

# Cytological studies on diploid and autotetraploid ginger (*Zingiber officinale* Rosc.)

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## ABSTRACT

Cytology of diploid and induced autotetraploid of ginger (*Zingiber officinale* Rosc.) was studied. The diploid ( $2n=22$ ) showed one or two associations of four chromosomes at first metaphase. The tetraploid formed a high frequency of quadrivalents at first metaphase. Both showed bridge-fragment configurations at first anaphase. Pollen fertility was 13% in the diploid and 85% in the tetraploid.

Key words : ginger, *Zingiber officinale*, autotetraploidy, cytology.

## Introduction

The somatic chromosome number of ginger (*Zingiber officinale* Rosc.) was reported by several workers (Federov 1969; Ramachandran 1969; Omanakumari & Mathew 1985). All the cultivars hitherto studied showed  $2n=22$ . Autotetraploids of ginger ( $2n=44$ ) were produced by colchicine treatment of sprouting buds on rhizomes (Ramachandran 1982; Ramachandran & Nair 1992). Cytological observations on the diploid and the induced autotetraploid ginger are presented here.

## Materials and methods

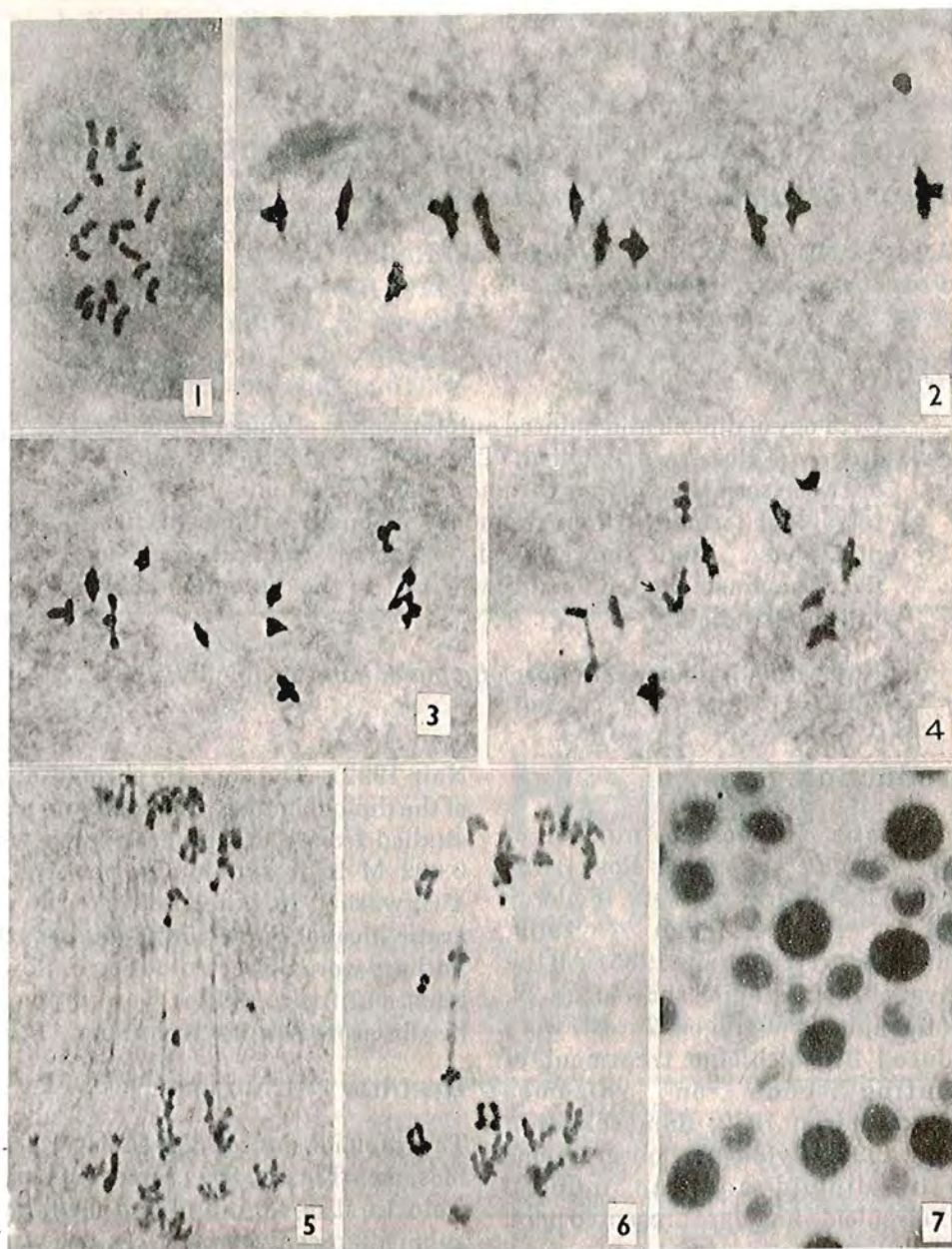
The diploid used in this study is a widely grown cultivar 'Maran'. Tetraploids of this clone were induced by colchicine treatment of sprouting buds on rhizomes as described earlier (Ramachandran &

Nair 1992). The somatic chromosomes of the diploid and tetraploid plants were studied from root tips pretreated with 0.002 M hydroxyquinoline for 4 h at 4°C, washed in water and fixed in 1:3 acetic alcohol. For meiotic studies, the anthers were dissected out from flower buds and fixed. Slides were prepared by the acetocarmine technique.

## Results

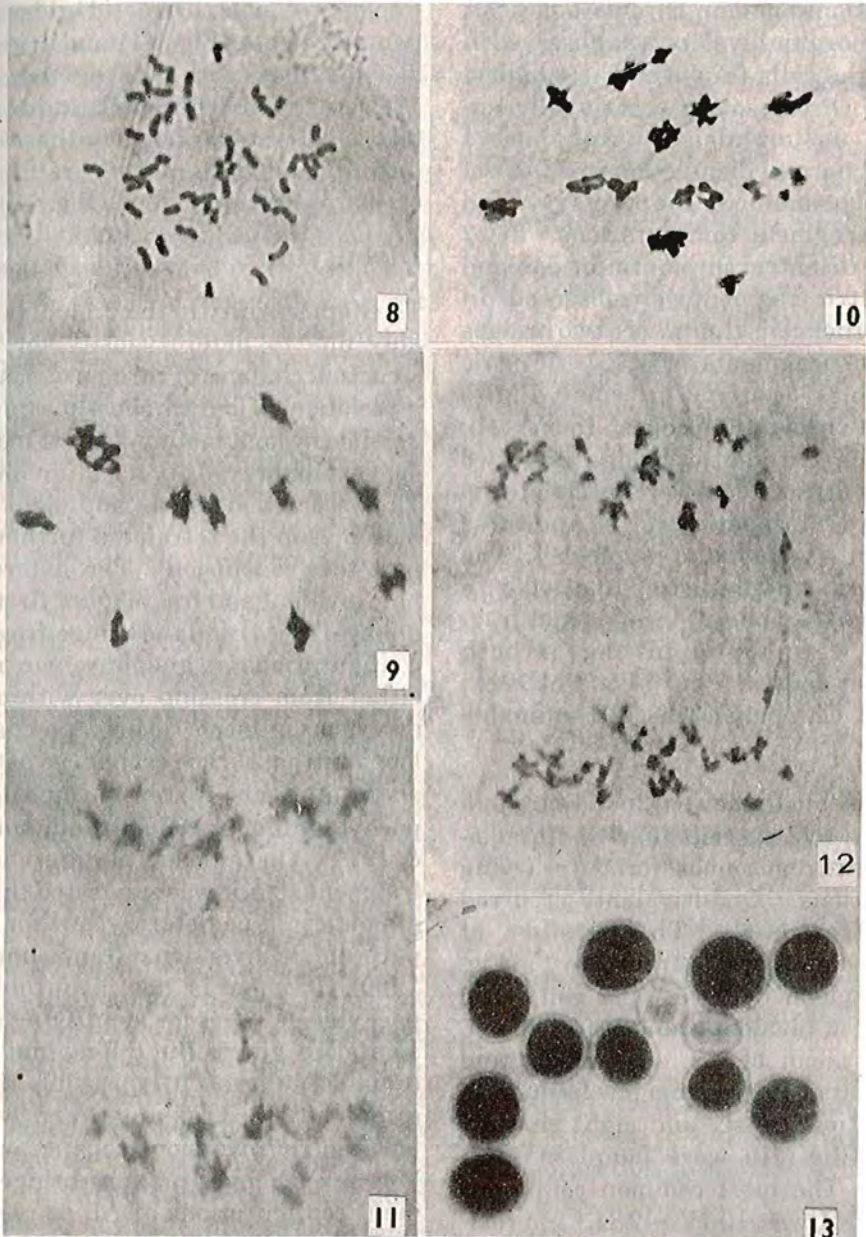
The diploid showed 22 somatic chromosomes (Fig. 1). They ranged in length from 1.6  $\mu$  to 4.3  $\mu$  and had median or submedian centromeres.

At meiosis the chromosomes were paired as 11 bivalents (Fig. 2) in 36 out of 68 Pollen Mother Cells (PMCs) examined at first metaphase. Multiples of four chromosomes, either as close side by side associations of bivalents or as chains



Figs. 1-7. *Zingiber officinale*, diploid (Figs. 1-6 : x 950; Fig. 7 : x 320)

1. Somatic chromosome ( $2n=22$ ) 2. MI showing 11 II 3. Chain of four chromosomes and 9 II at MI 4. MI showing a heteromorphic bivalent and fragment (arrow) 5. Two chromatid bridges and two fragments at AI 6. AI cell with a double chromatid bridge and two fragments 7. Pollen grains of diploid



Figs. 8-13. *Zingiber officinale*, autotetraploid (Figs. 8-12 : x 950; Fig. 13 : x 320)

8. Somatic chromosomes ( $2n=44$ ) 9. MI cell showing 11 IV 10. MI showing 1 VIII, 8 IV, 2 II 11. Double chromatid bridge and two fragments at AI 12. Tricentric double bridge and two fragments at AI 13. Pollen grains of tetraploid

(Fig. 3) were observed in 26 cells. Five cells showed seven bivalents and two associations of four chromosomes. A heteromorphic bivalent was observed in one of the cells (Fig. 4). The smallest pair of chromosomes occasionally appeared as univalents. Anaphase I showed normal disjunction in 32 out of 72 PMCs examined. The others revealed bridge-fragment configurations. In 27 cells, a dicentric chromatid bridge and an acentric fragment were observed. In seven other cells there were two bridges and two fragments (Fig. 5). Acentric fragments occurred in some PMCs without chromatid bridges. In one cell, a double chromatid bridge and two chromatid fragments were observed (Fig. 6). Three chromatid bridges and three fragments were found in one cell. The fragments varied in size ( $1.0 \mu - 2.4 \mu$ ). At second anaphase, bridges and fragments were found in one or both daughter cells in 7 out of 50 PMCs examined. The pollen was 13% stainable (Fig. 7).

The tetraploid showed  $2n=44$  chromosomes in root tip cells (Fig. 8). At meiosis the chromosomes formed varying associations. Quadrivalents occurred most frequently. The number of quadrivalents varied from 7 to 11 (Fig. 9), the mean being 9.7 per cell. The number of bivalents ranged from 0 to 6 with a mean of 1.8. Trivalents and univalents occurred in a low frequency. Associations of six and eight chromosomes (Fig. 10) were found in some PMCs. The most common configuration at MI was 10 IV + 2 II. Approximately 80% of the total of 124 PMCs at AI exhibited 22-22 disjunction, the others showing 21-23 (8%) and 20-24 (5%). One or two lagging chromosome were observed in 7% of the PMCs. The tetraploid showed chromatid bridges and

fragments in 47% of the PMCs at AI. Single chromatid bridges with one fragment, double chromatid bridges with two fragments (Fig. 11) and tricentric double bridges with two fragments (Fig. 12) were found. The pollen grains of the tetraploid were larger than those of the diploid, and 85% of the grains were stainable and well filled (Fig. 13).

## Discussion

The high sterility in the diploid is probably due to heterozygosity for gross structural changes of chromosomes. The associations of four chromosomes, as they persist through metaphase I of meiosis, suggest heterozygosity for interchanges. The associations of six and eight chromosomes in the tetraploid further support this conclusion. The heteromorphic bivalent and fragment at first metaphase (Fig. 4) and the bridge-fragment configurations at anaphase can result either from crossing over within heterozygous paracentric inversion pairing loops or from U-type exchanges between sister and non-sister chromatids at pachytene (Jones 1968; Brandham 1969 & 1977). On the basis of differences in fragment size, it was suggested that the diploid could be heterozygous for three paracentric inversions (Ramachandran 1969). Ginger is not favourable for pachytene studies for identifying inversion loops due to the diffuse nature of prophase stages. It is possible that in ginger, both crossing over within paracentric inversion loops as well as U-type exchanges produce bridge-fragment configurations at AI, as reported in *Podophyllum* (Newman 1966) and *Leontodon* (Finch 1967). Inversions are widespread in plants with vegetative propagation (Muntzing 1961).

The high pollen fertility in the artificially induced tetraploid may be a con-

sequence of the high frequency (87.3%) of quadrivalents followed by the regular two-by-two AI separation. Quadrivalent frequency depends on chromosome size and the number and distribution of chiasmata (John & Lewis 1965; Hazarika & Rees 1967). The chromosomes in ginger are of medium size (1.6  $\mu$  - 4.3  $\mu$ ) and are metacentric or submetacentric. In the diploid all the bivalents commonly form at least two chiasmata, one in each arm. The shapes of some of the bivalents with one arm opened and the other unopened suggest that probably two chiasmata, one proximal and the other distal are formed in the unopened arm. In view of the number and distribution of chiasmata in the diploid, a high frequency of quadrivalent association may be expected in the autotetraploid.

In spite of high pollen fertility, the tetraploid did not set seeds. This confirms the sporophytic nature of the incompatibility system in ginger, as it is shown that gametophytic incompatibility usually breaks down in induced polyploids (Lewis 1956; Pandey 1983). If, however, androgenesis can be induced in cultures of tetraploid anthers, diploid plants showing large genetic variation can directly be obtained, as reported in *Pelargonium roseum* (Tokumasu & Kato 1979). In *P. roseum* the diploid is male-sterile whereas the induced tetraploid has fertile pollen. The androgenic diploid plants raised through anther culture of tetraploids showed large variation in essential oil content. If androgenesis from cultured anthers of induced tetraploids of ginger proves successful, new improved varieties can be produced by this technique.

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## Pot-Pourri

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