

Research Paper

Quantitative characterization of fruit shape and its differentiation pattern in diverse persimmon (*Diospyros kaki*) cultivars

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ABSTRACT

There is considerable diversity in fruit shapes among persimmon (*Diospyros kaki*) cultivars. Although fruit shape is one of the most important traits affecting the commercial quality of fruits, quantitatively characterizing fruit shape features remains difficult. The objective of this study was to develop a reliable method for quantitatively analyzing persimmon fruit shapes, and to clarify the fruit development patterns as well as their relationships to the shapes of other organs across cultivars. Using 153 persimmon cultivars and two wild *Diospyros* relatives, we evaluated two-dimensional pictures of longitudinal and transverse fruit sections with elliptical Fourier descriptors (EFDs) and the SHAPE program. Principal component analysis (PCA) using each EFD generated representative vectors, which elucidated the fruit shape diversity among the cultivars. Additionally, a PCA of the EFDs quantified two major components of the variability in the shapes of longitudinal fruit sections, namely the ratio of length to diameter and the fruit apex shape. Seasonal characterizations of the first principal component revealed the fruit shape development patterns. The fundamental shapes in the longitudinal direction were coordinately determined during the early developmental stages across cultivars. Furthermore, a quantitative characterization of persimmon fruit, seed, and leaf shapes unveiled correlations between these traits. The data presented herein indicate that combining EFDs and PCA enables the quantitative analysis of persimmon organ shapes, which may be related to various quantitative indices.

1. Introduction

Among horticultural crops, traits related to the external appearance of fruits, including size, color, and shape, are important considerations for consumers. Many indices have defined fruit size and color as quantitative values. For example, a simple color-chart test or quantification of the causal compounds are appropriate for quantitatively analyzing fruit colors. In contrast, there are relatively few assays available for quantifying fruit shape, presumably because fruit shape is a three-dimensional characteristic and must be defined using pleiotropic explanatory variables rather than a simple/single index. Thus, novel insights into the genetic diversity associated with fruit shapes and the fruit shape differentiation processes have been very limited.

Recent improvements in computer performance along with decreases in the cost of digital imaging hardware and software have contributed to the increased application of digital image processing for agriculturally relevant morphological analyses. One of the effective approaches for defining a complex shape requires a set of coordinate values or descriptors obtained in a Fourier analysis. An example of one

of these methods involves a combination of elliptic Fourier descriptors (EFDs) and a principal component analysis (PCA) (Kuhl and Giardina, 1982; Rohlf and Archie, 1984; Rohlf and Archie, 1984). This method determines the overall shape based on image data by first transforming coordinate information regarding the image contours into EFDs, which are then summarized by a PCA. Analyses based on EFDs and PCA have been completed using the SHAPE program (Iwata and Ukai, 2002), including studies evaluating the shapes of horticultural crops such as Japanese radish (Iwata et al., 1998; Iwata et al., 2000), cucumber (Shimomura et al., 2016), and bottle gourd (Morimoto et al., 2005). However, only a few studies have investigated fruit tree crops.

Oriental persimmon (*Diospyros kaki*), which is hereafter simply called persimmon, is one of the main fruit tree crops in East Asia. The diversity in fruit shapes is greater for persimmon cultivars (Fig. 1A) than for other major fruit tree crops (e.g., grape or apple). To date, persimmon fruit shapes have been characterized mainly using a few criteria, including the ratio of length to diameter (L/D ratio), fruit apex shape, and the grooving along the side of the fruit. Although these indicators are considered to affect the commercial grade of fruit,

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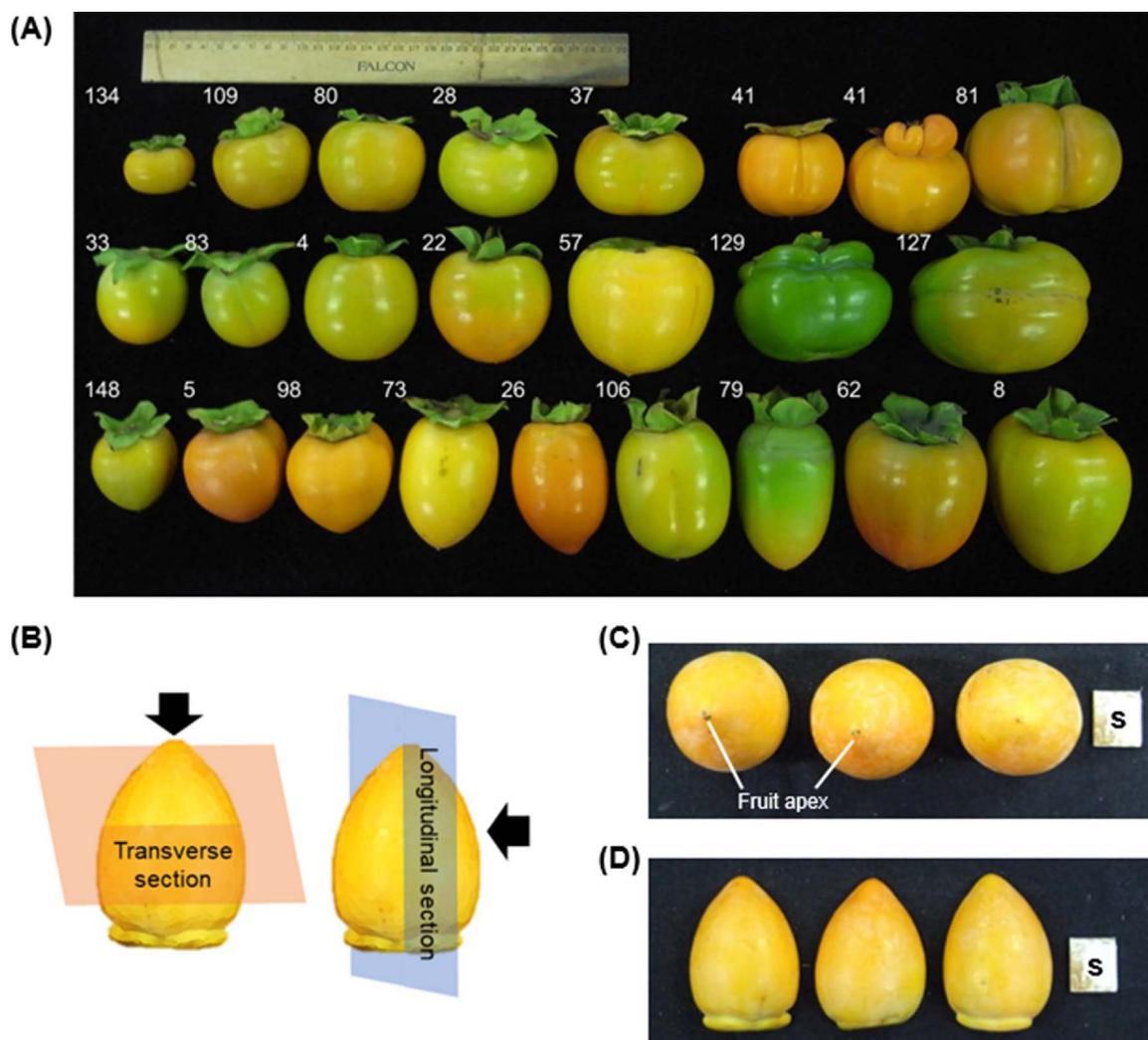


Fig. 1. Two-dimensional evaluation of variations in persimmon fruits shapes. (A) Diversity in persimmon (*D. kaki*) fruit shapes among cultivars. Numbers on the left of fruits refer to cultivars. (B) Diagram of the analyzed longitudinal and transverse fruit sections. Arrows indicate the direction of photographs. (C) Representative transverse view of fruits. (D) Representative longitudinal view of fruits. S: 2.4 cm × 2 cm scale.

persimmon fruit shapes are usually evaluated qualitatively. For example, in a previous survey of 306 persimmon cultivars, the shapes of the longitudinal and lateral sections were categorized into nine and six classes, respectively (Fruit Tree Experiment Station of Hiroshima Prefecture, 1979). These classifications were based on rough visual estimates, which resulted in frequent errors. To circumvent human errors, a computational study was conducted in which the three-dimensional fruit shapes of 11 F₁ progenies from 11 persimmon cultivars were characterized (Ding et al., 2000). However, studies evaluating broad genetic backgrounds have not been conducted.

In this study, we used the SHAPE program to clarify the differentiation of fruit, leaf, and seed shapes in 153 persimmon cultivars during different developmental stages. Seasonal characterizations elucidated the fundamental patterns of fruit shape development among the diverse cultivars. The resulting new insights into the quantitative characterization of fruit shape development can be linked to various quantitative indicators, which may be useful for developing new persimmon cultivars that produce desirable fruit shapes.

2. Materials and methods

2.1. Plant materials

Fruit and leaf shapes were assessed for 153 *D. kaki* cultivars, two

Diospyros lotus accessions, and one *Diospyros rhombifolia* accession, which were maintained in the experimental farm of Kyoto University in Kyoto, Japan. Most of the 153 *D. kaki* cultivars were hexaploid ($2n = 6x = 90$), while six were nonaploid and the ploidy of one was unknown (Table 1). The *D. lotus* and *D. rhombifolia* accessions were diploid and tetraploid wild relatives of *D. kaki*, respectively (Yonemori et al., 2008). Seed shapes were assessed using 41 *D. kaki* cultivars (Table 1). Three fruits were harvested from each cultivar every 2 weeks, starting from the flowering stage in mid-May until the mature fruit stage in mid-September 2016 (i.e., a total of nine times). Additionally, for each cultivar, six mature leaves were harvested in June, while six seeds were collected during the mature fruit stage. A single leaf was removed from the middle of the leaf-bearing zone of each branch.

2.2. Quantitative evaluation of fruit, leaf, and seed shapes

Fruit and seed samples were photographed using a GX200 digital camera (Ricoh Inc., Tokyo, Japan), with a black background and an index scale of 2.4 cm × 2 cm. Meanwhile, leaf samples were photographed with a light blue background and an index scale of 2 cm × 2 cm. To assess fruit shapes, photographs of longitudinal and transverse sections were evaluated independently. Longitudinal sections were prepared by cutting fruits along the central line to obtain the

Table 1
Persimmon cultivars and accessions included in this study.

No.	Cultivar	Seed	No.	Cultivar	Seed	No.	Cultivar	Seed	No.	Cultivar	Seed
1	Aburatsubo	s	40	Houkisuishi		79	Nagara	s	118	Soushu	
2	Aizumishirazu	s	41	Ibogaki	s	80	Nanshi		119	Sugitawase	
3	Akazu		42	Ichidagaki	s	81	Nanyousuishi		120	Suishi	s
4	Amahyakume	s	43	Ichiryu		82	Niikura		121	Sunami	
5	Amayotsumizo	s	44	Inayama		83	Nijuugaki	s	122	Suruga	
6	Aoso		45	Iwasedo	s	84	Nikorokonashiba	s	123	Taishu	s
7	Aoso (Seikan)		46	Izaemon		85	Nitari	s	124	Taiwan-koushi	
8	Atago	s	47	Jiro	s	86	Obishi		125	Takase	
9	Atagobou		48	Jisha	s	87	Okugosho		126	Takeda	
10	Beniemon		49	Jouren		88	Okujisha		127	Ta-mo-pan	s
11	Benigosho		50	Kakiyamagaki	s	89	Okumyoutan		128	Tenjingosho	
12	Benisakigake		51	Kanro		90	Okusaijyo		129	Tenpougai	
13	Beniwase		52	Kanshu		91	Onihei		130	Tenryubou	
14	Bongaki		53	Karasumi		92	Oogosho		131	Tohachi	
15	Butsushigano		54	Kawabata		93	Oohachiya		132	Tokudagosho	
16	Cal. Fuyu		55	Keizanbanshi	s	94	Ookouzu		133	Tonewase ^a	
17	Chagone		56	Kikuhira		95	Oonaga		134	Totsutanenashi ^a	
18	Chousengaki		57	Kiyosu-tanenashi ^a		96	Ooniwa		135	Toushi	
19	Daishiro		58	Koedagaki		97	Ootanenashi ^a		136	Toyoka	
20	Dennai		59	Kojimawase		98	Ooyotsumizo	s	137	Wakamiya	
21	Deshimaru		60	Komino	s	99	Oshoro		138	Wasamatsuo	
22	Doujouhachiya	s	61	Koudagosho		100	Oumidansu		139	Wasejisha	
23	Eboshi	s	62	Koushuhyakume	s	101	Pansu		140	Wasemyoutan	
24	Egosho	s	63	Kouyouwase		102	Pa-shi		141	Watarisawa ^z	
25	Emon	s	64	Kunitomi		103	Rashoutomoe		142	Yamagata-benigaki	
26	Fudegaki	s	65	Kuramitsu	s	104	Rokuzaemon		143	Yamato	
27	Fujiwaragosho		66	Kurogaki	s	105	Saburouza		144	Yamatogosho	
28	Fuyu	s	67	Kyara		106	Saijyo	s	145	Yashima	
29	Gionbou		68	Luo-tian-tian-shi		107	Saisho		146	Yatsudera	
30	Gobangaki		69	Maekawajiro	s	108	Sakata		147	Yokono	s
31	Gofu		70	Midai		109	Sangokuichi	s	148	Yomizo	
32	Hacchiuri		71	Mie		110	Sanja		149	Yosaburou	
33	Hagakushi		72	Mikatanigosho		111	Shakokushi		150	Yoshino	s
34	Hanagosho	s	73	Mino	s	112	Shibumyoutan		151	Yotsumizo	s
35	Hazegosho		74	Miyazaki-tanenashi ^a		113	Shimamyoutan		152	Yotsuyasaijyo	
36	Hiragaki	s	75	Mizushimagosyo		114	Shimokitahagakushi	s	153	Zenjimaru	s
37	Hiratanenashi ^a		76	Monpei		115	Shinpei		154	<i>D. lotus</i> Kunsenshi	
38	Hiroshima-shimofuri		77	Mukaku ^b		116	Shinshu		155	<i>D. lotus</i> Budougaki	
39	Houkikoushi		78	Muraya		117	Shouro		156	<i>D. rhombifolia</i>	

^a Nonaploid cultivar.
^b Cultivar with unknown ploidy.

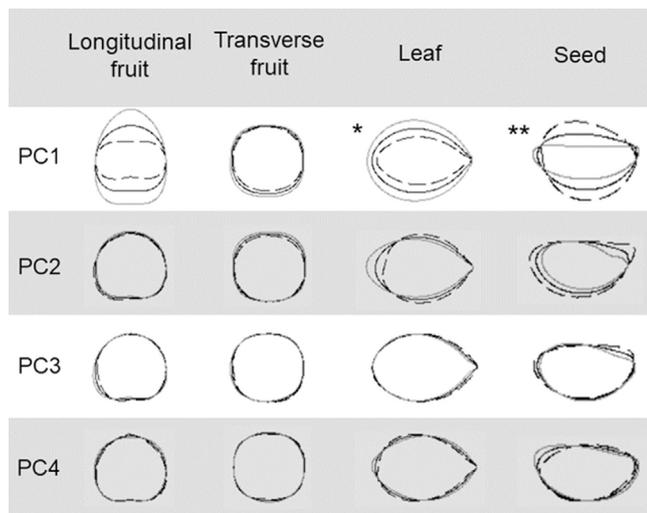


Fig. 2. Shape variations in a longitudinal fruit section, transverse fruit section, leaf, and seed as visualized by contour images based on the four most influential components. *The left end of each leaf represents the leaf petiole end. **The right end of each seed represents the calyx side. Each shape was reconstructed from the EFD coefficients, which were calculated with the score for a principal component equal to the mean \pm 2 standard deviations (SD), and the scores for the remaining components equal to zero. The broken, thin solid, and thick solid lines represent the mean $-$ 2 SD, mean, and mean $+ 2$ SD, respectively.

widest area, while transverse sections were prepared by cutting fruits to obtain the maximum diameter (Fig. 1B–D). The data for each image were saved as RGB color images (BMP format), with 256 gray levels per channel (red, green, and blue). Fruit shapes were analyzed based on EFDs and a PCA using the SHAPE program package developed by Iwata and Ukai (2002). The color images were converted into binary images according to a threshold. From these binary images, the closed contours of the samples were extracted and converted into a chain code (Freeman, 1974). The EFD coefficients, which were normalized to avoid variations related to the size, rotation, and starting point of the contour trace, were then calculated using the chain code data as described by Kuhl and Giardina (1982). During this procedure, we approximated the shape of each fruit using the first 20 harmonics. Thus, we calculated 80 (i.e., 4×20) standardized EFDs per sample. To summarize the information contained in the coefficients, a PCA was completed based on a variance–covariance matrix. To determine the effect of each principal component (PC) on fruit shape, we recalculated the EFD coefficients, with the score of a particular PC equal to the mean \pm 2 standard deviations (SD), while setting the scores of the remaining components as the means. We used the PC scores as the fruit shape characteristics in subsequent analyses. To evaluate the varietal effects on the PC scores among cultivars, an analysis of variance was completed using R ver. 3.3.2 (R Core Team, 2016). The same procedures were used to assess leaf and seed shapes. The analyses of fruits, leaves, and seeds were completed with three, six, and six biological replicates, respectively, at each sampling time point. Individual fruits, leaves, and seeds were

Table 2
Contribution of principal components (%) and significance of F according to an analysis of variance.

Component	Longitudinal fruits		Transverse fruits		leaves		seeds	
	Proportion (%)	Significance of F	Proportion (%)	Significance of F	Proportion (%)	Significance of F	Proportion (%)	Significance of F
1	88.49	***	44.79	***	56.16	***	46.99	***
2	4.67	***	28.80	***	25.42	***	32.02	***
3	1.74	***	6.24	ns	8.84	**	6.15	***
4	1.27	***	4.28	***	4.14	***	5.46	***

***, **: significance level at 0.1% and 1%, respectively; ns, not significant.

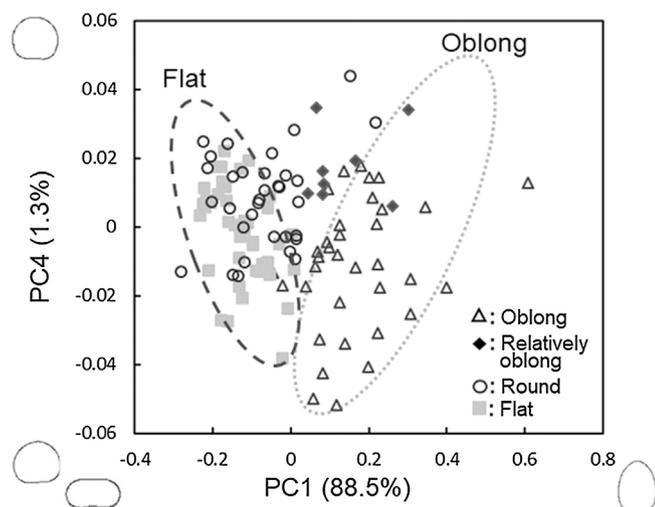


Fig. 3. Comparison between a quantitative evaluation of longitudinal fruit sections and a qualitative fruit classification completed in 1979. The two components of the principal component analysis (PC1 and PC4) are plotted on the axes. The proportion of variance explained by each principal component is provided in parentheses along each axis.

considered separate replicates.

To investigate the associations between fruit shape and leaf or seed shapes, a Pearson's product-moment correlation analysis was conducted using the first PC (PC1) scores for fruit, leaf, and seed shapes.

2.3. Seasonal characterization of fruit shape differentiation patterns

To characterize the fruit shape differentiation patterns, fruit PC1 scores at each sampling time point underwent a cluster analysis. Longitudinal and transverse sections were examined independently. Because the PC1 scores were relative scores for each sampling time point, they were standardized by being converted to Z-scores prior to the cluster analysis. The complete clustering algorithm of R was applied and the results were visualized using heatmaps prepared with the gplots package (Warnes et al., 2016).

To clarify when the fundamental tendency of fruit shapes was determined among cultivars, a Spearman's rank correlation analysis was conducted using the PC1 scores for longitudinal and transverse fruit sections at each sampling time point, including in mid-September.

3. Results and discussion

3.1. Quantitative characterization of fruit, leaf, and seed shapes

To analyze the shape of fully mature fruits, we first calculated the standardized EFD coefficients for the fruits of 156 cultivars and

accessions harvested in mid-September. Reconstructed fruit contours were drawn using the four most influential PCs and their respective means ± 2 standard deviations (Fig. 2). A significant percentage of the shape variability was extracted using the first PC for longitudinal (88.49%) and transverse (44.79%) fruit sections (i.e., the first PCs accounted for 88% and 45% of the diversity in the 156 cultivars included in this study). The same tendencies were observed for the leaves (56.16%) and seeds (46.99%) (Fig. 2; Table 2). A visual interpretation of these reconstructed contours implied that for the mature fruit longitudinal section, PC1 was a good indicator of the L/D ratio (Fig. 2). In fact, the PC1 scores were strongly correlated with the L/D ratios calculated using the actual measured values ($r = 0.97$). While PC2 and PC3 did not appear to substantially reflect any major persimmon fruit shape features, PC4 was associated with the flatness of the fruit apex, which is an important consideration when categorizing persimmon fruit shapes (Fruit Tree Experiment Station of Hiroshima Prefecture, 1979). We observed that PC4 accounted for 1.27% of the fruit shape variation among the analyzed cultivars. In contrast, for the transverse section of mature fruit, PC1 mainly reflected fruit flatness, whereas PC2 represented the sharpness of fruit corners (Fig. 2; Table 2).

A comparison of the evaluations using the SHAPE program or empirical definitions (Fruit Tree Experiment Station of Hiroshima Prefecture 1979) (Fig. 3) suggested that in the longitudinal section, the fruit shape tendencies were consistent between the two evaluation methods to some extent. This was expected because both methods for evaluating the longitudinal fruit sections considered the L/D ratio as a major criterion indicator. A drawback of the empirical method was that it could determine the shape only based on the criterion, while fruit shape is a quantitative trait. Meanwhile, the evaluation using the SHAPE program could determine the shape quantitatively, with results that were consistent with those based on the empirical criteria. In contrast, for the transverse sections, there were considerable discrepancies between the results of the analyses using the empirical method and the SHAPE program. This is presumably because the previously developed empirical evaluation method uses distinct classes for which the scale does not represent a single quantitative feature, but instead reflects shape categories. These results imply that it is difficult to evaluate multi-dimensional and quantitative fruit shapes using qualitative and one-dimensional criteria. Additionally, our attempt to determine the two-dimensional shape using EFDs with the SHAPE program may be useful for expressing fruit shapes using one-dimensional and quantitative values for each PC.

Regarding leaf shape, PC1 contributed 56.16% of the observed variability, and the first four PCs cumulatively accounted for 94.56% of the total variation among the examined cultivars. The constructed contours indicated that PC1, PC2, PC3, and PC4 were good indicators of the L/D ratio, position of the leaf centroid along the midrib, shape of the leaf apex, and shape of the leaf base, respectively (Fig. 2). For seed shape, the contours revealed by the EFDs indicated that PC1 mainly

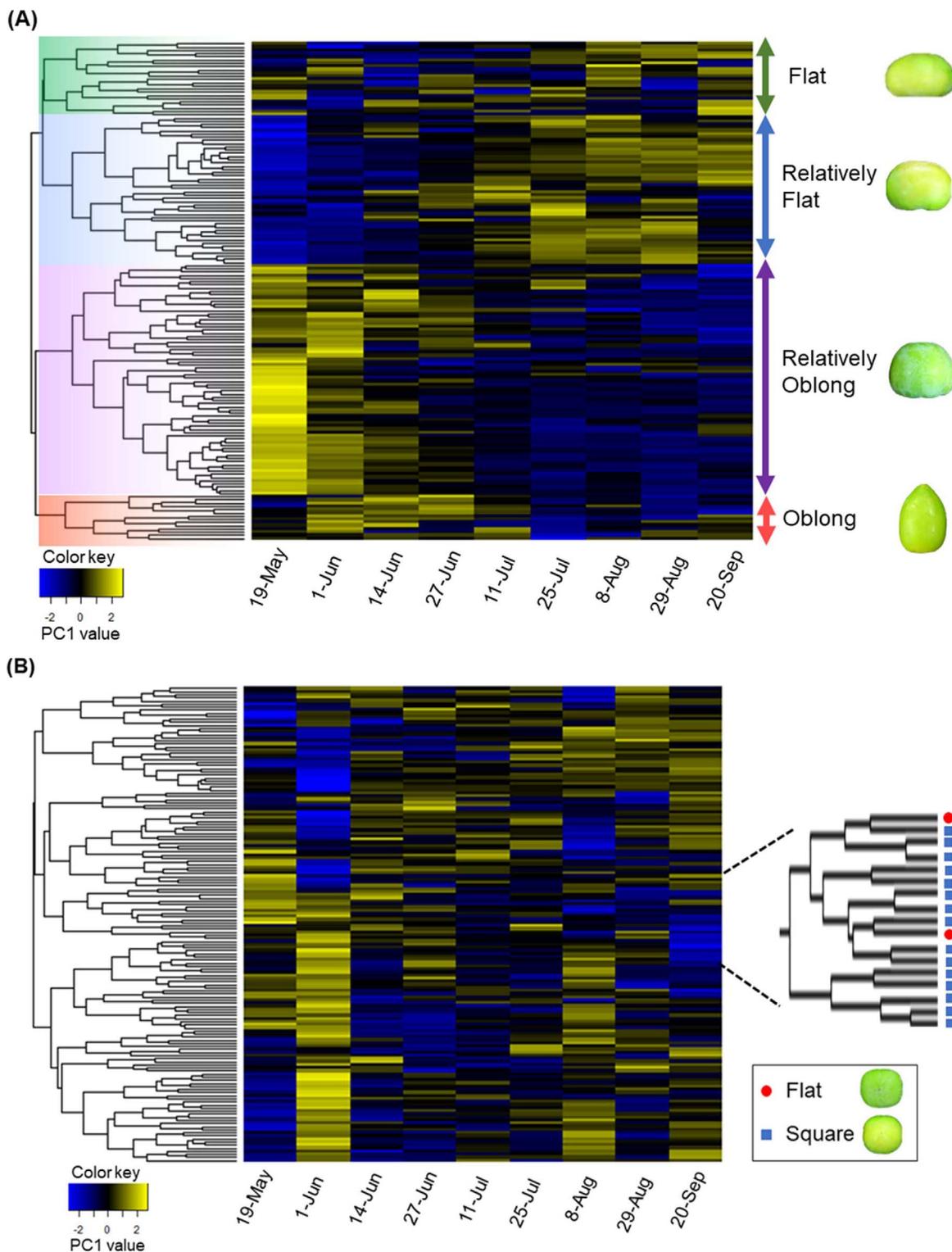


Fig. 4. Seasonal characterization of longitudinal and transverse fruit sections. (A) The PC1 scores for longitudinal sections at each sampling time point underwent a cluster analysis. (B) The PC1 scores for transverse sections at each sampling time point underwent a cluster analysis. (C) Spearman's rank correlation analysis of longitudinal sections. (D) Spearman's rank correlation analysis of transverse sections.

represented the L/D ratio, while PC2 and PC3 represented the seed end and calyx side shapes, respectively. Although the first four PCs significantly contributed to the observed variations among the analyzed cultivars ($p < 0.01$), PC1 explained approximately 47% of the total variability (Fig. 2). Therefore, the variations among leaf and seed shapes may be explained by only the PC1s. Consequently, we used the

PC1s for the correlation tests involving fruit shapes.

3.2. Seasonal fruit shape development patterns

To investigate the fruit shape development patterns, PC1 scores for longitudinal and transverse fruit sections at each sampling time point

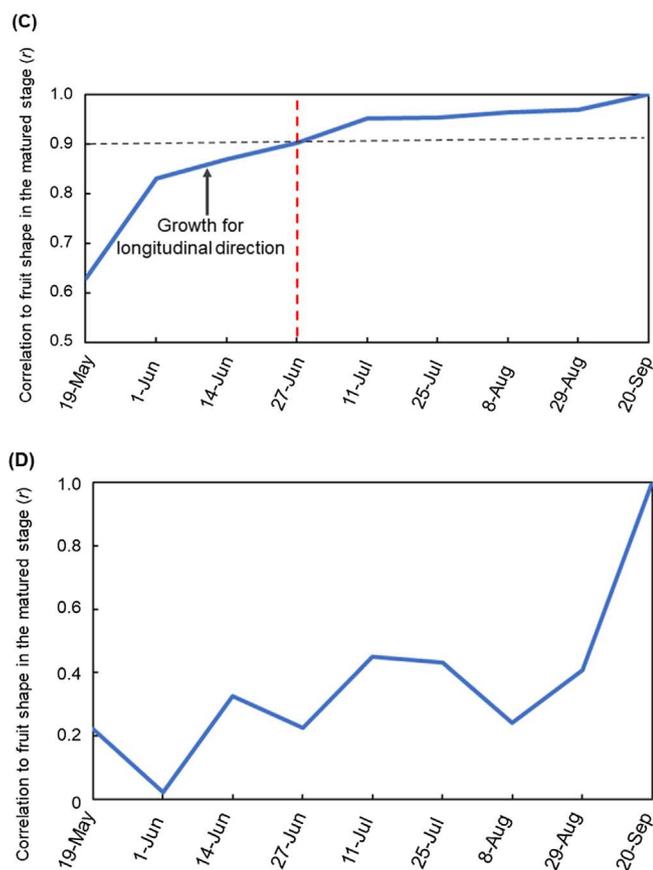


Fig. 4. (continued)

underwent a cluster analysis (Fig. 4). The PC1s for the longitudinal fruit shapes were clustered into at least four clades, which reflected the tendencies of their mature shapes (Fig. 4A). This result suggests that the development of persimmon fruit in the longitudinal direction is determined coordinately across diverse cultivars. Additionally, Spearman's rank correlation analysis indicated that the diversity of the PC1s for longitudinal sections of mature fruits was essentially determined ($r = 0.9$) during the early developmental stages (i.e., by the end of June) (Fig. 4C). This result is consistent with the findings of a previous study by Sugiura et al. (1977), in which an ethanol treatment in late May to late June affected the L/D ratio. In persimmon fruits, the early developmental stage is reportedly mainly associated with cell proliferation (Habu et al., 2016). These observations imply that in persimmon cultivars, the diversity in the longitudinal fruit shape might be determined by the direction of cell proliferation rather than by cell shape or elongation pattern. Regarding the transverse fruit sections, unlike the longitudinal sections, the clustering of PC1s did not reflect the shapes of mature fruits (Fig. 4B). This suggests that the transverse fruit shape is not determined coordinately across cultivars. Moreover, Spearman's rank correlation analysis (Fig. 4D) indicated that even in late August, the diversity of the PC1s for the transverse section of mature fruits had still not been determined. This may be because, unlike the longitudinal fruit sections, there was minimal variability in the transverse sections of the examined cultivars. Our data indicate that the fruit shape differentiation patterns differ between the longitudinal and transverse sections.

3.3. Characteristics associated with fruit shapes

To assess the association between mature fruit and leaf shapes, their

PC1 scores were aligned in a scatter diagram (Fig. 5A). Pearson's product-moment correlation and Spearman's rank correlation tests detected weak correlations between leaf and fruit shapes [$r = -0.30$ ($p < 0.01$) and $r = -0.29$ ($p < 0.01$), respectively]. These results imply that fruit and leaf shapes are not highly correlated. However, we observed that cultivars that produced flat fruits tended to have wide leaves. Persimmon fruits are derived from carpels. Interestingly, carpels and leaves have been described as homologous organs (Sobajima et al., 1974). In *Arabidopsis thaliana*, some genes affecting the orientation of organs, such as *JAGGED*, exhibit similar expression patterns between flowers and leaves (Dinnyen et al., 2004; Ohno et al., 2004). However, our results suggest that gene expression patterns specific to developing fruits are responsible for the considerable diversity in fruit shapes across persimmon cultivars.

The PC1 scores of mature fruits and seeds were highly correlated (Pearson's product-moment correlation $r = 0.73$; Fig. 5B), which is consistent with the results of an earlier study by Katayama-Ikegami et al. (2013). This implies that there are certain relationships between the L/D ratios of fruits and seeds in F_1 progenies. Yamamura and Naito (1973) reported that immature seeds may produce large amounts of a gibberellin-like substance, which is supplied to developing fruits. These results as well as ours, suggest that variations in fruit and seed shapes among cultivars are coordinately controlled, possibly by the same compounds (e.g., gibberellin). According to a morphological analysis, we determined that the number of locules differed between some cultivars, which may contribute to the diversity in fruit shapes in the longitudinal direction. Persimmon fruits normally have eight ovules (four locules), although some cultivars, such as 'Onihei', 'Hirakaki', and 'Keizanbanshi' produce fruits with more than eight ovules. The fruits of these three cultivars are relatively flat (Fig. 5B and D for 'Onihei'). In contrast, some 'Fudegaki' fruits, which are considered to have an ob-long shape (Fig. 5B), have only six ovules (three locules) (Fig. 5C). In tomato, fruit flatness is proportional to the number of locules, which is controlled by the *fascinated* gene (i.e., encoding a YABBY-like transcription factor) (Cong et al., 2008). This observation is consistent with the results for the persimmon cultivars described above, and may imply that the genes regulating locule numbers (or ovule numbers) also influence fruit shape development in these cultivars. However, the differences in the number of locules were based on a limited number of cultivars. Thus, although the fruit shape development patterns, at least in the longitudinal direction, are coordinated among diverse persimmon cultivars, there might be various molecular mechanisms affecting fruit shapes.

4. Conclusion

In this study, we quantitatively evaluated fruit shapes in 153 persimmon cultivars with a diverse genetic background. A quantitative analysis of the first major component explaining the diversity in fruit shapes revealed the fruit shape development patterns. We observed a coordinated development of fruit shapes across the analyzed cultivars, especially in the longitudinal direction. Additionally, the fruit shape tendencies were mostly determined in the early stages. A quantitative characterization of fruit shapes also revealed a clear association between fruit and seed shapes. The most effective use of this quantitative evaluation of fruit shapes involves association tests with quantitative environmental or genetic parameters, including temperatures or gene expression levels. Future studies should analyze whole genome expression levels. Doing so may help to elucidate the molecular mechanisms affecting fruit shape, which may have implications for attempts at artificially controlling this important trait.

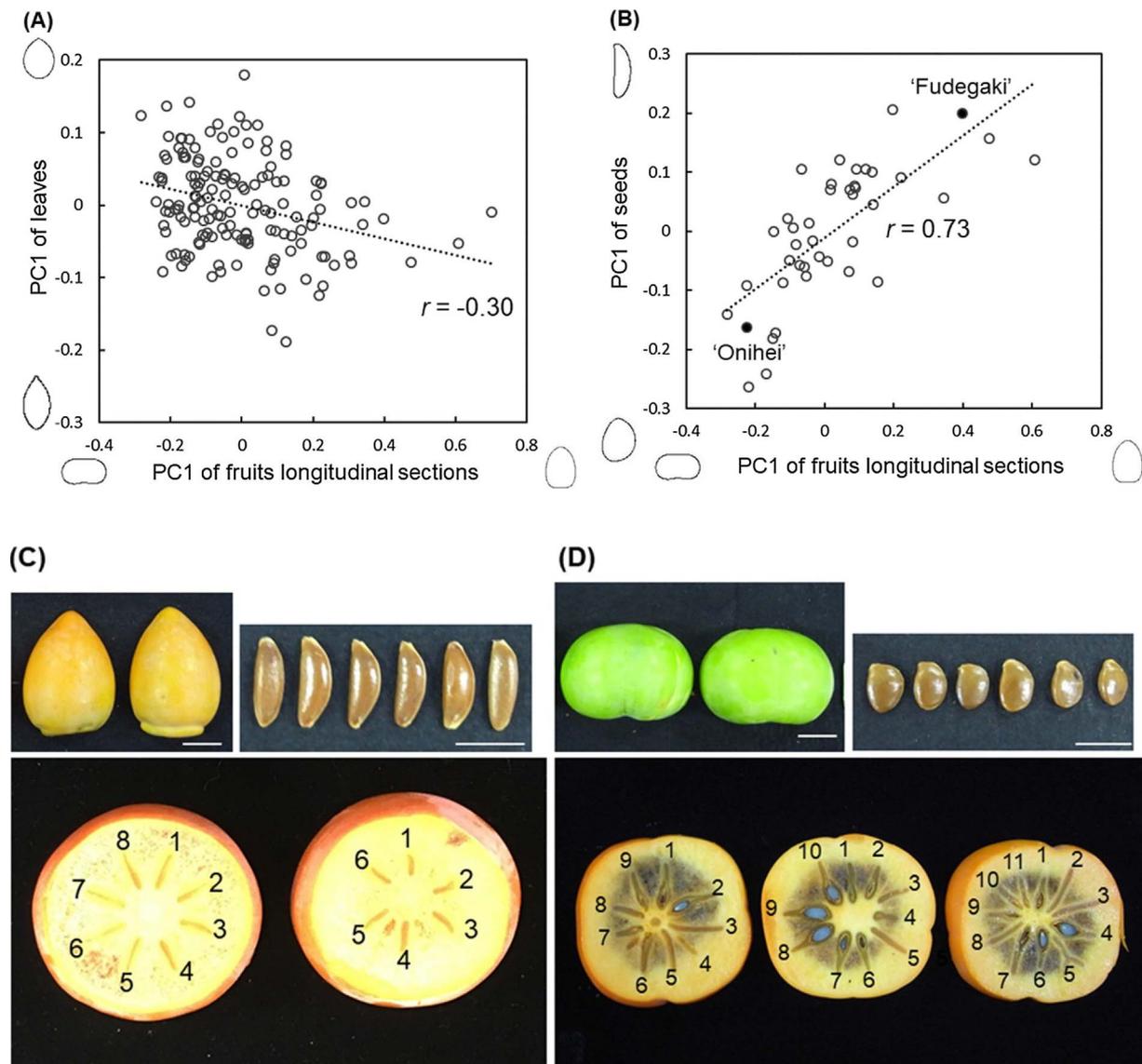


Fig. 5. Correlation between fruit shape and leaf or seed shape or locule number. (A) Correlation between fruit shape and leaf shape. (B) Correlation between fruit shape and seed shape. (C) 'Fudegaki' fruits had six locules and produced oblong seeds. (D) 'Onihei' fruits had more than eight locules and produced flat seeds.

Conflicts of interest

The authors declare that they have no conflict of interest.

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