Chromosome stickiness impairs meiosis and influences reproductive success in intraspecific *Panicum maximum* (Poaceae) hybrid plants

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Introduction

According to Singh (1993), male sterility is divided into three categories: (i) genetic, (ii) cytoplasmic, and (iii) cytoplasmic-genetic. Genetic male sterility produces nonfunctional androeciums or absence of pollen in plants. The *ms* genes act with remarkable precision at definitive meiotic stages (pre-meiotic, meiotic, and pos-meiotic) (CHAUDHURY, 1993). Some genes are effective during meiotic divisions, disturbing the normal sequence of meiosis or affecting a particular stage of meiosis, causing abnormal chromosome behavior and microspore formation (HORNER; PALMER, 1995).

Genetic male sterility exhibits Mendelian inheritance. The majority of *ms* genes represent monogenic recessive genes (KODURU; RAO, 1981; ALBERTSEN; PHILLIPS, 1981). Mutation in any of these genes that govern micro- or megasporogenesis from pre-meiotic to pos-meiotic events can lead to serious anomalies in the whole process resulting in genetically aberrant end products having adverse impact on fertility and overall reproductive efficiency of the species (LATTOO et al., 2006). Among the genes affecting microsporogenesis causing changes in structural organization of chromosomes, Singh (1993) cited the sticky (*st*) gene. Chromosome stickiness has been the subject of cytological studies in several species. It was recorded for the first time in intraspecific *Panicum maximum* hybrid plants, a forage grass belonging to the family Poaceae, widely used as pasture in the tropics. The present investigation deals with the consequences of chromosome stickiness in relation to meiotic behavior and reproductive success of the hybrids.

Material and Methods

Plant material. The material under analysis consisted of progenitors (*Panicum maximum*, line S12, as female and cv. Tanzania, as male), and 12 single hybrid plants. Two cloned plants of S12 were used.

Cytological analyses. Inflorescences for meiotic studies were collected from individual plants growing in the field, were fixed in ethanol 95%, chloroform and propionic acid (6:3:2, v/v) during 24 h, and stored at 4°C. Anthers containing microsporocytes were isolated from the flowers and slides containing cells spreads were obtained after squashing and staining with 0.5% propionic carmine. The number of pollen mother cells (PMCs) analyzed among hybrids ranged from 1360 to 2054. Images were obtained using Kodak Imagelink – HQ, ISO 25 black and white film.

Results

Anther and pollen development were evaluated in both fertile genitors and in the male-sterile hybrids plants. In the genitors, panicle development was normal in size and in number of flowers. However, in the hybrid plants, the first expression of mutation was observed in the aspect of panicles. Although with normal length, only a few flowers in the racemes presented normal development. In general, in the hybrids, developed flowers were found only in the apex of the panicle. In a fertile raceme of the genitors, all the flowers were well developed, while in the sterile hybrid, a few or no flowers occurred. Compared to the normal fertile plants of both genitors, male-sterile hybrid plants possess smaller, shrunken, and non-dehiscent anthers. In the majority of flowers, meiocytes were difficult to be found in the anthers, because the mutation impaired the meiotic division. In some anthers it was impossible to pick out the sporogenous tissue. The high number of cells analyzed was obtained from a large number of flowers.

Careful analysis of meiosis showed a quite normal meiosis in the genitors; only a few abnormalities related to irregular chromosome segregation was recorded in both. In the hybrids, however, besides irregular chromosome segregation observed in some meiocytes, the majority of them presented abnormal meiosis since prophase I, with chromosomes clumping in several groups. Pachytene, diplotene, and diakinesis did not present normal configuration. After prophase I, condensed sticky chromosomes were dispersed in the cytoplasm or clumped into amorphous masses. Anaphase I and telophase I was not observed. These amorphous masses of chromatin were separated by the occurrence of the first cytokinesis. After this stage, the amorphous masses of chromatin gave rise to several micronuclei of different sizes. Metaphase II and anaphase II also did not occur. Following this phase, the second cytokinesis took place and polyads with

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microspores of different sizes were formed. The percentage of meiocytes affected by chromosome stickiness ranged from 16 to 52.3% among hybrid plants.

Discussion

The general pattern of the sticky meiotic behavior is somewhat similar in several species analyzed in that the chromosomes start to assemble at prophase I or metaphase I, forming sticky clumps which do not orient themselves on the equatorial plate; anaphase I disjunction is irregular or frequently fails, and chromosome fragmentation occurs from prophase I onwards resulting in sterility. In *Panicum maximum*, chromosomes were clumped since prophase I, forming groups inside the meiocyte that failed to orient in metaphases and segregate in anaphases.

Chromosome stickiness was early identified as an effect of ionizing radiation. Stickiness has been the subject of numerous cytological studies, but so far the molecular basis of this type of aberration remains largely unknown (AL ACHKAR et al. 1989; TATUM; RAYBURN, 2006). Gaulden (1987) hypothesized that chromosome stickiness results from changes in specific non-histone proteins (topoisomerase II and peripheral proteins) that are integral components of the chromosome and which function is necessary for separation and segregation of chromatids.

Spontaneous mutations for chromosome stickiness were independently obtained in rye populations (SOSNIKHINA et al., 2003). The mutation recorded in the present *P. maximum* hybrid was also spontaneous in nature and emerged among several experimental single hybrids growing in the same field.

Male-sterile mutations are an important tool in the investigation of anther and pollen development and for obtaining hybrid seeds in plant breeding (ATANASSOVA, 2000). In *P. maximum*, cytogenetic studies on meiosis are rare and male sterility was not yet reported. This finding can open a new opportunity in the breeding program devoted to hybridization where manual cross-pollination is difficult and time consuming. However, the efficiency of this mutation might be investigated for breeding purposes, since less than 50% of meiocytes were affected.

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