Print ISSN: 2287-9358 Online ISSN: 2287-9366
Plant Breed. Biotech. 2026 (November) 14.320~330
https://doi.org/10.9787/PBB.2026.14.320

Plant Breeding and Biotechnology

Research Article

Cytogenetic Characterization of Seven *Zoysia* Cultivars using Fluorescent *in situ* Hybridization and Flow Cytometry

Saraswathi Umavathi[1], Byung Yong Kang[1], and Hyun Hee Kim[1*]

[1]Chemistry & Life Science Department, Chromosome Research Institute, Sahmyook University, Seoul, 01795, Republic of Korea

*Corresponding to
Hyun Hee Kim
TEL. +82-2-3399-1715
E-mail. kimhh@syu.ac.kr

Abstract *Zoysia* spp. are economically important warm-season turfgrasses widely used in landscaping. Despite their horticultural importance, cytogenetic differentiation among *Zoysia* cultivars remains poorly characterized. This study compared seven cultivars using pre-labeled oligonucleotide fluorescence *in situ* hybridization (PLOP-FISH) and flow cytometry. We investigated chromosome number and morphology, 5S and 45S rDNA distribution and signal intensity, telomeric repeat distribution, and nuclear DNA content. All seven cultivars possessed $2n = 40$ chromosomes, consistent with previously reported tetraploidy. 5S rDNA distribution was highly conserved, with two loci in every cultivar. However, their signal intensity differed: 'Halla Green 10', 'Halla Green 7', 'Anyang Joonggi', 'Jangsung Chorok', and 'Zenith' exhibited two strong 5S rDNA signals; whereas 'Sechan' and 'Jangsung Saetbyeol' exhibited one major and one minor 5S rDNA signal. In contrast, 45S rDNA was more varied: 'Halla Green 10', 'Jangsung Chorok', 'Jangsung Saetbyeol', and 'Zenith' had two major signals; 'Halla Green 7' exhibited one major and one minor signal; while 'Sechan' and 'Anyang Joonggi' had two major and two minor signals. The latter two cultivars possessed a colocalized pair of 5S-45S rDNA loci, with the colocalized 45S signal weaker than the major 45S signal. Each chromosome contained telomeric repeats at its termini. Flow cytometry revealed limited variation in nuclear DNA content (0.80 – 0.85 pg). Overall, *Zoysia* cultivars exhibited highly conserved chromosome complements and genome size but varied in rDNA signal number and intensity. These rDNA features may provide useful cytogenetic characteristics for cultivar differentiation and warrant evaluation across larger germplasm panels.

Key words Cytogenomic, Genome size, PLOP-FISH, rDNA, *Zoysia* cultivars

Introduction

Zoysiagrass (*Zoysia* spp.) is a perennial warm-season turfgrass widely cultivated in temperate and subtropical regions. Valued for drought tolerance, low maintenance, dense turf-forming ability, and adaptability to environmental stress, they are extensively used in residential and commercial landscaping, including golf courses, parks, and gardens. *Zoysia* comprises 11 known species distributed mainly across East Asia and the Pacific region (Bae et al. 2024; Chen et al. 2011; Loch et al. 2017; Tanaka et al. 2016). *Zoysia japonica*, *Z. matrella*, and *Z. pacifica* (also known as *Z. tenuifolia*) are particularly important in turfgrass production.

Breeding programs have generated numerous *Zoysia* cultivars with desired characteristics, including winter hardiness, texture, growth habits, and extended green-up periods. Existing properties such as drought tolerance and turf density have also been enhanced. This study investigated seven cultivars developed for the local climatic conditions of South Korea, namely 'Halla Green 10', 'Halla Green 7', 'Zenith', 'Jangsung Chorok', 'Jangsung

Received: September 28, 2026. Revised: October 20, 2026. Accepted: November 6, 2026. Published: November 20, 2026.

Copyright © 2026 by the Korean Society of Breeding Science
This is an Open-Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Saetbyeol', 'Sechan', and 'Anyang Joonggi' (Cho et al. 2007; Choi et al. 2018; Choi et al. 2020; Yang et al. 2021a; Yang et al. 2021b). These cultivars are commercially and horticulturally relevant *Zoysia* germplasm; however, their substantial phenotypic similarities can hamper accurate morphological identification. Thus, analyses of differences in growth characteristics and breeding backgrounds are necessary to accurately characterize these cultivars.

Previous genomic research on *Zoysia* have identified substantial conservation and structural variations (Tanaka et al. (2016). Comparative analyses of zoysiagrass sequences highlight the importance of genome-level information in understanding the chromosomal and overall evolutionary history of this genus (Tanaka et al. 2016). Cytogenetic characterization can complement these genomic studies, providing direct information on chromosome structure and physical distribution of repetitive sequences. The integration of these two techniques enables cultivar-level comparisons even in germplasm without whole-genome assemblies, facilitating the translation of genome-level insight into practical chromosomal markers.

Most *Zoysia* possess $2n = 40$ chromosomes, with a basic chromosome number of $x = 10$ (Chen et al. 1962; Forbes 1952; Gould 1968; Loch et al. 2017; Yaneshita et al. 1999). Cytological and molecular genetic evidence, including restriction fragment length polymorphism (RFLP) linkage mapping (Yaneshita et al. 1999), typically classifies *Zoysia* as an allotetraploid genus. The highly conserved chromosome count facilitates interspecific hybridization and breeding. However, individual chromosomes often cannot be distinguished through morphology alone, because metaphase chromosomes are relatively small and similar to each other.

Ribosomal DNA (rDNA) provides useful landmarks for cytogenetic characterization. In particular, the 5S and 45S rDNA families are tandemly repeated sequences that occupy specific chromosomal regions but vary in copy number, distribution, and signal intensity. Although rDNA loci are generally conserved within related taxa, changes in rDNA copy number, amplification, deletion, and chromosomal rearrangement can generate detectable differences across species, populations, and cultivars. Such variations are valuable for distinguishing between genetically similar germplasms. Another important cytogenetic marker is telomeric repeats, key stabilizers of chromosomal integrity located at the terminal ends. Detecting telomeric repeats can also reveal interstitial telomeric sequences that reflect historical chromosome fusion or rearrangement.

Fluorescence *in situ* hybridization (FISH) enables localization of repetitive DNA sequences on chromosomes. The development of pre-labeled oligonucleotide probes (PLOPs) eliminated post-labeling procedures in FISH, thus increasing the speed of a reproducible and high-resolution technique for detecting target sequences (Waminal et al. 2018). The PLOP-FISH innovation is particularly advantageous in cases when conventional chromosome identification is inadequate. However, to the best of our knowledge, PLOP-FISH has not yet been applied to characterize rDNA and telomeric repeats across multiple *Zoysia* cultivars.

Flow cytometry provides a rapid and reliable method for estimating nuclear DNA content and assessing genome size and ploidy (Bourge et al. 2018). Previous flow cytometric studies have found relatively small differences in genome size across *Zoysia* species and their cultivars (Arumuganathan et al. 1999; Schwartz et al. 2010). However, we do not know whether these minor differences translate to detectable variations in rDNA locus organization.

Therefore, this study aimed to establish comparative cytogenomic profiles of seven *Zoysia* cultivars, using

a combination of PLOP-FISH and flow cytometry. We investigated chromosome number and morphology, 5S and 45S rDNA distribution and signal intensity, telomeric repeat localization, and nuclear DNA content. The resulting cytogenetic profiles provide chromosome-level characteristics for cultivar characterization and may provide valuable data for *Zoysia* breeding and germplasm management.

Materials and Methods

Plant materials

The study investigated the following *Zoysia* cultivars: 'Halla Green 10', 'Halla Green 7', 'Zenith', 'Jangsung Chorok', 'Jangsung Saetbyeol', 'Sechan', and 'Anyang Joonggi', spanning multiple breeding backgrounds and horticultural characteristics (Table 1). Plant materials were obtained from the National Forest Seed Variety Center of the Korea Forest Service (Chungju, Korea). Plants were grown and maintained in a greenhouse at 25 ± 2℃ under 60-80% relative humidity.

Table 1. Characteristics and origin of seven selected *Zoysia* cultivars.

Cultivar	Origin	Characteristics	Reference
'Halla Green 10'	*Z. matrella* × *Z. japonica*	Dwarf with extended greening periods in low-temperature conditions	Yang et al. 2021a
'Halla Green 7'		Dwarf with a greening period-extending phenotype in low-temperature conditions	Yang et al. 2021b
'Sechan'		Short with a greening period-extending phenotype in low-temperature conditions	Bae et al. 2024
'Anyang Joonggi'	*Z. japonica*	Heat resistance, drought resistance, and dense growth and soft texture	Choi et al. 2020
'Jangsung Chorok'		Highly adapted to the Korean climate, excellent winter hardiness with the ability to withstand common turf diseases and environmental stressors	Choi et al. 2018
'Jangsung Saetbyeol'		Early spring green-up and faster growth rate	Choi et al. 2018
'Zenith'		Dark green, medium-textured, warm-season grass with excellent drought tolerance and heat resistance	Choi et al. 2006

Chromosome preparation

Fresh root tips were collected from growing plants and pretreated with 2 mM 8-hydroxyquinoline for 5 hours at 18℃. Root tips were fixed overnight in freshly prepared 1:3 (v/v) aceto-ethanol solution and stored in 70% ethanol at 4℃ until chromosome preparation, which followed Waminal et al. (2012). Briefly, fixed root tips were washed with distilled water and digested (90 minutes, 37℃) in a solution containing 1% pectolyase and 2% cellulase. The digested material was fixed in a chilled aceto-ethanol solution and centrifuged at 13,000 rpm for 30 seconds. After removing the supernatant, the pellet containing chromosomal material was resuspended in a 9:1 (v/v) aceto-ethanol solution. Suspensions were dropped onto slides in a humid chamber at 80℃. After air drying, slides were treated with 2% formaldehyde for 5 minutes, rinsed with distilled water, and dehydrated with 100% ethanol. Well-spread metaphase chromosomes were selected for counting and karyotyping.

Pre-labeled oligonucleotide probe for FISH

Pre-labeled oligonucleotide probes targeting 5S rDNA, 45S rDNA, and telomeric repeats were used for FISH (Supplementary Table 1). Probe design used four oligonucleotide sequences targeting the 5S rDNA region, as well as eight and four oligonucleotide sequences targeting the 18S and 5.8S regions of 45S rDNA, respectively. The telomeric probe was designed based on the common *Arabidopsis*-type telomeric repeat $(TTTAGGG)_4$. Oligonucleotide probes were labeled at the 5′ end with Alexa Fluor 488 for 5S rDNA, Cy3 for 18S/5.8S rDNA, and Atto 425 for telomeric repeats (Bioneer Corporation, Daejeon, South Korea). PLOP-FISH was performed as described by (Waminal et al. 2018), with minor modifications. The FISH master mixture (32 μL) contained 50% formamide, 10% dextran sulfate, and 2× SSC with 25 ng of each PLOP; nuclease-free water was added to achieve a final volume of 40 μL per slide.

After adding the hybridization mix, chromosomal DNA on glass slides was denatured at 75℃ for 3 minutes and incubated overnight at 37℃. Stringency washes were conducted using 2× SSC for 15 minutes at room temperature (22 ± 2℃), 0.1× SSC for 25 minutes at 42℃, and 2× SSC for 5 minutes at room temperature. The slides were dehydrated using an ethanol series (70%, 90%, 100%; 3 minutes each). Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) and mounted on Vectashield antifade medium. At least 30 well-spread metaphase cells per cultivar were used for karyotype analysis. FISH signals were categorized as major or minor based on relative fluorescence intensity assessed consistently across representative metaphase spreads.

Karyotype analysis

Metaphase chromosomes were observed under a BX53 fluorescence microscope (Olympus, Tokyo, Japan) equipped with a DFC365 FS CCD camera (Leica Microsystems, Wetzlar, Germany). Images were analyzed in Cytovision (version 7.2; Leica Microsystems), then merged and processed in Adobe Photoshop CS6. Chromosome lengths were measured in ImageJ (version 1.51 K). Chromosomes were paired according to their relative length, morphology, and FISH signal distribution. Chromosome counts per cultivar, along with positions of 5S rDNA, 45S rDNA, and telomeric repeats were recorded.

Flow cytometric estimation of genome size

Flow cytometry was used to estimate relative nuclear DNA content (2*C*) following (Bourge et al. 2018). For nuclear preparations, five fully expanded leaves were randomly collected from each cultivar. *Zea mays* was used as the reference standard. Approximately 1-2 cm^2 of leaf tissue per cultivar was crushed in 500 μL ice-cold LB01 buffer, then filtered through 50 μm and 20 μm nylon mesh filters. After staining with propidium iodide and RNase A to visualize nuclei, samples were incubated at 4℃ and analyzed in a CytoFLEX flow cytometer (Beckman Coulter, Brea, CA, USA). Measurements were performed in triplicate. Nuclear DNA content was calculated from the ratio of sample to standard fluorescence intensity. The conversion factor was 1 pg DNA = 0.978×10^9 bp. The coefficient of variation (CV) was maintained below 5% for all measurements. Relative genome size (2*C*) was calculated as follows:

$$Sample\ \ 2C\ DNA\ \ (pg) = \frac{Sample\ \ G_0/G_1\ \ Peak\ \ Mean}{Maize\ \ G_0/G_1\ \ Peak\ \ Mean} \times 5.43\ \ pg \quad (1)$$

Results

Results from PLOP-FISH indicated that all seven cultivars possessed $2n = 40$ somatic chromosomes, confirming the presence of a highly conserved chromosome complement among the examined germplasm (Fig. 1; Table 2). Chromosome morphology was generally symmetrical and did not differ between cultivars, although chromosome lengths did, ranging from 0.57 to 3.92 μm (Table 2). 'Anyang Joonggi' had the shortest chromosomes (range = 0.57-1.74 μm), whereas 'Sechan' chromosomes had the greatest range (1.87-3.92 μm).

5S rDNA loci were highly conserved across all seven cultivars (each with two signals), but signal intensity varied (Fig. 1; Table 2). 'Halla Green 10', 'Halla Green 7', 'Anyang Joonggi', 'Jangsung Chorok', and 'Zenith' all had two major (strong) 5S rDNA signals. In contrast, 'Sechan' and 'Jangsung Saetbyeol' had one major and one minor 5S rDNA signal. These PLOP-FISH results suggest that between-cultivar variation may involve differences in the relative abundance or organization of rDNA arrays rather than differences in locus number. 'Sechan' and 'Jangsung Saetbyeol' may possess a lower copy number of one 5S rDNA locus or exhibit differential organization of the corresponding 5S rDNA array.

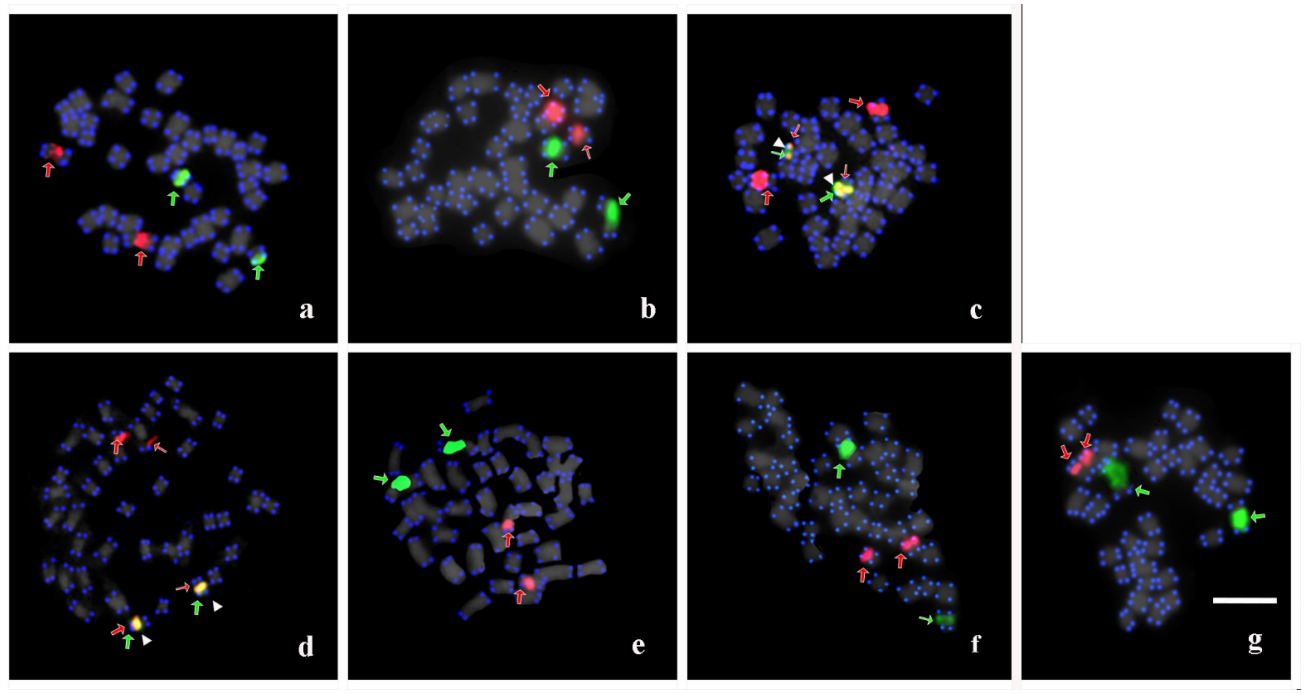


Fig. 1. Triple-color metaphase chromosome spreads of seven *Zoysia* cultivars, obtained from PLOP-FISH using universal probes of 5S rDNA, 45S rDNA, and *Arabidopsis*-type telomeric repeats. Respectively, 5S rDNA, 45S rDNA, and telomeric repeats were labeled with Alexa Fluor 488 (green), Cy3 (red), and Atto 425 (blue) probes. a) 'Halla Green 10', b) 'Halla Green 7', c) 'Sechan', d) 'Anyang Joonggi', e) 'Jangsung Chorok', f) 'Jangsung Saetbyeol', and g) 'Zenith'. Thick and thin arrows indicate major and minor loci of 5S rDNA and 45S rDNA. White arrowheads denote 5S-45S rDNA colocalization. Scale bar = 5 μm.

Table 2. Triple-color PLOP-FISH of seven *Zoysia* cultivars using universal probes of 5S rDNA, 45S rDNA, and telomeric repeat.

Cultivar	Chromosome composition		Signal intensity (chr. position)[z)]		5S-45S co-localization	Tel	Genome size (2*C*)	
	Number (2*n*)	Length (μm)	5S	45S			Mbp	pg
'Halla Green 10'	40	0.64 - 1.37	2s (#7^{pi})	2s (#7^{pi})	-	40 (c)	831	0.85
'Halla Green 7'	40	1.19 - 2.63	2s (#7^{pi})	1s+1w (#7^{pt})	-	40 (c)	812	0.83
'Sechan'	40	1.87 - 3.92	1s+1w (#7^{pst})	2s+2w (#7^{pst})	2	40 (c)	822	0.84
'Anyang Joonggi'	40	0.57 - 1.74	2s (#7^{pst})	2s+2w (#7^{pst})	2	40 (c)	802	0.82
'Jangsung Chorok'	40	1.26 - 3.39	2s (#7^{pi})	2s (#7^{pst})	-	40 (c)	831	0.85
'Jangsung Saetbyeol'	40	0.66 - 2.06	1s+1w (#7^{pi})	2s (#7^{pst})	-	40 (c)	782	0.80
'Zenith'	40	1.04 - 2.61	2s (#7^{pi})	2s (#7^{pst})	-	40 (c)	802	0.82

Note: 5S: 5S rDNA, 45S: 45S rDNA, Tel: telomeric repeats, c: common *Arabidopsis*-type
[z)] p: short arm, i: interstitial region, st: sub-telomeric region, t: telomeric region, s: major loci, w: minor loci

In contrast to 5S rDNA conservation, 45S rDNA signals varied considerably more in both signal number and intensity. 'Halla Green 10', 'Jangsung Chorok', 'Jangsung Saetbyeol', and 'Zenith' exhibited two major 45S rDNA signals. 'Halla Green 7' showed one major and one minor 45S rDNA signal. 'Sechan' and 'Anyang Joonggi' exhibited four (two major and two minor) 45S rDNA signals (Fig. 2; Table 2). In summary, although rDNA distribution in chromosomes was broadly conserved, variations in 45S rDNA signal intensity and the presence of minor loci provided greater cytogenetic differentiation between cultivars than 5S rDNA.

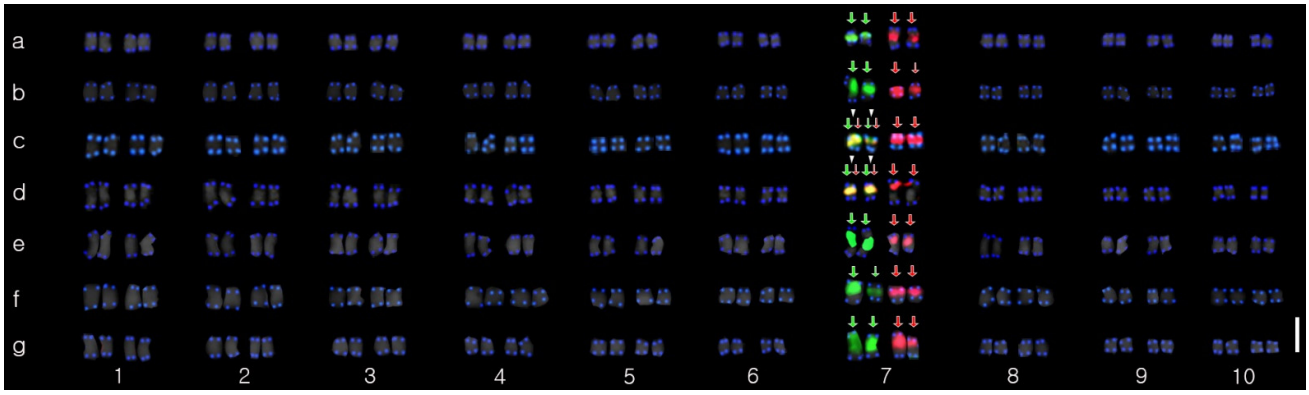


Fig. 2. Triple-color karyogram of seven *Zoysia* cultivars, obtained from PLOP-FISH with universal probes of 5S rDNA (Alexa Fluor: green), 45S rDNA (Cy3: red), and *Arabidopsis*-type telomeric repeats (Atto 425: blue). a) 'Halla Green 10', b) 'Halla Green 7', c) 'Sechan', d) 'Anyang Joonggi', e) 'Jangsung Chorok', f) 'Jangsung Saetbyeol', and g) 'Zenith'. Thick and thin arrows indicate major and minor loci of 5S rDNA and 45S rDNA. White arrowheads denote 5S-45S rDNA colocalization. Scale bar = 5 μm.

The chromosomal positions of the 5S and 45S rDNA loci were conserved across the seven *Zoysia* cultivars, with all loci detected on chromosome #7. However, their relative positions on the chromosome were cultivar-specific (Table 2). The chromosome bearing the rDNA loci was designated chromosome #7 based on relative chromosome length, morphology, and FISH signal distribution. In 'Halla Green 10', 'Halla Green 7', 'Jangsung Chorok', 'Jangsung Saetbyeol', and 'Zenith', 5S rDNA loci were situated in the interstitial region of the short arm. In 'Sechan' and 'Anyang Joonggi', they were located at the subtelomeric region of the short arm. 45S rDNA loci were found at the interstitial regions in 'Halla Green 10', the terminal region in 'Halla Green 7', and the subtelomeric

region in ‘Sechan’, ‘Anyang Joonggi’, ‘Jangsung Chorok’, ‘Jangsung Saetbyeol’, and ‘Zenith’. Consequently, the major between-cultivar differences in rDNA organization involved the relative positions of loci on chromosome #7, particularly the interstitial and subtelomeric regions.

The cultivars ‘Sechan’ and ‘Anyang Joonggi’ had a pair of colocalized 5S and 45S rDNA loci (Fig. 3). These colocalized 45S rDNA signals were consistently weaker than the major 45S rDNA signals, indicating that the colocalized region may represent a relatively small array. The repeated association of 5S and 45S rDNA in our plant material suggests that this chromosomal arrangement may represent a characteristic feature of the *Zoysia* genome, although its distribution and copy number varied across cultivars. We identified *Arabidopsis*-type telomeric repeat signals at the termini of all 40 chromosomes in every cultivar.

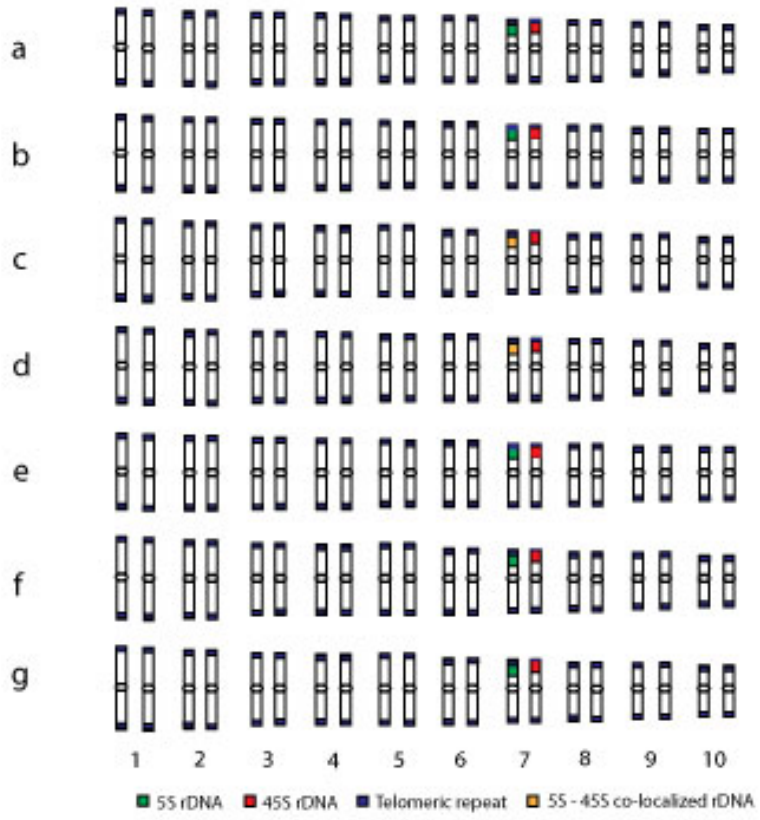


Fig. 3. Haploid idiogram of seven *Zoysia* cultivars using universal probes of 5S rDNA, 45S rDNA, and *Arabidopsis*-type telomeric repeats. a) ‘Halla Green 10’, b) ‘Halla Green 7’, c) ‘Sechan’, d) ‘Anyang Joonggi’, e) ‘Jangsung Chorok’, f) ‘Jangsung Saetbyeol’, and g) ‘Zenith’.

Flow cytometry revealed relatively limited variation in nuclear DNA content among the seven cultivars (Fig. 4; Table 2), reflecting a highly conserved genome size across cultivars. Nuclear DNA content ranged from 0.80 to 0.85 pg. Within this narrow range, ‘Jangsung Saetbyeol’ had the least nuclear DNA (0.80 pg), whereas ‘Jangsung Chorok’ and ‘Halla Green 10’ had 0.85 pg. These relatively small differences may reflect variation in repetitive DNA abundance, although the present data do not allow the contribution of rDNA to be determined specifically.

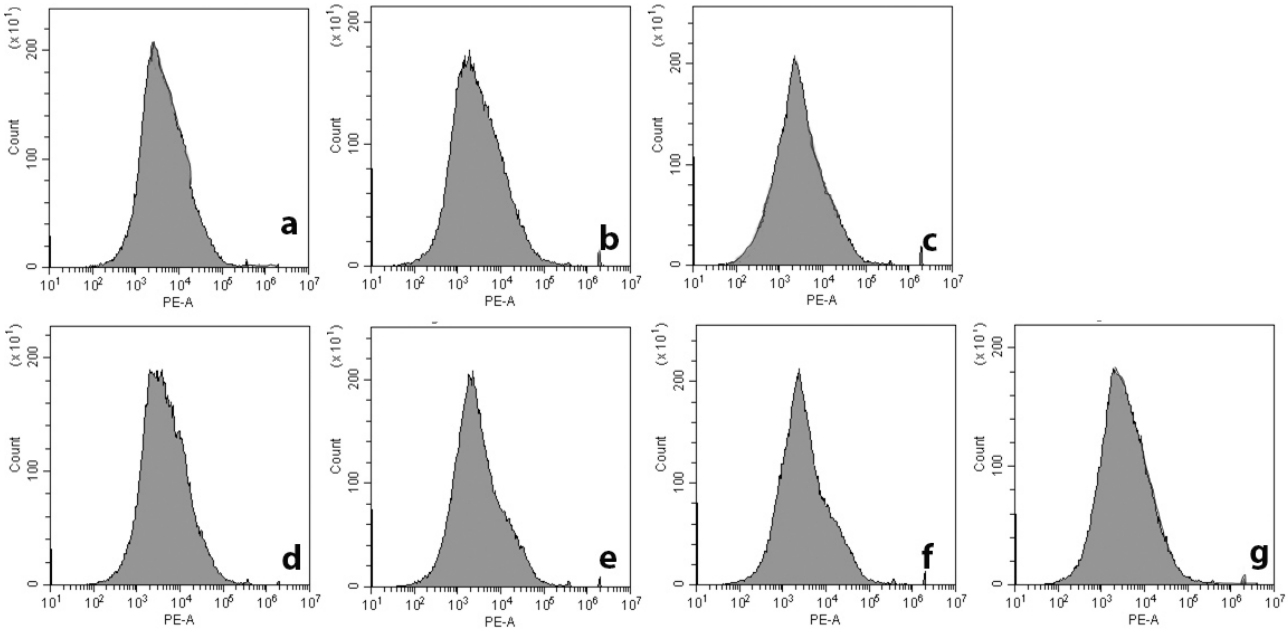


Fig. 4. Histogram of relative nuclear DNA content, with the dominant distribution corresponding to G1 nuclei of *Zoysia* cultivars obtained by flow cytometry. a) 'Halla Green 10', b) 'Halla Green 7', c) 'Sechan', d) 'Anyang Joonggi', e) 'Jangsung Chorok', f) 'Jangsung Saetbyeol', and g) 'Zenith'.

Discussion

Here, we demonstrated that the seven tested *Zoysia* cultivars were highly conserved in chromosome complement. The finding that every cultivar had $2n = 40$ chromosomes agrees with previous cytological and molecular studies characterizing *Zoysia* as predominantly tetraploid, with a basic chromosome number of $x = 10$ (Chen et al. 1962; Forbes 1952; Kole 2011; Loch et al. 2017; Yaneshita et al. 1999). Our analyses using FISH addressed weaknesses of previous research that verified allotetraploidy using techniques such as RFLP linkage maps (Yaneshita et al. 1999), but could not distinguish between cultivars because of an identical chromosome number. Here, we applied chromosome-level markers, particularly repetitive DNA sequences, to determine between-cultivar genomic variation. Our PLOP-FISH work confirms the importance of comparative genomic analyses for understanding chromosome organization and rDNA variation in zoysiagrasses (Tanaka et al. (2016). We demonstrated that the chromosome complement is highly conserved at the cytogenetic level, whereas rDNA signal intensity provides detectable distinctions across cultivars.

One of our most important findings was the contrasting behavior of 5S and 45S rDNA. All seven cultivars had two 5S rDNA loci, reflecting high conservation, although signal intensity differed between them. In contrast, 45S rDNA varied in both signal number and intensity. These PLOP-FISH results suggest that the major source of between-cultivar variation may involve differences in the relative abundance or organization of rDNA arrays rather than differences in locus number. In contrast, the presence of one major and one minor 5S rDNA signal in 'Sechan' and 'Jangsung Saetbyeol' indicates differences in relative array abundance or organization. Similar

outcomes in other genera include variations of highly repeated DNA sequences during *Secale* diversification (Cuadrado et al. 1997, 2002) and chromosomal variations in rDNA distribution among *Crocus* (Frello et al. 2000). Overall, rDNA arrays can undergo unequal recombination, amplification, deletion, and other structural changes that generate differences in copy number and FISH signal intensity (Jiang et al. 1994; Raskina et al. 2004). Despite this promising contribution to cultivar identification, a note of caution is needed regarding the interpretation of our findings. Because FISH signal intensity was not quantitatively calibrated against rDNA copy number, the observed differences should be considered relative variations in signal strength rather than direct evidence of copy number differences.

The consistent localization of rDNA loci on chromosome #7 indicates a highly conserved chromosomal organization within our *Zoysia* germplasm. Specifically, 5S rDNA loci were predominantly situated in the interstitial region of the short arm, while 45S rDNA loci were primarily in the subtelomeric region of the same arm, indicating differential localization of the two rDNA families. Notable exceptions were 'Sechan' and 'Anyang Joonggi,' where 5S rDNA was found in the subtelomeric region, as well as 'Halla Green 10' and 'Halla Green 7', the only cultivars with 45S rDNA in the interstitial and telomeric regions, respectively. These positional differences provide additional chromosomal characteristics for comparative identification. Nevertheless, our findings overall suggest that the basic chromosomal framework of rDNA has remained stable, with localized positional changes possibly occurring during cultivar diversification.

The observation of 5S-45S rDNA colocalization is particularly noteworthy because the two arrays are typically located at distinct chromosomal positions. This feature is not unique to *Zoysia*; several other plant groups also exhibit linked or colocalized 5S and 45S rDNA loci (Barros e Silva et al. 2013). Nevertheless, the current association observed among several of the *Zoysia* cultivars examined in the present study suggests that this chromosomal configuration may represent a relatively conserved feature within the studied germplasm (Umavathi et al. 2026a). Although not sufficiently distinctive to serve as a universal marker for *Zoysia* cultivar identification, this colocalization may provide a useful cytogenetic reference for comparative characterization of the germplasm examined here. Simultaneously, the difference in signal intensity indicates that copy number may vary within the locus. Further investigations using chromosome-specific probes and sequence-level analyses would help determine whether the colocalization is ancestral to the examined cultivars or arose through subsequent chromosomal rearrangements.

The seven cultivars exhibited a remarkably narrow range of nuclear DNA content, ranging from 0.80 to 0.85 pg. Thus, although previous reports have found genome size variation among *Zoysia* species and cultivars (Schwartz et al. (2010), the relatively narrow range observed in the present study indicates limited variation in nuclear DNA content among the seven cultivars examined. Similarly, Arumuganathan et al. (1999) reported a 2*C* nuclear DNA content of approximately 0.86 pg for *Z. japonica* cultivar 'Zenith'. Additionally, variation in repetitive DNA sequences, including rDNA, may contribute to the observed differences in total nuclear DNA content. However, the present flow-cytometry data do not allow attribution specifically to rDNA, and the overall genomic size remained relatively stable. In general, genome size can change following polyploidization through the loss or amplification of repetitive sequences. This "genome downsizing" has been documented in many polyploid species (Leitch et al. 2004). Further investigation using whole-genome sequencing and repeatome analysis will be necessary to determine whether the small variation in genome size across *Zoysia* cultivars reflects differential accumulation

or elimination of repetitive DNA.

The seven *Zoysia* cultivars examined here exhibited considerable structural stability, given the consistent conservation of chromosome number, telomeric organization, and genome size. Concurrently, variation in rDNA copy number and signal intensity indicates that localized repetitive regions can evolve while the overall chromosome complement remains conserved, similar to prior observations in zoysiagrasses (Tanaka et al. 2016). Thus, our cytogenetic findings provide additional evidence that chromosome-level conservation coexists with localized variations in repetitive DNA sequences. This combination may benefit breeding programs by supporting cultivar identification, informed parental selection, and monitoring of chromosome inheritance through rDNA variation. Cytogenetic markers, such as 5S rDNA and 45S rDNA, can facilitate the confirmation of parental identity, track chromosome inheritance, identify unusual germplasm, and investigate chromosome behavior in inter- and intra-specific hybrids (Umavathi et al. 2026a; Umavathi et al. 2026b). However, the present study primarily demonstrates variation in rDNA signal number, intensity, and position, rather than validated chromosome-specific markers. Precise chromosome tracking would require further chromosome-specific marker development.

Our study has several limitations. First, the use of universal rDNA and telomeric repeat probes constrains precise identification and discrimination of individual homologous chromosomes. Additionally, variations in FISH signal intensity are likely relative differences in rDNA abundance, meaning we lack direct quantification of rDNA copy number. Furthermore, the analysis was confined to seven cultivars and does not represent the complete cytogenomic diversity of *Zoysia* germplasm. Nevertheless, we successfully established a comparative cytogenomic framework for *Zoysia* that can serve as a starting point for future research. Specifically, PLOP-FISH should be incorporated with chromosome-specific oligonucleotide probes, chromosome-level genome assemblies, repeatome analysis, and quantitative rDNA copy number estimation to yield more comprehensive insight into chromosome organization, genome evolution, and cultivar differentiation. Such advancements would also support the development of chromosome-specific cytogenetic markers, enhance accurate cultivar identification, and facilitate future *Zoysia* breeding programs.

References

Arumuganathan K, Tallury S, Fraser M, Bruneau A, Qu R. 1999. Nuclear DNA content of thirteen turfgrass species by flow cytometry. Crop Sci. 39(5): 1518-1521.

Bae E, Jung J. 2024. Development of the New Zoysiagrass Cultivar 'Sechan'. Weed Turf. Sci. 13(4): 153-161.

Barros e Silva A, dos Santos Soares Filho W, Guerra M. 2013. Linked 5S and 45S rDNA sites are highly conserved through the subfamily Aurantioideae (Rutaceae). Cytogenet. Genome Res. 140(1): 62-69.

Bourge M, Brown SC, Siljak-Yakovlev S. 2018. Flow cytometry as tool in plant sciences, with emphasis on genome size and ploidy level assessment. Genet. Appl. 2(2): 1-12.

Chen C-C, Hsu C-C. 1962. Cytological studies on Taiwan grasses. 2. Chromosome numbers of some miscellaneous tribes. J. Jpn. Bot. 37: 300-313.

Chen Z, Lin Z, Guo C. 2011. Grass Flora of Taiwan: Pharoideae, Ehrhartoideae, Pooideae, Centothecoideae, Arundinoideae, Aristidoideae, Chloridoideae. Endemic Species Research Institute.

Cho YS, Ahn SH, Yang GM, Choi JS. 2007. Establishment Speed of 'Zenith' Zoysiagrass by Plugging Compared to Commercial Cultivars in Korea. Korean J. Turfgrass Sci. 21(1): 9-21.

Choi J, Yang G, Bae E, Lee K, Shon Y. 2020. Seed yields and germinabilities of Zoysiagrass species, cultivars and seed type breeding lines. Weed Turf. Sci. 9: 379-387.

Choi J-S, Yang G-M, Oh C-J, Lee G-J, Bae E-J, Lee K-S. 2018. Development of new cultivars 'Jangsung Chorok' and 'Jangsung Saetbyeol'' in zoysiagrass. Weed Turf. Sci. 7(3): 231-238.

Cuadrado A, Jouve N. 1997. Distribution of highly repeated DNA sequences in species of the genus Secale. Genome. 40(3): 309-17.

Cuadrado A, Jouve N. 2002. Evolutionary Trends of Different Repetitive DNA Sequences During Speciation in the Genus Secale. J. Hered. 93(5): 339-345.

Forbes I Jr. 1952. Chromosome numbers and hybrids in *Zoysia*. Agron. J. 44(4): 194-199.

Frello S, Heslop-Harrison JS. 2000. Chromosomal Variation in Crocus vernus Hill (Iridaceae) Investigated by *in situ* Hybridization of rDNA and a Tandemly Repeated Sequence. Ann. Bot. 86(2): 317-322.

Gould FW. 1968. Grass systematics. McGraw-Hill Book Company, New York, NY.

Jiang J, Gill BS. 1994. New 18S.26S ribosomal RNA gene loci: Chromosomal landmarks for the evolution of polyploid wheats. Chromosoma. 103(3): 179-185.

Kole C. 2011. Wild crop relatives: Genomic and breeding resources. Springer.

Leitch I, Bennett M. 2004. Genome downsizing in polyploid plants. Biol. J. Linn. Soc. 82(4): 651-663.

Loch DS, Ebina M, Choi JS, Han L. 2017. Ecological Implications of *Zoysia* species, distribution, and adaptation for management and use of zoysiagrasses. Int. Turfgrass Soc. Res. J. 13(1): 11-25.

Raskina O, Belyayev A, Nevo E. 2004. Quantum speciation in *Aegilops*: Molecular cytogenetic evidence from rDNA cluster variability in natural populations. Proc Natl Acad Sci U S A. 101(41): 14818-14823.

Schwartz BM, Kenworthy KE, Engelke M, Genovesi AD, Odom RM, Quesenberry KH. 2010. Variation in 2*C* nuclear DNA content of *Zoysia* spp. as determined by flow cytometry. Crop Sci. 50(4): 1519-1525.

Tanaka H, Hirakawa H, Kosugi S, Nakayama S, Ono A, Watanabe A, et al. 2016. Sequencing and comparative analyses of the genomes of zoysiagrasses. DNA Res. 23(2): 171-180.

Umavathi S, Kang BY, Kim HH. 2026a. Triple-color PLOP-FISH analyses and genome size estimation in three *Zoysia* species. Hortic. Environ. Biotechnol. 67(3): 523-529.

Umavathi S, Kang BY, Shin H, Kwon H-Y, Kim HH. 2026b. Triple-color PLOP-FISH and Genome Size Analyses of Three *Hibiscus syriacus* F1 Hybrids and Parental Cultivars. Plant Molecular Biology Reporter. 44(1): 1-9.

Waminal N, Pellerin R, Kim N, Jayakodi M, Park J, Yang T, et al. 2018. Rapid and efficient FISH using pre-labeled oligomer probes. Sci Rep. 8: 8224.

Waminal NE, Kim HH. 2012. Dual-color FISH karyotype and rDNA distribution analyses on four Cucurbitaceae species. Hortic. Environ. Biotechnol. 53: 49-56.

Yaneshita M, Kaneko S, Sasakuma T. 1999. Allotetraploidy of *Zoysia* species with 2*n*= 40 based on a RFLP genetic map. Theor. Appl. Genet. 98(5): 751-756.

Yang D-H, Jeong O-C, Sun H-J, Kang H-G, Jin I-D, Lee H-Y. 2021a. Development of Cultivar 'Halla Green 10' in Zoysiagrass. Korean J. Breed. Sci. 53(4): 467-474.

Yang D-H, Sun H-J, Jeong O-C, Kang H-G, Song I-J, Jin I-D, et al. 2021b. Development of 'Halla Green 7': A Zoysiagrass Cultivar with Greening Period Extending Phenotype at Lower Temperatures. Korean J. Breed. Sci. 53(4): 458-466.