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Comparative Karyomorphology and DNA Estimation Studies in Ginger Cultivars (*Zingiber officinale* Rosc.)[1]

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Summary Chromosome studies and *in situ* estimation of nuclear DNA content were carriedout in two different cultivar of *Z. officinale* Rosc. Somatic chromosome number 2n=22 was found to be constant in both the cultivars. Karyomorphological analysis indicated high heterogenity. DNA content of these two cultivars were highly varied and a correlation could be obtained between the amount of 4C DNA and the total chromosome length. Average packing ratio was workedout from which the relation between the 4C nuclear DNA content and the total chromosome length were established.

Ginger (*Zingiber officienale* Rosc.) is a herbaceous, perennial rhizomatous spice of the family Zingiberaceae. It has originated probably in South-East Asia, though the definite origin is not known (Purseglove *et al.* 1981). The genus *Zingiber* (Boehm) has about 80–90 species, and ginger the most important member of the genus is grown in many countries in the tropics and subtropics. Though considerable cytogenetical studies had been carried out in this species (Sugiura 1928, Sharma and Bhattacharya 1959, Ramachandran 1969, Ratnambal 1979, Omnakumari and Mathew 1985), comparative karyomorphological studies and estimation of 4C nuclear DNA content correlation studies with reference to Average packing ratio of different cultivars have not been done in this taxon except, for a study on Scitaminae (Mandi 1981) and estimation of DNA and karyotype analysis in north eastern Indian cultivars of ginger (Rai *et al.* 1997). Moreover, most of cultivars exhibit high similarity in their plant morphotype and rhizome characters. In such cases, cytological characterisation studies and comparative 4C DNA content estimation would help to identify the individual cultivars and also to estimate the genetic variability among the cultivars. Present investigation had been undertaken to compare the karyomorphology and 4C nuclear DNA content of ginger cultivar Maran, an indigenous promising line and China an exotic high yielder, in the light of average packing ratio.

Materials and methods

Rhizomes of ginger were collected from ginger germplasm repository, Indian Institute of Spices Research, Peruvannamuzhi and planted in earthen pots at IISR, Chelavoor in the month of April 1996. The somatic chromosome studies were carried out from actively growing root tips pretreated with 0.05% colchicine at 10–15°C for 4 hr and fixed in 3 : 1 alcohol acetic acid mixture for overnight. The root tips were washed with distilled water and hydrolysed with 1 N HCl for 15–20 min at 60°C and stained with 2%. Lacto propionic orcein for overnight and squashed in 45% propionic acid. Five metaphase plates from each plant were taken in to account for calculating the total chromosome length and average packing ratio. The total forma percentage (TF%) has been calculated (Huziwara 1962) using the formula,

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$$TF\% = \frac{\text{Total sum of short arm length}}{\text{Total sum of chromosome length}} \times 100$$

For the estimation of 4C nuclear DNA content studies, actively growing root tips were fixed in 1 : 2 propionic acid and alcohol mixture for overnight, hydrolysed in 1 N HCl at 60°C for 15–20 min, stained in Schiff's reagent for one hour at 18°C to 22°C and squashed in 45% propionic acid.

Microspectrophotometric analysis were carried out in Leitz MPV microscope with photometric attachment using single wave length method (Sharma and Sharma 1980). The DNA values calculated in terms of relative arbitrary unit of absorbance which were converted in to picograms (pg) using 4C nuclear value for *Allium cepa* L. as a standard (Von't Hof 1965). Average packing ratio of DNA was calculated (Sinha *et al.* 1996) using the formula,

$$\text{Average Packing Ratio} = \frac{\text{Length of 4C DNA content at metaphase/cell } (\mu m)}{2 \times \text{length of total chromosome at metaphase/cell } (\mu m)}$$

Where,

$$\text{The length of DNA content} = \frac{\text{Amount of 4C DNA present/cell (pg)} \times 0.965 \times 10^9 \times 3.4}{10^4}$$

as equivalence of 1 pg $=0.965 \times 10^9$ bp (Lewin 1985). The distance between two bases $=3.4$ Å and 1 μm $=10^4$ Å. Therefore, the average packing ratio of DNA (at metaphase 4C) can be calculated by the formula,

$$APR = \frac{\text{4C DNA content/cell (pg)} \times 0.965 \times 10^9 \times 3.4}{*2 \times \text{length of total chromosome at metaphase/cell} \times 10^4}$$

(* metaphase chromosome consists of two identical chromatids, therefore to compute the APR, 4C DNA content is divided by 2.)

Results

The somatic cells of ginger cultivars showed $2n=22$ chromosomes. The chromosome length of Maran and China vary from 0.67 to 3.92 respectively. Karyotype classification was done following the system proposed by Stebbins (1971). Based on the centromeric position the somatic chromosomes of both ginger could be classified in to four distinct morphological types.

Type A: Median chromosomes

Type B: Sub-Median chromosomes

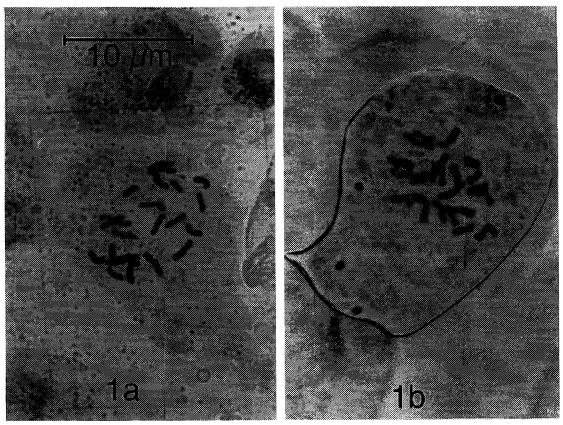
Type C: Terminal chromosomes

Type D: B chromosomes

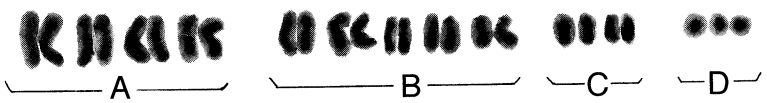
The karyogram and karyomorphological analysis of the cultivar Maran revealed 5 pairs of median chromosome and 6 pairs of sub-median chromosomes. Where as in cv. China, there are 4 pairs of median, 5 pairs of sub-median and 2 pairs of terminal chromosomes. The cultivar Maran consists of one SAT-chromosome in the first pair and the secondary constriction was observed in all the five pairs of median chromosomes. In case of cv. China, the SAT-chromosome was absent and the secondary constriction was observed in the first pair of the long arm. Three B chromosomes were identified in the cultivar China. The total chromosome lengths and arm ratios (short arm/long arm) were calculated. Microphotographs showing metaphase plates and karyogram were given in Figs. 1a, b and 2a, b. The total chromosome lengths of cv. Maran and cv. China were observed as 45.19 μm and

Table 1. Chromosome complements and 4C nuclear DNA content of *Z. officinale* Rosc.

Cultivar	Somatic chromosome number (2n)	Karyotype formula	TF%	4C DNA content in pg (mean±SE)
Maran	22	10A+12B	41.15	5.49±0.35
China	22	8A+10B+4C+3 'B'	33.42	3.96±0.59



2a



2b

Figs. 1–2. 1a, b) Somatic metaphase plate of ginger cv. Maran and cv. China showing 2n=22 chromosome respectively. 2a, b) Karyogram of ginger cv. Maran and cv. China.

38.12 µm respectively. TF% of cv. Maran and cv. China were 41.15 and 33.42 respectively (Table 1). The 4C nuclear DNA content of the two cultivars of ginger showed marked variation ranging from 5.49 pg in cv. Maran to 3.96 pg in cv. China (Table 1). The test of significance at 1% level between the mean DNA values shows significant variation.

Discussion

Every variety/cultivar is known to be characterised by its own karyotype (Lavania 1985). Chromosome morphology is useful in the identification of individual variety/cultivar and to establish the relationship among the related variety/cultivar (Lavania 1985, Stebbins 1971). In the present study the somatic chromosome number 2n=22 (Fig. 1a, b) is found to be constant in both the

Table 2. Average packing ratio of DNA in *Z. officinale* Rosc.

Cultivar	Total chromosome length at metaphase (μm)	Length of 4C DNA content (μm)	Average packing ratio
Maran	45.19±8.06	18.0×10^5	$1.99\times10^4\pm0.032$
China	38.12±4.02	13.0×10^5	$1.71\times10^4\pm0.673$

cultivars which confirmed the earlier reports (Ratnambal 1979 and Omanakumari *et al.* 1985). Though both the cultivar of ginger showed symmetrical karyotype, they highly differ in their chromosome morphology, particularly with regards to the distribution of secondary constriction and SAT-chromosome. Three 'B' chromosome were identified in the ginger cultivar China. "B' chromosomes were documented by Janakiammal *et al.* (1945) in *Zingiber officinale.* However in general, there is variation in the overall chromosome morphology in both ginger types. Difference in the chromosome morphology, inspite of the similar plant type and rhizome character, suggest chromosomal variations due to genomic difference that might have accumulated during the course of evolution. Estimation of nuclear DNA amount (4C) and chromosome morphology may be useful criteria for cultivar identification. Rai *et al.* (1997) studied the estimation of nuclear DNA content and karyotype analysis of 6 different North-East Indian ginger cultivars suggest the significant variation in the DNA amount at cultivar level may be due to structural alteration as well as loss or addition of highly repetitive sequences in the genome. Karyological studies with reference to average packing ratio was reported in other plant species like *Vetiveria Zianioides* L. (Lavania 1985), *Memordica cochinchinensis* Lour (Sinha *et al.* 1996). These current earlier reports proved that the average packing ratio is highly useful to establish relationship between nuclear DNA content and total chromosome length. On the basis of available data it is clear that the increase in the amount 4C nuclear DNA is highly associated with the increase in chromosome length and thereby obtaining a linear relationship between the 4C DNA content and the total chromosome length. The average packing ratio of cv. Maran and cv. China differ as 1.99×10^4 and 1.71×10^4 respectively (Table 2). The average packing ratio clearly indicates that overall compaction of the DNA molecule in chromosome complements of cv. Maran is comparatively high ($P<0.001$) there by revealing the differential condensation involved with the organisation of somatic chromosome in two cultivars of ginger.

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References

Huziwara, Y. 1962. Karyotype analysis in some genera of *Aster.* Amer. J. Bot. **49**: 116–119.

Janakiammal, E. K. 1945. Chromosome Atlas of Flowering Plants. George allen and Unwin Ltd. London. pp. 397.

Lavania, U. C. 1985. Nuclear DNA and Karyomorphological studies in *Vetiver.* Cytologia **50**: 177–185.

Lewin, B. 1985. Genes. Wiley Eastern Ltd. New York. pp. 769.

— 1994. Genes V. Oxford University press Inc. New York. pp. 769.

Mandi, Kalyan Kumar 1981. Studies on chromosomes, DNA estimation and chemical constituents of different members of Scitaminae. Ph.D. thesis. University of Culcutta.

Omana Kumari, N. and Mathew, P. M. 1985. Karyomorphological studies on four species of *Zingiber* Adms. Cytologia **50**: 445–451.

Purseglove, J. W., Brown, E. G., Green, C. L. and Robbins, S. R. J. 1981. Spices. Vol. 2. Longman group Ltd. pp. 447–449.

Rai, S., Das, A. B. and Das, P. 1997. Estimation of 4C DNA and karyotype analysis in ginger (*Zingiber officinale* Rosc.)—I. Cytologia **62**: 133–141.

Ramachandran, K. 1969. Chromosome numbers in Zingiberaceae. Cytologia **34**: 213–221.

Ratnambal, M. J. 1979. Cytogenetical studies in ginger. Ph.D. thesis. University of Bombay.

Sharma, A. K. and Bhattacharya, M. K. 1959. Cytology of several members of Zingiberaceae and a study of the inconstancy of their chromosome complement. Cellule **59**: 299–345.

— and Sharma, A. 1980. Chromosome Techniques. Theory and Practice. Third edition Butterworthe Ltd. London.

Sinha, S., Debnath, B. and Sinha, R. K. 1996. Karyological studies in Dioecious *Memordica Cochinchinensis* Lour. with reference to average packing ratio. Cytologia **61**: 297–300.

Stebbins, G. L. 1971. Chromosomal Evolution in Higher Plants. Edward Arnold Ltd. London.

Sugiura, T. 1928. Chromosome numbers in some higher plants. I. Bot. Mag. **42**: 504–506.

Von't Hof, J. 1965. Relationship between mitotic cycle duration, S period duration and Average rate of DNA synthesis in the root meristem cells of several plants. Expt. Cell Res. **39**: 48.