POLLEN VIABILITY OF *Eragrostis plana* GENOTYPES FROM DIFFERENT GEOGRAPHIC POPULATIONS IN RIO GRANDE DO SUL

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ABSTRACT

Livestock production in southern Brazil is largely sustained by the production of natural pastures, whose distinct botanical compositions, adapted to different climatic and soil nuances, have allowed combining animal production and environmental preservation over time. Among the main problems related to vegetation management, is the disturbing expansion of the exotic grass *Eragrostis plana* Ness., which deserves to be highlighted due to its high capacity for colonization of the grasslands and the trend of excluding the native plant community. Despite the importance of this species for the region, information on its biology are insufficient, which impedes the establishment of efficient mechanisms for invasion control. In this paper, the estimation of pollen viability of *E. plana* was studied by staining, using acetic orcein 2% and Alexander reactive. Three different populations were used, evaluating 6 genotypes per population. The young inflorescences were collected and fixed in ethanol: acetic acid (3:1) for 24 hours room temperature, then stored in 70% ethanol under refrigeration. To evaluate pollen viability, then 300 pollen grains of each blade were analyzed. All studied genotypes showed high pollen viability for both stains, and the Alexander reactive proved to be more appropriate to estimate the pollen viability of *E. plana*.

KEYWORDS: Acetic orcein 2%, Alexander reactive, pasture invaders, pollen viability, reproductive characteristics.

VIABILIDADE POLÍNICA DE GENÓTIPOS DE *Eragrostis plana* DE DIFERENTES POPULAÇÕES GEOGRÁFICAS DO RIO GRANDE DO SUL

RESUMO

A produção pecuária na Região Sul é sustentada em grande parte pela produção das pastagens naturais, cujas distintas composições botânicas, adaptadas

às diferentes nuances climáticas e edáficas têm permitido aliar a produção animal e a preservação do ambiente ao longo do tempo. Entre os principais problemas relacionados ao manejo da vegetação, merece destaque a expansão preocupante da gramínea exótica *Eragrostis plana* Ness., devido à elevada capacidade de colonização dos campos naturais e à tendência de exclusão da comunidade vegetal nativa. Apesar da importância dessa espécie para a região, as informações sobre sua biologia são insuficientes, o que dificulta o estabelecimento de mecanismos eficientes de controle da invasão. No presente trabalho, estudou-se a estimativa da viabilidade polínica do capim-annoni (*E. plana*) através da metodologia de coloração, utilizando os corantes orceína acética 2% e o reativo de Alexander. Utilizou-se 3 populações diferentes, sendo avaliados 6 genótipos por população. As inflorescências jovens foram coletadas e fixadas em solução de etanol: ácido acético (3:1) por 24 horas a temperatura ambiente, em seguida armazenadas em etanol 70% sob refrigeração. Para avaliação da estimativa da viabilidade polínica foram preparadas lâminas pela técnica do esmagamento, sendo analisados 300 grãos de pólen de cada uma das lâminas, totalizando 600 grãos de pólen por genótipo para cada corante. A análise das lâminas foi realizada utilizando microscópio ótico com aumento de 40x, os dados foram analisados quanto à sua normalidade e submetidos à análise de variância e as médias foram comparadas pelo teste de Tukey a 5% ($p \leq 0,05$) com o auxílio do programa Assistat versão beta 7.5. Todos os genótipos estudados apresentaram estimativa da viabilidade polínica alta com ambos os corantes, sendo que o reativo de Alexander demonstrou ser o mais indicado para estimar a viabilidade polínica de capim-annoni.

PALAVRAS-CHAVE: Características reprodutivas, invasora de pastagens, viabilidade do pólen, orceína acética 2%, reativo de Alexander

INTRODUCTION

Eragrostis plana Ness. is a perennial, estival grass, native to Africa, introduced to Rio Grande do Sul (RS) in the 1950s (Reis, 1993). During this period the native fields of RS, especially in the middle plateau region, were dominated by the grassy *Aristida* spp., which reduced the nutritional value of the pastures. In the 1970s farmers from this region began using *E. plana*, capable of competing and overcoming *Aristida* spp.; thus it was multiplied and commercialized, also in other states, such as Santa Catarina and Parana (Kissmann, 1997). Promoted by the Grupo Rural Annoni until the late 1970s, the species became popularly known in Brazil as ‘capim-annoni’, only later becoming regarded as a weed.

The invasive characteristic of the plant is due to large production of seeds with high physiological quality (Silveira and Medeiros, 2006), to easy establishment, to high capacity for colonization of the native grasslands and road networks, to allelopathic activity, and the tendency to exclude the natural plant community. Different studies have demonstrated that the *E. plana* does not present more nutritional benefits for livestock production in relation to native plants, showing high fiber content and low levels of protein (Figueiro, 1976; Nascimento and Hall, 1978; Reis and Coelho, 2000; Medeiros et al., 2006).

E. plana expansion is troubling since control after its establishment is difficult, requiring the use of herbicides, mowing, and burning, which generates environmental and social damage, and because the weeds are now the second largest global threat to biodiversity, following habitat destruction by human exploitation (Ziller, 2001),

studies that contribute to the practical control of *E. plana* are needed. The estimation of pollen viability is an important parameter in the analysis of gene flow in plant studies, highlighting the male reproductive potentiality of the species and also contributing to taxonomic, ecological, genetic and palynological studies (Alexander, 1980). According to Alexander (1980), using tests with Alexander reactive stain, it is possible to differentiate the aborted pollen grains from those non-aborted. Aborted pollen grains do not have a nucleus, thus staining only the cellulose in the wall. However, studies with 2% acetic orcein stain may overestimate the viability of pollen grains (Biondo and Battistin, 2001; Auler et al., 2006).

Paula (2009) studied pollen viability of *Conyza bonariensis* L. Cronquist using acetic orcein 2% stain, acetic carmine 1%, blue amman and Alexander reactive, with the results indicating that Alexander reactive was the most appropriate for estimating pollen viability, due to its capability to stain viable and unviable pollen with different colors. Meanwhile, other stains may overestimate the viability of pollen grains, because they stain viable and unviable pollen with the same color, distinguishing them only by shades of red.

The aim of this paper was to evaluate the viability of pollen grains of *E. plana* genotypes by using two staining methods, providing data to support genetic, autoecology, ecophysiology and control studies of *E. plana*.

MATERIALS AND METHODS

The approaches used in this study were previously analyzed in regards to genetic diversity within the framework of the network project “Strategies for the Containment of the Invasion of Southern Fields by *E. plana”*, coordinated by Empresa Brasileira de Pesquisa Agropecuária (Embrapa), Pecuaria Sul. The evaluated plant material originated from *E. plana* seeds collected in 3 different regions in Rio Grande do Sul, in the municipalities of Bage, Mostardas, and Tupancireta. These seeds represented the study’s three populations, B, M, and T, respectively. Once germinated, the plants were kept in a greenhouse in pots with a capacity for 3 kg of soil.

For the study of pollen viability, carried out at the Laboratory of Cytogenetics and Genotoxicity of the Department of Biology, Center for Natural and Exact Sciences (CCNE) of the Universidade Federal de Santa Maria (UFSM), six individuals of each of the 3 populations were evaluated, analyzing a total of 18 genotypes. For the staining of pollen grains, two distinct stains were used: acetic orcein 2% and Alexander reactive (malachite green + acid fuchsin).

At the beginning of flowering, plant inflorescences grown in a greenhouse were collected and fixed in ethanol: acetic acid (3:1) over a period of 24 hours at room temperature. They were then kept in 70% ethanol and refrigerated until slide preparation. The slides were prepared using the squashing technique (Guerra and Souza, 2002).

In the case of acetic orcein 2%, the analysis was performed immediately, considering viable pollen grains those with dark-red tonality and unviable the ones with light-red tonality, indicating that the unviable ones did not show a protoplasm. On the other hand, with the Alexander reactive, the material was covered with a coverslip, sealed with glue, refrigerated and, only after 24 hours the slides analysis was performed. During analysis, viable pollen grains were those with a purplish color and unviable those greenish ones, because the distinguishing coloring of Alexander reactive stains purple pollen grains with protoplasm (viable) and the ones without a

protoplasm only have the cell wall stained.

Four slides per plant were evaluated, two for each stain, with a light microscope at 400x. To obtain a random sampling of viable and unviable pollen grains, the scanning method was used until reaching the total number of 300 pollen grains per slide. After counting, calculations were made to obtain the percentage staining of pollen grains.

The statistical analysis was performed with the aid of the program Assistat® beta 7.5. The data was analyzed for their normality and the averages were compared using the Tukey test at 5% ($p \leq 0.05$).

RESULTS AND DISCUSSION

Table 1 shows the results of pollen viability of the 18 genotypes of the 3 populations evaluated with acetic orcein 2%, while Table 2 shows the results using Alexander reactive.

Table 1 – Result of the pollen viability with acetic orcein 2%.

Genotypes	Viables pollens	Unviable Pollens	Mean genotype (%)	Mean population (%)
B4	600	0	100[a]	
B9	600	0	100[a]	
B10	600	0	100[a]	
B24	600	0	100[a]	100[a]
B28	600	0	100[a]	
B34	600	0	100[a]	
M3	600	0	100[a]	
M5	600	0	100[a]	
M11	600	0	100[a]	
M12	600	0	100[a]	100[a]
M18	600	0	100[a]	
M30	600	0	100[a]	
T2	600	0	100[a]	
T4	600	0	100[a]	
T5	600	0	100[a]	100[a]
T14	600	0	100[a]	
T16	600	0	100[a]	
T17	600	0	100[a]	

[a]Values with different superscripts are statistically different according to Tukey test ($P<0.05$).

Table 2 – Result of the pollen viability with Alexander reactive dye.

Genotypes	Viables pollens	Unviable Pollens	Mean genotype (%)	Mean population (%)
B4	579	21	96,5[a]	
B9	548	52	91,33[ab]	
B10	525	75	87,5[ab]	
B24	473	127	78,83[ab]	90,22[a]
B28	584	16	97,33[a]	
B34	539	61	89,83[ab]	
M3	465	135	77,5[ab]	
M5	510	90	85[ab]	
M11	509	91	84,83[ab]	
M12	417	183	69,5[b]	84,33[a]
M18	592	8	98,66[a]	
M30	543	57	90,5[ab]	
T2	577	23	96,16[a]	
T4	528	72	88[ab]	
T5	575	18	95,83[a]	
T14	538	62	89,66[ab]	92,88[a]
T16	567	33	94,5[a]	
T17	559	41	93,16[a]	

[a]Values with different superscripts are statistically different according to the Tukey test (P<0.05).

The comparison of pollen viability among the populations showed no difference between the populations studied (B, M and T) in any of the stains used (Tables 1 and 2).

Between the stains used, it was possible to verify that the acetic orcein 2% showed the highest pollen viability (100%) when compared to the Alexander reactive (89.14%). Between these two stains, a significant difference occurred in estimating pollen grain viability (Table 3).

Table 3 – Result of the comparison of viability estimate between acetic orcein 2% and Alexander reactive.

Stains	Viable pollen grains	Unviable pollens grains	Pollen grains observed	Mean Satin (%)
Acetic orcein 2%	10.800	0	10.800	100[a]
Alexander reactive	9.628	1.172	10.800	89,14[b]

[a]Values with different superscripts are statistically different according to the Tukey test(P<0.05).

There was no significant difference in pollen grain viability among the genotypes studied with acetic orcein 2% (Table 1), however, with the Alexander reactive stain, there was a difference in pollen viability among the genotypes (Table 2).

This study showed that between the two stains used, acetic orcein 2% did not

have the discriminatory power to resolve the pollen grain viability of the genotypes studied (Table 1). This stain resulted in greater pollen viability, totaling in 100% of pollen grains stained (Fig1A and 1B), being that this high viability may be due to the difficulty in distinguishing between shades of colors to sort viable and unviable pollen grains, thus overestimating pollen grain viability. Of the two stains used to estimate pollen viability of *E. plana*, we found Alexander reactive stain to be most suitable from the two used because it was possible to distinguish viability by differential staining of viable and unviable pollen grains (Fig 1C, 1D, 1E and 1F).

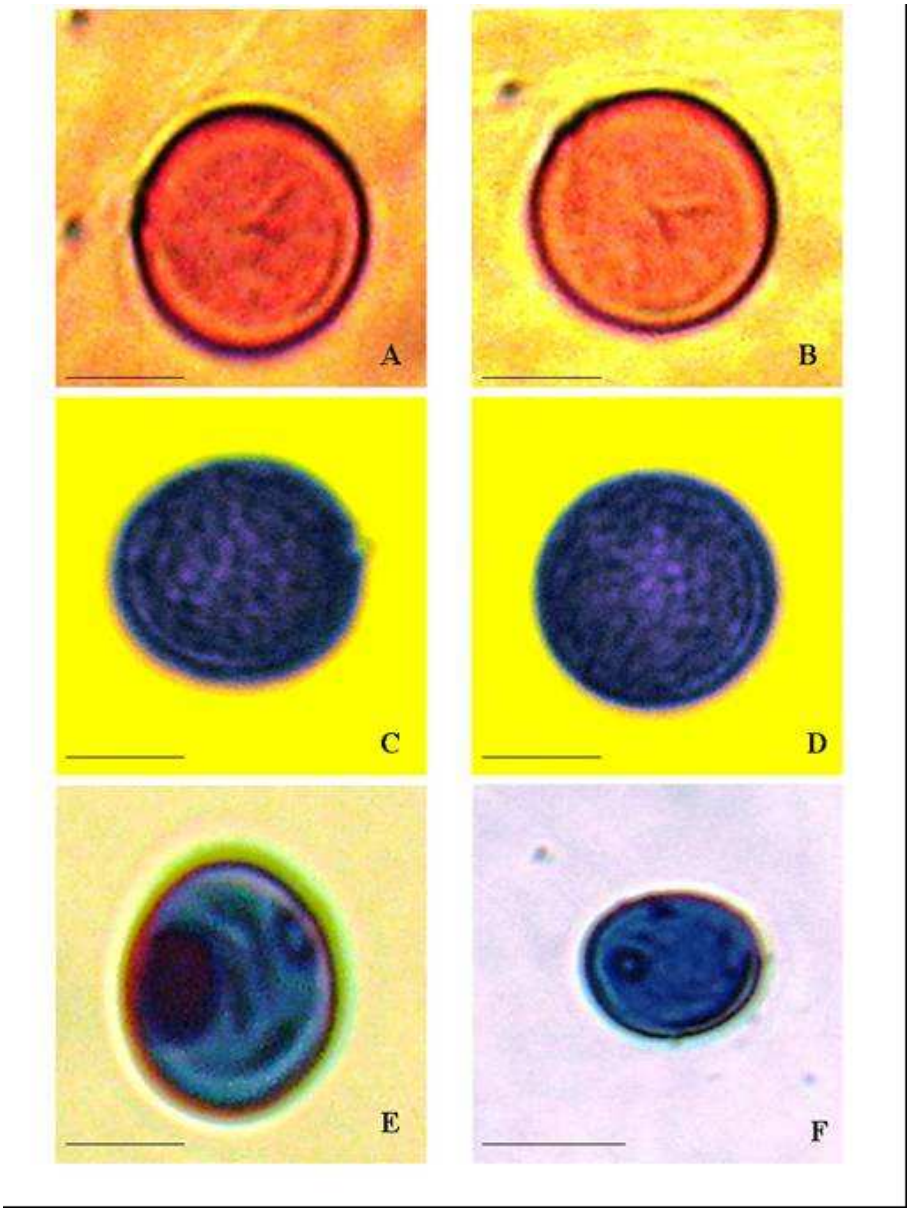

Figure 1: *Eragrostis plana* pollen grains. A) Viable pollen of the genotype B4 stained with acetic orcein; B) Viable pollen of the genotype M30 stained with acetic orcein 2%; C) Viable pollen of the genotype B28 stained with Alexander reactive; D) Pollen of the genotype T2 stained with Alexander reactive; E) Unviable pollen of the genotype B24 stained with Alexander reactive; F) Unviable pollen of the genotype M12 stained with Alexander reactive. Scale= 5 µm.

According to Alexander (1980), the analysis of pollen viability using the Alexander reactive stains provides more accurate data, because there is a color difference among the viable and unviable pollens, and this occurs due to the simultaneous use of malachite green and acid fuchsin, which show reverse coloring. The cell protoplasm of the pollen chemically reacts with the acid fuchsin giving a purple color (Fig 1C and 1D). The unviable pollen (aborted) shows the cell wall, blue-green and the protoplasm, poorly distributed, slightly stained and often absent (Fig 1E and 1F).

Working with *Pennisetum purpureum* Schumach., Techio (2006) also considered the Alexander reactive superior in evaluating pollen viability. The author found that although the stains acetic orcein 1% and propionic carmine 2% distinguish between viable and unviable pollens of the studied accessions, the Alexander reactive (malachite green + acid fuchsin) showed more accurate data due to its differential staining.

In the present study, the Alexander reactive showed differences in estimating of pollen viability in some of the genotypes studied. The genotype M12 differed from the genotypes B4, B24, M18, T2, T5, T16 and T18 (Table 2), and this characteristic may be related to the genetic variability within and among populations, since the collection of pollen was obtained from plants grown under controlled conditions. Souza et al. (2002), Meletti et al. (2003) and Corrêa et al. (2005) report that pollen viability can vary considerably among individuals of a single species and among different samples of an individual species.

Still, it was possible to observe that there was no consistent difference among the populations studied, both with acetic orcein 2% and the Alexander reactive (Tables 1 and 2), indicating low variability in the genotypes studied.

Among the genotypes studied, the pollen viability was considered high, above 70%, according to Souza et al. (2002). With the results obtained, it was evident that the *E. plana* has high pollen viability, because in all genotypes stained with acetic orcein 2% a viability of 100% was observed and with the Alexander reactive the average was of 89.14% (Table 3).

Pollen viability was high for *Crotalaria spectabilis*, *C*. *zanzibarica* and *C*. *micans,* above 98%, with the different stains, including Alexander reactive stain (Ferreira et al, 2009). According to Twell (1995), pollen can become nonviable during microgametogenesis, where errors in meiotic behavior result in gametes with unbalanced, or anucleate, chromosomes or in pollen grains with a retracted cytoplasm.

CONCLUSION

From the results obtained, it can be concluded that *E. plana* has high pollen viability and no consistent difference among the populations studied, and Alexander reactive was the stain that showed best discriminatory ability for estimating the viability of *E. plana* pollen grains.

The difference in pollen viability among genotypes revealed the occurrence of genetic variability within and between populations.

The high pollen viability of *E. plana* is possible for adaptability of species to environmental conditions in southern Brazil.

These results should be considered when determining strategies for controlling this weed species.

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