DETERMINATION OF THE NATURE OF TETRAPLOIDY IN CASSAVA THROUGH MEIOTIC ANALYSIS

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ABSTRACT

The prevalence of normal bivalent pairing at diakinesis and metaphase I and the high percentage of cells having regular meiosis suggest that cassava (*Manihot esculenta* Crantz) is an allotetraploid and not an autotetraploid. Based on the results, cassava seems to be a segmental rather than a true allotetraploid as indicated by the presence of multivalents and univalents in 29% of the total meiotic cells observed. This implies partial homology between the chromosomes of the species resulting in segmental pairing of homologous chromosomes. Therefore, cultivated cassava is a segmental allotetraploid with a basic chromosome number of x = 9 and a chromosome number of 2n = 4x = 36.

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KEY WORDS: Cassava. *Manihot esculenta.* Polyploidy. Chromosome number. Meiosis. Cytology. Genetic improvement.

INTRODUCTION

Several workers have found no variation in the diploid number of 2n = 36 on cassava (Perry, 1943; Sohmer, 1968; Magoon, Krishnan and Vijaya-Bai, 1969; Abraham, 1970). On the basis of cytological analysis, however, evidences support the polyploid origin of *Manihot* and the fact that cassava is a tetraploid with a basic chromosome number of x = 9 (Perry, 1942; Jennings, 1963; Magoon, Krishnan and Vijaya-Bai, 1969; Martin, 1970; Krishnan, Magoon and Vijaya-Bai, 1970; Umanah and Hartman, 1973).

As an initial step in the genetic improvement of cassava, this cytological investigation determined whether *Manihot esculenta* is an autotetraploid or an allotetraploid.

MATERIALS AND METHODS

Cultivated varieties of cassava, namely: Niña Girl Bonita, Forastera, Sip 24-2, Matalin Collection and

McCol 1684 were obtained from the Institute of Plant Breeding germplasm collection. Male inflorescences were collected in 30-min intervals at different stages of development of cassava. The materials were immediately fixed in Carnoy's solution (3 parts 95% ethanol, 1 part glacial acetic acid) for 12 to 24 hr and then transferred to 70% ethanol until they were ready for use.

The iron acetocarmine squash technique was used for studying meiotic stages. The microsporocytes were squeezed out of the anthers in a drop of iron acetocarmine and the anther debris was removed. A coverslip was then placed on the slide after which pressure was applied. The slides were heated gently, until steam rose from the edges of the coverslip, and then sealed with paraffin.

RESULTS AND DISCUSSION

All the five varieties had a chromosome number of 2n = 36. Although a greater number of microsporocytes had normal meiosis, irregularities were observed as to the presence of multivalents, laggards, non-simultaneous separation of homologues, unoriented chromosomes and consequently micronuclei at telophase I and II. Of all the meiotic cells observed, only 29% exhibited meiotic irregularities (Table 1).

Forastera variety showed the highest number of meiotic irregularities (Fig. 1). Multivalents, as hexavalents, quadrivalents and trivalents as well as univalents were observed. At anaphase I, trivalents resembling a chain of three chromosomes as well as the late disjunction of multivalents were evident. The non-simultaneous disjunction of chromosomes is believed to be the cause of the scattered appearance of chromosomes (Fig. 1d). In addition, laggards observed were possibly formed by the production of acentric fragments resulting from deletions due to segmental pairing. Some segments may have stronger linkages with their homologue than the other segments. As a consequence, a relatively large percentage of cells had micronuclei ranging from 1 to as

Table 1. Meiotic configurations in five varieties of *Manihot esculenta* showing 2n = 36.

	Frequency (%) of Diakinesis & Metaphase I configurations								Telophase I & Anaphase I laggard	
Variety	18 II	16 II 1 IV	14 II 1 III 1 IV 1 I	16 II 1 I 1 III	13 II 1 III 7 I	12 II 6 I 2 III	13 II 1 I 1 VI 1 III	11 II 5 I 3 III	Frequency (%)	Number
Forastera	60		10	10		12	8		10	1-3
Matalin Collection	72				4	24			8	1-3
MCol 1684	68	24	4					4	10	1-2
Niña Girl Bonita	78					22			6	1-2
Sip 24-2	74				2	24			4	1-2

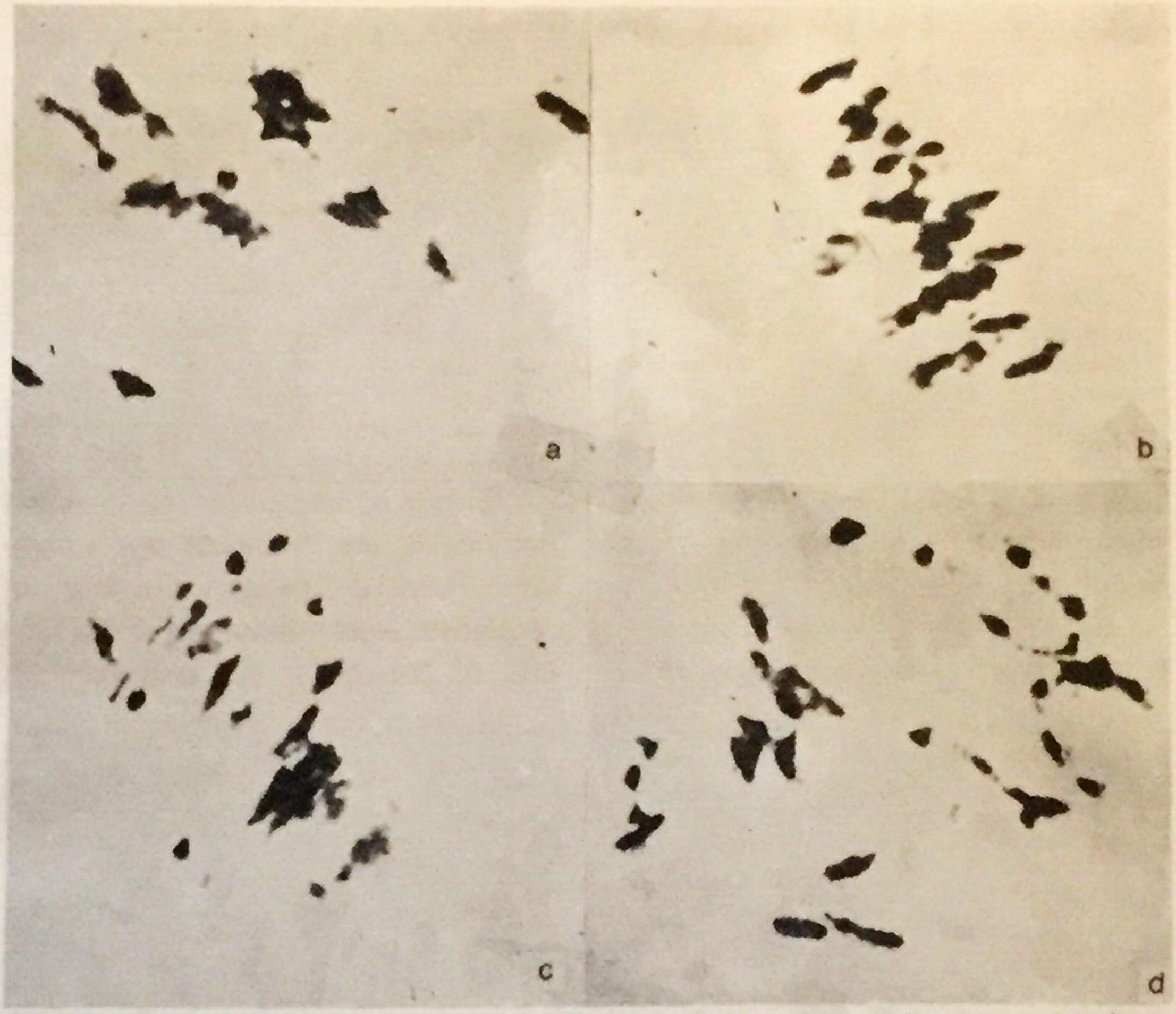

Fig. 1. Meiosis of Forastera variety. A. Diakinesis of prophase I showing multivalents B. Normal metaphase I C. Late disjunction of some multivalents at early anaphase D. Anaphase I showing scattered appearance of chromosomes due to non-simultaneous disjunction.

high as 10 at telophase I and II (Table 2).

Meiosis of Matalin Collection was regular with bivalent pairing at diakinesis and very low incidence of laggards at anaphase I and II (Fig. 2). At diakinesis, bivalent pairing was predominant except in about 28% of the cells where trivalents and univalents were found aside from bivalents. Early disjunction of a few trivalents and some bivalents and consequently laggards was observed at anaphase and metaphase II, respectively (Fig. 2b and 2d).

MCol 1684 was second to Forastera in having the highest frequency of meiotic irregularities reaching to about 32%. There were quadrivalents and trivalents observed aside from bivalents. Non-simultaneous disjunction of chromosomes at early anaphase was also observed just like in the other varieties (Fig. 3b). This is one of the possible reasons for the appearance of laggards and of the micronuclei at anaphase and telophase, respectively. However, laggards might have caught up together with the other late disjoining multivalents and trivalents so

Table 2. Number and frequency (%) of micronuclei at Telophase I and tetrads.

Variety	Total No. of Cells Observed	Number and Frequency (%) of Micronuclei per Cell							
		0	1	2	3	4	5	6	7-up
Forastera	50	46	10	14	12	8	4	2	4
Matalin Collection	50	52	6	4	10	6	12	2	8
MCol 1684	50	48	8	8	6	10	10	4	6
Niña Girl	50	62	6	6	2	4	6	4	10
Sip 24-2	50	60	4	4	6	8	4	8	6

that only a few or no laggard was observed at late anaphase and telophase I.

Seventy-eight per cent of the total meiotic cells observed in Niña Girl Bonita exhibited regular bivalent pairing. A few trivalents and univalents and a greater number of bivalents were observed from the rest of the cells. No pentavalents

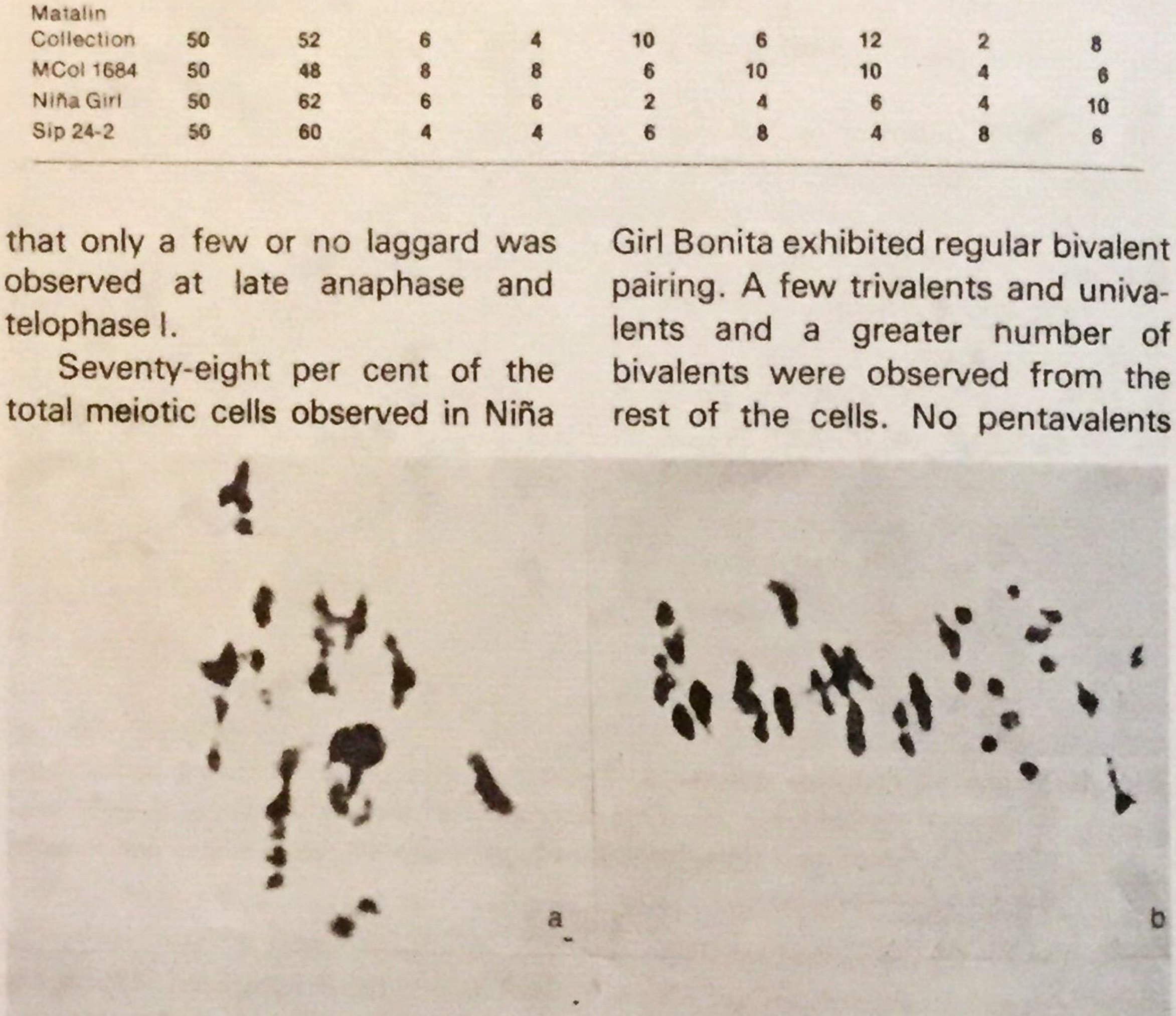

Fig. 2. Meiosis of Matalin Collection variety. A. Diakinesis of prophase I showing univalents and trivalents B. Early disjunction of a few trivalents and bivalents at anaphase I C. Normal prophase II D. Metaphase II showing laggards due to non-simultaneous disjunction of chromosomes.

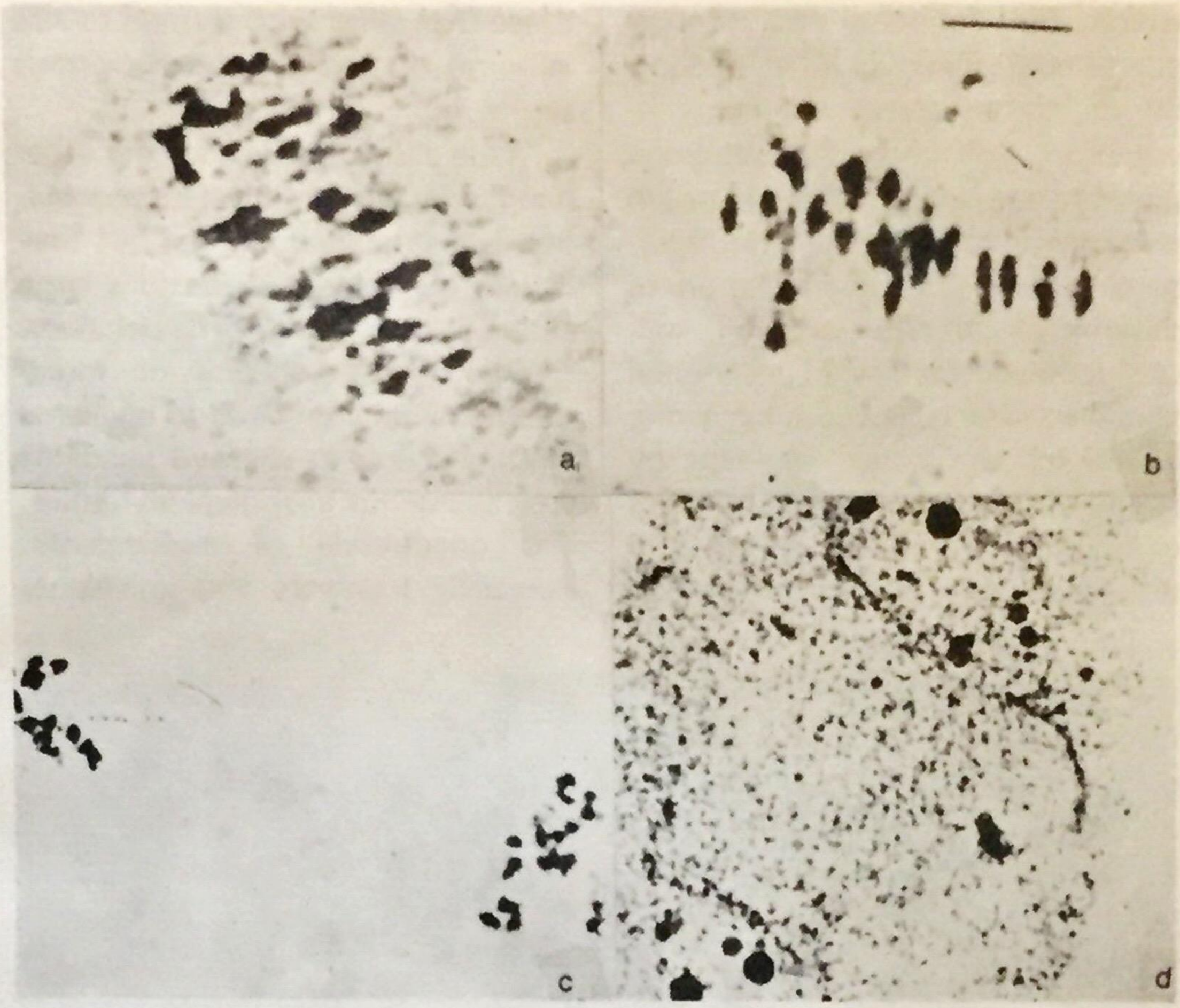

Fig. 3. Meiosis of MCol 1684 variety. A. Diakinesis of prophase I showing several multivalents B. Non-simultaneous disjunction of chromosomes at anaphase I C. Normal prophase II D. Telophase cell showing several micronuclei (arrow) in each of the daughter nuclei.

and quadrivalents were noted (Table 1). Like the other varieties, laggards were also observed in this variety at anaphase I and II except that it had a lower frequency as compared to the others.

Fig. 5 shows some meiotic stages of the variety Sip 24-2 with observed abnormalities. About 74% of the total number of meiotic cells observed exhibited normal meiosis with bivalent pairing at diakinesis and metaphase I. However, a fourth of the observed cells showed some irregularities like the appearance of trivalents and univalents at diakinesis and metaphase I and non-simultaneous disjunction of chromosomes leading to the formation of laggards at anaphase I and II.

Autotetraploids have four homologous sets of chromosomes or genomes. Since pairing at meiosis is a function of homologous segments rather than of whole chromosomes, all four homologues tend to be associated at different levels. They are usually characterized by the presence of multivalents at meiosis of tetrasomic ratios and reduced fertility.

Segmental allopolyploids, in

which by definition all of the component genomes have a majority of chromosomal segments in common, will resemble autopolyploids to a greater or lesser degree in possessing multivalents and tetrasomic ratios. The phenomenon of differential affinity is the most characteristic feature of segmental allopolyploids. It is caused by pairing of the chromosomes segment by segment, so that those which are completely homologous have a greater affinity for each other than those that differ with respect to size as large or small non-homologous segments.

True allopolyploids, on the other hand, may have multivalent association and tetrasomic ratios, but they usually resemble diploids to a large extent in their cytogenetic behavior.

The low frequency of multivalents when compared to bivalents and univalents in cassava indicates their segmental allopolyploid nature. The occurrence of multivalents, especially trivalents and univalents

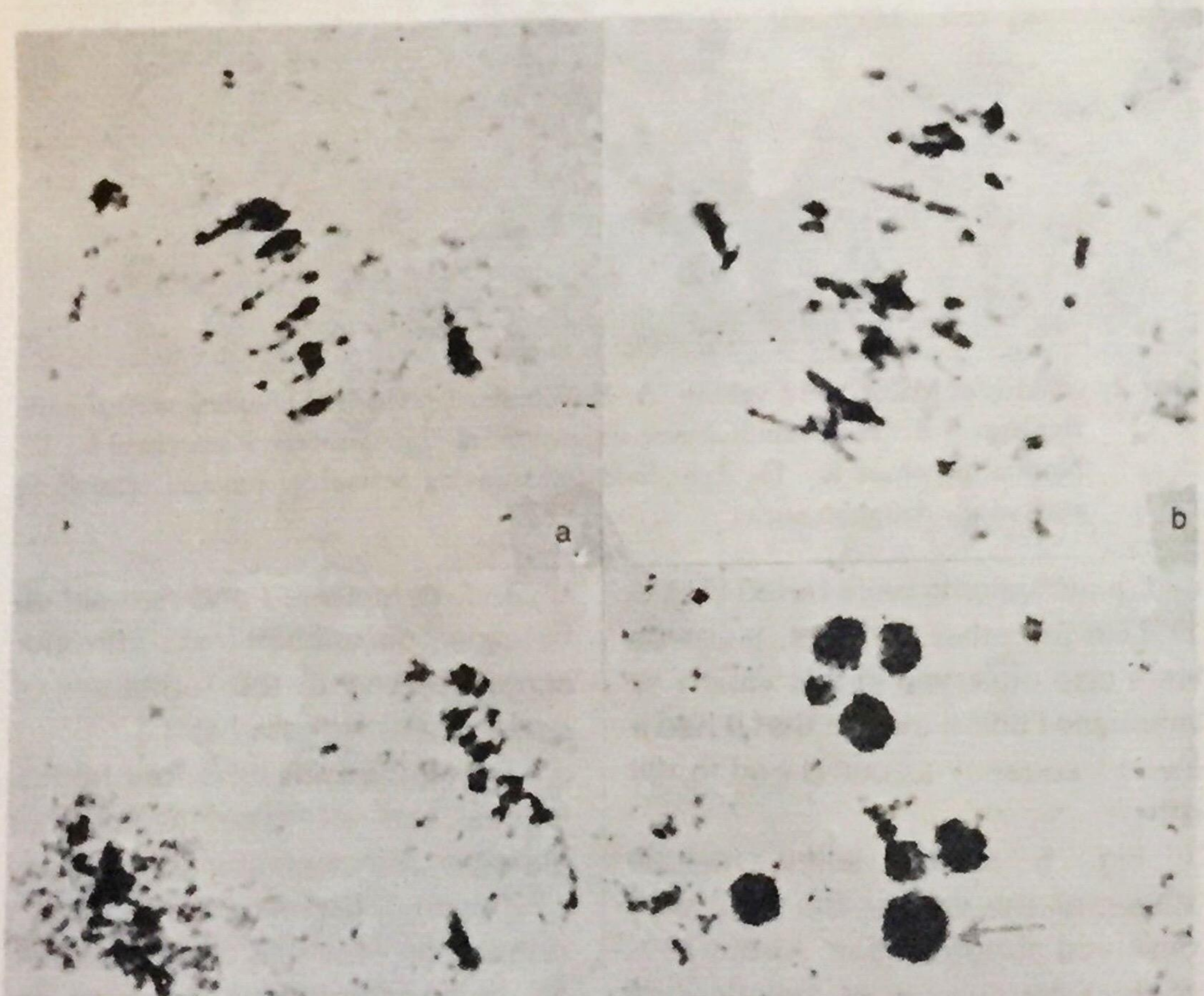

Fig. 4. Meiosis of Niña Girl Bonita variety. A. Metaphase I showing a few trivalents and univalents B. Non-simultaneous disjunction of chromosomes at early anaphase I C. Normal anaphase I D. Daughter nuclei at telophase I showing several micronuclei (arrow).

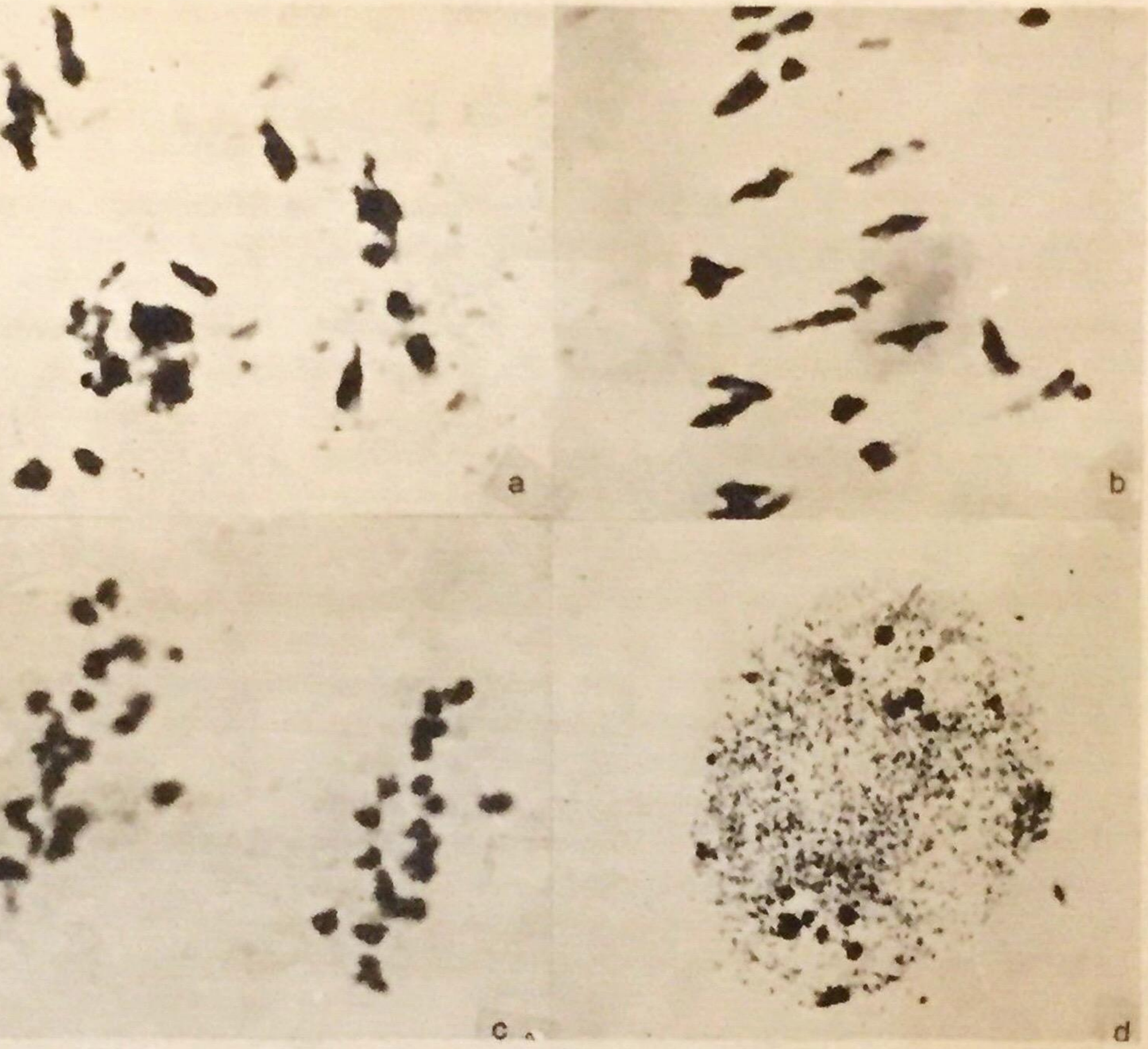

Fig. 5. Meiosis of Sip 24-2 variety. A. Diakinesis of prophase I showing multivalents B. Late disjunction of some multivalents at anaphase I C. Late anaphase I cell showing a laggard D. Telophase I cell showing several micronuclei (arrow) in each of the daughter nuclei.

in all the varieties studied, indicated allosyndesis or partial homology among the chromosomes. It further shows the occurrence of segmental allopolyploidy wherein the component genomes have chromosome segments common to them.

Bivalent formation in this species prevailed due to the preferential pairing of completely homologous chromosomes. The occurrence of trivalents and univalents may also be an indication of the homoeologous pairing of chromosomes. Considering the ploidy of cassava, it is possible that aside from homologous pairing of chromosomes coming from the same set, a third chromosome coming from the other set paired segmentally with the homologues due to partial homology of the two chromosomes from different sets. The univalent was thus formed because its homologue was already paired with a homoeologue. Since the multivalents paired only segmentally, the pairing was loose so that at the very start of anaphase, they easily separated and distributed randomly to the opposite poles.

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