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Flow Cytometric Assessment of Ploidy in Native Resources of *Actinidia* in Japan

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Abstract

Ploidy of accessions of *Actinidia arguta* and *A. rufa* in the collections conserved at Kagawa Prefectural Agricultural Experiment Station and Kagawa University was assessed by flow cytometric analysis. In *A. arguta*, intraspecific variation of ploidy in the ranges of 2x, 4x, 6x and 7x was observed. One wild type; 'Gassan' and locally selected cultivar; 'Mitsuko' were hexaploid. Ploidy of two accessions of Issai varied between 6x and 7x. All of the 11 accessions of *A. rufa* were diploid. Introduced accessions of *A. chinensis* contained diploid and tetraploid forms, whereas all the cultivars of *A. deliciosa* were hexaploid.

Introduction

Among 66 species of *Actinidia* distributed in East Asia, several species are indigenous to Japan; *A. arguta, A. rufa, A. kolomikta* and *A. polygama* (6, 15). *A. arguta* (Sieb. et Zucc.) Planch. ex Miq., called "Sarunashi", is commonly found in mountainous areas all over the country. The fruits showing high total soluble solid and vitamin C contents with excellent flavor have been locally utilized for eating in the fresh state and processing. Additionally, durable vines had been used as materials for suspension bridges in the past (15).

A. rufa (Sieb. et Zucc.) Planch. ex Miq., called "Shima-Sarunashi" grows only in the warm coastal regions of the southwestern part of Japan (15). The edible fruits are characterized by a low actinidin content compared with those of *A. deliciosa* (A. Chev.) Liang et Ferguson and *A. arguta* (13). Besides its potential for rootstock use (14), the adaptability to warm climate has been recently suggested, as *A. rufa* displayed a low-chilling requirement for the breaking of endodormancy (18).

In view of these valuable characteristics of the native *Actinidia* resources, selection and interspecific cross-breeding were attempted to develop new commercial crops (7, 16, 19, 21).

When performing cross breeding, especially interspecific hybridization, information about the ploidy of the materials is essential. It has been reported that in *Actinidia*, the basic chromosome number is x=29 (11) and the ploidy varies widely in the ranges of 2x, 4x, 6x and 8x (2). Differences in parental ploidy may lead to partial sterility in the hybrid, even though fruit can be set and develop (3, 5).

The objective of this study was to determine the ploidy of *A. arguta* and *A. rufa* accessions in the collections conserved at both Kagawa Prefectural Agricultural Experiment Station and Department of Horticulture, Kagawa University, Kagawa, Japan by flow cytometric analysis, which is a highly convenient method to estimate the ploidy level for the species such as *Actinidia* which contain small and numerous chromosomes (2). Additionally, the ploidy of introduced accessions of *A. deliciosa* and *A. chinensis* Planch. in our collections was determined. The strains of *A. deliciosa* except 'Koryoku' came from New Zealand, originally from China, and those of *A. chinensis* is ultimately from China.

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Materials and Methods

Plant materials. Cultivars and local accessions of *Actinidia* species in the collections conserved at Kagawa Prefectural Agricultural Experiment Station and Dept. of Horticulture, Kagawa University were used (Table 1). One clone of *A. arguta* Issai (Issai A) conserved at Junior College, Nihon University in Kanagawa was also used. Expanding young leaves were sampled from shoot tips. A leaf segment of 25 mm^2 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 a 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·l^{-1} 4′, 6-diamidino-2-phenylindole (DAPI), pH 7.5 (12). After incubating the mixture on ice for 30 sec., the fluorescence of the nuclei was measured using Ploidy Analyzer PA (Partech, Münster, Germany) equipped with a mercury arc lamp. Barley (*Hordeum vulgare*) 'Sanukihadaka' was used as an internal standard. The measurement was done by using three leaf samples collected from each plant.

Results and Discussion

By the flow cytometric analysis, intraspecific variation of ploidy was detected in our accessions of *A. arguta* and *A. chinensis*. In *A. arguta*, 2*x*, 4*x*, 6*x* and 7*x* forms were observed (Table 1, Figure 1). Three accessions with 2*x* ploidy, collected in Nagao and Shodoshima, Kagawa was characterized by the whitish lower surface of leaves, and was considered to

Table 1. Estimated ploidy of *Actinidia arguta*, *A. rufa* and other accessions by flow cyometry.

Species/Cultivar/ Accessions	Classification [z]	Flower type[y]	Ploidy level
A. arguta* var. *hypoleuca			
Nagao	lc. Kagawa Pref.	p	2x
Shodosima A	lc. Kagawa Pref.	p	2x
Shodosima B	lc. Kagawa Pref.	s	2x
A. arguta* var. *arguta			
Awaji	lc. Hyogo Pref.	s	4x
Hirano	lc. Fukushima Pref.	p	4x
Iya A	lc. Tokushima Pref.	p	4x
Iya B	lc. Tokushima Pref.	p	4x
Kochi	lc. Kochi Pref.	p	4x
Nagano	lc. Nagano Pref.	p	4x
Shimane	lc. Shimane Pref.	p	4x
Gassan	lc. Yamagata Pref.	p	6x
Mitsuko	cv. selected from lc in Yamagata	p	6x
Issai A [x]	cn.Unknown origin	p	6x
Issai B	cn.Unknown origin	p	7x
Elder	cn.Unknown origin	p	7x
Impal	cn.Unknown origin	p	7x
A. rufa			
Ashizuri A	lc. Kochi Pref.	p	2x
Ashizuri B	lc. Kochi Pref.	p	2x
Awaji	lc. Hyogo Pref.	p	2x
Iwaishima A	lc. Yamaguchi Pref.	p	2x
Iwaishima B	lc. Yamaguchi Pref.	p	2x
Iwaishima C	lc. Yamaguchi Pref.	p	2x
Iwaishima D	lc. Yamaguchi Pref.	p	2x
Kochi	lc. Kochi Pref.	s	2x
Nagano	Unknown origin	p	2x
Nakamura	lc. Kochi Pref.	p	2x
Okinawa	lc. Okinawa Pref.	p	2x

Table 1. (continued) Estimated ploidy of *Actinidia arguta*, *A. rufa* and other accessions by flow cyometry.

Species/Cultivar/ Accessions	Classification [z]	Flower type[y]	Ploidy level
A. chinensis			
FC1	ss. Kagawa Agr. Exp. Stn.	p	2x
FC2	ss. Kagawa Agr. Exp. Stn.	p	2x
FC3	ss. Kagawa Agr. Exp. Stn.	p	2x
FCM1	ss. Kagawa Agr. Exp. Stn.	s	2x
FCM2	ss. Kagawa Agr. Exp. Stn.	s	2x
FCM3	ss. Kagawa Agr. Exp. Stn.	s	2x
Kohi	cv.	p	2x
Rainbow Red (Hongyang) [w]	cv.	p	2x
Yellow Queen	cv.	p	2x
Apple (Kuimi)	cv.	p	4x
Golden King	cv.	p	4x
Golden Yellow (Jinfeng)	cv.	p	4x
Jumbo Yellow	cv.	p	4x
Red Princess (Lushan 79-1)	cv.	p	4x
A . deliciosa			
Abbot	cv.	p	6x
Bruno	cv.	p	6x
Gracie	cv.	p	6x
Hayward	cv.	p	6x
Koryoku	cv.	p	6x
Matua	cv.	s	6x
Monty	cv.	p	6x
Tomuri	cv.	s	6x
Interspecific hybrid			
Kosui	cv.(*A. arguta* 'Issai' *A. deliciosa* 'Matua') ?	p	2x
Shinzan	cv. (*A. arguta* _ *A. deliciosa* 'Tomuri')	p	5x
Sanryoku	cv. (*A. deliciosa* 'Koryoku' _ *A. chinensis* 'FCM1')	p	4x

[z] cv:cultivar, lc:localities for collection, ss:selected strain, cn:introduced from commercial nursery

[y] p:pistillate, s:staminate.

[x] Leaf sample was provided by Prof. Watanabe at Junior College, Nihon University, Fujisawa, Japan

[w] Original Chinese name was shown in parenthesis.

be var. *hypoleuca* Nakai, which is distributed in low-elevation mountains in the southern part of Japan. Watanabe et al. (20) also detected 2*x* form in a local accession of *A. arguta* collected at Hayama, Kanagawa Prefecture. Considering that Hayama locates at Miura peninsula under Pacific warm climate, the 2x form is suggested as *A. arguta* var. *hypoleuca*. Although morphological characteristics of these two varieties are very similar, except only lower surface color and leathery texture of the leaves, their geographic distribution was obviously separated. The specific character of ploidy found in var. *hypoleuca* may support the classification of this variety as a distinct species as *A. hypoleuca* Nakai.

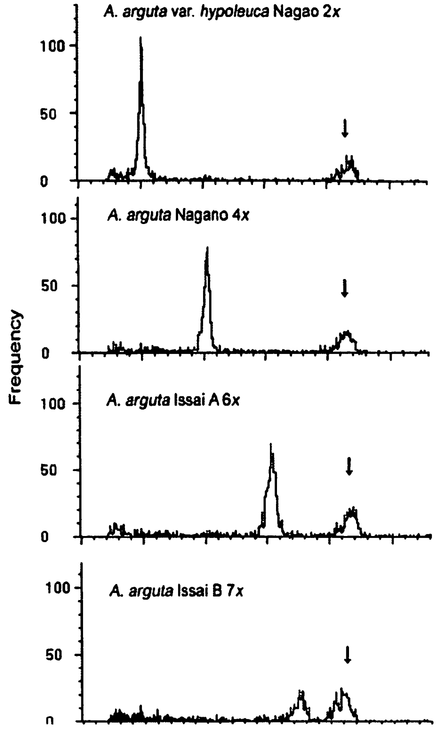
Seven accessions of *A. arguta* were estimated to be tetraploid. Gassan, a local accession in Yamagata Pref. and cv. Mitsuko, selected from the seedlings of native *A. arguta* were hexaploid. Previously, Watanabe et al. (20) observed 2*x* and 4*x* variation in the local accessions of *A. arguta* from Kanagawa, Kumamoto and Gunma based on chromosome counting. These results suggest that intraspecific variation in ploidy commonly occurs under natural conditions. Besides 2*x*, 4*x* and 6*x*, Ferguson et al. (2) found 8*x* forms in var. *purpurea*. However, we did not detect any octaploid forms in our accessions.

On the other hand, we found that the accessions of Issai contained 6*x* and 7*x* forms. Additionally, two commercial clones, Impal and Elder were also estimated to be 7*x* forms. Issai A conserved at Junior College, Nihon University was reported to be 6*x* by microscopic observation (20). In the present study, Issai A was confirmed to be hexaploid by flow cytometric analysis. Ferguson et al. (2) also confirmed that the two accessions introduced into New Zealand as Issai were hexaploid. Another accession of Issai B and two commercial clones estimated to be heptaploid are unique among the ploidy variation in *Actinidia*. Although the exact ploidy level of the accession should be determined by microscopic observation of the chromosomes, there are several possible explanations for the generation of heptaploid forms, namely intra- or interspecific crossing between maternal parent with 6*x* form and unreduced pollen from a 4*x* form.

The Japanese term "Issai" means "early flowering" or "early bearing", and is commonly used by nursery workers for woody ornamentals showing precociousness such as Issai sakura 'Asahiyama' (dwarf cherry) (4) or Issai Zakuro (dwarf pomegranate) (17). This suggests that "Issai" is a general term but not the name of the cultivar. It should be one of the reasons for the existence of Issai accessions having different characteristics.

In field observations, the fruit size of *A. arguta* slightly increased with the increase of ploidy from 2*x* to 6*x*. Differences in vine vigor were not appreciable among the

Figure 1. Frequency distribution histograms of fluorescence intensity obtained from DAPI-stained nuclei in *A. arguta* accessions. Arrows denote fluorescence intensity of Barley (*Hordeum vulgare*).



accessions with different ploidy levels up to 6*x*. Two clones of Issai had quite similar leaf morphology, having distinct soft prickles on the surface. Issai 7*x* was more vigorous than other *A. arguta* accessions, but its fruits were smaller and fewer seeds.

All accessions of *A. rufa* belonged to the 2*x* form. Although fruit size ranged from 5 to 30 g among accessions, and one accession from Kochi bore fruits with red flesh around the core, no differences were detected in ploidy. *A. rufa* displays some unique characters such as a low chilling requirement for the breaking of endodormancy (18), and low protease (actinidin) content in the fruit (13). *A. rufa* seems to be a valuable breeding material for specific objectives such as low-chilling character or low allergen content in the fruit.

As for the introduced cultivars, all the cultivars of *A. deliciosa* were hexaploid, while *A. chinensis* contained 2*x* and 4*x* forms (2). In field observation, 2*x* plants flowered 2 weeks earlier than 4*x* ones in *A. chinensis*. The fruit of 4*x* forms were larger than those of 2*x* ones.

In the interspecific hybrid cultivars formally registered in Japan, 'Shinzan', a hybrid between *A. arguta* native to Nagano Pref. and *A. deliciosa* 'Tomuri' (16) was pentaploid, implying that *A. arguta* used in this crossing might be tetraploid. 'Sanryoku' derived from the crossing of *A. deliciosa* 'Koryoku' with *A. chinensis* FCM1 was estimated to be tetraploid, showing an intermediate ploidy level between that of the parents. On the other hand, 'Kosui' reported to be a hybrid between *A. arguta* Issai and *A. deliciosa* 'Matua' (8), was diploid, which did not correspond to the expected ploidy. It was suggested that unexpected ploidy levels of 2*x* and 4*x* possibly occurred in the progenies from the crossing between *A. arguta* 4*x* and *A. deliciosa* 6*x* (1). However, in the case of 'Kosui', several characters were similar to those of *A. rufa* including well-developed winter buds, hairless brown skin of fruit, low actinidin content (13), and low chilling requirement for the breaking of endodormancy (9). Additionally, the similarity index of 'Kosui' obtained by RAPD analysis was higher in *A. rufa* rather than in *A. arguta* or *A. deliciosa* (10). These results suggest that *A. rufa* might be involved in the parentage of 'Kosui'.

The variation of ploidy among the species of our collection estimated by flow cytometry was mostly identical with the results by the chromosome counting done by several researchers (2, 20, 22, 23). Although the exact ploidy level of these accessions should be confirmed by the chromosome observation, the present information should facilitate breeding of *Actinidia*.

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