High Meiotic Association in a Monoploid Sugar Beet Plant

by L. CISTUE, I. ROMAGOSA and J.M. LASA

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A B S T R A C T

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Chromosome association at metaphase I was studied in a sugar beet haploid plant. All possible 30 different meiotic configurations, from nine univalents to a nonavalent-like structure, were observed at this stage. More than 80% of the pollen mother cells (PMC) showed from one to eight chromosome bounds. The average number of chromosomes associated per PMC varied from 2.17 to 3.25 among anthers. Association was found to be very environment dependent, and no pattern in the distribution of chromosome bounds per PMC was detected.

I N T R O D U C T I O N

Monoploids have only one basic chromosome complement, and consequently their meiosis is very irregular. Meiosis in monoploids has been reported in a large number of plant species. Ocurrence of bivalents or multivalents at first metaphase is uniformly low in most cases, however associations of more than two chromosomes have also been reported (for reviews see Kimber and Riley, 1963 and Schulz-Schaeffer, 1980). Sadasivaiah and Kasha (1971) observed quadrivalent-like structures in monoploid barley, and Puertas and Giraldez (1979) reported the formation of up to pentavalents in haploid rye (2n= 7).

Monoploid meiosis in sugar beet was first reported in 1945 by Levan. He found intragenomic associations in more than 40% of the PMC. Yu (1980) found a much lower frecuency of PMC showing chromosome associations (less than 15%). No quadrivalent, nor higher order chromosome associations were found in either study. The present work was undertaken when a routine examination of meiosis in a monoploid sugar beet plant showed a very high degree of chromosome associations at first metaphase.

MATERIAL AND METHODS

The haploid plant used for this study was produced by crossing diploid male-sterile plants with green hypocotyls, and tetraploid fodder beets homozygous for red hypocotyls (Bosemark, 1971).

For meiotic analysis, flower buds of appropiate size were collected at any time during the morning. Inmediately after collection, one of the five anthers was squashed in 1% aceto-carmine to determine the specific stage. When a desired stage was found, the other four anthers from the same flower were fixed in a 1:3 solution glacial acetic acid: 95% ethanol. After four to six hours in the fixative, they were transferred to 70% ethanol for at least one day before staining in an ethanol-HCl-carmine solution (Snow, 1963) for 2-3 days. The slides were prepared by the squash method in 45% acetic acid.

When numerical counts were needed, only PMC with broken cell wall and well spread chromosomes were used. This was done in order to assure that a minimum pressure has been applied and consequently to reduce the probability of error due to overlapping chromosomes.

RESULTS

Somatic chromosome analysis of the haploid plant was made to confirm its true monoploid condition (Fig. 1). No structural changes could be detected in this plant when compared with the standard sugar beet karyotype (Cistue et al., 1985).

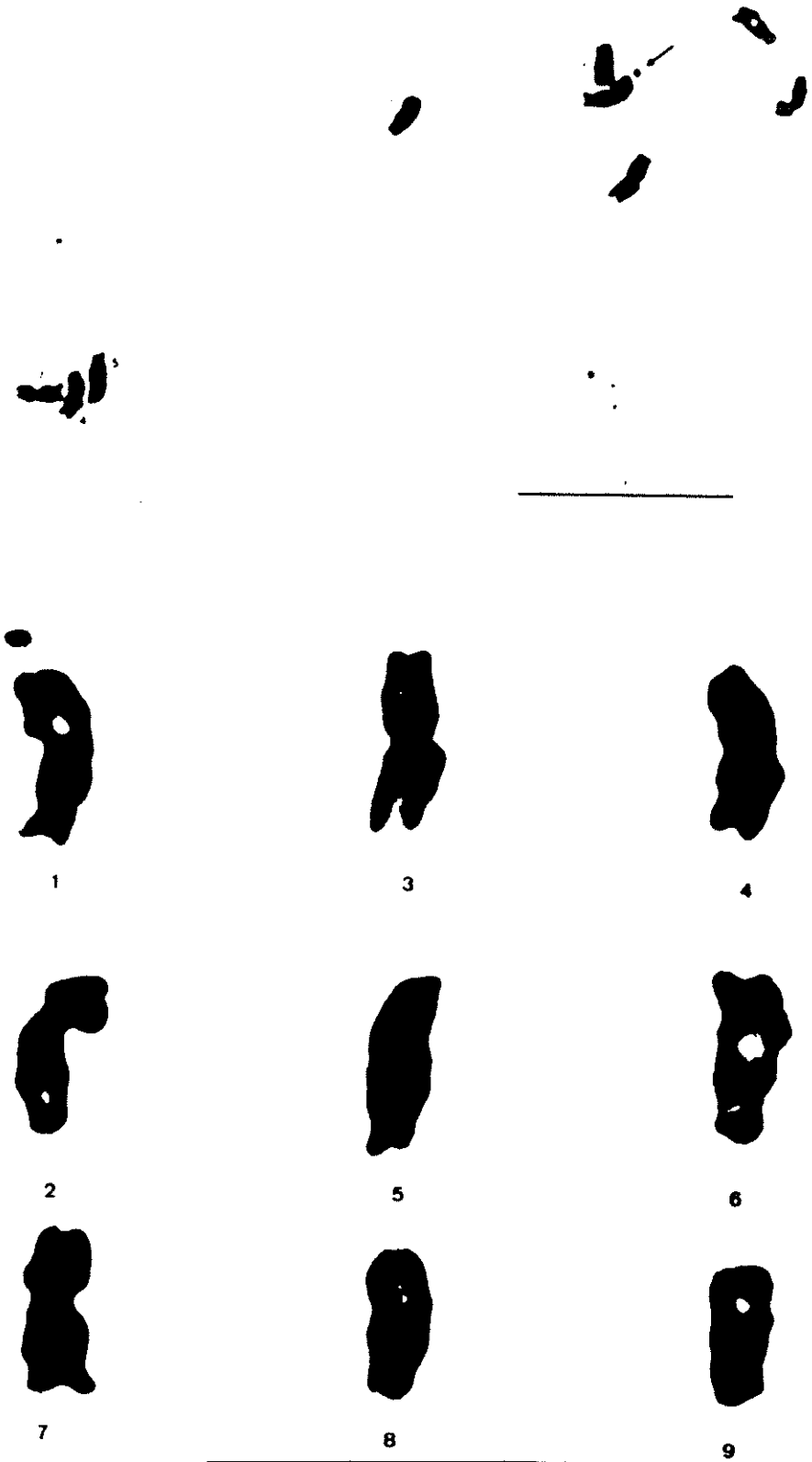

FIG. 1. —Somatic metaphase karyotype.

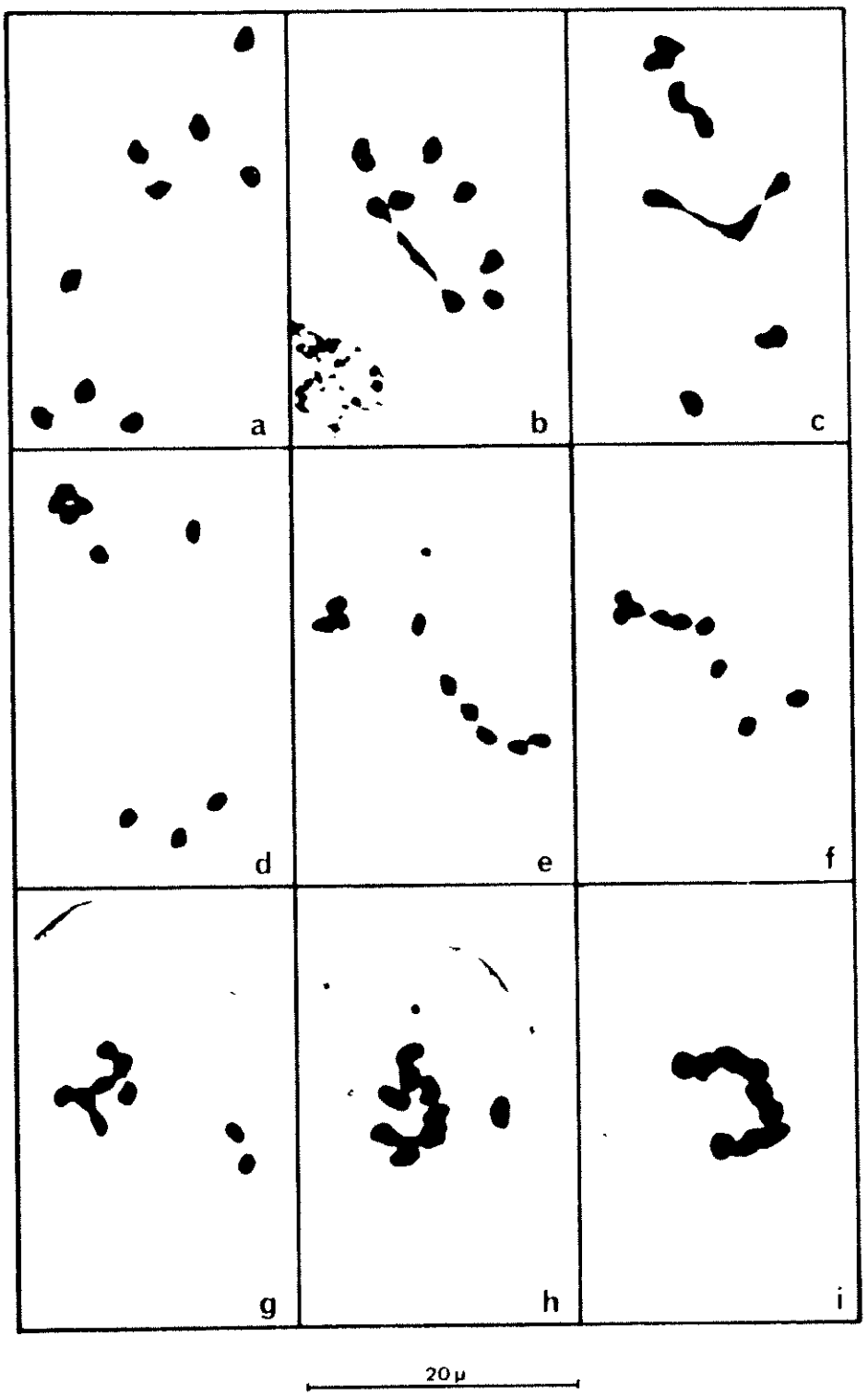

FIG. 2. —Meiotic configurations at Metaphase I — Showing meiotic figures from 0 to 8 chromosome bounds.

Chromosome associations at first metaphase were recorded for a total of 901 PMC. Data were taken from the best six slides to assure that most PMC in any microscope field could be easily checked. Again, this was made to eliminate possible sources of systematic error in the data collecting process.

All possible 30 different meiotic configurations were observed at first metaphase, ranging from nine univalents to one association of nine chromosomes or nonavalent-like structure. Hereafter the term multivalent-like structure will describe any meiotic figure involving a group of chromosomes that are associated. Unfortunately it was not possible to discriminate in most meiotic figures if conexions among chromosomes were due to chiasmata or to any other means. Figure 2 shows nine meiotic configurations, each one with different meiotic figures, in an increasing degree of multivalent-like organizations.

Table 1 summarizes the data of the 30 meiotic configurations, grouping them using as a single variable the number of chromosomes associations per PMC. That is, in the case of a configuration of 7I + 1II (Fig. 1b), there are two chromosomes forming a bivalent-like structure, but we defined only one chromosome association or bound. In the case of Fig. 1e, there is one univa-

TABLE 1. —*Frecuency (%), average per PMC (Av) and probabilities (p) of chromosome association in 6 anthers.*

Assoc	*1*	*2*	*3*	*4*	*5*	*6*
0	4.0	15.3	11.3	16.7	15.3	9.3
1	13.2	18.7	16.0	15.3	20.7	17.3
2	20.5	22.0	26.0	19.3	26.7	22.0
3	22.5	17.3	19.3	10.0	18.7	20.0
4	15.9	10.0	22.7	7.3	11.3	18.7
5	9.9	6.0	2.7	8.7	4.0	8.0
6	8.6	4.7	2.0	6.7	2.7	2.7
7	4.6	4.0	.0	10.0	.0	1.3
8	.7	2.0	.0	6.0	.7	.7
Av	3.25	2.57	2.42	3.15	2.17	2.67
p	.36	.29	.27	.35	.24	.30

Ho: Equal distribution of chromosome association per PMC

$$\chi^2_c = 118.8 <> \chi^2_c(35GL): p \leqslant 0.001$$

lent, one association of three and other of five chromosomes, that is a total of 1 x (1--1) + 1 x (3--1) + 1 x (5—1) =6 chromosome associations.

Table 1 also lists the average number of chromosome associations per PMC and dividing this last value by nine, the probability "p" of one chromosome being associated to others. The number of chromosome associations varied from 2.17 to 3.25 per PMC, corresponding to probabilities of chromosome association of 0.24 and 0.36 respectively.

The frecuency of PMC's showing any type of chromosome association was very high from 83 to 96%. These values were much more larger than the 15% (Yu, 1980) or 40% (Levan, 1945) reported in other monoploids in this species.

The number of chromosome associations in metaphase I was analyzed in relation to two aspects. First, to check if all anthers of a single genotype showed the same degree of chromosome association. And second, to determine if there was any pattern in the distribution of the number of chromosome bounds or associations within PMC's in each anther.

A chi-square test for a 6x8 contingency table of PMC x eight levels of association (grouping the classes of seven and eight bounds) was highly significant $\chi^2_c(35GL) = 118.75$, showing that the null hypothesis of equal degree of association could be strongly rejected $p \leqslant 0.001$. It should be mentioned that flowers were collected over a four week period under variable field-nursery conditions.

A second factor of interest was to study the number of associations or chromosome bounds per cell at first metaphase. By doing so we could indirectly test the hypothesis of random chromosome association *versus* discriminative type of association. The idea behind this test was that if there were a tendency of a small group of chromosomes to be associated (the case of residual homology due to archaic polyploidy or aneuploidy) we would expect a larger frecuency of PMC's showing low numbers of associations.

On the other hand, if associations were true random events, the number of chromosome bounds per PMC would follow a binomial distribution, given by the probability "p" of chromosome associations calculated from data on Table 1. The probability distribution for the formation of meiotic configurations of 0 to 8 chromosome associations would be, under the null hypothesis of random association, $[p+ (1—p)]^8$, This hypothesis was tested, given the strong environmental influence of chromosome association numbers, for the two cases (anthers 1 and 5, Table 1) which showed the largest and smallest probabilities of association. None of these two distributions followed a binomial probability $\chi^2_c (6GL) = 67.9$ **, and $\chi^2_c(5GL) = 24.8$ ** respectively . However if the observed *versus* expected frecuency are plotted on a histogram (Fig. 3) none of the anthers showed a higher frecuency of observed cells with low chromosome association than the expected as it was hypothesized before. Therefore, no explainable pattern in the distribution of chromosome bounds per PMC was detected.

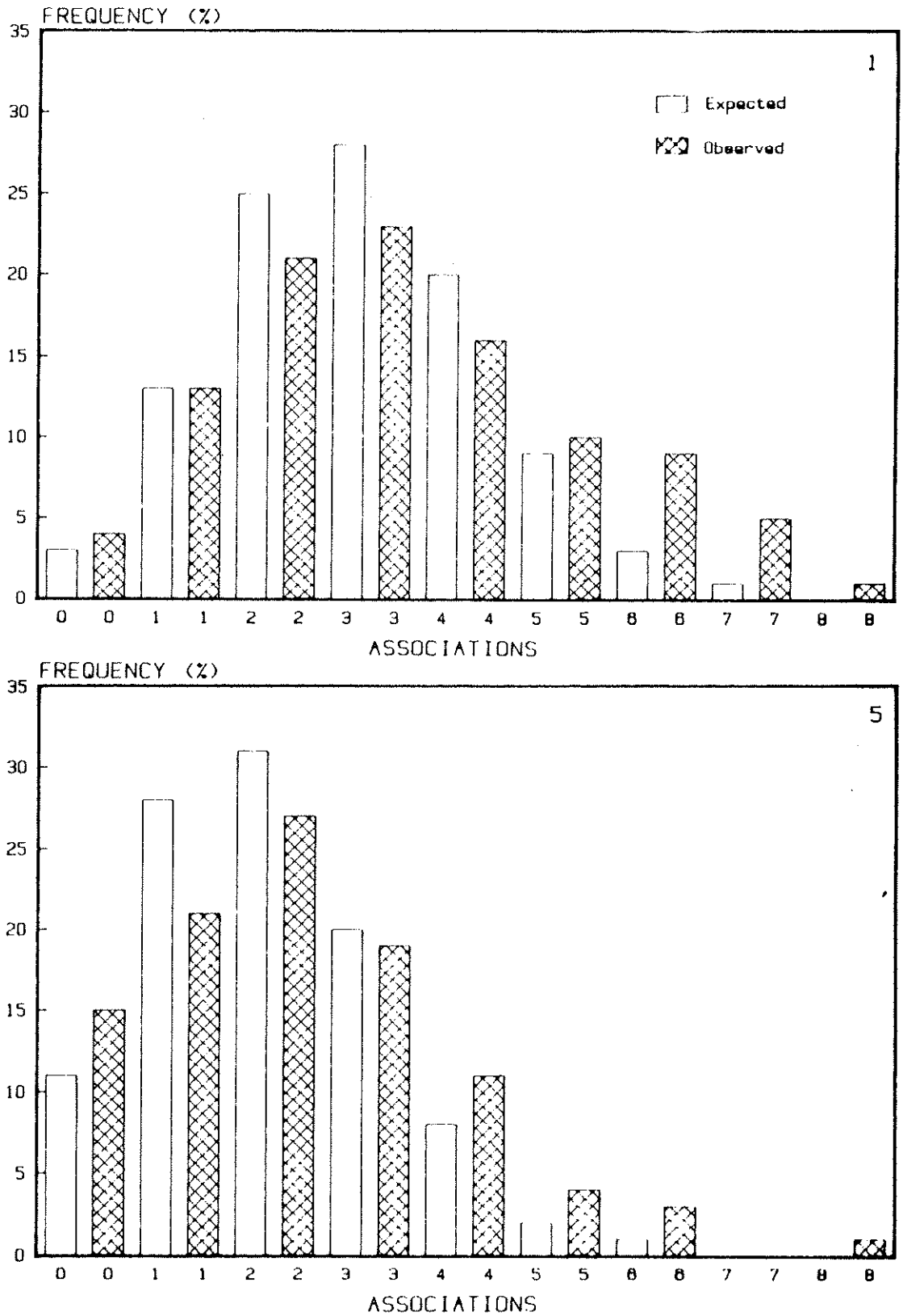

FIG. 3. —Observed and expected frecuencies of PMC with different number of chromosome associations, in two anthers (1 and 5).

DISCUSSION

The monoploid sugar beet plant studied showed a high degree of chromosome association compaired to other materials previously studied (Levan, 1945; Yu, 1980). These associations were seen during mid— to late-prophase and metaphase up to anaphase I. However, it was not believed that chiasmata alone was responsible for the high degree of chromosome association.

Chromosome interconnections have been frecuently reported in sugar beet meiosis at early— to mid-prophase and specially in interkinesis (Nakamura, 1979; Skaracis, 1980; Romagosa, 1980). But in any case they were found at first metaphase. However this type of association has been observed by the authors at an extremely low frecuency in sugar beet trisomic meiosis (Fig. 4). In this Figure quadrivalent-like structures and associations of up to nine chromosome are also easily seen in an apparently homozygous material.

Three types of cytological explanations have been given to the presence of chromosome associations in monoploids. Kimber and Riley (1963) mentioned the existance of intergenomic pairing governed strictly by homology. This homology could be caused by the presence of duplicated segments, archaic polyploidy, or aneuploidy. Sadasivaiah and Kasha (1971) reported association of non-homologous barley chromosomes from pachytene to metaphase I. The presence of synaptonemal complexes in haploid petunia and

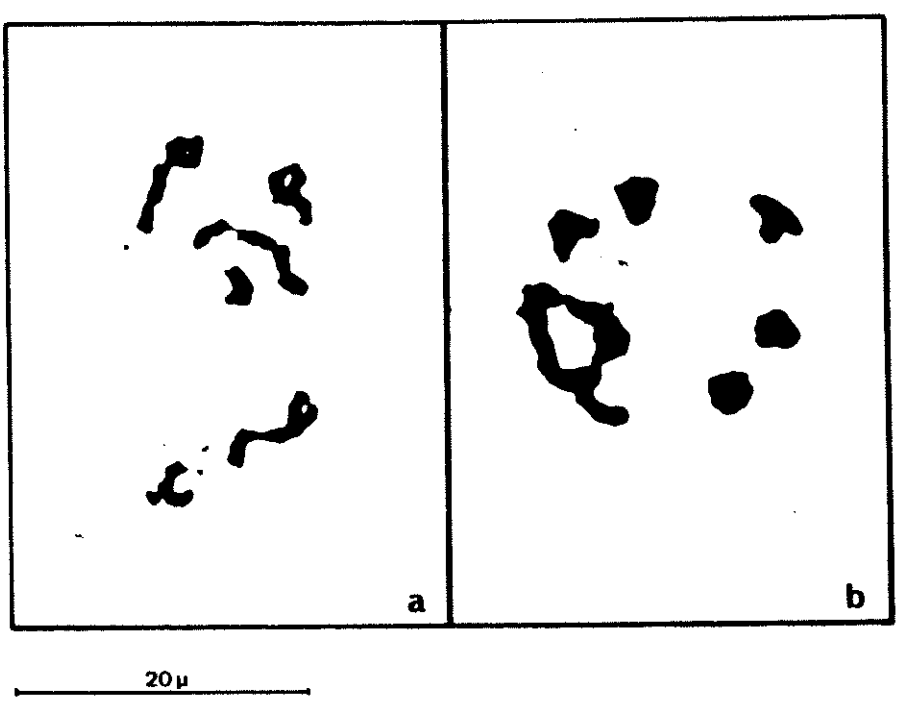

FIG. 4. —Meiotic configurations at late diakinesis in homozygous trisomic plants with multivalent-like structures.

snapdragon (Sen, 1970) leaded Sadasivaiah and Kasha (1971) to suggest that non-homologous chromosomes may pair as intimately as homologous do.

Stickiness of heterochromatic zones has also been mentioned as another cause of chromosome interconnections. Skaracis (1980) discussed the nature of interconnections found at various meiotic stages in diploid sugar beet. He mentioned as alternative causes, connections possesing a chromatine-like structure, artefacts due to the preparation techniques or as remnants of the nuclear membrane to which the chromosomes were attached.

The true nature of these chromosomes bounds was not determinate. It was believed that a combination of the mentioned possible explanations was responsible in different degree of the high level of association herein reported.

The strong influence of environmental factors on chromosome associations, together with the absence of a clear distribution pattern in the number of associations per PMC, and the fact that only one single genotype could be used, made not possible a further study of this type of associations. It is necessary to isolate and study more haploid plants for an overall study of this type of association in monoploids of this plant species.

RESUMEN

Se estudia la asociación cromosómica en metafase I, en una planta haploide de remolacha azucarera. En dicho estado, pudieron observarse la totalidad de las 30 configuraciones posibles, desde nueve univalentes a estructuras tipo-nonavalente. Mas del 80% de las células madres del polen (PMC) mostraron entre 1 y 8 asociaciones cromosómicas. Variando el número medio de asociaciones por PMC, entre 2.17 y 3.25 según anteras. Se encontró que las asociaciones dependían fuertemente del medio ambiente, no pudiéndose detectar ningún patrón de distribución de las mismas. Aún no se conoce la naturaleza verdadera de estas asociaciones cromosómicas. Se piensa que la combinación de apareamiento verdadero, apareamiento entre no homólogos y otras interconexiones, fue la causante de las asociaciones cromosómicas. Sin embargo, se considera necesario la realización de estudios similares en otros genotipos.

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