# Variation in 2C Nuclear DNA Content of *Zoysia* spp. as Determined by Flow Cytometry

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**ABSTRACT**

Little is known about the variation of 2C nuclear DNA content in zoysiagrass (*Zoysia* Willd.). Therefore, a glasshouse study including 20 cultivars and 16 experimental genotypes of *Zoysia japonica* Steud., *Z. macrantha* Desv., *Z. matrella* (L.) Merr., *Z. minima* (Colenso) Zotov, *Z. pacifica* (Goudswaard) M. Hotta & Kuroki, *Z. pauciflora* Mez, and select interspecific hybrids was conducted in 2008 at Gainesville, FL. Nuclei were stained with propidium iodide, and flow cytometry (FCM) analysis was conducted with a laser cytometer. Genotypes from *Z. minima* and Z. *matrella* had the largest (0.96 pg) and smallest (0.77 pg) 2C nuclear DNA content, respectively. The observed 0.19 pg spread between zoysiagrass species was less than variation reported in other tetraploid warm-season grasses within the same species. Results further suggest that midparent values for 2C nuclear DNA content of interspecific hybrids between *Z. minima* and other *Zoysia* spp. may be statistically detectable. Zoysiagrasses have one of the smallest genome sizes but are extremely diverse with respect to morphology, growth habit, and response to abiotic and biotic stresses. This information supports the large genome constraint hypothesis that species with small genome sizes are better able to adapt to environmental changes due to a lack of evolutionary limitations associated with large amounts of repetitive DNA.

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**Abbreviation:** AFLP, amplified fragment length polymorphism; CRBC, chicken red blood cell; FCM, flow cytometry; PMC, pollen mother cell; RFLP, restriction fragment length polymorphism.

Zoysiagrasses, *Zoysia* Willd., are perennial turf species that are adapted to warmer regions of the world but can persist through areas of the northern transition zone. This versatility, in combination with low fertility requirement, slow growth, and varying leaf textures, make them a viable choice for use in residential lawns, in commercial landscapes, and on golf courses. Breeding efforts are currently underway to improve abiotic and biotic stress responses, which will further improve the range of adaptation to environments that are unsuitable for older cultivars.

Anderson (2000) suggests that the genus *Zoysia* comprises 11 species that vary with respect to morphology and nuclear DNA constitution. Speciation appears to have occurred through geographic isolation (Kim, 1983; Weng et al., 2007) rather than by genetic changes associated with an increase or decrease in ploidy level (Forbes, 1952). All cytological studies of *Zoysia* spp. have determined that $2n = 4x = 40$ (Arumuganathan et al., 1999; Chen and Hsu, 1962; Christopher and Abraham, 1974; Forbes, 1952; Murray et al., 2005; Tateoka, 1955), except for an unexplained report of a diploid plant, $2n = 2x = 20$, collected from Sri Lanka (Gould and Soderstrom, 1974). Forbes (1952) theorized that *Zoysia* spp. behaved

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genetically diploid in nature but did not disregard the possibility that the basic chromosome number could be 5 or 10. Chen and Hsu (1962) suspected that the basic chromosome number was 10 based on cytological studies in other eragrostoid grasses. This was later confirmed by Gould (1968). Zoysiagrasses are currently described as allotetraploids based on restriction fragment length polymorphism (RFLP) linkage mapping and analysis (Yaneshita et al., 1999). The absence of anaphase bridges, fragments, lagging chromosomes, univalents, or multivalents during meiosis I in conjunction with the formation of 95% viable pollen (Forbes, 1952) supports the allotetraploid classification.

Taxonomic assignment in *Zoysia* has been predominately weighted toward morphological and molecular marker variation rather than by reproductive isolation as suggested by Forbes et al. (1955) in accordance with the biological species concept (Mayr, 1948). Genetic variability has been characterized through random amplification of polymorphic DNA (RAPD) (Choi et al., 1997; Weng et al., 2007), RFLP (Anderson, 2000; Yaneshita et al., 1999; Yaneshita et al., 1997), amplified fragment length polymorphism (AFLP) (Cai et al., 2004), and simple sequence repeat (SSR) (Cai et al., 2005; Tsuruta et al., 2005) marker analyses to discern relatedness among zoysiagrass species. Cross-compatibility does exist between a number of the Zoysia species (Forbes, 1952; Hong and Yeam, 1985), although Engelke and Anderson (2003) observed low germination percentages between a few interspecific combinations.

Flow cytometry (FCM) was first developed for analyzing animal cells. The techniques and instrumentation were modified for lysis of the plant cell wall and separation of the nucleus from plastid and mitochondrial DNA. The chopping method has largely replaced the primordial digestion maceration technique (Galbraith, 1990; Heller, 1973). This methodology provides rapid and accurate ploidy determination and DNA content analysis for plant breeding programs (Arumuganathan and Earle, 1991a; de Laat et al., 1987; Dolezel et al., 1989; Kron et al., 2007; Ochatt, 2008). A major application of FCM has been the general characterization of plant species for their DNA contents (Arumuganathan and Earle, 1991b; Bennett and Leitch, 1995), but other uses include analysis of plant cell cycles (Galbraith et al., 1983), sex identification in dioecious plants (Costich et al., 1991), the estimation of C-banded constitutive heterochromatin (Rayburn et al., 1992), and use as a taxonomic marker when the gain or loss of DNA is correlated with evolutionary relationships between species (Ohri, 1998). More recent innovative uses of FCM include the localization of supernumerary (B) chromosomes (Sharbel et al., 2004) and chromosome sorting for physical mapping of genomes (Dolezel et al., 2007).

Reports of variation in DNA content from *Zoysia* spp. are limited. In an analysis of native New Zealand grasses, Murray et al. (2005) concluded that, in general, tropical grasses ($C_4$) have lower DNA contents than those of temperate origin ($C_3$). *Zoysia pauciflora* Mez. ranked the lowest of all grasses surveyed with a 2C value of 0.97 pg. *Zoysia minima* (Colenso) Zotov was recorded to have a genome size of 0.99 pg. Arumuganathan et al. (1999) reported the 2C nuclear DNA content of 'Zenith', a seeded *Z. japonica* Steud. cultivar, to be $0.86 \pm 0.00$ pg.

Flow cytometry has become a useful tool for plant breeders to characterize the nuclear DNA content and ploidy level in other warm-season turfgrass species. Taliaferro et al. (1997) developed an accurate method to determine ploidy level and genome size in bermudagrass (*Cynodon* spp.). This methodology was used by Wu et al. (2006) to study the genetic diversity of Chinese bermudagrass germplasm. Triploid, tetraploid, pentaploid, and hexaploid genotypes were identified and further characterized with AFLPs. Johnson et al. (1998) used FCM to distinguish diploid, tetraploid, and hexaploid buffalograss (*Bouteloua dactyloides* [Nutt.] Columbus) germplasm for breeding purposes. In doing so they discovered three previously undescribed pentaploid clones. Jarret et al. (1995) surveyed the nuclear DNA contents of 35 *Paspalum* species and determined that there was insufficient variation to discriminate between genotypes within a species but that ploidy evaluation was accurate and repeatable. Vaio et al. (2007) observed genome sizes in natural dallisgrass (*Paspalum dilatatum* Poir.) tetraploids that were less than twice the size of their diploid progenitors. This reduction in DNA content was not present in synthetically created allotetraploid dallisgrasses. This phenomenon, documented in other polyploid species, was credited to "genome downsizing" (Leitch and Bennett, 2004).

Genome analysis by FCM has been classified as a rapid and reliable method to identify cytotypes with varying ploidy levels in Kentucky bluegrass (*Poa pratensis* L.) (Barcaccia et al., 1997; Huff and Bara, 1993). Eaton et al. (2004) were able to distinguish true hybrids derived from both intra- and interspecific hybridizations, and Wieners et al. (2006) differentiated genotypes with the four major reproductive pathways in Kentucky bluegrass according to the presence and position of peaks. Other cool-season grasses in which ploidy level variation has been researched utilizing FCM include fine fescue (*Festuca* spp.) (Huff and Palazzo, 1998), ryegrass (*Lolium* spp.) (Barker et al., 2001), and bentgrass (*Agrostis* spp.) (Bonos et al., 2002).

Comprehensive investigations of nuclear DNA content and ploidy level in the *Zoysia* genus are largely absent. Therefore, this research was initiated to (i) characterize the variation in 2C nuclear DNA content of available cultivars and experimental lines representing *Zoysia japonica*, *Z. macrantha* Desv., *Z. matrella* (L.) Merr., *Z. minima*, *Z. pacifica* (Goudswaard) M. Hotta & Kuroki, and *Z. pauciflora*, and select interspecific hybrids between these species through FCM and (ii) determine if sufficient variation exists between 2C nuclear DNA content within and

among *Zoysia* species to statistically identify intermediate nuclear DNA contents from $F_1$ hybrids.

## MATERIALS AND METHODS

### Plant Materials

Zoysiagrass materials consisted of 20 cultivars and 16 experimental lines from five species and five interspecific hybridizations (Table 1). Genotypes were assigned to their respective species based on floral (spikelet number and shape, pedicel length, raceme length, and peduncle length) and vegetative (leaf blade width, leaf blade shape, and culm internode length) morphology, not by genotypic characterization. Each genotype was vegetatively propagated into three 5-cm pots containing a 50% sand and 50% Metro-Mix 250 (Scotts-Sierra Horticultural Products Co., Marysville, OH) growing medium in January 2008. Plants were organized in a completely randomized design with three replications and grown in a glasshouse in Gainesville, FL, with supplemental overhead irrigation. Fertilizer was applied monthly with Peters Professional 20–20–20 General Purpose Water Soluble Fertilizer (Scotts-Sierra Horticultural Products Co., Marysville, OH) at a rate of 2.4 g $m^{-2}$. Leaf canopies were trimmed twice monthly except for overhanging, aerial stolons.

### Flow Cytometry

Flow cytometry analysis was initiated when at least one aerial stolon was present in each pot. The terminal node and tip from one stolon was removed from each experimental unit and stored on ice before FCM analysis. A CyStain PI Absolute P (05-5002, Partec North America, Inc., Mt. Laurel, NJ) nuclei extraction and DNA staining kit was used to prepare samples using a technique modified from Arumuganathan and Earle (1991a). The terminal end of each stolon was chopped with a double-edge razor blade in a glass petri dish with 500 μL of extraction buffer for approximately 60 s. The resulting solution, containing isolated nuclei, was pipetted into a 5-mL test tube through a 50-μm nylon mesh filter cap. One drop of chicken red blood cell (CRBC) standard (BioSure Inc., Grass Valley, CA) was then added to the test tube and nuclei were treated and stained with a 2-mL solution containing 0.3% ribonuclease (RNase) and 0.6% propidium iodide. Samples were incubated at 4°C for 60 min in the dark. Flow cytometry analysis was conducted at the University of Florida's Interdisciplinary Center for Biotechnology Research on a LSR-II cytometer (BD Biosciences, San Jose, CA) using a 100-mW solid-state laser emitting at 488 nm to excite the propidium iodide. DNA peak data, based on 10,000 scanned particles, were quantified using FACS DiVa v5.2 software (BD Biosciences, San Jose, CA). The mean 2C nuclear DNA content of each sample, measured in picograms (pg), was adjusted based on the peak of the CRBC internal standard by taking the ratio of the plant sample peak mean and the CRBC peak mean and then multiplying by the 2C nuclear DNA content of the internal standard, that is, 2.5 pg (Rasch, 1985; Tiersch et al., 1989). Genotypic 2C nuclear DNA contents are means of three replicates.

### Cytology

The chromosome numbers of 'Diamond', 'Meyer', and 5194-5 zoysiagrasses were determined using pollen mother cell (PMC) squashes. For preparation, racemes were harvested when one to three florets had emerged from the boot. The inflorescences were fixed by immersing the racemes in 95% ethanol, glacial acetic acid, and chloroform (6:3:1 v:v:v) for a period of 24 to 48 h. Fixed racemes were then washed twice in 70% ethanol and transferred to a staining solution (Snow's Carmine; Snow, 1963), fully immersed, and left to stain overnight. The stained racemes were preserved by transfer to 70% ethanol.

For observation of PMC chromosomes, the anthers were dissected from individual florets, placed on a slide, immersed in a drop of Aceto-orcein, and squashed beneath a slide cover. Chromosome numbers were determined by viewing cells in metaphase and anaphase using a phase contrast microscope (Wild Heerbrugg, Heerbrugg, Switzerland). Pictures of PMCs were taken using an Infinity 1-3C camera (Lumener Corp., Ottawa, ON, Canada) mounted on the microscope. Chromosome numbers were confirmed from the pictures.

### Morphological Measurements

Zoysiagrasses are commonly classified by their leaf width. A digital caliper was used to measure the widest section of three fully expanded and mature leaf blades in each experimental unit. The average value of the subsamples in each experimental unit was used for further analysis.

### Statistical Analysis

The distribution of data for both 2C nuclear DNA content and leaf blade width was assessed with a histogram and normal probability plot for normality. An analysis of variance was performed on each of the measured traits to test whether genotypes, species determined by morphology, and where appropriate, genotypes within species varied. Genotype and species means were separated using a Waller–Duncan *K*-ratio LSD when the main effect was found to be significant. Pearson correlation coefficients were computed to test whether 2C nuclear DNA contents were associated with leaf blade widths.

## RESULTS AND DISCUSSION

Consistent extraction and staining of nuclei was not achieved using young, freshly harvested leaves according to standard protocol (Arumuganathan and Earle, 1991a). This is probably due to the high silica content in the leaves (Ruemmele and Engelke, 1990) that renders physical chopping very difficult. Tissue from root and rhizome tips also resulted in inconsistent FCM analysis. Only analysis of plant material from the terminal node and tip of a stolon yielded consistent and repeatable results, producing peak CV < 6.0% for all genotypes.

Genotypes varied ($p \leq 0.01$) for 2C nuclear DNA content (Table 1). In our study, the 2C nuclear DNA contents of Zenith (0.83 pg $2C^{-1}$) and 5194-5 (0.96 pg $2C^{-1}$), a representative of Z. minima, were both ~3% lower than those reported by Arumuganathan et al. (1999) and Murray et al. (2005), respectively. These small differences could be attributed to sample preparation or between-lab instrumental variation (Dolezel et al., 1998). *Zoysia matrella* was the only species in which genotypes significantly differed

**Table 1. Means for 2C nuclear DNA content and leaf blade width of *Zoysia* genotypes for five species and five interspecific hybridizations.**

| | | 2C DNA content | | Leaf morphology | | | 2C DNA content | Leaf morphology |
|---|---|---|---|---|---|---|---|---|
| Developing institution | Genotype | Genotypic mean | SE | Blade width | Leaf texture¶ | *Zoysia* species | Species mean | Blade width |
| | | pg | pg | mm | class | | pg | mm |
| Texas A&M Univ. | 5194-5‡ | 0.96 a§ | 0.01 | 0.44 u§ | Very fine | *Z. minima* | 0.96 a§ | 0.44 e§ |
| Texas A&M Univ. | 5504-6 | 0.94 ab | 0.02 | 0.96 r | Very fine | *Z. matrella* × *Z. minima* | 0.92 b | 0.93 d |
| Texas A&M Univ. | 5458-39 | 0.90 bc | 0.00 | 0.90 rs | Very fine | | | |
| Texas A&M Univ. | 5335-3 | 0.89 cd | 0.02 | 3.90 e | Coarse | *Z. macrantha* × *Z. matrella* | 0.89 bc | 3.90 a |
| Texas A&M Univ. | 5193-19 | 0.88 c–f | 0.01 | 2.23 kl | Medium | *Z. macrantha*# | 0.88 c | 2.65 b |
| Texas A&M Univ. | 5186-16 | 0.88 c–g | 0.01 | 3.06 h | Coarse | | | |
| Texas A&M Univ. | 5463-9 | 0.88 c–g | 0.01 | 0.83 s | Very fine | *Z. matrella* × *Z. pauciflora*# | 0.87 cd | 0.75 d |
| Texas A&M Univ. | 5459-10 | 0.87 c–g | 0.00 | 0.67 t | Very fine | | | |
| Univ. of Florida | BA433 | 0.85 d–i | 0.03 | 0.46 u | Very fine | *Z. pacifica* | 0.85 cde | 0.46 e |
| Sod Solutions | Empire | 0.88 cd | 0.02 | 4.48 b | Coarse | *Z. japonica*# | 0.85 cde | 3.54 a |
| USDA and USGA† | Meyer‡ | 0.87 c–i | 0.01 | 3.52 g | Coarse | | | |
| Univ. of Florida | UFZ-10 | 0.86 c–i | 0.01 | 3.11 h | Coarse | | | |
| Univ. of Florida | Ultimate | 0.86 c–i | 0.02 | 2.51 j | Medium | | | |
| Texas A&M Univ. | Palisades | 0.86 c–i | 0.01 | 3.73 f | Coarse | | | |
| Seed Research of Oregon | Compadre | 0.86 c–i | 0.02 | 4.97 a | Coarse | | | |
| Univ. of California | El Toro | 0.85 d–k | 0.02 | 3.61 fg | Coarse | | | |
| Bladerunner Farms, Inc. | JaMur | 0.84 e–l | 0.01 | 4.17 c | Coarse | | | |
| Univ. of California | Victoria | 0.84 f–l | 0.01 | 2.57 j | Medium | | | |
| Sod Solutions | Empress | 0.84 g–l | 0.02 | 2.15 l | Medium | | | |
| Texas A&M Univ. | Crowne | 0.84 g–l | 0.00 | 4.07 cd | Coarse | | | |
| Patten Seed Company | Zenith | 0.83 h–m | 0.01 | 3.59 fg | Coarse | | | |
| Texas A&M Univ. | Cavalier | 0.87 c–h | 0.01 | 1.80 m | Fine | *Z. matrella*#†† | 0.84 de | 1.49 c |
| Texas Tech Univ. | Shadow Turf | 0.87 c–i | 0.01 | 1.35 q | Fine | | | |
| Texas A&M Univ. | Royal | 0.86 d–i | 0.01 | 1.60 no | Fine | | | |
| Pursley Turf Farms | Cashmere | 0.85 d–i | 0.02 | 1.66 n | Fine | | | |
| Bladerunner Farms, Inc. | Zeon | 0.85 d–j | 0.02 | 1.38 q | Fine | | | |
| USDA and USGA† | Emerald | 0.84 f–l | 0.02 | 1.49 p | Fine | | | |
| Univ. of Florida | Pristine | 0.83 g–m | 0.02 | 1.62 no | Fine | | | |
| Texas A&M Univ. | Zorro | 0.79 mn | 0.00 | 1.54 op | Fine | | | |
| Texas A&M Univ. | Diamond‡ | 0.77 n | 0.01 | 0.97 r | Very fine | | | |
| Texas A&M Univ. | 5337-46 | 0.83 i–m | 0.02 | 3.97 de | Coarse | *Z. japonica* × *Z. macrantha* | 0.83 e | 3.97 a |
| Texas A&M Univ. | 5343-52 | 0.86 c–i | 0.00 | 2.18 l | Medium | *Z. japonica* × *Z. matrella*# | 0.83 e | 2.33 b |
| Texas A&M Univ. | 5334-6 | 0.85 d–j | 0.00 | 2.78 i | Medium | | | |
| Texas A&M Univ. | 5332-52 | 0.81 j–n | 0.02 | 2.22 kl | Medium | | | |
| Texas A&M Univ. | 5283-5 | 0.81 k–n | 0.03 | 2.14 l | Medium | | | |
| Texas A&M Univ. | 5282-20 | 0.80 lmn | 0.02 | 2.34 k | Medium | | | |
| % CV | | 3.11 | | 1.87 | | | 3.71 | 10.19 |

†United States Department of Agriculture and United States Golf Association.

‡Chromosome counts confirmed $2n = 4x = 40$.

§Means within a column followed by the same letter are not different at $K = 100$ (approximates $Pp = 0.05$) according to Waller–Duncan LSD.

¶Leaf texture classifications assigned based on leaf width measurements (Very fine < 1.0mm; 1.0mm ≤ Fine < 2.0mm; 2.0mm ≤ Medium < 3.0mm; 3.0mm ≤ Coarse).

#Leaf blade widths were different for genotypes within species at the 0.01 level of probability.

††2C nuclear DNA contents were different for genotypes within species at the 0.01 level of probability.

($p \leq 0.01$) for 2C nuclear DNA content, although the 0.10 pg range between 'Cavalier' (0.87 pg 2C nucleus$^{-1}$) and Diamond (0.77 pg 2C nucleus$^{-1}$) is narrower than those found within species of other warm-season grass genera. The low measured values for Diamond and 'Zorro' were largely responsible for the detection of these differences.

After viewing a minimum of 20 meiotic cells of Diamond (Fig. 1), Meyer, and 5194-5, it was confirmed that these zoysiagrasses have $2n = 4x = 40$ chromosomes. This is the same number as reported for Zenith zoysiagrass (Arumuganathan et al., 1999) and other samples of *Z. japonica*, *Z. matrella*, and *Z. tenuifolia* (Forbes, 1952). Therefore, the observed 0.19 pg spread between Diamond, *Z. matrella* with 0.77 pg 2C nucleus$^{-1}$, and 5194-5, *Z. minima* with 0.96 pg 2C nucleus$^{-1}$ is representative of the naturally occurring evolutionary variability in 2C DNA content of tetraploid zoysiagrass. Similar ranges in 2C nuclear DNA content have been reported in other grasses within the same species and ploidy level. Taliaferro et al. (1997) and Wu et al. (2006) reported a range of 0.33 pg (2.03–2.36 pg 2C nucleus$^{-1}$) and 0.34 pg (1.96–2.30 pg 2C nucleus$^{-1}$), respectively, in tetraploid bermudagrasses. A 0.20 pg spread (2.23–2.43 pg 2C nucleus$^{-1}$) was reported for common dallisgrass (Jarret et al., 1995), whereas a larger range of 0.53 pg (1.77–2.30 pg 2C nucleus$^{-1}$) was observed in tetraploid buffalograss (Johnson et al., 1998). 2C nuclear DNA contents are typically higher in temperate than tropical grasses (Arumuganathan et al., 1999; Murray et al., 2005) resulting in proportionally larger ranges for this trait in cool season grasses of the same species and ploidy level (Barker et al., 2001; Bonos et al., 2002; Eaton et al., 2004; Huff and Palazzo, 1998).

Differences were found ($p \leq 0.01$) for 2C nuclear DNA content between *Zoysia* species and interspecific hybrids (Table 1). Only those between *Z. minima* and the rest of the group as a whole may be meaningful, although others were statistically significant. The ability to detect true interspecific hybrids between *Z. minima* and other *Zoysia* spp. is supported by the differences between *Z. minima*, *Z. matrella*, and the interspecific hybrids of the two. A representative *Z. pauciflora* genotype was unfortunately not available for FCM analysis. Murray et al. (2005) reported *Z. pauciflora* to have 0.97 pg 2C nucleus$^{-1}$. It may be inferred that differences between *Z. pauciflora*, *Z. matrella*, and interspecific hybrids of the two could be detected based on their respective 2C nuclear DNA contents.

Variation in leaf blade width was present between genotypes ($p \leq 0.01$) and species ($p \leq 0.01$). Large leaf widths were typically associated with *Z. macrantha* and *Z. japonica* genotypes, whereas those within *Z. minima* and *Z. pacifica* had the smallest leaves. This trait has been well characterized within and between *Zoysia* species (Anderson, 2000; Choi et al., 1997; Hong and Yeam, 1985; Kim, 1983; Yaneshita et al., 1997). Leaf blade width and 2C nuclear DNA content were not correlated ($r = -0.18$, $p > 0.05$). Knight et al. (2005) suggested that leaf anatomical trait (i.e., length, width, area, and mass) correlation with 2C nuclear DNA content is dependent on the species sampled. Leaf width was not correlated with 2C nuclear DNA content in buffalograss (Johnson et al., 1998), bentgrass (Bonos et al., 2002), napiergrass (*Pennisetum purpureum* Schumach.) (Taylor and Vasil, 1987), and switchgrass (*Panicum virgatum* L.) (Hultquist et al., 1997).

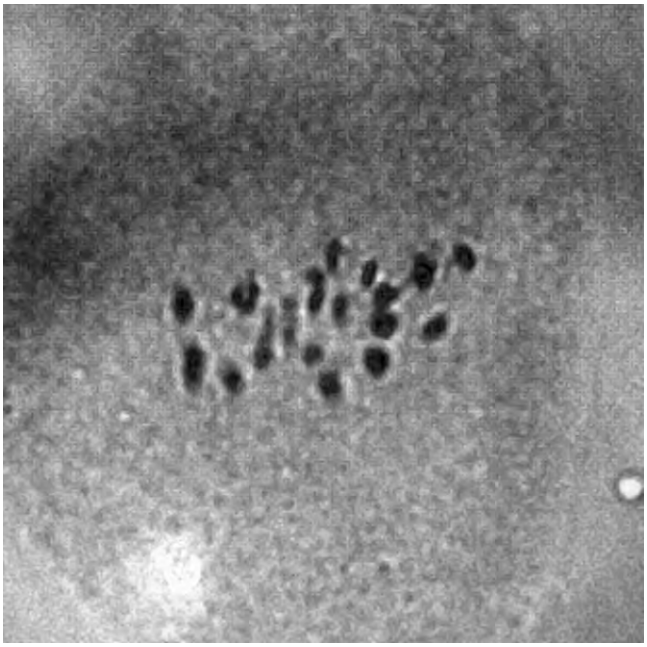

Fig. 1. Meiotic chromosome associations of Diamond zoysiagrass at late metaphase I to early anaphase I.

The speciation of zoysiagrass is partially linked to its dissemination over time throughout geographically isolated Pacific Rim countries (Weng et al., 2007). Greilhuber (1998) theorized that genome size variation between reproductively isolated populations will occur over time due to common chromosomal polymorphisms and spontaneous aberrations. *Zoysia* spp. have one of the smallest genome sizes in the Poaceae family (Murray et al., 2005). A tentative association has been made between species of increasing genome size and a decreased ability to adapt to extreme environments where conditions quickly change (Grime and Mowforth, 1982; Knight et al., 2005). Conceivably, the vast variation in morphological characteristics and establishment rates (Patton et al., 2007) in *Zoysia* spp. can be partially explained by its small genome size and the corresponding lack of evolutionary constraints associated with large amounts of repetitive DNA such as longer cell cycles and noncoding regions that can buffer the effects of mutation. This also could have a factor in the differential responses of zoysiagrasses to drought (Marcum et al., 1995; White et al., 2001), shade (Morton et al., 1991), salinity (Marcum et al., 1998; Qian et al., 2000), temperature (Patton and Reicher, 2007), disease (Green et al., 1994), insect (Braman et al., 2000; Reinert and Engelke, 2001), and nematode (Busey et al., 1982) pressures.

## CONCLUSIONS

Continuous variation for 2C nuclear DNA content across most of the *Zoysia* spp. studied herein was present but over a smaller range than in other warm-season grasses within the same species and ploidy level. Further quantification of 2C nuclear DNA content variation in additional *Zoysia* spp. and interspecific hybrids as they become available is needed before an association between genome size and species is deemed relevant. The rapid and accurate method for screening zoysiagrass germplasm using the terminal end of a stolon as described will allow for repeatable FCM analysis in the future.

An alternative viewpoint that merits reintroduction is the assignment of species based on reproductive compatibility (Mayr, 1948) rather than by genotypic and phenotypic variation that transverse multiple species within the genus. Murray (2005) emphasized the need to test for a reduction of fertility in hybrids between individuals that differed in C-value before the two are recognized as different species. This premise could apply to any plant characteristic, whether it is genome size or morphology. Leaf width was not correlated with 2C nuclear DNA content in the genotypes evaluated herein. Under the current taxonomic system, zoysiagrasses are often incorrectly distinguished as a species based on leaf texture alone. This confusion would be eliminated if the biological species concept was in use.

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