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TILLER PROPAGATION OF CERRADO GRAMINOIDS: NEW POSSIBILITIES FOR SEEDLING PRODUCTION (SCIENTIFIC NOTE)[1]

PROPAGAÇÃO DE PERFILHOS DE GRAMINÓIDES DO CERRADO: NOVAS POSSIBILIDADES PARA PRODUÇÃO DE MUDAS (NOTA CIENTÍFICA)

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ABSTRACT - The lack of knowledge and ecological challenges in the reproduction of Cerrado graminoids has prevent the commercial production of native seedling, complicating efforts to restore the Cerrado. This study aimed to assess the survival of vegetative tillers from clonal propagation of five native caespitose graminoid species from the Cerrado. We collected ten adult plants (matrices) per species in of a cerrado sensu stricto area, located at São Carlos – SP, to be used as source of clonal units (tillers). A total of 68 tillers per specie were separated and transplanted into containers, with survival monitored under nursery conditions for 60 days. *Axonopus pellitus*, *Bulbostylis hirtella* and *Aristida jubata* showed survival rates close to 70%, while *Aristida setifolia* and *Andropogon bicornis* had survival rates below 12%. These results suggest that tiller transplanting could be a viable method for commercial production of certain Cerrado graminoids, contributing to the of this biome.

Keywords: Grassy biomes; Seedling production; Vegetative propagation; Active restoration; Savanna restoration.

RESUMO - A falta de conhecimento e os desafios ecológicos na reprodução de graminóides do Cerrado têm impedido a produção comercial de mudas, dificultando os esforços para restaurar o Cerrado. Este estudo teve como objetivo avaliar a sobrevivência de perfilhos vegetativos provenientes da propagação clonal de cinco espécies nativas de graminóides cespitosos do Cerrado. Coletamos dez plantas adultas (matrizes) por espécie, em uma área de cerrado sensu stricto, em São Carlos – SP, para serem utilizadas como fonte de unidades clonais (perfilhos). Um total de 68 perfilhos por espécie foram separados e transplantados para tubetes, com a sobrevivência dos perfilhos sendo monitorada em condições de viveiro por 60 dias. *Axonopus pellitus*, *Bulbostylis hirtella* e *Aristida jubata* apresentaram taxas de sobrevivência próximas a 70%, enquanto *Aristida setifolia* e *Andropogon bicornis* tiveram taxas de sobrevivência abaixo de 12%. Esses resultados sugerem que o transplante de perfilhos pode ser um método viável para a produção comercial de parte das espécies graminóides do Cerrado, contribuindo para restauração desse bioma.

Palavras-chave: Biomas gramíneos; Produção de mudas; Propagação vegetativa; Restauração ativa; Restauração de savana.

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1 INTRODUCTION

Early Cerrado restoration focused on planting woody species, often neglecting the herbaceous layer vital for biodiversity and open physiognomies (Pilon et al. 2018a; Buisson et al. 2020). Recent methods to restore this layer include topsoil transfer and direct sowing of native seeds, but these approaches face practical limitations (Pilon et al. 2018a, 2018b; Buisson et al. 2020).

Seedling planting, a globally used method (Leverkus et al. 2021), allows reintroduction of various lifeforms and better control over species diversity and distribution. It enhances survival and establishment rates compared to direct sowing, especially for species with low germination rates or high seed predation (Palma and Laurance 2015; Leverkus et al. 2021).

Seedling production and transplant from seedbeds are suggested for reintroducing native grasses (Oliveira et al. 2020). However, studies show low germination rates for Cerrado grasses (Dairel and Fidelis 2020; Fontenele et al. 2020; Gorgone-Barbosa et al. 2020; Oliveira et al. 2020), but vegetative propagation (cloning) is proposed as an effective method for many Cerrado herbs, including grasses, by producing new tillers or stolons from adult plants (Zaidan and Carreira 2008).

Graminoids are among the largest groups of clonal plants in terrestrial angiosperms (Briske and Derner 1998). Plant propagation, including tiller transplanting, addresses challenges in non-forest ecosystems restoration, particularly in areas with difficult natural regeneration (Buisson et al. 2020). Tiller transplanting has shown success in coastal dunes (Hobbs et al. 1983), wet grasslands (Forbes 1993), and dry grasslands (du Toit 2009), indicating its potential for restoring Cerrado physiognomies. Although well-established in forestry and ornamental plant production (Franzon et al. 2010), tiller transplanting is novel for Cerrado graminoids. It offers a solution for species with low-quality seeds or poor initial survival by reintroducing mature individuals, enhancing survival and reducing invasive species competition (Huddleston and Young 2004).

This study aimed to evaluate the survival of vegetative tillers from clonal propagation of five native caespitose graminoid species – four Poaceae and one Cyperaceae. The goal was to determine if graminoids could be a viable option to produce seedlings to restore the Cerrado herbaceous layer.

2. MATERIAL AND METHODS

2.1. Plant collection

The study was conducted in a Cerrado remnant at the Federal University of São Carlos (UFSCar), São Carlos, Brazil (21º58' - 22º00'S; 47º51' - 47º52'W). This 95 ha area of *cerrado sensu stricto* physiognomy, with a Köppen Cwa subtropical humid climate (Alvares et al. 2013), features an average annual rainfall of 1362 mm, temperature of 21.5ºC, and sandy Oxisols. In July 2019, during dry season, we collected adult individuals of four native Poaceae species — *Andropogon bicornis* L., *Aristida jubata* (Arechav.) Herter, *Aristida setifolia* Kunth and *Axonopus pellitus* (Nees ex Trin.) Hitchc. & Chase — and one Cyperaceae species, *Bulbostylis hirtella* (Schrad.) Urb. All tussocks had green leaves at the time of collection.

The collected species are common caespitose graminoids found in neotropical non-forest ecosystems. Characterized by compact tiller clusters and lacking rhizomes or stolons, they dominate grasslands and savannas (Briske and Derner 1998). These graminoids produce numerous tillers within tussocks (Kraehmer 2019), each with the potential to become a new individual, making them ideal for clonal seedling production through tiller transplanting.

The tillers were collected from ten tussocks of each evaluated species (matrices) manually harvested from various sites and populations within the source area. Approximately 40 to 50% of each tussock, including its root system, was removed using a sharp shovel to a depth of about 20 cm. This partial removal allowed the remaining portion to regrow and minimized damage to the vegetation.

The matrices were pruned to prevent dehydration and to take then transported to the laboratory, where vegetative tillers were divided into clonal units. For each species, 68 tillers (10-15 cm in length with 5 cm roots) were placed in soil-filled containers and watered to prevent root damage and stress.

2.2. Experimental design

The experimental design consisted of four randomized blocks, each containing 17 tillers – referred to as individuals – of each graminoid species (*A. bicornis*, *A. jubata*, *A. setifolia*, *A. pellitus* and *B. hirtella*), totaling 68 individuals per species.

One tiller per tube was planted the day after field removal in 80 ml biodegradable cellulose fiber container tubes (Ellepot). Each tube was filled with commercial substrate (40% of sphagnum peat moss + 20% vermiculite + 40% carbonized rice husk) commonly used for seedling production.

Tillers were planted with roots below the substrate using a toothpick. The tubes were kept in a greenhouse with controlled irrigation (three times a day), with soil moisture was monitored and weeds removed daily. For 60 days after planting, individuals were checked daily for survival. Those completely dry and decomposing were considered dead, though initially dead individuals were kept confirming mortality.

2.3. Data analysis

The survival rates were calculated by the relation between the number of survivals and the total number of transplanted individuals. A binomial generalized linear mixed model (GLMM) with a logit link function was used to analyze species survival rates (R Core Team 2024). The model was fitted using the “glmer” function from the “lme4” package in R (Bates et al. 2015).

3. RESULTS AND DISCUSSION

The GLMM analysis showed significant differences in survival rates among the species. The reference species (*A. bicornis*) had a significant negative effect on survival (Table 1).

Compared to *A. bicornis*, *A. jubata* had a significantly higher survival rate. *A. setifolia* did not differ significantly from *A. bicornis*. Both *A. pellitus* and *B. hirtella* had significantly higher survival rates than *A. bicornis*. Thus, *A. jubata*, *A. pellitus*, and *B. hirtella* have notably higher survival, while *A. setifolia* does not significantly differ from *A. bicornis*. The highest mortality (%) for all species occurred in the intervals of 30 to 45 days (Figure 1).

The similar results for *A. jubata*, *B. hirtella* and *A. pellitus* may be attributed to their similar life form: they form vigorous tussocks, produce substantial biomass year-round and have comparable root systems (Wanderley et al. 2001). *A. pellitus* had high survival rates with tiller transplanting, despite low germination and emergence in seed sowing experiments, where it did not establish ground cover even after two rainy seasons (Pellizzaro et al. 2017).

The survival curves for *A. setifolia* and *A. bicornis* remained unstable and had lower rates compared to the other species (Figure 1). Many individuals, though senescent, did not fully die, suggesting higher mortality rates if evaluation had continued. These low survival rates may be due to harvesting during the dry season, a period of reduced growth and dormancy in neotropical species, especially in seasonal ecosystems like the Cerrado (Bosio et al. 2016).

Studies on graminoid survival via tiller transplantation show variable mortality rates by species. For instance, Sena et al. (2021) reported survival rates of 66-70% for *Schizachyrium tenerum*, while du Toit (2009) found rates of 39-92% in South Africa, with *Aristida junciformis* Trin. & Rupr. having the lowest survival. Similarly, Hobbs et al. (1983) observed 30-40% mortality for grass rhizomes in Scotland.

Table 1. Results of GLMM analysis for survival rates.

Tabela 1. Resultado da análise do modelo GLMM.

Variable	Estimate	Standard Error	z-value	Pr(>|z|)
(Intercept)	-4.971	0.3582	-13.877	< 2e-16*
Aristida jubata	1.8056	0.3846	4.694	0.00000268*
Aristida setifolia	-0.4727	0.5714	-0.827	0.408
Axonopus pellitus	1.6117	0.3903	4.13	0.0000363*
Bulbostylis hirtella	1.7603	0.3859	4.562	0.00000507*

*Significant at 0.001 level; Pr(>Z): p-value of the z-test.

*Significativo ao nível 0,001; Pr(>Z): valor p do teste z.

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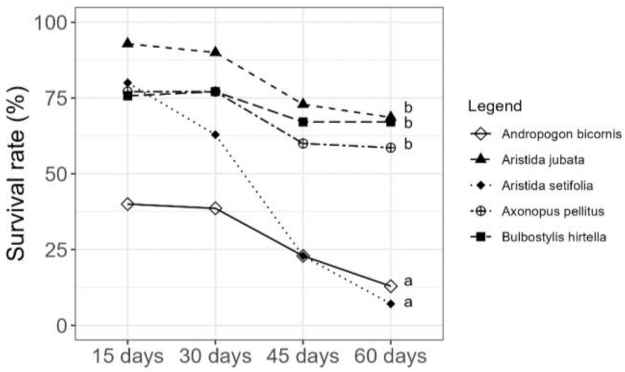

Figure 1. Survival rate (%) of evaluated graminoid species calculated every 15 days post-transplanting. At 60 days, rates with the same letter do not differ significantly (Z-test, p≤0.001). Author: Boschi, R.S.

Figura 1. Taxa de sobrevivência (%) das espécies de graminóides avaliadas, calculada a cada 15 dias após o transplante. Aos 60 dias, as taxas com a mesma letra não diferiram estatisticamente (teste-Z, p≤0.001). Autora: Boschi, R.S.

Tiller transplanting for species like *A. jubata*, *B. hirtella*, and *A. pellitus* is promising, but survival rates may decline in the stressful Cerrado environment with acidic, nutrient-poor soils and a dry season (Haridasan 2008). Despite this, field studies indicate potential (Sena et al. 2021). For effective large-scale clonal production, identifying high-survival species, optimal conditions, and key plant characteristics is crucial.

Maintaining genetic diversity when selecting matrices is essential for adapting to changing conditions (Van Groenendael et al. 1996; Young et al. 1996). Ensuring source area recovery and avoiding overexploitation are also important (Pilon et al. 2018b). Tiller transplantation can be as cost-effective as seedling production, making it viable for restoration projects lacking non-tree species (Kolb et al. 2016; Pilon 2016; Oliveira et al. 2020; Oliveira et al. 2021; Sena et al. 2021). Future research should focus on long-term studies and standardized growth parameters (Wigley et al. 2020) to assess feasibility.

4 CONCLUSIONS

Tiller transplanting can help increase graminoids seedlings availability in nurseries. This study highlights the potential of clonal technology for producing caespitose graminoids through tiller transplanting, despite lower survival rates for *Andropogon bicornis* L. and *Aristida setifolia* Kunth and challenges with dry season tiller collection. This technique could be scaled commercially, enhancing growth forms and restoration methods for the herbaceous layer in Neotropical grasslands and savannas, fostering more complex and diverse plant communities.

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6 AUTHORS' CONTRIBUTION

Lucas Dias Sanglade, Gabriela Strozzi, and Raquel Stucchi Boschi conceived and designed the research; L.D. Sanglade undertook the experiment; G. Strozzi and R.S. Boschi analyzed the data; L.D. Sanglade, G. Strozzi, R.S. Boschi, Dalva Maria da Silva Matos and Vânia Regina Pivello wrote and edited the manuscript; D.M.S. Matos and V.R. Pivello revised and contributed to the previous versions of this manuscript.

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