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Full Length Research Paper

Karyotype and nucleic acid content in *Zantedeschia aethiopica* Spr. and *Zantedeschia elliottiana* Engl.

Bimal Kumar Ghimire[1], Chang Yeon Yu[2], Ha Jung Kim[3] and Ill Min Chung[3]*

[1]Department of Ethnobotany and Social Medicine, Sikkim University, Gangtok- 737 102, Sikkim, India.
[2]Department of Applied Plant Sciences, Kangwon National University, Chuncheon 200-701, South Korea.
[3]Department of Applied Life Science, Konkuk University, Seoul 143-701, South Korea.

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Analysis of karyotype, nucleic deoxyribonucleic acid (DNA) content and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were performed in *Zantedeschia aethiopica* and *Zantedeschia elliottiana*. Mitotic metaphase in both species showed 2n=32. The chromosomes of both species were quite similar with medium length ranging from 1.55 ± 0.04 to 3.85 ± 0.12 µM in *Z. aethiopica* and 2.15 ± 0.04 to 3.90 ± 0.12 µM in *Z. elliottiana*. However, some differences were found in morphology and centromeric position among the chromosomes. Identification of individual chromosomes was carried out using chromosomes length, and centromeric positions. The karyotype of *Z. aethiopica* was determined to be 2n = 32 = 14 m + 18 sm and of *Z. elliottiana* to be 2n = 32 = 10 m + 22 sm. The 2C nuclear DNA content was found to be 3.72 ± 0.10 picograms (equivalent to 3638.16 mega base pairs) for *Z. aethiopica* and 1144.26 ± 0.05 picograms (equivalent to 1144.26 mega base pairs) for *Z. elliottiana*. Leaf protein analysis showed 11 and 9 bands for *Z. aethiopica* and *Z. elliottiana,* respectively, among which some were species specific. These results may provide useful information regarding *Zantedeschia* for the study of taxonomic relationships, genetics and breeding.

Key words: *Zantedeschia,* karyotype, mitotic metaphase, chromosomes, flow cytometry.

INTRODUCTION

Zantedeschia (Araceae), a genus constituting 28 species of herbaceous perennial plants is distributed mostly in tropical and temperate zones, and is native to South Africa (Mesquita et al., 2007). These species produces large, showy flower spathes with immense decorative value and are often grown both as ornamental plants and for cut flowers (Funnel, 1993; Kuehny, 2000). *Zantedeschia aethiopica* and *Zantedeschia elliottiana* have quite similar morphological and phenological characteristics, differing mainly in leaf characteristics and the number of dominant buds on the rhizome. *Z. aethiopica* (arum lily) with rich lustrous white spathe grows naturally in marshy areas, multiplying by rhizome-offsets. Under suitable growing conditions, it is potentially evergreen and flowers continuously (Funnell, 1993; Tjia, 1985). The tubers of *Z. aethiopica* have several dominant buds on the corns/rhizomes that produce several shoots on planting, and give a multi-branching growth habit (Funnel, 1993). On the other hand, *Z. elliottiana* is very beautiful for its remarkable, spacious, arrow-jaded green leaves which are frequently spotted and blotched with white color with rich lustrous yellow spathe and blooms in the summer (Tjia, 1985). The tubers of *Z. elliottiana* have one dominant bud exhibiting a monopodial growth habit and consequently low flowering potential (Tjia, 1985).

Chromosomal data have played an important role in the study of plant systematics and evolution at all levels (Mabberley, 1997). Study of chromosome size and morphology may help to indicate evolutionary relationship among plant species (Clark and Wall, 1996) and also in species identification and analysis of hybrid populations (Qu et al., 2004). It may also be useful for tracing chromosomes behaviors in interspecific hybrids and

*Corresponding author. E-mail: imcim@konkuk.ac.kr. Tel: +82-2-450-3730. Fax: +82-2-446-7856

backcross plants and in assigning linkage groups to chromosomes and mapping genes on the chromosomes arms (Lim et al., 2001). This family Araceae is well known for its extensive variations in karyotype. Inconsistencies in chromosome complement of somatic tissue in vegetatively producing species of *Zantedeschia* have been reported by Sharma and Datta (1961). However, precise descriptions of nucleic acid content of these two species have not been reported yet.

Therefore, to provide suitable information for germplasm preservation and genetic breeding, we described and compared herein the karyotypes of *Z. aethiopica* and *Z. elliottiana*. Any change in the somatic chromosome complement of a taxon might well be expected to have a corresponding change in the nucleic acid content. We also determined the cellular nucleic acid content and leaf protein content by biochemical studies to find the correlation if any, between alterations in somatic chromosome complement and change in nucleic acid level.

MATERIALS AND METHODS

Z. aethiopica and *Z. elliottiana* of the Araceae were collected from Bioherb Research Institute, Kangwon National University, Chuncheon, Korea, and maintained in a greenhouse at 28°C, with a 12 to 16 h photoperiod. Three plants growing in greenhouse were used as a ready source of materials for the cytological analysis and flow cytometric study of plants.

Preparation of somatic chromosome counting and karyotype analysis

Chromosomal study and biochemical studies were performed using three replicates plantlets each of *Z. aethiopica* and *Z. elliottiana*. Fresh and healthy root apical meristems were pretreated in saturated solution of para-dichlorobenzene for 4 h at 20°C with an initial treatment at 0°C for 5 min followed by fixation in glacial acetic acid ethanol (1:3) overnight. The root tips were then warmed for a few seconds in a mixture of 2% aceto-orcein and 1 N HCl (9:1), kept as such for 2 to 3 h and then squashed on a dry grease-free and clean slide in 45% acetic acid, sealed and observed under microscope. The slides were then made permanent on the following day by detachment of cover glass and dehydration in ethanol and mounted in digital picture exchange (DPX).

Nuclear DNA content

Flow cytometry

All the plant materials used for chromosomal study were also included in the biochemical studies. Briefly, about 0.5 cm^2 of leaf sample was chopped by razor blade in Petri dish (96 mm) with 0.5 ml ice-cold nuclei-isolation buffer A of the Partec high resolution DNA kit (Partec, Munster) and filtered through a 45 µM mesh nylon membrane into a 5 ml cytometry tube and centrifuged for 5 min at 150 g for 5 min at room temperature. Subsequently, 1.2 ml of staining propidium iodide (PI) solution was added and after incubation for 30 to 45 min at room temperature, fluorescence intensity of isolated nuclei was measured using a Partec CA-II flow cytometer. At least three different samples were measured for each plant. The absolute DNA amount of the sample was calculated by running it together with the standard *Lycopersicon esculentum* cv ‘‘Stupicke´ ’’ (2C=1.96 pg), according to method describe by Dolezel et al. (1992). 2C DNA amounts are expressed in mega base pairs (Mbp) according to the method described by Bennett et al. (2000). Nuclear DNA content (2C) was calculated as:

$$\text{DNA content (pg)} = \frac{\text{Sample peak mean} \times \text{2C DNA content of standard (pg)}}{\text{Standard peak mean}}$$

Leaf protein electrophoresis

Briefly, 50 mg of clean leaves for each sample was homogenized in a pre-cooled mortar and pestle with liquid nitrogen and 1 ml of extraction buffer (0.063 M Tris-HCl (pH-6.8), 5% mercaptoethanol, 2% sodium dodecyl sulphate (SDS) and 10% glycerol). The crude extracts were mixed by vortexing and boiled for 5 min in a water bath. The resulting mixture was centrifuged at 10,000 g for 20 min. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method described by Laemmli (1970), using a 4% stacking gel and 15% acrylamide gel in a water-cooled electrophoresis apparatus (Bio-Rad® instruments, USA). Protein electrophoresis by SDS-PAGE used 20 µg of protein in each lane. Gels were stained with Coomassie brilliant blue G and fixated by trichloroacetic acid as described by Blakesley and Boezi (1977).

Statistical analysis

All the experiments were repeated at least three times. The data shown represent the mean ± SD. The data were statistically analyzed using the one-way analysis of variance (ANOVA) and significant differences between the means were assessed by Duncan’s multiple comparison tests at $P<0.05$.

RESULTS AND DISCUSSION

The present investigations have been undertaken to investigate the chromosomal and bio-chemical background of two species of *Zantedeschia* of Araceae, *Z. aethiopica* and *Z. elliottiana*, respectively. A detailed karyotype analysis of two species of *Zantedeschia* reveals gross similarity of the chromosome complement. However, the species can be distinguished from one another by minor differences in chromosome structure, including chromosome length and position of primary and secondary constriction.

The somatic chromosome number for the material investigated has been verified from more than 20 metaphase plates. The stained chromosomes of both species of *Zantedeschia* are presented in Figures 1a and b, respectively. A detailed karyotype study revealed that both species are diploid, consisting of somatic chromosome number 2n = 32. This 2n chromosome number of *Z. aethiopica* is consistent with the findings of Wang et al. (2011), Zhang et al. (2011), Wu et al. (2008) and Yau et al. (1994a). In contrast to our result, Mohamed et al. (2006) and Darlington and Wylie (1955) reported 2n = 26 chromosomes number for *Z. aethiopica*.

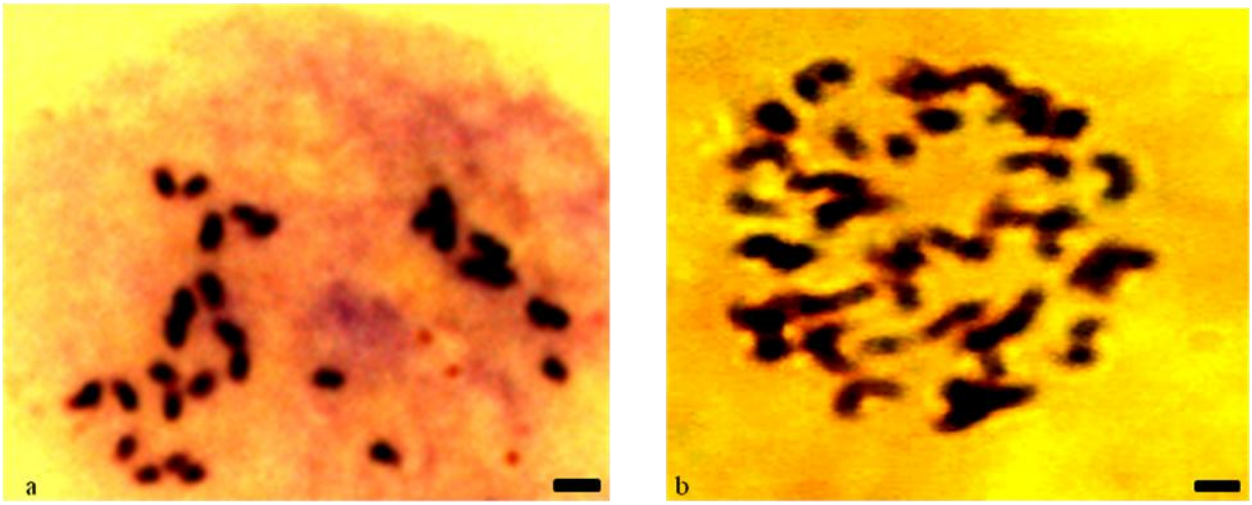

Figure 1. Somatic cells of (a) *Z. aethiopica* and (b) *Z. elliottiana* with chromosome number 2n=32. Bar = 50 µM.

Table 1. Mean of chromosome lengths and centromeric position of *Z. aethiopica.*

Chromosome type	Number of chromosome	Average length of chromosome[2] (µM)	Centromeric position[1]
A	1	3.85 ± 0.12[d]	SM
B	2	2.85 ± 0.15[c]	M
C	4	2.90 ± 0.19[c]	SM
D	3	2.90 ± 0.01[c]	M
E	5	2.50 ± 0.02[b]	M
F	1	1.55 ± 0.04[a]	M

[1]SM, Sub median region centromere; M, median region centromere. [2]Data having the same letter in a column were not significantly differed by Duncan's multiple comparison test (P<0.05).

Intra-specific variation in basic chromosome number in other members of Araceae has also been reported (Okada and Hambali, 1989; Coates et al., 1988; Cotias-de-Oliveira, 1999; Yi, 2002). This variability has often been attributed to rearrangement, amplification, or loss of heterochromatin, processes that finally result in speciation (Martel et al., 1997).

The intraspecific variation of nuclear DNA amount has previously been interpreted as ecological adaptation (Grime and Mowforth, 1982). However, a great stability of the nuclear genome has been reported for geographically isolated population of *Sesleria albicans* (Lysak et al., 2000), *Setaria* (Le Thierry et al., 1998) and in *Capsicum* (Moscone et al., 2003). The karyotype structure of *Z. aethiopica* and *Z. elliottiana* displays distinct differences in chromosomal organization. In this study, based on two characteristics discussed below, six chromosomes, type A, B, C, D, E and F were present in both species (Tables 1 and 2). Type E chromosomes were the most frequent type in both species and had different lengths. The total chromosomes length varied significantly in *Z. aethiopica* and *Z. elliottiana* with average length of 16.55 and 16.10 µM, respectively (Tables 1 and 2).

Furthermore, based on the position of the centromere, the chromosomes were divided into two groups. Group I consisted of chromosomes pairs with median centromere and group II consists of chromosomes with sub median centromere. The karyotype for Z. *aethiopica* and *Z. elliottiana* was formulated as 2n = 32 = 10 m + 22 sm and 2n = 32 = 14 m+18 sm, respectively (Table 3). Though the karyotypes analysis reveals the presence of mostly sub medium sized chromosomes, both groups can be further divided into three groups on the basis of length viz. comparatively long, medium and short. Using both parameters give six chromosomes types. In terms of arm ratio, chromosome types A, B and C have a primary constriction in the sub median region, whereas those of chromosome type D, E and F have a primary constriction at median region in *Z. elliottiana*. However, chromosome type A and C have primary constriction at the sub median region, while, chromosome type B, D, E and F have a primary constriction at the median region in *Z. aethiopica*.

Table 2. Mean of chromosome lengths and centromeric position of *Z. elliottiana*.

Chromosome type	Number of chromosome	Average length of chromosome[2] (µM)	Centromeric position[1]
A	1	3.90 ± 0.12[d]	SM
B	2	2.85 ± 0.15[c]	SM
C	4	2.50 ± 0.19[c]	SM
D	3	2.45 ± 0.01[c]	M
E	5	2.25 ± 0.02[b]	M
F	1	2.15 ± 0.04[a]	M

[1]SM, Sub median region centromere; M, median region centromere. [2]Data having the same letter in a column were not significantly differed by Duncan's multiple comparison test (P<0.05).

Table 3. Genomic size analysis of two *Zantedeschia* species.

Plant	Somatic chromosome number	Karyotype formula[1]	2C DNA content[2] (pg)	Mbp
Z. aethiopica	32	2n = 10 M + 22 SM	3.72 ± 0.10[b]	3638.16
Z. elliottiana	32	2n = 14 M + 18 SM	1.17 ± 0.05[a]	1144.26

[1]SM, Sub median region centromere; M, median region centromere. [2]Data having the same letter in a column were not significantly differed by Duncan's multiple comparison test (P<0.05).

The members of genus *Zantedeschia* are quite distinct not only in morphological characters (Letty, 1973) but also in chromosome karyotypes (Yao et al., 1994a). According to the previous review of cytology of the Araceae (Grayum, 1990; Mayo et al., 1997; Peterson, 1989), the chromosomes number can vary greatly between and also within genera, from 2n = 14 in *Ulearum* to 2n = 168 in *Ariseama*.

Flow cytometry

The absolute nuclear DNA content of two *Zantedeschia* species was calculated by flow cytometry by running sample of the internal standard (*L. esculentum, 2n* = 24) together with nuclei of sample leaves. The histogram of relative DNA content showed three distinct peaks corresponding to G0/G1 nuclei of all the tested plant species (Figure 2). The *L. esculentum* peak was used as an internal standard with 2C = 1.96 pg DNA. The 2C DNA content of two *Zantedeschia* species varied. The average DNA content (2C) was significantly lower in G1 nuclei of diploid *Z. aethiopica* (3.72 pg) than for the G1 nuclei of diploid *Z. elliottiana* (1.71 pg), which is equivalent to 3638.16 and 1144.26 mega base pair for *Z. aethiopica* and *Z. elliottiana*, respectively (Table 3). The average DNA content was highly correlated to total average chromosome length. Interspecific variation in DNA amount is not unique to angiosperm species (Laurie and Bennet, 1985; Rayburn et al., 1989). It has been reported that DNA content in the angiosperms varies as a consequence of the change and proportion of repeated DNA sequences in the nuclear genome, particularly tandem repeats or satellite DNAs that make up heterochromatic C-bands on the chromosomes (Flavell, 1986; Bennett et al., 2000; Greilhuber, 1995).

SDS-PAGE

The SDS polyacrylamide gel electrophoresis of leaf proteins of the *Z. aethiopica* and *Z. elliottiana* is shown in Figure 3. Electropherogram of two *Zantedeschia* species revealed lower level of similarity which may be attributed to a significant difference in karyotype, including morphological variation of chromosomes and chromosome lengths of two *Zantedeschia* species. The most number of protein bands were 11 and nine for *Z. aethiopica* and *Z. elliottiana*, respectively, ranging from 20 to 97.2 kDa. In comparison, eight of them were common, while the other differed in band pattern with varied molecular weight. *Z. aethiopica* has strong dark bands but *Z. elliottiana* has faint dark bands. The size and number of protein bands reported by Mohamed et al. (2006) on *Z. aethiopica* vary with present investigation.

The nucleic acid content differed appreciably between the two species. The structural alternation in somatic chromosomes complement is evidently reflected in the nucleic acid level and size of protein bands. Thus, this study might be useful for further analysis of DNA and cytogenetic investigation of *Zantedeschia* species.

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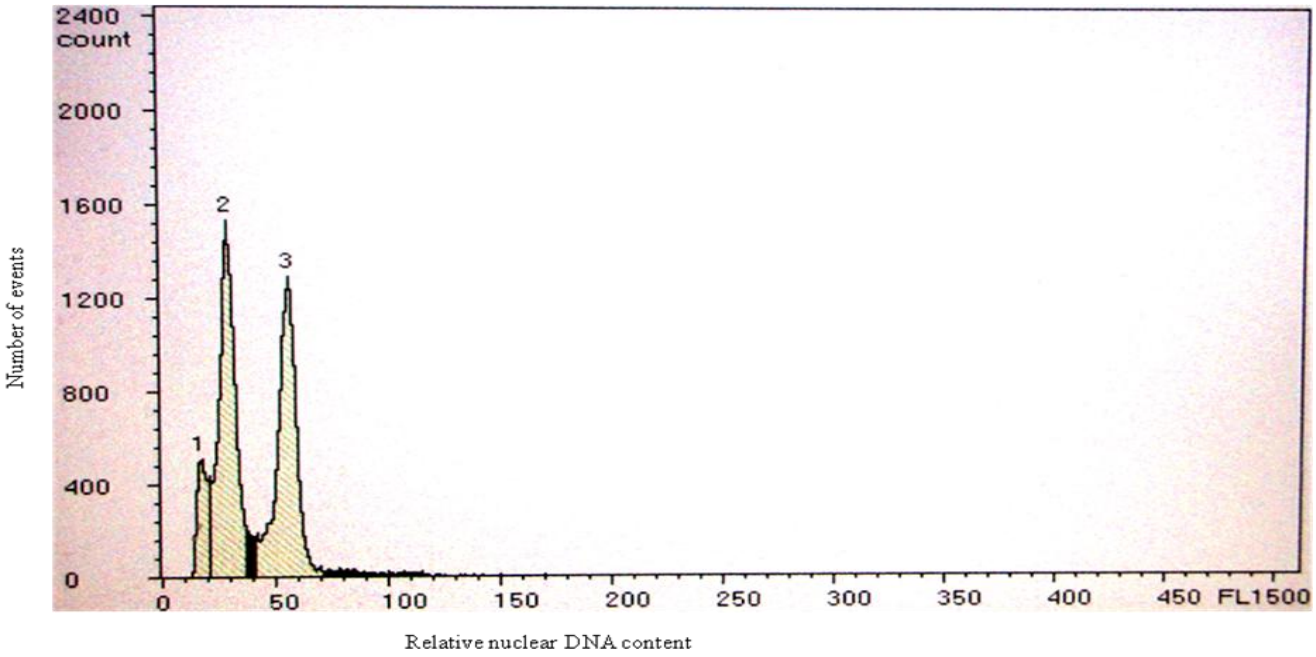

Figure 2. Estimation of absolute nuclear DNA amount (genome size) in *Z. elliottiana* and *Z. aethiopica.* The histogram of relative DNA content was obtained after flow cytometric analysis of propidium iodide-stained nuclei of *Z. elliottiana*, *Lycopersicon esculentum* and *Z. aethiopica,* which were isolated, stained and analyzed simultaneously. *Lycopersicon esculentum* (2C = 1.96 pg DNA) served as internal reference standard. Peak 1, *Z. elliottiana*; Peak 2, *Lycopersicum esculentum*; Peak 3, *Z. aethiopica.*

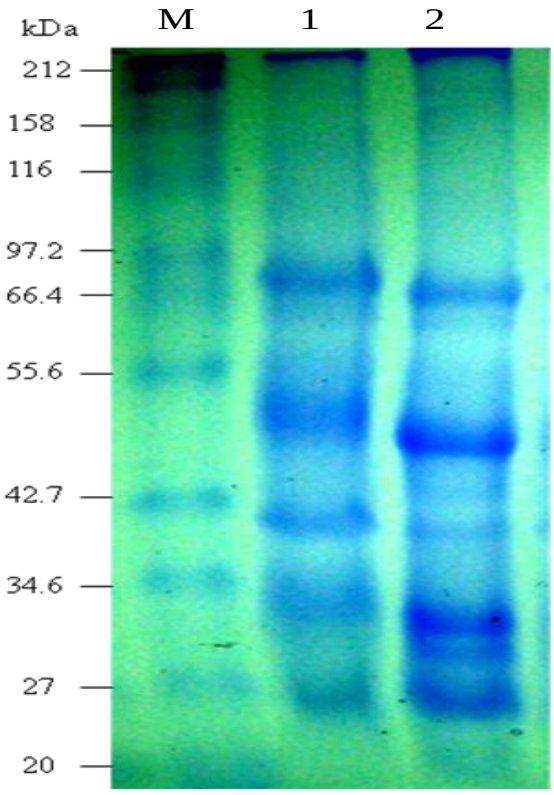

Figure 3. Polyacrylamide gel showing leaves protein bands of two *Zantedeschia* spp. Lane M, Marker; lane 1, *Z. elliottiana*; lane 2, *Z. aethiopica*.

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