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Cytogenetics of Tropical Bulbous Ornamentals III. Mitotic Mosaicism in 3x *Crinum augustum*

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Summary. Triploid *Crinum augustum* contains two types of complements ($2n = 33$, $32 + Te$) in the meristems of primary and secondary roots, together with a small amount of aneusomaty. This condition is maintained by efficient vegetative reproduction. The normal complement ($2n = 33$) contains 11 triplets. The $32 + Te$ complement tallies with the former except that one of the small submedian chromosomes of the XIth triplet is replaced by a telocentric which more or less tallies in size with the short arm of the missing chromosome. The telocentric probably arose through misdivision early in ontogeny in which the complete centromere was in the smaller arm. The longer acentric arm, lost in a few subsequent cell generations, is not expected to exercise any deleterious effect because of the polyploid nature of the taxon. Only cells containing the full complement of 33 chromosomes are selected in the male germ line.

Introduction

Crinum augustum Roxbg., a native of the Indian Ocean region (Koshimizu, 1938), has been reported as diploid ($2n = 22$) by Bose (1965), while Miege (1962) has recorded two numbers, $2n = 32$ or 33. Whether he found the two numbers in the same or in different individuals is not apparent from his account. In any case, the occurrence of numbers such as 32 or 33 in a species of *Crinum*, a genus almost exclusively based on $x = 11$ (Jones and Smith, 1967; Raina and Khoshoo, 1970), is extremely interesting. The present authors, while not confirming Bose's findings, have, however, found two types of chromosome complements ($2n = 32 + Te$ and 33) in different root tip cells in one plant of the species, while only balanced cells ($2n = 33$) are selected in male meiosis.

Material and Methods

Plants of the species, originally obtained through the courtesy of Mr. S. Percy Lancaster from the (Royal) Agri-Horticultural Society, Alipore, Calcutta, are a part of the *Crinum* collection in the Bulb Section of this Garden. They were investigated following the methods given in an earlier paper (Khoshoo and Raina, 1968).

Observations

I. Root tip mitosis

The present study is based on an analysis of 158 root tip mitoses, of which 81 cells were from primary and 77 from secondary roots. The results are summarized in Table 1 and 2.

About 52% of the cells contained 33 chromosomes (Fig. 1). This complement resolves, as usual, into 11 triplets, one large, 6 medium and 4 short in size (Fig. 6). Triplet numbers I and VII show heteromorphicity. Two chromosomes of the former have almost equal long arms but differ in the length of the short arms, while the third member is significantly shorter than the other two. Only one of the chromosomes of the triplet VII possesses a secondary constriction in the longer arm (Figs. 1, 6); this appears to have been suppressed in the other two chromosomes. Such a karyotype fits well within the pattern found in the genus as a whole (Jones and Smith, 1967; Khoshoo and Raina, 1968; Raina and Khoshoo, 1970).

In nearly 46% of cells, instead of the regular complement of 33 chromosomes, there were 32 normal plus a small, apparently telocentric, chromosome (Figs. 2–3). An analysis reveals that these cells tally with the normal complement in every way except for the last (XIth) triplet (compare Figs. 6 and 7). In this triplet, while two chromosomes are present as usual, the third more or less corresponds in size to the short arm of the third small submedian chromosome of this triplet (Fig. 7, Table 2).

These two complements together constituted the bulk (about 97.5%) of the cells, and, of the two types,

Table 1. *Chromosome variation in root tips of C. augustum* (*Te = telocentric*)

Chromosome numbers ($2n$)	Number of cells		Total number	Percentage
	Primary roots	Secondary roots		
33	40 (49.39%)	42 (54.55%)	82	51.898
32 + Te	38 (46.92%)	34 (44.16%)	72	45.569
33 + Te	—	1 (1.29%)	1	0.632
37 + Te	1 (1.23%)	—	1	0.632
34	1 (1.23%)	—	1	0.632
32	1 (1.23%)	—	1	0.632
Total:	81	77	158	99.995

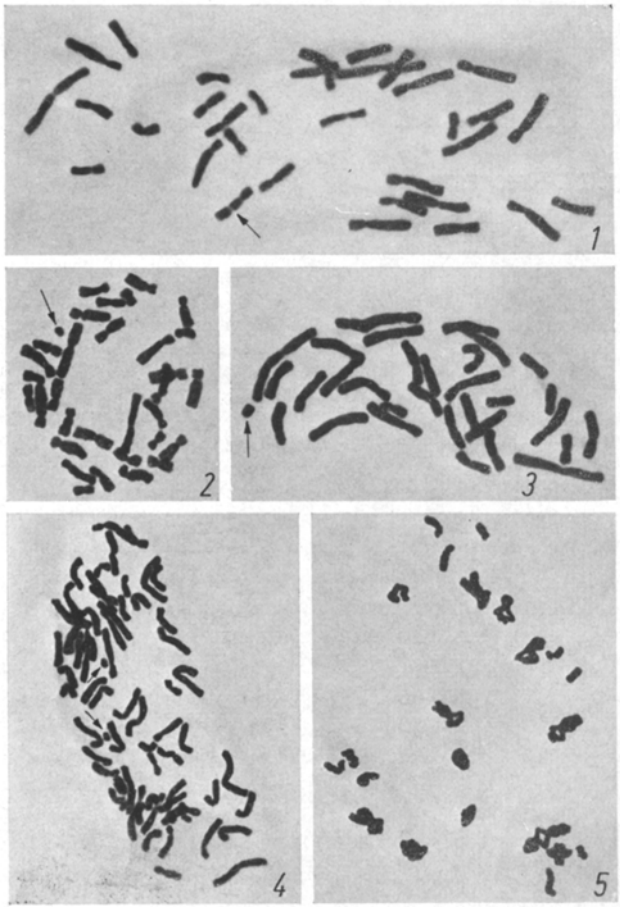
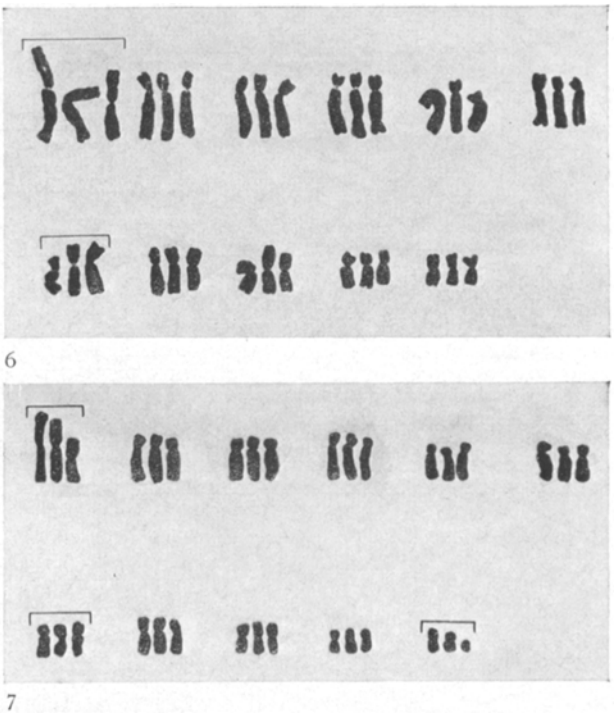


Fig. 1. $2n = 33$
Figs. 2—3. $2n = 32 + Te$
Fig. 4. Pretreated anaphase containing 64 biarmed and 2 telocentric chromosomes
Fig. 5. Metaphase I in p.m.c. with 11 II + 11 I × 1500

there was some preference for the normal complement.

Four deviant cells were found, 3 in the primary and one in secondary roots. Their analysis is given in Table 2. Two of the cells contained the small telocentric chromosome, while the other two lacked it. Of the former, one cell contained the telocentric in addition to the normal complement of 33 chromosomes, while the second contained 37 other chromosomes. A closer analysis has shown (Table 2) that while there are 4 chromosomes of type I, five of X and 6 of XI, this cell is deficient in type VIII, containing only 1 instead of the normal 3 chromosomes. The remaining two cells, with 32 and 34 chromosomes, had 2 chromosomes of type VIII, and 4 of XI, respectively.



Figs. 6—7. Photo-idiograms of the two complements, $2n = 33$ and $32 + Te$

The telocentric chromosome was found to be perfectly feulgen-positive throughout its length and there is no indication of its being heterochromatic. Its division and anaphase movement was investigated in PDB treated and untreated cells. While most metaphases showed normal behaviour of the chromosomes including the telocentric, a few metaphases appeared to have late congression of the telocentric chromosome. The treated anaphases showed 32 chromosomes and one telocentric each on either side (Fig. 4). In 45 out of 50 untreated anaphases, the telocentric divided normally like all other chromo-

Table 2. *Karyotypic analysis of cells with deviant numbers*

Chromosome number ($2n$)	Chromosome type										
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
33	3	3	3	3	3	3	3	3	3	3	3
32 + Te	3	3	3	3	3	3	3	3	3	3	2 + Te
33 + Te	3	3	3	3	3	3	3	3	3	3	3 + Te
37 + Te	4	3	3	3	3	3	3	1	3	5	6 + Te
34	3	3	3	3	3	3	3	3	3	3	4
32	3	3	3	3	3	3	3	2	3	3	3

somes of the complement. In four anaphases, its separation and movement was slower, and often when division of all the other chromosomes was more or less complete, the telocentric continued to remain in the middle. In the remaining one cell, when the anaphase movement was complete for all the other chromosomes, the telocentric had formed a micronucleus in the centre.

II. Male Meiosis

Although in root meristems the species is a chromosomal mosaic and shows a reasonable amount of aneusomaty, the present observations show conclusively that only cells with the full chromosome complement ($2n = 33$) are selected in the male line. Average chromosome associations in pollen mother cells at metaphase I are 0.46 ± 0.09 III $+10.51 \pm 0.18$ II $+10.6 \pm 0.23$ I per cell. There is a very low trivalent frequency and the number never exceeds two per cell; the most common association found was 11 II + 11 I (Fig. 5).

Discussion

The present investigation has revealed the occurrence of two numerically equal, but morphologically somewhat different, chromosome complements ($2n = 33$ and 32 + Te), in almost equal numbers in this taxon. The occurrence of 32 or 33 chromosomes reported by Miege (1962) can be reconciled with the present observations if we assume that Miege overlooked the small telocentric chromosome in the cells containing 32 + Te. An analysis of his Fig. 6 has shown the presence of only 2 small chromosomes of the XIth triplet.

The normal complement of 33 chromosomes resolves into 11 triplets, as is the case in all *Crinum* triploids reported so far, but the 3 genomes involved are not identical in their karyomorphology (Raina and Khoshoo, 1970). Analysis of a large number of cells with 32 + Te shows that this complement tallies with the normal one except that, in the XIth triplet, one of the small submedian chromosomes is replaced by a telocentric which is more or less equal to the short arm of the missing chromosome (Figs. 1—3, 6—7).

The telocentric is feulgen-positive for its entire length. It is clear that it does not represent a portion of a chromosome which got separated by pressure due to squashing because, in a large number of treated and untreated cells in which hardly any pressure was applied, the telocentric could be spotted easily. Furthermore, if it were a part of a chromosome, then it should have been detected in a large number of cells with $2n = 33$. Such a possibility had to be examined because Markarian and Schulz-Schaeffer (1958) found that supernumerary telocentrics reported by various authors in *Alopecurus pratensis*, *Anthoxanthum odoratum* and *Secale montanum* are actually a part of their nucleolar chromosomes which got separated from the main body of the chromosome. In all these cases the number was always more than $2n$, and not exactly $2n$ as in the present case.

The other possibility is that the telocentric is a B chromosome, but B's are more often present in addition to the full complement of A chromosomes and there is no conclusive case where a B has replaced an A chromosome. The writers are familiar with only 2 cases where B's are present when the number is less than the $2n$ number. Müntzing (1948) observed one cell in *Poa alpina* ($2n = 14$) with the probable constitution of 13A + 3B. However, he adds that such a cell is "remarkable" and feels that one of the B's may be actually an A chromosome which contracted more than the normal. Another case is a cell in *Allium stracheyi* (Sharma and Aiyanger, 1961) showing a subdiploid complement with B chromosomes. It thus appears that the presence of B's is more often the result of an addition to, rather than a replacement of, the A chromosomes.

The mode of origin of the 32 + Te complement is not very clear. There are two possibilities. The telocentric may have arisen by unequal translocation in which the longer arm got translocated to other chromosome(s) while in return the small submedian received a very minute segment, so minute that the reconstituted chromosome appears to be telocentric

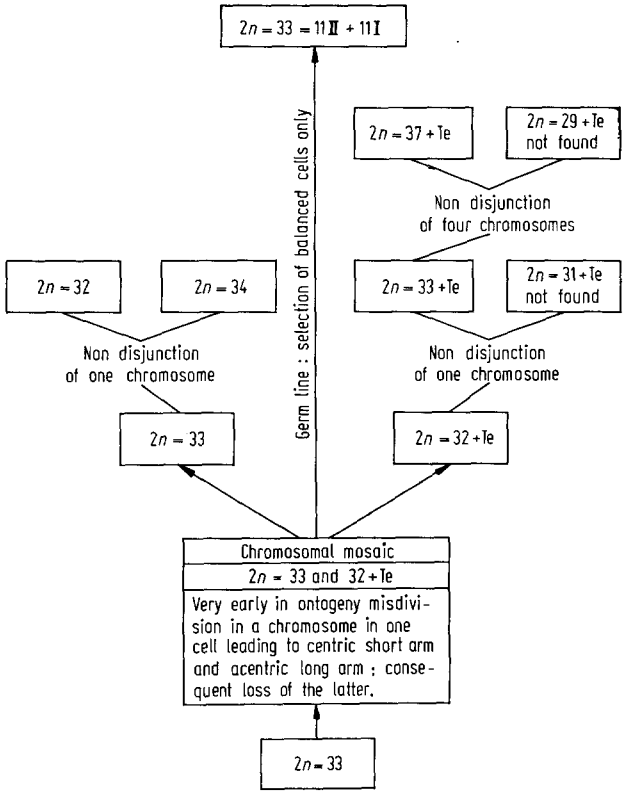


Fig. 8. Chart showing the possible origin of telocentric chromosome and the deviant complements

instead of acrocentric. However, a critical comparison between the 33 and 32 + Te complements produced no evidence of such a translocation having occurred. The chromosome morphology in the two complements, except for the replacement of a small submedian by a small telocentric, was exactly identical. Perhaps the "erosion" of the long arm of this chromosome did not take place in this manner.

The other and rather more plausible possibility is that the telocentric arose through misdivision of the 2B type of Marks (1957, Fig. 2) in which the complete centromere was in the smaller arm. The longer arm was acentric and got lost in a few subsequent cell generations. Since the taxon is a polyploid, such an event would not have any deleterious effect because this chromosome is fully represented by two others of the XIth triplet. The observations at anaphase support this view, because in 90% of cells, the telocentric divides and moves perfectly normally and, as Marks (1957) has remarked, it is not the position but the completeness of the centromere that is necessary for movement of the telocentric. In other words, the small telocentric is perfectly stable.

Since the two types of complements are found in about equal numbers in the bulbs and bulblets of the plant, it appears that misdivision may have taken place in one of the early divisions of the zygote, with the result that in subsequent development the two types of cells became intricately mixed (Fig. 8). This is further corroborated by the observations of Miege (1962) on plants of this taxon obtained from Darkar Garden in Senegal. If the assumption is correct that Miege missed the telocentric chromosome in the complement which he reported as 32 (in addition to the normal cells with $2n = 33$), then the present situation may be true for the triploid cytotype as a whole.

Only the normal complement of 33 chromosomes takes part in the male meiosis. Evidently, rigorous selection is operating in which all deviant cells are eliminated and rejected (Fig. 8). Whether the same situation is characteristic of female meiosis is not known. Furthermore, in view of the almost equal numbers of the two complements in roots, it would be most interesting to see the manner in which progressive elimination of the 32 + Te complements takes place in the different tissues leading to the germ line. This contrasts with the complex translocations in *Lilium callosum* that occurred in somatic cells but were carried to the germ line along with the normal cells (Kayano, 1969). However, both in that species and the present case the chimeral condition is perpetuated by efficient vegetative reproduction (cf. Sharma, 1969).

The four cells containing deviant complements, 33 + Te, 37 + Te, 34 and 32, may have arisen by a simple process of non-disjunction at anaphase (Fig. 8). 33 + Te could have arisen from 32 + Te by the non-disjunction of one chromosome of the XIth triplet. In turn perhaps a deviant cell such as 33 + Te underwent non-disjunction of one chromosome of type I, two each of VIII and X and three of XI (Table 2). From such an event a deviant complement like 37 + Te could be derived if the additional chromosomes of I, X and XI went to the same pole, while those of VIII went to the other. This situation would give rise to the particular karyotype of 37 + Te. The origin of $2n = 34$ can be explained by non-disjunction of one chromosome of type XI from a balanced cell of 33. Again, $2n = 32$ is derivable from $2n = 33$ by non-disjunction of one member of type VIII. If non-disjunction is the physical basis of origin of the four deviant cells, then the complementary karyotypes were for some reason not found. From Table 2 it is clear that all deviant cells including 32 + Te involve, in all but one case, small chromosomes of the complement. This is in line with the observations of Khoshoo and Narain (1967).

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References

1. Bose, S.: Polyploidy in the genus *Crinum*. Cytologia **30**, 349–353 (1965). – 2. Jones, K., Smith, J. B.: Chromosome evolution in genus *Crinum*. Caryologia **20**, 163 to 179 (1967). – 3. Kayano, H.: A chromosomal chimera in a plant of *Lilium callosum* with a complex translocation. Heredity **24**, 490–495 (1969). – 4. Khoshoo, T. N., Raina, S. N.: Cytogenetics of tropical bulbous ornamentals I. Heterozygosity in *Crinum latifolium*. Cytologia **33**, 209–219 (1968). – 5. Khoshoo, T. N., Narain, P.: Aneusomaty in an *Amaryllis* hybrid. Indian J. Genet. and Pl. Breeding **27**, 322–333 (1967). – 6. Koshimizu, T.: Phytogeographical distribution of *Crinum* throughout the world. Bot. Mag., Tokyo, **52**, 32–39 (1938). – 7. Markarian, D., Schulz-Schaeffer, J.: A possible origin of supernumerary fragment chromosomes. J. Hered. **49**, 3–7 (1958). – 8. Marks, G. E.: Telocentric chromosomes. Am. Nat. **91**, 223–232 (1957). – 9. Miege, J.: Quatrième liste de nombres chromosomiques d'espèces d'Afrique occidentale. Rev. Cytol. et de biologie végétale **24**, 149–159 (1962). – 10. Müntzing, A.: Accessory chromosomes in *Poa alpina*. Heredity **2**, 49–61 (1948). – 11. Raina, S. N., Khoshoo, T. N.: Cytogenetics of tropical bulbous ornamentals II. Variation in mitotic complement in *Crinum*. The Nucleus (1970, in press). – 12. Sharma, A. K., Aiyanger, H. R.: Occurrence of B chromosomes in diploid *Allium stracheyi* Baker and their elimination in polyploids. Chromosoma **12**, 310–317 (1961). – 13. Sharma, A. K.: Evolution and Taxonomy of Monocotyledons. Chromosomes To-day **2**, 241–249 (1969).

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