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B chromosomes in popcorn (*Zea mays* L.)

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ABSTRACT. Cytological analysis of microsporogenesis in 72 popcorn plants, comprising nine from the original population (CMS-43, S_0) and 63 from seven cycles of self-fertilization (S_1 to S_7), one plant of S_0 generation (plant 2) was identified with B chromosomes. The number of B chromosomes varied from two to three in the same anther. The pattern of chromosome pairing and meiotic behavior of Bs were similar to those found in other plant species. The presence of B chromosomes did not affect chiasma frequency and chiasma distribution in A chromosomes. This is the first report of B chromosomes in popcorn.

Key words: Popcorn, B chromosome, Meiotic behavior,
Chiasma frequency, Chiasma distribution

INTRODUCTION

B chromosomes are a class of supernumeraries which may be present as non-homologous additions to the basic complements in diploid or polyploid eukaryotes (Jones, 1981). They are recognized by three criteria: i) they are dispensable and may be present or absent in individuals within a population; ii) they do not pair or recombine with any members of the standard diploid (or polyploidy) set of A chromosomes at meiosis; iii) their inheritance is non-Mendelian and irregular (Jones and Houben, 2003). Whether B chromosomes are genetically inert or not is still widely discussed (Müntzing, 1974; Jones, 1975; Jones and Matthews, 1983; Jones and Houben, 2003). According to Jenkins and Jones (2004), the concept that B chromosomes are nuclear parasites places them in the arena with other forms of selfish DNA in the A chromosome genome, e.g., retro-elements.

B chromosome occurs frequently not only in hundreds of flowering plants, but also in gymnosperms and in other lower forms such as ferns, bryophytes and fungi. They occur also in animals, including mammals (Müntzing, 1974; Jones, 1975; Jones and Houben, 2003). B chromosomes are widely distributed in maize in which numerical polymorphisms are frequently found (Jones, 1975; Carlson, 1978; González-Sánches et al., 2003). In maize, they have been extensively used in genetic analysis involving A-B translocations for mapping (Beckett, 1991) and for the identification of centromere structure and size (Kaszas and Birchler, 1998; Carlson, 2006). In maize and other species, there is an interest in their capacity to behave as diploidizing agents for chromosome pairing in certain allopolyploid hybrids (Jenkins, 1986) and in their influence on recombination through the modulation of chiasma frequency and distribution in A chromosomes (Jones and Rees, 1982).

In maize, B chromosomes are non-essential, highly heterochromatic and accumulate by a non-disjunction process during the second-pollen mitosis (Jones and Houben, 2003). B chromosomes may accumulate several copies without any reduction in plant vigor. Although they do not contain known genes, the short euchromatic tip is necessary for non-disjunction during the second-pollen mitosis (Phelps-Durr and Birchler, 2004; Carlson, 2006). B chromosomes have not been reported in popcorn. The present study reports its occurrence in one population chosen for a breeding program involving hybridization.

MATERIAL AND METHODS

The microsporogenesis process of one population of popcorn (S_0) (CMS-43) from the National Research Center of Maize and Sorghum (CNPMS/Embrapa) at Sete Lagoas, MG, Brazil, and seven self-fertilized generations (S_1 to S_7) obtained from S_0 were cytologically characterized for breeding purposes. Nine plants were analyzed in each generation. Plants from S_0 to S_7 generations were cultivated in the Experimental Farm of the State University of Maringá, Maringá, PR, Brazil, in 2005.

Inflorescences for meiotic study were collected and fixed in a mixture of 95% ethanol and acetic acid (3:1) for 24 h, transferred to 70% alcohol and stored under refrigeration until use. Microsporocytes were prepared by squashing and staining with 0.5% propionic carmine. Photomicrographs were taken with a Wild Leitz microscope using Kodak Imagelink - HQ, ISO 25 black and white film.

RESULTS AND DISCUSSION

Seventy-two plants from S_0 to S_7 were cytologically analyzed. Only one plant (plant 2) of the S_0 generation showed B chromosomes. All the meiocytes had two or three B chromosomes in plant 2. Table 1 shows the percentage of cells with two or three B chromosomes. The supernumerary chromosomes were easily detected during pachytene (Figure 1a), diplotene, and diakinesis by their small size and stainability in relation to A chromosomes. Numerical variation of B chromosomes among cells of the germ-line has been reported in several animals (Carrol, 1920; Nur, 1963, 1969; Jones, 1975; Peters, 1981; López-Fernández and Gosálvez, 1983; Kolomiets et al., 1988) and in plants (Jones, 1975; Rao and Pantulu, 1978). Variation in all cases has been attributed to a non-disjunction of B chromosomes in early cell mitosis which has given rise to follicles that constitute male testes in animals or pollen mother cells in plants.

Table 1. Meiotic behavior of B chromosomes from diakinesis to tetrad in 100 meiocytes of the plant 2 from the S_0 generation.

Phase	Behavior of B chromosomes
Diakinesis	B pairing as II (55%) B pairing as 2 I (30%) B pairing as 3 I (10%) B pairing as II + I (5%)
Metaphase I	2 Bs migrating precociously to the same pole (18%) 1 B migrating precociously to each pole (2%) Bs migrating together with A chromosomes (80%)
Anaphase I	Bs migrating precociously to the pole (8%) Bs behaving as laggards (20%) Bs migrating together with A chromosomes (72%)
Telophase I	Bs eliminated in micronucleus (14%)
Prophase II	Bs in micronucleus (8%)
Metaphase II	Bs inserted in the A metaphase plate (100%)
Anaphase II	Bs migrating together with A chromosomes (100%)
Telophase II	Bs inserted in the nucleus (100%)
Tetrad	Bs inserted in the nucleus (100%)

Chromosome pairing patterns were typical of those observed in animals and plants with B number. In meiocytes with two B chromosomes, they paired preferentially as bivalents (Figure 1b). However, when three Bs were present, they were found to be preferentially univalent (Table 1). B chromosomes in maize are structurally homologous, pair strictly *inter se* and are able to form multivalents when more than two are present (Jones, 1975). In the plant under analysis, however, a considerable number of meiocytes exhibited the two (Figure 1c) Bs as

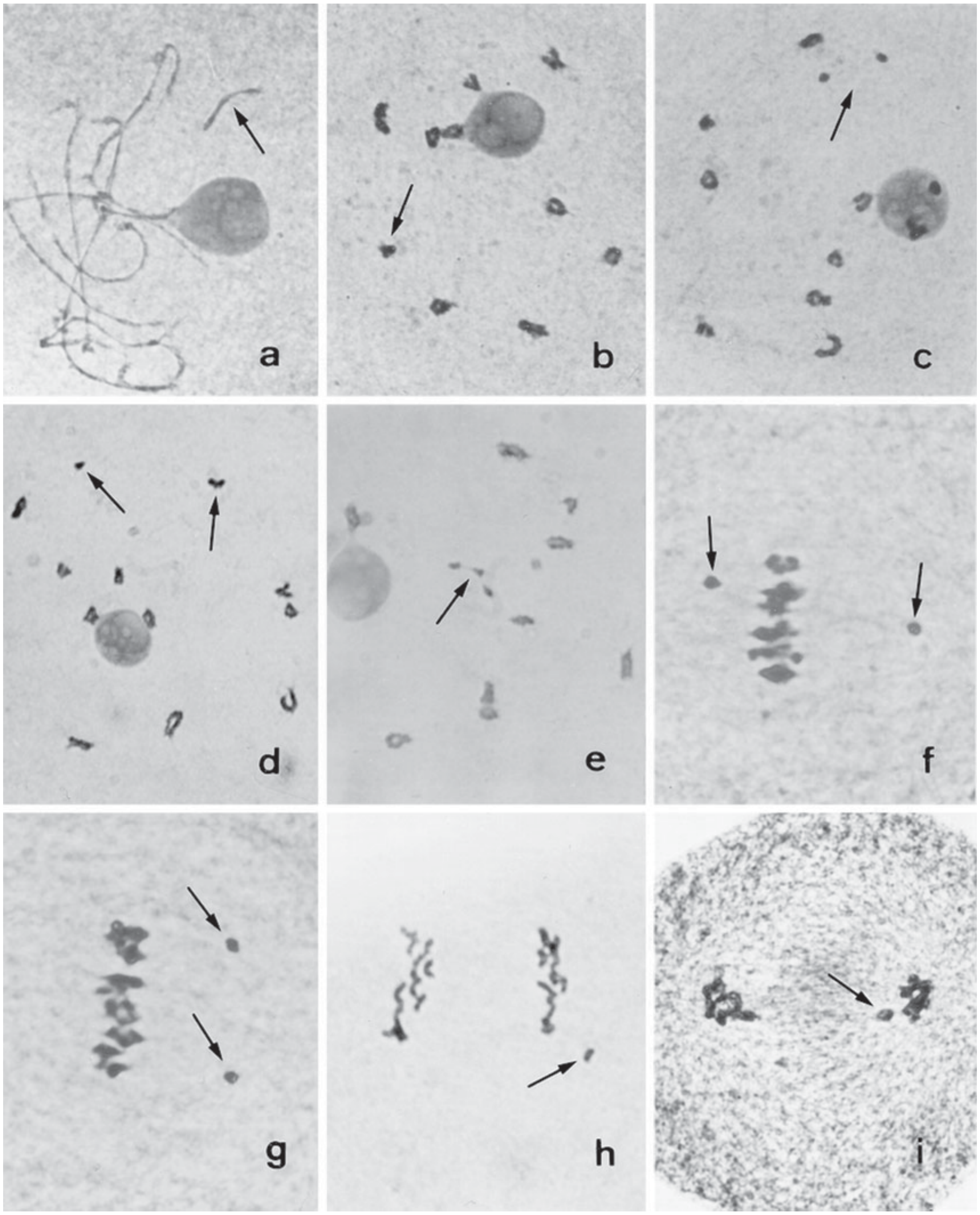

Figure 1. Aspects of meiotic behavior of B chromosomes. a) Meiocyte in pachytene with two B chromosomes pairing in bivalent (arrow). b, c) Diakinesis with the two B chromosomes associated in bivalent (b) and as two univalents (c) (arrow). c, d) Meiocytes with three Bs: II + I in d and III in e (arrows). f, g) Metaphase II with two Bs showing precocious chromosome migration to the poles (arrows). h) Anaphase I with a B chromosome migrating precociously to the pole (arrow). i) Telophase I with B chromosomes behaving as laggards (arrow). Magnification: 400X.

univalent. A few numbers of trivalents were found among meiocytes in diakinesis (Figure 1e). The pairing relationship among B chromosomes is highly dependent on A genetic background, whereas the frequency of bivalents in plants with 2 Bs varies strongly among different species (Jones and Rees, 1967; Jones, 1975; Henriques-Gil et al., 1984; Cano et al., 1986).

Segregation meiotic behavior of popcorn B chromosomes was similar to that in maize and other species reported by Jones (1975). In 80% of meiocytes, they showed normal orientation, congression, and disjunction, following behavior of A chromosomes at metaphase I and anaphase I. In 2% of meiocytes at metaphase I they migrated to distinct poles (Figure 1f), while in 18% they did so to the same pole (Figure 1g). Precocious migration was caused by their univalent status. In anaphase I, B chromosomes were found migrating precociously in 8% of meiocytes (Figure 1h) or behaving as laggard in 20%. The precocious or laggard migration to the poles led to their elimination from the A nucleus in 14% of meiocytes at telophase I (Figure 1i). The B micronuclei remained in the cell until the end of prophase II when they migrated to the second-metaphase plate and rejoined the A chromosomes, accompanying them in the second division. This behavior ensured their permanence in the cell. There is much evidence that the meiotic behavior of B chromosomes is genetically controlled (Jones, 1975; Chiavarino et al., 1998, 2001). Recently, González-Sánches et al. (2003) demonstrated that one gene determines maize B chromosome accumulation by preferential fertilization while another(s) determines their meiotic loss. The numerical polymorphism frequently found in maize is mediated by three cytological process: i) B non-disjunction at the second pollen mitosis, which produces sperm nuclei with different B number (Jones, 1975; Carlson and Chou, 1981; Rusche et al., 1997; Carlson, 2006); ii) preferential fertilization by sperm nucleus carrying Bs after the non-disjunction process (Carlson, 1978, 2006), and iii) the suppression of meiotic loss when Bs are unpaired (Carlson and Roseman, 1992).

In species of plants and animals that carry B chromosomes, those individuals of a population with and without Bs cannot generally be phenotypically distinguished from one another. However, in some species, there are instances in which B chromosomes change certain morphological characteristics (Jones, 1975; Jones and Rees, 1982; Jones and Houben, 2003) or cause some selective advantageous effects (Müntzing, 1974; Jones, 1975; Teoh and Jones, 1978; Jones and Rees, 1982; Plowman and Bougourd, 1994). The most common influence of B chromosomes on A chromosomes is their effect on chiasma frequency. Jones (1975) and Jones and Rees (1982) reported several cases, including maize, in which B chromosomes increase recombination in A chromosomes. This effect, however, is not consistent. In other species, their presence causes a reduction in A chromosome chiasma frequency or fails to affect it. B chromosomes may also modify the distribution of chiasma along A chromosomes (Jones and Rees, 1982; Carlson, 1994). B chromosomes in popcorn affected neither chiasma frequency in A chromosomes nor their distribution (Table 2). There is no significant difference among the plant with Bs (plant 2) and the plants without Bs with regard to chiasma frequency and chiasma localization.

Only one plant of S_0 generation had B chromosomes in the 72 popcorn plants analyzed (from S_0 to S_7). This plant could not be distinguished phenotypically from others without the B chromosome. According to Jones (1975), B chromosomes are rarely found in inbreeding species and there is a strong correlation between the occurrence of B chromosomes and a tendency toward out-breeding. The reasons for this fact have never been adequately explained, although it is known that forced inbreeding of a naturally out-crossing species with Bs rapidly leads to a decline in their frequency. The current study shows that although B chromosomes have not been found in endogamous popcorn plants (from S_1 to S_7), the cause of their absence may not be explained merely by elimination caused by inbreeding, but perhaps by the selection of plants with agronomic characteristics that will constitute the new generation.

Table 2. Chiasma frequency in the nine plants of the original population (CMS-43) where B chromosomes were found.

Chiasmata	1	2	3	4	5	6	7	8	9	Mean
Interstitial	5.45	5.80	5.45	6.10	6.00	4.40	5.20	6.10	5.75	5.58
Terminal	14.75	14.95	15.05	14.75	16.20	14.65	16.45	15.80	15.25	15.31
Total	20.20	20.75	20.50	20.90	22.20	19.05	21.65	21.90	21.00	20.90

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