.5	20.1	Sucrose	1.24	21.2	0.36
Early-Dec.											
Okugosho	9.43	2.14	1.88	13.5	70.1	15.9	14.0	Sucrose	2.35	43.0	0.24
Suruga	8.86	2.26	2.13	13.3	66.9	17.1	16.1	Sucrose	2.02	35.4	0.26
Hanagosho	9.03	2.20	1.96	13.2	68.5	16.7	14.9	Sucrose	2.17	37.1	0.25

Fruit ripening time (*Late-Sep., Early- to Late-Oct., Early- to Late-Nov., Early-Dec.*) was classified according to Yamada *et al.* (1995).
SH ratio= sucrose [g 100 g^{-1} FW] / hexoses (glucose + fructose) [g 100 g^{-1} FW].
Sweetness value= soluble solids content (reference value in Table S1) x SH ratio.

In the PVNA-type cultivars (Table 2), 'Nishimurawase' (SH = 0.47) was hexose accumulator, whereas the sucrose accumulators had SH ratios, varing from 1.83 ('Akagaki') to 2.77 ('Rendaiji'). The SH ratio of intermediate accumulators ranged from 0.86 ('Fudegaki') to 1.22 ('Zenjimaru'). PCA-type cultivars (Table 3) were all sucrose accumulators, except for 'Kawazokogaki' in SH ratio of 1.12 (intermediate accumulator). Conversely, most PVA-type cultivars (Table 3) could be classified as hexose accumulators, with SH ratios ranging from 0.39 ('Tonewase') to 0.67 ('Hiratanenashi') with the exception of 'Koshu-

Table 2 - Sugar composition and other fruit traits of pollination-variant non-astringent (PVNA) type of Japanese persimmon cultivars in 2016

Fruit ripening time/ cultivar or selection	Sugar composition (g 100 g^{-1} FW)				Sugar composition (%)			Type of sugar accumulation	SH ratio	Sweetness value	Flesh juiciness (mL g^{-1} FW)
	Sucrose	Glucose	Fructose	Total	Sucrose	Glucose	Fructose				
Late-Sept.											
Nishimurawase	3.47	3.22	4.16	10.9	32.0	29.7	38.3	Hexose	0.47	7.1	0.16
Early-Oct.											
Akagaki	8.13	2.24	2.2	12.6	64.7	17.8	17.5	Sucrose	1.83	29.3	0.20
Mid-Oct.											
Fudegaki	5.45	3.29	3.06	11.8	46.2	27.9	25.9	Intermediate	0.86	14.0	0.21
Ganzan	8.56	2.38	2.11	13.1	65.6	18.2	16.2	Sucrose	1.91	31.1	0.18
Oomiyawase	10.17	1.90	1.83	13.9	73.2	13.7	13.2	Sucrose	2.73	46.1	0.20
Saefuji	7.12	3.93	3.55	14.6	48.8	26.9	24.3	Intermediate	0.95	16.4	0.28
Late-Oct.											
Rendaiji	8.45	1.58	1.47	11.5	73.5	13.7	12.8	Sucrose	2.77	41.0	0.20
Early-Nov.											
Zenjimaru	8.05	3.41	3.20	14.7	54.9	23.3	21.8	Intermediate	1.22	21.5	0.25
Early-Dec.											
Shogatsu	9.84	1.90	1.80	13.5	72.7	14.0	13.3	Sucrose	2.66	46.3	0.19

Fruit ripening time (*Late-Sep., Early- to Late-Oct., Early- to Late-Nov., Early-Dec.*) was classified according to Yamada *et al.* (1995).
SH ratio= sucrose [g 100 g^{-1} FW] / hexoses (glucose + fructose) [g 100 g^{-1} FW].
Sweetness value= soluble solids content (reference value in Table 1S) x SH ratio.

Table 3 - Sugar composition of pollination-constant astringent (PCA) and pollination-variant astringent (PVA) type of Japanese

Fruit ripening time /cultivar or selection	Type of astringency	Sugar composition (g 100 g^{-1} FW)				Sugar composition (%)			Type of sugar accumulation	SH ratio	Sweetness value	Flesh juiciness (mL g^{-1} FW)
		Sucrose	Glucose	Fructose	Total	Sucrose	Glucose	Fructose				
Mid-Oct.												
Ichidagaki	PCA	10.43	3.59	3.13	17.1	60.8	20.9	18.2	Sucrose	1.55	30.5	0.35
Tonewase	PVA	3.77	5.13	4.48	13.4	28.2	38.3	33.5	Hexose	0.39	5.9	0.25
Late-Oct.												
Saijo	PCA	8.69	2.12	1.97	12.8	68.0	16.6	15.4	Sucrose	2.12	34.8	0.28
Kawazokogaki	PCA	7.80	3.80	3.15	14.8	52.9	25.8	21.4	Intermediate	1.12	18.1	0.31
Early-Nov.												
Hiratanenashi	PVA	5.54	4.50	3.81	13.8	40.0	32.5	27.5	Hexose	0.67	11.3	0.22
Koshuhyakume	PVA	9.62	2.18	1.88	13.7	70.3	15.9	13.7	Sucrose	2.37	41.0	0.31
Hagakushi	PCA	8.56	2.32	2.34	13.2	64.8	17.5	17.7	Sucrose	1.84	32.4	0.26
Taigetsu	PVA	4.34	4.74	4.25	13.3	32.6	35.6	31.9	Hexose	0.48	7.5	0.43
Taiten	PVA	5.93	3.80	2.61	12.3	48.1	30.8	21.2	Intermediate	0.93	15.7	0.46
Mid-Nov.												
Aizumishirazu	PVA	5.85	3.52	3.40	12.8	45.8	27.6	26.6	Intermediate	0.85	12.7	0.21
Atago	PCA	8.15	2.02	1.78	12.0	68.2	16.9	14.9	Sucrose	2.14	33.8	0.23

Fruit ripening time (*Late-Sep., Early- to Late-Oct., Early- to Late-Nov., Early-Dec.*) was classified according to Yamada *et al.* (1995).
SH ratio= sucrose [g 100 g^{-1} FW] / hexoses (glucose + fructose) [g 100 g^{-1} FW].
Sweetness value= soluble solids content (reference value in Table 1S) x SH ratio.

hyakume' (SH = 2.37), which was classified as a sucrose accumulator. 'Taiten' was an intermediate accumulator in SH = 0.93.

Despite the small number of cultivars examined in the present study, there are no general relationships between astringency type (PCNA, PVNA, PVA, and PCA) and sugar accumulation type. In terms of biochemical consideration, varietal difference in the SH ratio can be explained by the degree of sucrose cleavage due to the activity of vacuolar acid invertase (Hirai *et al.*, 1986; Zheng and Sugiura, 1990). Furthermore, recent transcriptional studies on the sugar accumulation-related key genes postulated that varietal differences in the SH ratio may be resulted from the balance between sucrose synthase and vacuolar acid invertase activities in persimmon fruit (Suzuki *et al.*, 2010; Shiraishi and Asakuma, 2019). To date, the genetic mechanisms controlling sucrose accumulation in persimmon fruit remain unclear. However, we hypothesize that dominance of sucrose accumulation over hexose accumulation in persimmon fruit on the basis of our ongoing breeding program (unpublished data).

Environmental variance in sugar composition, flesh juiciness and SSC

Table 4 shows the contribution of variance from each trait to the total variance. The variance of genotype (σ_g^2) was high for SH ratio in 66.9% and flesh juiciness in 61.7%. As a whole, the variance of year (σ_y^2), among trees within genotype (σ_t^2), genotype × year (σ_{gy}^2) and tree × year (σ_{ty}^2) interactions were small or negligible, varying from 0.0 to 6.6% of the total variance. Similar to previous report (Asakuma and Shiraishi, 2017), the present results indicated that adding year or tree replications will not be efficient in reducing the environmental variance for SH ratio and flesh juiciness. Mitani *et al.* (2015) also showed that genotypic effect of flesh juiciness is significantly high. It is thus considered that the genotypic effect on the SH ratio and flesh juiciness is high with negligibly small environmental variance, and that these traits can be determined by a one-year trial without tree replication. In contrast, σ_g^2 of SSC was small in 14.6%, followed by σ_y^2 in 12.5% and σ_{gy}^2 in 8.8%. Other variance components, σ_t^2 and σ_{ty}^2 of SSC were small or negligible with 1.5 and 4.2%, respectively. Furthermore, the ratio of $\sigma_t^2/(\sigma_y^2 + \sigma_{gy}^2)$ is calculated as 0.07. If the ratio exceeds 1.0, tree replications should be required. However, our results indicate that repeated yearly measurements are more efficient than replicated trees to estimate the genetic variance of SSC as observed in grape (Sato *et al.*, 2000) and persimmon (Yamada *et al.*, 1993)

Using variance components in Table 4, the error variance (σ_E^2) of each trait can be obtained by the following equation (cf. Yamada *et al.*, 1993):

$$(\sigma_y^2/3) + (\sigma_{gy}^2/3) + (\sigma_t^2/3) + \{\sigma_{ty}^2/(3\times3)\} + \{\sigma^2/(3\times3\times8)\}.$$

The σ_E^2 of SH ratio, flesh juiciness, and SSC is 0.01366, 0.00014, and 0.16003, respectively. Broad-sense heritability ($\sigma_g^2/\{\sigma_g^2+\sigma_E^2\}$) results in high for SH ratio in 0.95 and flesh juiciness in 0.97, whereas low for SSC in 0.63. In general, a high broad-sense heritability means that most of the variation among genotypes is caused by genetic variation and not environmental variation. Knowing the heritability can be of value when the breeder will make an effective selection. In this study, the high heritability of SH ratio and flesh juiciness is useful to discriminate genetic sweetness and juiciness of persimmon fruit, respectively. Yamada *et al.* (1993) elucidated that an increase in yearly repetition instead of tree replications substantially reduced σ_E^2 in the measurements for SSC and fruit weight to clarify the genetic properties of genotypes. In the present study, SSC of each

Table 4 - Estimates of variance component and their percentage to total variance obtained from the analysis of variance

Variance components	SH ratio	Flesh juiciness	Solube solids content (SSC)
σg^2 (genotype)	0.2636 (66.9%)	0.0052 (61.7%)	0.2725 (14.6%)
σy^2 (year)	0.0260 (6.6%)	0.0000 (0.0%)	0.2326 (12.5%)
σgy^2(genotype× year)	0.0000 (0.0%)	0.0003 (3.5%)	0.1642 (8.8%)
σt^2(among trees within genotype)	0.0062 (1.6%)	0.0000 (0.0%)	0.0285 (1.5%)
σty^2(tree × year)	0.0161 (4.1%)	0.0000 (0.0%)	0.0791 (4.2%)
σ^2(among fruit within tree)	0.0819 (20.8%)	0.0029 (34.8%)	1.0869 (58.3%)

Negative value was assumed to be zero
SH ratio= sucrose/hexoses (glucose + fructose)

cultivar/selection was evaluated as the reference value based on one tree with more than five years field trials (Table S1).

Varietal difference in sweetness value and flesh juiciness

Sugars represent a crucial component of fruit edible quality, principally conferring sweetness, one of the main attributes influencing the degree of consumer acceptance. The ratio of constitutive sugars determines the sweetness of fruits; the higher the sucrose percentage, the stronger the organoleptic perception of sweetness in Asian pear (Kajiura *et al.*, 1979), oriental melon (Zhang and Li, 2005), peach (Cirilli *et al.*, 2016) and strawberry (Sone *et al.*, 2000). In this study, we proposed a new index entitled "sweetness value" evaluating fruit sweetness by the equation: SSC × SH ratio. In place of Table S3, SSC value in Table S1 was used for calculation of sweetness value because of the above-described environmental error. As shown in Tables 1-3, the sweetness value varied due to the propotional level of sucrose content, ranging from 3.1 to 58.9 in PCNA-, 7.1 to 46.3 in PVNA-, and 5.9 to 41.0 in PVA- and PCA-type cultivars. Corresponding to sugar accumulation type, hexose accumulators exhibited lower sweetness value in 3.1 ('Soushu') to 11.1 ('Taiga'), whereas sweetness values of sucrose accumulators were higher in 20.8 ('Uenishiwase') to 58.9 ('Sodawase'). These results indicate that sweetness value seems to be a useful predictor of fruit sweetness in persimmon genotype. In our previous sensory tests (Asakuma and Shiraishi, 2017), the less-sweet genotypes exhibited SH ratios below 0.3, while highly-sweet genotypes had SH ratios exceeding 1.0. Given that the SSC of genotype is around 16 (average value in 16.6 of 43 persimmon cultivars/selections in Table S1), sweetness value of highly- and less-sweet genotype is expected as exceeding 16 and below 4.8, respectively. For instance, its sensory sweetness of 'Kishu' has been evaluated to be lower than that of 'Fuyu' by several persimmon breeders and growers, although 'Kishu' fruit has normally around 16 in SSC, which is comparable to 'Fuyu' (Yamada *et al.*, 2009). In fact, sweetness value of 'Kishu' was 3.4 in contrast to that of 'Fuyu' in 31.7 (Table 2), which is in agreement with above-mentioned sensory sweetness.

However, there can be inconsistencies when evaluating the eating quality between sweetness scores and cultivars having different flesh juiciness, particularly less juice content (data not shown). In the present study, highly varietal difference in flesh juiciness was observed, ranging from 0.13 ('Uenishiwase') to 0.38 ('Akiou') mL g^{-1} FW of PCNA type (Table 2), 0.16 ('Nishimurawase') to 0.28 ('Saefuji') mL g^{-1} FW of PVNA type (Table 3), and 0.21 ('Aizumishirazu') to 0.46 ('Taiten') mL g^{-1} FW of PVA and PCA type (Table 4). In general, the harder the flesh of fruits, the more chewing is required to breakdown the tissue and the longer it takes to release the juice (Harker *et al.*, 2003). In peach (Suzuki *et al.*, 1990), sweet cherry (Dever *et al.*, 1996), and carrot (Horie and Hiramoto, 2009), flesh sweetness is significantly promoted by high juiciness. Similarly, Ban *et al.* (2010) and Mitani *et al.* (2015) revealed that flesh juiciness is considered to be crucial mouth-feel attribute in the overall taste of persimmon fruit. Asakuma and Shiraishi (2017) proposed that the new descriptor of flesh juiciness of persimmon fruit as "very juicy" (≥0.30 mL g^{-1} FW), "juicy" (0.21-0.29 mL g^{-1} FW) and "slightly juicy" (≤0.20 mL g^{-1} FW) based on the sensory juiciness. From this perspective, three sucrose accumulating PCNA-type cultivars/selections ('Akiou', 'Okitsu 20', and 'Taiho') are considered to be promising breeding stocks because of the high sweetness value (21.2 to 27.6) and high juiciness (0.31 to 0.38 mL g^{-1} FW) together with large fruit size and brilliant fruit color (Tables S1 and S3). Thus, in terms of palatability, persimmon cultivar's improvement will be effectively performed using a combination of sweetness value and flesh juiciness.

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