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Cytogenetic analysis of the artificial tetraploid *Lycopersicon esculentum* var *cerasiforme*

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Abstract

The artificial tetraploids obtained from *Lycopersicon esculentum* var *cerasiforme* (2n = 4x = 48), were taller than diploids (2n = 2x = 24), and had larger flowers, leaves, and more hairs. All pollen grains were empty. At diakinesis was observed a low quadrivalent and a high bivalent frequency, which indicates normal pairing at pachytene, but beyond metaphase I some genes or environmental factors must disrupt the meiotic spindle in some way, producing genetically unbalance gametes with 5, 6 and 7 microspores. The low quadrivalent and the high bivalent frequency may be due to the presence of pairing control different genes in the original parent A^oA^1, which by doubling the genomes produce more bivalents than expected.

Key words: Autotetraploid; bivalents; chiasmata; *Lycopersicon esculentum* var *cerasiforme;* meiosis; quadrivalents.

Análisis citogenético del tetraploide artificial *Lycopersicon esculentum* var *cerasiforme*

Resumen

Los tetraploides artificiales obtenidos de *Lycopersicon. esculentum* var *cerasiforme* (2n = 4x = 48) presentaron mayor altura, flores, hojas y pelos más grandes que los organismos diploides (2n = 2x = 24). Los granos de polen no presentaron citoplasma. En diacinesis se observó una baja frecuencia de tetravalentes y una alta frecuencia de bivalentes que indicó un apareamiento normal de los cromosomas homólogos en paquiteno, pero a partir de la metafase I las fibras meióticas se desprenden, desconociéndose si por efecto de factores genéticos o ambientales, produciendo gametos genéticamente no balanceados con 5, 6 y 7 microsporas. La baja frecuencia de tetravalentes y la alta frecuencia de bivalentes puede ser debida a la presencia de genes diferentes que controlan el apareamiento en los padres originales A^oA^1, que por duplicación de los genomas producen más bivalentes que los esperados.

Palabras clave: Autotetraploides; bivalentes; *L. esculentum* var *cerasiforme*; meiosis; quiasmas; tetravalentes.

Introduction

Poliploids have three or more genomes and are considered more important than diploid organisms because they may have some characteristics such as wider ecological tolerance, larger cell sizes, and higher

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secondary metabolite production. These characteristics are important for economic plants (1-5). The creation of new tomato varieties has increased in the last fifty years by artificial selection, mutation, hybridization, and induced autotetraploids. The tomato artificial autotetraploids obtained so far have a high sterility (6). *Lycopersicon esculentum* var *cerasiforme* (2n = 24), a natural tomato variety from Venezuela is important for food, and it has high genetic variability. In order to get a tomato fruit from *L. esculentum* var *cerasiforme* artificial tetraploids, it was carried out this study in order to know the meiotic behaviour, causes of the high sterility and the genetic inestability in these artificial tetraploids.

Materials and Methods

The artificial autotetraploids were obtained by placing the *Lycopersicon esculentum* var *cerasiforme* diploid seeds in a colchicine 0.1% in Hoagland solution (1:3), for three days in order to disrupt the mitotic spindle fibers in fast division somatic cells. Once the seeds germinated they were grown into adult plants under normal light conditions.

Meiotic analysis. Inmature heads from adult plants were collected between 13:45 and 14:10 hours and fixed in carnoy solution (ethanol:acetic acid:cloroform, 6:3:1) for 48 hours. Sixty microsporocytes from the diploid parent and thirty two from the polyploid were squashed and stained with FLP orcein and analyzed at meiosis. The pollen grains were stained with Buffalo Black B in 45% propionic acid. Pollen was considered normal and fertile if the cytoplasm was uniformly stained, while those incompletely or lightly stained blue were considered abnormal. Approximately 500 pollen grains per floret were sample and scored for fertility/infertility.

The diakinesis configurations for diploids and tetraploids behavior were testing for normal pairing and nonrandom chiasmata distribution according to Jackson and Hauber (7).

The equations for normal diploids are: oII = (C - x) · number of cells; cII = x - (C - x) · number of cells.

The following equations were used for autotetraploid testing:

- $oIV = 0.6667(P^4)\cdot[1 - 2Q/P^3(1 + Q)]\cdot x\cdot$ number of cells
- $cIV = 2.6667(P^3Q)\cdot[1 - 2Q/P^3(1 + Q)]\cdot x\cdot$ number of cells
- $oII = 0.6667(1 - 2Q)\cdot x\cdot$ number of cells
- cII = [(number of cells · 4x) - (sum of chromosomes in oII, cIV, and oIV)]·0.5.

When they occur, a trivalent-univalent configuration is converted to two chain bivalents because they are equivalent in terms of chiasmata and chromosomes. For reasons as yet unknown, chiasmata occur in opposite arms of quadrivalents, so the III,I class usually does not occur. If it does occur, its frequency is usually less than expected (8).

Symbols use throughout this paper and their meaning are as follow: oII = a bivalent with two chiasmata; cII = a bivalent with one chiasma; cIV = a quadrivalent chain of four chromosomes; oIV = a quadrivalent circle of four chromosomes; III = a trivalent chain of three chromosomes; I = a univalent; C = mean chiasmata number per cell; x = a basic chromosome number; P = number of chiasmata per cell divided by the maximun number expected, which is 2 in this model. The Q value is 1-P. In nonrandom methods, chiasmata are allocated by binomial distribution. In the derived coefficient and terms, P and Q replace p and q. Exponents of p represent the number of chiasmata and those of q the lack thereof.

Results

In *Lycopersicon esculentum* var *cerasiforme* (2n = 2x = 24) several meiotic stages were analyzed. At diakinesis, bivalents were

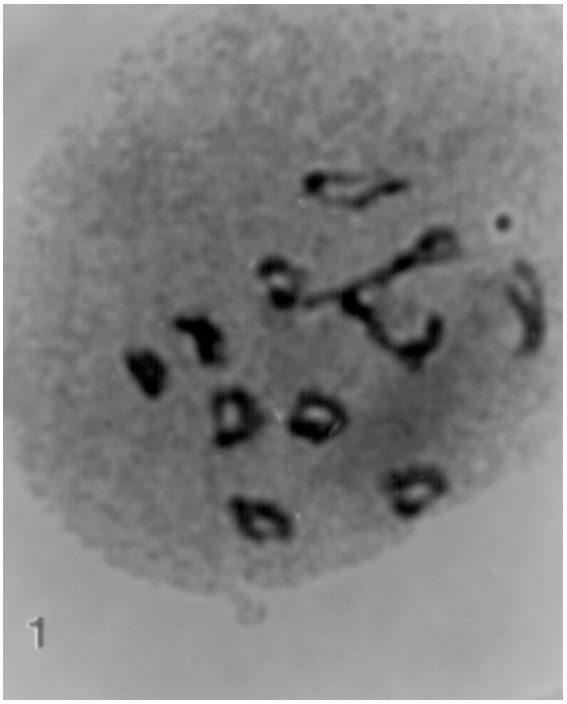

Figure 1. Diakinesis with seven circle bivalents and five chain bivalents. *Lycopersicon esculentum* var *cerasiforme* (2n =2x = 24).

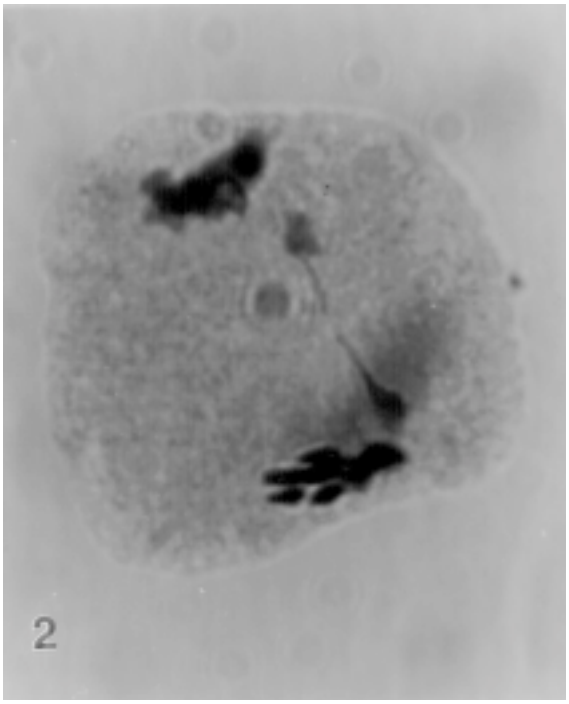

Figure 2. Anaphase I with a dicentric bridge. *Lycopersicon esculentum* var *cerasiforme* (2n =2x = 24).

Table 1
Meiotic configurations in *Lycopersicon esculentum* var *cerasiforme* 2n = 2x = 24

Configurations	Meiotic stage diakinesis
8 oII, 4 cII	4
7 oII, 5 cII	18
6 oII, 6 cII	26
5 oII, 7 cII	12
Number of cells	60
C	18.23

observed with one and two chiasmata (Figure 1; Table 1). A dicentric chromosome bridge was observed in five cells (Figure 2), but disjunction in all other anaphase I and II cells was normal. Normally stained pollen was 97.00%. A mean chiasma frequency per cell was 18.23 at late diakinesis. The P and Q values were 0.7597 and 0.2403, respectively. This probably most accurately reflects the chiasmata value because coiling makes a count more difficult at diplotene. A zero chi-square value was found for diakinesis when observed bivalent numbers were tested against theoretical expectations for normal diploids (Table 2).

The proportions of artificial tetraploids obtained from colchicine treatment were 4.08%. These tetraploids were taller than diploids and had larger flowers, leaves, and more hairs. All pollen grains were empty. Chromosome configurations in diakinesis showed a low quadrivalents and a high bivalents frequency (Figure 3, Table 3), which indicates normal chromosome pairing at pachytene, but beyond metaphase I some genes or enviromental factors must disrupt the meiotic spindle fibers in some way. Chromosome disjunction at anaphase I and II was abnormal (Figure 4). Telophase I, in-

Table 2
Observed (Obs) and expected (Exp) bivalents in *Lycopersicon esculentum* var *cerasiforme* 2n = 2x = 24

			Configuracions		
Meiotic stage	Nº cells		oII	cII	X^2
Diakinesis	60	Obs	374.00	346.00	
		Exp	374.00	346.00	0

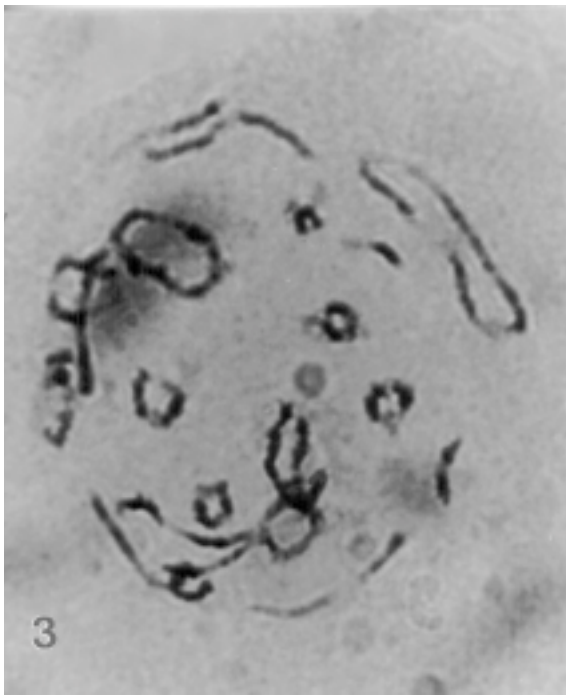

Figure 3. Diakinesis with four circle quadrivalents eight circle bivalents and eight chain bivalents. *Lycopersicon esculentum* var *cerasiforme* (2n = 4x = 48).

terkinesis, metaphase II, and telophase II showed that not all chromosomes were aligned on the metaphase plane, and some univalents were present (Figures 5-7). Some pollen mother cells had 5, 6 and 7 microspores (Figure 8). Mean chiasmata number per cell was 38.67, and P and Q values were 0.8014 and 0.1986, respectively. There was no agreement between observed and expected for the tetraploid ($P < 0.001$; Table 4).

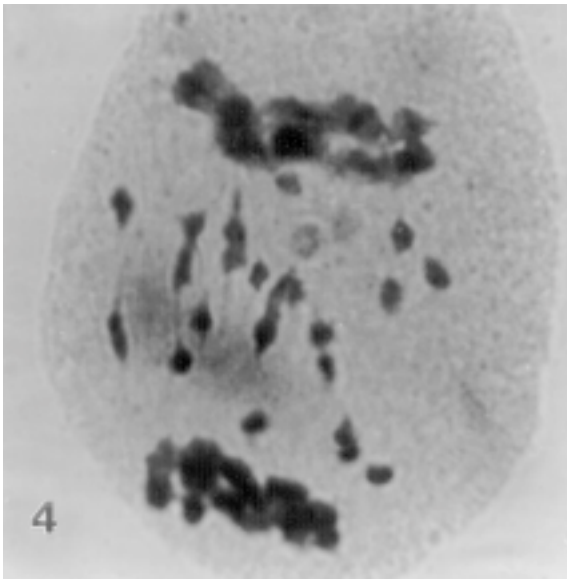

Figure 4. Anaphase I with lagging chromosomes. *Lycopersicon esculentum* var *cerasiforme* (2n = 4x = 48).

Discussion

The 2n = 2x = 24 chromosome complement of *Lycopersicon esculentum* var *cerasiforme* agree with previous reports for *Lycopersicon esculentum* (9). The mean chiasmata per cell (18.23), may be related to the chromosome morphology observed (10 metacentric pairs, one submetacentric pair and one telocentric pair).

A chi-square value of zero was calculated for a fit oII and cII configurations at diakinesis which indicates that *L. esculentum* var *cerasiforme* chromosomes has at least one and a maximun of two chiasmata, and the bivalents has equal chance to form a single crossing over.

Table 3
Diakinesis configurations in *Lycopersicon esculentum* var *cerasiforme* 2n = 4x = 48

	Configurations				
Nº cells	oIV	cIV	III,I	oII	cII
4	2	1	0	9	9
2	3	0	0	8	10
2	4	0	0	8	8
4	5	0	0	5	9
2	3	0	0	10	8
1	4	0	1	11	3
1	3	1	0	5	11
1	2	1	1	11	5
3	2	1	0	9	9
1	5	0	0	5	9
1	5	0	0	6	8
3	3	0	0	9	9
1	0	5	0	6	8
1	3	1	0	6	10
2	1	3	0	8	8
1	4	1	0	5	9
1	4	2	0	3	9
1	4	0	0	5	11

A dicentric bridge observed in five cells at anaphase I, may be due to a single crossover at the four-strand stage involving two nonsister homologous chromatids or a double crossover at the four-strand stage involving three strand chromatids within a heterozygous paracentric inversion loop (10, 11). This would reduce from a theoretical 100% to 95.84% pollen fertility which is not significantly different from the 97.00% observed.

Meiosis in the *L. esculentum* var *cerasiforme* artificial tetraploid (2n = 4x = 48) was abnormal in all post diakinesis stages and gave unbalance genetic gametes as meiotic products. The zero observed fertility is usually common among tomato artificial tetraploids (12, 13, 6, 14). The bivalents and quadrivalents disjunction at anaphase I and later meiotic stages was abnormal with chromosomes distributed randomly in the nuclei (Figures 5-7). These events were not expected because these tetraploids have normal pairing except for two trivalents and two univalents. The lagging chromosomes at anaphase I and II may produce extra micronuclei which should produce small, abnormal pollen grains.

There was no agreement between observed and expected diakinesis chromosome configurations for tetraploids, this is probably due to the total low quadrivalent numbers and the high circle bivalent numbers (Table 4). The low quadrivalent and the high bivalent frequencies may be due to a) the preferential pairing of two homologous

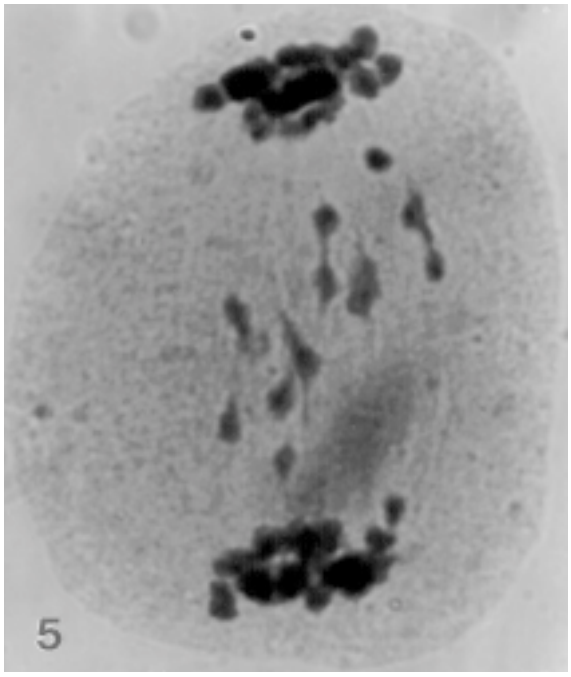

Figure 5. Telophase I with lagging chromosomes. *Lycopersicon esculentum* var *cerasiforme* (2n = 4x = 48).

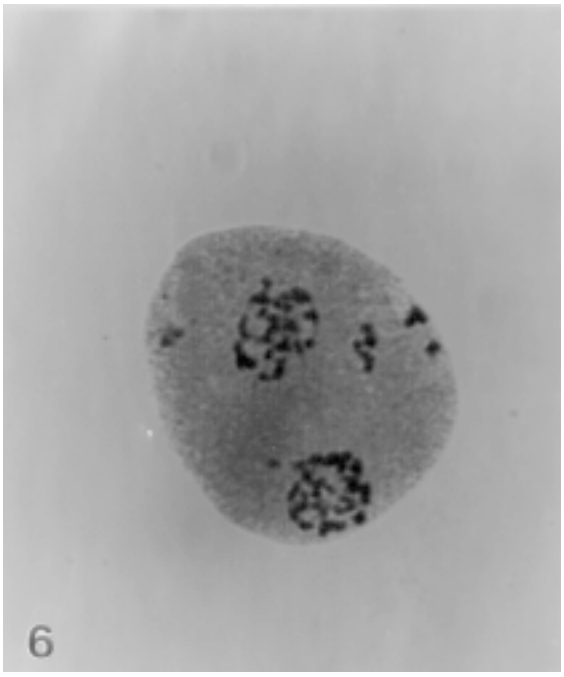

Figure 6. Interkinesis with two nucleoli and three chromosome groups. *L. esculentum* var *cerasiforme* (2n = 4x = 48).

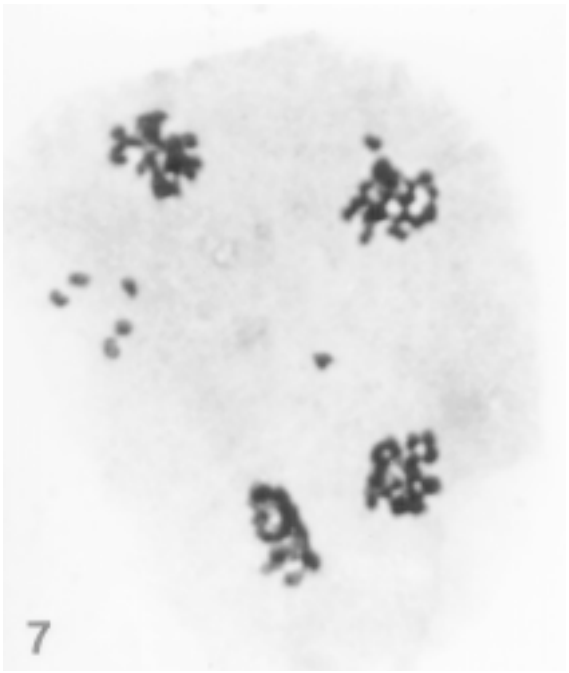

Figure 7. Telophase II with six different number chromosome groups. *L.* var *cerasiforme* (2n = 4x = 48).

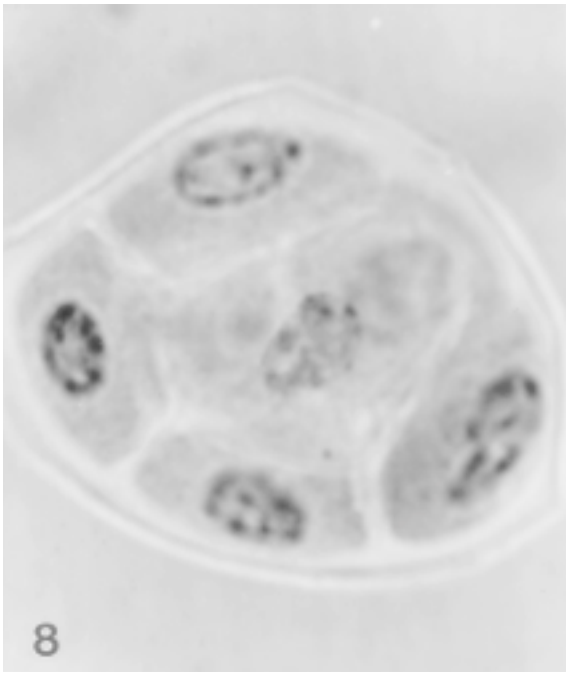

Figure 8. Tetrad stage with five microspores. *Lycopersicon esculentum* var *cerasiforme* (2n = 4x = 48).

Table 4
Test of observed (Obs) and expected (Exp) diakinesis configurations in *Lycopersicon esculentum* var *cerasiforme* 2n = 4x = 48 for a fit to tetraploid expectation. Sample size is 32 cells

		Configurations				
Meiotic stage		oIV	cIV	oII	cII	X^2
Diakinesis	Obs	99.00	24.00	241.00	281.00	
	Exp	103.18	102.30	154.33	202.71	138.93

chromosomes giving a high number of bivalent, b) the associations of chromosomes before pachytene at a specific nuclear membrane place in which pairing involve only two homologous chromosomes, c) presence of pairing control different genes A^oA^1 in the original parent, which by doubling the genomes produces $A^oA^oA^1A^1$ and therefore produce more bivalents than expected (15, 16). Similar results have been found in other Compositae artificial tetraploids obtained by colchicine diploid seeds treatment in *Machaeranthera tagetina*, *M. tanacetifolia*, *M. parthenium*, *M. viscida* and *M. aquifolia* (17).

Conclusions

1. *L. esculentum* var *cerasiforme* (2n = 2x = 24), showed normal meiosis with 97.00% pollen fertility. At diakinesis, bivalents has nonrandom chiasma distribution with one chiasma and a maximum of two.

2. *L. esculentum* var *cerasiforme* artificial tetraploid (2n = 4x = 48), showed normal pairing at pachytene, but abnormal meiosis in all post diakinesis stages with genetic unbalance gametes as meiotic products.

3. There was no agreement between observed and expected diakinesis chromosome configurations for artificial tetraploid, according to normal pairing and nonrandom chiasmata distribution.

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