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Genetic Diversity in *Costus speciosus* (Koen.) Sm.

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A member of the family Zingiberaceae—*Costus speciosus* (Koen.) Sm. is widely distributed in India and has received considerable attention due to its diosgenin content (Sarin *et al.* 1974, 1976).

The profusion of growth of Zingiberaceae in different areas of the Eastern Himalayas is suspected as prognostic of the existence of cytotypes and polyploid forms, specially in view of the importance of polyploidy in speciation in Zingiberaceae (Chakravorti 1948, Sharma and Bhattacharyya 1959).

In course of the present investigation, diploid, triploid and tetraploid cytotypes have been recorded from different ecological locations and even from the same ecological zone. Lately, the occurrence of polyploids in this species has been reported from Assam, Arunachal Pradesh and peninsular India, as also Andamans (Subrahmanyam 1978, Nagendra and Abraham 1981). These records mainly deal with the chromosome numbers without any details of the karyotypes. In view of the absence of any such previous records from the Himalayas and also in order to study the chromosome structure and nature of the genotypes with the aid of improved methods, the present investigation was undertaken.

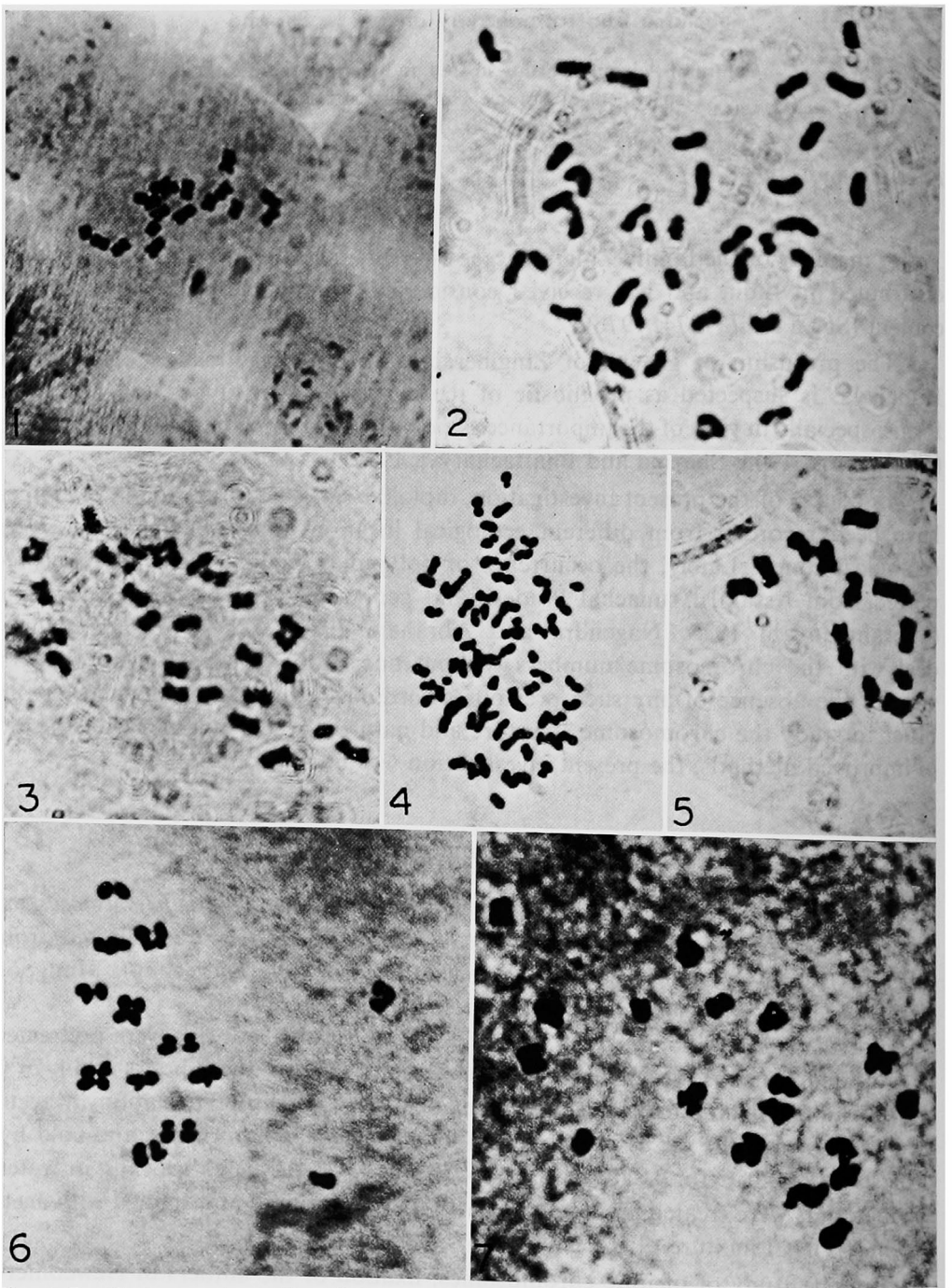
Materials and methods

The present study is mainly restricted to populations of *Costus speciosus* exhibiting intraspecific chromosomal races. The populations were collected from different districts of West Bengal, namely Dhalgawn (Dt. Darjeeling), Mungpoo (Dt. Darjeeling), New Jalpaiguri (Dt. Darjeeling) and Burdwan.

For the study of somatic chromosomes, fresh healthy root tips were pretreated in saturated aqueous paradichlorobenzene-aesculin mixture for 2 hours at 14–16°C with an initial 5 minutes shock treatment at 0–5°C. Overnight fixation in acetic acid–ethanol mixture (1 : 3) was followed by washing of the root tips and cold hydrolysis at 18–20°C for 15 minutes in 5N HCl. After thorough washing in water, the materials were treated for 5 minutes with 45% acetic acid and stained with aceto orcein- (N)HCl mixture (9 : 1).

For the study of meiotic chromosomes, fixation of the anthers in Newcomer's fluid for 2–4 days was followed by overnight treatment in 45% propionic acid. The anthers were then fixed in 1 : 3 propionic acid–ethanol mixture for 4 hours and transferred to distilled water for overnight. They were subsequently kept in a N/10

NaOH and water (1 : 2) mixture for 2 days; thoroughly washed with distilled water; kept in water for overnight and then subjected to overnight treatment in 1 : 3 pro-



Figs. 1–7. *Costus speciosus* (Koen.) Sm. 1–3, somatic metaphase of populations I, 2n=18 (×1000); V, 2n=36 (×1600) and II, 2n=27 (×1600) respectively. 4 and 5, variation plates, 2n=72 (×1300) and 2n=13 (×1650) respectively. 6 and 7, meiotic metaphase I of populations II showing 9II+6I+1III (×1600) and V showing 18II (×1600) respectively.

pionic acid–ethanol mixture and 15 minutes treatment in 45% propionic acid. Temporary smears were prepared with 2% propionic–carmine.

Observations

The somatic chromosome numbers of the different populations investigated have been found to be multiples of nine.

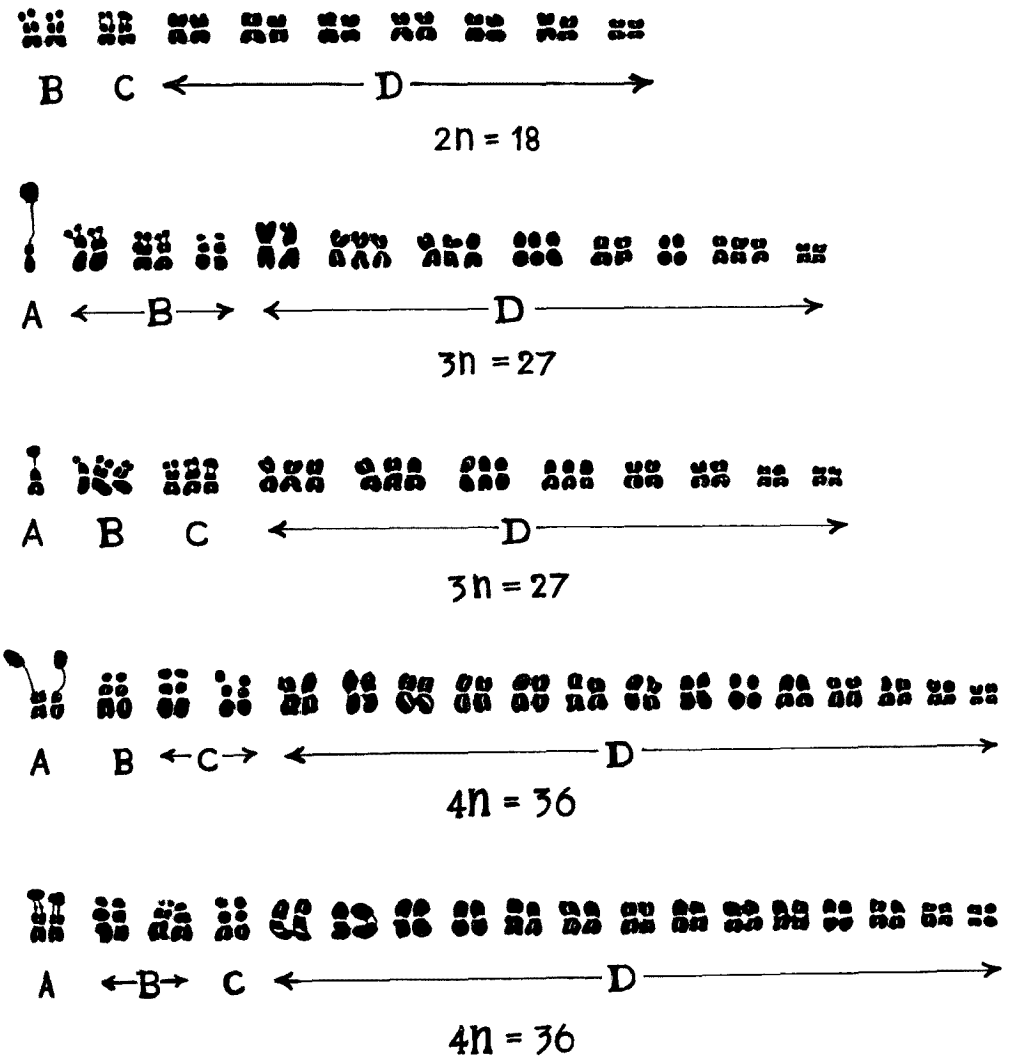


Fig. 8. Karyograms of the five different populations of *C. speciosus* (Koen.) Sm.

The plants collected from Dhalgawn represent a diploid population consisting of 18 chromosomes (Fig. 1), while those from Burdwan are triploids, bearing 27 chromosomes (Fig. 3). The Mungpoo population is interesting in that, a single plant has been found to be triploid bearing 27 chromosomes while all the remaining plants are tetraploid with 36 chromosomes. The plants from New Jalpaiguri represent a regular tetraploid population (Fig. 2).

A detailed chromosomal analysis revealed a gross morphological similarity between complements of different populations which can be distinguished from each other by minute karyotypic differences.

On the basis of gross morphological similarities, four chromosome types have been found to occur in these populations (Fig. 8).

Type A bears a secondary constriction whose distal part is widely separated from the rest and remains connected by a DNA positive thread, the exact nature of which is unknown. It is clear that the distal segment of this type 'A' chromosome is prominently large. This is a characteristic chromosome of *C. speciosus*, found in pairs in all tetraploid populations so far studied. In triploid plants, this chromosome remains unpaired. In the diploid plants of Dhalgawn population, this particular type is absent.

Type B also bears a secondary constriction. The arm after primary constriction is larger than that after secondary constriction.

Type C also bears secondary constriction. Here the two other arms are equal.

Type D includes all the remaining chromosomes having mostly median to nearly median primary constrictions.

The chromosome numbers and karyotype formulae of the different populations investigated are given below:

Pop. I (Dhalgawn) $2n=2x=18=B_2+C_2+D_{14}$

Pop. II (Burdwan) $2n=3x=27=A_1+B_6+D_{4(III)}+4(II)$

Pop. III (Mungpoo A) $2n=3x=27=A_1+B_3+C_3+D_4(III)+4(II)$

Pop. IV (Mnugpoo) $2n=4x=36=A_2+B_2+C_4+D_{28}$

Pop. V (New Jalpaiguri) $2n=4x=36=A_2+B_4+C_2+D_{28}$

These can also be represented as follows:

Popluation I (diploid) $2n=18=2B+2C+14D=2(B+C+7D)$

Population II (triploid) $2n=27=A+6B+20D=(A+B+7D)+2(B+C+7D)+3B-2C-D$

Population III (triploid) $2n=27=A+3B+3C+20D=(A+B+7D)+2(B+C+7D)+C-D$

Population IV (tetraploid) $2n=36=2A+2B+4C+28D=2(A+C+7D)+2(B+C+7D)$

Population V (tetraploid) $2n=36=2A+4B+2C+28D=2(A+B+7D)+2(B+C+7D)$

So, the basic karyotype (genome)

$X_1=A+B+7D$

$X_2=A+C+7D$ are assumed, and as such the karyptype

$X_3=B+C+7D$

formulae appear to be

Pop. I $2n=18=2(B+C+7D)=X_3X_3$

Pop. II $2n=27=(A+B+7D)+2(B+C+7D)+3B-2C-D=X_1X_3X_3+3B-2C-D$

Pop. III $2n=27=(A+B+7D)+2(B+C+7D)+C-D=X_1X_3X_3+C-D$

Pop. IV $2n=36=2(A+C+7D)+2(B+C+7D)=X_2X_2X_3X_3$

Pop. V $2n=36=2(A+B+7D)+2(B+C+7D)=X_1X_1X_3X_3$

In these populations, chromosome size ranges from 1.5 to 4.07 μ. Variation numbers such as 2n=13, 72, have been noted to occur in very low frequency in some of the populations (Figs. 4 and 5). Meiotic studies in all the tetraploid populations reveal 18 bivalents at metaphase I (Fig. 7). Triploid plants show varying numbers of bivalents and univalents. Trivalents, though infrequent, yet have been recorded (Fig. 6). The different types of chromosomal configurations observed in the triploid plants are, 9II+9I, 8II+1II, 7II+13I, 8II+8I+1III, 9II+6I+1III, 6II+9I+2III. Among these, the first one occurs in 50% of the cells.

In the diploid, due to nonavailability of flowers, meiotic study could not be carried out.

Discussion

The normal somatic chromosome number, so far reported to occur in majority of populations of *Costus speciosus*, is 2n=36. The existence of 2n=18 and 27 chromosomes had been reported earlier from Japan and Trinidad (Sato 1948, Simmonds 1954). Such individuals have been recorded from Assam, Arunachal Pradesh and peninsular India and also Andamans (Ramachandran 1969, Subrahmanyam 1978, Nagendra and Abraham 1981). These reports however mainly cite the chromosome count without any details of karyotype. The present study deals for the first time with a detailed karyotype analysis of this particular species. Furthermore, the reports of the occurrence of variable cytotypes, so far unreported from the Himalayas, are very significant. Meiotic data revealing the absence of quadrivalents in the tetraploids (Banerji 1940, Ramachandran 1969) and rare occurrence of trivalents in the triploids, can be regarded to indicate the allopolyploid nature and the occurrence of structural alterations of chromosomes in evolution. The importance of structural alteration is also evidenced in the presence of satellited chromosomes (Type A), which is absent in the diploid cytotype.

There are two possiblities of accounting for this chromosome—

1) There has been uncoiling of the intervening thread, possibly needed for gene expression.
2) There has been non-homologous translocation between 'B' and 'C' so that the distal part of 'C' is replaced by large proximal part of 'B'. However, more evidences should be forthcoming to account for such a process.

Moreover, the populations differ with respect to other details of the karyotype. For example, the two triploid and two tetraploid types have dissimilar karyotypes. In view of these facts, it appears that structural alterations have played an important role in the evolution of different strains, and populations as well. The occurrence of a triploid plant in a tetraploid population is quite interesting. Such an individual in a population may originate out of the union of a haploid and a diploid gametes. Diploid populations have been reported in different locations within the same climatic zone. It is likely that further exploration may reveal diploid populations near the tetraploids, thus providing further evidence of the possibility of union of haploid and diploid gametes.

The existence of different cytotypes with structurally altered chromosomes and the occurrence of different ploidy levels between 2500 ft. to 6000 ft. in the Himalayas clearly suggest a wide genetic diversity existing in the species (see Sharma 1956, 1970). Such wide genetic diversity offers scope for exploitation of medicinal principles from different genotypes.

Summary

Chromosome studies have been carried out on diploid, triploid and tetraploid populations of *Costus speciosus* distributed in the Eastern Himalayas. Detailed karyotype analysis with suitably modified techniques shows a wide range of structural alterations of chromosomes even within populations of the same ploidy level. Meiotic data reveals 18 bivalents in the tetraploid and varying numbers of bi- and univalents, with rare occurrence of trivalents, in the triploids. These observations suggest the role of allopolyploidy and structural alterations of chromosomes in the evolution of these cytotypes.

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References

Banerji, I. 1940. A contribution to the life-history of *Costus speciosus* Smith. J. Indian Bot. Soc. **19**: 181–196.

Chakravorti, A. K. 1948. Multiplication of chromosome numbers in relation to speciation in Zingiberaceae. Sci. Cult. **14**: 137–140.

Nagendra, P. and Abraham, P. Z. 1981. Polyploidy and speciation in *Costus speciosus* (Koen.) Sm. Curr. Sci. **50**: 26–28.

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

Sarin, Y. K., Bedi, K. L. and Atal, C. K. 1974. *Costus speciosus* rhizome as source of diosgenin. Curr. Sci. **43**: 569–570.

—, Kapahi, B. K., Kapur, S. K. and Atal, C. K. 1976. Studies on *Costus speciosus* Sims. Part I: Variability in diosgenin content. Curr. Sci. **45**: 688–690.

Sato, D. 1948. Jap. J. Genet. **23**: 44. Cited in Darlington, C.D. and Wylie, A.P. Chromosome Atlas 2nd ed. 1955. George Allen & Unwin Ltd., London.

Sharma, A. K. 1956. A new concept of a means of speciation in plants. Caryologia **9**: 93–130.

— 1970. Polyploidy and chromosome size. Chromosomes Today **3**: 248–252, 1st print 1972. Longman Group Ltd. Journals division, London.

— and Bhattacharyya, N. K. 1959. Cytology of several members of Zingiberaceae and a study of the inconstancy of their chromosome complements. La Cellule **59**: 299–346.

Simmonds, N. W. 1954. Chromosome behaviour in some tropical plants. Heredity **8**: 139–146.

Subrahmanyam, G. V. 1978. Intraspecific polyploidy in *Costus speciosus*. Curr. Sci. **47**: 435–436.