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Cytogenomic evaluation of regenerated *Aralia elata* using PLOP-FISH and flow cytometry

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Abstract

Aralia elata is closely related to *Panax ginseng* and contains high levels of saponins and other medicinal compounds. Successful *A. elata* micropropagation is commercially significant; however, the genomic stability of tissue culture-derived regenerants is unclear. In this study, callus-derived regenerated *A. elata* plants were obtained, and their cytogenomic constitutions were assessed. Using RepeatExplorer, pre-labeled oligonucleotide probes (PLOPs) were developed with newly mined tandem repeats from $<1\times$ NGS whole-genome short reads, fluorescence *in situ* hybridization (FISH) was performed using six repeat probes, including three universal PLOPs, and genomic DNA content was estimated using flow cytometry. Regenerated *A. elata* plants (50) exhibited consistent ploidy, repeat distribution, and genome sizes compared with those exhibited by the mother plant. Six repeat probes were detected using FISH. Tandem repeat AeTR49 was identified as an excellent cytogenetic marker for homologous chromosomes, and AeTR161 and AeTR178 were localized in the centromeric and telomeric sections, respectively. Genomic DNA content (2C) was estimated at 2.46 ± 0.04 pg in the mother plant and 2.41 ± 0.05 pg in regenerated plants, with no significant variations in genome size or chromosome length. These results demonstrate that cytogenomics can be used to effectively evaluate chromosome-level genomic stability in regenerated *A. elata* plants.

Keywords: *Aralia elata*, cytogenomic study, FISH, flow cytometry, regenerated plants

1 **Introduction**

2 *Aralia elata* (Miq.) Seem. is a deciduous perennial shrub in the
3 Araliaceae (ginseng) family that is primarily found in Northeast Asian
4 countries, such as Japan, Korea, and China [1,2]. *A. elata* has been used
5 for centuries as a medicinal and edible plant because of its abundance of
6 valuable active secondary metabolites, including triterpenes, diterpenes,
7 flavonoids, coumarins, phenols, and protopectins [3]. Aralia saponins, also
8 known as aralosides, are fundamental constituents of *A. elata* responsible
9 for its medicinal properties [4]. Saponins isolated from the roots and bark
10 of *A. elata* have been used in traditional folk medicine to treat
11 neurodegenerative diseases, arthritis, and diabetes [3,5,6]. Furthermore,
12 the shoots are widely regarded for their culinary appeal and nutritional
13 benefits, and they are increasingly popular in micropropagation research
14 for this species [7–10].

15 Micropropagation is extensively used to meet the growing demand for
16 fresh and processed *A. elata* products [2,3]. This technique is useful
17 because it facilitates rapid multiplication of selected plants using *in vitro*
18 culture techniques [11,12]. Tissue culture-derived micropropagation is
19 more effective than natural and conventional propagation approaches such
20 as seedlings, cuttings, layering, and grafting [1,13]. It has already been
21 used to regenerate medicinal plants, orchids, cereals, vegetables, root
22 crops, and trees [14]. Successful micropropagation of *A. elata* using the
23 winter buds of 10-year-old trees [9], leaflet petioles [7], hairy roots [8], cell
24 suspensions [1], leaves, shoots, stems, and root segments [5], and mature
25 embryos [10] has been exploited for commercial purposes. However, the

genomic stability of tissue culture-derived *A. elata* regenerants has not yet been evaluated.

Cytogenomic information such as chromosomal composition, karyotype, repeat distribution, and genome size is essential for investigating the genomic stability of micropropagated plants [15–17]. Fluorescence *in situ* hybridization (FISH) is a promising technique that can be used to physically observe the distribution of specific DNA segments and/or genome rearrangements in regenerated plants [18–20]. In addition, estimating genomic DNA content using flow cytometry is effective for screening ploidy, including mixoploidy and aneuploidy [21,22].

In this study, chromosome composition, genomic repeat distribution, and genome size were analyzed in 50 tissue culture-derived regenerated *A. elata* plants. This is the first cytogenomic report on tissue culture-derived *A. elata* regenerants and provides helpful information for further cytogenomic research and breeding programs involving the ginseng family.

Methods

Plant materials

A. elata plants were provided by the Herbal Garden at Seoul National University, Korea, and were grown in a greenhouse at Sahmyook University to harvest fresh leaves. Leaf explants were washed with a detergent solution for 5 min and rinsed under running tap water for 10 min [23]. To ensure sterilization, the explants were treated with 70% ethanol and 2% sodium hypochlorite supplemented with two drops of

Tween-20 for 1 and 15 min, respectively. Finally, the explants were rinsed meticulously with autoclaved distilled water five times [5,24].

Tissue culture

Callus induction was performed on leaf explants cut into 2–10-mm sections [23]. The culture medium and incubation conditions were as previously described [5,24,25]. To initiate plant regeneration, callus sections were transferred to MS medium supplemented with 2.9 μ M gibberellic acid (GA), 3% sucrose, and 0.7% plant agar to encourage shoot development. Once tiny shoots emerged, root induction was performed in another MS medium supplemented with 1.2 μ M IBA, 2% sucrose, and 0.2% gelrite. These cultures were maintained at 25°C [8,13] with a photoperiod of 16/8 h light/dark during shoot and root development [5].

Plantlets were acclimatized in a local greenhouse for optimal adaptation to the natural environment. Plantlets (approximately 5–8 cm) were transplanted into 128-cell seedling trays filled with coco peat and moistened with water. The relative humidity of the greenhouse ranged from 50% to 70%, and the light intensity reached 130,000 lx. These environmental conditions were gradually introduced to the young regenerants until they were fully acclimated. Subsequently, the plants were placed in larger pots and watered every alternate day to ensure healthy growth [5,13].

Repeat mining and probe preparation

The < 1 × NGS whole-genome sequences of *A. elata* (NN-0919-000001) were obtained from the National Agricultural Biotechnology Information Center (NABIC) (<https://nabic.rda.go.kr/>). Subsequently, read quality trimming, sampling, and read clustering were performed using the Tandem Repeat Analyzer (TAREAN) workflow in the RepeatExplorer tool [26]. To identify genome-specific repeats, three tandem repeat sequences (AeTR49, AeTR63, and AeTR162) were selected to prepare probes.

A pre-labeled oligonucleotide probe (PLOP) labeled with Texas Red at the 5'-end was designed exclusively for the 49-bp AeTR49 minisatellite to facilitate FISH analysis and procured from Bioneer Corporation, South Korea (<https://www.bioneer.co.kr/>). For the AeTR161 and AeTR178 minisatellites, reverse and forward primers were designed using Primer3Plus (<http://www.primer3plus.com/cgi-bin/dev/primer3plus.cgi>), and subsequent PCR amplification was conducted as previously described [27]. The PCR amplicons of both repeats were labeled using a nick-translation labeling method with digoxigenin-11-dUTP and biotin-16-dUTP (Roche, Germany) as FISH probes. DNA amplicons (approximately 2 µg) for AeTR161 and AeTR178 were separately labeled with biotin-16-dUTP and digoxigenin-11-dUTP, respectively (Table 1). The labeling mixtures were incubated at 15.6°C for 3 h and this was then terminated by adding 0.5 M ethylenediaminetetraacetic acid (EDTA) and incubating for 10 min.

PLOPs targeting 5S rDNA, 45S rDNA, and *Arabidopsis*-type telomeric repeats have previously been synthesized and labeled with FAM, Cy3, and Texas Red, respectively, at the 5'-end [18].

99 ***Slide preparation***

100 Chromosome spreads were prepared according to established
101 protocols [18,28,29]. Root meristems were collected from 50 of the
102 regenerated plants cultivated in the greenhouse. The roots were
103 preliminarily treated with nitrous oxide at 0.5–0.6 MPa for 3 h. They were
104 then fixed in an aceto-ethanol solution (1:3 v/v) overnight at room
105 temperature (RT), transferred to a 70% ethanol solution, and stored at 4°C.
106 The meristematic root tips were carefully dissected and subjected to
107 enzymatic digestion using 1% pectolyase and 2% cellulase for 45 min at
108 37°C. Following the enzymatic treatment, the hydrolyzed root tips were
109 immersed in acetoethanol (1:3, v/v) and resuspended in a solution
110 containing 9:1 (v/v) acetic acid and ethanol. The suspension
111 (approximately 20 µL) was pipetted onto pre-warmed slides and air dried.
112 To further process the chromosomes, glass slides were fixed in 2%
113 formaldehyde for 5 min and dehydrated in an ethanol series (70, 90, and
114 100%) for 3 min each before air drying.

115

116 ***Fluorescence in situ hybridization***

117 Fluorescence *in situ* hybridization (FISH) using PLOPs based on
118 tandem repeats was performed as previously described [18,20,30]. Briefly,
119 a hybridization mixture (40 µL) was prepared and applied to the prepared
120 slides, followed by denaturation at 80°C for 5 min and overnight
121 hybridization at 37°C in a humid chamber. Subsequently, slides were
122 washed sequentially in 2× SSC at RT for 10 min, 0.1× SSC at 42°C for 25
123 min, and again in 2× SSC at RT for 5 min, followed by dehydration in an

ascending ethanol series (70, 90, and 100%) at RT for 3 min each, and then air-dried. Post air drying, chromosomes were counterstained with a 1:100 dilution of 4',6-diamidino-2-phenylindole (DAPI) (from a stock concentration of 100 $\mu\text{g}\cdot\text{mL}^{-1}$) in Vectashield (Vector H1000, Vector Labs, USA).

To detect nick-translated labeled minisatellite DNA, chromosomes were washed and reprobed according to a previously reported protocol [27]. For the hapten-labeled probes, an additional probe detection step was performed. After hybridization and stringent washes with $2\times$ SSC, slides were treated with a blocking buffer (0.1 M Tris-HCl, 0.15 M NaCl, 1% [w/v] blocking reagent) for 5 min at RT. Subsequently, digoxigenin- and biotin-labeled transposable elements were detected using anti-digoxigenin FITC conjugate (Sigma) and streptavidin-Cy3 conjugate (Zymed, USA), respectively. Slides were then washed three times with TNT (0.1 M Tris-HCl, 0.15 M NaCl, 0.2% [v/v] Tween-20) at 37°C for 5 min each, dehydrated with an ethanol series, air-dried, and counterstained as described above.

Chromosomal images were captured using an Olympus BX53 fluorescence microscope (Olympus, Tokyo, Japan) equipped with a Leica DFC365 FS CCD camera (Leica Microsystems Inc., Germany) and analyzed using Cytovision ver. 7.2 (Leica Microsystems, Wetzlar, Germany). Further image contrast and level adjustments were performed using Adobe Photoshop CS6 (Adobe Inc., California, USA). Chromosome length was measured using ImageJ software (Wayne Rasband, Maryland, USA) and statistically analyzed using GraphPad Prism v7 (GraphPad Software, La

Jolla, CA, USA). Homologous pairings and arrangements were determined based on chromosome length, satellite presence, and FISH signals, as described previously [31,32].

Flow cytometry

Dendropanax morbifera was used as the internal reference standard to estimate nuclear DNA content, with a known genome size of $2C = 4.09$ pg DNA [33]. The genomic DNA content of the mother plant and tissue culture-derived regenerants was measured to assess variations in genome size. Young leaves of the reference standard and regenerated plants were simultaneously chopped in a glass Petri dish with LB01 buffer (500 μ L) [22,34]. Nuclear suspensions were sequentially filtered through 50 and 30 μ m nylon mesh into a 1.5 mL microtube on ice. Propidium iodide (PI; 50 μ g $\cdot$ mL⁻¹) was added to stain the nuclear DNA, and RNase (10 μ g $\cdot$ mL⁻¹) was used to degrade RNA contamination [34]. A CytoFLEX BC04039 flow cytometer (Beckman Coulter Inc. USA) was used to measure the PI fluorescence of the stained nuclei (Fig. 3). More than 10,000 nuclei were counted for each measurement and analyzed using the built-in CytExpert software (Beckman Coulter, CA, USA). The sample 2C DNA content was estimated using standard computations [21]. The process was replicated three times on different days to obtain averages and standard deviations.

Results

Regeneration of the plants

Regenerated plants were successfully derived from callus tissue. Callus induction was observed after 4 weeks of inoculation, with the appearance of white-to-pale-yellow friable calli in the explants (Fig. 1A). Within 9 weeks, calli fragments were suspended in liquid MS medium to develop somatic embryos (Fig. 1B). After 8 weeks of culture, the calli generated globular-to-cotyledonary embryos (Fig. 1C). Two weeks later, complete plantlets with fully formed organs were observed (Fig. 1D) and transplanted into pots in a greenhouse. Fifty regenerated plants were obtained and used for cytogenomic analysis (Fig. 1E).

Novel repeats and probe development

Repetitive DNA elements in *A. elata* were analyzed to identify suitable FISH markers for karyotyping. RepeatExplorer analysis estimated that the repeats accounted for 52% of the *A. elata* genome. Three satellite repeats were identified (AeTR49, AeTR161, and AeTR178), showing typical satellite clustering graphs (Table 1 and Supplementary Fig. S1). The consensus sequences of AeTR49, AeTR161, and AeTR178 were 49, 161, and 178 bp long, representing 0.09, 0.33, and 0.05% of the *A. elata* genome, respectively.

Chromosome constitution

All 50 regenerated *A. elata* plants exhibited diploid $2n = 24$ chromosome complements, demonstrating uniformity with no variation in chromosome number (Supplementary Fig. 1). Both the mother plant and regenerants exhibited consistent chromosomal constitutions and FISH

karyotypes. The chromosome lengths of the mother plant ranged from 2.93 ± 0.50 to $4.79 \pm 0.72 \mu\text{m}$, and the short and long arms had average lengths of $1.77 \pm 0.30 \mu\text{m}$ and $2.20 \pm 0.39 \mu\text{m}$, respectively (Table 2). The chromosome lengths of the regenerants ranged from 2.58 ± 0.39 to $4.45 \pm 0.75 \mu\text{m}$, and the short and long arms had average lengths of $1.58 \pm 0.32 \mu\text{m}$ and $2.00 \pm 0.41 \mu\text{m}$, respectively (Table 3). No significant variation in chromosome length was observed. The mother plant and regenerants both had 11 pairs of metacentric chromosomes and one pair of submetacentric chromosomes. The total chromosome length (TCL) of the 50 regenerants varied slightly from that of the mother plant but not significantly (Supplementary Fig. 2).

Fluorescence in situ hybridization analysis

Multicolor FISH karyograms of both the *A. elata* mother plant and its regenerants were analyzed using six repeat probes: three newly developed AeTR49 (orange), AeTR161 (yellow), and AeTR178 (purple), and three additional universal probes for 5S rDNA (green), 45S rDNA (red), and telomeric repeats (blue) (Fig. 2). FISH probes exhibited clear signals within the chromosomes of the mother plant (Fig. 2A, B) and its regenerants (Fig. 2C, D). The FISH karyotypic idiogram in Fig. 2E illustrates the distribution of the repeat probes.

All plants exhibited distinct 45S rDNA signals in the distal region of the short arm of chromosome 3 and 5S rDNA signals in the intercalary region of chromosome 1. Telomeric signals were present across all chromosome ends. AeTR49 was consistently detected along the intercalary

regions of both the short and long arms of chromosomes, except in chromosomes 2 and 7, where the signals were restricted to the short arms. In contrast, AeTR161 was predominantly located at the centromeric regions, whereas AeTR178 was co-localized with *Arabidopsis*-type telomeric repeats at all chromosome ends (Fig. 2B, D and Tables 2, 3).

AeTR49 exhibited chromosome-specific distribution and was identified as an excellent cytogenetic marker for each chromosome. In contrast, AeTR161 and AeTR178 were distributed throughout the genome as centromeric and telomeric repeats, respectively. FISH signal distribution using the six repeat probes confirmed the chromosome-level constitution of *A. elata* and its regenerated plants (Supplementary Fig. 3).

The FISH signal distribution patterns of the mother plant and all regenerants were consistent, and no chromosome-level variations were detected. The genomic stability of the regenerated plants was confirmed, at least for specific sequence-related chromosome constitutions.

Estimation of nuclear DNA content

A histogram of the relative 2C DNA content was obtained via simultaneous analysis of *D. morbifera* as an internal reference and individual *A. elata* plants. The G0/G1 peaks of the reference standard were located in channel 200 (Fig. 3). Simultaneous flow cytometry analyses of the mother and each selected regenerated plant revealed minor variations in DNA content, with 2C values of 2.46 ± 0.04 and 2.41 ± 0.05 pg, respectively. Regenerated plants had lower relative 2C nuclear DNA content than native plants. However, according to Tukey's multiple

comparison test, this variation was not statistically significant at the 95% (Table 4). In addition, the genome sizes of the regenerants were not correlated with the observed variation in their TCL (Supplementary Fig. S3).

Discussion

Micropropagation can enhance the regeneration frequency and survival rates of plants, and well-developed regenerated *A. elata* plants have been documented [1,5,10,35]. However, cytogenomic information such as ploidy, chromosomal constitution, specific sequence distribution on the chromosome, and genomic DNA content, which are useful for confirming the genomic stability of micropropagated plants, have not yet been reported for regenerated *A. elata* plants. This multistep regeneration process, which involves somatic embryo formation and maturation, can induce somaclonal variations, leading to genomic and epigenomic reprogramming [1,14,36]. Therefore, evaluation of the cytogenomic constitutions of regenerated plants is imperative because variations in ploidy, chromosome constitution, and genome size can significantly influence the phenotypic characteristics and overall quality of regenerated plants. Furthermore, cytogenomic information is crucial to genomic research and breeding programs for crop improvement [37,38].

Flow cytometry and FISH have emerged as valuable tools for evaluating cytogenetic stability, as demonstrated in various studies on different plant species [17,39]. Specifically, PLOP-FISH enables comprehensive physical mapping, facilitating the monitoring of genomic

changes and discrimination of homologous chromosomes [18,20,28,32,33,40]. FISH results for the 5S and 45S rDNA probes in this study were consistent with those of a previous report [32]. However, clear telomeric repeat signals were observed at the ends of the short and long arms of chromosomes 1 and 3, respectively, which differed from those previously reported (Fig. 2). Considering that the chromosome end sequence is essential for telomere formation across all chromosomes, the results of the FISH study appear to be more accurate. This discrepancy might be due to the metaphase chromosome quality, telomeric probe concentration, and choice of the labeled fluorochrome ATTO425, which has lower emission and excitation wavelengths than Texas Red, which was used in our experiment [41].

FISH results using universal repeat probes of 5S and 45S rDNAs can provide useful information for identifying and pairing homologous chromosomes during karyotyping [28,31] and telomeric repeats when investigating genomic differentiation in plant species [18,40]. In addition, three more tandem repeat probes were developed in this study, which appeared to be excellent chromosome-specific repeat markers that could be used to discriminate homologous chromosome pairs in the *A. elata* genome and to examine the chromosome-level genomic constitution of regenerated plants. A pre-labeled oligonucleotide probe was developed from the newly mined tandem repeat AeTR49A, but not from the tandem repeats of AeTR161 and AeTR178 because these two repeats consist of long repeat sequences; thus, PLOP development is impractical [15]. These

newly developed repeat markers are expected to be useful in broader genomic research and breeding programs [36,42].

The correlation between genome size (GS) and total chromosome length (TCL) has been studied in various plant species. In particular, this correlation has been highlighted in *Triticum* and rye species [37], whereas similar observations have been made in Bromeliaceae [38] and Fabaceae [16]. However, this study found no significant correlation between GS and TCL. The relationship between GS and TCL may be affected by different pretreatment agents, treatment durations, and chromosome stages [43]. Further research involving different plant taxa is required to clarify the correlation between GS and TCL.

Given the challenges associated with maintaining the integrity of genetic material during *in vitro* propagation, achieving genetic stability in tissue cultures represents a significant milestone in plant biotechnology [11,44]. The unpredictable nature of genetic variation among plant species presents a formidable obstacle to the reliable production of genetically stable regenerants. Numerous factors contribute to these variations, from the initial choice of culture method to the complexity of plant genetics, including culture period, explant type, and ploidy [35,36,42]. Therefore, developing a customized micropropagation protocol and regularly evaluating the genetic stability of regenerants is essential.

This study presents cytogenomic tools for evaluating the chromosome-associated genetic stability of regenerated *A. elata* plants, which will aid in the development of breeding strategies for crop improvement.

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Author contribution statement

EAP performed the experiments, analyzed the data, and wrote the initial manuscript draft. HHK and NEW designed and conceptualized the study, analyzed the data, and revised the manuscript. HTN revised the manuscript. KHH and TJY supervised the study and revised the manuscript. All authors have approved the final manuscript.

Data availability

The authors confirm that all data supporting the findings of this study are available in the article and supplementary material.

Conflict of interest

The authors declare no conflicts of interest regarding the submitted work.

Figure legends

Fig. 1. Tissue culture-derived regenerants of *Aralia elata*. A) Callus formation from leaf explants in solid medium and B) liquid medium (bottom of Erlenmeyer flask). C) Somatic cotyledonary embryos developed from suspended calli, D) young regenerants, and E) fully developed regenerants of *A. elata*.

Fig. 2. Distribution of repeat markers in *Aralia elata* mother plant and the regenerants. Respective fluorescence *in situ* hybridization (FISH) metaphase spread and karyogram of *A. elata* mother plant (A, B) and its regenerants (C, D) elucidate the distribution of the repeat marker in the chromosome complement of $2n = 24$. The three repeat probes of 5S (green) and 45S (red) rDNAs, telomeric repeat (blue), and three genome-specific repeat sequences of AeTR49 (orange), AeTR161 (yellow), and AeTR178 (purple) were revealed as cytogenetic markers for chromosome pairing and identification. Chromosome pairing and identification were based on the distribution of the repeats, and they were arranged in decreasing length. E) The ideogram illustrates the detailed distribution of the six repeats. The telomeric probe was illustrated equally regardless of the signal intensity. Scale bar = 10 μ m.

525 **Fig. 3. Flow cytogram of *Aralia elata*.** A, B) Estimation of relative
526 genomic DNA content from the mother plant (M) and tissue culture-
527 derived regenerants (R) of *A. elata* (1st peak) using *Dendropanax morbifera*
528 as the reference standard (2nd peak). n = 9.

529 **Tables**

530

531 **Table 1.** Summary of major repeats identified in this study through RepeatExplorer to evaluate the cytogenetic stability of
532 the regenerants.

Repeat	Unit length h (bp)	AT Content t (%)	PLOP/prime r	Sequences	Genome Proportion (%)	PLOP/ primer length (bp)	Fluorochrome	Distribution
AeTR49	49	40	AeTR49 (PLOP)	AACAAGATGGTGAGCAA CCCTGGGAAGGGCTGA GATCCACGCGAACCAC	0.09	49	Texas red	Intercalary
AeTR161	161	62.7	AeTR161_F	AGC AAT CTC ATC ACT ATC GCG T	0.33	22	FITC	Centromeric
			AeTR161_R	TCG AGA CTT GTG TGA GAT GT		20	FITC	Centromeric
AeTR178	178	55.1	AeTR178_F	CGG AAT CGA GTC CCG TAA GT	0.05	20	Cy3	Telomeric
			AeTR178_R	GGC GTC AAA CCA ACA CGA AA		20	Cy3	Telomeric

534 **Table 2. Chromosome constitution and fluorescence *in situ* hybridization (FISH) karyotype analysis of the *Aralia***
535 ***elata* mother plant.**

Chr. No	Chromosome length (µm)			Arm ratio l/s	Chr. type	FISH signals					
	Short arm (s)	Long arm (l)	Total (s + l)			5S rDNA	45S rDNA	Telomeri c repeat	AeTR49	AeTR161	AeTR178
1	1.57 ± 0.30	3.22 ± 0.48	4.79 ± 0.72	2.05 ± 0.22	sm	-	s	t	s + l	c	t
2	2.08 ± 0.34	2.41 ± 0.52	4.50 ± 0.77	1.32 ± 0.40	m	-	-	t	s	c	t
3	1.89 ± 0.35	2.43 ± 0.45	4.32 ± 0.68	1.38 ± 0.30	m	s	-	t	s + l	c	t
4	2.01 ± 0.37	2.22 ± 0.42	4.23 ± 0.66	1.12 ± 0.23	m	-	-	t	s + l	c	t
5	1.90 ± 0.35	2.21 ± 0.32	4.11 ± 0.60	1.18 ± 0.18	m	-	-	t	s + l	c	t
6	1.79 ± 0.32	2.23 ± 0.34	4.02 ± 0.62	1.26 ± 0.16	m	-	-	t	s + l	c	t
7	1.79 ± 0.28	2.14 ± 0.36	3.94 ± 0.60	1.20 ± 0.14	m	-	-	t	s	c	t
8	1.84 ± 0.32	2.02 ± 0.31	3.86 ± 0.61	1.10 ± 0.10	m	-	-	t	s + l	c	t
9	1.78 ± 0.36	1.98 ± 0.36	3.77 ± 0.61	1.13 ± 0.21	m	-	-	t	s + l	c	t
10	1.65 ± 0.22	1.98 ± 0.41	3.63 ± 0.61	1.21 ± 0.15	m	-	-	t	s + l	c	t

11		1.83 ± 0.34	3.46 ± 0.56	1.14 ± 0.13	m	-	-	t	s + l	c	t
	1.62 ± 0.26										
12		1.65 ± 0.37	2.93 ± 0.50	1.31 ± 0.23	m	-	-	t	s + l	c	t
	1.27 ± 0.20										
Average:		2.20 ± 0.39	3.96 ± 0.63	1.28 ± 0.20							
	1.77 ± 0.30										

536 s: short arm, l: long arm, sm: submetacentric, m: metacentric, c: centromeric, t: telomeric

537 **Table 3. Average chromosome constitution and fluorescence *in situ* hybridization (FISH) karyotype analysis in**
538 **50 *Aralia elata* regenerants.**

Chr. No	Chromosome length (µm)			Arm ratio l/s	Chr. type	FISH signals					
	Short arm (s)	Long arm (l)	Total (s + l)			5S rDNA	45S rDNA	Telomere	AeTR49	AeTR161	AeTR178
1	1.42 ± 0.24	3.02 ± 0.59	4.45 ± 0.75	2.14 ± 0.35	sm	-	s	t	s + l	c	t
2	1.90 ± 0.40	2.27 ± 0.46	4.13 ± 0.81	1.24 ± 0.17	m	-	-	t	s	c	t
3	1.85 ± 0.42	2.17 ± 0.49	4.02 ± 0.82	1.19 ± 0.23	m	s	-	t	s + l	c	t
4	1.77 ± 0.44	2.11 ± 0.40	3.88 ± 0.78	1.26 ± 0.38	m	-	-	t	s + l	c	t
5	1.77 ± 0.36	2.02 ± 0.50	3.79 ± 0.74	1.17 ± 0.29	m	-	-	t	s + l	c	t
6	1.69 ± 0.27	1.98 ± 0.50	3.67 ± 0.67	1.18 ± 0.25	m	-	-	t	s + l	c	t
7	1.66 ± 0.40	2.00 ± 0.44	3.66 ± 0.81	1.22 ± 0.15	m	-	-	t	s	c	t
8	1.59 ± 0.36	1.84 ± 0.35	3.43 ± 0.63	1.19 ± 0.24	m	-	-	t	s + l	c	t
9	1.45 ± 0.31	1.87 ± 0.32	3.32 ± 0.57	1.32 ± 0.22	m	-	-	t	s + l	c	t
10	1.45 ± 0.30	1.77 ± 0.35	3.22 ± 0.57	1.25 ± 0.24	m	-	-	t	s + l	c	t

11		1.66 ±	2.98 ±	1.28 ±	m	-	-	t	s + l	c	t
	1.32 ± 0.26	0.30	0.50	0.55							
12		1.39 ±	2.58 ±	1.17 ±	m	-	-	t	s + l	c	t
	1.19 ± 0.15	0.27	0.39	0.18							
Average:	1.58 ±0.32	2.01 ±0.41	3.59 ±0.67	1.30 ±0.24							

539 s: short arm, l: long arm, sm: submetacentric, m: metacentric, c: centromeric, t: telomeric

540 **Table 4. Chromosome length and genomic DNA content estimation in *Aralia elata* mother plant and regenerants.**

Plant Samples	Chr. no. (2n)	Chromosome length	DNA content (2C, pg) ^a		
			Min.	Max.	Mean $\pm$ SD ^b
Mother plant	24	2.93 $\pm$ 0.50 - 4.79 $\pm$ 0.72 μ m	2.40	2.51	2.46 $\pm$ 0.04
Regenerant s	24	2.58 $\pm$ 0.67 - 4.45 $\pm$ 0.75 μ m	2.31	2.47	2.41 $\pm$ 0.05

541 ^an = 9.

542 ^bMean + Standard deviation, $P > 0$.

Figures



Figure 5



Figure 6



Figure 7

Supplementary Files

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