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Comparative microsporogenesis between diploid and tetraploid plants of *Brachiaria ruziziensis and their progenies*

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ABSTRACT

Microsporogenesis studies in *Brachiaria* have been concentrated in the species *B. ruziziensis, B. decumbens* and *B. brizantha* and hybrids because they have great importance as forage. Besides the sexual behavior, *B. ruziziensis* is among a minority of *Brachiaria* diploid species ($2n = 2\times = 18$), while most are tetraploid ($2n= 4\times = 36$) and apomictic. *Brachiaria ruziziensis* is used in intra and interspecific crossbreedings. For the success of interspecific hybridization, artificial tetraploidization of *B. ruziziensis* is necessary. The aim of this study was to compare the microsporogenesis in diploid and synthetic tetraploid *B. ruziziensis* plants and their respective progenies. The slides were prepared by squash technique and stained with 1% propionic carmine for meiocyte analysis and Alexander's stain for pollen viability. Abnormalities were found in all phases of meiosis in the families of the diploid and tetraploid plants and, in general, the rate was considered low, with the percentage varying according to the genotypes. The diploid family was considered more stable. For both families, there is a tendency to reduce the total frequency of abnormalities in the progenies compared to the mother plants. This response indicates that combinations of random crosses or self-fertilization among plants with the same ploidy level were favorable. For the diploid accessions, the pollen viability index was high, reaching up to 88.3%, whereas the tetraploidized plants presented lower values, reaching a maximum of 61.1%. The results will be of interest to researchers involved in tropical forage breeding or conducting intraspecific crosses, since the occurrence of regular meiosis in progenies ensures the formation of viable pollen grains.

1. Introduction

Since their domestication, plants have played a crucial role in satisfying the demand for food and non-food products. Plants form the basis of food, clothing, cosmetic, pharmaceutical and other industries. Forage plants also contribute to the development of agribusiness in meat and milk, which plays a relevant role in the supply of food to the market, generation of employment and income for the population, and, in recent years, also began to make up the export agenda of many countries.

For the success of this economic activity, it is necessary to cultivate high quality forages. The forages predominantly used in pastures are grasses of African origin of the genus *Brachiaria* (Trinius) Grisebach (syn. *Urochloa* P. Beauv.), which, in 2006, for example, already occupied 85% of the total planted area in Brazil (IBGE, 2006). This is because members of the genus do not require high input of nutrients to be very productive, which facilitates the farming of livestock on any of the types of soil that occur in Brazil. The most cultivated species are *B. brizantha* (Hochst. ex A. Rich.) Stapf, *B.humidicola* (Rendle) Schweick, *B.decumbens* Stapf and *B. ruziziensis* R. Germ & C.M. Evrard (Valle and Pagliarini, 2009).

The breeding programs, through interspecific and intraspecific hybridization, have been working to obtain plants with higher agronomic characteristics, such as higher nutritional value, high productivity and resistance to their main pest, the spittlebug, aiming at increasing pasture quality (Souza Sobrinho, 2005).

However, most species, including *B.brizantha* and *B.decumbens*, present polyploidy ($2n = 4\times = 36$) and an apomictic reproduction cycle, whereas *B. ruziziensis* is diploid ($2n = 2\times = 18$) with sexual reproduction. This variation in the ploidy level among genotypes is one of the limitations in the breeding program through interspecific hybridization. In this way, the artificial tetraploidization of diploid *B. ruziziensis* is performed to allow crossing with the tetraploid and apomictic species (Pereira, 2001; Souza Sobrinho, 2005). Timbo et al. (2014) induced polyploidy in *B. ruziziensis* from seeds germinated *invivo,* using colchicine. The tetraploidized plants and the obtained intra and interspecific hybrids have been evaluated to verify their reproductive efficiency and to observe their cytogenetic characteristics. In relation to the characteristics of fodder interest, Simeão et al. (2016) showed that tetraploid progenies of *B. ruziziensis* presented higher percentage of crude protein, higher digestibility, lower neutral detergent fiber (NDF) and

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lignin concentrations than teraploids of *B.brizantha* cv. Marandu and *B.decumbens* cv. Basilisk. Timbo et al. (2014) reported that tetraploidized *B. ruziziensis* displays productivity similar to that of commercial cultivars of *B. ruziziensis* available in Brazil as well as tolerance/ resistance to stress caused by aluminum and spittlebugs.

The procedure of observation of the chromosomal behavior is important because, depending on the dosage and exposure time of the genotype to colchicine, genotoxic effects can be observed, causing alterations as chromosomal losses, mixoploidy, impairment in the segregation and even sterility, making it more difficult to use them in breeding programs (Luckett, 1989; Pereira et al., 2012).

Considering this scenario, the ideal is that artificially tetraploidized *B. ruziziensis* plants exhibit a regular microsporogenesis, that is, they present low rates of meiotic abnormalities, resulting in the formation of viable pollen grains, both in the mother plants and in their progenies, ensuring the success of future crossings. Studies performed by Paglianini et al. (2008) in others genotypes of *B. ruziziensis* showed an average of meiotic abnormalities that ranged from zero to 24.46% in the diploid plants and from 5.20 to 54.71% in the tetraploidized plants. In another study conducted by Paula et al. (2016), an artificially tetraploidized *B. ruziziensis* genotype, a *B. ruziziensis* genotype from a population obtained from the crossbreeding of eight tetraploidized plants and four genotypes from the self-fertilization of the tetraploidized plant of *B. ruziziensis*, was evaluated. The meiotic behavior of these genotypes was considered little affected (4.43 to 11% of abnormalities) and pollen viability was considered high (61 to 85%).

Thereby, the aim of the present study was to compare the microsporogenesis in diploid and tetraploid plants of *B. ruziziensis* with their respective progenies.

2. Material and methods

2.1. Plant material

For the meiotic analyses, eight *B. ruziziensis* plants were evaluated, divided into two families: diploid (mother plant -M-779 genotype and progenies: P1-779, P2-779 and P3-779) and tetraploid (mother plant -M-686 genotype and progenies P1-686, P2-686 and P3-686) from the breeding program conducted by Embrapa Gado de Leite - Juiz de Fora, Minas Gerais State, Brazil. The tetraploid mother plant was obtained by Timbo et al. (2014). Progenies are the result of polycross blocks among plants with the same ploidy level, where the female parent is known, but the male parent is unknown, and anemophily or self-pollination may occur.

Young inflorescences were collected and fixed in absolute ethyl alcohol: acetic acid: propionic acid (6: 3: 2)and stored at −4 °C until the time of evaluation. Under the stereoscopic microscope, the anthers were removed and macerated on a slide with a drop of 1% propionic carmine stain for meiotic analysis, and Alexander's stain for analysis of pollen viability. The slides were prepared by squash technique and evaluated on a light microscope (Carl Zeiss, AxioLabA1) equipped with microcamera (AxioCam ICc1). The meiotic behavior was evaluated on about 2800 cells/plant. All meiotic phases were evaluated and the abnormalities recorded for the families of the diploid and tetraploid plants.

For pollen viability, five slides/plant and 200 pollen grains/slide were evaluated. The pollen grains were considered viable when they stained purple and had no deformations, and nonviable when stained green. The percentage of viability was obtained as a function of the total number of evaluated pollen grains and subjected to the Scott-Knott statistical test of the Sisvar software (Ferreira, 2000).

3. Results

In the evaluations of the meiosis of plants M-779, P1-779, P2-779 and P3-779, the diploidy ($2n = 2\times = 18$) was confirmed (Fig. 1), with diakinesis and metaphases I showing nine bivalents, one, or occasionally two, of them being connected to the nucleolus (Fig. 1C and D). A terminal knob located in the nucleolar bivalent and a subterminal knob (Fig. 1B) were observed in two pachytenic chromosomes. Other smaller heterochromatic regions, called chromomeres, were also observed interstitially positioned (Fig. 1B).

For the plants M-686, P1-686, P2-686 and P3-686, the tetraploidy ($2n = 4\times = 36$) was confirmed (Fig. 2), with diakinesis and metaphases I showing configurations in univalents, bivalents and multivalents. Due to the variation in the occurrence of these configurations, it was not possible to determine the exact number of bivalents connected to the nucleolus (Fig. 3A and B).

Regarding the abnormalities, both the diploid (M-779) and tetraploid (M-689) mother plants of *B. ruziziensis* showed regular meiosis and rates similar to their progenies (Table 1 and Figs. 2 and 3). Most of the abnormalities were observed from metaphase I, but univalent and multivalent configurations were observed in the diakinesis of tetraploid plants (Table 1).

In the diploid genotypes, the percentage of metaphases I with non-oriented chromosomes ranged from 0 to 2.93% for the three progenies and 5.3% for the mother plant (M-779). The progeny P2-779 showed no abnormal behavior at this phase. In anaphase I, with the exception of progeny P1-779, which did not show alterations, the average percentage of laggards chromosomes was 3%. During telophase I and prophase II, bridges and micronuclei were observed only in the P3-779 progeny. The presence of non-oriented chromosomes in metaphase II ranged from 5 to 21%, the latter value being observed in the mother plant (M-779). In relation to anaphase II, telophase II and tetrads, the percentage of abnormalities was low (less than 1%), represented by few cells altered only in the genotypes P1-779 and P3-779(Table 1 and Fig. 2).

In tetraploid genotypes, the highest rate of abnormalities was observed in metaphase I due to the non-orientation of several chromosomes in the metaphase plate (Fig. 3 B)and stickiness (Fig. 3C), ranging from 7.32 to 14.97% among the progenies and 32.04% for the mother plant (M-686). Among the anaphases I and prophases II, the rates of alterations (including laggards chromosomes, micronuclei and asynchronous nuclei) reached about 7%. In the metaphases II, all the genotypes of the tetraploid family showed high numbers of alterations due to the rate of asynchronous nuclei, varying from 13.82% to 27.11% in the progenies P1-686 and P3-686, respectively. With the exception of telophases II that did not show abnormalities, the percentage of alterations in the anaphases II and tetrads were variable, especially the progeny P2-686 that showed 10.34% of laggards chromosomes/chromatids in anaphase II and P3-686, with 8.15% of alterations in tetrads and formation of triads (Table 1 and Fig. 3). In relation to the total number of cells quantified by genotype, the M-686 mother plant showed the highest rate of abnormality in meiosis, reaching 6.22%.

The pollen viability test indicated the genotypes of the diploid family with rates varying from 37.8 (P3-779) to 88.3% (P1-779). The P3-779 progeny (37.8%) showed lower viability of the pollen grain ($p < 0.05$), significantly different from the M-779 mother plant and the other two progenies (P1-779 and P2-779) (Table 2).

In the tetraploid genotypes, the values ranged from 35.5 (M-686) to 61.1% (P3-686). The progeny (P3-686) showed the highest viability rate, statistically different in relation to the other progenies and the mother plant (Table 2).

4. Discussion

Microsporogenesis studies in *Brachiaria* have concentrated on the species of greater forage importance (*B. ruziziensis, B. decumbens* and *B.brizantha*) and their respective hybrids. Emphasis has been on the description and frequency of the abnormalities, as well as their consequences (Mendes-Bonato et al., 2002; 2006a; Risso-Pascotto et al., 2003a; 2005; Paglianini et al., 2008; Paula et al., 2016). In this study, diploid and tetraploid plants of *B. ruziziensis* and their progenies were