Ploidy variation of hardy kiwifruit (*Actinidia arguta*) resources and geographic distribution in Japan

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Abstract

Survey on the wild genetic resources of hardy kiwifruit (*Actinidia arguta*) in Japan was conducted to determine the ploidy variation and its geographic distribution. Among the 127 wild plants collected from different geographic locations, 15 plants were diploid, 87 were tetraploid, and 22 were hexaploid. Additionally, 2 plants were heptaploid and one plant was octaploid. The tetraploid plants were distributed all over the country, whereas the diploid and

hexaploid plants were geographically localized, in the warm Pacific hill areas of the south western part and in the deep-snow region of the mid-northern part of Honshu, respectively. The diploid plants could be clearly distinguished from other plants with ploidy variation by the morphological characteristics of the leaf and fruit. Hexaploid plants showed a relatively larger L/D ratio of the leaf blade, a greenish petiole, and pubescence on the petiole and lower leaf vein, whereas the tetraploid plants exhibited a reddish petiole and callose hairs on the vein of the lower leaf surface. Fruit shape of the tetraploid plants varied largely, from round to ellipsoidal, whereas that of the hexaploid plants was mostly ellipsoidal. These results indicate that the hexaploid plants of *A. arguta* as well as the diploid and tetraploid ones, naturally grow in a certain size of population in the restricted region of Japan.

Keywords: *A.arguta*; Hardy kiwifruit; Distribution; Flow cytometry; Morphology; Polyploidy; Sarunashi

1. Introduction

Actinidia arguta known as hardy kiwi or bower vine is a cold-hardy species native to East-Asia (Ferguson and Huang; 2007). The fruit of *A. arguta* displays a hairless edible skin and a well-balanced sweet and sour taste with excellent flavor. It contains a larger amount of vitamin C and polyphenols, and shows a high activity of a cysteine protease, actinidin (Nishiyama and Oota, 2007; Kim et al. 2009), which promotes digestion and laxation in human body (Rush et al., 2002; Nishiyama and Oota, 2007). At present, *A. arguta* is

commercially cultivated in Oregon in US, Chile and New Zealand, and small-scale production for local consumption is conducted in many regions under a relatively cool climate (Kabaluk et al., 1997; Ferguson and Huang, 2007). Due to the readiness in eating and the abundance of the functional components for health, the market value of *A. arguta* fruit is increasing and selection of superior cultivars is being attempted in several countries (Williams et al., 2003; Jo et al. 2007; Latocha and Krupa, 2007; Stănică and Zuccherelli, 2007).

In the taxon of *A. arguta*, it had been recognized that the ploidy varied largely, including diploid, tetraploid, hexaploid, heptaploid and octaploid plants (Ferguson and Huang, 2007). Tetraploid plants are most commonly distributed throughout East-Asia. *A. arguta* var. *hypoleuca* found mainly in Japan was reported to be diploid (Watanabe et al., 1990), and *A. arguta* var. *purpurea* growing in central China contains teteraploid and octaploid forms (Ferguson and Huang, 2007). 'Issai' (Japanese name), which has been introduced to other countries is estimated to be hexaploid (Watanabe et al., 1990; Yan et al., 1997, Kabaluk et al., 1997). However, 'Issai' is not a true cultivar in Japan and its origin has not been determined (Phivnil et al., 2005).

In Japan, *A. arguta* is called "Sarunashi". It occurs in mountainous areas all over the country and the fruit has been locally utilized from old times. However, the evaluation of these natural resources has not been systematically conducted. Watanabe et al. (1990) counted the chromosome number of one wild plant of *A. arguta* var. *hypoleuca* and two wild plants of

A. arguta, and of 'Issai' obtained from a commercial nursery, and determined that the ploidy included diploid, tetraploid and hexaploid forms, respectively. This finding suggests the existence of polyploidy in *A. arguta* resources in Japan. However, ploidy variation in the population of wild plants has not been elucidated.

In a previous study, we determined the ploidy variation in the cultivar and local collections of *A. arguta* in Japan and implied the existence of localized distribution of hexaploid plants (Phivnil et al., 2005). Detailed information on the ploidy of natural resources is essential to analyze their origin and dissemination, and is practically valuable for selecting materials for breeding programs (Ferguson and Huang, 2007). In the present report the results of field surveys on the ploidy variation and its geographic distribution in the wild resources of *A. arguta* in Japan are outlined.

2. Materials and Methods

2.1 Plant materials.

127 wild plants of *A. arguta* collected from the natural growing sites representing different environmental areas in Japan, from north (Hokkaido, average yearly temperature; 8.0 ℃) to south (Miyazaki in Kyushu, 16.9 ℃). Local collections conserved at the regional research stations were also used. Among them, 78 plants were propagated by cutting and conserved in the research field of Kagawa University for morphological observation of leaves and fruit.

2.2 Morphological observation

Five expanded leaves per plant were collected from the mid-position of the shoots and the morphological characteristics were observed. Fully mature 10 fruit per plant were harvested in mid-September, and the morphological characteristics of the fruit were observed, After ripening of the fruit with ethylene treatment (Kim et al., 2009), total soluble solid content (TSS) of the juice was measured using a hand refractometer. Standard deviation was calculated for each parameter in ploidy variance.

2.3 Flow cytometric analysis

Flow cytometric analysis was performed by the method described by Phivnil et al. (2005). Expanding new leaves were collected from the shoot tips. A leaf segment 25 mm^2 in size without midribs was chopped with a razor blade in a plastic Petri dish with 0.5 ml of ice-cooled nucleus buffer (solution A of high resolution DNA kit, Partech, Münster, Germany) and kept on ice for 5 min. After filtration through 20 μm nylon mesh, the samples were treated with 2.5 ml of a staining solution containing 10 ml Tris, 50 mM sodium citrate, 2 mM $MgCl_2$, 1% (w/v) PVP 30 (Wako Chemicals, Tokyo), 0.1 % (v/v) Triton X-100 and 2 $mg \cdot l^{-1}$ 4', 6-diamidino-2-phenylindole (DAPI), pH 7.5. After incubation of the mixture on ice for 30 sec., the fluorescence of the nuclei was measured using Ploidy Analyzer PA (Partech, Munster, Germany) equipped with a mercury arc lamp. As an internal standard, Barley (*Hordeum vulgare*) 'Sanukihadaka' was used. The measurements were tripricated by using three leaf

samples collected from each plant.

3. Results

3.1 Variation in ploidy and geographic distribution of the wild plants of A. arguta

The ploidy levels of *A. arguta* plants were clearly determined by flow cytometry (Fig. 1). Among the 127 wild plants tested, 15 plants were diploid, 87 were tetraploid, and 22 were hexaploid. Additionally, 2 plants were heptaploid and one plant was octaploid (Table 1). The diploid plants were collected in the Pacific hill areas in the middle-part of Honshu and southwards (Fig. 2). Tetraploid plants were found in 21 prefectures from Hokkaido in the North and Kyushu in the South. In contrast, hexaploidy was observed only in the wild plants collected in 4 prefectures in the mid-northern region of Honshu; Yamagata, Fukushima, Niigata and Nagano (northern area). Additionally, two heptaploid and one octapoid plants were identified in Yamagata and Fukushima prefecture.

3.2 Leaf and fruit characteristics of ploidy variants in the wild plants of A. arguta

Leaf size was apparently smaller in the diploid plants than in the tetraploid and hexaploid plants (Fig. 3, Table 2). The leaves of the diploid plants were also characterized by the glaucousness of the lower surface. The shape of the leaves in the diploid, tetraploid and hexaploid plants varied from ellipsoidal to ovate within the same ploidy. The L/D ratio was slightly larger in the diploid and hexaploid plants than in the tetraploid ones. Hexaploid plants showed a relatively larger L/D ratio of the leaf blade, a greenish petiole, and pubescence on

the petiole and lower leaf vein, whereas the tetraploid plants exhibited reddish petiole and callose hairs on the vein of the lower leaf surface. Petiole was relatively longer in tetraploid and hexaploid plants than diploid ones.

Fruit size in the diploid plants was relatively smaller than in the tetraploid and hexaploid ones (Fig. 4, Table 3). Fruit shape varied largely from round to ovoid and ellipsoidal in the tetraploid plants, whereas that of the hexaploid plants was mostly ellipsoidal. The fruit of the two heptaploid plants were ellipsoidal and seedless. The fruit of the octaploid plant was ovoid. TSS contents of the juice hardly differed among the ploidies.

4. Discussion

Through the survey on the wild plants collected in Japan, it was confirmed that *A. arguta* displays a wide range of variation in ploidy namely diploidy, tetaraploidy, hexaploidy, heptaploidy, and octaploidy. Except for the heptaploid and octaploid plants which might occur incidentally, the diploid, tetraploid and hexaploid plants are likely to be naturally grown in a certain size of population. Interestingly, the distribution of the diploid and hexaploid plants was geographically localized.

Although, the systematics of *A. arguta* in Japan had been found to be complex (Koizumi, 1940; Uehara, 1960; Kitamura and Murata, 1979), Ohba (2006) lately described that *A. arguta* (Siebbold et Zucc.) Planch. ex Miq. contained var. *arguta* and var. *hypoleuca* (Nakai) Kitam. The diploid plants observed in the present survey were identified as var.

hypoleuca by distinct morphological traits consisting of a whitish lower leaf surface and a localized distribution in relatively warm Pacific regions. Additionally, Ohba (2006) described that var. *arguta* contained a forma *platyphylla* (A. Gray) H. Ohba which is characterized by the callose hairs on the lower leaf surface, and put Japanese name: Ko-kuwa. However, the geographic distribution of the forma has not been distinguished from the basic typical variety *arguta*.

In an empirical observation, Osawa (1993) identified a group of local collections grown in the deep-snow region of Honshu that differed from the common type of *A. arguta*, and designated it as "Dewa-no-Matatabi" following Nakai (1913), meaning "native *Actinidia* grown in Dewa, old name of northwestern mountainous area in Yamagata" in Japanese. He noted that this group could be characterized by relatively larger fruit and leaves with greenish petioles. The localization of the distribution of the hexaploid plants displaying similar morphological characteristics to those described by Osawa (1993) strongly suggests that populations with a higher ploidy are distributed in this area. The hexaploid 'Issai' (Fig. 3-M, Fig. 4-T) might have been derived from this population.

The geographic segregation among the populations with different ploidy levels has been reported in many plant species (Lewis, 1980). Generally, polyploids have been suggested to be more adaptive to the new environment than the ancestral diploid. The geographic distribution in *A. arguta* found in the present study implies the plants enhanced their

adaptability to colder climate by reaching a higher ploidy level. Additionally, the hexaploid plants may be evolved specifically in the deep-snow area of Japan Sea region.

The detailed mechanisms of occurrence of polyploidy and their phylogenetic evolution in *A. arguta* in Japan should be investigated by cytogenetic and molecular analysis. Further surveys on the ploidy variation of resources with superior horticultural traits and their conservation are also important for breeding programs by intra- and interspecific hybridization.

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Table 1. Estimation of ploidy level of local collections of *A.arguta* in Japan by flow cytometry.

Collection sites (Prefectures)	Number of collections				
	2x	4x	6x	7x	8x
A. arguta var. *hypoleuca*					
Chiba	2 (100)[z]				
Kanagawa	2 (100)				
Shizuoka	2 (100)				
Tokyo	1 (100)				
Hyogo	3 (100)				
Wakayama	1 (100)				
Kagawa	4 (100)				
A. arguta					
Hokkaido		2 (100)			
Aomori		16 (100)			
Akita		26 (100)			
Iwate		1 (100)			
Yamagata		3 (21.4)	9 (64.3)	1 (7.1)	1(7.1)
Fukushima		4 (28.6)	9 (64.3)	1 (7.1)	
Niigata			3 (100)		
Nagano		3 (75)	1 (25)		
Tochigi		1 (100)			
Gunma		1 (100)			
Yamanashi		14 (100)			
Toyama		2 (100)			
Ishikawa		2 (100)			
Fukui		2 (100)			
Gifu		1 (100)			
Chiba		1 (100)			
Shizuoka		2 (100)			
Shimane		1 (100)			
Tokushima		2 (100)			
Kochi		1 (100)			
Oita		1 (100)			
Miyazaki		1 (100)			
Total	15 (11.8)	87 (68.5)	22 (17.3)	2 (1.6)	1(0.8)

z: number of plants (percentage to the total)

Table 2. Leaf characteristics of ploidy variants in *A. arguta* native to Japan.

Ploidy level	Leaf			Petiole
	Length (cm)	Width (cm)	L/D ratio	Length (cm)
Diploid plants	6.9 ± 3.0	4.1 ± 2.4	1.7 ± 0.5	2.4 ± 1.3
Tetraploid plants	11.5 ± 2.4	7.5 ± 1.9	1.5 ± 0.2	4.0 ± 1.5
Hexaploid plants	11.4 ± 2.4	6.4 ± 1.5	1.8 ± 0.2	3.4 ± 1.4

Means ±SD, n=15(diploid), n=87(tetraploid), n=22(hexaploid)

Table 3. Fruit characteristics of ploidy variants in *A. arguta* native to Japan

Ploidy level	Weight (g)	Length (cm)	Width (cm)	L/D ratio	TSS (%)
Diploid plants	4.8 ± 1.0	2.3 ± 0.2	1.8 ± 0.1	1.2 ± 0.2	11.1 ± 0.4
Tetraploid plants	8.1 ± 3.0	2.7 ± 0.3	2.4 ± 0.3	1.3 ± 0.2	12.8 ± 1.2
Hexaploid plants	8.9 ± 2.9	2.9 ± 0.5	2.1 ± 0.3	1.4 ± 0.1	12.2 ± 2.3

Means ±SD, n=3 (diploid), n=8 (tetraploid), n=11(hexaploid)

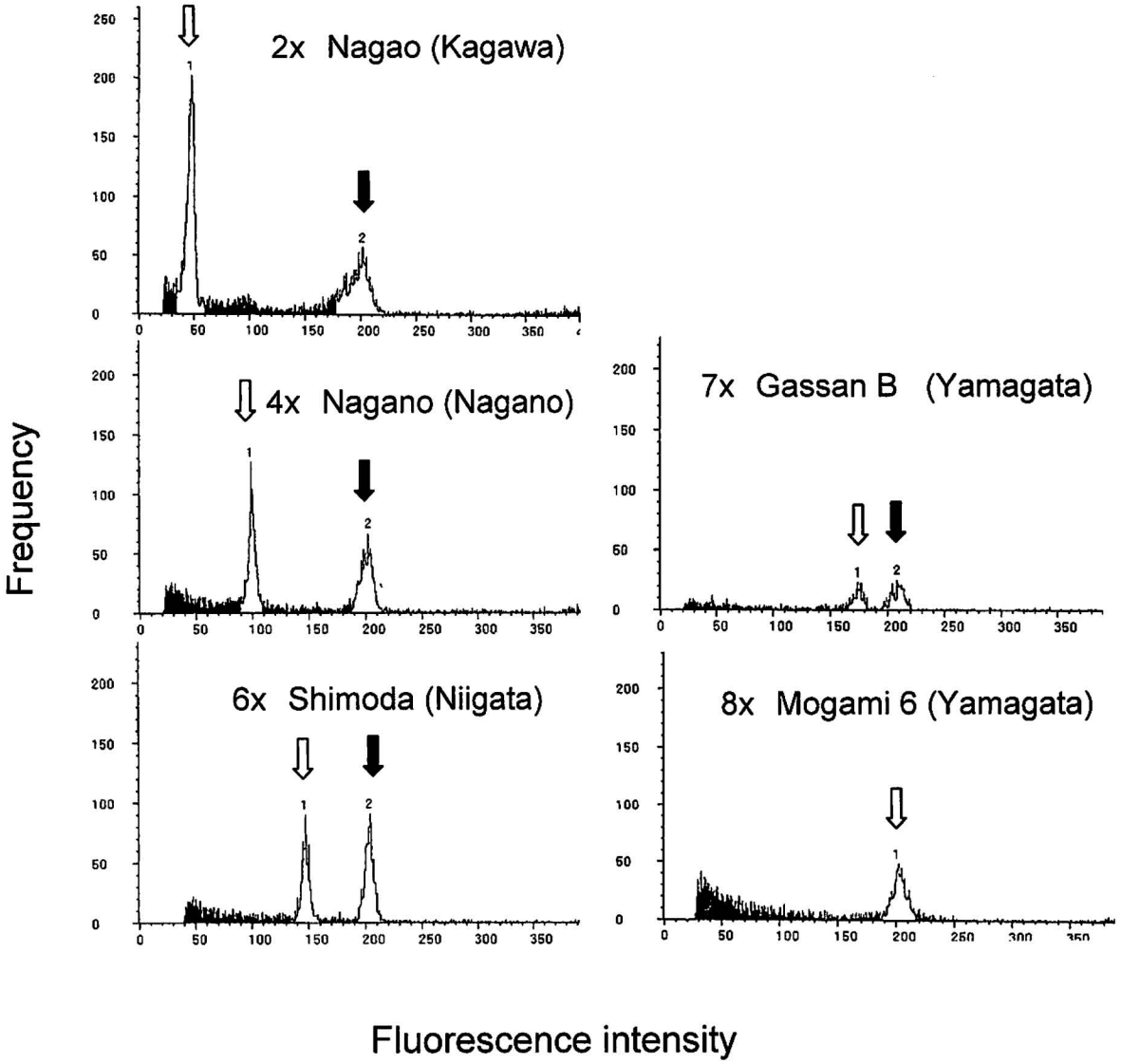

Fig.1. Histograms of frequency distribution of fluorescence intensity obtained from DAPI-stained nuclei in *A. arguta* collections. White and black arrows denote fluorescence intensity of sample and Barley (*Hordeum vulgare*), respectively.

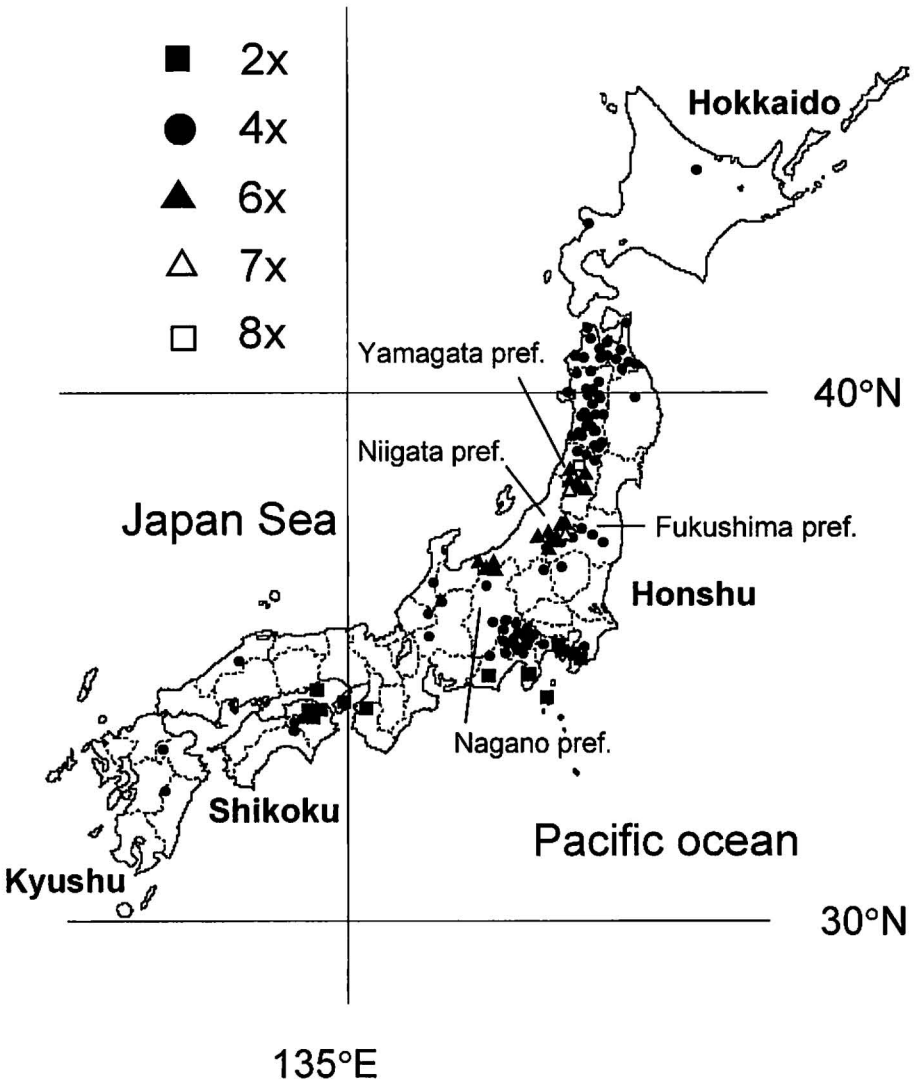

Fig.2. Geographic distribution of ploidy variants in *A. arguta* in Japan

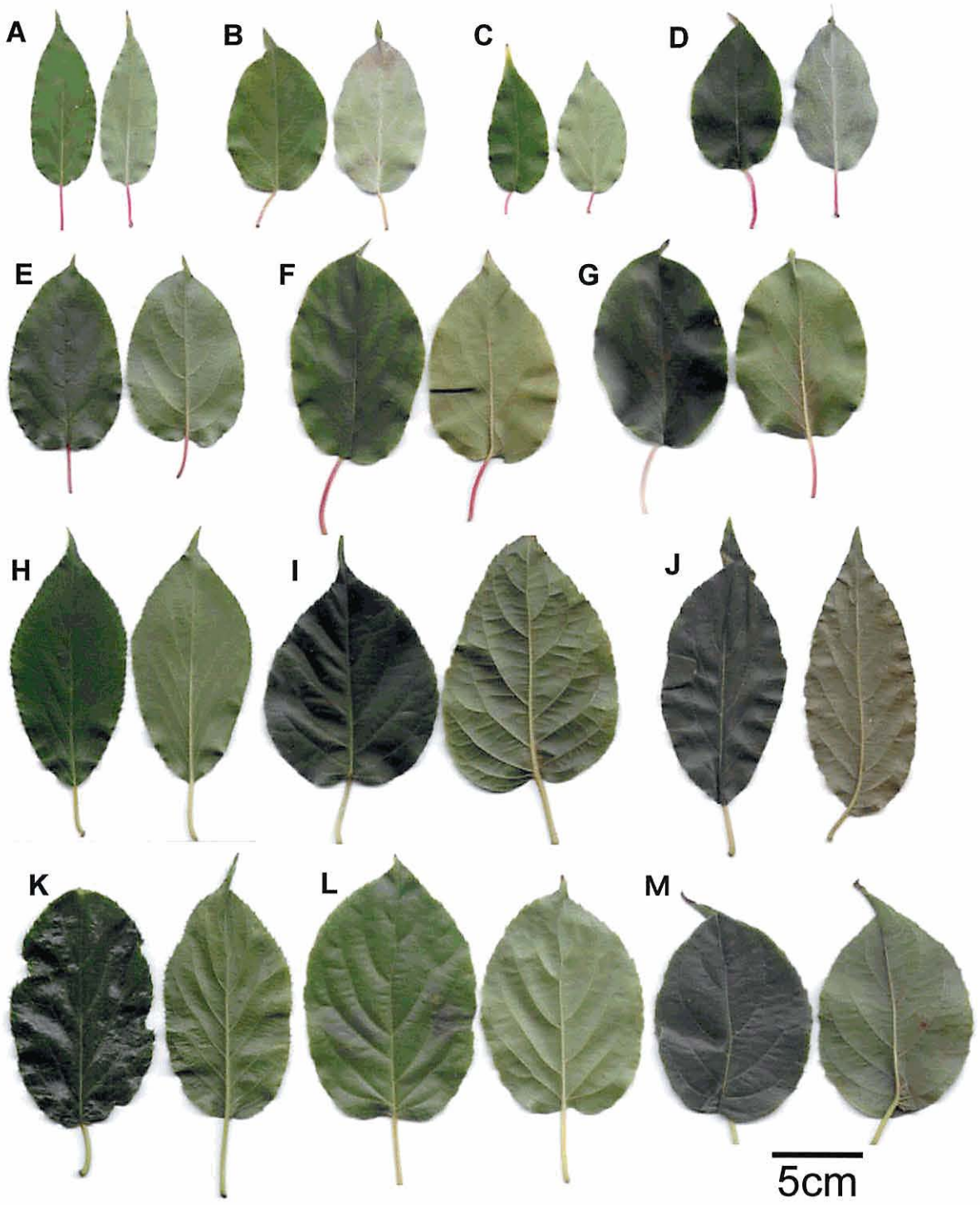

Fig. 3. Leaf morphology of ploidy variants in *A.arguta* in Japan

Diploid plants; A: Zushi (Kanagawa), B: Myojogatake (Kanagawa), C: Oishi (Wakayama)
D: Nagao (Kagawa)
Tetraploid plants; E: Misaka (Yamanashi), F: Nagano (Nagano), G: Hirano (Fukushima)
Hexaploid plants; H: Gassan A (Yamagata), I: Shimoda (Niigata), J:Otari (Nagano)
Heptaploid plants; K: Gassan B (Yamagata) Octaploid plants; L: Mogami 6 (Yamagata)
M:Issai (Hexaploid)

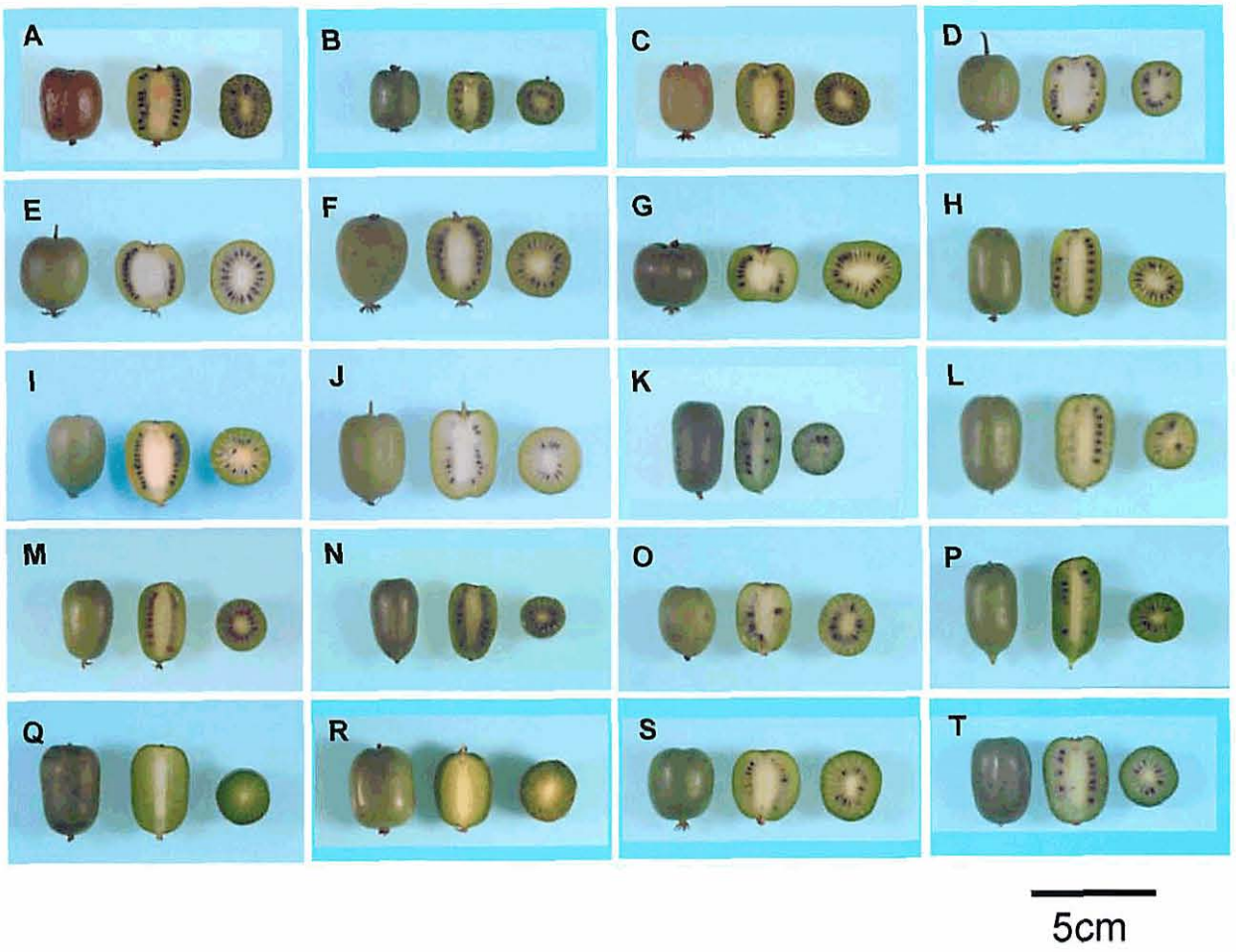

Fig. 4. Fruit of ploidy variants in *A. arguta* in Japan

Diploid plants; A: Myojogatake (Kanagawa), B: Miki (Kagawa), C: Izu (Shizuoka),
Tetraploid plants;D: Hirano (Fukushima), E: Nagano (Nagano), F: Otoyo (Kochi),G: Mogami 1 (Yamagata), H: Mogami 2 (Yamagata), I; Sanbe (Shimane)
Hexaploid plants; J: GassanA (Yamagata), K: Otari (Nagano), K: Shimoda (Niigata), L: M:Hinoemata (Fukushima), N: Tadami (Fukushima), O: Gassan04A (Yamagata), P: Mogami 4 (Yamagata),
Heptaploid plants; Q: GassanB (Yamagata), R: Aizu (Fukushima),
Octaploid plants; S: Mogami 6 (Yamagata)
T: Issai (Hexaploid)