

Recurrence of multiple meiotic abnormalities in maize genotypes from the same origin and their influence on productivity

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ABSTRACT

The frequency of meiotic abnormalities among single-, double- and three-way cross experimental hybrids and their parental inbred lines is studied. Among the sixteen inbred lines examined, fifteen originated from populations developed at the National Center of Research in Maize and Sorghum (CNPMS/Embrapa) and one from a population developed at ESALQ (Faculty of Agriculture Luiz de Queiroz of the University of São Paulo). Previous analyses of homozygous and heterozygous maize genotypes from CNPMS populations demonstrated high meiotic instability. The present investigation aims at analyzing the meiotic behavior of different inbred lines obtained from CNPMS populations by another breeding enterprise to verify the presence of the same abnormalities and determine whether the abnormalities might compromise productivity. Analyses showed the presence of some abnormalities described previously, albeit at a lower frequency. The most common abnormalities in homozygous and heterozygous genotypes from CNPMS populations were related to irregular chromosome segregation and to chromosome stickiness. An inbred line from the ESALQ population showed only abnormalities related to irregular chromosome segregation caused by the presence of univalent chromosomes. The Pearson correlation procedure indicated high negative correlation between meiotic abnormalities and productivity.

KEY WORDS: Maize, meiotic abnormalities, productivity.

INTRODUCTION

Meiosis is an event of high evolutionary stability that culminates in the reduction of chromosome number. Cytological events of gametogenesis are controlled by a large number of genes acting from pre-meiotic to the post-meiotic mitoses. Mutation of these genes may cause anomalies that impair plant fertility (Golubovskaya, 1979, 1989; Albertsen and Phillips, 1981; Curtis and Doyle, 1991). When an allogamous plant is submitted to self-fertilization, many genes affecting plant characteristics, including those involved in the control of the meiotic process, experience homozygosis causing inbreeding depression. Production of hybrid maize requires inbred lines of good agronomic characters which are normally obtained by successive selfing cycles. Since many lines produced during the inbreeding process are inferior, they are eliminated. Hallauer and Miranda-Filho (1981) estimated 0.01% as the number of lines that may be used in hybrid production.

Studies have been carried out by Pagliarini and her

group to evaluate the meiotic behavior in homozygous and heterozygous genotypes of various Brazilian maize breeding enterprises in different regions of the country. All genotypes originally came from populations of the National Center of Research on Maize and Sorghum (CNPMS/Embrapa). Analyses with genotypes provided by Organization of Cooperatives of the State of Paraná (Taschetto and Pagliarini, 1993; Defani-Scoarize et al., 1995a, 1995b, 1996) and genotypes provided by PLANAGRI S.A., a private breeding enterprise (Caetano-Pereira et al., 1995a, 1995b; 1998a, 1998b, 1999; Caetano-Pereira and Pagliarini, 1996, 1997), showed high meiotic instability. Although the two cultivation sites, i.e., Cascavel in the south-western region of Paraná, Brazil, and Goianésia in the center-western state of Goiás, Brazil, are separated by more than 1500 km, the distinct genotypes analyzed had similar meiotic abnormalities. Most meiotic abnormalities found in these genotypes have been described as responsible for plant sterility in maize and in other plant species.

CNPMS/Embrapa is a governmental breeding

enterprise that provides basic genetic material for Brazilian maize breeding programs developed in different regions of Brazil. Results found by our group in the material analyzed from the above-mentioned breeding enterprises and their possible consequences for breeding programs have caught the attention of Brazilian maize breeders that use CNPMS populations to obtain inbred lines. Researchers are now requesting cytological evaluations for some promising genotypes. In this paper we have evaluated 30 genotypes of the Cooperativa Agrícola de Cotia, including homozygous and heterozygous genotypes from some CNPMS populations and from one population of distinct origin. Our aim was to determine whether they present the same meiotic abnormalities found previously in those materials of the same origin and the influence of these abnormalities on productivity.

MATERIAL AND METHODS

Sixteen inbred lines with seven or more cycles of self-fertilization from nine different populations were analyzed. Ten single cross, two double-cross and two three-way cross hybrids were obtained from inbred lines crossing. Fifteen inbred lines were selected from CNPMS populations (CMS 04: lines 1 and 2; CMS 05: lines 3, 5 and 7; CMS 06: lines 9, 10, 11 and 14; CMS 07: line 4; CMS 11: line 6; CMS 12: line 8; CMS 28: lines 15 and 16; CMS 50: line 13); line 12 was selected from ESALQ PB-1 population. Plants were cultivated in Maringá PR, Brazil, where soils are adequate for maize cultivation.

Male inflorescences were fixed in 3:1 alcohol/acetic acid solution, transferred to 70% alcohol and stored under refrigeration. Slides were prepared by the squash technique, followed by staining with 1% propionic carmine. All the meiotic phases in 300 cells per plant and in seven plants per genotype were analyzed. A comparative study of productivity among genotypes was performed taking into account the grain weight (gr.) of five ears. The frequency of meiotic irregularities was compared with productivity by the Pearson correlation procedure. The more frequent meiotic abnormalities, such as irregular chromosome segregation and stickiness, were also specifically correlated with productivity.

RESULTS AND DISCUSSION

Cytological analyses showed a certain meiotic instability among the genotypes tested (Table 1).

Inbred lines were more affected than hybrids. Among hybrids, single-crosses were more affected than double-crosses, while these, in their turn, showed more meiotic instability than three-way crosses. Among inbred lines, the meiotic behavior differed. Some lines showed a high frequency of cells with abnormalities, e.g., lines 2, 4, 6, 9, 10, 11, 15 and 16. While productivity among inbred lines was lower than that of single-cross hybrids, in the latter it was lower than double- and three-way cross hybrids. On the other hand, the frequency of meiotic abnormalities was inverted, or rather, it was high in inbred lines and lower in single-cross hybrids, while in double- and triple-cross hybrids it was much lower than in single-cross hybrids (Table 1). In other words, results suggest that there is a negative correlation between meiotic abnormalities and productivity. The higher yielding genotype (three-way cross hybrid ((15x16)x11) had the lowest level of meiotic abnormality. As a rule, similar behavior was shown among inbred lines: the least productive lines usually gave very high frequencies of abnormalities and the most productive lines presented very low frequencies. This apparent negative correlation between meiotic abnormalities and productivity was confirmed by Pearson correlation procedure for the 30 genotypes ($r = -0.73$; $p < 0.01$) and for the 16 inbred lines ($r = -0.54$; $p < 0.05$). This finding indicates that, in general, meiotic irregularities impair grain production. The normal and harmonious course of meiosis ensures gamete viability. Inbred lines usually show reduced vigor and fertility. Although many other factors may negatively affect the fertility of inbred plants, data indicate that at least part of the depression of this trait may be attributed to meiotic irregularities (Smith and Murphy, 1986; Pagliarini, 1989). The most frequent abnormalities, irregular chromosome segregation and micronuclei formation, give rise to aneuploid gametes. According to Curtis and Doyle (1992), maize as a diploid species shows strong selection against aneuploidy. Unbalanced gametes in the lines are unable to compete with normal gametes, reducing productivity.

The most common meiotic abnormalities were those related to chromosome segregation. The Pearson correlation procedure also showed a negative correlation among irregular chromosome segregation and productivity for all genotypes ($r = -0.72$; $p < 0.01$). One cause of irregular segregation is the formation of univalent chromosomes. Univalents appear in diplotene/diakinesis as a result of the absence of chiasmata in some bivalents. Chiasma formation is a

characteristic under polygenic control (Rees and Thompson, 1957; Pagliarini, 1980; Lein and Lelley, 1987). Selfing of allogamous plants leads to gene segregation, so that different chiasma frequency may appear among lines of the same origin. Different frequencies of univalents were observed among lines selected from the same population (see Table 1). A correlation between low chiasma frequency and high univalent chromosome frequency in inbred maize lines has already been studied by Pagliarini (1989). Dependence of chiasma formation on heterozygosity is also evident in genotypes analyzed by the authors, since the hybrids showed fewer frequencies of univalents than their parental inbred lines.

Meiosis is a multiple step process controlled by a number of single genes as well as polygenes. Successive selfing cycles in allogamous plants may put major single gene mutations in homozygosity, producing meiotic abnormalities. When a meiotic process is controlled by a single gene mutation, all plant cells are affected. However, uniform expression is usually not found among inbred lines selected from CNPMS populations. In these lines and in some hybrids, meiotic behavior is highly unstable. Many different types of meiotic abnormalities may occur in the same plant and they do not affect all cells. For example, in one anther we may find different anomalies, characterized as typical meiotic mutations, which do not affect all cells. Similar variation in expression of some meiotic mutations among

genotypes originated from CNPMS populations have already been described (see Taschetto and Pagliarini, 1993; Defani-Scoarize et al., 1995a, 1995b, 1996; Caetano-Pereira et al., 1995a, 1995b, 1998a, 1998b, 1999; Caetano-Pereira and Pagliarini, 1996, 1997).

Chromosome stickiness, another frequent anomaly in the genotypes analyzed has been extensively described in genotypes selected from CNPMS populations (Caetano-Pereira et al., 1995; Defani-Scoarize et al., 1995a, 1998b, 1996). This abnormality in the studied genotypes showed a negative correlation with productivity ($r=-0.52$; $p < 0.01$), especially in inbred lines ($r=-0.55$; $p < 0.05$). Such correlation may be explained by chromosome stickiness action leading to picnosis and culminating in total chromosome degeneration. Absence of cytokinesis, leading to dyad and triad formation, was another common abnormality found among the CNPMS genotypes (Defani-Scoarize et al., 1995 a b, 1996; Caetano-Pereira et al., 1998). Chromosome fragmentation (Caetano-Pereira et al., 1995), cell fusion (Caetano-Pereira et al., 1999), cytomixis (Caetano-Pereira and Pagliarini, 1997), abnormal cell shape (Caetano-Pereira and Pagliarini, 1996), although of lower frequency were also found in those materials previously analyzed.

It was interesting to observe that inbred line 12 selected from the ESALQ PB-1 population, which had an origin different from all other inbreds, also had a unique pattern of abnormalities. Only

Table 1. Number of cells with meiotic abnormalities in inbred lines, single, double and three-way cross experimental hybrids scored among 2100 microsporocytes analyzed in each genotype and grain weight (gr) of five ears.

Meiotic abnormalities	Inbred Lines																Single-cross Hybrids								Double-cross Hybrids		Triple-cross Hybrids			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	4x2	4x6	8x13	14x6	3x9	3x11	14x7	5x12	3x12	15x16	(5x12) x (3x10)	(5x12) x (3x11)	(15x16) x 11	(15x16) x 1
Univalent chromosomes	35	58	8	78	31	158	8	39	34	29	13	48	25	8	43	30	0	5	5	4	2	0	1	1	0	5	0	1	0	1
Irregular segregation	100	161	72	138	84	224	71	68	140	139	135	137	49	80	124	200	55	69	28	66	36	38	54	54	30	77	39	25	21	31
Micronuclei	7	65	10	32	0	144	13	24	44	46	32	27	4	4	48	44	20	4	3	1	5	7	14	0	4	2	1	24	4	5
Stickiness	8	13	19	142	64	144	13	9	97	04	113	0	8	4	10	35	14	21	0	0	0	4	0	10	0	4	1	24	4	5
Bridges + fragments	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Chromosome fragmentation	0	0	0	0	0	0	0	2	0	2	8	0	3	0	2	0	0	0	0	0	17	1	5	2	3	2	0	7	2	0
Cell fusion	0	1	0	0	0	0	0	0	0	1	0	0	6	0	0	11	0	0	0	0	0	0	2	0	2	9	0	0	0	0
Giant cells	0	5	0	0	0	0	0	0	0	0	0	0	13	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anucleated cell	2	0	0	0	0	0	0	0	0	0	0	1	0	1	0	4	0	0	0	3	0	0	0	0	0	0	0	0	0	0
Cytomixis	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Abnormal cell shape	0	0	0	0	0	0	0	0	0	0	9	0	3	0	0	0	0	0	0	0	0	6	0	4	6	0	0	0	0	0
Absence of cytokinesis	8	37	1	6	2	6	13	4	18	16	1	0	21	6	20	27	0	4	3	3	10	0	6	0	0	0	1	8	0	4
Dyads + triads	10	9	4	12	5	12	3	4	11	10	4	0	10	9	9	5	3	5	9	3	0	0	6	4	1	4	3	6	0	8
Total	172	350	114	417	186	544	121	150	344	336	317	212	131	112	256	357	92	108	48	82	70	56	88	75	46	103	44	83	27	48

abnormalities related to chromosome segregation occurred. This kind of abnormality is caused, in general, by homozygosis in the polygenic system that controls chiasma frequency. This irregularity is frequently found among inbred lines. The line was unique among the sixteen analyzed in that it did not display the other abnormalities commonly found among those originated from CNPMS populations.

According to Hawley (1988), during meiosis genetic control reduces the error rate by about 10^{-3} per generation; thus, spontaneous meiotic mutants are rare in nature. Consequently, the causes of high frequency of meiotic abnormalities found in genotypes from CNPMS populations are being speculated on the basis of transposons activation. According to McClintock (1984), among the many known and explored responses of genomes to stress in maize, there is the activation of potentially transposable elements. Under adverse conditions, a genome may modify itself, being abnormally reprogrammed or restructured. Transposable elements, often carried in a silent state in the maize genome, may modify gene action and restructure the genome at several levels, from small changes involving a few nucleotides to gross modifications involving large segments of chromosomes. While restructuring the genome, it may give rise to a wide range of altered phenotypic expressions. There is a transposable element in maize held to be a probable candidate for the increase of the frequency of meiotic abnormalities to such high levels as those observed in genotypes originated from CNPMS populations. The TE *Mutator* (*Mu*), originally identified in lines exhibiting very high frequencies of mutations (Robertson, 1978), is capable of provoking mutations simultaneously at many loci (Robertson, 1985). The mutator activity of *Mu* seems to be restricted to a time shortly before and/or during meiosis and may increase the mutation rate by as much as one hundred percent (Robertson, 1980).

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