Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v36i2.19440

Intra-inflorescence pollen viability in accessions of *Brachiaria ruziziensis*

Cristina Maria Pinto de Paula[1], Vânia Helena Techio[1*], Flávio Rodrigo Gandolfi Benites[2] and Fausto de Souza Sobrinho[2]

*[1]Departamento de Biologia, Universidade Federal de Lavras, Cx. postal. 3037, 372000-000, Lavras, Minas Gerais, Brazil. [2]Empresa Brasileira de Pesquisa Agropecuária, Setor Gado de Leite, Juiz de Fora, Minas Gerais, Brazil. *Author for correspondence. E-mail: vhtechio@dbi.ufla.br*

ABSTRACT. Knowledge about the pollen viability is important to ensure success in controlled hybridizations and, consequently, support breeding programs. The aims of this study were to evaluate the pollen viability in progenies of artificially induced tetraploid accessions of *Brachiaria ruziziensis*, and to verify if the position of the flower buds on the raceme affects the pollen viability rate. Staining of aborted and non-aborted pollen (Alexander's technique) was used to determine the viability of the pollen grains. Tetraploid accessions of *B. ruziziensis* plants had high pollen viability (x = 76.8% to x = 99.6%). Some of these plants had viability rates similar to diploid *B. ruziziensis*, showing that the induction of chromosome duplication by colchicine did not result in abnormalities in production and morphology of pollen grains. Pollen grains from middle and apical regions of the raceme presented higher viability rates (x = 97.9% and x = 97.7% respectively). The viability of pollen grains in artificially induced tetraploid accessions of *B. ruziziensis* plants was high, which may favor obtaining fertile descendants in possible crosses.

Keywords: fertility, pollen grain, forage breeding, forage grass.

Viabilidade de pólen intra-inflorescência em acessos de *Brachiaria ruziziensis*

RESUMO. O conhecimento da viabilidade de grãos de pólen é imprescindível para assegurar o sucesso nas hibridações controladas e, consequentemente, auxiliar programas de melhoramento genético. O objetivo deste estudo foi avaliar a viabilidade de grãos de pólen em progênies de *Brachiaria ruziziensis* tetraploidizadas artificialmente, além de verificar se há efeito da posição dos botões florais no racemo sobre a taxa de viabilidade de pólen. A coloração de pólen abortado e não abortado (técnica de Alexander) foi usado para determinar a viabilidade dos grãos de pólen. Plantas de *B. ruziziensis* apresentaram elevada viabilidade de pólen (x = 76,8% a x = 99,6%). Algumas dessas plantas apresentaram taxas de viabilidade similares a *B. ruziziensis* diploide, mostrando que a indução de duplicação cromossômica usando colchicina não resultou em anormalidades na produção e na morfologia dos grãos de pólen. Grãos de pólen das regiões mediana e apical do racemo apresentaram maiores taxas de viabilidade (x = 97,9% e x = 97,7%, respectivamente). A viabilidade dos grãos de pólen em plantas de *Brachiaria ruziziensis* tetraploidizadas artificialmente foi alta, o que pode favorecer a obtenção de descendentes férteis em eventuais cruzamentos.

Palavras-chave: fertilidade, grãos de pólen, melhoramento de forrageiras, gramíneas forrageiras.

Introduction

Grasses of the genus *Brachiaria* (Trin.) Griseb. [(syn. *Urochloa* Hochst. ex A. Rich.) R. D. Webster] gather around 100 species (RENVOIZE et al., 1996), some of great economic importance as forage crops (SOUZA SOBRINHO, 2005; VALLE et al., 2009). It is estimated that approximately 85% of the areas planted to pasture in Brazil use *Brachiaria* cultivars (MACEDO, 2006), among which those of greatest importance are the apomictic and generally polyploid *B. brizantha*, *B. decumbens*, *B. humidicola* and the sexual diploid, *B. ruziziensis*.

The main strategy of *Brachiaria* breeding programs is the evaluation and selection of promising genotypes, as well as interspecific crosses involving *B. brizantha*, *B. decumbens* and *B. ruziziensis* (SOUZA SOBRINHO, 2005). Another interesting strategy has been artificial tetraploidization of *B. ruziziensis* to allow undertaking interspecific hybridizations with genotypes of *B. brizantha* and *B. decumbens* (ISHIGAKI et al., 2009) and thus exploit the genetic variability of the genus for selection of superior materials (PEREIRA et al., 2001; SOUZA SOBRINHO, 2005). Induction of polyploidy is performed using antimitotic substances and, in forages, there is the search to maximize expression of characteristics of agronomic interest, as well as to make crosses between plants with distinct ploidies possible (PEREIRA et al., 2012).

Among the basic studies necessary at all the stages of a breeding program, such as that of *Brachiaria*, the evaluations of aspects related to pollen fertility and viability are essential to ensure success in controlled hybridizations (VALLE et al., 2009). A pollen viability study is commonly used in plant breeding for diverse species due to its ease, fast and low financial cost and the reliability of the technique (CARDOSO et al., 2009; CORRÊA et al., 2005). It is also important to determine the fertility rates and the availability of pollen grains depending on the position of the anthers in the inflorescences; i.e., to check if the viability is consistent throughout the inflorescence. Viability data allow correlations to be made with genetic factors such as chromosome imbalances caused by meiotic abnormalities or a high percentage of normal tetrads as a consequence of meiotic stability (RISSO-PASCOTTO et al., 2006). These data may assist in selection of genotypes and in determination of the best regions of the raceme of *Brachiaria* for pollen collection, which will be reflected in the efficacy of the crosses.

The aims of this study were to evaluate the viability of pollen grains in artificially induced tetraploid accessions of *B. ruziziensis* progenies, as well as to verify possible effects of the position of the flower buds on the raceme in regard to the pollen viability rate.

Material and methods

The study was performed with the following treatments on 27 tetraploid *B. ruziziensis* progenies (2n = 4x = 36) derived from a population obtained from intercross 8 plants from the Embrapa Gado de Leite breeding program that were duplicated with colchicine. Plants of *B. decumbens* Stapf. cv. Basilisk (2n = 4x = 36), *B. brizantha* (Hochst. ex A. Rich.) Stapf. cv. Marandu (2n = 4x = 36) and *B. ruziziensis* Germain & Evrard. cv. Comum (2n = 2x = 18) were used as controls.

For mounting the slides, pollen grains were collected directly from the anthers of inflorescences which were stored in a becker with distilled water. Three slides/treatment were prepared by the squash technique and staining was performed with Alexander's stain (ALEXANDER, 1980). After 24 hours, counting of 1000 pollen grains/slide was carried out in an light microscope. Pollen grains were considered viable when they had a purple coloring and were free of deformities, and unviable when stained green. The percentage of viable pollen was obtained in accordance with the total number of pollen grains evaluated.

A second analysis considered collections from the basal, middle and apical regions of the raceme of five diploid *B. ruziziensis* clones (Identification: 1, 13, 17, 18 and 85). The methodology used was the same as described previously. Five slides per plant were prepared from each region of the raceme (basal, middle and apical), for a total of fifteen slides/treatment.

In both experiments, the percentage of viable pollen was obtained in accordance with the total number of pollen grains evaluated. A completely randomized statistical design was used, and comparison of the means were carried out by the Scott-Knott test ($p < 0.05$).

Results and discusssion

The result of analysis of variance detected significant differences for pollen grain viability among the plants derived from the tetraploid *B. ruziziensis* population and also in comparison with the control plants (Table 1). The mean grouping of Scott-Knott test ($p < 0.05\%$) divided the treatments into three groups. Group 1 was formed of *B. decumbens* and *B. brizantha* control plants which had low pollen viability, with a mean value of 57.8% (Table 1). Group 2, formed of 11 plants, had mean pollen viability of 82.2%, and group 3 gathered most of the plants, including diploid *B. ruziziensis*, with a mean value of 96.7% (Table 1).

Table 1. Mean pollen grain viability using the staining test with Alexander's stain in tetraploid *B. ruziziensis* (4 to 30) progenies and genotypes of diploid *B. ruziziensis* (3), *B. decumbens* (2) and *B. brizantha* (1) grouped according to the Scott-Knott test ($p < 0.05$).

Treatment	Mean pollen viability (%)	Treatment	Mean pollen viability (%)
2 (*B. decumbens*)	56.3 a	23	94.2 c
1 (*B. brizantha*)	59. 3 a	10	94.6 c
13	76.8 b	29	94.9 c
7	78. 3 b	14	96.2 c
6	79.7 b	21	96.5 c
5	80.6 b	20	97.0 c
30	81.0 b	19	97.6 c
8	81.7 b	17	97.7 c
25	84.0 b	24	98.1 c
26	84.2 b	9	98.2 c
28	84.5 b	16	98.4 c
27	86.2 b	15	98.4 c
22	87.0 b	11	98.6 c
12	91.2 c	3 (*B. ruziziensis*)	99.3 c
4	93.9 c	18	99.6 c
Groups		Mean values (%)	
Group 1		57.8	
Group 2		82.2	
Group 3		96.7	
Overall mean		88.8	

*Mean values followed by the same letter are not significantly different by the Scott-Knott test ($p < 0.05$).

The results show that the plants of the artificially induced tetraploid accessions of the *B. ruziziensis* population exhibited high pollen viability, with

mean values ranging from 76.8 to 99.6% (Table 1). Some of these plants had viability rates similar to diploid *B. ruziziensis* (treatment 3), showing that the induction of chromosome duplication using colchicine did not lead to abnormalities in the production or morphology of pollen grains.

The mean percentage of viability in the progenies under evaluation was greater than by Risso-Pascotto et al. (2005) in tetraploid *B. ruziziensis* that exhibited 38.6%. Considering that part of the success of crosses depends on donators of fertile pollen grains, the progenies evaluated in this study, based on this information, have potential for being incorporated in breeding programs, there being the need, however, for complementing with evaluations of agronomic performance.

Brachiaria decumbens and *B. brizantha* control plants exhibited mean values of 56.3 and 59.3%, respectively. This lower viability is probably because these species are polyploids and apomictic. Apomixis is normally associated with polyploidy which, in turn, often results in meiotic irregularities, leading to low pollen fertility (PEREIRA et al., 2001). Although these values are low when compared to the mean value of the other groups (b and c), they cannot yet be considered critical for assisted selection in breeding programs of a determined crop (ZANOTTO et al., 2009).

According to Valle and Savidan (1996), naturally diploid plants of *B. brizantha*, *B. decumbens* and *B. ruziziensis* exhibit regular meiosis, with the formation of nine bivalents and sexual mode of reproduction. In contrast, tetraploid plants show quite irregular meiosis, with frequent formation of univalents and quadrivalents, and apomixis as the reproductive mode. These data correspond with the fact of tetraploid *B. ruziziensis* exhibiting high pollen fertility as a probable consequence of meiotic stability.

In the microsporogenesis study on accessions of diploid and tetraploid *B. ruziziensis,* some meiotic irregularities were observed, such as formation of univalents to tetravalents and non-orientation of chromosomes on the metaphase plate. Nevertheless, the more frequent alterations in both accessions were related to the irregular segregation of chromosomes. These data were associated with low pollen viability (PAGLIARINI et al., 2008; RISSO-PASCOTTO et al., 2005).

The differences in viability among individuals of a single species, as occurred in *B. ruziziensis*, may be explained, according to Shivanna and Rangaswamy (1992), by the flowering period, the environmental changes and the genotype differences. Another aspect that also needs to be considered is that the viability of the pollen grain may vary considerably among individuals of a species and among samples of a single individual (TECHIO et al., 2006).

By means of the analysis of variance for pollen viability in different regions of the raceme, it was possible to observe no significant differences ($p < 0.05$) among the clones. Nevertheless, significant differences were detected for position of pollen grains on the raceme (basal, middle and apical regions) and significant interaction between clones and position, indicating that the location of the flower within the raceme has an effect on pollen viability and also that this influence is not consistent for all the clones.

On average, the genotypes showed high pollen grain viability, ranging from 93 to 98.9% (Table 2 and Figure 1).

Table 2. Mean pollen viability (%) for the basal, middle and apical regions of the raceme of diploid *B. ruziziensis* clones.

Clones	Mean pollen viability in the regions (%)		
	Basal	Middle	Apical
1	95.6 a	97.2 b	97.9 b
13	93.0 a	98.6 b	98.9 b
17	95.3 a	98.8 b	97.0 b
18	96.8 a	96.7 b	97.5 b
85	95.0 a	98.5 b	97.1 b

Region	(%)
Basal	95.1
Middle	97.9
Apical	97.7
Overall mean	96.9

*Mean values followed by the same letter in the column are not significantly different by the Scott-Knott test ($p < 0.05$).

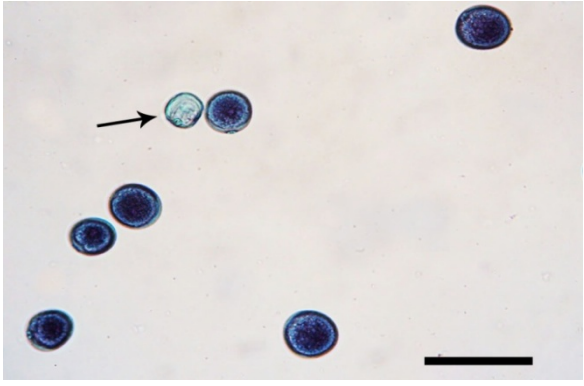

Figure 1. Diploid *B. ruziziensis* pollen grains stained with Alexander's stain. Pollen with purple coloring are considered viable, and with green coloring as unviable (arrow). The bar represents 50 μm.

This high viability rate is in agreement with that observed for tetraploid *B. ruziziensis* and also with the commercial cultivar Comum, used as a control (Table 1), which showed mean viability of 96.7%. From a practical point of view, this means that there are no restrictions in regard to the option for any of these clones as donators of pollen grains for the breeding program. This information is corroborated by Souza et al. (2002) upon affirming that pollen

viability is considered high for values above 70%, and these percentages would not cause losses in breeding studies on the species. Higher mean values in viability rate were observed in pollen grains in the middle and apical regions, with 97.9 and 97.7% respectively. Pollen viability was lower in the basal region with a mean value of 95.1%. Despite of this small variation, viability may be considered high since it was greater than 90%, regardless of the position of the flower bud on the raceme, i.e., the pollen can be collected from any of the regions of the raceme without a reduction in efficiency of the crosses. This variation may be attributed to the difference in the anthesis period over the raceme. Normally, the anthers of the middle region, followed by the apical region, are displayed before than the basal region. According to Zanotto et al. (2009), the viability of the pollen grain may vary significantly throughout its development; as time goes on, the viability of the pollen grain diminishes, reducing its efficiency in fertilization.

Considering the clone x position interaction, the mean value of pollen viability was greatest for clones 13 (98.6%), 17 (98.8%) and 85 (98.5%) in the middle region and for clone 13 (98.9%) in the apical region. On the other hand, the lowest mean value of viability was observed in clone 13 (93%) in the basal region.

To ensure success in the use of selected superior individuals, and especially production of new cultivars by means of recombination of traits by controlled hybridization, it is important that the pollen to be used has good viability. High meiotic instability, associated with genetic abnormalities and/or chromosome aberrations that result in the formation of atypical or male-sterile plants, or those unable of formation of pollen grains, may hinder achieving the minimum standards required for seed production as well as affecting pollination (POZZOBON et al., 2011). In the case of forage plants, there is the need for production of a large amount of viable seeds to meet the demand for extensive pasture areas. Thus, the results obtained here, by means of analysis of pollen grain viability, may be used to indicate more favorable future crosses that ensure adequate production of seeds within the *Brachiaria* breeding program.

Conclusion

The viability of pollen grains in artificially induced tetraploid accessions of *B. ruziziensis* plants was high, which may favor obtaining fertile descendants in possible crosses. Greater viability rates were observed in pollen grains collected from the middle and apical regions of the raceme of *B. ruziziensis*.

Acknowledgements

The authors thank the National Council of Scientific and Technological Development (*Conselho Nacional de Desenvolvimento Científico e Tecnológico* - CNPq) and the Research Support Foundation of the State of Minas Gerais (*Fundação de Amparo à Pesquisa do Estado de Minas Gerais* - Fapemig) for financial support and the Coordination for the Improvement of Higher Education Personnel (*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* - Capes) for granting a scholarship to the first author.

References

ALEXANDER, M. P. A versatile stain for pollen from fungi, yeast and bacteria. **Stain Technology**, v. 55, n. 1, p. 13-18, 1980.

CARDOSO, R. D. L.; GRANDO, M. F.; MS-BASSO, S.; SEGEREN, M. I.; AUGUSTIN L.; SUZIN M. Caracterização citogenética, viabilidade de pólen e hibridação artificial em gérbera. **Horticultura Brasileira**, v. 27, n. 1, p. 40-44, 2009.

CORRÊA, M. G. S.; VIÉGAS, J.; SILVA, J. B.; ÁVILA, P. F. V.; BUSATO, G. R.; LEMES, J. S. Meiose e viabilidade polínica na família *Araceae*. **Acta Botânica Brasilica**, v. 19, n. 2, p. 295-303, 2005.

ISHIGAKI, G.; GONDO, T.; SUENAGA, K.; AKASHI, R. Induction of tetraploid ruzigrass (*Brachiaria ruziziensis*) plants by colchicines treatment of *in vitro* multiple-shoot clumps and seedlings. **Japanese Society of Grassland Science**, v. 55, n. 3, p. 164-170, 2009.

MACEDO, M. C. M. Aspectos edáficos relacionados com a produção de *Brachiaria brizantha* cultivar Marandu. In: BARBOSA, R. A. (Ed.). **Morte de pastos de braquiárias**. Campo Grande: Embrapa Gado de Corte, 2006. p. 35-65.

PAGLIARINI, M. S.; RISSO-PASCOTTO, C.; SOUZA-KANESHIMA, A. M.; VALLE, C. B. Analysis of meiotic behavior in selecting potential genitors among diploid and artificially induced tetraploid accessions of *Brachiaria ruziziensis* (Poaceae). **Euphytica**, v. 164, n. 1, p. 181-187, 2008.

PEREIRA, A. V.; VALLE, C. B.; FERREIRA, R. P.; MILES, J. W. Melhoramento de forrageiras tropicais. In: NASS, L. L.; VALOIS, A. C. C.; MELO, I. S.; VALADARES-INGLIS, M. C. (Ed.). **Recursos genéticos e melhoramento de plantas**. Cuiabá: Fundação Mato Grosso, 2001. p. 549-602.

PEREIRA, R. C.; DAVIDE, L. C.; TECHIO, V. H.; TIMBÓ, A. L. O. Duplicação cromossômica de gramíneas forrageiras: uma alternativa para programas de melhoramento genético. **Ciência Rural**, v. 42, n. 7, p. 1278-1285, 2012.

POZZOBON, M. T.; SOUZA, K. R. R.; CARVALHO, S. I. C.; REIFSCHNEIDER, F. J. B. Meiose e viabilidade polínica em linhagens avançadas de pimenta. **Horticultura Brasileira**, v. 29, n. 2, p. 212-216, 2011.

RENVOIZE, S. A.; CLAYTON, W. D.; KABUYE, C. H. S. Morphology, taxonomy and natural distribution of *Brachiaria* (Trin.) Griseb. In: MILES, J. W.; MAASS, B. L.; VALLE, C. B. (Org.). ***Brachiaria***: Biology, agronomy, and improvement. Cali: CIAT/Campo Grande: Embrapa-Cnpgc, 1996. p. 1-15.

RISSO-PASCOTTO, C.; PAGLIARINI, M. S.; VALLE, C. B. Multiple spindles and cellularization during microsporogenesis in an artificially induced tetraploid accession of *Brachiaria ruziziensis* (Gramineae). **Plant Cell Reports**, v. 23, n. 8, p. 522-527, 2005.

RISSO-PASCOTTO, C.; PAGLIARINI, M. S.; VALLE, C. B. Microsporogenesis in *Brachiaria dictyoneura* (Fig. & De Not.) Stapf (Poaceae: Paniceae). **Genetics and Molecular Research**, v. 4, n. 5, p. 837-845, 2006.

SHIVANNA, K. R.; RANGASWAMY, N. S. **Pollen biology**. A laboratory manual. Berlin: Springer-Verlag, 1992.

SOUZA SOBRINHO, F. Melhoramento de forrageiras no Brasil. In: EVANGELISTA, A. R. (Ed.). **Forragicultura e pastagens**: Temas em evidência. Lavras: Editora UFLA, 2005. p. 65-120.

SOUZA, M. M.; PEREIRA, T. N. S.; MARTINS, E. R. Microsporogênese e microgametogênese associadas ao tamanho do botão floral e da antera, e viabilidade polínica em maracujá amarelo (*Passiflora edulis* f. *flavicarpa* Degener). **Ciência e Agrotecnologia**, v. 26, n. 6, p. 1209-1217, 2002.

TECHIO, V. H.; DAVIDE, L. C.; PEDROZO, C. A.; PEREIRA, A. V. Viabilidade dos grãos de pólen de acessos de capim-elefante, milheto e híbridos interespecíficos (capim-elefante x milheto). **Acta Scientiarum. Biological Sciences**, v. 28, n. 1, p. 7-12, 2006.

VALLE, C. B.; SAVIDAN, Y. Genetics, cytogenetics and reproductive biology of *Brachiaria*. In: MILES, J. W.; MAASS, B. L.; VALLE, C. B. (Ed.). ***Brachiaria***: biology, agronomy, and improvement. Cali: CIAT/Campo Grande: Embrapa-CNPGC, 1996. p. 147-163.

VALLE, C. B.; JANK, L.; RESENDE, R. M. S. O melhoramento de forrageiras tropicais no Brasil. **Revista Ceres**, v. 56, n. 4, p. 460-472, 2009.

ZANOTTO, M.; BRAMMER, S. P.; JUNIOR, A. N.; SCAGLIUSI, S. M. Viabilidade polínica como seleção assistida no programa de melhoramento genético de Triticale. **Ciência e Agrotecnologia**, v. 33, ed. esp., p. 2078-2082, 2009.

Received on December 13, 2012.
Accepted on August 28, 2013.