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In vitro germination of pollen grains of three native species from Pampa biome with ornamental potential

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Abstract

The aim of this work was to verify the *in vitro* germination of pollen grains of *Angelonia integerrima* L., *Campomanesia aurea* O. Berg and *Sesbania punicea* (Cav.) Benth in different culture medium and temperatures. For this purpose, flower buds from which pollen was collected and sprayed on plates containing the three evaluated culture medium: M1 - agar and sucrose; M2 - agar, sucrose and H_3BO_3; M3 - agar, sucrose, H_3BO_3, $Ca(NO_3)$, $MgSO_4$ and KNO_3; and two incubation temperatures (20 °C and 30 °C). Data was subjected to analysis of variance after its transformation to square root and means were compared by Fisher's test (LSD). For the three species, the temperature of 30 °C provided the highest percentage of pollen grain germination. For *A. integerrima*, M1 and M3 promoted the highest germination percentages (40.7 % and 56.5 %, respectively). On the other hand, for *C. aurea*, M2 provided the highest germination average (43.7 %). At last for *S. punicea*, M3 was the one that provided the highest average (31.62 %). It was concluded that the evaluated species differ in micronutrient requirements for *in vitro* germination of pollen grains. The temperature of 30 °C was suitable for all three species.

Keywords: *Angelonia integerrima* L., *Campomanesia aurea* O. Berg, floriculture, pollen tube, *Sesbania punicea* (Cav.) Benth

The Pampa biome is characterized by its high species richness, and Boldrini et al. (2015) described the existence of approximately 2,150 higher plant species in this biome. In a study by Stumpf et al. (2012), at least 250 species from the Pampa biome were recognized due to their ornamental potential for using in floral art and landscaping.

The usage of native species as ornamental plants has emerged as a new niche in the floriculture market, showing a high potential for production and commercialization. These species have some advantages over the exotic ones, such as greater adaptation to local edaphoclimatic conditions (Oliveira Junior et al., 2013), besides showing a singular beauty within a market already saturated by traditional crops. In addition, floriculture can directly contribute to the *in situ* preservation of native germplasm (Nahoum & Fraga, 2015), especially in species that are the target of extractivism, since if adequately propagated and marketed, the indiscriminate collection of these material in the wild will decrease.

Among the native species of the Pampa biome with ornamental potential, some have been highlighted because of their characteristics such as the size, architecture, color and aroma of flowers, such as: *Angelonia integerrima* Spreng. (Plantaginaceae), *Campomanesia aurea* O. Berg (Myrtaceae) and *Sesbania punicea* (Cav.) Benth (Fabaceae). These three species and their attributes were introduced by Stumpf et al. (2009) in the book "Colors and Shapes in the Pampa Biome: Native Ornamental Plants".

For commercial purposes, studies on the *in vitro* germination capacity of genotype pollen grains of a species may presuppose the success of their use in further crosses (Chagas et al., 2010), with the objective of obtaining a material with even more interesting characteristics.

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The conditions required for pollen germination differs among species, especially concerning the culture medium components, temperature and incubation time (Stanley & Linskens, 1974). Regarding the culture medium, it must be composed of organic and inorganic elements, in order to provide to pollen with a condition similar to that found in the flower stigma (Sousa et al., 2010; Silva et al., 2017).

So far, there was a lack of knowledge about adequate conditions for *in vitro* germination tests for pollen of *A. integerrima*, *C. aurea* and *S. punicea*. Thus, the aim of this work was to observe the *in vitro* germination of pollen grains of these three species in different culture medium and temperatures.

The plant material used was flower buds collected in November 2018 of 15 individuals from a population of *C. aurea* and of 25 individuals from a population of *A. integerrima*, both located in Parque Natural Morro do Osso, in Porto Alegre city – state of Rio Grande do Sul. Also was collected flower buds of 15 individuals from a population of *S. punicea* located in an area adjacent to the road in the municipality of Palmares do Sul, state of Rio Grande do Sul, in December 2018. The project is registered with SisGen under number A685CD8.

Once collected, the material was immediately packed in Styrofoam boxes and transported to the laboratory, where flower buds were manually sorted, keeping only those that were in a balloon stage (pre-anthesis). The buds were then placed on trays lined with absorbent paper in a B.O.D. (Biochemical Oxygen Demand) chamber at 30 °C in the dark for 24 hours for pollen drying. Subsequently, the petals were removed and the anthers were exposed to incandescent light for 20 minutes for complete dehiscence and pollen release.

Three culture medium expressed in a concentration for 100 ml of autoclaved deionized water were tested: Medium 1 (M1) – 10 g sucrose + 1 g agar (standard culture medium); Medium 2 (M2) – 10 g sucrose + 1 g agar + 0.08 g H_3BO_3 (Franzon et al., 2006); and Medium 3 (M3) – 15 g sucrose + 1 g agar + 0.01 g H_3BO_3 + 0.1 g Ca (NO_3) $.4H_2O$ + 0.03 g $MgSO_4.7H_2O$ + 0.01 g KNO_3 (Sahar & Spiegel-Roy, 1984); and two incubation temperatures: 20 °C and 30 °C.

For the preparation of the culture medium, the constituent elements were dissolved in deionized water and heated on a magnetic stirrer at a temperature of approximately 90 °C and then poured into cell culture plates (35 mm × 12 mm) coupled to microscopy slides. After solidification of the medium, the pollen was removed from the anthers with the aid of a number-4 brush and sprayed all over the surface of the plate. The plates/slides were then kept in gerbox-type boxes with moist Germitest paper simulating a wet chamber and incubated in B.O.D. chamber for 12 hours in the dark at both temperatures.

The germinated pollen grains (which emitted the longest pollen tube, twice the pollen diameter, at least) were counted with the aid of optical microscope with 20-x magnification, on which 500 pollen grains per plate/slide were observed.

The study used a completely randomized design in a 3 x 2 factorial arrangement, with three culture medium (M1, M2 and M3) and two incubation temperatures (20 °C and 30 °C), with four replications (each plate/slide corresponding to one repetition). Data was subjected to analysis of variance after transformation to square root, and then means were compared by Fisher's test (LSD) at 5 % probability of error. The analysis was performed using Sigmaplot 11.0 software.

For *A. integerrima* (Figure 1A), interaction was found between culture medium and incubation temperature (p-value <0.001). The germination percentage did not differ between culture medium at 20 °C, with an average of only 0.9 % of germinated pollens (Table 1). However, when the temperature was 30 °C, a difference between the media was found, in which M2 medium was the one with the lowest average, while the M1 and M3 medium increased the germination by 6.2 and 8.6 times compared to M2, respectively (Table 1).

For *C. aurea* (Figure 1C), no pollen grain germinated at 20 °C (Table 1). At 30 °C, M2 provided the highest average of germinated pollens, increasing the germination by 6.6 times when compared to M3 (which resulted in the lowest average), but both did not differ from M1, which provided a germination of 20 % (Table 1).

On the other hand, no interaction was found between the factors (p-value: 0.384) for *S. punicea* (Figure 1E); only the effect of the isolated factors. The highest germination means were obtained with M3, which provided a germination increment of about 50 times in germination in comparison with M1 and the temperature of 30 °C, which caused twice the germination in relation to the temperature of 20 °C (Table 2).

The M1, composed only by agar and sucrose, and the M3, the most complete, provided the highest percentages of pollen grain germination for species *A. integerrima* in this study, therefore, indicating that these species do not need addition of micronutrients for pollen germination.

For the species *Syagrus romanzoffiana* (S.) Cham (jerivá - Arecaceae), Sousa et al. (2010) found that the

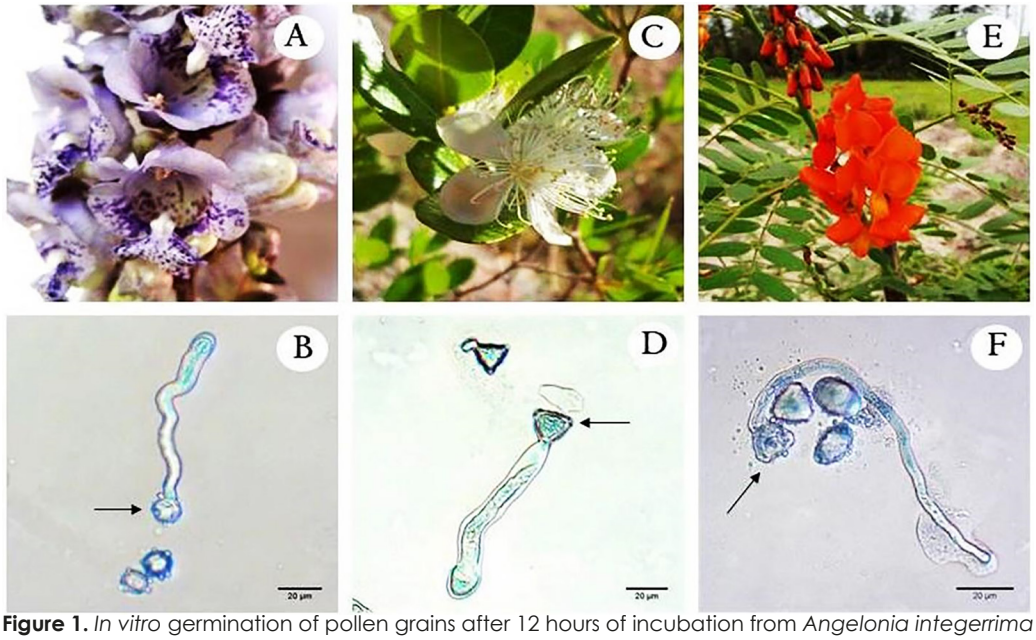

Figure 1. *In vitro* germination of pollen grains after 12 hours of incubation from *Angelonia integerrima* Spreng., *Campomanesia aurea* O. Berg and *Sesbania punicea* (Cav.): A) detail of inflorescence *A. integerrima*; B) germinated (arrow) and non-germinated pollen grains of *A. integerrima*; C) detail of *C. aurea* flower; D) germinated (arrow) and non-germinated pollen grain of *C. aurea*; E) detail of *S. punicea* inflorescence; F) germinated (arrow) and non-germinated pollen grains of *S. punicea*.

Table 1. Average percentage of *in vitro* pollen germination of *Angelonia integerrima* Spreng. and *Campomanesia aurea* O. Berg in different culture medium and temperatures

Species	Culture medium	Incubation temperature	
		20°C	30°C
Angelonia integerrima	M1	1.2 aB	40.7 aA
	M2	0.5 aB	6.55 bA
	M3	1.0 aB	56.5 aA
Campomanesia aurea	M1	0 aB	20 abA
	M2	0 aB	43.7 aA
	M3	0 aB	8.05 bA

* Means followed by the same lowercase letter in the column and capital letter in the row do not differ from each other by the test of Fisher (LSD) at 5% significance.

Table 2. Average percentage of *in vitro* germination of pollen from *Sesbania punicea* (Cav.) Benth in different culture medium and temperatures

	Culture medium	Germination (%)
Sesbania punicea	M1	0.65 c
	M2	3.85 b
	M3	31.62 a
	Incubation temperature	
	20°C	8.2 b
	30°C	15.9 a

medium composed only of agar and sucrose provided the highest percentage of *in vitro* germination of pollen grains.

When boron is added to the medium as boric acid, it promotes the formation of a sugar-borate ionizable complex which interacts with cell membranes, resulting in an increase in the germination percentage and pollen tube length (Thompson & Batjer, 1950). This element may have been responsible for the increased germination in *C. aurea*, since M2, composed of agar, sucrose and boric acid, provided the highest germination mean in these species. Although M3 also contains boric acid in its constitution, the interaction with the other constituent elements of this medium may have been detrimental to the germination of pollen grains of *C. aurea*.

Regarding native species of the Myrtaceae family, differences are found in relation to the pollen behavior with regard to culture medium. For *Campomanesia xanthocarpa* Mart. ex O. Berg (guabirobeira), three different culture medium were tested and did not differ, indicating that boron did not influence the average pollen germination for this species, while for *Eugenia uniflora* L. the medium composed by sucrose and agar provided the best *in vitro* germination averages (Franzon et al., 2006). For *jabuticaba* trees of genus *Plinia* L., the addition of boric acid in the culture medium increased *in vitro* pollen germination (Danner et al., 2011).

In addition do agar, sucrose and boric acid, M3 is composed of calcium nitrate, magnesium sulfate and potassium nitrate. It provided the highest germination percentage in *S. punicea*, suggesting that this species

needs micronutrients to stimulate germination of pollen grains. Calcium, one of the constituent elements of this medium, may have been responsible for this result, as it is especially important for pollen tube growth (Sousa et al., 2010).

It can be seen on Figures 1B, 1D and 1F that, after 12 hours of incubation, the pollen tubes reached about 8 to 10 times the pollen grain size, indicating that this time was sufficient for germination evaluation.

Plants, being sessile organisms, are more frequently influenced by environmental factors such as drought, cold, salinity and high temperatures, which can considerably affect the success of reproduction and fertilization processes (Giorno et al., 2013).

For the three species studied, the temperature of 20 °C provided a minimum percentage of germinated pollens, including the absence of grains germinated for *C. aurea*. These results show that low temperatures are not suitable for pollen grain germination in these species. In pollen grains, the effect of low temperatures is related to the reduction of cellular metabolism (Cuchiara et al., 2012), which ends up affecting the essential processes that initiate the germination of the pollen tube.

According to Karni & Aloni (2002), the development and germination of pollen depend on the uptake and metabolism of carbohydrates by it, and the temperature can interfere in this process (Aloni, 2001). In the present study, the temperature of 30 °C may have promoted an increase in metabolic activity and a concomitant decrease in the internal potential of the pollen, promoting greater absorption of water, sucrose and nutrients from the culture medium, thus facilitating the germination process of the pollen tube. Furthermore, the three species bloom in the spring months (between September and November), where the maximum average temperatures approach 30 °C (in the referred collection municipalities), this may explain the requirement of higher temperatures for the in vitro germination of pollen grains.

For many species, temperatures between 25 °C and 30 °C are considered ideal for pollen germination, such as for *Eugenia involucrata* DC. (Myrtaceae) (Franzon et al., 2007), *Campomanesia xanthocarpa* (Myrtaceae) (Franzon et al., 2006), *Olea europaea* L. (Oleaceae) (Silva et al., 2016), among others.

For the three species studied in the present work, pollen grain germination ranged from 31.6 to 56.5 % for the medium that provided the highest averages, values considered satisfactory for *in vitro* germination tests (Franzon et al., 2006; Danner et al., 2011). Considering that several factors influence pollen grain germination, some adjustments may be made in further works, such as the evaluation of different temperatures, micronutrient concentrations and flower stage, which may further increase the germination percentage.

It was concluded that the evaluated species differ in micronutrient requirements for *in vitro* germination of pollen grains, in which M1 and M3 were the best medium for *A. integerrima*, M2 for *C. aurea* and M3 for *S. punicea*, and temperature 30 °C was suitable for all three species.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.