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ORIGINAL ARTICLE

Estimation of nuclear DNA content of various bamboo and rattan species

Prakash P. Kumar · Ian M. Turner · A. Nagaraja Rao · K. Arumuganathan

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Abstract We determined the nuclear DNA content (genome size) of over 35 accessions each of bamboo and rattan species from Southeast Asia. The 2C DNA per nucleus was quantified by flow cytometry. The fluorescence of nuclei isolated from the leaves and stained with propidium iodide was measured. The genome size of the bamboo species examined was between 2.5 and 5.9 pg DNA per 2C nucleus. The genome size of the rattan species examined ranged from 1.8 to 10.5 pg DNA per 2C nucleus. This information will be useful for scientists working in diverse areas of plant biology such as biotechnology, biodiversity, genome analysis, plant breeding, physiology and molecular biology. Such data may be utilized to attempt to correlate the genome size with the ploidy status of bamboo species in cases where ploidy status has been reported.

P. P. Kumar (✉) · I. M. Turner · A. Nagaraja Rao
Department of Biological Sciences,
National University of Singapore, 10 Science Drive 4,
Singapore 117543, Singapore
e-mail: dbskumar@nus.edu.sg

P. P. Kumar
Temasek Life Sciences Laboratory,
National University of Singapore, 1 Research Link,
Singapore 117604, Singapore

Present Address:
I. M. Turner
P.O. Box 20, Winchelsea TN36 4WA, UK

A. Nagaraja Rao
131 Cavenagh Road, #12-01, Singapore 229627, Singapore

K. Arumuganathan
Flow Cytometry and Imaging Core Laboratory,
Benaroya Research Institute at Virginia Mason,
1201 Ninth Avenue, Seattle, WA 98101, USA

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Introduction

Knowledge of the nuclear DNA content (genome size) is useful for plant scientists engaged in genome analysis, biotechnology, plant breeding, physiological and molecular biological studies. In addition, valuable information can be gained from such studies for cataloguing species in studies on biodiversity. While discussing the value of genome size data for angiosperm species, Bennett and Leitch (1995) deplored the fact that such data exist for only about 1% of the known angiosperm species. They highlighted the need for more work along these lines for many of the plant species from the tropics. The nuclear DNA content has been established to be a constant for a given species—both between cells of an individual and between various individuals within the same species (Bennett and Leitch 1995). Based on this observation, the 'C' value ('C' for Constant) concept was established by Swift in the early 1950s (see Bennett and Leitch 1995). The C value of DNA represents the amount of DNA per haploid, unreplicated genome. Thus, the newly formed cells at the end of meiosis contain the '1C' value of DNA, and the somatic cells after completion of mitosis contain the '2C' value of DNA. We have used this convention in the present study and the genome size reported here corresponds to the 2C DNA content.

Flow cytometry is currently being employed to quantify nuclear DNA content (Arumuganathan and Earle 1991a, b). Nuclei from plant tissues produce two peaks, one corresponding to the 2C value and the other corresponding to the 4C value. The latter emit twice the fluorescence because they contain twice the DNA amount as 2C nuclei (DNA is

replicated prior to mitosis). In some cases, as in *Arabidopsis thaliana*, multiple peaks may be observed (see below). These correspond to various ploidy levels. Therefore, if multiple peaks are observed, the ploidy levels can be correlated with the fluorescence, and flow cytometry can be used in these cases for estimating ploidy. In some species, e.g., *Arabidopsis thaliana* (about 0.3 pg/2C nucleus), multiploidy (2C, 4C, 8C, 16C and 32C) was detected in the mature tissues such as leaves, root and stem (Arumuganathan and Earle 1991a, b). This suggests that, in some species, the developmental state may influence the ploidy level. Such information will be valuable for scientists working with these species.

Bamboos grow in about 3% of the world's forest area and they provide incomes and subsistence for over a billion people (INBAR 2010). Also, being one of the fastest growing species of plants, bamboos have been shown to be an effective carbon sink. The overall carbon sequestration by managed bamboos was estimated to be between 10 and 20% higher than that of other fast-growing species such as eucalyptus or Chinese fir plantations (INBAR 2010). Despite the significant economic importance of these forestry species, there is inadequate information on the genetic composition of bamboo and rattan. A recent report on Chinese moso bamboo (Gui et al. 2007) seems to be the only report so far on bamboo genome size. More recently, sequence analysis was performed on over 10,000 cDNA from moso bamboo, which led to the conclusion that bamboo has diverged from rice, wheat and other grasses possibly through adaptive radiation (Peng et al. 2010). Other than these two reports, there seem to be no published data on genome size or genetic structure of bamboos and rattans.

As part of a survey, we determined the nuclear DNA contents of several species of bamboo and rattan of Southeast Asia. We also compared the genome sizes of a given species of bamboo/rattan collected from different countries, namely, Singapore, Malaysia and Thailand. Despite the absence of chromosome counts for the species studied, the data are valuable because they provide a good estimate of genome size for these important tropical forestry resources for which very little genetic information is available.

Materials and methods

Sample collection

Young, fully expanded leaves without any visible signs of infection or necrosis were collected from the field. Specimens of some of the bamboo species were obtained from the collection in the Singapore Botanic Gardens, while others were collected from the region. Several rattan samples were collected from Malaysia (Forest Research Institute) and Thailand (Royal Forest Department).

The leaves were thoroughly cleaned and wrapped in moist paper towels, and packed in resealable plastic bags. Rattan leaflets that had high levels of algal growth on the surface were surface-sterilized with 10% Clorox solution after thorough cleansing with a mild detergent. The samples thus prepared could be maintained fresh for over 5 days without refrigeration. This permitted easy transport of samples from the remote sites of collection to the laboratory for analysis.

Isolation of nuclei and flow cytometry

Flow cytometry procedures described by Arumuganathan and Earle (1991a, b) were used to determine nuclear DNA content. The procedure consisted of preparing suspension of intact nuclei by slicing plant tissues in a $MgSO_4$ buffer (van den Engh et al. 1984) mixed with DNA standard and staining with propidium iodide (PI) in a solution containing DNase-free RNase. Fluorescence intensities of the stained nuclei were measured by a flow cytometer. Values for nuclear DNA content were estimated by comparing fluorescence intensities of the nuclei of the test population with those of an appropriate internal DNA standard which was included with the tissue being tested.

Approximately 50 mg of healthy leaf tissue was excised and placed on ice in a 35 × 10 mm plastic petri dish. About 20 mg of tobacco leaf was added to the petri dish as an internal standard for DNA quantification. The tissue was chopped into thin strips (0.25–0.5 mm wide) in 1 ml of propidium iodide-$MgSO_4$ buffer solution (10 mM $MgSO_4 \cdot 7H_20$, 50 mM KCl, 5 mM Hepes), 20 mg ml^{-1} polyvinyl pyrrolidone (PVP-40), 1.5 mg ml^{-1} dithiothreitol, 20 µl ml^{-1} propidium iodide stock (5 mg ml^{-1}), 25 µl ml^{-1} Triton X-100 stock (10% w/v) and 2.5 µl ml^{-1} DNase-free RNase. The suspension of nuclei was collected with a pipet and filtered through 30-µm nylon mesh into a 1.5-ml microfuge tube. The samples were incubated at 37°C for 15 min before flow cytometric analysis.

The prepared material was analyzed on a standard FACscan flow cytometer (Becton–Dickinson Immunocytometry System, San Jose, CA, USA) using 15 mW of 488 nm laser. For each measurement, propidium iodide fluorescence area signal (FL2-A) from 1,000 nuclei were collected using CellQuest software (Becton–Dickinson). A live gate was set using the FL2-A and FSC parameters allowing the fluorescence measurements from nuclei to generate a histogram of FL2-A. The mean position of G0/G1 (nuclei) peak of the sample and the internal standard were determined by analyzing the data using the

CellQuest software. The mean DNA content of the plant was based on the 1,000 scanned nuclei. The internal standard used for comparison was *Nicotiana tabacum* which has a DNA content of 9.24 pg/2C.

The formula used for converting fluorescence values to DNA content was:

Nuclear DNA content = [(mean position of sample peak)/(mean position of the peak of standard)] × DNA content of the standard.

Also, 1 pg = approximately 965 Mb (Arumuganathan and Earle 1991b; Bennett and Leitch 1995).

Results and discussion

Prior to the development of microdensitometry and flow cytometry, chemical analysis and reassociation kinetics were employed for DNA quantification. The trend of common use (over 87% of all estimations) of microdensitometry until about 1990, and flow cytometry beyond 1990 (over 50% of all estimations after 1990), for DNA quantification has been recorded (Bennett and Leitch 1995). Further, in a study of several woody plant species, it was found that the values reported for several of the species studied by the older method varied considerably and a better estimate could be obtained by flow cytometric measurement (Arumuganathan and Earle 1991a, b).

Bennett and Leitch (1995) highlighted the fact that, despite the occurrence of intraspecific variation in the DNA content per cell in some cases, the concept of 'C' value is largely in use for practical reasons. Some of the variations are likely to be due to the presence of repeated DNA (non-genic DNA) in angiosperms and other higher eukaryotes. Some of the parameters affected by DNA content include length and volume of chromosomes, duration of meiosis, ecological behavior, optimum environment and range of cultivation of crop (see Bennett and Leitch 1995). The varied uses of genome size data, the adaptive significance and other phenotypic effects due to the DNA content per nucleus have been discussed previously (Bennett and Leitch 1995).

Bamboo

We determined the 2C nuclear DNA content of 37 taxa of bamboo from Singapore, Malaysia and Thailand (Table 1). The genome size of most of the bamboo species examined varied from 2.5 to 5.9 pg DNA per 2C nucleus. Among the bamboo species tested, there was no correlation between the genome size and the culm size. Furthermore, different specimens of the same species of bamboo collected from various locations gave comparable results.

The 2C DNA amount per nucleus of a given species and the ploidy status will be directly proportional to each other. The limitation to establish correlation between the ploidy status and the DNA content measured is primarily the lack of cytogenetic information (chromosome number) for many of the bamboo species. Also, when a range of chromosome numbers is reported for certain species of bamboo, e.g., 70–72 chromosomes for *Bambusa bambos* (Devi and Sharma 1993), it becomes difficult to ascribe ploidy based on the DNA content. However, *Dendrocalamus brandisii* and *Dendrocalamus giganteus* (the latter has perhaps the largest stem among bamboos) are both known to be hexaploid, with a chromosome number of 72, and they have 3.4 pg DNA per 2C nucleus. Interestingly, the 2C DNA amount per nucleus in *Dendrocalamus strictus* was 3.1 pg (Table 1), which is lower than that of the data for the other three species under this genus. The reported chromosome number for this species varies: 56, 70 or 72; and this species is also claimed to be a hexaploid (Devi and Sharma 1993). Based on our data, we can speculate that the individual from which the samples were obtained might have the chromosome number of 70 (aneuploid?), thus accounting for the slightly lower DNA amount than the other three *Dendrocalamus* species examined. Only if cytological studies are carried out can this fact be established unequivocally.

Bambusa vulgaris is known to have chromosome numbers ranging from 64 to 72 (Devi and Sharma 1993). There are two morphologically distinct varieties ('Vittata'—yellow with green stripes; and 'Wamin'—"Buddha's belly" bamboo) of this species besides the wild-type, and all of them yielded the same DNA amount per 2C nucleus (namely, 2.9 pg; Table 1). This supports the view that they should be grouped under the same species, but they merit the status of different varieties based on their vegetative phenotypes.

All the *Gigantochloa* species studied (7) had comparable DNA amounts, but *Phyllostachys* species showed some differences among their 2C DNA amounts (Table 1). Also, most of the *Bambusa* species (14 studied) had approximately 3 pg DNA per 2C nucleus, with the exception of *B. bambos*. The latter is reported to have chromosome numbers of 70–74; again, the individual from which the sample was obtained might have had the lower chromosome number, which is yet to be confirmed. One of the few studies on the genome size of bamboos (Gui et al. 2007) reported that moso bamboo (*Phyllostachys pubescens*) of China has 2,034 Mb (equivalent to about 2.1 pg DNA per nucleus), which is about half of the DNA content in the tropical *Phyllostachys* spp. we screened (Table 1). The Chinese moso bamboo is reported to be a tetraploid (4x = 48; Gui et al. 2007) and it remains to be seen if the tropical varieties have become octaploid.

Table 1 Comparison of genome size of different taxa of bamboo

Species	Mean (pg/2C) ± SD	Chromosome no. and (ploidy)
Bambusa bambos	2.83 ± 0.01	70–74
Bambusa blumei	3.13 ± 0.02	
Bambusa glaucescens (00-1271.1)	3.15 ± 0.00	
Bambusa heterostachya (06/13/1996)	3.08 ± 0.08	
Bambusa multiplex (06/13/1996)	3.11 ± 0.02	72
Bambusa sp. (BG extension, 07/8–9/1996)	3.32 ± 0.02	
Bambusa sp. (SEAsia w260B)	3.02 ± 0.01	
Bambusa sp. ("*Bambusa variegata*" 00-1272.1)	3.12 ± 0.02	
Bambusa textilis	3.28 ± 0.01	56, 64, 72
Bambusa tulda	3.07 ± 0.02	70, 72 (aneuploid)
Bambusa tuldoides (06/13/1996)	2.97 ± 0.02	
Bambusa vulgaris (06/13/1996)	2.94 ± 0.04	64–72
Bambusa vulgaris cv Vittata (06/13/1996)	2.93 ± 0.01	64–72
Bambusa vulgaris cv Wamin (06/13/1996)	2.94 ± 0.01	64–72
Cephalostachyum pergracile	3.19 ± 0.01	48, 54, 60, 72 (6x)
Dendrocalamus asper (06/13/1996)	3.32 ± 0.06	
Dendrocalamus brandisii (55-95–4813)	3.40 ± 0.01	72 (6x)
Dendrocalamus giganteus (07/8/1996)	3.41 ± 0.05	72 (6x)
Dendrocalamus strictus (KL, 08/28/96)	3.11 ± 0.02	56, 70, 72 (6x)
Dendrochloe levis (KL, 08/28/96)	3.42 ± 0.02	
Gigantochloa apus (07/8/1996)	3.21 ± 0.01	
Gigantochloa atroviolacea (00-1263.1)	3.29 ± 0.03	
Gigantochloa ligulata (KL, 08/28/96)	3.19 ± 0.01	
Gigantochloa ridleyi	3.28 ± 0.00	
Gigantochloa rostrata (07/8/1996)	3.14 ± 0.00	
Gigantochloa scertechnii (KL, 08/28/96)	3.34 ± 0.01	
Gigantochloa sp. 00-1278.1 BGext	3.31 ± 0.01	
Gigantochloa verticillata (00-1265.1)	3.30 ± 0.04	
Melocanna baccifera	2.51 ± 0.02	72
Phyllostachys pubescens (KL, 08/28/96)	4.65 ± 0.01	48
Phyllostachys sp. (00-1279.1)	5.85 ± 0.04	
Phyllostachys glauca (KL, 08/28/96)	4.55 ± 0.02	
Phyllostachys viridi-glaucescens (KL, 08/28/96)	4.73 ± 0.02	
Schizostachyum brachycladum	3.14 ± 0.01	
Schizostachyum jaculans (00-1267.1)	3.10 ± 0.18	
Teinostachyum dullooa (07/8/1996)	2.98 ± 0.03	56 (aneuploid)
Thyrsostachys siamensis (07/8/1996)	2.44 ± 0.02	

The 2C DNA content was estimated by flow cytometry. The error limit for data presented is within 10%

Rattan

We collected 19 species of rattan in Singapore. Several more specimens were obtained from Thailand (14 species) and Malaysia (9 species) as indicated in "Materials and methods". The genome size of the rattan species examined varied between 1.8 and 10.5 pg DNA per 2C nucleus (Tables 2, 3 and 4). Interestingly, the larger canes such as *Plectocomia* sp. had a relatively large genome (over 9 pg DNA per nucleus; Table 2). It is known that some palms have tissues with high ploidy levels (e.g., endosperm cells of coconut;, Kumar et al. 1985). However, information on the ploidy status of the rattans is not readily available. Therefore, we are unable to compare the DNA content with ploidy status in rattans.

We compared leaves from seedlings and mature rattans in some cases to determine if there are any developmentally associated changes in ploidy levels. Such situations were not encountered in rattans—in that both the seedling leaves and leaves from the mature palms had comparable amounts

Table 2 Comparison of genome size of different taxa of rattans collected from Singapore

Species	2C DNA content (pg per nucleus; mean ± SD)
Calamus sp.	2.80 ± 0.01
Calamus sp.	3.18 ± 0.11
Calamus javensis	4.00 ± 0.05
Calamus oxleyanus	2.39 ± 0.02
Calamus speciosissimus	3.02 ± 0.03
Daemonorops angustifolia	3.31 ± 0.02
Daemonorops didymophylla	4.29 ± 0.04
Daemonorops grandis	3.28 ± 0.08
Daemonorops hystrix	3.76 ± 0.13
Daemonorops hystrix	4.71 ± 0.04
Daemonorops longipes	4.12 ± 0.07
Daemonorops longipes (?)	3.45 ± 0.03
Daemonorops periacantha	4.27 ± 0.07
Daemonorops sabut	3.69 ± 0.01
Daemonorops sp.	3.51 ± 0.23
Daemonorops sp.	7.52 ± 0.10
Korthalsia scaphigera	1.83 ± 0.02
Myrialepis paradoxa	10.50 ± 0.02
Plectocomia elongata	9.11 ± 0.08

The error limit for data presented is within 10%

of DNA per nucleus (Table 3). In particular, *Calamus javensis*, *C. latifolius*, *C. longisetus*, *C. manan* and *C. peregrinus* were subjected to such analysis. This is in contrast to the observation that in some species, such as *Arabidopsis thaliana* (Arumuganathan and Earle 1991a), tissues from a given plant are reported to contain nuclei of different ploidy levels. The vegetative and reproductive tissues of *Arabidopsis* also showed differences, leading to the conclusion that multiploidy in *Arabidopsis* was under developmental control, with younger tissues showing no or fewer cases of multiploidy than the older. Also, cells of the flower bud contained only 2C and 4C amounts of DNA in *Arabidopsis*.

We examined if there were any differences between male and female individuals of *C. javensis*. Cells from both the sexes yielded comparable amounts of DNA per 2C nucleus (Table 3). Furthermore, the 9 species of *Calamus* collected from Malaysia showed significant variation in their 2C DNA amounts (Table 4), suggesting that these different species are genetically farther apart from each other. Also, the 2C DNA amounts estimated in our study of six rattan species collected from two or three locations (Singapore, Thailand or Malaysia) were compared (Table 5). The values obtained for the corresponding species from the different locations were comparable because they were within the acceptable error limit of 10%.

Table 3 Comparison of genome size of 22 taxa of rattans collected from Thailand. The error limit for data presented is within 10%

Species	2C DNA content (pg per nucleus; mean ± SD)
Calamus caesius	2.97 ± 0.06
Calamus insignis	3.17 ± 0.02
Calamus javensis (female)	3.89 ± 0.06
Calamus javensis (male)	3.80 ± 0.05
Calamus javensis (seedling)	3.86 ± 0.02
Calamus latifolius (youngest lf seedling)	3.71 ± 0.01
Calamus latifolius (oldest lf seedling)	3.64 ± 0.02
Calamus longisetus	3.55 ± 0.03
Calamus longisetus (oldest lf seedling)	3.53 ± 0.01
Calamus longisetus (youngest lf seedling)	3.61 ± 0.02
Calamus manan (adult)	3.75 ± 0.02
Calamus manan (oldest lf seedling)	3.70 ± 0.01
Calamus manan (youngest lf seedling)	3.71 ± 0.08
Calamus oxleyanus	2.70 ± 0.01
Calamus palustris (seedling)	4.38 ± 0.01
Calamus peregrinus	4.17 ± 0.15
Calamus peregrinus (seedling)	3.94 ± 0.01
Calamus rudentum	3.60 ± 0.01
Calamus blumei	3.59 ± 0.01
Daemonorops angustifolia	3.49 ± 0.15
Daemonorops didymophylla (seedling)	4.11 ± 0.06
Korthalsia rigida	1.52 ± 0.02

Table 4 Comparison of genome size of different taxa of rattans collected from Malaysia. The error limit for data presented is within 10%

Species	2C DNA content (pg per nucleus; mean ± SD)
Calamus axillaris	4.34 ± 0.02
Calamus caesius	2.84 ± 0.16
Calamus manan	3.76 ± 0.01
Calamus merrillii	2.50 ± 0.02
Calamus oxleyanus	2.54 ± 0.01
Calamus palustris	4.49 ± 0.02
Calamus subinermis	3.88 ± 0.01
Calamus tetradactylus	2.96 ± 0.42
Calamus tumidus	3.86 ± 0.01

The origins of the following three species are: *Calamus merrillii:* The Philippines; *Calamus subinermis*: Borneo; and *Calamus tetradactylus*: China

Lastly, on an ad hoc basis, the genome sizes of the following plant species were determined: rubber tree *Hevea brasiliensis* (3 pg), a fast-growing social forestry species *Paulownia kawakamii* (1.12 pg), an orchid *Dendrobium* sp.

Table 5 Comparison of genome size of different species of rattans collected from Singapore, Thailand and Malaysia

Species	2C DNA content (pg per nucleus ± SD)		
	Singapore	Thailand	Malaysia
Calamus caesius	–	2.97 ± 0.06	2.84 ± 0.16
Calamus javensis	4.00 ± 0.05	3.80 ± 0.05	–
Calamus oxleyanus	2.39 ± 0.02	2.70 ± 0.01	2.54 ± 0.01
Calamus manan	–	3.75 ± 0.02	3.76 ± 0.01
Calamus palustris	–	4.38 ± 0.01	4.49 ± 0.02
Daemonorops angustifolia	3.31 ± 0.02	3.49 ± 0.15	–

Only those species for which leaf samples were obtained from all three countries are presented in this table. For a more extensive list of species from each country, please refer to Tables 2, 3, and 4. Values differing by less than 10%, which is the error limit, may be treated as comparable to each other

(2.4 pg) and a fern *Platycerium coronarium* (gametophyte 17 pg, sporophyte 39 pg). It is hoped that the genome size values (2C DNA content) for the various species reported here will form a useful reference for interested plant scientists.

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