Rodriguésia 73: e00942021. 2022
http://rodriguesia.jbrj.gov.br
DOI: http://dx.doi.org/10.1590/2175-7860202273088

Original Paper

Strategies for reintroduction and conservation of *Gymnopogon doellii*, an endemic grass at risk of extinction

Carlos Romero Martins[1], *Fabian Borghetti*[2,7], *Márcio de Carvalho Moretzsohn*[3,4], *Sérgio Eustáquio de Noronha*[3,5] & *José Francisco Montenegro Valls*[3,6]

Abstract
The Brazilian savanna, regionally known as Cerrado, is characterized by a great diversity of physiognomies and holds the highest species diversity and level of endemism among world savannas. However, due mainly to agribusiness, this vegetation is among the most threatened ones, currently presenting alarming rates of extinction. Among the species present in the "red list" we find the endemic *Gymnopogon doellii*, a grass whose distribution is restricted to a few sites. In the Federal District and surroundings, for example, only one population, with scattered subpopulations, is known, within the limits of the Brasilia National Park. By this study we raised information related to the biology of *G. doellii*, as population size and distribution, genetic variability and germination characteristics. Besides, we produced seedlings in greenhouse, planted in different physiognomies of the Cerrado and followed them for almost four years to check for their survival, growth and fruiting under natural conditions. Seedlings transplanted to the field presented high rates of recruitment (> 25%), growth patterns similar to wild plants and produced viable caryopses. We recommend transplanting of individuals of *G. doellii* for both in situ conservation as well as for the revegetation of degraded areas of the Cerrado.
Key words: conservation, endangered species, genetic variability, recruitment, reintroduction, savanna.

Resumo
A savana brasileira, também conhecida como Cerrado, é caracterizada por sua grande diversidade de fitofisionomias e detém a maior diversidade de espécies e nível de endemismo entre as savanas mundiais. Contudo, devido principalmente às atividades agropecuárias essa vasta vegetação está entre as mais degradadas, apresentando elevadas taxas de extinção. Entre as espécies atualmente presentes na "lista vermelha" encontra-se a endêmica *Gymnopogon doellii*, uma gramínea cuja distribuição é extremamente restrita a algumas poucas áreas. No Distrito Federal e arredores apenas uma população é conhecida, localizada dentro dos limites do Parque Nacional de Brasília. Neste estudo levantamos informações relevantes relacionadas a biologia dessa população de *G. doellii*, como sua área de distribuição, variabilidade genética e características de germinação. Mudas a partir de suas cariopses foram produzidas em casa de vegetação, plantadas em diferentes fisionomias do Cerrado, incluindo áreas degradadas, e suas taxas de sobrevivência e desempenho fisiológico acompanhados por quase quatro anos. A população apresenta baixa variabilidade genética. As mudas apresentaram altas taxas de sobrevivência (> 25%), padrões de crescimento similar aos indivíduos selvagens e produziram cariopses viáveis. Recomendamos o plantio de indivíduos de *G. doellii* tanto para sua conservação in situ como para recuperação de áreas degradadas do Cerrado.
Palavras-chave: conservação, espécie ameaçada, variabilidade genética, recrutamento, reintrodução, cerrado.

See supplementary material at <https://doi.org/10.6084/m9.figshare.21349773.v1>

[1] Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA, Brasília, DF, Brazil. ORCID: <https://orcid.org/0000-0001-9884-4313>.
[2] Universidade de Brasília, Depto. Botânica, Lab. Termobiologia, Brasília, DF, Brazil. ORCID: <https://orcid.org/0000-0001-7141-265X>.
[3] Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica, Brasília, DF, Brazil.
[4] ORCID: <https://orcid.org/0000-0003-1708-1508>. [5] ORCID: <https://orcid.org/0000-0002-5839-6357>. [6] ORCID: <https://orcid.org/0000-0002-4586-5142>.
[7] Autor for correspondence: borghetti.fabian@gmail.com

Introduction

It is well established that Brazil is home of the world's greatest biodiversity but, at the same time, among the most threatened countries in respect to species conservation (Amaral *et al.* 2017; Françoso *et al.* 2020). In respect to grasses, around 1,410 species occur within its territory, of which some 115 are at risk of extinction and 67 are currently on the Red List of Threatened Species. *Gymnopogon doellii* Boechat & Valls is among the grasses at risk of extinction, classified as "CR" - in critical danger - by the Red Book of Brazilian Flora (Filgueiras *et al.* 2013).

The genus *Gymnopogon* belongs to the family Poaceae Barnhart, subfamily Chloridoideae Kunth *ex* Beilschm., tribe Cynodonteae Dumort., subtribe Hubbardochloinae (Soreng *et al.* 2017), and occurs in South America, Central America, North America and Southeast Asia. It is composed of 14 described species (Cialdella & Zuloaga 2011), seven of which are found in Brazil (Boechat & Valls 1990a, b). The species are megathermic (Burkart 1975), preferring sandy and dry soils, though some species, e.g., *G. burchellii* (Munro *ex* Döll) Ekman and *G. fastigiatus* Nees, can occur in humid areas, such as natural fields, marshes and plains. *Gymnopogon doellii* is exclusive to the Brazilian flora, and its occurrence is restricted to the states of Minas Gerais (MG), Goiás (GO) and the Federal District (DF) (Boechat & Valls. 1990a, b; Filgueiras 1992; Cialdella & Zuloaga 2011; Vinícius-Silva *et al.* 2020).

Filgueiras (1992) classified *G. doellii* as a species of rare occurrence within the Federal District. The great devastation of the Cerrado by agribusiness (Lahsen *et al.* 2016) most likely contributed to the reduction of its distribution over native areas. Besides, the invasion of natural areas by exotic plants has become a particular threat to the conservation of the native flora (Sühs *et al.* 2020), and alien species represent the second most common threat associated with species extinction since AD 1500 (Bellard *et al.* 2020). At the Brasilia National Park, for example, 28 species of alien grasses were registered, which represent 19% of the total number of grasses (147) in this protected area. For the effective preservation of the native vegetation, a monitoring and control/eradication program should be elaborated including all alien species in this protected area (Martins *et al*. 2007).

The Brasília National Park has the largest known protected population of *G. doellii* within a Conservation Unit. To date, the extent of its area of occurrence and population size, physiological attributes, genetic variability of the remaining plants, germination capability and recruitment potential over native areas remain unknown.

Previous studies revealed a low production of, and a high level of dormancy among viable caryopses collected from natural populations of *G. doellii* (Martins *et al*. 1997), suggesting that these attributes may have contributed to its low occurrence and distribution over natural areas. As far as we know, there is no other study on germination characteristics, initial plant growth and development of this endangered species neither conducted in the laboratory nor under field conditions.

Within this context, the present study aimed at contributing for a better knowledge of the endangered native grass *G. doelli* by mapping its occurrence areas, determining its seed germination characteristics, analyzing the genetic variability of these remaining populations and evaluating growth parameters and establishment rates of individuals introduced in different savanna physiognomies occurring within the Brasília National Park. By this study we also intend to contribute to unraveling limitations for its reproductive capacity and determining efficient management strategies for its reintroduction in natural.

Material and Methods

Occurrence of *G. doellii* in the Brasília National Park

The survey of *G. doellii* populations at the Brasília National Park was carried out from February 2014 to June 2017, totaling 287 individuals sampled. Fieldwork was combined with a Global Positioning System (GPS) and laboratory signal receiver, with the support of Geoprocessing techniques. The areas of occurrence of *G. doellii* were georeferenced and, subsequently, they were spatialized using the ArcGIS SIG program. Areas of influence with a radius of 50 m were generated from each point of occurrence.

Seed germination experiments

Our field observations at the Brasilia National Park revealed that individuals of *G. doellii* reach the reproductive period between late April and May. The experiments of germination were conducted with mature caryopses collected between May-June of 2015 at the Brasilia National Park. Around

40 inflorescences from as many individuals as possible were randomly harvested from natural areas of open savanna as well as from dense grasslands, stored in paper bags and dried under natural conditions (~23 ºC) in the Laboratório de Termobiologia of the University of Brasilia, DF. The spikelets were manually separated to remove the dispersal units (caryopses), which were stored in Gerbox at lab temperature (~23 ºC) in a dark room for up to one year. Full spikelets (*i.e.*, those containing embryos) could be easily differentiated from the empty ones by their color and size; while embryo-bearing spikelets are bigger and reddish, the empty ones are smaller and yellowish (Carmona *et al.* 1997).

Germination tests started in September 2015, four months after the dispersal units were collected. The caryopses were placed in transparent polystyrene (6 cm) plates lined with three sheets of qualitative filter paper moistened with distilled water. Tests were performed in a germination chamber set for alternating temperature of 22 ºC/28 ºC (12/12 hours) under a photoperiod of 12 hours (white, fluorescent light). This temperature regime was set according to the average minimum and maximum temperatures recorded during the wet season, which represents the growing season for most savanna species in the Cerrado, including grasses (Ramos *et al.* 2017). Tests in the dark were carried out at the same temperature regime, however the dark treatment was applied by wrapping the plates with two sheets of aluminum foil. Five replicates of twenty embryo-bearing caryopses were used for each light and dark treatments. Germination was monitored daily and distilled water was added as needed. For experiments conducted in the dark, observations were made under a green safelight (490–560 nm) (Labouriau 1983). The germination experiments lasted 83–95 days, according to the treatment, and ended when no germination was detected for 15 consecutive days. Germinability (%) and its standard deviation were calculated according to Labouriau (1983).

DNA extraction and genetic analyses

Genetic variability of *G. doellii* populations was assessed using RAPD markers.

Samples were collected from 96 individuals of *G. doellii* in the following areas: open savanna, dense grassland, and disturbed area. Total genomic DNA was extracted from freeze-dried leaf tissue using a modified CTAB protocol (Grattapaglia & Sederoff 1994). DNA was quantified comparing the fluorescence intensities of the samples to those of lambda DNA standards in ethidium bromide-stained 1% agarose gels under UV light.

RAPD reactions were performed in a 13 µl solution, containing 10X PCR buffer, 1 µg/µl purified BSA (New England Biolabs), 200 µM of each dNTP, 0.4 µM 10-base primer (Operon Technologies Inc.), 7.5 ng of genomic DNA and 1 unit of *Taq* DNA polymerase. Amplification reactions were performed in a Veriti Thermal Cycler (Applied Biosystems) programmed for 40 cycles of 1 min at 92 ºC, 1 min at 35 ºC and 2 min at 72 ºC, and a final DNA extension cycle at 72 ºC for 7 min. RAPD products were analyzed by electrophoresis in 1.5% agarose gels in 1X TBE and 1.5 µg/µl ethidium bromide.

PCR amplification products of the 96 samples were scored as presence (1) or absence (0) of bands. The data matrix was used to calculate Jaccard's similarity coefficient. Dendrograms were constructed using the unweighted pair-group method analysis (UPGMA). These analyses were performed using NTSYS-pc software, version 2.21 (Rohlf 2009).

Management strategies for reintroduction into the National Park of Brasilia

Seedlings of *G. doellii* were produced in a greenhouse covered by a shade cloth blocking 50% of solar radiation of the state company NOVACAP/Federal District, located near the Brasília National Park. In the greenhouse, plastic trays (20 × 30 × 10 cm) were filled with a substrate consisting of a mixture of 25 kg of Bioplant fertilizer (agricultural substrate with coconut fibers) plus 2 kg of the NPK Osmocote fertilizer (18–5–9). In the first week of June 2015, seeds were sown in the trays and covered with a thin layer of the substrate (~1 mm). The trays were watered frequently and around 30 days after sowing the growing seedlings were transplanted into 50cm^3 tubules (conical tube of rigid plastic) filled with the same substrate as the germination trays.

The transplanting of *G. doellii* seedlings into natural areas started on November 2015 in the following areas: a) open savanna; b) dense grassland; and c) disturbed area. Seedlings were planted one per each corner and one in the center of four plots of 16 m^2 (4 × 4 m) previously demarcated, so resulting in 20 seedlings transplanted in each studied area.

The survival and grow of the transplanted *G. doellii* seedlings were followed during the next four years. In December 2015, May 2017 and May 2019, the number of living individuals, the number of tillers per individual and length of the longest tiller (as a proxy for shoot height) per individual were accessed. In order to compare the performance of the transplanted seedlings with the performance of those naturally occurring in the Park, in May 2019, the same growth parameters were measured on 40 adult individuals randomly selected from native subpopulations of *G. doellii*.

Statistical analyses

Analysis of Variance (ANOVA) followed by Tukey HSD was performed to detect differences in the germination percentages of freshly harvested or one-year stored caryopses of *G. doellii,* incubated under both light and dark conditions. Two-tailed Kruskal-Wallis - Bonferroni posteriori test was performed to compare the number of tillers and the shoot height of individuals measured in December 2015, May 2017 and May 2019 in the three areas under study (Rahardja 2017).

Results and Discussion

The area of potential occurrence of *G. doellii* in the Brasília National Park was estimated in 104.87 ha (Fig. 1). Our exhaustive field surveys revealed that the spatial distribution of individuals of *G. doellii* was generally clumped, and subpopulations were detected in the Cerrado physiognomies of open savanna, dense grassland, and grassland (sensu Ribeiro & Walter 2008). Individuals of *G. doellii* were also detected in an area formerly subjected to mining (disturbed area), but now going through spontaneous vegetation recovery. The population mapping shows that this native species can occur in a wide range of physiognomies of the Cerrado, as well as in disturbed areas (Fig. 2). The identification of areas of spontaneous, natural occurrence of *G. doellii* is critical to most effectively allocate efforts in reintroduction of native grasses for restoration purposes (Schmidt *et al*. 2019).

Freshly harvested caryopses of *G. doellii* were shown to present a considerable level of dormancy and absolute light requirement for germination (Tab. 1), as already reported

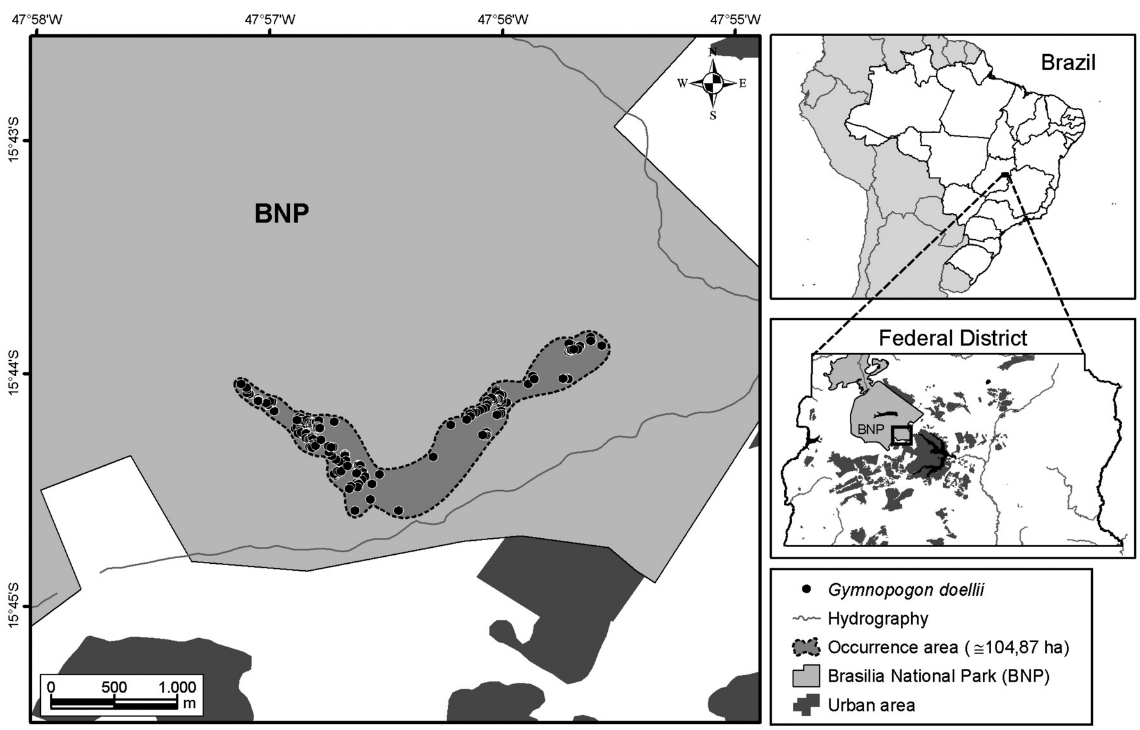

Figure 1 – Populations of *Gymnopogon doellii* recorded (black dots) and area of occurrence estimated (circled dark grey area) at the Brasília National Park (BNP), Federal District.

by Carmona *et al.* (1997). However, after one year of storage, the germination percentage increased significantly, and the light-dependence for germination virtually disappeared (Tab. 1). Moreover, the results indicate that the caryopses can maintain a high level of viability up to one year of storage.

Dormancy among caryopses of native grass species has been previously reported (Ramos *et al.* 2017, 2019; Saraiva *et al.* 2020) and different cues, as smoke and heat shock (Ramos *et al.* 2019) as well as KNO_3 and alternating temperatures (Carmona *et al*. 1998; Saraiva *et al*. 2020) were reported to improve their germination. Caryopses of *G. doellii* are dormant at harvest, but alternating temperature treatments, coupled or not with KNO_3 and light were shown to considerably improve germination (Carmona *et al*. 1997).

The level of dormancy and considerable longevity of *G. doellii* caryopses seem to represent a recruitment strategy to reduce the probability of failed germination during the dry season and, at the same time, to keep a seed bank until the following rain season, as already reported among other grass species of the Cerrado (Ramos *et al.* 2017). In fact, previous studies have reported a

Figure 2 – Individuals of *Gymnopogon doellii* of spontaneous occurrence at the Brasília National Park, Federal District.

Table 1 – Germination percentage and standard deviation of freshly harvested (Sept 2015) and one year stored (Sept 2016) caryopses of *Gymnopogon doellii* at alternating temperature of 22 ºC/28 ºC under light or dark conditions.

Treatment	Sept 2015		Sept 2016	
	Germination (%)	**Standard deviation**	**Germination (%)**	**Standard deviation**
Light	57 *,**	21.1	81 ^{*,ns}	11.4
Darkness	0 **,**	0	88 ^{**,ns}	7.58

First symbol represents comparison within rows, second symbol represents comparison within columns. Shapiro-Wilk test did not reject data normality (W = 0.93908, p-value = 0.2304), and Levene's Test did not reject variance homogeneity (F-value = 2.5106, p-value = 0.0956).

ANOVA two-way test followed by Tukey HSD test: * = significance level of 0.05; ** = significance level of 0.01; ^{ns} = no significant difference among treatments).

higher level of dormancy among grass caryopses dispersed early in comparison to those dispersed late in the dry season (Ramos *et al.* 2017). Due to the presence of a positive photoblastism, it is also expected that caryopses of *G. doellii* germinate primarily on or near the soil surface, so reducing their probability of germination either buried or in shaded environments.

A total of 60 RAPD primers were screened for polymorphism using eight samples. Of these, only 10 revealed at least one polymorphic fragment and were used to analyze the 96 individuals. The 10 primers amplified 51 fragments, which were used for the genetic variability analysis. All primers produced identical RAPD patterns for the selected fragments during the primer-screening step and in the final analysis.

An UPGMA dendrogram based on Jaccard's similarity index was constructed for the 96 individuals (Fig. S1, available on supplementary material <https://doi.org/10.6084/m9.figshare.21349773.v1>). Some groups were evident, but they were not associated neither with the area nor with the vegetation type in which the samples were collected. The average genetic similarity indices between the 96 plants were very high, estimated at 0.92. These results revealed a very low genetic variability of *G. doellii* within the National Park of Brasilia, proving the urgency of finding new populations, due to the high risk of extinction of this species.

Well planned reintroduction of new individuals has been viewed as a promising strategy for conserving rare plant species worldwide (Maschinski & Albrecht 2017), and conservation units have emerged as relevant areas for in situ conservation of threatened ones (Costa *et al*. 2018). One month after transplanting the seedlings to the selected areas, all individuals of *G. doellii* were still alive. This high survival rate is very probably associated to the fact that they were transplanted in November, a period when rains are relatively regular and frequent in the region. After 18 months, survival was still high among individuals planted in the open savanna physiognomy (90%) but dropped to 50% and 65% in dense grassland and disturbed areas, respectively (Tab. 2). After 42 months of transplanting, the survival dropped to 40%, 30% and 25% in the open savanna, dense grassland and disturbed area, respectively. The reduction in survival rates among transplanted individuals was very likely related to the periods of water shortage which plants face every year during the dry season, when the grass-herb component of the vegetation usually dries out in the open physiognomies of the Cerrado (Silva *et al*. 2008). The establishment rates reached in our transplanting approach are within the range observed in similar studies; for example, in a study of reintroduction of *Artemisia tridentata* seedling in communities subjected to fire, Davies *et al*. (2020) reported survival rates between 46% and 7%, depending on microsite type, after four growing seasons of planting. A survival rate above 25% as reached by our study is very promising, since at least one out of four individuals survive in disturbed area, revealing that this strategy of seedling transplanting may be effective both for reintroduction efforts of endangered grasses as well as for revegetation of open and disturbed areas with native species.

Differently from scleromorphic forests and even savanna physiognomies, which are likely to regenerate naturally following low- or medium-intensity land use due to extensive resprouting of woody plants, the restoration of grassland physiognomies may require reintroduction of grass

Table 2 – Survival (in %) of individuals of *Gymnopogon doellii* after three years of transplanting into different types of vegetation at the Brasilia National Park, Federal District. Six-month old individuals produced under greenhouse conditions were transplanted to the areas in November 2015.

Physiognomy	**Survival (%)**		
	December 2015	**May 2017**	**May 2019**
open savanna	100	90	40
dense grassland	100	50	30
disturbed area	100	65	25

and forb species that do not tolerate soil disturbance and exotic grass competition (Schmidt *et al*. 2019). Although this approach may be unsuccessful for forest tree species (Souza *et al.* 2020), direct seeding could work for open physiognomies. In fact, direct seeding effectively established many native neotropical savanna species of different life forms (including grasses) in tropical savanna-grassland mosaics (Sampaio *et al.* 2019) and may reach up to 30% of soil cover for grass species (Pellizzaro *et al*. 2017). On the other hand, the establishment success of our seedling transplanting approach (> 25%) was very close to those studies and shows that the use of grass seedlings for revegetation of open areas and reintroduction of endangered species may be effective.

It is worth mentioning that, over the first year after transplanting, four out of 20 seedlings originally transplanted reached their reproductive stage in the open savanna physiognomy. Laboratory tests conducted at the same experimental conditions described above with caryopses collected from these individuals revealed that 55% were fertile (bearing viable embryos) and reached a germination of 43% under the experimental conditions described above. In the second year of monitoring, we observed that six, eight and four individuals reached their reproductive stage in the open savanna, dense grassland and disturbed areas, respectively, representing 30%, 40% and 20% of the total individuals transplanted. These results revealed that, within its first years of life, this species presents a great potential to recruit and reproduce over different physiognomies of the Cerrado, what is promising in terms of recovery of natural grasslands (Overbeck *et al.* 2013).

Besides survival and reproduction, growth parameters can also provide evidence of successful recruitment and establishment of a species at new sites (Monks *et al*. 2012). The monitoring of the transplanted seedlings revealed a decline in the number of tillers per individual over the experimental period, however, the average shoot height increased in all experimental areas, in particular between the first and second years of monitoring (Tab. S1, available on supplementary material <https://doi.org/10.6084/m9.figshare.21349773.v1>). Taking together, these results indicate that the growth of the seedlings started with a higher investment in the number of tillers, later supplanted by an increase in the average shoot height, in particular among individuals transplanted in the open savanna and dense grassland areas (Tab. S1, available on supplementary material <https://doi.org/10.6084/m9.figshare.21349773.v1>). After four years of monitoring the number of tillers and average shoot height was the lowest among individuals transplanted into the disturbed area. We have not intended to check for effects of spatial variability in our planting responses (Davies *et al*. 2020), but the establishment and grow performance of seedlings cleared differed across the areas of study (Tab. S1, available on supplementary material <https://doi.org/10.6084/m9.figshare.21349773.v1>). The low growth performance of seedlings observed in the disturbed area may be related to edaphic factors: the soil in this anthropized area is much more compacted than in the undisturbed, natural areas (C.R. Martins 2018, personal communication).

A field survey conducted over native subpopulations of *G. doellii* revealed an average number of four tillers per clump, and an average shoot height of 70.4 cm (± 19.8 cm) among adult individuals (n = 40). These observations allowed us to conclude that the individuals of *G. doellii* transplanted to the open savanna and dense grassland areas showed a physiological performance comparable to individuals growing spontaneously in these physiognomies.

Our study revealed and characterized a remnant population of *G. doellii* thriving in the Brasília National Park. This population spread over physiognomies of grassland and open savanna, coexisting with a large number of herbs and grasses. The considerably high survival rates after transplanting, comparable physiological performance and capability to produce viable caryopses show that the production and transplanting of *G. doellii* seedlings into natural areas may represent a viable alternative both for restoration purposes as for the in-situ conservation of this endangered grass in the Cerrado.

The analyses using molecular markers revealed that the genetic variability among subpopulations of *G. doellii* occurring spontaneously at the Brasília National Park is low, which highlights the urgency of finding new areas of occurrence of this species. After 149 years since its first collection in Minas Gerais State (Lagoa Santa locality), new occurrences of *G. doellii* were recorded in that state, some 650 km far from the Brasília National Park (Vinícius-Silva *et al*. 2020). This discovery is extremely relevant for the conservation of *G. doellii* and renews the hope that new populations be found in areas where this species was formerly registered.

Acknowledgements

Fabian Borghetti and José Francisco Montenegro Valls gratefully acknowledge the Conselho Nacional de Desenvolvinento Científico e Tecnológico - CNPq, Brazil, for their fellow grants (PQ number 312152/2018-3 and 310026/2018-0, respectively). Studies conducted at the Laboratório de Termobiologia were partially supported by a research grant from the Fundação de Apoio à Pesquisa do Distrito Federal - FAP-DF (process number 193.000.922/2015). We thank the staff of the Parque Nacional de Brasilia who provided research facilities for the field work.

References

Amaral AG, Munhoz CBR, Walter BMT, Aguirre-Gutiérrez J & Raes N (2017) Richness pattern and phytogeography of the Cerrado herb-shrub flora and implications for conservation. Journal of Vegetation Science 28: 848-858.

Bellard C, Cassey P & Blackburn TM (2016) Alien species as a driver of recent extinctions Biological Letters 12: 20150623.

Boechat SC & Valls JFM (1990a) *Gymnopogon doellii* Boechat & Valls (Gramineae; Chloridoideae), espécie crítica da flora brasileira. Bradea 5: 313-318.

Boechat SC & Valls JFM (1990b) O gênero *Gymnopogon* Palisot de Beauvois (Gramineae; Chloridoideae) no Brasil. Iheringia, Série Botânica 40: 3-43.

Burkart A (1975) Evolution of grasses and grasslands in South America. Taxon 24: 53-66.

Carmona R, Camilo MGB & Martins CR (1997) Estímulo à germinação de *Gymnopogon doellii* - uma gramínea ameaçada de extinção. Revista Brasileira de Fisiologia Vegetal 9: 125-130.

Carmona R, Martins CR & Fávero AP (1998) Fatores que afetam a germinação de sementes de gramíneas nativas do Cerrado. Revista Brasileira de Sementes 20: 16-22.

Cialdella AM & Zuloaga FO (2011) Taxonomic study of *Gymnopogon* (Poaceae, Chloridoideae, Cynodonteae). Annals of the Missouri Botanical Garden 98: 301-330.

Costa MLMN, Fernandes PWJRA & Peixoto AL (2018) Conservation of threatened plant species in botanic garden reserves in Brazil. Oryx 52: 108-115.

Davies KW, Bates JD & Clenet D (2020) Improving restoration success through microsite selection: an example with planting sagebrush seedlings after wildfire. Restoration Ecology 28: 859-868.

Filgueiras TS (1992) Gramíneas forrageiras nativas no Distrito Federal, Brasil. Revista Brasileira de Botânica 27: 1103-1111.

Filgueiras T, Oliveira RP, Sfair JC, Monteiro NP & Borges RAX (2013) Poaceae. *In*: Martinelli G & Moraes MA (eds.) Livro vermelho da flora do Brasil. Andrea Jakobsson / Instituto de Pesquisas Jardim Botânico, Rio de Janeiro. Pp. 858-881.

Françoso RD, Dexter KG, Machado RB, Pennington RT, Pinto JRR, Brandão RA & Ratter JA (2020) Delimiting floristic biogeographic districts in the Cerrado and assessing their conservation status. Biodiversity and Conservation 29: 1477-1500.

Grattapaglia D & Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudotestcross: mapping strategy and RAPD markers. Genetics 137: 1121-1137.

Labouriau LG (1983) A germinação das sementes. Secretaria Geral da Organização dos Estados Americanos, Washington, D.C. 174p.

Lahsen M, Bustamante MMC & Dalla-Nora EL (2016) Undervaluing and overexploiting the Brazilian Cerrado at our peril. Environment 58: 4-15.

Martins CR, Carmona R & Leite LL (1997) Fenologia e qualidade de sementes da gramínea *Gymnopogon doellii* - uma gramínea ameaçada de extinção. *In*: Leite LL & Saito CH (eds.) Contribuição ao Conhecimento Ecológico do Cerrado. Congresso de Ecologia do Brasil. Brasília. Universidade de Brasília, Brasília. Pp. 71-74.

Martins CR, Hay JDV, Valls JFM, Leite LL & Henriques RPB (2007) Study on alien gramineous of the Brasilia National Park, Federal District, Brazil. Natureza & Conservação 5: 93-100.

Maschinski J & Albrecht MA (2017) Center for plant conservation's best practices guidelines for the reintroduction of rare plants. Plant Diversity 39: 390-395.

Monks L, Coates D, Bell T & Bowles M (2012) Determining success criteria for reintroductions of threatened long-lived plants. *In*: Maschinski J & Haskins KE (eds.) Plant reintroduction in a changing climate: promises and perils. Island Press, Washington, D.C. Pp. 189-208.

Overbeck GE, Hermann JM, Andrade BO, Boldrini II, Kiehl K, Kirmer A, Koch C, Kollmann J, Meyer ST, Müller SC, Nabinger C, Pilger GE, Trindade JPP, Vélez-Martin E, Walker EA, Zimmermann DG & Pillar VD (2013) Restoration ecology in Brazil - time to step out of the forest. Natureza & Conservação 11: 92-95.

Pellizzaro KF, Cordeiro AOO, Alves M, Motta CP, Rezende GM, Silva RRP, Ribeiro JF, Sampaio AB, Vieira DLM & Schmidt IB (2017) Cerrado restoration by direct seeding: field establishment and initial growth of 75 trees, shrubs and grass species. Brazilian Journal of Botany 40: 681-693.

Rahardja D (2017) A review of the multiple-sample tests for the continuous-data type. Journal of Modern Applied Statistical Methods 16: 127-136.

Ramos DM, Diniz P, Ooi MKJ, Borghetti F & Valls JFM (2017) Avoiding the dry season: dispersal time and syndrome mediate seed dormancy in grasses in Neotropical savanna and wet grasslands. Journal of Vegetation Science 28: 798-807.

Ramos DM, Valls JFM, Borghetti F & Ooi MKJ (2019) Fire cues trigger germination and stimulate seedling growth in grass species from Brazilian savannas. American Journal of Botany 106: 1190-1201.

Ribeiro JF & Walter BMT (2008) As principais fitofisionomias do Bioma Cerrado. *In*: Sano SM, Almeida SP & Ribeiro JF (eds.) Cerrado: ecologia e flora. Embrapa Cerrados, Planaltina. Pp. 151-212.

Rohlf F (2009) NTSYS-pc: numerical taxonomy system. Ver. 2.21c. Exeter Software, Setauket.

Sampaio AB, Vieira DLM, Holl KD, Pellizaro KF, Alves M, Coutinho AG, Cordeiro AOO, Ribeiro JF & Schmidt IB (2019) Lessons on direct seeding to restore Neotropical savanna. Ecological Engineering 138: 148-154.

Saraiva DF, Paula CC, Moraes PJ, Vinícius-Silva R, Silva MM, Dias DCFS & Botelho AS (2020) Seed germination and dormancy break in *Eragrostis polytricha*, a native Brazilian grass species with potential for recovery of degraded lands. Acta Botanica Brasilica 34: 497-504.

Schmidt IB, Ferreira MC, Sampaio AB, Walter BMT, Vieira DLM & Holl KD (2019) Tailoring restoration interventions to the grassland-savanna-forest complex in central Brazil. Restoration Ecology 27: 942-948.

Silva FAM, Assad ED, Ercília TS & Müller AG (2008) Clima do Bioma Cerrado. *In*: Albuquerque ACS & Silva AG (eds.) Agricultura tropical: quatro décadas de inovações tecnológicas, institucionais e políticas. Embrapa Informações Tecnológicas, Brasília. Pp. 93-148.

Soreng RJ, Peterson PM, Romaschenko K, Davidse G, Teisher JK, Clark LG, Barber P, Gillespie LJ & Zuloaga FO (2017) A worldwide phylogenetic classification of the Poaceae (Gramineae) II: an update and a comparison of two 2015 classifications. Journal of Systematics and Evolution 55: 259-290.

Souza DC, Engel VL & Mattos EC (2020) Direct seeding to restore tropical seasonal forests: effects of green manure and hydrogel amendment on tree species performances and weed infestation. Restoration Ecology 29: e13277.

Sühs RB, Giehl ELH & Peroni N (2020) Preventing traditional management can cause grassland loss within 30 years in southern Brazil. Scientific Reports 10:783.

Vinícius-Silva R, Paula CC & Saraiva DF (2020) New records of *Gymnopogon doellii* (Poaceae, Chloridoideae, Cynodonteae, Hubbardochloinae), a Brazilian endangered species. Phytologia 435: 56-72.

Area Editor: Dr. Pedro Viana
Received in July 01, 2021. Accepted in February 25, 2022.