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Meiotic behavior of economically important plant species: the relationship between fertility and male sterility

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Abstract

Meiosis is an event of high evolutionary stability which culminates in a reduction of chromosome number. The normal and harmonious course of meiosis ensures gamete viability. The cytologic events of gametogenesis are controlled by a large number of genes that act from premeiotic to postmeiotic mitosis. Mutations in these genes cause anomalies that may impair fertility, and many abnormalities affecting plant fertility or causing total male sterility have been detected during the evaluation of meiotic behavior in some species. Some of these abnormalities have been frequently described in the literature, while others have not been previously reported. The most frequent abnormalities found in the species analyzed were irregular chromosome segregation, cytomixis, chromosome stickiness, mixoploidy, chromosome fragmentation, syncyte formation, abnormal spindles, and failure of cytokinesis. Uncommon abnormalities, such as chromosome elimination during microsporogenesis, were found in one species. Original meiotic mutations affecting different steps of meiosis were also observed in these species, especially in maize, *Paspalum* and soybean. Some mutants present characteristics that may be exploited successfully in breeding programs because they cause total male sterility.

INTRODUCTION

Meiosis is a seemingly paradoxical process in which universality and uniqueness are harmoniously combined. All organisms, irrespective of their evolved complexity, meiotically reduce the chromosome number at the start of sexual reproduction, compensating for fertilization and maintaining the diploid chromosome set from generation to generation (Golubovskaya, 1979).

Micro- and megasporogenesis comprise three sequential stages that culminate with gamete formation, that is pre-meiosis, meiosis and post-meiosis, which are controlled and coordinated by a diversity of genes. Meiosis, in addition to being the stage of longest duration, is also the stage that consumes the most cellular energy and is controlled by a larger number of genes than the other stages. Although consisting of individual components, suggesting independent hierarchical gene control at each step, meiosis is a highly coherent integrated process, although the steps may be changed by the presence of mutant genes resulting in abnormal meiotic products that prevent gamete formation and impair plant fertility. Similarly, pre- and post-meiosis may also be affected by the action of mutant genes, but these events, in contrast to meiosis, are controlled by a relatively small number of genes. The major mutations causing male sterility are post-meiotic, and sterile male mutants provide a potential starting point for the genetic and molecular investigation of anther and pollen development in higher plants. In addition, such mutants have often been investigated for their applications to plant breeding, and in particular for their potential use in the production of hybrid seed (Kaul, 1988). Seventeen years ago, the Laboratory of Plant Cytogenetics of the State University of Maringá (PR, Brazil) started some studies aimed at the evaluation of meiotic behavior in plant species economically important to Brazil. About 50 species were analyzed and, in general, most of them presented some problem related to seed production.

MATERIAL AND METHODS

Various species were investigated, including those of agronomic value (*Zea mays*, *Brassica napus*, *Brassica campestris*, *Glycine max*, *Saccharum officinarum*, *Cedrela fissilis*, *Hevea brasiliensis*, *Euphorbia heterophylla*, *Vitis vinifera*, *Avena sativa* and more than 30 *Paspalum* species), medicinal value (*Centella asiatica*, *Ochna* sp., *Pilocarpus pennatifolius*, *Aloysia lycioides*, *Cissus* sp.), and ornamental value (*Chlorophytum comosum*, *Thunbergia mysorensis*, *Aptenia cordifolia*, and *Boungainvillea*). Species of agronomic value (usually represented by commercial cultivars, inbred lines or accessions from germplasm collections) were donated by private breeding companies or governmental enterprises involved in plant breeding, especially the national research centers for specific crops (*Embrapa*). Medicinal species were collected in the Medicinal Garden of the State University of Maringá, whereas those of ornamental value were collected in Maringá city.

The number of plants analyzed in each study was variable, depending on the objectives and the availability of the material. For all species, the methodology employed for meiotic analysis was the same. Flower buds in the ideal stage for meiotic studies were fixed in 3:1 alcohol:acetic acid, transferred to 70% alcohol and stored under refrigeration. Slides were prepared by the squash technique followed by

staining with 1% propionic carmine. Starting from the pachytene, all meiotic phases were analyzed. When necessary and possible, pollen viability was also tested.

RESULTS AND DISCUSSION

Maize is the crop most frequently analyzed in our laboratory, with studies having been started twenty years ago to assess the genetic control of chiasma frequency (Pagliarini, 1980) which we showed to be under polygenic control, suggesting that inbred lines may present differences in chiasma frequency after inbreding. This fact was later confirmed by Aguiar-Perecin *et al.* (1984), who showed that a low chiasma frequency leads to the appearance of univalent chromosomes. In order to correlate chiasma frequency with combining ability many inbred lines of maize were analyzed (Pagliarini, 1983, 1989; Pagliarini *et al*., 1986), and in all cases there was a correlation between the two traits, showing that univalents lost during the meiotic process impair pollen fertility and seed production.

The most common meiotic abnormality found was irregular chromosome segregation, characterized by precocious chromosome ascension and laggards. This irregularity was widely observed in inbred lines of maize (Pagliarini, 1983, 1989; Pagliarini *et al*., 1986; Defani-Scoarize *et al*., 1995a,b, 1996), *Chlorophytum comosum* (Pagliarini *et al*., 1993), sugarcane (Pagliarini *et al*., 1990), *Bougainvillea* (Adamowski *et al.*, 1996), *Aloysia lycioides* (Corazza-Nunes *et al*., 1993, 1995), *Hevea brasiliensis* (Pagliarini *et al*., 1992a), *Ochna* sp. (Pagliarini *et al*., 1992b), *Pilocarpus pennatifolius* (Pagliarini and Pereira, 1992), *Aptenia cordifolia* (Pagliarini, 1990a), *Centella asiatica* (Consolaro and Pagliarini, 1996a), *Brassica napus* and *B. campestris* (Souza *et al.*, 1997), and in many *Paspalum* species (Freitas *et al.*, 1997; Adamowski *et al.*, 1999). The causes of this abnormality can be diverse. Univalent chromosomes at diakinesis or metaphase I may result from low chiasma frequency, precocious chiasma terminalization or by the presence of asynaptic or desynaptic genes in prophase I (for review, see Gottschalk and Kaul, 1980a,b; Koduru and Rao, 1981). Irrespective of their origin the meiotic behavior is always the same, with univalents showing precocious ascension at metaphase I or remaining as laggards at anaphase I. In both cases they may originate micronuclei at telophase I and in meiosis II. In all species in which irregular chromosome segregation occurred a correlation between this characteristic and pollen fertility or seed production was observed. Another more rare segregational abnormality observed was non-congressed bivalents at the equatorial plate in metaphase I, only found in *Chlorophytum comosum* (Pagliarini *et al*., 1993), *Glycine max* (Bione *et al*., 1999) and *Avena sativa* (Baptista-Giacomelli, 1999).

Chromosome transfer from cell to cell through cytoplasmic connections, a phenomenon known as cytomixis, has been observed in *Pilocarpus pennatifolius* (Pagliarini and Pereira, 1992), *Centella asiatica* (Consolaro and Pagliarini, 1995), *Brassica napus* and *B. campestris* (Souza and Pagliarini, 1997), maize (Caetano-Pereira and Pagliarini, 1997), and *Glycine max* (Bione *et al*., 1999). Although cytomixis has been reported in several plant species, its origin is not clear, but among the factors thought to cause it are the influence of genes, abnormal cell wall formation during premeiotic divisions, chemicals, pathological conditions, herbicides, radiation, temperature, mechanical injury, hybridization and polyploidy. The causes of cytomixis in the species cited above were not identified, although the affected plants were grown under the same environmental conditions as normal ones. Although the role of cytomixis in plant evolution is considered an additional mechanism for the origin of aneuploidy and polyploidy, the deviation in chromosome number affects pollen fertility. In all the species investigated the number of cells showing cytomixis was low, although it, along with other cellular abnormalities, contributed to increased pollen sterility.

Chromosome stickiness was another abnormality observed in some plants, occurring in maize (Caetano-Pereira *et al*., 1995a), *Centella asiatica* (Consolaro and Pagliarini, 1996b), *Brassica napus* and *B. campestris* (Souza and Pagliarini, 1996) and *Glycine max* (Bione *et al*., 1999). Stickiness is characterized by chromosome clustering during any phase of the cell cycle. The phenotypic manifestation of stickiness in these species was highly variable, ranging from a mild phenomenon involving only a few chromosomes in the genome, to an extensive one involving the entire chromosome complement. Chromosome stickiness may be caused by genetic and environmental factors, and several agents have been reported to cause chromosome stickiness. In maize cultivated in Brazilian *cerrado* (Brazilian savanna) soils, where aluminum saturation is naturally high, Caetano-Pereira *et al*. (1995a) observed intense chromosome stickiness in microsporocytes, with inbred lines being more affected than hybrid lines, while in the other species, the results suggested that interactions between the plant genotype and the environment caused chromosome stickiness. In severe cases of stickiness, the lack of chromosome separation provoked the formation of single or multiple pycnotic nuclei which culminated in full chromatin degeneration. Depending on the intensity of chromosome stickiness, pollen fertility may be partial or totally affected. Although many studies have reported the occurrence of chromosome stickiness, the primary cause and biochemical basis of the phenomenon are unknown. Gaulden (1987) postulated that stickiness may result from the defective functioning of one or two types of specific non-histone proteins involved in chromosome organization which are needed for chromosome separation and segregation. The altered functioning of these proteins is caused by mutation in the structural genes coding for them (hereditary stickiness) or by the direct action of mutagens (induced stickiness).

Chromosome fragmentation, chromosome degeneration, mixoploidy and cell fusion were also observed among

the species investigated, especially in maize. Some inbred lines, single-cross and double-cross hybrids, cultivated in *cerrado* soils and in the south of the country, presented intense chromosome fragmentation (Caetano-Pereira *et al.*, 1995b), closely resembling the chromosome shattering reported in the literature. The spontaneous appearance of this abnormality in plants is unusual, and previous descriptions have indicated that it was due to induction by radiation, sometimes associated with chemicals and agents known to be clastogenic and mutagenic (Cremer *et al.*, 1981; Albanese, 1982; Cremer and Cremer, 1986). The chromosome fragmentation observed in maize microsporocytes may be associated with damaged of DNA repair mechanisms caused by genetic and/or environmental factors. This assumption is due to the fact that inbred lines from distinct locations, but of common origin, were much more affected than heterozygous genotypes, and the phenotypic expression of this abnormality was much higher when the lines were cultivated on acid soils. Mixoploidy also occurred in this group of genotypes (Caetano-Pereira *et al*., 1998a), and 13 genotypes out of 43 evaluated presented pollen mother cells with different levels of ploidy in the same anther, ranging from diploidy to octaploidy. In plants cultivated in southern Brazil all cells with altered ploidy levels presented nuclear fusion, i.e., all chromosomes shared the same nucleus, but in plants cultivated in the center western region, in addition to this type of behavior for 4n cells, other cells (4n, 6n and 8n) only showed cytoplasm fusion, with the nuclei remaining individualized. Although mixoploidy is widely reported in somatic tissues (for a review, see Nirmala and Rao, 1996), it is rare in anthers. Mixoploidy is often associated with the occurrence of polyploidy, hybridization, chemicals and, in same cases, it is genetically controlled. In the affected maize genotypes, the results suggest that the abnormality is under genotypic control. Mixoploidy is a cytogenetic event of great importance with practical and evolutionary implications. In higher plants lacking sexual reproduction, mixoploidy is a potential force in evolution, while in sexually propagated species, the chromosomal instability in reproductive tissue can produce gametes with variable chromosome numbers with the addition or loss of chromosomes and the formation of gametes that can produce aneuploids in subsequent generations which may present low fertility due to meiotic irregularities. In the maize genotypes affected by mixoploidy, unbalanced gametes side-by-side with normal ones cause impaired seed production. Also in this group, the majority of genotypes showed syncyte formation (Caetano-Pereira *et al*., 1998b); although for most genotypes the number of syncytes was small, in some it was higher. These syncytes must have resulted from cell fusion in premeiotic mitosis because they were observed from the early stages of meiosis. In another group of genotypes, also cultivated in *cerrado* soils, one inbred line showed syncyte formation (Caetano-Pereira *et al*., 1999). In some plants the syncytes showed progressive nuclear degeneration at the beginning of meiosis, producing pycnotic micronuclei and incomplete cell cycle, while in other plants, meiosis was complete despite syncyte formation. Chromosome degeneration did not occur. Microspores and pollen grains anomalous in terms of shape, size and number of nuclei were observed, but the nuclei always remained separate, and we could not identify the cause of the abnormality. According to Nirmala and Rao (1996) cell fusion and chromatin degeneration may be caused both by environmental and genetic factors. Cell fusion, with or without chromatin degeneration, is considered to be a source of male sterility.

Abnormalities related to abnormal spindles and failure of cytokinesis were also frequently observed. Failure of cytokinesis was found in maize genotypes (Defani-Scoarize *et al*., 1995a,b, 1996; Caetano-Pereira *et al*., 1998b) and in many accessions of *Paspalum* species (Pagliarini *et al*., 1999). In maize, two genes have been reported to disrupt cytokinesis, the *va* (variable sterile) gene (Beadle, 1932) and the *el* (elongate) gene (Rhoades and Dempsey, 1966), whereas the absence of cytokinesis has not been reported in *Paspalum*. The absence of cytokinesis in maize occurred at the end of both meiotic divisions, with a prevalence for meiosis II, and as a consequence, tetranucleate monads, binucleate dyads and triads were observed. In some dyads, a restitutional nucleus was formed. In *Paspalum*, only dyads and triads were formed, and there was no cytokinesis at the end of the first meiosis. Abnormal spindles were found in *Thunbergia mysorensis* (Pagliarini, 1990b), *Ochna* (Pagliarini *et al.*, 1992b), *Aloysia lyciodes* (Corazza-Nunes *et al*., 1993), maize (Defani-Scoarize *et al.*, 1995a,b, 1996; Caetano-Pereira *et al*., 1998b), and canola (Souza *et al.*, 1999). Abnormal spindles are also an important meiotic irregularity, because depending on its shape, the spindle can rejoin or split the chromosome complement. Tripolar spindles, for example, produce 2n gametes, while multipolar spindles produce unbalanced and sterile gametes. The formation of 2n gametes has been investigated both for studies of evolution (Harlan and De Wet, 1975) and for breeding programs (Veilleux, 1985). Most species with a polyploid series have relied upon 2n gametes for the evolution of cultivated forms through sexual polyploidization. Breeders of many vegetatively propagated crops have been taking advantage of the occurrence of 2n gametes from wild diploid species for their cultivated polyploid crops through ploidy manipulation (Peloquin and Ortiz, 1992). Unilateral or bilateral polyploidization through the action of 2n gametes has been used for genetic improvement in many crops (Ortiz, 1997).

Chromosome elimination during microsporogenesis, an uncommon abnormality in plants, was detected in *Paspalum subciliatum* (Adamowski *et al*., 1998). Chromosome elimination occurs preferentially during the early stages of embryo development (Davies, 1974; Laurie and Bennett, 1986). Few reports have described this phenomenon during gametogenesis and many mechanisms have been described showing how the chromosomes can be eliminated

(Singh, 1993; Fuge, 1997). The mechanism of elimination found in *P. subciliatum* has never been reported in any other species. In this species meiosis was normal until diakinesis, but starting at metaphase I meiosis was very peculiar because, while ten bivalents were clustered in the equatorial plate, the other ten were still dispersed in the cytoplasm. In anaphase I the chromosomes showed different abilities to migrate to the poles and while one genome reached the poles in telophase I, the laggard was in metaphase or anaphase and was engulfed by extra nuclei. In the second division, behavior of the chromosomes was the same, showing clear asynchrony in the cell cycle. The chromosomal behavior of this tetraploid (2n = 40) accession suggests that it is an allotetraploid resulting from a cross between species whose chromosomes did not have the same rhythm in cell division. Chromosome elimination in interspecific hybrids is a powerful tool in breeding programs. Differential chromosome elimination has facilitated the production of additional lines, while total elimination of one genome permits the formation of haploids.

Meiotic division is characterized by the occurrence of a series of mechanical and biochemical phenomena of considerable complexity which culminate in the reduction of chromosome number. Extensive evidence obtained for different animal and plant species has demonstrated that each step of meiosis is genetically controlled (see Gottschalk and Kaul, 1974; Baker *et al.*, 1976; Golubovskaya, 1979; Kaul and Murthy, 1985). Many meiotic mutants were found during these years, especially in maize (Albertsen and Phillips, 1981; Golubovskaya, 1989) and *Arabidopsis thaliana* (Dawson *et al.*, 1993). Some of these mutants were known, whereas others had never been described before. Among well-known maize mutants are those displaying chromosome stickiness, failure of cytokinesis, syncyte formation, absence of first division, abnormal spindles, polymitotic division in microspores, etc. These mutants have been found and described by Defani-Scoarize *et al*. (1995 a,b, 1996) and Caetano-Pereira *et al*. (1995a, 1997, 1998b) along with some new maize mutants. Taschetto and Pagliarini (1993) have reported an unusual mutation affecting spindle formation in some plants of a double-cross hybrid, in which all meiotic steps were normal up to diakinesis, when disorganization started due to the lack of spindle formation. In the absence of a spindle the bivalents did not organize in the equatorial region, but remained scattered at random in the cytoplasm. The distance among bivalents was extremely variable, with the bivalents clustering into a few groups. Homologous chromosome segregation in anaphase I and sister chromatid segregation in anaphase II did not occur and meiosis went directly from diakinesis to the telophase, skipping the other phases, and telophases with the number of nuclei ranging from 3 to 10 were observed. As a consequence of these irregularities, microspore triads and polyads occurred producing sterile pollen grains of different sizes.

Another original meiotic mutation related to cell shape was found by Caetano-Pereira and Pagliarini (1996) in several inbred lines and hybrids cultivated in *cerrado* soils and which presented pollen mother cells with cytoplasmic projections similar to the pseudopods of amoebae. This abnormality occurred in all phases of meiosis, including pollen grains, but did not affect meiotic division or pollen fertility. Another original meiotic mutation was recently discovered by Caetano-Pereira and Pagliarini in which a premature and additional cytokinesis occurred in metaphase I, sometimes fractionating the genome. Despite this premature cytokinesis, normal cytokinesis occurred at the end of the first and second division. At the end of the first division, telophase I was represented by a tetrad, and at the end of the second division, telophase II was represented by a polyad, generally with microspores of different sizes.

Another original meiotic mutation was described in *Paspalum regnellii* by Pagliarini *et al*. (1998), which, in many aspects, is similar to that described by Taschetto and Pagliarini (1993) in maize. In the *P. regnellii* mutant there was spindle formation with the bivalents arranged at the equatorial plate as in normal metaphase I, but the spindle fibers did not converge towards the poles, instead there was degeneration of spindle fibers at the end of metaphase I and chromosome segregation did not occur, with the bivalents remaining scattered at random in the cytoplasm and remnants of chromosome fibers could be seen close to the centromere during this stage. In telophase I the bivalents gave rise to micronuclei with an extremely wide variation in number and size. With the absence of spindles in the second meiosis, metaphase and anaphase II were not observed. Second cytokinesis occurred in prophase II cells after the occurrence of the first cytokinesis. The final product of meiosis was completely abnormal, with a predominance of polyads with microspores of different sizes that resulted in abortive pollen grains. Also in *Paspalum*, an unidentified accession (BRA-014176) presented a sticky mutation that affected all meiotic phases, impairing chromosome segregation and reducing pollen fertility.

Some meiotic mutants present characteristics that may be successfully exploited in breeding programs, among which are those that cause male sterility in which male gametophytic function is prevented although the potential for female reproduction remains. Among the meiotic mutants identified some caused total male sterility. The maize mutant identified in a double-cross hybrid by Taschetto and Pagliarini (1993) was totally male sterile and its discovery led the breeders to remove it from the breeding program. In the last year, we began to analyze male sterile Brazilian lines of soybean and observed some interesting mutations. One line showed normal meiosis up to telophase II, when some irregularities started to occur, such as total or partial failure of cytokinesis, irregular cell shape and cell degeneration resulting in microspore degeneration in the tetrad and total pollen sterility. In another line meiosis was completely normal until tetrad formation, but the microspores isolated from the tetrad did not undergo total transformation into pollen grains. During pollen development, differentiation

was affected, culminating in total sterility. In another soybean line the mutation affected prophase I, causing chromosome asynapsis which affected all meiotic phases, producing a polyad with many microcytes and sterile pollen. Although some meiotic mutants causing male sterility have been described in American soybean lines (Palmer *et al.*, 1978, 1980; Albertsen and Palmer, 1979; Skorupska and Palmer, 1989), the mutants found in the Brazilian lines are original and are being tested for use in hybridization breeding programs.

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RESUMO

A meiose é um evento de alta estabilidade evolucionária que culmina na redução do número de cromossomos. O curso normal e harmonioso da meiose garante a viabilidade gamética. Os eventos citológicos da gametogênese são controlados por um grande número de genes que atuam desde as mitoses pré-meióticas até as pós-meióticas. Mutações nestes genes causam anomalias que podem afetar a fertilidade. Durante a avaliação do comportamento meiótico em algumas espécies de plantas, muitas anormalidades que afetaram a fertilidade ou causaram total macho-esterilidade foram detectadas. Algumas das anormalidades encontradas já eram descritas na literatura, enquanto outras eram totalmente desconhecidas. As anormalidades mais freqüentes nas espécies analisadas foram segregação irregular de cromossomos, citomixia, aderências cromossômicas, mixoploidia, fragmentação cromossômica, formação de sincícios, fusos anormais e falta de citocinese. Eliminação de cromossomos, uma anormalidade meiótica rara, foi observada em uma espécie de *Paspalum*. Mutantes meióticos nunca antes descritos, afetando diferentes passos da meiose, foram observados em algumas espécies, especialmente em milho, soja e *Paspalum*. Alguns mutantes apresentam características que podem ser exploradas com sucesso em programas de melhoramento, pois causam total macho-esterilidade.

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