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Karyomorphological analysis on different populations of *Costus speciosus* (Koen.) Sm. under different ploidy levels

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Abstract

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Detailed investigations involving cytological, morphological, anatomical and palynological studies were carried out on 20 different populations of *Costus speciosus* (Koen.), Sm., collected from different parts of India and Nepal. The populations studied represented three distinct ploidy levels, namely diploid, triploid and tetraploid. In relation to the morphological, anatomical and palynological characters, the populations differed both within and between ploidy levels.

Detailed karyotype analysis with the aid of improved methodology revealed a wide range of structural changes both within and outside the ranges of the same chromosome number, but they did not affect meiosis regularity. All these observations suggest a role for allo-rather than autopolyploidy as well as for structural alterations of chromosomes in the evolution of the polyploid cytotypes.

Résumé

Vingt populations différentes de *Costus speciosus* (Koen.) Sm., récoltées dans plusieurs régions de l'Inde et du Népal, ont été étudiées du point de vue cytologique, morphologique, anatomique et palynologique. Trois degrés de polyploïdie ont été mis en évidence, soit diploïde, triploïde et tétraploïde. Les tétraploïdes offrent la plus large distribution. Les triploïdes ont été trouvés essentiellement à des altitudes élevées, mais également dans les plaines du Bengale, alors que le diploïde semble confiné au Khasia et à l'Himalaya. Le polymorphisme morphologique, anatomique et palynologique ne résulte pas exclusivement de la polyploïdie, mais affecte aussi chaque cytodème.

Dedicated to Professor Claude Favarger as a token of admiration and respect on his 70th birthday

L'analyse détaillée du caryotype, à l'aide d'une méthode améliorée, a mis en évidence un large gradient de mutations structurales affectant chaque cytotype. Toutefois, elles paraissent sans incidence notable sur le déroulement de la méiose. En effet, les diploïde et tétraploïde offrent respectivement 9 et 18 bivalents. Le triploïde, en revanche, présente des bi- et univalents, plus rarement des trivalents. La présence d'univalents a également été observée dans certaines populations tétraploïdes. L'ensemble des observations parle en faveur d'une allopolyploïdie plutôt que d'une autopolyploïdie et révèle l'importance des altérations structurales dans l'évolution des cytotypes polyploïdes.

Introduction

The availability of diosgenin – the steroidal precursor, has made *Costus speciosus* (Koen.) Sm. an important material for investigation (Sarin et al. 1974, 1976, Sadasivan 1975, Chandra et al. 1977, Kapahi et al. 1977). This species of Zingiberaceae grow in different forms in various parts of India. Individuals showing diploid, triploid and tetraploid chromosome numbers were obtained. An intensive and extensive analysis of the species collected from different parts of India and Nepal has been undertaken during the present course of study.

It was also desirable to find out the extent to which the various polyploid cytotypes differ in other characters as well. In the present investigation, twenty populations have been included in the analysis on which detailed cytological and morphological studies have been carried out. To find out the correlation, if any, with the different parameters studied, emphasis has been laid on the variants having the same chromosome number. Of the different cytotypes, those with $2n = 36$ chromosomes or tetraploids occur in abundance ranging from plains of Bengal, United Provinces and southern parts of India to the subtemperate zone of the Himalayas (Ramachandran 1969, Subrahmanyam 1978, Khoshoo 1979).

Material

During the course of the present investigation dealing with the morphological and cytological studies on *C. speciosus* (Koen.) Sm., 20 populations were taken into account. Both wild and cultivated populations were collected from different localities of West Bengal and other States of India. One population was obtained from Nepal. The localities from where the plants were collected along with the altitude and mean annual rainfall of those areas are listed in Table 1.

The rhizomes were first grown in earthenware pots in the experimental garden of the Department of Botany, Calcutta, and finally shifted to the field when they sprouted. The plants generally sprout in the months of April/May and wither away during the months of November/December. Flowering usually occurs during July–September, but it varies depending on the onset of monsoon.

Methods

Morphological studies were carried out in all the populations of *C. speciosus* (Koen.) Sm. The characters which were studied included leaf index, length of internode, length of inflorescence, number of flowers per inflorescence and length of bracts. For each character at least 20 readings from different plants of each population were taken at random and mean values as well as standard errors were calculated.

Somatic chromosomes of all the 20 populations were studied from root tip cells. The time for maximum meristematic activity was determined following several trials and the optimum period was found to be between 10:40 A.M. to 12:30 P.M. during which fresh healthy root tips were collected and washed thoroughly. For better penetration of the pretreatment chemicals, the root tips were split longitudinally, prior to pretreatment. For clarification of the chromosome morphology and scattering of the chromosomes, several pretreatment chemicals were used for varying periods at different temperatures. In general, pretreatment in saturated aqueous solution of PDB with trace of aesculine for 2 hours at 14–16 °C was found to be very effective for all the populations. Prior to this, an initial shock treatment for 3–5 minutes at 0–5 °C was found to produce uniform scattering of chromosomes. Overnight fixation at room temperature in acetic acid – ethanol mixture (1:3) was quite effective in all the populations. – Heavy cytoplasmic content is the main problem for cytological observations in this particular species and for clearing of the cytoplasm, several trials were made with different chemicals for varying durations and at various temperatures. Cold hydrolysis with 5(N) HCl at 14–20 °C for 15 minutes was found to produce the required result in all the populations. This was followed by thorough washing with distilled water and 5 minutes treatment with 45% acetic acid. The root tips were then warmed in 2% acetic-orcein staining solution for 3–5 seconds and subsequently kept in the staining solution for 3–4 hours. The tip portion was finally squashed in 45% acetic acid.

Meiotic chromosomes were studied from microsporocytes. Flower buds of different sizes were collected during different times of the day. The optimum time for obtaining maximum metaphase stages was found to be from 9 A.M. to 11 A.M. Anthers from flower buds of suitable size were fixed in different fixatives for varying periods of time. Fixation of the anthers in Newcomer's fluid for 2–4 days at room temperature was found to be very effective, but heavy cytoplasmic content hampered microscopic observations and clearing of the cytoplasm still remained as the main problem. The technique adopted in case of somatic chromosome studies could not be applied here as 5(N) HCl was found to be too strong for the microsporocytes. In contrast, alkaline solution was noted to clear the cytoplasm to a considerable extent. – For effective results, prior to this alkaline treatment, the anthers were transferred from Newcomer's fluid to 45% propionic acid and kept overnight. This was followed by 4 hours treatment with 1:3 propionic acid – ethanol mixture. The anthers were then kept in distilled water overnight and were subsequently treated with N/10 NaOH and water mixture (1:2) for 2 days; thoroughly washed with distilled water, kept in water overnight and then subjected to overnight treatment in 1:3 propionic acid – ethanol mixture. This was followed by 15 minutes treatment with 45% propionic acid. Temporary smears were prepared in 2% propionic – carmine staining solution.

Total chromosome length of each population was determined by adding the whole lengths of all the chromosomes present in a complement.

Chromosome volumes were determined from the karyogram plates by measuring the breadths of individual chromosomes and then applying the following formula considering the chromosomes as cylindrical:

$$\text{Chromosome volume (v)} = \pi r^2 h$$

where, r = radius of the chromosome = breadth/2, and h = whole length of the chromosome. Total chromosome volume of each population was calculated by summing up the volumes of all the chromosomes of the complement.

Pollen sterility was investigated in all the populations and cytotypes excepting a few populations in which flowering stage could not be obtained. To determine the percentage of pollen sterility, mature anthers from unopened buds were stained in 2% acetic-carmine for 3–4 hours. Pollen, which did not take up stain and were irregularly shaped, were considered as sterile. At least 400 pollen were examined for each population and the sterility percentage and standard errors were calculated.

The anatomical characters of the cytotypes studied included stomatal frequency and stomatal index. Six different populations belonging to three different cytotypes, collected from six distinctly different geographical locations, were observed. For such studies, the epidermal layers were

peeled from the apex, base, margin and middle portion of both upper and lower surfaces of leaves. The peelings were then boiled in 70% ethanol in waterbath, stained in safranin staining solution for 15 to 30 seconds and washed in 70% ethanol for a short while. Suitable stained portions were mounted in a drop of glycerine, covered with a coverglass and sealed. The numbers of epidermal cells and stomata were counted under the microscope for stomatal index study which was calculated as follows:

$$\text{Stomatal index (S.I.)} = \frac{S \times 100}{S + E}$$

where, S = Number of stomata, and E = Number of epidermal cells. The stomatal frequency per unit area was then calculated as follows:

$$\text{Stomatal frequency} = \frac{\text{no. of stomata per field area}}{\text{area of the field}}$$

In both these two cases, the values were expressed as minimum, mean and maximum values.

Observations

(a) Morphological data

C. speciosus (Koen.) Sm., an erect perennial herb with tuberous rhizome, showed certain unique morphological characters of which the phyllotaxy is worth mentioning. The oblong to lanceolate leaves were arranged in two developmental spirals on thick cane-like stems (Antony 1979, Asolkar and Chadha 1979). The inflorescence, a dense terminal head, also showed similar radial symmetry in respect to the sequence of blooming of individual flowers and aestivation of corolla.

Five different morphological characters were taken into consideration during the present study. But those related with flowering could not be considered in populations XII, XVI, XVIII and XIX, due to non-availability of the flowering stage unde the same experimental set-up. The data of morphological measurements are represented in Table 2.

In the diploid populations the leaf indices varied within a very narrow range, whereas in the tetraploids, it differed to a considerable extent, the highest being 4.002 in population VIII from New Jalpaiguri and the lowest 2.466 in population VI from Bankura. Amongst the triploids, population XX from Kathmandu with 3.541 index, slightly differed from the two other triploid populations with more or less similar indices. However, the magnitude of variation in the different cytotypes so far studied, was not very distinct (Table 2).

The internodal lengths were, in general, shorter in the diploid populations with the exception of population X from Dhalgawn, where it was 5.06 cm. The triploids differed in this respect amongst themselves to a certain extent. In the tetraploids, the maximum length (i.e. 4.62 cm) was observed in population IX from Naxalbari and the minimum (i.e. 1.94 cm) in population XIX from Coimbatore, while the rest showed considerable variations within this range (Table 2).

The inflorescence lengths as well as number of flowers per inflorescence differed quite distinctly within the different populations and cytotypes (Table 2).

The lengths of floral bracts were almost the same in all the different populations and cytotypes, with the exception of populations X and XVII where the mean lengths were 1.04 cm and 3.7 cm respectively (Table 2).

Tab. 1.

Pop. number	Localities	Altitude (in metres)	Mean annual rainfall (in mm)
I.	Jammu, Jammu & Kashmir	366.0	1148.3
II & IV.	Lucknow, Uttar Pradesh	111.0	992.4
III.	Mohanlalgunge, Lucknow, Uttar Pradesh	128.0	992.4
V.	Shibpur, West Bengal	5.5	1581.8
VI.	Bankura, West Bengal	98.0	1422.0
XIV.	Burdwan, West Bengal	32.0	1403.9
VII.	Sukna, West Bengal	152.4	3352.7
VIII.	New Jalpaiguri, West Bengal	134.0	3352.7
IX.	Naxalbari, West Bengal	152.0	3352.7
X.	Dhalgawn, West Bengal	980.0	3352.7
XI. XII. & XIII.	Mungpoo, West Bengal	1011.0	2758.4
XV.	Devrali, Sikkim	1749.0	1250.0
XVI.	Saramsa, Sikkim	1225.0	1250.0
XVII.	Rongpoo, Sikkim	913.0	1250.0
XVIII.	Shillong, Meghalaya	1500.0	2415.3
XIX.	Coimbatore, Tamil Nadu	402.0	612.2
XX.	Kathmandu, Nepal	1402.08	1250.0

Tab. 2. A comparative representation of different morphological features in different populations of *Costus speciosus* (Koen.) Sm.

Pop. number	2n	Leaf index	Percentage of pollen sterility	Length of the internode (in cm.)	Length of the inflorescence (in cm.)	Length of the floral bract (in cm.)	No. of flowers per inflorescence
I.	36	3.830±0.07	6.50±0.25	3.18±0.37	6.90±0.66	2.06±0.11	19.2 ±1.44
II.	36	3.055±0.07	5.00±0.14	2.56±0.16	7.37±0.46	2.17±0.11	22.8 ±1.86
III.	36	2.909±0.04	0.50±0.07	2.78±0.19	3.88±0.23	2.01±0.03	14.0 ±0.99
IV.	36	2.905±0.05	2.00±0.13	2.65±0.23	4.10±0.22	2.04±0.07	15.6 ±1.40
V.	36	2.669±0.11	0.50±0.12	2.54±0.24	6.30±0.39	2.16±0.12	19.2 ±1.37
VI.	36	2.466±0.06	0.50±0.02	2.78±0.19	3.88±0.23	2.01±0.03	14.0 ±0.99
VII.	36	2.706±0.07	3.00±0.25	2.31±0.28	5.94±0.28	2.26±0.06	13.4 ±1.21
VIII.	36	4.002±0.04	1.00±0.09	2.66±0.21	8.00±0.26	2.18±0.15	24.0 ±1.71
IX.	36	3.101±0.06	20.00±0.95	4.62±0.93	7.34±0.31	2.38±0.11	18.3 ±1.13
X.	18	3.460±0.08	9.25±0.43	5.06±0.52	5.60±0.58	1.04±0.21	8.33±0.88
XI.	18	3.720±0.18	12.50±0.34	1.90±0.19	5.27±0.25	2.29±0.28	8.00±0.58
XII.	27	2.822±0.06	–	1.46±0.17	–	–	–
XIII.	36	3.157±0.07	3.75±0.19	3.54±0.68	10.03±0.84	2.53±0.12	27.1 ±0.88
XIV.	27	2.735±0.08	63.50±1.32	3.17±0.46	6.12±0.12	2.20±0.12	13.60±1.21
XV.	18	3.924±0.48	53.50±1.04	1.88±0.17	5.62±0.28	2.71±0.32	7.80±0.63
XVI.	18	3.640±0.07	–	1.54±0.14	–	–	–
XVII.	36	3.235±0.11	6.00±0.52	2.76±0.29	9.31±1.48	3.70±0.06	26.6 ±3.71
XVIII.	36	2.929±0.15	–	2.24±0.33	–	–	–
XIX.	36	2.921±0.06	–	1.94±0.31	–	–	–
XX.	27	3.541±0.05	45.00±1.62	2.84±0.28	7.25±0.75	2.41±0.10	26.2 ±2.66

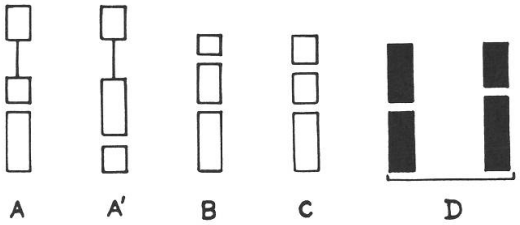

Fig. 1. Diagrammatic representation of common chromosome types present in different populations.

(b) Cytological data

In the present investigation, somatic chromosomes of 20 populations of *C. speciosus* (Koen.) Sm. from different habitats were studied. The meiotic chromosome analysis had to be restricted to 10 populations due to the non-availability of flowers as well as proper divisional stages in others.

The somatic chromosome numbers of the different populations were found to be multiples of 9 and as such the observed intraspecific chromosomal races were diploids ($2n=2x=18$), triploids ($2n=3x=27$) and tetraploids ($2n=4x=36$).

Variation numbers were noted both at the diploid, triploid and tetraploid levels in significantly low frequency.

Normally, the chromosome size did not differ significantly with the different ploidy levels (Figs. 2 and 3), though in some cases the size decreased considerably with a notable increase in chromosome number. In the diploids, the chromosome length ranged from 1.85 μm to 4.63 μm. In the triploids, the range was from 1.67 μm to 3.89 μm and in the tetraploids it was from 1.48 μm to 4.63 μm (Table 3).

The total chromosome length did not differ significantly within a cytotype. However, such lengths at the triploid and tetraploid levels were not exact multiples of the diploid set (Fig. 3).

The chromosome complements of the different populations, however, revealed a gross morphological similarity on the basis of which the general chromosome types were formulated. The chromosome types were classified mainly on the basis of centromeric position.

The different types of chromosomes classified on the basis of number and position of centromeres are as follows (Fig. 1):

Type A: Relatively long chromosome with two constrictions dividing the chromosome into three segments. One constriction is much longer than the other and is traversed by a satellite thread. In most cases, the distal segment is prominently large and the middle segment is either equal or relatively smaller than the proximal part.

Type A′: Similar to type A, only the middle segment is larger than the end ones.

Type B: Chromosome with two constrictions, one median and the other submedian in position.

Type C: Chromosome with two constrictions, one nearly median and the other dividing the relatively longer arm into two equal segments.

Type D: All the remaining chromosomes having mostly median to nearly median primary constriction.

Tab. 3. A comparative representation of different chromosomal parameters in different populations of *Costus speciosus* (Koen.) Sm.

Pop. number	2n	*	Karyotype formula	Total chromosome length (μm)	Range of chromosome length (μm)	TF (%) value	Total chromosome volume (cu. μm)	Range of chromosome volume (cu. μm)
I.	36	8	$A_2B_4C_2D_{28}$	95.44±0.08	1.67–3.70	47.23	82.43±0.10	0.80–3.65
II.	36	8	$A_2B_4C_2D_{28}$	89.46±0.07	1.67–3.15	45.85	105.58±0.17	1.16–5.27
III.	36	8	$A_2B_4C_2D_{28}$	79.04±0.06	1.48–2.59	44.89	102.39±0.14	1.16–4.46
IV.	36	8	$A_2B_4C_2D_{28}$	99.80±0.08	1.48–3.52	44.57	110.59±0.12	1.26–4.67
V.	36	10	$A_2B_4C_4D_{26}$	109.22±0.07	2.22–4.26	45.47	122.30±0.15	2.19–5.73
VI.	36	8	$A_2B_4C_2D_{28}$	96.62±0.08	1.67–3.70	45.91	108.48±0.13	1.80–4.91
VII.	36	8	$B_6C_2D_{28}$	101.08±0.09	1.67–4.07	48.40	107.54±0.12	1.65–4.46
VIII.	36	8	$A_2B_4C_2D_{28}$	104.40±0.09	1.85–3.50	45.79	97.93±0.10	1.57–4.42
IX.	36	6	$A'_2B_2C_2D_{30}$	107.80±0.10	2.04–4.44	44.49	108.70±0.37	2.55–9.92
X.	18	4	$B_2C_2D_{14}$	45.18±0.06	2.04–2.96	43.25	78.84±0.30	3.01–7.04
XI.	18	4	$A_2B_2D_{14}$	57.92±0.17	2.04–4.63	45.03	78.31±0.30	2.27–6.37
XII.	27	7	$A_1B_3C_3D_{4(3)+4(2)}$	69.54±0.09	1.67–3.15	44.48	73.70±0.23	1.13–5.28
XIII.	36	8	$A_2B_2C_4D_{28}$	112.90±0.10	1.85–4.07	45.74	138.63±0.17	1.23–5.09
XIV.	27	7	$A_1B_6D_{4(3)+4(2)}$	81.83±0.11	1.85–3.89	43.00	97.92±0.22	1.57–6.07
XV.	18	4	$A_2B_2D_{14}$	44.80±0.08	1.85–2.96	45.60	78.34±0.33	2.46–6.48
XVI.	18	4	$A_2B_2D_{14}$	58.76±0.14	2.22–3.89	44.79	65.96±0.23	1.74–5.16
XVII.	36	8	$A_2B_4C_2D_{28}$	103.64±0.10	1.85–4.07	46.72	113.88±0.17	1.54–6.07
XVIII.	36	8	$A_2B_2C_4D_{28}$	101.64±0.11	1.85–4.07	46.97	74.16±0.14	0.88–4.93
XIX.	36	10	$A_2B_6C_2D_{26}$	112.94±0.10	2.22–4.63	45.44	123.05±0.17	1.67–6.06
XX.	27	6	$A_1B_3C_2D_{3(3)+6(2)}$	81.24±0.11	2.04–3.89	46.34	72.80±0.13	1.39–3.58

* Number of chromosomes bearing secondary constructions

The karyotype formulae of the different populations are expressed on the basis of numbers of these four types of chromosomes (Table 3).

Structural and numerical alterations of the nucleolar chromosomes were very well marked in the different populations. In the diploids, though the number of nucleolar chromosomes was four in all the four populations studied, population X from Dhalgawn differed from the others in having 'C' type chromosome in place of 'A' type (Table 3, Fig. 2). Among the three triploid populations, two were with 7 nucleolar chromosomes, while the third one from Nepal showed only 6 such chromosomes. Furthermore, those having 7 nucleolar chromosomes differed amongst themselves, for example, in population XIV from Burdwan the 'C' type was totally replaced by the 'B' type

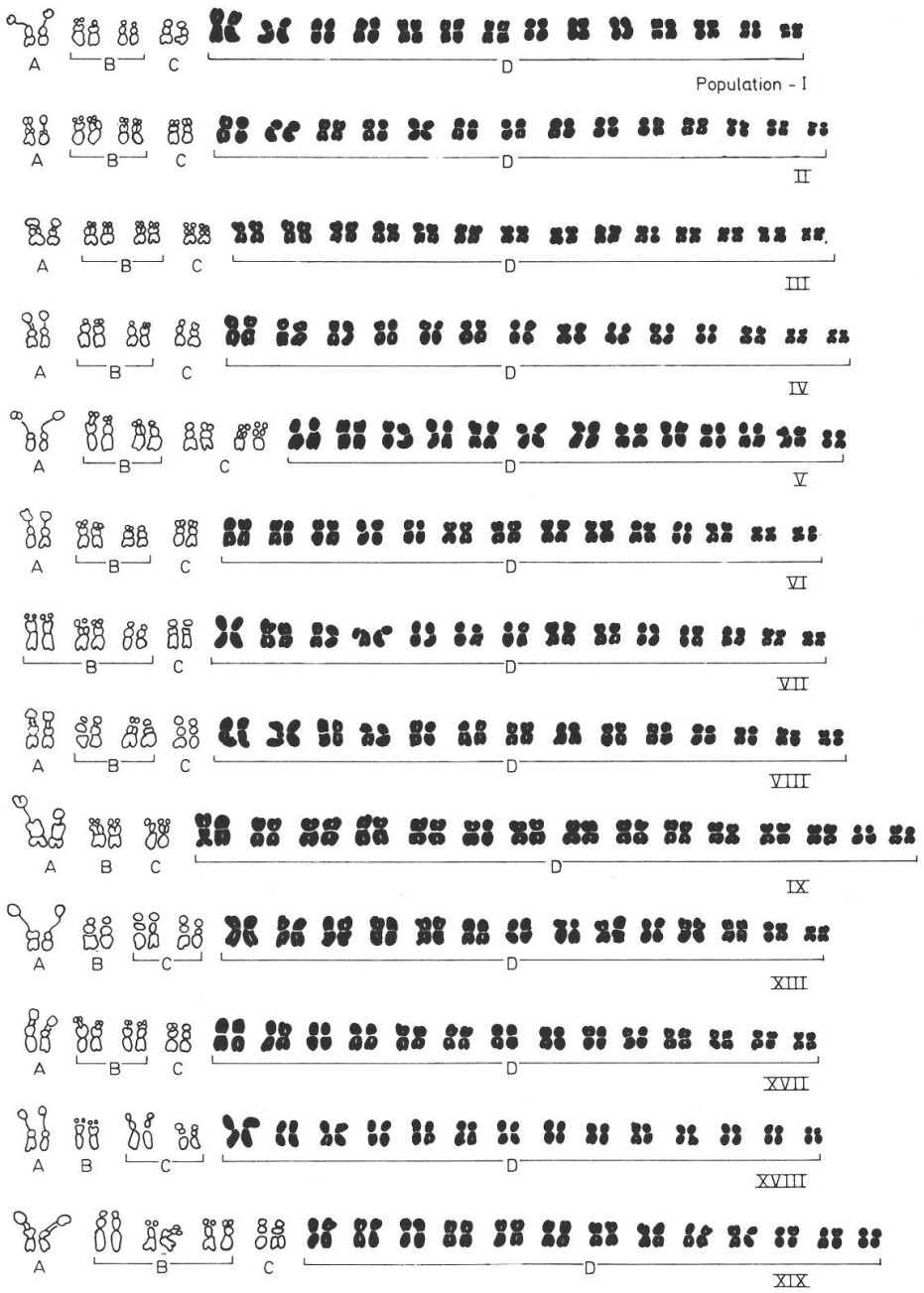

Fig. 2

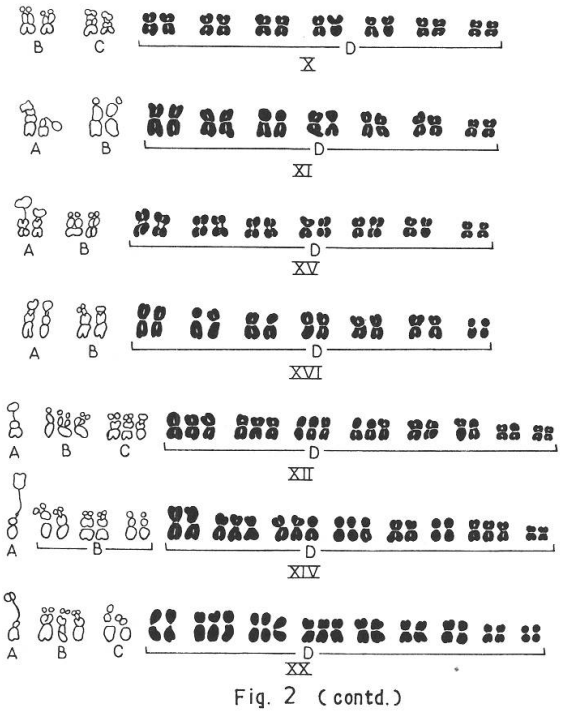

Fig. 2. Comparative representation of karyograms of different populations of *Costus speciosus* (Koen.) Sm.

chromosome, while in population XII from Mungpoo both 'B' and 'C' types were present. Amongst the tetraploids, the number of nucleolar chromosomes ranged from 6 to 10 with wide diversity in the number of different types (Table 3).

The total chromosome volume in the diploids ranged from 65.96 cu μm to 78.84 cu μm whereas in the triploids and tetraploids the ranges were from 72.80 cu μm to 97.92 cu μm and from 74.16 cu μm to 138.63 cu μm respectively. The chromosome volumes differed quite significantly both at the intra- and intercytotypic levels (Table 3 and Fig. 3).

At meiotic metaphase I, 18 bivalents were noted in all the tetraploid populations, although the rare occurrence of univalents had also been noticed in extremely low frequencies in some of the populations.

Meiotic analysis of the triploid population from Burdwan revealed varying numbers of bi- and univalents at metaphase I. Trivalents, though infrequent, were also recorded.

In the diploid population XV from Devrali, Sikkim, regular 9 bivalents were observed. Both ring and rod-shaped bivalents were observed, the ring shaped ones being more prevalent.

The chromosome numbers of *C. speciosus* (Koen.) Sm., collected from different ecological habitats, as reported by previous authors and in the present paper, have been represented in Tables 4 and 5.

(c) Palynological data

The pollen sterility percentage was studied in all the different populations in which flowering stage was obtained under the experimental set-up. The results are presented in Table 2. The sterility percentage was rather high in the triploids as compared to the

Tab. 4. List of previous chromosome number reports of *Costus speciosus* (Koen.) Sm.

Author	Year	Chromosome number		Locality
		n	2n	
Sato, D.	1948	–	18	Japan
Subrahmanyam, G. V.	1978	–	18	Jorhat, Assam
Subrahmanyam, G. V.	1978	–	18	Arunachal Pradesh
Nagendra, P. and Abraham, P. Z.	1981	–	18	Nedumboyil, Kerala
Nagendra, P. and Abraham, P. Z.	1981	–	18	Shoranur, Kerala
Simmonds, N. W.	1954	9II + 9I	27	Trinidad
Subrahmanyam, G. V.	1978	–	27	Andaman
Nagendra, P. and Abraham, P. Z.	1981	–	27	Kakkayam, Kerala
Banerji, I.	1940	18II	36	University College Experimental Garden, Calcutta
Raghavan, T. S. and Venkatasubban, K. R.	1943	–	36	Tenmalai Forests, Tamil Nadu
Chakravorti, A. K.	1948	–	36	
Sharma, A. K. and Bhattacharyya, N. K.	1959	–	36	Khumani, Darjeeling
Mitra, K. and Datta, N.	1967	18II	–	Shibpur, West Bengal
Ramachandran, K.	1969	18II	36	South India
Subrahmanyam, G. V.	1978	18II	36	Jammu, Jammu and Kashmir
Subrahmanyam, G. V.	1978	18II	36	Kangra, Himachal Pradesh
Subrahmanyam, G. V.	1978	–	36	Dehra Dun, Uttar Pradesh
Subrahmanyam, G. V.	1978	–	36	Bamboo Forest, Andaman
Subrahmanyam, G. V.	1978	18II	36	Mohanlalganj Gopalkhera, Lucknow.
Nagendra, P. and Abraham, P. Z.	1981	–	36	Shoranur, Keralan
Nagendra, P. and Abraham, P. Z.	1981	–	36	Kottayam, Kerala
Nagendra, P. and Abraham, P. Z.	1981	–	36	Jammu, Jammu and Kashmir

Tab. 5. List of present chromosome number reports of *Costus speciosus* (Koen.) Sm.

Chromosome number		Locality	Chromosome number		Locality
n	2n		n	2n	
–	18	Dhalgawn, West Bengal	–	36	Bankura, West Bengal
–	18	Mungpoo, West Bengal	18II	36	Sukna, West Bengal
9II	18	Devrali, Sikkim	–	36	New Jalpaiguri, West Bengal
–	18	Saramsa, Sikkim	18II	36	Naxalbari, West Bengal
–	27	Mungpoo, West Bengal	18II	36	Mungpoo, West Bengal
9II + 9I	27	Burdwan, West Bengal	17II + 2I	36	Rongpo, Sikkim
–	27	Kathmandu, Nepal	–	36	Shillong, Meghalya
18II	36	Jammu, Jammu and Kashmir	–	36	Coimbatore, Tamil Nadu
18II	36	Lucknow, Uttar Pradesh	18II	36	Lucknow, Uttar Pradesh
18II	36	Shibpur, West Bengal	–	36	Lucknow, Uttar Pradesh

diploids and tetraploids, with the exception of population XV – a diploid one from Devrali, Sikkim, in which the percentage was 53.5. Non-viable seeds were also observed in this diploid population.

Fusion of the pollen grains was noted in the diploid populations XV and in the tetraploid population XIII.

Dimorphic pollen grains were quite distinct in the triploid populations, whereas, amongst the diploids, the size variation, though present, was not so prominent. The tetraploids did not show any such size difference.

Binucleate pollens were observed in low frequency in population XV from Devrali.

(d) Anatomical data

The stomatal indices and frequencies of both upper and lower surfaces of leaves of 6 populations of *C. speciosus* (Koen.) Sm. were studied and the data are represented in Table 6. These 6 populations belong to 3 different cytotypes and were collected from six different geographical regions of varied altitudes (Table 2). In general, the stomata were found to be 'paracytic' in nature.

The mean stomatal frequency of the upper surface of leaves was significantly less in the diploids as compared to the triploids and tetraploids. Both the triploid populations showed the same mean values though their minimum and maximum values differed to a certain extent. The tetraploids showed wide diversity in his respect, the frequencies being 8 in population II from Lucknow and 15 in population XVII from Rongpo (Table 6).

In regard to the stomatal frequency of the lower surface of leaf, the variations were more prominent at the intracytotypic level than at the intercytotypic level.

In the stomatal indices, the values of the upper leaf surfaces differed considerably both at the intra- and intercytotypic levels, whereas the variations were less prominent in the case of the indices of the lower leaf surfaces.

Discussion

Chromosome number – diploids and polyploids

The present study on 20 different populations of *Costus speciosus* (Koen.) Sm., the diosgenin yielding species, covers 13 tetraploids (with 36 chromosomes), 3 triploids (with 27 chromosomes) and rest diploids (with 18 chromosomes).

The diploids, so far found, are mostly restricted to Sikkim, Dhalgawn and Mungpoo, that is the Khasia and Himalayan belts. However, the diploid number has also been reported from Kerala, possibly from the Western ghat ranges (Nagendra and Abraham 1981) and from the eastern parts of India (Subrahmanyam 1978).

The triploids too, so far studied, are found to be restricted to comparatively higher altitudes, though a population has also been reported from Burdwan, the plains of Bengal. The occurrence of triploidy has previously been reported from the Andaman islands (Subrahmanyam 1978) and also from the humid tropics of Kerala and adjoining places (Nagendra and Abraham 1981).

Tetraploids, on the other hand, have more or less a sort of cosmopolitan distribution – from Mungpoo in the Himalayas to Lucknow in the United Provinces. In fact, from Mungpoo area all the three cytotypes – diploid, triploid and tetraploid – have been recorded. A similar sort of distribution has also been observed at Kerala including the Western ghat ranges by Nagendra and Abraham (1981).

Evidently, high rainfall, humidity and comparatively subtemperate climate have permitted the profusion and survival of diploid and polyploid cytotypes. As far as the wide distribution of tetraploids is concerned, ample cases are on record where polyploids are endowed with a wide range of tolerance as compared to diploids (Gottschalk 1976, Stebbins 1950, Stott 1981).

Polyploidy as indicated through meiotic analysis, does not necessarily show mere duplication of the diploid set. Multivalents are comparatively rare. This is also indicated in the karyotypes where the chromosomes are not present in exact multiples (vide Table 3).

As far as chromosomes with secondary constriction are concerned, similar indications have been obtained. For example, in population IX from Naxalbari with 36 chromosomes there are six chromosomes with secondary constriction as compared to four of diploids. All these facts suggest allo- rather than autopolyploidy or polyploidy associated with structural alteration of chromosomes which have been responsible for the evolution of the polyploid cytotypes.

Cytotypes having same chromosome number

Cytotypes with the same chromosome number have been recorded at the diploid, triploid and tetraploid levels, the majority belonging to the tetraploid. All these populations at the intraspecific level differ in minute karyotypic details, indicating the role of

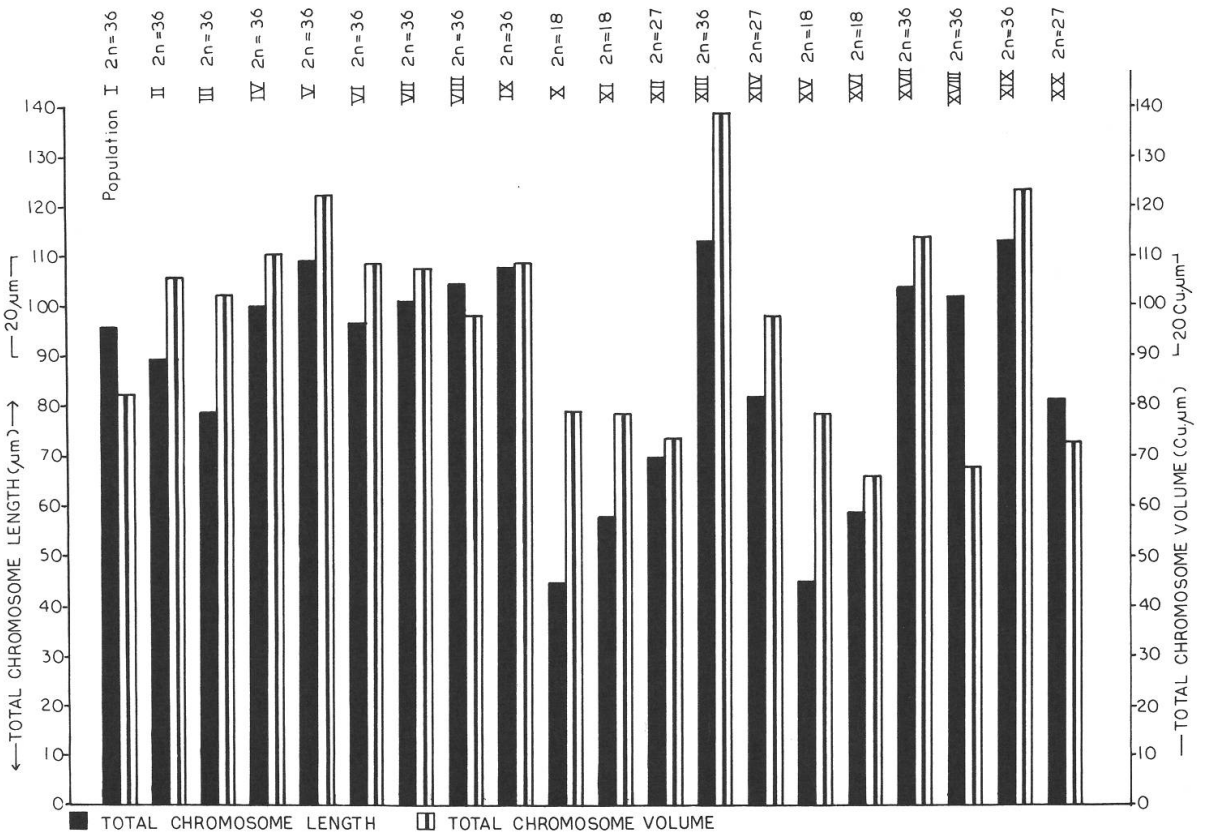

Fig. 3. Comparative histogram plate representing the total chromosome length and volume in different populations of *Costus speciosus* (Koen.) Sm.

structural alteration of chromosomes in bringing out the diversity of genotypes. The extent to which such genotypes are adapted to narrow ecological natures is yet to be established. However, meiotic analysis does not reveal inhibition of bivalent formation to a significant extent. This fact may be taken to suggest that minute structural changes have not been so extensive as to affect bivalent formation.

Pollen sterility is lower in diploids and tetraploids than triploids (vide Table 2). The high percentage of pollen sterility in triploids is a rather common feature in other plants and in *C. speciosus* (Koen.) Sm. as well (Simmonds 1954, Khoshoo 1979, Nagendra and Abraham 1981). Amongst the diploids, only in population XV from Devrali, Sikkim ($2n = 18$) has a very high percentage of pollen sterility been recorded. Evidently, minute genetic changes might have been responsible for such high frequency of sterile grains. The heavy frequency of sterility on this diploid population can also be attributed to the high degree of abnormalities in pollen where fusion and binucleate pollen have often been recorded.

The presence of such wide occurrence of genotypes at the intraspecific level opens up the possibility of the exploration and utilisation of their active principles.

Chromosome characteristics – size, number and volume

Amongst the populations with the same chromosome number in general, the total chromosome length does not show a heavy difference, notwithstanding with minute karyotypic differences (vide Table 3 and Fig. 2). Amongst the tetraploid populations, it ranges from 79 μm in population III from Lucknow to 112 μm in population XIX from Coimbatore. The differences, so far observed, can be attributed to karyotypic differences as well.

An analysis of the total chromosome lengths between diploids, triploids and tetraploids gives an indication of the extent to which duplication has been involved. Evidently, as revealed from karyotypes too, the length does not necessarily involve exact multiplication indicating the importance of structural changes as well as allopolyploidy.

In relation to chromosome size, the range also more or less remains the same if one takes into account a large number of genotypes in its totality. However, the slight difference in chromosome size between the different cytotypes including the range of size are further indications of the influence of structural changes of chromosome in the origin of different genotypes (vide Table 3).

There has, however, been a marked difference in chromosome volume in the complements of different populations both within and outside the ranges of the same chromosome number (vide Table 3 and Fig. 3). However, the chromosome volume is determined not only by its nucleic acid content, but also by the protein moiety and the extent of spiralization. All these factors are under genetic control and are quite distinctive for the genotype concerned.

Correlation between chromosome complements, morphological and anatomical characters

As far as the phenotypic characters are concerned, studies have been carried out in all the 20 populations, but in regard to the flower characters, studies had to be restricted to 15 populations in view of the absence of profuse flowering in several of them under the same experimental set up.

In general, diploids are comparatively smaller and weaker in overall growth as compared to the tetraploids. However, in population X with $2n = 18$ chromosomes, the

growth has been found to be rather luxurious. The cause of high percentage of pollen sterility in the diploid population XV has also been attributed to genic causes. Even the overall data indicate that, associated with the karyotypic changes which the populations represent, there has been observable differences in phenotypic and anatomical characters, mainly the stomatal index (vide Table 6). It is of significance that the diploids in general of the 6 populations studied, have very low number of stomata on the upper surface as compared to that of the triploids and tetraploids (vide Table 6). This is quite unusual, as in general polyploidy has so far been recorded to be associated mostly with increase in stomatal size but not necessarily in numbers (Cain 1944). However, this anatomical difference in relation to the number of chromosomes is quite remarkable.

Tab. 6. The ranges of stomatal frequency and index values of different cytotypes of *Costus speciosus* (Koen.) Sm.

Pop. no.	Altitude (in metres)	2n	Stomatal frequency						Stomatal index					
			Upper surface			Lower surface			Upper surface			Lower surface		
			min.	mean	max.	min.	mean	max.	min.	mean	max.	min.	mean	max.
X.	980	18	3	4	5	34	60	91	0.77	1.07	1.27	5.83	7.84	9.71
XV.	1749	18	1	3	5	46	75	103	1.69	3.06	4.25	8.76	9.47	10.53
XIV.	32	27	5	9	16	45	64	74	1.17	1.90	2.92	6.58	8.09	8.93
XX.	1402	27	3	9	11	96	100	111	1.08	2.10	3.93	8.57	8.98	9.53
II.	111	36	5	8	9	39	56	64	1.22	2.22	3.85	7.35	8.94	10.53
XVII.	913	36	12	15	18	54	99	137	3.59	4.81	5.58	7.09	7.82	8.66

In the stomatal frequency of the lower surface, the populations at the intra-diploid, intra-triploid and intra-tetraploid levels show remarkable differences in the mean number. Such a difference, with chromosome number remaining the same, is the clear indication of the association of minute karyotypic changes with anatomical characters. It is quite likely however, that such changes have been associated with the adaptive features under specific environmental conditions (Singh and Srivastava 1980).

These different parameters, taken as a whole may be utilised as identifying characteristics of the populations as the observations are based on all the individuals of the populations and reproduceable constancy in results were obtained.

The present data was able to bring out the association of minute chromosomal changes at diploid, triploid and tetraploid levels with the change in phenotypic characters. These results may serve as markers for populations to be explored for commercial exploitation.

Summary

Detailed investigations involving cytological, morphological, anatomical and palynological studies were carried out on 20 different populations of *Costus speciosus* (Koen.) Sm., collected from different parts of India and Nepal. The populations studied, represented three distinct ploidy levels, namely diploid, triploid and tetraploid.

The tetraploids predominated having a wider distribution. The triploids were obtained from comparatively higher altitudes as well as from the plains of Bengal, whereas the diploids were found to be restricted mostly to the Khasia and Himalayan belts. In relation to the morphological, anatomical and playnological characters, the populations differed both within and between ploidy levels.

Detailed karyotype analysis with the aid of improved methodology revealed a wide range of structural changes both within and outside the ranges of the same chromosome number. The total chromosome length did not differ significantly within a cytotype. However, such alterations in length and total chromosome volume at the triploid and tetraploid levels did not necessarily involve exact multiplication of the diploid set. Evidently chromosome volumes differed quite significantly both between and within cytotypes.

Meiotic data revealed nine regular bivalents in the diploid, varying numbers of bi- and univalents with rare occurrence of trivalents in the triploid and 18 bivalents in the tetraploids. However, occurrence of univalents had also been noted in extremely low frequencies in some of the tetraploid populations. All these factors are under genetic control and suggest the role of allo- rather than autopolyploidy and structural alteration of chromosomes in the evolutions of the polyploid cytotypes.

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