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Seed germination of *Ornithogalum saundersiae*, under different temperatures

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ABSTRACT – Ornitogalo is a bulbous plant with ornamental and medicinal potential, which verifies the lack of information on how to assess the viability of its seeds. The goal of this work was to study the germination of the species and define the evaluation criteria of its seedlings. Three germination temperatures were tested, analyzed the percentage and speed germination, seedlings characterization, beyond defining the data of the first and last counts in the evaluation of germination. The ideal temperature for germination was 25 ° C, with high percentage of normal seedlings (92 %). The first counting must be performed at 15 days after sowing and the last at 31 days. Normal seedlings showed protrusion of the root, roots hair, early bulb, and leaf. Abnormal seedlings were those that showed absence or deformities in the described parameters, important characterization to assist the genetic improvement of the species.

Keywords: evaluation criteria, ornamental bulbous, ornitogalo, germination temperature, seed viability.

RESUMO – Germinação de sementes de *Ornithogalum saundersiae*, sob diferentes temperaturas. Ornitogalo é uma planta bulbosa com potencial ornamental e medicinal, para qual se verifica carência de informações sobre forma de avaliação da viabilidade de suas sementes. O objetivo deste trabalho foi estudar a germinação da espécie e definir os critérios de avaliação das suas plântulas. Três temperaturas de germinação foram testadas, analisando a porcentagem e a velocidade da germinação, caracterização das plântulas, além da definição das datas de primeira e última contagem na avaliação da germinação. A temperatura ideal para a germinação foi 25 °C, com alta porcentagem de plântulas normais (92%). A primeira contagem deve ser realizada aos 15 dias após a semeadura e, a última, aos 31 dias. Plântulas normais apresentaram protrusão da raiz, pelos radiculares, primórdios de bulbo e foliar. Foram consideradas plântulas anormais as que apresentavam ausência ou deformidades nos parâmetros descritos, caracterização importante para auxiliar o melhoramento genético da espécie.

Palavras-chave: bulbosa ornamental, critério de avaliação, ornitogalo, temperatura de germinação, viabilidade de sementes.

Ornithogalum saundersiae Baker., known as ornitogalo, is a bulbous plant, belonging to the Hyacinthaceae family. It is cultivated commercially as a cutted flower, potted plant, and garden plant. The species has pharmacological properties, with cytotoxic activity and anticarcinogenic effect (Salachna *et al*. 2015). The small-scale commercial propagation is from adventitious bulbs (Gitonga *et al*. 2015). However, this method has a low yield as well as the possible dissemination of diseases such as soft rot - *Pectobacterum carotovora* (Joshi & Yedida 2017). Genetic improvement has already been carried out, but authors cite the need for the sexual propagation of this improved material (Joshi & Yedida 2017).

The efficiency of the ornitogalo 'sexual propagation depends on the development of studies on this species' germination, such as knowledge of the ideal germination temperature, of seed and seedling morphology. This knowledge enables helping seed analysts in developing germination tests. However, to date, only information about *in vitro* ornitogalo propagation (Salachna & Zawadzinska 2014, Gitonga *et al*. 2015) is known, and information of ornitogalo seedlings from seeds are unknown.

The objective of this work was to study the germination of the species and define the evaluation criteria of its seedlings.

The ornitogalo bulbs were planted in March, in a fiberglass greenhouse covered with diffuser agricultural film (ELVD), with 150 μm thickness, average temperature of 23.2 °C during the cultivation period in pots. 10 L plastic, containing commercial substrate based on Pinus bark, vermiculite, and carbonized rice shells. The sprouting of flower stems began 80 days post planting, with full bloom starting approximately 110 days, in August (Fig. 1A). Plants remained in vases until October, when flowering, pollination by insects, and fruits formation happened. After dehiscence of the fruit was observed a visible separation of loculi with dry aspect (Fig. 1B).

Branches containing the dry fruits were taken to the Seed Analysis Lab., where the fruits (Fig. 2A) and the loculi were separated (Fig. 2B) and manual extraction of the seeds (Fig. 2C). After cleaning, the seeds were homogenized, separated into four sub-samples using a mechanical sorter (statistical repetitions), and stored at 17 ± 2 ºC.

The water content and the weight of a thousand seeds, recently harvested, were determined according to the Rules for Seed Analysis (Brasil 2009). The number of seeds by locule and seeds per fruit were established by counting seeds from 50 fruit, obtaining the mean for the repetitions.

Figure 1. Reproductive structure of *Ornithogalum saundersiae* (ornitogalo). **A.** inflorescence at the point of maximum floral opening; **B.** dehiscent fruits with opening of the loculi. Scale bar: A-B = 2.5 cm.

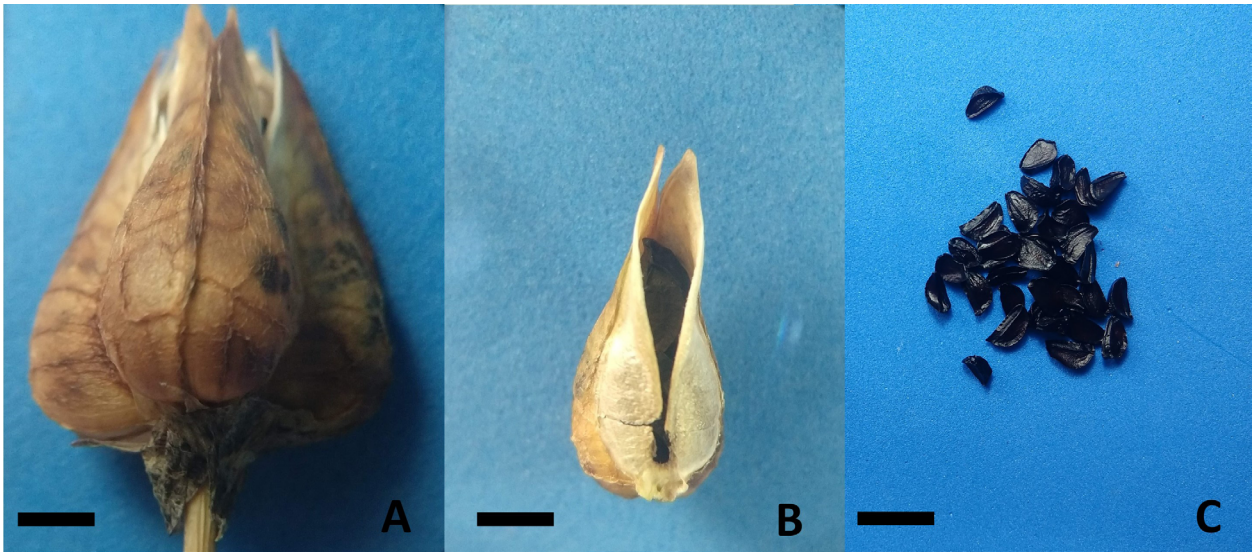

Figure 2. Obtaining the seeds of *Ornithogalum saundersiae*. (ornitogalo). **A.** dry fruits; **B.** open the fruit locule with the seeds inside; **C**. seeds. Scale bar: A-B = 1.0 cm, C = 2.5 cm.

For studying of germination, expressed in percentage of normal seedlings, four 50 seed repetitions were evaluated, seeded in transparent plastic crates (11.0 x 11.0 x 3.5 cm) on two sheets of blotter paper, moistened with a volume of water equivalent to 2.5 times the mass of the dry substrate (Brasil 2009). Germination temperatures of 20, 25 and 30 ºC were tested, with continuous lighting, in a germination Mangelsdorf® type. Counts were performed daily, characterizing normal and abnormal seedlings, starting the observation of the first normal seedling, until germination became constant (Brasil, 2009). The Germination Speed Index (GSI) in each temperature was assessed using the mathematical expression proposed by Maguire (1962). The experimental design was complete randomized, with four repetitions of fifty seeds per repetition, and means compared using the Tukey test, at 5 % probability.

Ornitogalo seeds water content post-harvest was 13.8 %, similar to the one observed in *Ornithogalum longibracteatum* seeds of 13.3 % (Kulkarni *et al*. 2005). The weight of a thousand seeds was 23 g. The number of seeds per locule was 7.39 ± 1.89 and the number of seeds per fruit was 22.17 ± 5.67. Ornitogalo seeds are irregularly shaped, they are contracted at the base, smooth, dark in color; and length of 6 to 8 mm (Fig. 2A).

Despite temperatures affecting percentage and speed of germination of ornitogalo seeds, the seeds displayed no dormancy in any temperature tested (Tab. 1). Germination at 20 and 25 °C was similar; however, the highest GSI was obtained at 25 °C. This outcome is certainly related to the occurrence of temperatures in the 25 °C range (Luria *et al*. 2002) at the specie's Center of Origin, at the time of seed maturation.

Table 1. Germination percentage (G), germination speed index (GSI), germination start (GS) and germination end (GE) of *Ornithogalum saundersiae* (ornitogalo) seeds at different controlled temperatures (T).

T (ºC)	G (%)	GSI	GS (days)	EG (days)
20	95 a	3,5 b	23	36
25	92 a	4,6 a	15	31
30	84 b	1,5 c	43	69
CV (%)	2,03	3,82	-	-

Means followed by the same letter in the column do not differ by Tukey's test, at 5% probability. Coefficient of Variation (CV).

The lowest percentage of germination were observed for the temperature of 30 ºC, showing that the species are not adapted to higher temperatures, which may cause the denaturation of essential proteins, directly affecting the enzymatic reactions in the seeds, reducing the percentage and speed of germination (Qu *et al*. 2013, Flores *et al*. 2014).

The highest GSI occurred at 25 °C (Tab.1). All seeds had germinated 31 days after seeding. When seeds were maintained at 20 or 30 °C, them germinated after 36 and 69 days, respectively. It is worth highlighting that, at a temperature of 25 °C, the germination started 15 days after seeding, whereas at a temperature of 30 °C, this could only be observed at 43 days. Therefore, in the seed germination test, we suggest the temperature of 25 °C, with the first seedling count on the 15th day and ending on the 31st day.

Kulkarni *et al*. (2005), working with *Ornithogalum longibracteatum*, obtained a higher germination rate at a temperature of 25 ° C, a result similar to the one obtained in this paper, where the highest germinations rates were obtained at temperatures of 20 and 25 °C, with no statistical difference. On the other hand, for temperature of 30 °C, both papers showed a drop in germination (Tab. 1).

Knowledge of seed morphology and initial growth stages of seedling promotes accurate interpretation of seed quality testing. Figure 3 shows the seed and seedlings morphology of ornitogalo; a recently harvested seed (Fig. 3A); the protrusion of the radicle (Fig. 3B); the appearance of radicular hairs (Fig. 3C); the appearance of the bulb primordium (Fig. 3D); the development of radicular structures and the bulb primordium (Fig. 3E); the leaf primordium formation (Fig. 3F) and the normal seedling, per seed (Fig. 3G), with all essential structures appropriately formed and the minimum size for its differentiation (2 cm leaf length). Normal seedlings are those that display potential to continue developing and to give rise to normal plants, when developing under favorable conditions (Brasil 2009). Normal seedlings display protrusion of the root, radicular hairs, bulb primordium and leaf primordium formation, resulting in establishing a complete seedling. Abnormal seedlings are characterized by absence or deformities of the vital structures (primary root, hypocotyl and plumule).

The occurrence of abnormal seedlings may be the consequence of genetic, environmental, or handling practices factors. These factors result in the absence, atrophy, or deformity of the vital structures (primary root, hypocotyl and plumule) (Brasil 2009). The most common abnormalities found in the germination test were seeds with primary infection, transmitted by the seed, (Fig. 3H); seeds with secondary infection (Fig. 3I), which interrupted the seedlings' development; and seedlings with atrophied roots, forming a short, thick root without root hair (Fig. 3J). Other abnormalities identified were deformed bulb beginnings and the no primary bulb formation.

The ideal temperature for the *Ornithogalum saundersiae* seeds germination is 25 °C, providing a high percentage of normal seedlings (92 %). The first count should be performed at 15 days after seeding and the last, at 31 days.

Figure 3. Normal and abnormal seedlings of *Ornithogalum saundersiae* Baker. (ornitogalo). **A.** recently harvested seed; **B.** protrusion of the radicle; **C.** radicular hair; **D.** The appearance of the bulb primordium; **E.** development of root structures and the bulb primordium; **F.** leaf primordium formation; **G.** normal seedling, per seed; **H.** abnormal seedling with primary fungal infection; **I.** abnormal seedling with secondary fungal infection; **J.** abnormal seedling with atrophied roots. Scale bar: A = 0.25 cm; B-G = 0.50 cm, H-J = 1.0 cm.

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