

Ploidy and phenotype variation of a natural *Actinidia arguta* population in the east of Daba Mountain located in a region of Shaanxi



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ABSTRACT

Ploidy levels of a natural population of *A. arguta* from east of Daba Mountain located in a region of Shaanxi province were determined by flow cytometric analysis. Decaploids of *A. arguta* were the first time found in nature. The frequencies of tetraploid, hexaploid, octaploid and decaploid in the population were 36%, 11%, 33% and 20% respectively. The haploid size of *A. arguta* was estimated to be 776.4 ± 94.82 Mb. Fruits of the female plants are purple or red color with a range of color intensity. The typical fruit shapes of those individuals vary from round, ovoid, oblong, ellipsoidal to spindle-shaped. Fruits of female plants with higher ploidy levels had smaller diameter of cross section and higher *L/D* ratio (length/width). Contents of sugars and organic acids in fruits of the population varied largely, and D-21 was found to have extremely high accumulation of sugars and organic acids. Present data evidenced natural distribution of decaploid and the co-existence of multi-ploidy of the purple *A. arguta* in the east of Daba Mountain.

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1. Introduction

Actinidia arguta is the second *Actinidia* species commercially cultivated in the world, although the production remains small-scale at present (Kabaluk et al., 1997; Williams et al., 2013; Ferguson and Huang, 2007; Latocha and Jankowski, 2011). The species displays not only horticultural advantages like cold-tolerance and short vegetation period, also highly nutritional values, hairless edible skin as well as wellness to human being health, which makes *A. arguta* a novel favorable fruit and grown commercially in more and more regions (Chat, 1994; Park et al., 2005). Breeding of superior *A. arguta* cultivars is being conducted to improve the comprehensive characteristics for meeting the increasing requirements in the market.

A. arguta is likewise the most widespread one among all *Actinidia* species, being found in eastern Russia (as far north as the southwestern part of Sakhalin), Korea, Japan and throughout much of China from Changbai Mountain in the north-east of China to Dawei Mountain of Yunnan Province in the south-west of China (22° N–47° N latitude) (Vorobiev, 1939; Liang, 1983; Cui, 1993). Diploid *A. arguta* var. *giraldii* was recorded to have distribution in western, central and eastern mountains of China (Cui, 1993;

Li et al., 2007). Another example of diploid *A. arguta* distribution was found in Japan (Watanabe et al., 1990; Phivnil et al., 2005). Tetraploid was extensively distributed from North Korea, central China, northeast China, Japan and Far-east of Russia (Bowden, 1945; Xiong et al., 1985; Deng and Sen, 1986; Watanabe et al., 1990; Baranec and Murin, 2003; Phivnil et al., 2005). Until now, hexaploid *A. arguta* was found only in Japan (Watanabe et al., 1990; Phivnil et al., 2005). Sporadic octaploid was also reported by Yan et al. (1997) and Kataoka et al. (2010), which means that high ploidy levels of *A. arguta* exist widely in central China and Japan Island. In addition, it was well documented that at least three ploidy levels of *A. arguta* are naturally distributed in Japan in certain sizes of populations (Phivnil et al., 2005; Kataoka et al., 2010). However, information from natural population of this species in China is too limited to understand the genetic and phenotype variations systematically.

Purple kiwifruit is one group of special genotypes of *A. arguta* var. *arguta* widely distributed in western mountains of China (Li et al., 2007). Fruits of the genotypes change into purple color at maturity and are mostly edible. But these resources have been scarcely evaluated on the level of population.

In this study, we investigated the ploidy variation of a natural *A. arguta* var. *arguta* population located in the eastern part of Daba Mountain area located in a region of Shaanxi province and evidenced the existence of decaploid within *A. arguta* species. Purple fruits of this population were evaluated in morphology and their relationship with ploidy levels as well.

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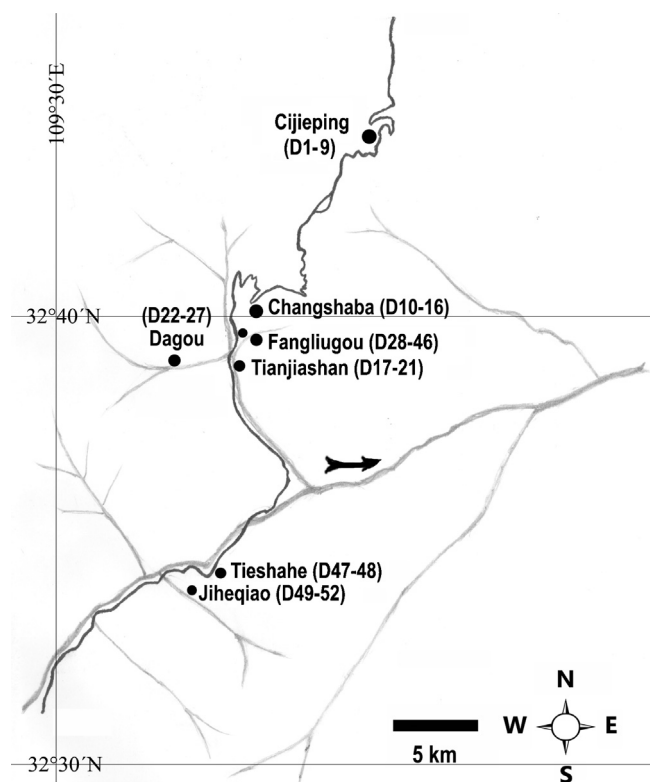


Fig. 1. Geographic distribution of the *A. arguta* population in the east of Daba Mountain located in a region of Shaanxi province. Plants sampled from Cijieping and Changshaba distributed on the hillsides. Plants sampled from Fangliugou, Tianjiashan, Dagou, Tieshahe and Jiheqiao were in valleys along the four streams respectively.

2. Materials and methods

2.1. Plant materials

A population of *A. arguta* var. *arguta* located in eastern part of Daba Mountain area located in Ankang of Shaanxi province was investigated and 52 wild individuals distributed in 5 small valleys and 2 hillsides were collected in late September of 2010 (Fig. 1). The individual plants located in the latitude 32°35'–32°45'N and the longitude 109°33'–109°38'E, and altitudes between 1470 m and 1899 m. Vigorous and lignified shoots of 25 cm in length and 1–1.5 cm in diameter were selected for sampling and transported to lab in Wuhan Botanical Garden, Chinese Academy of Sciences for further sprouting. Twenty to fifty fruits were harvested from each female plant, packaged with ice bag and transported to lab. All the fruits were sampled for measurement of sugar and organic acids at the eating ripeness stage.

2.2. Flow cytometric analysis

For flow cytometric analysis, tiny and newly expanding leaves were collected from the shoot tips. A leaf segment about 100 mg in weight was chopped with a sharp razor blade in a culture Petri dish with 1 ml of ice-cooled nucleus buffer containing 10 mM MgSO₄, 1% (w/v) DTT, 0.1% (w/v) Triton X-100, 50 mM KCl, 5 mM HEPES, pH = 8.0. After filtration through 33 μm nylon mesh, the samples were treated with 1 μl RNase for 15 min at 37 °C. Then 5 μl 5 mg/ml propidium iodide (PI) staining solution was added. After incubation of the mixture on ice, the fluorescence of the nuclei was measured using FACSARIA™ III High Speed Sorting Flow Cytometer (BD Biosciences, United States).

Tomato (*Solanum lycopersicum*) 'Stupiché' was used as a standard for estimating genome size of the samples. The known diploid *A. chinensis* var. *chinensis* 'Hongyang' ($2n = 2x = 58$) (Wang et al., 1996) and another known diploid *A. polygama* from sympatric areas of the *A. arguta* population were used as two references for deciding the ploidy levels. All the measurements were repeated for 3 times by using leaf samples collected from each individual.

2.3. Morphological evaluation

Length and width of fruits from each female individual plant were measured at the outdoor when sampling in Daba Mountain area. The parameters of fruit size were compared using unbalanced analysis of variance (ANOVA) with SAS software (Version 9.1; SAS Institute Inc.) for Windows.

2.4. Analyses of sugars and organic acids

Around 10 g of flesh tissues sampled from 2–3 fruits were used to analysis sugars and organic acids. And the sampling was repeated for 3–4 times for each plant. Extraction and derivatization of sugars (sucrose, fructose and glucose) and organic acids (malic acid, citric acid and quinic acid) were conducted by the protocols described by Wang et al. (2012). Chromatography was performed using an Agilent 6890N GC (Agilent Technology, Palo Alto, CA) with an HP-5 capillary column (30 mm × 0.25 mm × 0.1 mm). Sugars and organic acids were quantified based on standard curves generated for each metabolite and internal standard (methyl-α-D-glucopyranoside). All chemical standards were obtained from Sigma–Aldrich (St. Louis, MO). Analysis of variance (ANOVA) was conducted to compare the differences among the individual plants. For some female plants, sugars and organic acids were not measured because of the limited number of fruits. Content of quinic acid were not obtained because no area values were output, although its peak could be seen in the chromatogram.

3. Results

3.1. Ploidy variation and estimated genome size of the wild *A. arguta* population

Tetraploid, hexaploid, octaploid and decaploid were observed in this wild *A. arguta* population (Fig. 2). The individual's frequencies of each ploidy level were 36%, 11%, 33% and 20% respectively (Fig. 3). The wild population consisted of 7 geographical subgroups distributed along 4 streams or on 2 hillsides (Fig. 1). Interestingly, ploidy variation was also observed within each subgroup. Two subgroups (Cijieping and Changshaba) were dominated by tetraploid, and three subgroups (Fangliugou, Tieshahe and Jiheqiao) consisted of octaploid and decaploid. While the other two subgroups (Dagou and Tianjiashan) contained several ploidy levels (Fig. 1).

Based on data from different individuals with equal ploidy level, DNA content and genome size of *A. arguta* were estimated. The haploid size of *A. arguta* (776.4 ± 94.82 Mb) was shown to be higher than that of *A. chinensis* (651.81 ± 68.21 Mb) and *A. polygama* (546.3 ± 133.73 Mb), (Table 1).

3.2. Fruit morphology and its relationship with ploidy level

Fruits of this population are purple or red color with different intensity. And tiny black spots can be observed on skins of some fruits. Fruit color of some individuals like D-18, D-21 and D-30 were found to turn from green to red, then purple during storage (Fig. 4). The typical fruit shapes of those individuals vary from round, ovoid, oblong, ellipsoidal to fusiform (Fig. 4). The shape in cross section of

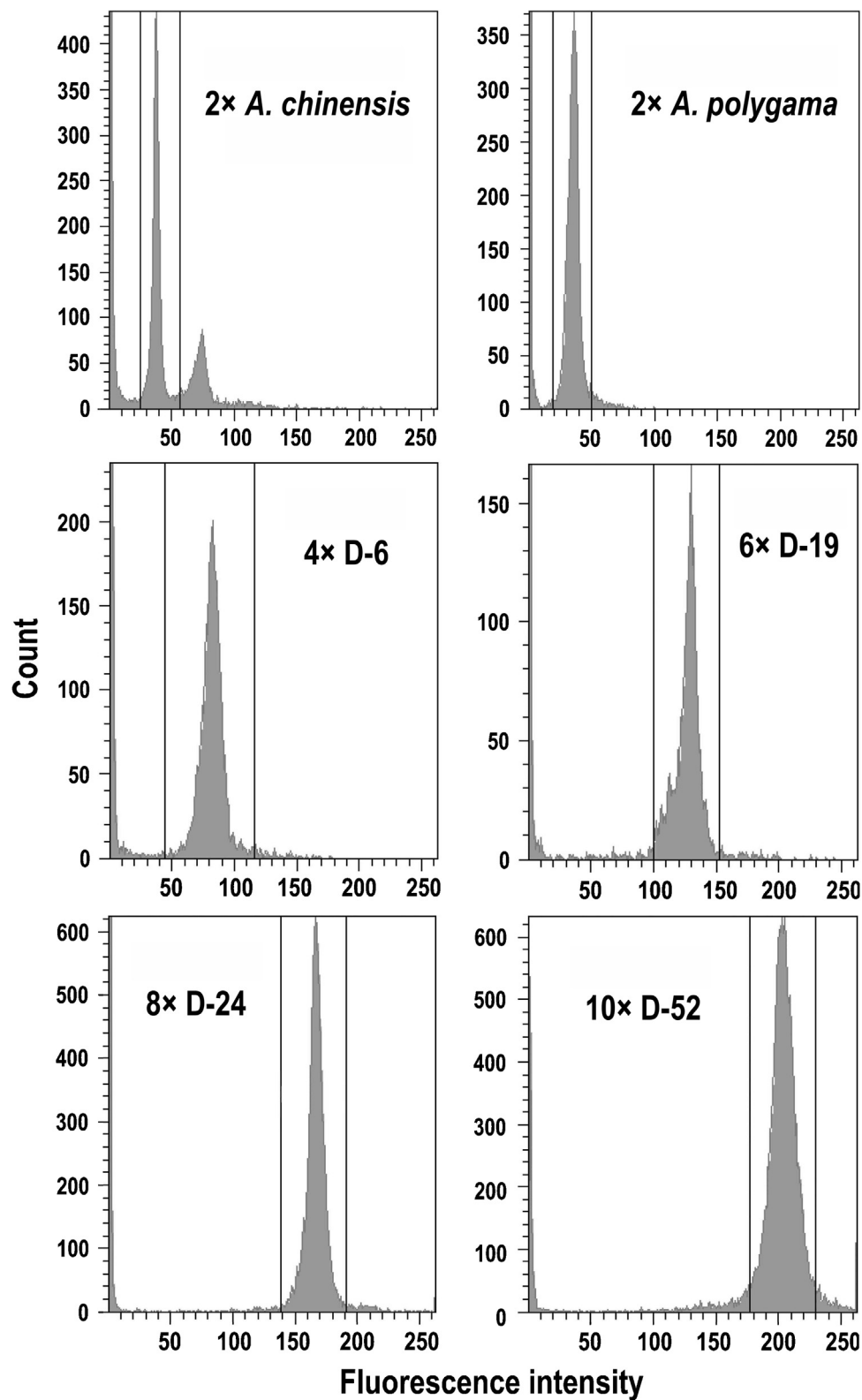


Fig. 2. Flow cytometric histograms show the relative fluorescence intensity obtained from PI-stained nuclei in *Actinidia arguta*. D-6, D-19, D-24 and D-52 indicate the individual plants of tetraploid, hexaploid, octaploid and decaploid respectively.

the fruits is mostly circular or oblate. Some fruit apices are round or flat, but most of them are protruding because of the remaining stylar end (Fig. 4).

Fruit of D-2 had longer length and wider cross section than others (Table 2). Fruit of D-5, D-18 and D-46

showed wider cross section than others (Table 2). No significant correlation was observed between the length of fruit and the ploidy level. Linear regression analysis showed that fruit width decreased with increase of ploidy level (Fruit width = $-0.07915 \times \text{ploidy} + 1.91638$, $R^2 = 0.4888$, $P = 0.0004$), but

Table 1Estimated genome size of different ploidy levels of *Actinidia arguta* plants sampled from the east of Daba Mountain, a region of Shaanxi province.

Species (ploidy level)	DNA content (pg/2C)	Estimated genome size (Mb/2C)	Estimated haploid size (Mb)
<i>A. chinensis</i> 2x	1.33 ± 0.14	1303.63 ± 122.02	651.81 ± 68.21
<i>A. polygama</i> 2x	1.12 ± 0.27	1092.6 ± 267.47	546.3 ± 133.73
<i>A. arguta</i> 4x	3.01 ± 0.32	2945.71 ± 304.4	736.43 ± 76.1
<i>A. arguta</i> 6x	4.12 ± 0.84	4032.36 ± 819.61	672.06 ± 136.6
<i>A. arguta</i> 8x	6.57 ± 0.34	6427.37 ± 331.69	803.42 ± 41.46
<i>A. arguta</i> 10x	7.31 ± 0.49	7149.63 ± 478.36	893.7 ± 59.8
Average			776.4 ± 94.82

DNA content = DNA content of standard species (*Solanum lycopersicum* var 'Stupiché') × (relative fluorescence intensity of sample/relative fluorescence intensity of standard).

Estimated genome size = 978 Mb/pg × DNA content of sample.

Estimated haploid size = Estimated genome size/ploidy level.

Means ± SD, for *A. chinensis* and *A. polygama*, n = 4; for *A. arguta* 4x, 6x, 8x and 10x, n = 20, 5, 10 and 12 respectively.**Table 2**Fruit length (*L*), width (*D*) and *L/D* ratios of *Actinidia arguta* accessions sampled from the east of Daba Mountain in a region of Shaanxi province.

Accession names	Ploidy level	Mean length (cm)	Mean width (cm)	<i>L/D</i> ratio
D-1	4x	2.11 ± 0.36	1.92 ± 0.3*	1.1
D-2	4x	3.1 ± 0.54*	1.77 ± 0.28*	1.75
D-3	4x	1.3 ± 0.11	1.37 ± 0.2	0.95
D-5	4x	2.51 ± 0.62	1.79 ± 0.27*	1.4
D-10	4x	1.96 ± 0.58	1.39 ± 0.21	1.41
D-12	4x	2.21 ± 0.62	1.80 ± 0.34*	1.23
D-13	4x	2.33 ± 0.53	1.24 ± 0.2	1.88*
D-14	4x	2.18 ± 0.55	1.61 ± 0.21	1.35
D-16	4x	2.37 ± 0.57	1.73 ± 0.39*	1.37
D-18	6x	2.76 ± 0.71	1.69 ± 0.26	1.63
D-19	4x	1.7	1	1.7
D-21	10x	2.23 ± 0.7	1.17 ± 0.21	1.91*
D-23	8x	2.33 ± 0.25	1.20 ± 0.08	1.94*
D-27	4x	2.52 ± 0.49	1.60 ± 0.22	1.58
D-30	8x	2.85 ± 0.31*	1.29 ± 0.13	2.21*
D-33	8x	2.26 ± 0.22	1.09 ± 0.08	2.07*
D-37	10x	2.75 ± 0.3	1.13 ± 0.08	2.43**
D-39	8x	2.52 ± 0.5	1.25 ± 0.14	2.02*
D-41	8x	1.89 ± 0.15	1.12 ± 0.09	1.69
D-42	4x	2.58 ± 0.38	1.63 ± 0.11	1.58
D-45	10x	2.59 ± 0.28	1.36 ± 0.11	1.9*
D-46	4x	2.57 ± 0.24	1.84 ± 0.11*	1.4
D-50	8x	3.4	1.30	2.62
D-52	10x	2.73 ± 0.32	1.23 ± 0.14	2.22*

All data shown are average ± SD, sample numbers n ≥ 20, but for D-19 and D-50, each only one fruit were available.

*significant at 0.05 level.

**significant at 0.01 level./

L/D ratio was positively affected by ploidy level in this population ($L/D \text{ ratio} = 0.1317 \times \text{ploidy} + 0.92043$, $R^2 = 0.5822$, $P < 0.0001$).

3.3. Variation of sugars and organic acids contents in fruits of wild *A. arguta*

At the soft mature stage, content of malic acid varied between 1.31 and 2.55 mg/g.FW (Table 3). All the checked samples had much higher content of citric acid than malic acid. D-21 showed extremely high content of citric acid, up to 30.67 mg/g.FW (Table 3). The soft fruit of the female plants contained approximate contents of three types of sugar (Table 3). In addition, fructose, glucose and sucrose content in fruit of D-21 were the highest among these checked samples (Table 3).

4. Discussion

A natural population of *A. arguta* was investigated on ploidy level and fruit characteristics. Using flow cytometric analysis, decaploids of *A. arguta* were the first time found in nature. Moreover, diverse ploidy levels of tetraploid, hexaploid, octaploid and decaploid were shown to co-occur in the population located in eastern part of Daba Mountain area.

As far as we know, the *A. arguta* accessions in Japan, Korea and northeastern China should be attributed to *A. arguta* var. *arguta* (Li et al., 2007). Based on the data from this variety throughout Japan Island, frequencies of tetraploid, hexaploid and diploid are 68.5%,

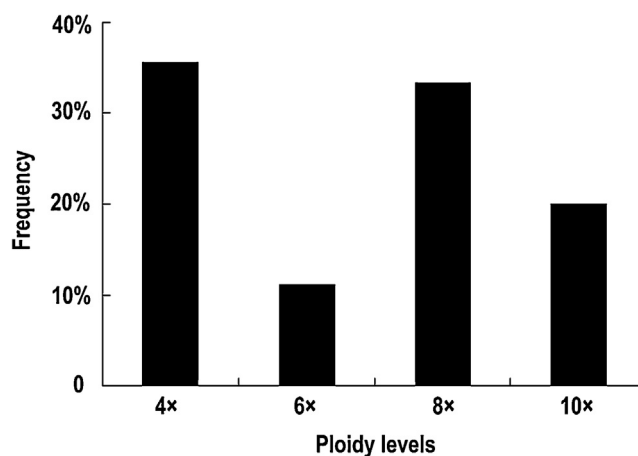


Fig. 3. Frequencies of individual plants with different ploidy levels in the *Actinidia arguta* population from the east of Daba Mountain in a region of Shaanxi province.

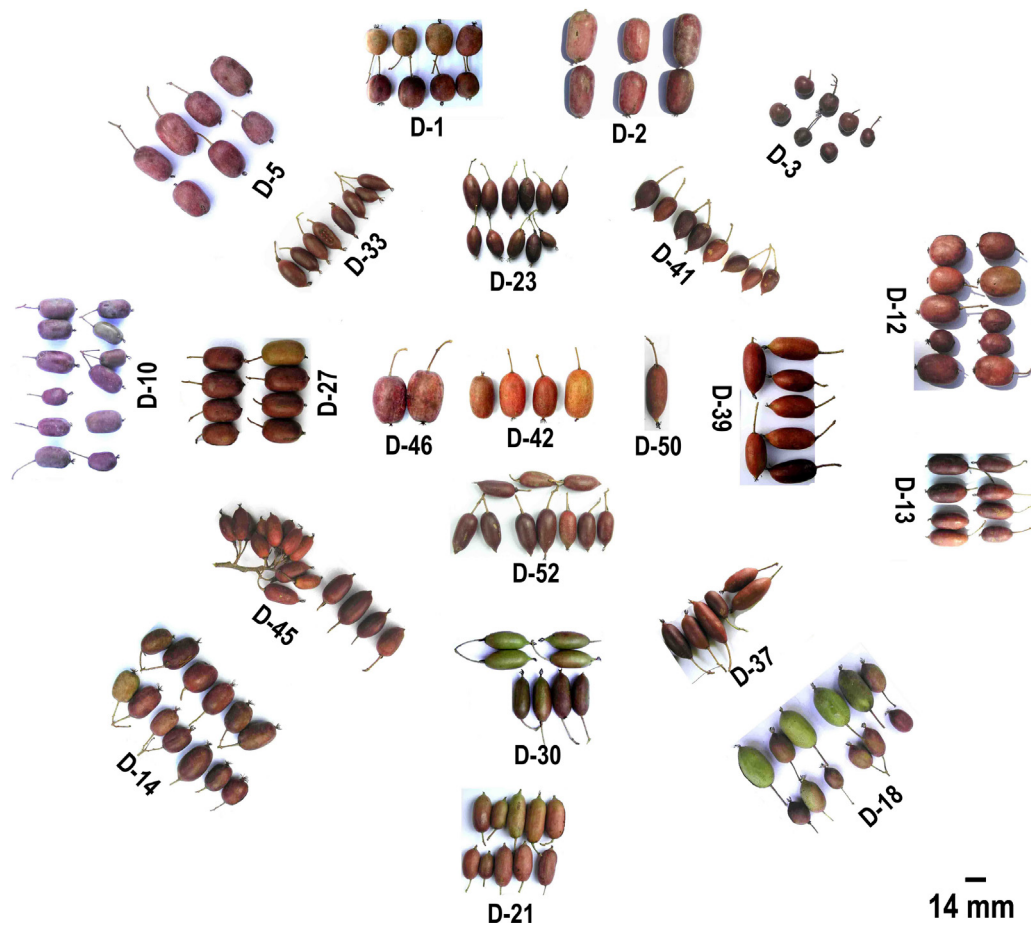


Fig. 4. Fruits of female *Actinidia arguta* plants in the population located in the east of Daba Mountain in a region of Shaanxi province. Tetraploid plants: D-1, D-2, D-3, D-5, D-10, D-12, D-13, D-14, D-18, D-27, D-46; Hexaploid plants: D-19; Octaploid plants: D-30, D-33, D-39, D-41, D-50; Decaploid plants: D-21, D-37, D-45, D-52.

17.3% and 9.6% respectively (Kataoka et al., 2010). In present study, no diploid was found in the investigated population located in such a smaller geographical area, but more octaploid and decaploid individuals were observed. It still suggests the possibility that *A. arguta* var. *arguta* in central China displays a trend of polyploidization comparing to that in Japan. Regardless of other ploidy, tetraploid *A. arguta* were found to have the widest distributions in Taiwan Island, central and south-west China, and across the cold climate in north-eastern Asia (Bowden, 1945; Xiong et al., 1985; Deng and Sen, 1986;

Baranec and Murín, 2003; Liu et al., 2011; Hsieh, 2012). However, ploidy levels of *A. arguta* have been sporadically investigated using the wild plants from Mainland of China (Bowden, 1945; Xiong et al., 1985; Deng and Sen, 1986; Yan et al., 1997; Liu et al., 2011). Ploidy data from wild populations of *A. arguta* still needs to be collected for elucidating the pattern of ploidy variation in a large-scale.

Multi-ploidy of *A. arguta* and *A. chinensis* complex has been reported to distribute in sympatric areas of Japan, Wuling-Xuefeng Mountain areas and Dalaoling National Nature Reserve respectively

Table 3

Contents of sugars and organic acids in fruits of *Actinidia arguta* accessions sampled from the east of Daba Mountain in a region of Shaanxi province.

Accession names	Ploidy level	Sucrose (mg/g-FW)	Glucose (mg/g-FW)	Fructose (mg/g-FW)	Malic acid (mg/g-FW)	Citric acid (mg/g-FW)
D-1	4×	10.58 ± 0.83	15.87 ± 0.88	11.68 ± 0.67	1.50 ± 0.18	7.94 ± 0.86*
D-2	4×	9.90 ± 0.65	12.61 ± 2.09	10.03 ± 0.87	1.39 ± 0.08	8.75 ± 0.97*
D-10	4×	9.70 ± 0.93	17.47 ± 1.44	13.32 ± 1.54	1.44 ± 0.22	5.56 ± 0.38
D-12	4×	11.30 ± 0.76	15.38 ± 0.69	11.59 ± 2.12	1.36 ± 0.12	10.53 ± 1.09*
D-13	4×	11.00 ± 1.05	13.99 ± 1.33	11.25 ± 1.08	1.53 ± 0.17	6.93 ± 0.76
D-14	4×	10.92 ± 0.96	12.98 ± 1.86	11.31 ± 1.25	1.61 ± 0.23	7.07 ± 0.92
D-18	6×	11.42 ± 1.17	14.57 ± 2.13	11.94 ± 0.95	1.54 ± 0.27	8.20 ± 1.02*
D-21	10×	19.08 ± 1.22*	130.57 ± 15.37**	81.38 ± 5.45**	2.55 ± 0.25*	30.67 ± 2.47**
D-27	4×	11.34 ± 0.64	15.60 ± 2.19	12.37 ± 1.79	1.31 ± 0.16	9.83 ± 1.06*
D-30	8×	11.41 ± 1.08	20.21 ± 2.88*	14.52 ± 1.21	1.45 ± 0.24	9.29 ± 1.29*
D-39	8×	10.35 ± 0.89	14.94 ± 0.97	11.89 ± 0.43	1.37 ± 0.21	10.45 ± 0.99*
D-45	10×	10.79 ± 1.06	16.56 ± 0.79	13.32 ± 1.58	1.48 ± 0.09	6.63 ± 0.75
D-46	4×	11.20 ± 0.99	13.23 ± 1.35	11.02 ± 0.97	1.47 ± 0.22	8.65 ± 0.93*
D-52	10×	10.77 ± 2.11	18.60 ± 3.02*	14.21 ± 2.33	1.58 ± 0.17	5.88 ± 0.54

D-1–D-52, 14 female plants with enough samples were used.

All data shown are average ± SD; FW means fresh fruit weight.

*significant at 0.05 level.

**significant at 0.01 level.

(Kataoka et al., 2010; Li et al., 2010a; Yan et al., 2012). Present results showed another sympatric occurrence of *A. arguta* cytotypes from 4× to 10× in Daba Mountain area of China. It is worthy of investigating whether the ploidy variation within *A. arguta* is also a result of natural interploidy hybridization, which has been provided to occur in *A. chinensis* complex in Wuling-Xuefeng Mountain areas (Li et al., 2010a). If so, evaluation and screening of *A. arguta* resources from Daba Mountain area should be a good choice for improvement of this germplasm because interploidy hybridization can combine diverse traits with commercial potential (Yan et al., 1994; Li et al., 2010b; Wu, 2012). Moreover, discovery of this population with co-existence of diverse ploidy levels will contribute to exploring how the *A. arguta* polyploidy originates (Wu, 2012).

The 2C-values for tetraploid or octaploid purple var. *arguta* estimated by other researchers were a bit lower than our result (Blanchet et al., 1992; Ollitrault-Sammarcelli et al., 1994; Ferguson et al., 1997), while the values for hexaploid var. *arguta* obtained by Blanchet et al. (1992) and Ferguson et al. (1997) were slightly higher than that of present samples (Table 1). The difference is probably derived from the diverse sampling numbers and/or experimental conditions used in those studies. In present study, we used the other two species, *A. chinensis* and *A. polygama*, as the reference of diploid. However, they were shown to have small difference in genome sizes with *A. arguta*. Cytogenetic technology should be applied to confirm ploidy levels of these *A. arguta* resources.

In this result, ratio of fruit length to width (L/D) of wild *A. arguta* was positively correlated with ploidy level. It is quite different from the recent evaluation of colchicine-induced tetraploid *A. chinensis* in which fruit had lower ratio of length to width (cross section) than that of diploid progenitors as fruit cross section increased and became round in cross section after chromosome doubling (Wu et al., 2012). Is this related with species or just specific to this wild *A. arguta* population? More individuals and more populations of this species need to be tested before answering the question. It is possible that fruit growth in the two species is different in genetic mechanism. All of these are interesting to uncover their genetics and evolution process from genomics (Wu, 2012).

Fruit quality in kiwifruit becomes much more important in germplasm evaluation for commercial breeding. Today color of skin and flesh is the important horticultural traits and always attract interest of consumers (Wu et al., 2013). *A. arguta* with purple fruits is the only wild genotype of kiwifruit germplasm characterizing of totally red fruits with edible flesh. Evaluation of this genotype is of great significance for developing a novel kiwifruit that maturity can be indicated by visual coloration. The contents of sugars and organic acids in fruits of the *A. arguta* population were similar to the results reported by Boyes et al. (1996), in which the purple fruit had higher content of citric acid than malic acid. A purple-fleshed cultivar, 'Purpurowaja Sadowaja', had the highest average yield among several green-fleshed genotypes and the hybrids between the purple and green genotypes of this species, although its average fruit weight was reported to be only 5.09 g (Bieniek, 2012). However, there's no definite difference in fruit size and contents of sugars and organic acids between the green and purple genotypes (Boyes et al., 1996; Latocha, 2007; Wang et al., 2011; Kim et al., 2012). D-21 was an interesting individual with extremely high accumulation of sugars and organic acids. It may be useful for research and deserved to be further characterized. Some of the variations in fruit characteristics are possibly induced by the different environmental conditions because the evaluation was conducted using fruit samples from wild conditions. Characterization of these resources under sympatric conditions remains to be conducted.

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References

- Baranec, T., Murín, A., 2003. Karyological analyses of some Korean woody plants. *Biologia* 58, 797–804.
- Bieniek, A., 2012. Yield, morphology and biological value of fruits of *Actinidia arguta* and *Actinidia purpurea* and some of their hybrid cultivars grown in north-eastern Poland. *Acta Sci. Pol. Hortorum Cultus* 11 (3), 117–130.
- Blanchet, P., Brown, S., Hirsch, A.-M., Marie, D., Watanabe, K., 1992. Détermination des niveaux de ploïdie dans le genre *Actinidia* Lindl. par cytométrie en flux. *Fruits* 47, 451–460.
- Bowden, W.M., 1945. A list of chromosome numbers in higher plants. I. Acanthaceae to Myrtaceae. *Am. J. Bot.* 32, 81–92.
- Boyes, S., Strübi, P., Marsh, H., 1996. Sugar and organic acid analysis of *Actinidia arguta* and rootstock-scion combinations of *Actinidia arguta*. *Lebensm. Wiss. Technol.* 30, 390–397.
- Chat, J., 1994. Screening *Actinidia* species germplasm for frost tolerance. In: Schmidt, H., Kellerhals, M. (Eds.), *Progress in temperate fruit breeding*. Kluwer Academic Pub, Dordrecht, The Netherlands, pp. 459–461.
- Cui, Z.X., 1993. *Zhong Guo Mi Hou Tao (Actinidia in China)*. Shangdong Science and Technology Publisher, Jinan, China.
- Deng, X.X., Sen, H.M., 1986. Studies on the chromosome numbers of *Actinidia*. *Acta Hortic.* 19, 13, 80.
- Ferguson, A.R., O'Brien, I.E.W., Yan, G.-J., 1997. Ploidy in *Actinidia*. *Acta Hortic.* 444, 67–71.
- Ferguson, R., Huang, H., 2007. Genetic resources of kiwifruit: domestication and breeding. In: Janick, J. (Ed.), *Horticultural Reviews*, 33, pp. 1–121.
- Hsieh, T.Y., 2012. Taxonomy and distribution of indigenous *Actinidia* in Taiwan. *National Chung Hsing University, Taiwan* (Dr. diss.).
- Kabaluk, J.T., Kempler, C., Toivonen, P.M.A., 1997. *Actinidia arguta* characteristics relevant to commercialization production. *Fruit Var. J.* 51, 117–122.
- Kataoka, I., Mizugami, T., Kim, J.G., Beppu, K., Fukuda, T., Sugahara, S., Tanaka, K., Satoh, H., Tozawa, K., 2010. Ploidy variation of hardy kiwifruit (*Actinidia arguta*) resources and geographic distribution in Japan. *Sci. Hortic.* 124, 409–414.
- Kim, J.G., Beppu, K., Kataoka, I., 2012. Physical and compositional characteristics of 'Mitsuko' and local hardy kiwifruits in Japan. *Hortic. Environ. Biotechnol.* 53 (1), 1–8.
- Latocha, P., 2007. The comparison of some biological features of *Actinidia arguta* cultivars fruit. *Ann. Warsaw Univ. Life Sci.* 28, 105–109.
- Latocha, P., Jankowski, P., 2011. Genotypic difference in post-harvest characteristics of hardy kiwifruit (*Actinidia arguta* and its hybrids), as a new commercial crop. Part II. Consumer acceptability and its main drivers. *Food Res. Int.* 44, 1946–1955.
- Liang, C.F., 1983. On the distribution of *Actinidia* Guihaia, 3., pp. 229–248.
- Li, D., Liu, Y., Zhong, C., Huang, H., 2010a. Morphological and cytotype variation of wild kiwifruit (*Actinidia chinensis* complex) along an altitudinal and longitudinal gradient in central-west China. *Bot. J. Linn. Soc.* 164, 72–83.
- Li, D., Zhong, C., Liu, Y., Huang, H., 2010b. Correlation between ploidy level and fruit characters of the main kiwifruit cultivars in China: implication for selection and improvement. *New Zeal. J. Crop Hortic. Sci.* 38, 137–145.
- Li, J.Q., Li, X.W., Soejarto, D.D., 2007. Actinidiaceae. In: Wu, Z.Y., Raven, P.H., Hong, D.Y., eds. *Flora of China*. Vol. 12. Beijing: Science Press & Saint Louis, Missouri: Missouri Botanical Gardens, pp. 334–360.
- Liu, C., Sun, X., Dai, H., Zhang, Z., 2011. In vitro induction of octaploid plants from tetraploid *Actinidia arguta*. *Acta Hortic.* 913, 185–190.
- Ollitrault-Sammarcelli, F., Legave, J.M., Michaux-Ferrière, M., Hirsch, A.M., 1994. Use of flow cytometry for rapid determination of ploidy level in the genus *Actinidia*. *Sci. Hortic.* 57, 303–313.
- Park, J.H., Lee, Y.J., Choi, J.K., 2005. Pharmacognostical study on the Korean folk medicine 'Da Rae Ip'. *Kor. J. Pharmacogn.* 36, 26–33.
- Phivnil, K., Beppu, K., Takamura, T., Fukuda, T., Kataoka, I., 2005. Flow cytometric assessment of ploidy in native resources of *Actinidia* in Japan. *J. Am. Pom. Soc.* 59, 44–49.
- Vorobiev, D.P., 1939. Review of Far East species of genera *Actinidia*. Lindley. Bulletin of Gornotaiyngny Station of Far East Branch of the Academy of Sciences of USSR. *Chabarovsk* 3, 5–38.
- Wang, M.Z., Li, M.Z., Wu, B.L., Hou, S.X., 1996. Study on the breeding of new kiwifruit cultivar. *Resour. Dev. Market* 12, 51–54.
- Wang, Z., Mi, J., Ji, H., Wang, B., 2011. Preliminary study on fruit quality of wild *Actinidia arguta*, Shanxi forest. *Sci. Technol.* 40, 326–327.

- Wang, Y.C., Zhang, L., Man, Y.P., Li, Z.Z., Qin, R., 2012. Phenotypic characterization and SSR identification of red-fleshed kiwifruit germplasm accessions. *HortScience* 47 (8), 1–8.
- Watanabe, K., Takahashi, B., Shirato, K., 1990. Chromosome numbers in kiwifruit (*Actinidia deliciosa*) and related species. *J. Jpn. Soc. Hortic. Sci.* 58, 835–840.
- Williams, M.H., Boyd, L.M., McNeilage, M.A., MacRae, E.A., Ferguson, A.R., Beatson, R.A., Martin, P.J., 2013. Development and commercialization of 'Baby Kiwi' (*Actinidia arguta* Planch.). *Acta Hortic.* 610, 81–86.
- Wu, J.-H., 2012. Manipulation of ploidy for kiwifruit breeding and the study of *Actinidia* genomics. *Acta Hortic.* 961, 539–546.
- Wu, J.-H., Ferguson, A.R., Murray, B.G., Duffy, A.M., Jia, Y., Cheng, C., Martin, P.J., 2013. Fruit quality in induced polyploids of *Actinidia chinensis*. *HortScience* 48, 701–707.
- Wu, J.-H., Ferguson, A.R., Murray, B.G., Jia, Y., Datson, P.M., Zhang, J., 2012. Induced polyploidy dramatically increases the size and alters the shape of fruit in *Actinidia chinensis*. *Ann. Bot.* 109, 169–179.
- Xiong, Z.T., Huang, R.H., Wu, X.W., 1985. Observations on the chromosome numbers of 4 species in *Actinidia*. *J. Wuhan Bot. Res.* 3, 219–223.
- Yan, G., Ferguson, A.R., McNeilage, M.A., 1994. Ploidy races in *Actinidia chinensis*. *Euphytica* 78, 175–183.
- Yan, G., Yao, J., Seal, A.G., Murray, B.G., 1997. New reports of chromosome numbers in *Actinidia* (Actinidiaceae). *N.Z. J. Bot.* 35, 181–186.
- Yan, L., Liu, Y., Huang, H., 2012. Genetic and epigenetic variation in the cytotype mixture population of *Actinidia chinensis*. *Chin. Bull. Bot.* 47 (5), 454–461.