

Meiotic and mitotic behaviour of B chromosomes of ryegrass

Comportamento dos cromossomos B na mitose e na meiose de azevém

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ABSTRACT

Mitotic and meiotic analyses using conventional and fluorescent stains were employed in plants (accession ETBAZ 055 – origin: Capão do Leão, Rio Grande do Sul State, Brazil) from the Germplasm Active Bank of Ryegrass (Banco Ativo de Germoplasma de Azevém) of Embrapa, for observing the behaviour of B chromosomes. In several meiotic stages, there were observed up to two B chromosomes, which have presented an unstable behaviour regarding their precocious ascension to metaphase I or delays during anaphase I. At the end of the process, the Bs showed predominantly segregation for the nuclei under formation in order to guarantee their propagation. Concerning the cells that comprise the anther tapetum and the root meristems, the B chromosomes have presented a more stable behaviour.

Key words: *Lolium multiflorum*, accessory chromosome, preferential segregation.

RESUMO

Análises mitóticas e meióticas com coloração convencional e fluorescente foram realizadas em plantas (acesso ETBAZ 055 – procedência Capão do Leão, Rio Grande do Sul) provenientes do Banco Ativo de Germoplasma de Azevém da Embrapa para observar o comportamento dos cromossomos B. Em vários estágios da meiose, foram observados um a dois cromossomos B, os quais apresentaram um comportamento instável, com ascensão precoce na metáfase I ou atrasos na anáfase I. Ao final do processo, os Bs exibiram predominantemente segregação para os núcleos em formação, a fim de garantir a sua propagação. Nas células que constituem o tapete da antera e nos meristemas da raiz, os cromossomos B apresentaram um comportamento mais estável.

Palavras-chave: *Lolium multiflorum*, cromossomos acessórios, segregação preferencial.

INTRODUCTION

B chromosomes also named accessory or supernumerary chromosomes represent one of many cases of numeric variation described in more than 1300 species of plants, 500 species of animals, and some fungi (JONES & REES, 1982; TRIVERS et al., 2004; JONES & HOUBEN, 2003). JONES & PASAKINSKIENE (2005) include them in the group of selfish genic elements with transposons and centromere (specially the kinetocore ones), which present their own mechanisms of perpetuation and may cause conflicts with the host's genomes. This relation, denominated by FRANK (2000) as co-evolution of the genomic conflict is characterized by the perpetuation ability of Bs through generations and the attempt of the host's genome to eliminate or suppress their effect.

The B chromosomes show instability during meiosis, but present preferential segregation for the nuclei which form gametes, and normal behaviour during the mitotic process, except in the second division of the microspores in the endosperm and tapetal cell, where they show high nondisjunction rates (RANDOLPH, 1941; RHOADES et al., 1967; CARLSON, 1986; GONZÁLEZ-SÁNCHEZ et al., 2004). In the last

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case, Bs induce instability in the As chromosomes (RHOADES et al., 1967; RHOADES & DEMPSEY, 1972; RHOADES & DEMPSEY, 1973; ALFENITO & BIRCHLER, 1990; CHIAVARINO et al., 2000). Such alterations may induce deleterious quantitative phenotypical effects, particularly related to seed fertility reduced, seed germination and flowering time delayed, heading date delayed and seed weight increased (RHOADES et al., 1967; BOUGOURD & JONES, 1997; GONZÁLEZ-SÁNCHEZ et al., 2004).

In the genus *Lolium*, the normal chromosomal complement is characterized by seven pairs of chromosomes, which present disjunction and regular segregation during meiosis (KLEIJER, 1982; FARUQI et al., 1987; DEVESA et al., 1990a; GOHIL & KOUL, 1990; DUCKERT-HENRIOD, 1991). In some varieties of *L. multiflorum*, *L. perenne* (DELAY & PETIT, 1972; FARUQI et al., 1987) and *L. rigidum* (HOVIN & HILL, 1966; DEVESA et al., 1990b), *L. persicum*, *L. remotum*, *L. strictum* (HOVIN & HILL, 1966), there were described B chromosomes varying in number from one to eight in microsporocytes of the same plant. There is, as expected, no evidence of homologies and translocation among these chromosomes and those that integrate the A complement, as one of the well-known characteristics of B-chromosomes is the absence of homology with the chromosomes of the normal complement (JONES, 1995). The only exception to the rule is maize, in which translocations can be induced between B's and chromosomes of the normal complement same as reported for all cases of B-chromosomes (JONES, 1995).

The interest in the study of B chromosomes in *Lolium* is supported by the usefulness of the knowledge regarding the origin of the polymorphism and investigation of the somatic and germinative cells. Furthermore, their number varies, within certain limits, amongst individuals of a population, and as such generate a spectrum of DNA amounts, and thus nucleotypes, within the species (BOUGOURD & JONES, 1997). In the present context, in according to JONES et al. (2008), we are especially interested in the way in which they contribute to intraspecific variation in nuclear DNA amounts, as well as to the qualities of the additional DNA which they contribute.

B chromosomes offer the potential for modifying and exploring the A genomes of their host species. They have utility in mapping the A genome, in maize, modulating recombination, exploring the structure of the centromere and the process on nondisjunction, as well as other aspects of genome evolution (JONES et al., 2008). Furthermore, their have physiological implications and their effects, in general,

are not seriously contemplated in plant breeding. There is interest in their capacity to behave as diploidizing agents for chromosome pairing in certain alopolyloid hybrids, such as *Lolium temulentum* x *Lolium perenne* hybrids (JENKINS, 1986; JONES et al., 2008), and their influence on recombination through the modulation of chiasma frequency and distribution in the A chromosome (e. g. in rye) (BEUKEBOOM, 1994).

In according to BURT & TRIVERS (2006) in a minority of species with Bs, the Bs are not harmful to the creatures they inhabit, nor are they maintained by drive. They either show no transmission ratio distortion or they modest drag. They often have the usual negative effects on phenotypic characters (especially with a high B number) but typically show strong positive effects on early survival, often under extreme conditions (e.g. competition or drought). It is not know whether Bs in *Lolium perenne* drive, but several studies have shown that the more densely plants with B chromosome are planted, the better they survive. Because overall survival is reduced, the B is giving a benefit under adverse conditions (HUTCHINSON, 1975; TEOH et al., 1976).

Thus, the objective of this study was the assessment and comparison of the number and behaviour of B chromosome during the meiosis and mitosis of an accession of *Lolium multiflorum*.

MATERIAL AND METHODS

Thirty seven accessions of *Lolium multiflorum* from Germoplasm Active Bank of Ryegrass of Embrapa, Brazil were cytologically analysed. Only one accession (ETBAZ 055 – from *Capão do Leão*, Rio Grande do Sul State, Brazil) showed B chromosome. Ten plants from this accession were analysed. This plants are available at the *Estação Experimental Terras Baixas* of *Embrapa Clima Temperado* in the township of *Capão do Leão*, Rio Grande do Sul State, Brazil.

For the analyses of the meiocytes and the tapetal cells, the inflorescences were collected in early stages of development between 8h30min and 10h, fixed in Carnoy (ethylic alcohol: acetic acid, 3:1) and kept at -4°C until the moment of use. The slides were prepared according to the conventional protocol for meiotic studies by smear and staining using Giemsa at 2% (GUERRA & SOUZA, 2002). Moreover, the fluorescent staining with 4'-6-diamidino-2-phenylindole (DAPI) was used according to the descriptions of the protocol of GUERRA & SOUZA (2002). Three thousand five hundred and fifty meiocytes in prophase I, metaphase I, anaphase I and 1635 tetrads and microspores were analysed.

Regarding the mitotic analyses, besides the tapetal cells, meristematic cells obtained from the roots of germinated seeds were analysed. The metaphases were obtained using the smear technique and staining with Giemsa as described in the protocol of GUERRA & SOUZA (2002).

The photomicrographs were made using Nikon® microscope by capturing images with the CCD Nikon® camera linked to a computer. The slides stained with fluorochromes were analysed using Leica® fluorescence microscope and captured by CCD camera and with the Leica Q Fish® software.

RESULTS AND DISCUSSION

The presence of one or two B chromosomes in the same anther was detected in several stages of meiosis I and II using conventional staining and confirmed using fluorescent staining (Figure 1). Such

variations in the number of B chromosome between cells have been reported already for *Lolium* by HOVIN & HILL (1966) and for other species by POWELL & BURTON (1966); JONES (1975); RAO & PANTULU (1978) and RICCI et al. (2007).

In meiocytes with one B chromosome, they (chromosomes) appear as univalent in diakinesis and metaphases I; when in number of two, they form bivalents. This behaviour was also observed by RICCI et al. (2007) in popcorn and was already been described in other species by JONES & REES (1982) and JONES (1995). There was not observed any multivalent configuration, indicating that there is no homology between the A and B chromosomes in the accession of *L. multiflorum* under study.

In 69.5% of 3,550 meiocytes analysed, the B chromosomes show themselves detached from the chromatin especially during the pachytene (431 cells), in early ascension in the metaphase I (938 cells), or

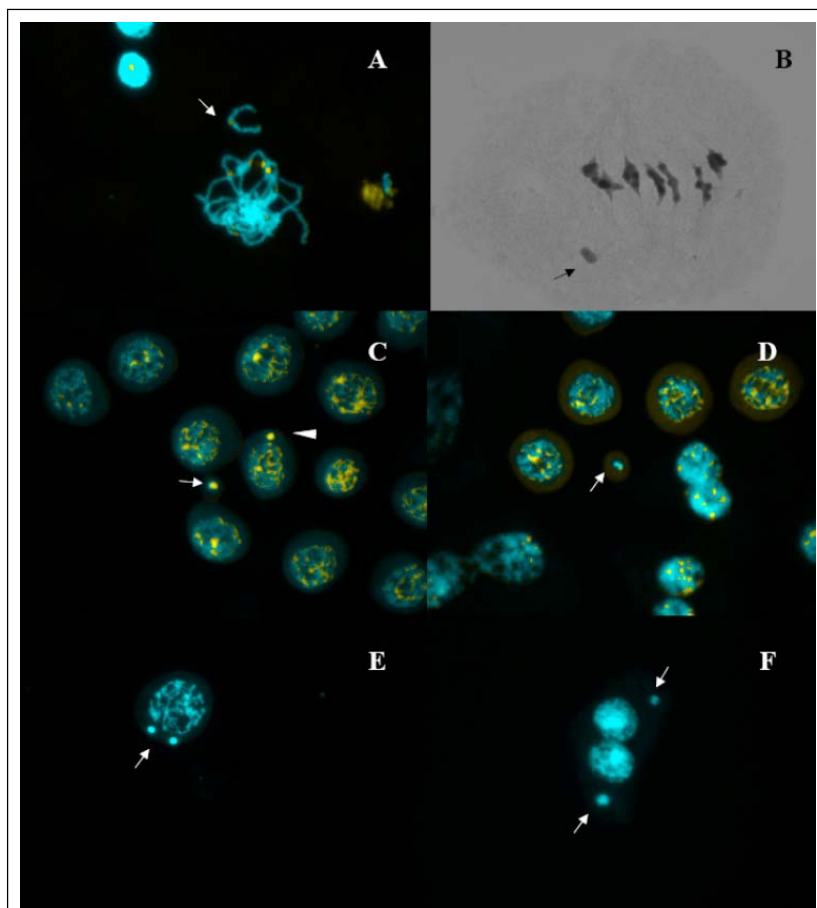


Figure 1 - B chromosomes in the accession ETBAZ 055 of *Lolium multiflorum* Lam. A. Pachytene with B chromosomes forming bivalent (arrow); B. Metaphase I with seven bivalents and an univalent (B chromosome) in early ascension (arrow); C. Microspore with one micronucleus (top of the arrow) and microcyte (arrow); D. Microcyte (arrow); E. Microspore with two micronuclei (arrow); F. Tapetal Cells with two micronuclei (arrow).

delayed in the anaphase I (1098 cells) (Figures 1A and B). The distinction of the B chromosomes from the A chromosomes was possible due to its reduced size, variable number, and strong staining using Giemsa, which revealed its heterochromatic nature.

The meiotic analyses performed by HOVEN & HILL (1966) in *Lolium persicum*, *L. remotum*, *L. rigidum* and *L. strictum*, revealed that the B chromosomes were detected in the metaphase I and anaphase I, but they were absent in the diakinesis. The evidence of preferential segregation of B chromosomes in the accession ETB AZ 055 is the reduction of the tetrads and microspore with micronuclei (14.4%) (Figures 1C and E), indicating that the majority of Bs were included in the nuclei to guarantee their propagation or the expulsion from the cytoplasm, therefore originating the microcytes (Figures C and D). The expulsion of a micronucleus is a way of chromosomal elimination, which is common in interspecific and intergeneric hybrids, but there is no similar report to *L. multiflorum*.

Results of several studies have demonstrated that the behaviour and the mechanism of transmission of B chromosomes may be, in some cases, controlled by genes located in the A chromosomes (NUR & BLETT, 1987; CHIAVARINO et al., 2000; JONES & HOUBEN, 2003; JONES et al., 2008). The B chromosome of maize has a complex genetic organization with at least four different regions that influence the nondisjunction process, which happens on the male side only. In addition, unpaired univalent B chromosomes can suppress their meiotic loss and enhance their transmission potential. The four genetic regions are (a) distal euromatic tip and (b) distal euromatin, are *trans-acting* and essential for nondisjunction; (c) centromeric chromatin is a *cis-acting* receptor for nondisjunction, and (d) short arm and centromere region enhances nondisjunction (CARLSON & ROSEMAN, 1992; JONES & HOUBEN, 2003). Subsequent experiments (CHIAVARINO et al., 2001) determined that a single major gene in an A chromosome controls B-chromosome transmission rate in maize, and that it acts in the haploid egg cell at the time of fertilization. However, concerning rye, PUERTAS et al. (1998) and JONES & HOUBEN (2003) described that the genes that control its mode of polymorphism are located in the Bs and suggested that such genes are sites where there are formations of chiasma.

In *Lolium perenne* and *Festuca pratensis* (genetically related species with *Lolium*) and other 260 species belonging to the Poaceae family, the B's disjunction control and mitosis seem to be involved in

a similar genetic controlling system presenting a *trans-acting* element as its base (BOSEMARK, 1956; JONES & REES, 1982; JONES, 1995; JONES & PASAKINSKIENE, 2005).

In the somatic tissues analysed (tapetal cells and the root meristems), there was observed that the B chromosomes present a more stable behaviour. In the meristematic cells, there were observed up to two chromosomes, which have presented regular segregation. Regarding the 1075 binucleated tapetal cells analysed, approximately 17% represented one or two micronuclei (Figure 1F), which may be originated from the non-integration of Bs to the nuclei or even from the chromosomes of the A complement. In some corn lineage, CHIAVARINO et al. (2000), using FISH, detected the formation of three types of micronuclei in the tapetal cells that were formed according to the decreasing order: micronuclei with B chromosomes, micronuclei with chromosomes containing knobs, and micronuclei with chromosomes without knobs. Such results show that Bs tend to form more micronuclei than chromosomes of the A complement.

According to CHIAVARINO et al. (2000), the nondisjunction of A chromosomes and the formation of micronuclei in the tapetal cells are events that happen regularly during the process of maturation of the anther, but the presence of B chromosomes increase the frequency of both events, therefore, indicating that these chromosomes and the genotype influence the instability of the A chromosomes.

Although the sporogenous and tapetal cells have a common origin from the archesporial cells (ECHLIN, 1973; CANALES et al., 2002), and, in *Poaceae* family, remain in close contact during their all developmental stages (KIRPES et al., 1996), there is a tendency of more stability of Bs in the somatic tissue.

CONCLUSIONS

In several meiotic stages, there were observed up to two B chromosomes, which have presented an unstable behaviour regarding their precocious ascension to metaphase I or delays during anaphase I. At the end of the process, the Bs showed predominantly segregation for the nuclei under formation in order to guarantee their propagation. Concerning the cells that comprise the anther tapetum and the root meristems, the B chromosomes have presented a more stable behaviour.

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