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Article

Fruit and Seed Morphometry and in Vitro Germination of Hollyhock Seeds

Talita dos Santos Ferreira [1], Rogério Gomes Pêgo[2], Rosária da Costa Faria Martins [3]

[1] Mestra em Fitotecnia. Universidade Federal Rural do Rio de Janeiro. ORCID: 0000-0001-6796-1656. E-mail: talirafalau@gmail.com

[2] Doutor em Fitotecnia. Universidade Federal de Viçosa Professor da Universidade Federal Rural do Rio de Janeiro. ORCID: 0000-0002-2122-6442.. E-mail: engagropego@yahoo.com.br

[3] Mestra em Fitotecnia. Universidade Federal Rural do Rio de Janeiro. ORCID: 0000-0001-7590-2939. E-mail: rosaria.cfmartins@gmail.com

ABSTRACT

Alcea rosea L. is an ornamental shrubs that has many possible uses, including medical purposes. This species is mainly propagated by seeds, however, there are few studies that describe their morphometric characteristics, in addition, previous reports show that they have dormancy that makes the germination difficult. Therefore, this work aimed to determine the morphological characteristics of the fruit and seeds of *A. rosea* and to evaluate its in vitro seeds germination under different pregerminative methods in different culture media. For the morphological description, 25 fruits or seeds were evaluated. For the in vitro germination in the MS and WPM culture media, the pregerminative treatments were: T1 - untreated seeds (control); T2 - mechanical scarification (cut in the seed coat); T3 - thermal scarification (immersion of the seeds in water at 60 ºC for 16 min). The experimental design was completely randomized, in a 3x2 factorial scheme (pregerminative treatments x culture media), with eight replications and twenty seeds per replication. A visual scale was proposed to classify the seedlings vigor. The schizocarp hollyhock fruit is flattened and its diameter is greater than its height, and each fruit contains 33 seeds, on average. The seeds have approximately 4.2 mm in length and 100 seeds weight, on average, 0.89 g. The culture medium does not influence the seeds germination and the pregerminative treatments are not necessary for the germination. However, the mechanical scarification promoted the best germination speed index and the production of more vigorous plants.
Keywords: *Alcea rosea* L., seeds dormancy, scarification, vigor scale, medicinal.

RESUMO

A *Alcea rosea* L. é um arbusto ornamental que tem muitas possibilidades de usos, incluindo fins medicinais. Essa espécie é propagada principalmente por sementes, no entanto, existem poucos estudos que descrevem suas características morfométricas, ademais, relatos anteriores mostram que elas têm dormência que dificulta a germinação. Portanto, este trabalho teve como objetivos determinar as características morfológicas dos frutos e sementes de *A. rosea* e avaliar a germinação in vitro de suas sementes sob diferentes métodos pré-germinativos em diferentes meios de cultura. Para a descrição morfológica, foram avaliados 25 frutos ou sementes. Para a germinação in vitro nos meios de cultura MS e WPM, os tratamentos pré-germinativos foram: T1 - sementes não tratadas (controle); T2 - escarificação mecânica (corte no tegumento); T3 - escarificação térmica (imersão das sementes em água a 60 ºC por 16 min). O delineamento experimental foi inteiramente casualizado, em esquema fatorial 3x2 (tratamentos pré-germinativos x meios de cultura), com oito repetições e vinte sementes por repetição. Uma escala visual foi proposta para classificar o vigor das mudas. O fruto esquizocarpo da alcea é achatado e seu diâmetro é maior que sua altura, e cada fruto contém, em média, 33 sementes. As sementes têm comprimento aproximado de 4,2 mm e 100 sementes pesam, em média, 0,89 g. O meio de cultura não influencia a germinação das sementes e não é necessário o uso de tratamento pré-germinativo para a germinação. Entretanto, a escarificação mecânica promoveu o melhor índice de velocidade de germinação e a produção de plantas mais vigorosas.
Palavras-chave: *Alcea rosea* L., dormência de sementes, escarificação, escala de vigor, medicinal.

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1. Introduction

Alcea rosea L., popularly known as hollyhock, is an ornamental shrubs traditionally grown under full sun, which can be used in massive beds, as border along walls or as isolated plants in gardens (LORENZI, 2015; EVIK & RUSSAK, 2019). Hollyhock plants are 2-3 m tall and produce up to 40 flowers daily; they have higth stately ornaments and produce large single, semi-double, or double flowers of many different colors; moreover, they have a long period of flowering (LI *et al.,* 2012; FAHAMIYA *et al.,* 2016; ORAEE *et al.,* 2019).

Beyond beauty, the hollyhock produces edible flowers that are used to make tea, which taste is slightly bitter (NEWMAN & O'CONNOR, 2009); besides that, it has a great folkloric medicinal history in the East Mediterranean, where all parts of the plants are used due to their medicinal properties such as their antioxidant, antimicrobial, antiviral and hepatoprotective actions, among others (AZAB, 2016; ABDEL-SALAM *et al.,* 2018; KALEMBA-DROŻDŻ & CIERNIAK, 2019).

The hollyhock is acropetal (basal flowers open first) and the seeds must be harvested from ripe fruits (JOHRI & RAGHUVANSHI, 2014). However, there are few works showing the morphometric description of its fruit and seeds, and it is very important for the management of seeds technology and breeding (BRASIL, 2009; LI *et al.,* 2012; FAHAMIYA *et al.,* 2016).

This species is often propagated by seeds, however, there are reports that indicate the presence of dormancy that inhibits its germination (BRASIL, 2009; FAHAMIYA *et al.,* 2016; BATTAL *et al.,* 2019). This fact may happen due to the impermeability of the integument to water, considered one of the main causes of dormancy in some species of the Malvaceae family (ENSCONET, 2009).

Different pregerminative methods have been used to overcome seeds dormancy such as scarification (mechanical, chemical or thermical) and the soaking of seeds in water (SHOOSHTARIAN & SALEHI, 2010; TIWARI *et al.,* 2016). Among the pregerminative treatments in hollyhock seeds, the scarification can be used through a pin-pricking or by cutting the seed coat with the aid of a scalpel, enabling the water absorption to increase the germination rates (BRASIL, 2009). Hot water (60-80 °C) is an alternative to improve the seed germination of others species of the same botanic family, nevertheless, boiling water (100 °C) can cause sensitive damage to the seeds (McDONNELL *et al.,* 2012).

The in vitro seeds germination can be a technique to optimize the propagation that is limited by other methods, and, it has been widely used to obtain seedlings of species of limited germination (LEE et al., 2013; PÊGO *et al.,* 2015; PRUDENTE *et al.,* 2015; BATTAL *et al.,* 2019). Many different basic media formulations have been used for the in vitro propagation of ornamental plants. The MS medium (MURASHIGE & SKOOG, 1962) is the most used one in the micropropagation of many species, however, for the seeds germination stage, it has been modified or diluted. On the other hand, the WPM medium (LLOYD & MCCOWN, 1980) has shown good results in the seed germination of native species or of other species that are sensitive to high salt concentrations, possibibly due to the fact that it contains neither cobalt nor iodine micronutrients and it has 4 times less nitrogen in comparison to MS (LARRABURU, 2012, KOENE et *al.,* 2019).

Previous studies have shown that the in vitro seed germination of *Alcea sp.* is influenced by the composition of the substrate; and it seems that these seeds are sensitive to the osmotic variations of the culture medium. Negative responses to *A. officinalis* seed germination were observed when sucrose was added in the medium (YOUNESIKELAKI et *al.,* 2016). The use of adequate methods to overcome the seed dormancy as well as the appropriate germination in the culture media, enable to obtain high quality and to achieve the classification of vigorous plants. The seedlings classification is a parameter that helps to choose the methodology or the plants for stages of the in vitro multiplication or even for the conservation of species.

There are few studies on the propagation of hollyhock, therefore, this work aimed to determine the morphological characteristics of the fruit and seeds of *A. rosea*; and to evaluate the in vitro germination of its seeds under different pregerminative methods in different culture media.

2. Material And Methods

Ripe fruits of hollyhock were harvested and taken to the laboratory for their morphological characterization and biometric analysis. The metric dimensions of fruits and seeds were carried out with the aid of a graduated caliper. The biometric data were obtained from 25 replications of fruits or seeds and subjected to quantitative analysis. For the morphological description, it was observed the form and the color of the fruits. The 1000-seeds weight was determined according to BRASIL (2009).

The seeds were selected and those visibly empty or attacked by pests were retained. The selected ones were used in the in vitro germination, and for it, the following pregerminative methods were tested: T1 - untreated seeds (control); T2 - mechanical scarification by cutting the seed coat with a scalpel, according to BRASIL (2009); T3 - thermal scarification by the immersion of the seeds in hot water (60 ºC) for 16 min, which were later kept still until they cooled naturally and reached room temperature (25 ºC).

After the pre-treatments, the seeds were sterilized under flow cabinet with 70% ethanol for 5 min, followed by sodium hypochlorite 2.5% (v/v) for 15 min and washed three times with sterile distilled water. The sterilized seeds were placed in sterile flasks containing 20 mL of culture medium. The MS (MURASHIGE & SKOOG, 1962) and WPM - Woody Plant Medium (LLOYD & MCCOWN, 1981) mediums were used. Both media were prepared with the addition of 30 g L^{-1} of sucrose and 8 g L^{-1} of agar, and the pH was adjusted to 5.8 before being sterilized in autoclave for 20 min at 121 °C. After the inoculation, the seeds were kept in a growth chamber at 30 $\mu mol.m^{-2}.s^{-1}$ of photonsirradiance, 25 °C ± 2 °C of temperature, and under 16-hour photoperiod provided by fluorescent lamps (OSRAM®, similar to daylight).

The germination data were registered daily and at the end of the observation period (seven days after the inoculation), the germination (%) and the germination speed index (GSI) were calculated according to MAGUIRE (1962). A classification to discriminate the vigor of seedlings was proposed, as follows: high vigor (HV) - seedlings with the presence of primary root and with completely open cotyledon leaves; medium vigor (MV) - seedlings with short primary root and shoot in the beginning of development; and low vigor (LV) - seedlings with the beginning of the radicle emission and with no shoot emitted.

The experimental design was completely randomized, in a 3 x 2 factorial scheme (three pregerminative treatments and two culture media), with eight replications and twenty seeds per replication. The data were subjected to the analysis of variance and the means were compared by the Tukey's test at 5% probability level, using the statistical program Sisvar (FERREIRA, 2011).

3. Results and Discussion

The hollyhock fruits are schizocarp (Figure 1A), with a persistent cálix that turns brown on ripening; which occurs at different periods over the cycle because of the acropetal flowering (JOHRI & RAGHUVANSHI, 2014). Inside the fruits are produced many mericarps arranged radially (Figure 1B), with a black seed in each one of them (Figure 1C).

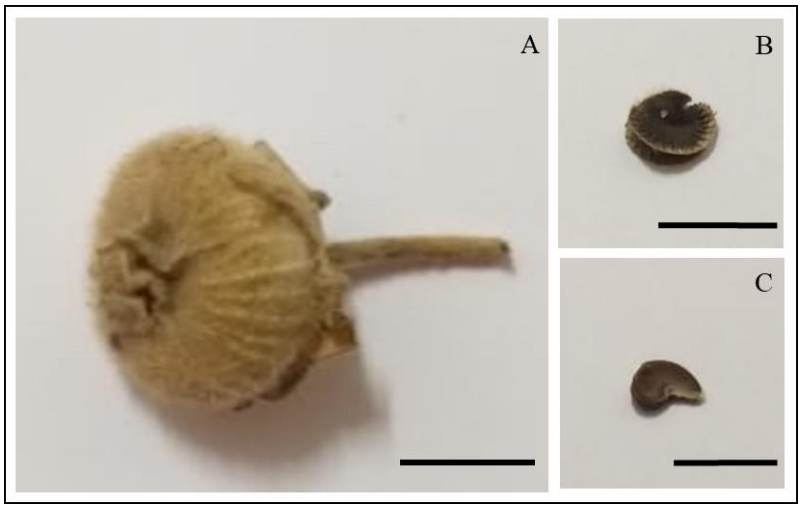

Figure 1. Schizocarpic fruit (A), mericarp (B) and seed (C) of hollyhock. Bars: A = 1.0 cm; B and C = 0.5 cm.

Hollyhock fruits (schizocarp) are small and flattened and their diameter is greater than their height, and each fruit contains 33 seeds, on average. The seeds are homogeneous with approximately 4.2 mm in length and 100 seeds weight, on average, 0.89 g (Table 1), equivalent to 113 seeds per gram.

Table 1. Fruit diameter (FD), fruit height (FH), number of seeds per fruit (NSF), seed length (SL), seed width (SW) and 1000-seeds weight (1000-SW) of hollyhock.

	FD (mm)	FH (mm)	NSF -	SL (mm)	SW (mm)	1000-SW (g)
Maximum	22.92	8.75	37.00	4.48	3.26	9.04
Average	20.70	7.95	32.52	4.19	2.97	8.88
Minimum	18.70	7.00	22.00	3.87	2.64	8.61
CV (%)	4.74	6.66	8.41	3.97	5.89	1.61

The seed germination of *A. rosea* started two days after the inoculation on the MS and WPM culture media. In this study, it was observed that, even though the culture medium does not influence the hollyhock seeds germination, it seems that there is no dormancy, as frequently reported in the literature, because even in the untreated seeds, the germination was higher than 93% (Figure 2A).

Despite the fact that previous studies reported that hollyhock has coat dormancy, through the observation of 1% of germination when no pregerminative treatment was used (SHOOSHTARIAN & SALEHI, 2010), a study shows that the in vitro germination improves the germination of hollyhock seeds (*A. rosea*), when the tegument is partially removed with a scalpel, reaching 80% of germination (KEW, 2019). Similarly, the germination rates of *Alcea kurdica* can reach 90% after the treatment with a cut in the seed (BATTAL *et al.,* 2019).

The pregerminative treatments influenced significantly the GSI (Figure 2B). There was a significant interaction among the pregerminative treatments and the culture media. It was observed that the highest GSI was achieved when the seeds were treated by the mechanical scarification, regardless the culture medium. However, when the seeds were treated with the thermal scarification, the GSI was slightly higher in the WPM medium compared to MS. There was no significant difference in the GSI between culture media in the untreated seeds. It seems that the mechanical scarification enables the quick absorption of water, in order to

allow the faster germination process in comparison to the other pregerminative treatment methods (URSULINO *et al.,* 2019).

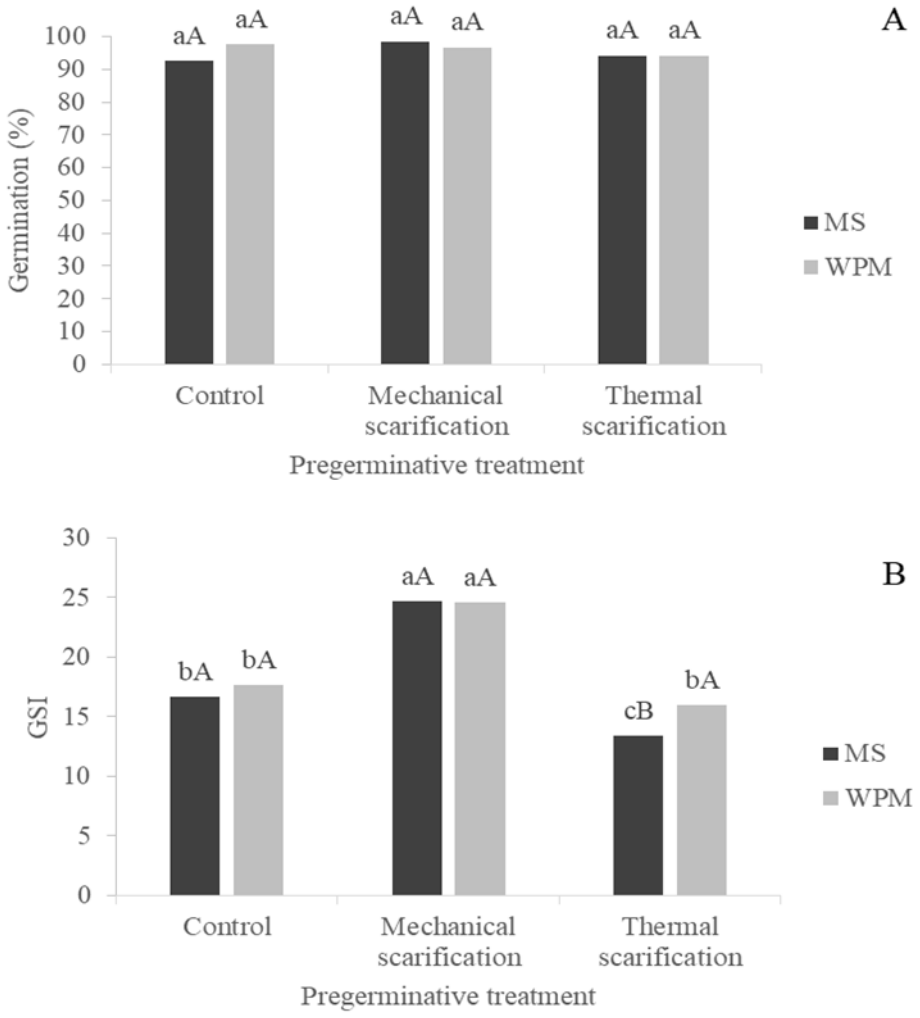

Figure 2. Germination (A) and germination speed index (GSI) (B) of hollyhock seeds grown in different culture media. Mean values followed by different capital letters between the media and lowercase letters among the pregerminative treatments significantly differ from each other by the Tukey's test at 5%.

A scale to classify the quality of the seedlings was established (Figure 3). According to the rules for seed analysis (BRASIL, 2009), a seedling is normal when it has all the essential structures and when it is properly developed and healthy. The standardization of the quality of seedlings has a wide application in agriculture because it ensures that vigorous materials with physiological quality are efficiently classified. There are no visual standards to classify the hollyhock seedlings quality, therefore, the development of a visual scale may help in future studies of this species, whether for in vitro propagation or for seed technology and analysis, in which other types of seeds are used. The development of visual scales helps to minimize mistakes during the seeds classification, minimizing the subjectivity in the essay (BRASIL, 2009; MARCOS FILHO, 2015).

Figure 3. Visual scale of the vigor of hollyhock seedlings: high vigor (A); medium vigor (B) and low vigor (C). Bars = 1 cm.

There was a significant interaction among the pregerminative treatments and the culture media. The mechanical scarification allowed the production of high vigor seedlings in the MS and in the WPM media (Table 2). However, the highest percentages of low vigor seedlings were found in the intact seeds or in the ones treated with hot water (60 ºC), regardless the medium used.

Table 2. Vigor classifications of in vitro hollyhock seedlings obtained from seeds submitted to pregerminative treatments and grown on different culture media.

Treatment	HV (%)		MV (%)		LV (%)	
	MS	WPM	MS	WPM	MS	WPM
Control	11 bA*	19 bA	5 bB	17 bA	78 aA	59 aA
Mechanical scarification	51 aA	39 aA	21 aB	33 aA	26 bA	23 bA
Thermal scarification	12 bA	6 bA	6 bB	22 bA	77 aA	67 aA
CV (%)	7.1		5.8		3.3	

* Mean values followed by different capital letters between the media and lowercase letters among the pregerminative treatments significantly differ from each other by the Tukey's test at 5%.

There was a relationship between the GSI (Figure 2B) and the vigor classifications of the seedlings detected through the visual scale of hollyhock. This happened because plants that germinate quickly have a better capacity to show their physiological potential in optimal conditions of seed germination (MARCOS FILHO, 2015). The classification of vigor, based on the visual scale, allowed to detect more accurate physiological expressions than those only presented by the maximum seed germination. Besides that, many studies correlate plant morphology with the successful micropropagation of many species, suggesting the selection of plants with stronger morphological characteristics (YOUNESIKELAKI *et al.,* 2016; SILVA *et al.,* 2019; KOENE et al., 2019).

In practice, this new scale of vigor proposed for hollyhock allows to identify the seedlings that have a greater potential to be selected for future stages of seedlings production, such as in vitro multiplication. In addition, this methodology is the first proposal for rating the vigor of seedlings of hollyhock, which contributes to other areas of research, such as seed technology.

4. Conclusions

The schizocarp hollyhock fruit is flattened and its diameter is greater than its height, and each fruit contains 33 seeds, on average. The seeds have approximately 4.2 mm in length and 100 seeds weight, on average, 0.89 g.

The culture medium does not influence the seeds germination and the seeds do not need any pregerminative treatment to germinate. However, the mechanical scarification promoted the best germination speed index and the production of more vigorous plants.

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