Estimation of 4C DNA and Karyotype Analysis in Ginger (*Zingiber officinale* Rosc.)—I

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Ginger (*Zingiber officinale* Rosc.) a herbaceous perennial of the family Zingiberaceae, is an important spice of South Asian origin (Baily 1949, Parry 1969). It is grown extensively in the tropical and sub-tropical parts of the world. The dried rhizome of ginger is widely used in the preparation of soft drinks and beverages such as gingerale, ginger beer, ginger tea, ginger wine, bitters, cordials and liquors and candies, pickles, sauces, preserves, bakery products and also used as vitaminized effervescent. Ginger forms a major ingredient in the traditional medicine of India; the oil and oleoresin are used in pharmaceutical preparations as a carminative, rubefacient and stimulant for alcoholic gastritis, dyspepsia, flatulent colic *etc.* It is propagated vegetatively by rhizomes and cultivated commercially in India, China, Taiwan, Philippines, Sierra Leone, Jamaica, Fiji and Nigeria. India produces 14, 85, 200 tonnes of rhizome annually from an estimated area of 53,300 hectares (Ravindran *et al.* 1994) and has a lucrative export market (Peter 1994). In India, the production trend registered a decline with 6.7% less annual yield in 1994–95 as compared to that of 1993–94 (Peter 1996) mainly due to the rhizome rot disease caused by *Pythium* spp. and the bacterial wilt caused by *Pseudomonas solanacearum*. Many cultivars and local races are facing the threat of extinction for many reasons other than these two major problems.

However, the genetic improvement of this crop through conventional breeding is handicapped due to incompatibility and high pollen sterility resulting in no seed set. The somatic chromosome number $2n=22$ was reported in *Zingiber officinale* (Sugiura 1928, Sharma and Bhattacharyya 1959, Ramachandran 1969) but $2n=24$ and 66 was reported by Kihara *et al.* (1931) and Bisson *et al.* (1968) respectively. Detailed karyotype analysis and 4C DNA estimation in different cultivars and species of ginger have not been reported so far, which is a prerequisite for ginger genetic improvement programme. The study of DNA content in different taxa has much importance in different aspects of chromosome research (Price 1976, Sharma 1983). In order to ascertain precisely the importance of DNA in genetic diversity and phylogeny, an understanding of the variations at generic/specific and cultivar level, if any, is necessary. The present study principally deals with the DNA estimation of 9 cultivars of *Zingiber officinale* to find out the extent to which the values of DNA are indicative of the relationship with karyotype, chromosome volume, chromosome size and to what extent they have a selective advantage.

Materials and methods

Estimation of nuclear DNA content and karyotype analysis were made on 9 different cultivars of ginger (*Zingiber officinale* Rosc.) namely 'Sichhey', 'Turia local', 'S-541', 'Z-17', 'V 2E52', 'S-558', 'V3S18', 'Suravi', 'Gorubathaney'. Rhizomes were collected from Pottangi of Orissa, Kalimpong of West Bengal and Sichhey of Sikkim, India. The rhizomes were grown in the experimental gardens of Regional Plant Resource Centre, Bhubaneswar, Orissa.

Young and healthy root tips were pretreated in saturated paradichlorobenzene and

oxiquinoline mixture (1 : 1) for 3.5 hr at 14°C followed by overnight fixation in 1 : 3 propionic ethanol. Chromosome staining was made in 2% lacto-propionic orcein after cold hydrolysis in 5 N HCl for 7 min. Root-tips were squashed in 45% propionic acid. Ten well scattered metaphase plates were selected for karyotype analysis of each species. Total chromosome length of the genome was ascertained by adding the length of all chromosomes in the karyotype; total chromosome volume of a karyotype was calculated by applying the formula $\frac{4}{3}\pi r^2h$, where r and h represent the radius and the length of the chromosome respectively. Form percentage (F%) of individual chromosome was calculated following the method of Das and Mallick (1993b). Total form percentage (TF%) of a karyotype was the average of sum total of F% of a karyotype. Mean values of total genomic chromosome length and total genomic chromosome volume with standard error were calculated.

For feulgen cytophotometric estimation of 4C DNA, ten fixed root-tips from each cultivar were hydrolysed in 1 N HCl for 12 min at 60°C, washed in distilled water and stained in Schiff's reagent for 2 hr at 14°C; each root-tip squash was prepared in 45% acetic acid. Ten scorings were made from each slide and 4C DNA was estimated from metaphase chromosomes using Nikon Optiphot microscope with microspectrophotometer following the method of Sharma and Sharma (1980) with monochromatic light at 550 nm. *In situ* DNA values were obtained on the basis of optical density which were converted to picograms (pg) by using Van't Hof's (1965) 4C nuclear DNA value (67.1 pg) for *Allium cepa* as standard. The correlation coefficient analysis between different chromosomal parameters was done to find out the relationship of genomic characteristics. ANOVA were performed (Sokal and Rohlf 1973) among the different cytological parameters following Duncan's multiple range test (Harter 1960); t-test between different chromosomal parameters was done to find out the relationship between different genomic characteristics at the family level. The karyogram and idiogram of individual species were prepared through the application of CHROMEX software, courtesy Dr. Sour, University of Tübingen, Germany.

Results

Chromosome characteristics

The somatic chromosome number $2n=22$ was observed in all the cultivars of ginger studied. Some of the somatic cells of 'Gorubathaney' and 'Suravi' showed $2n=24$ which were not significant statistically. On the basis of the size of the chromosome and the position of the constrictions, a number of chromosome types were found to be common with the cultivars studied though they differed from each other in the minute details of the karyotype. The general characteristics of different types of chromosomes are presented in Fig. 1. The karyotype formula of all the cultivars revealed definite differences in the structural alterations of chromosomes (Table 1, Figs. 2–10, 2a–10a). A remarkable feature was noted in 'S-542' where all the types of chromosomes, i.e. A, B, C, D, and E were found in the genome. The A type of chromosome having secondary constriction in the long arm was observed in 'Turia local', 'S-541', 'Z-17' and 'V2E52'. The satellite constricted C types with different doses were common in all the cultivars except in 'Turia local'. Absence of B type of chromosome was noticed in all the cultivars except in 'S-541'. Furthermore, dose differences in the of submedian and the median constricted D and E types of chromosome revealed the genome specific characteristics. The total genomic chromosome length ranged from 55.62–85.23 μ in 'Sichhey' and 'Gorubathaney' respectively. The total chromosome volume varied from 140.29 μ^3 in 'Turia local' to 180.28 μ^3 in 'S-541' (Table 1, Fig. 11). The total form percentage (TF%) varied from 35.53 in 'S-541' to 42.70 in 'Gorubathaney' respectively. Significant variations in chromosome length, volume and TF% were observed among the studied cultivars of ginger.

Fig. 1. Types of chromosomes present in different cultivars of *Zingiber officinale* Rosc. Figs. 2–10. Somatic metaphase plates of different cultivars of *Z. officinale* Rosc. with $2n=22$ chromosomes $\times 2940$. 2. 'Sichhey', 3. 'Turia local', 4. 'S-541', 5. 'Z-17', 6. 'V2E52', 7. 'S-556', 8. 'V3S18', 9. 'Suravi', 10. 'Gorubathaney'.

4C nuclear DNA amount

The nuclear DNA amount along with the other cytological parameters have been presented in Table 1 and Fig. 11. The 4C DNA varied significantly in different cultivars of ginger from 19.663 pg in 'Z-17' to 24.280 pg in 'S-541' where the genomic chromosome volume and

Table 1. 4C DNA values in different cultivars of ginger (*Zingiber officinale* Rosc.) along with other cytological parameters

Cultivars	Somatic chromosome number (2n)	Karyotype formula	NSC	Genomic chromosome length (μ) $\pm$ SE	Genomic chromosome volume (μ^3) $\pm$ SE	4C DNA content (pg) $\pm$ SE	GCL per chromosome (μ)	GCV per chromosome (μ^3)	4C DNA per chromosome (pg)	TF%
Sichhey	22	6C + 6D + 10E	6	55.62 $\pm$ 1.20	146.83 $\pm$ 2.34	21.437 $\pm$ 0.101	2.528	6.674	0.974	36.19
Turia local	22	2A + 10D + 10E	2	57.55 $\pm$ 1.14	140.29 $\pm$ 3.40	20.146 $\pm$ 0.220	2.615	6.376	0.915	38.53
S-541	22	2A + 4B + 2C + 6D + 8E	8	63.73 $\pm$ 2.00	180.28 $\pm$ 2.04	24.280 $\pm$ 0.099	2.896	8.194	1.103	35.57
Z-17	22	2A + 2C + 4D + 14E	4	65.40 $\pm$ 0.90	135.98 $\pm$ 2.56	19.663 $\pm$ 0.067	2.972	6.180	0.893	40.49
V2E52	22	2A + 6D + 14E	2	67.72 $\pm$ 1.23	170.83 $\pm$ 1.29	23.041 $\pm$ 0.111	3.078	7.765	1.047	42.46
S-558	22	2C + 4D + 16E	2	68.49 $\pm$ 0.50	174.77 $\pm$ 3.46	23.943 $\pm$ 0.123	3.113	7.944	1.088	42.48
V3S18	22	2C + 12D + 8E	2	68.75 $\pm$ 1.11	179.74 $\pm$ 2.00	24.102 $\pm$ 0.104	3.125	8.170	1.095	37.24
Suravi	22	2C + 2D + 18E	2	80.08 $\pm$ 1.02	161.65 $\pm$ 1.20	22.962 $\pm$ 0.068	3.640	7.347	1.043	42.24
Gorubathaney	22	2C + 4D + 16E	2	85.23 $\pm$ 1.89	164.01 $\pm$ 1.88	22.201 $\pm$ 0.034	3.874	7.455	1.009	42.70

NSC = Number of secondary constricted chromosome, GCL = genomic chromosome length, GCV = genomic chromosome volume.

Figs. 2a-10a. Idiograms of corresponding metaphase plates in different cultivars of *Z. officinale*.

length ranged from $135.98\text{--}180.28\ \mu^3$ and $65.40\text{--}63.73\ \mu$ respectively. The nuclear DNA content was directly proportional to the genomic chromosome volume in the ginger cultivars studied. The ANOVA and Duncan's multiple range test showed significant variation in nuclear DNA among the cultivars of ginger at 1% level (Tables 2, 3).

Discussion

Karyotype, genome length and nuclear DNA amount

A detailed karyotype analysis of 9 cultivars of ginger revealed some important findings.

Fig. 11. Comparative bar diagram representing total chromosome volume and 4C DNA content in different cultivars of *Z. officinale*.

The metaphase chromosome number $2n=22$ chromosomes were constant in all the cultivars but the type of chromosomes present and also number of secondary constricted chromosomes varied with the cultivars. The sporadic occurrence of $2n=24$ in some cultivars might be due to abnormal spindle function in metaphase during clonal multiplication or due to the residual effects of mutagens during synthetic establishment of the ginger cultivars. But the non-stability of the aneuploid number might be directly related with the natural selection. The B type of chromosomes was present only in 'S-541'. Absence of A type of secondary constricted chromosomes was recorded in 'Siçhey', 'V2E52', 'S-558', 'Suravi', 'Gorubathaney'. Though

Table 2. ANOVA of 4C DNA content in different cultivars of ginger (*Zingiber officinale* Rosc.)

Source	Df	SS	MS	F
Between species	8	954.234	119.279	457.007**
Within species	81	21.142	0.261	—
Total	89			

**=significant at $P \geq 0.01$, DF=degrees of freedom, SS=sum of squares, MS=mean squares, F=variance ratio.

the karyotype formula in 'S-558' and 'Gorubathaney' showed no difference, the genomic chromosome volume and genomic chromosome length varied significantly between the cultivars. Again, a gradual numeric alteration of D and E type of chromosomes was evident in all the cultivars except 'Siçhey' and 'S-541' where alterations were more prominent between the super-numeric constricted chromosomes. The structural

Table 3. Multiple comparisons of the means of 4C DNA amounts for 9 cultivars of ginger (*Zingiber officinale* Rosc.). Values are different between pairs of means (pg)

Cultivars	Turia local	Siçhey	Gorubathaney	Suravi	V3E52	S-558	V3S18	S-541
Z-17	0.483**	1.774**	2.538**	3.299**	3.378**	4.280**	4.439**	4.617**
Turia local		1.291**	2.055**	2.816**	2.895**	3.797**	3.956**	4.134**
Siçhey			0.764**	1.525**	1.604**	2.506**	2.665**	2.843**
Gorubathaney				0.761**	0.840**	1.742**	1.901**	2.079**
Survi					0.981**	0.981**	1.140**	1.318**
V2E52						0.902**	1.061**	1.239**
S-558							0.159ns	0.337**
V3S18								0.178ns

**=significant at 1% level, ns=not significant $P \geq 0.05$, C.D. values at 5%=0.188, C.D. values at 1% level 0.247.

changes as well as the variations in the parts of heterochromatins might have played a vital role (Mukhopadhyay and Sharma 1987, Das *et al.* 1995) to effect these differences in the sequences in the chromosomes.

The gradual changes and shifting of TF% values (Table 1) might be due to the chromosomal alterations in the genome. However, detailed structural alteration of the chromosome morphology as well as variation of secondary constricted chromosomes in different cultivars might be due to duplication of chromosomes or translocation between the chromosomes with or without secondary constriction at a very early stage of evolution (Das 1991, Das and Mallick 1993a, b, Das and Das 1994). The genome length varied from 2.528μ to 3.874μ per chromosome in 'Sichhey' and 'Gurubathaney' respectively. The nuclear DNA content was rather constant i.e. 24.280 pg and 24.102 pg in 'S-541' and 'V3S18' respectively where the karyotype and genome length showed quite significant differences. The correlation coefficient between genome length and genomic DNA content did not show significant positive relation ($r=0.372$). This clearly suggests that the DNA content is not positively correlated with the total chromosome length. The differences in length may be attributed to the differential condensation and spiralization of chromosome arm.

Nuclear DNA amount in relation to genomic chromosome volume

A critical analysis revealed significant variations in the total chromosome volume ranging from $6.376 \mu^3$ in 'Turia local' to $8.194 \mu^3$ in 'S-541' (Table 1). The DNA content showed a high degree of correlation with their genome volume ($r=0.915$). However, in eukaryotic system chromosome volume is determined not only by DNA but by the basic and non-basic proteins as well. The cultivars and species specific compaction of DNA threads along with nucleosomes or the additional gene sequences (Das and Mallick 1989a) with altered non-histone proteins (Chattopadhyay and Sharma 1990) in the chromosome played an important role for chromosomal architecture of the species.

Varietal diversification in DNA amount

The estimated 4C DNA values were reported for the first time in the 9 studied cultivars of ginger. Significant differences of 4C DNA were recorded between the cultivars; however, such differences are in agreement with the findings of other workers (Nishikawa 1970, Furuta *et al.* 1975, Narayan and Rees 1976, Price *et al.* 1980, Ressler *et al.* 1981, Raina and Rees 1983, Banerjee and Sharma 1987, Das and Mallick 1989b, 1993a, b, Das and Das 1994). The constancy in the DNA amount at the cultivar level in repeated experiments revealed the stable 4C DNA content in each cultivar. The DNA amount, though, differed significantly at the intercultural level, the differences in the DNA content, however, greatly depended on the repetitive DNA amount (Flavell *et al.* 1977). The maximum (24.280 pg) 4C DNA content was noted in 'S-541' with all chromosomal types and the minimum (19.663 pg) in cv. Z-17 among the studied cultivars of ginger. The chromosome volume, however, showed a high correlation with 4C DNA amount (0.979). The variability in the stable DNA content in different cultivars might be attributed to the loss or addition of many repeats in the genomes' micro- and macro-environment during evolution of new cultivars (Price *et al.* 1980, Das and Das 1994). The variability of DNA amount has often been attributed to loss or addition of highly repetitive DNA sequences in a genome which reached a certain level and got stabilized through gradual selection.

Summary

Extensive karyotype analysis including determination of somatic chromosome number,

total chromosome length and volume and estimation of 4C DNA amount were carried out on 9 different cultivars of ginger (*Zingiber officinale* Rosc.) for the first time. A significant variation in nuclear DNA amount was recorded at the cultivar level. The 4C DNA amount varied from 19.663–24.102 pg in the cultivars studied. The correlation coefficient studies showed that the 4C DNA content and genomic chromosome volume were interdependent. The structural alteration of chromosomes as well as loss or addition of highly repetitive sequences in the genome showed variation in the DNA amount at cultivar level, but a marginal variation in nuclear DNA content at the cultivar level indicated a close relationship between them.

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