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Physical and physiological characterization of heteromorphic seeds of *Erythrina velutina* Willd.

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ABSTRACT: *Erythrina velutina* Willd. is a xerophytic plant native to the Caatinga, whose seeds show heteromorphism in seed coat color, varying between reddish and orangish. This study aims to analyze the influence of seed coat color on the physical characteristics, dormancy and physiological potential of the seeds of this species. The seeds were evaluated for length, width, moisture content, hundred-seed weight, water absorption curve, first count, germination, hard seeds, shoot and root length, and shoot and root dry mass. In addition, cross-sectional fragments of 10 seeds of each color were examined by means of a scanning electron microscope (SEM), which was used to visualize the epidermis, palisade layer and parenchyma cell layers. The experimental design was completely randomized with four replications of 25 seeds in a 2 x 2 factorial scheme, considering seed coat color (orangish and reddish) and dormancy overcoming (intact and scarified seeds). The data were subjected to analysis of variance and compared by Tukey test at 5% probability level. There was no significant influence of seed coat color on the physical characterization of the seeds. It was found that reddish seeds have greater dormancy and lower physiological potential when compared to orangish seeds.

Index terms: dormancy, Fabaceae, forest seeds, heteromorphism.

RESUMO: *Erythrina velutina* Willd. é uma planta xerófila nativa da Caatinga, cujas sementes apresentam heteromorfismo na coloração do tegumento, variando entre avermelhadas e alaranjadas. Este estudo objetiva analisar a influência da cor do tegumento nas características físicas, dormência e potencial fisiológico das sementes dessa espécie. As sementes foram avaliadas quanto ao comprimento, largura, umidade e peso de cem sementes, curva de absorção de água, primeira contagem, germinação, sementes duras, comprimento da parte aérea e raiz, e massa seca da parte aérea e raiz. Além disso, foram examinadas seções transversais de 10 sementes de cada cor por meio de microscópio eletrônico de varredura (MEV) em que foram visualizadas a epiderme, camada paliçádica e camadas de células parenquimáticas. O delineamento experimental foi o inteiramente casualizado com quatro repetições de 25 sementes em esquema fatorial 2 x 2, considerando a cor do tegumento (alaranjada e avermelhada) e a superação de dormência (sementes intactas e escarificadas). Os dados foram submetidos à análise de variância e comparados pelo teste de Tukey a 5% de probabilidade. Não houve influência significativa da cor do tegumento na caracterização física das sementes. Constatou-se que as sementes avermelhadas possuem maior dormência e menor potencial fisiológico quando comparadas às alaranjadas.

Termos para indexação: dormência, Fabaceae, sementes florestais, heteromorfismo.

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INTRODUCTION

Erythrina velutina Willd., Fabaceae family, is a xerophytic species native to the Caatinga with occurrence in the semi-arid region of Brazil. It can reach 12 to 15 m in height and is characterized as an arboreal deciduous plant, occurring in forest fragments of Caatinga and Cerrado. Widely known in Brazil as "mulungu", "mulungu-velutina", "canivete", "corticeira" and "suinã", it stands out in the production of wooden artifacts (toys, rafts and crates), with medicinal and ornamental applications, besides being used in agroforestry systems (Alves-Junior et al., 2016; Macêdo et al., 2018). Its seeds show seed coat dormancy, which leads to very slow and uneven germination (Silva et al., 2021a).

Dormancy intensity in seeds can vary due to the influence of the genotype, as well as their position within the fruit, and also unevenness of maturation and climatic changes during maturation (Marcos-Filho, 2015). This difference in dormancy intensity plays an important ecological role, since it distributes germination over time (Silva et al., 2018). Although dormancy is an important evolutionary strategy to ensure germination over time under natural conditions, this characteristic can hinder the cultivation and commercial production of quality seedlings, posing a challenge for nurseries and reforestation programs.

In addition to showing dormancy, *E. velutina* seeds are heteromorphic, being found in the final stage of maturation with reddish or orangish colors. Many species produce heteromorphic seeds to ensure a safe propagation of the next generation; for this, these species adopt different adaptive strategies and modify morphological characteristics of the seeds, for example changing seed coat color (Liu et al., 2018). In addition, it can also be considered an adaptive strategy that helps species survive in changing and unpredictable environments, especially in deserts or salinized soils (Xu et al., 2016).

Heteromorphism can also be used as a visual morphological index to inform about the maturity and physiological potential of seeds (Soares et al., 2016), as most species show changes in seed coat color along their development process. The physiological potential combines information on viability (germination) and vigor of seeds, which can influence the production of quality seedlings (Marcos-Filho, 2015; Medeiros et al., 2019). For *E. velutina*, no information was found on the influence of heteromorphism on physiological potential and dormancy. Such information is particularly important for nurserymen, who will be able to classify seeds based on seed coat color to ensure quality and success in seedling production.

Research studies with other forest species show that heteromorphism influences the physiological quality and dormancy of seeds, as in *Bowdichia virgilioides* Kunth, whose results indicated that seeds with dark red color had greater physiological potential (Silva et al., 2021b). For *Parahancornia fasciculata* (Poir) Benoist, it was found that brown seeds performed better than yellow ones (Duarte et al., 2021). Seeds of *Medicago denticulata* with cream-colored seed coat have seed coat dormancy, while brownish seeds do not (Sethi et al., 2020).

Studies on seed heteromorphism can contribute to better regeneration and protection of biodiversity, especially of arid and semi-arid ecosystems (Moulay et al., 2023). In addition, knowledge about the morphological characteristics associated with the ecology of native species enables a better understanding of the functionality of these structures in ecosystems, contributing to the development of environmental conservation and restoration programs (Cosmo et al., 2017).

In view of the above and also considering that the results of this study can help nurserymen in the management and selection of seeds, the aim was to assess the influence of seed coat color on the physical characterization, dormancy and physiological potential of *E. velutina* seeds.

MATERIAL AND METHODS

The experiment was conducted at the Seed Analysis Laboratory (LAS) of the *Universidade Federal Rural do Semi-Árido* (UFERSA), in Mossoró, RN, Brazil. The seeds were collected from three parent trees located at UFERSA, campus of Mossoró, RN, in June 2019, where the climate is hot and dry, with an average temperature of 27.5 °C and irregular rainfall (Silva, 2020). The fruits were in the final stage of maturation, with a dark brown color, starting dehiscence.

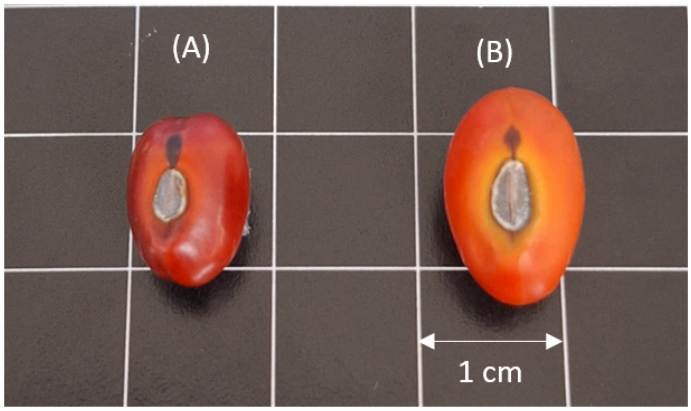


Figure 1. *Erythrina velutina* seeds Willd. with reddish (A) and orangish (B) seed coat.

After extracting the seeds from the fruits, they were separated based on seed coat color into orangish and reddish (Figure 1). The seeds were stored for three years in plastic bottles in a controlled environment (± 17 °C; 55% RH) until the experiment was carried out.

Initially, the following determinations were made with *E. velutina* seeds of both colors:

Hundred-seed weight: determined using 100 seeds of each color, by individually weighing each sample on a precision scale (0.0001 g), with results expressed in grams.

Moisture content: performed by the oven method at 105 °C (Brasil, 2009) for 24 hours, with two replications of approximately 6.0 g for each color, with results expressed as a percentage.

Seed biometrics: a sample of 100 seeds of each color was randomly collected and their length and width were measured with a digital caliper (accuracy of 0.01), with results expressed in mm.

Imbibition curve: part of the seeds of each color was kept intact (without overcoming dormancy) and the other part was cut at the opposite side of the hilum with pliers (Brasil, 2013). Four replications of 25 seeds were used for each combination (intact orangish seeds; scarified orangish seeds; intact reddish seeds; scarified reddish seeds). Prior to imbibition, the initial mass of the seeds was measured on a precision scale. For water absorption, the seeds were placed on two sheets of paper towels, arranged in the form of an envelope, previously moistened with distilled water with a volume of 2.5 times the weight of the sheets, and placed in Biochemical Oxygen Demand (B.O.D.) germination chambers at 30 °C. The weighing procedures started from 1 hour of imbibition, with an initial interval of 1 hour for the first 12 hours and then every 12 hours until the end of the test, when at least 50% of the seeds of each replication had at least 2 mm of primary root. The data obtained were then used to create graphs in the Excel program.

Electron micrographs of the seed coat: performed under a scanning electron microscope (SEM) using 10 seeds of each color. Cross-sectional cuts were made in the median region of the dry seeds with steel blade. Then, the material was metallized with palladium gold, the palisade layer and parenchymal cell layer were measured, and the results were expressed in µm.

Germination test: set up to assess the physiological potential of the seeds with different colors, in a completely randomized design in a 2 x 2 factorial scheme, totaling four treatments, with four replications of 25 seeds each. The first factor refers to seed coat color (orangish and reddish) and the second factor refers to dormancy overcoming (intact and scarified). The substrate used was paper towel, previously moistened with distilled water in a volume equivalent to 2.5 times its dry weight. The seeds were distributed on two sheets and covered by a third sheet, arranged in the form of a roll and placed to germinate in a B.O.D. germination chamber at 30 °C under photoperiod of 8 h. The first and last counts were performed at 7 and 14 days, respectively (Brasil, 2013), and the results were expressed in percentage of normal seedlings.

Shoot and root length: at the end of the germination test, 10 normal seedlings of each treatment were randomly chosen to measure shoot and root with a ruler graduated in centimeters. Root was measured considering the distance from the insertion of the collar to the end of the main root. Shoot was measured considering the distance from the insertion of the collar to the insertion of the first leaves. The results were expressed in centimeters.

Shoot and root dry mass: the normal seedlings previously measured for length were separated into shoot and root, placed in a kraft paper bag and dried in an oven with forced air circulation at 65 °C for 72 hours. Then, the material was weighed on an analytical scale (0.001 g), and the results were expressed in grams.

Hard seeds: at the end of the germination test, the number of hard seeds was also counted, considering as hard seeds those that did not absorb water. The results were expressed as a percentage.

The data were subjected to analysis of variance and the means were compared by Tukey test at 5% probability level, with the aid of the statistical program Sisvar (Ferreira, 2019).

RESULTS AND DISCUSSION

Reddish and orangish seeds had similar values of moisture content and hundred-seed weight (Table 1). Moisture content was equal to 8.3% in orangish seeds and 8.4% in reddish seeds. According to Ribeiro and Dantas (2019), *E. velutina* seeds exhibit orthodox (anhydrobiotic) behavior regarding tolerance to desiccation and, consequently, storage. In relation to hundred-seed weight, an average of approximately 48 grams was obtained for both colors (Table 1). This result makes it possible to infer that there are on average 2,127 seeds per kilogram, which is within the range reported by Brasil (2013), from 1,000 to 3,700 seeds.kilogram^{-1}. The number of seeds per kilogram is important information for farmers, as it allows them to calculate the amount of seeds needed for planting a given area.

During the biometric characterization of the seeds, similarities were found between their measurements. In orangish seeds, the length and width ranged from 10.5 to 18.9 mm and from 6.2 to 9.0 mm, respectively, while in reddish seeds, the length and width ranged from 11.2 to 15.9 mm and from 6.2 to 9.6 mm, respectively (Table 2 and Figure 2). These values are close to those found by Bezerra et al. (2022) when they emphasized that the environmental conditions of the different regions of Paraíba influenced the biometric characteristics of seeds of this same species, with length measurements ranging from 9.00 to 16.84 mm, and width from 5.0 to 12.99 mm. In seeds of *Retama sphaerocarpa*

Table 1. Moisture content and hundred-seed weight of orangish and reddish seeds of *Erythrina velutina* Willd.

Seed coat color	Moisture content (%)	Hundred-seed weight (g)
Orangish	8.3	47.98
Reddish	8.4	47.69

Table 2. Mean, maximum, minimum values and standard deviations of orangish and reddish seeds of *Erythrina velutina* Willd.

Seed coat color	Dimension	Mean (mm)	Maximum (mm)	Minimum (mm)	Standard deviation
Orangish	Length (mm)	13.5	18.9	10.5	1.293
	Width (mm)	7.9	9.0	6.2	0.674
Reddish	Length (mm)	13.7	15.7	11.2	0.952
	Width (mm)	7.7	9.6	6.2	0.745

with different seed coat colors, no differences in morphometry were found between yellowish and greenish seeds, but yellowish seeds germinated faster than greenish seeds (Moulay et al., 2023).

Biometric analyses are important parameters for determining intrinsic and extrinsic variability between populations and constitute tools for defining the relationship between factors related to environmental issues and genetic variability. Seed size is an important basic characteristic for understanding the survival, dispersal, and establishment of seedlings of a species (Pontes et al., 2018). In addition, the size of fruits and seeds can affect the germination process, as well as aspects related to the vigor of the seedlings formed, as verified by Leão-Araújo et al. (2020) in seeds of *Campomanesia adamantium*, whose size did not affect germination, but large seeds produced larger seedlings. Larger seeds with more reserves are capable of generating seedlings of greater vigor and with better physical and physiological characteristics (Carvalho and Nakagawa, 2012).

The results obtained in the imbibition curve of *E. velutina* indicate that, within the same time interval, orangish seeds showed greater weight gain than reddish seeds, regardless of scarification (Figure 3). Consequently, scarified orangish seeds produced the primary root earlier, with 108 hours of imbibition, while reddish seeds produced it only at 120 hours. Intact orangish seeds took 204 hours for at least 50% to germinate, while reddish seeds required 216 hours

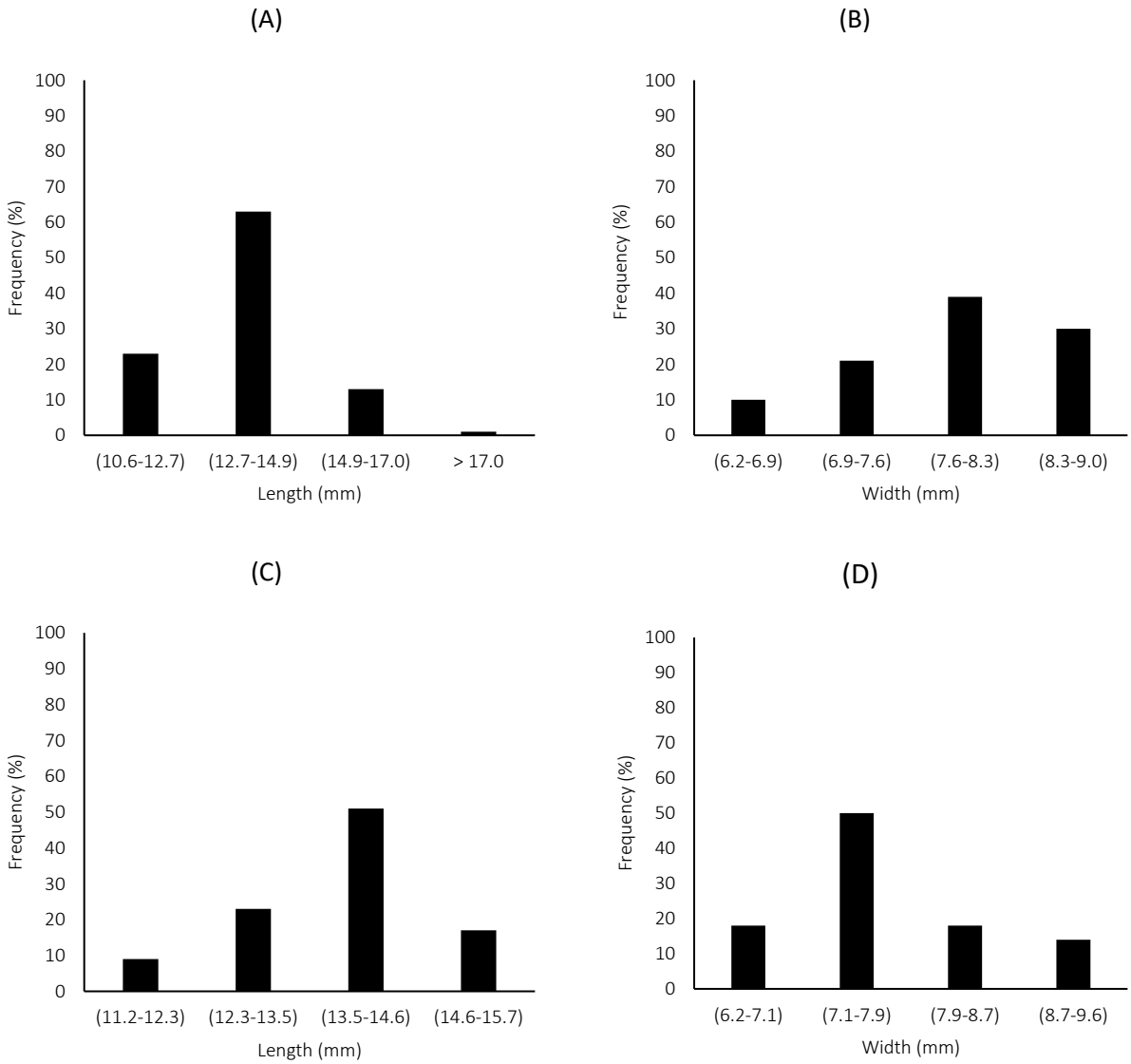


Figure 2. Frequency of length and width of orangish (A and B) and reddish (C and D) seeds of *Erythrina velutina* Willd.

of imbibition and only 18% had produced the primary root (Figure 3). With this, it can be inferred that reddish seeds have a more rigid seed coat compared to orangish seeds, so by opting for orangish seeds, the producer will reduce costs and time with treatments to overcome dormancy. These seeds possibly have a higher concentration of substances that confer greater hardness, such as lignin, cutin and suberin.

Studies carried out with water absorption curve in seeds of the Fabaceae family have reported that water gain is slower with the seed coat intact, due to the cell layers with thickened walls present in the seeds, which confer impermeability and resistance to water entry (Matos et al., 2015).

The results presented previously are consistent with the images obtained by scanning electron microscopy, as seed coat thickness measurements varied as a function of seed coat color (Figures 4A and 4B). The results

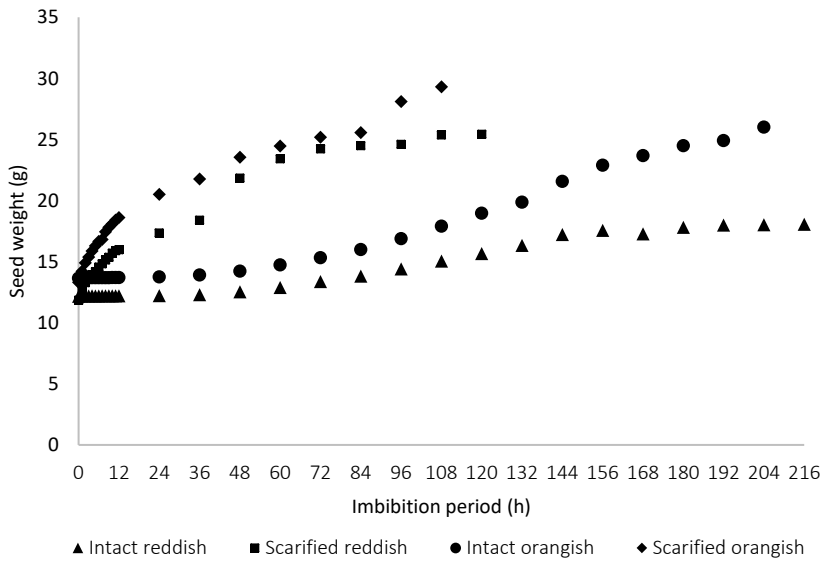


Figure 3. Water absorption curve in intact and scarified orangish and reddish seeds of *Erythrina velutina* Willd.

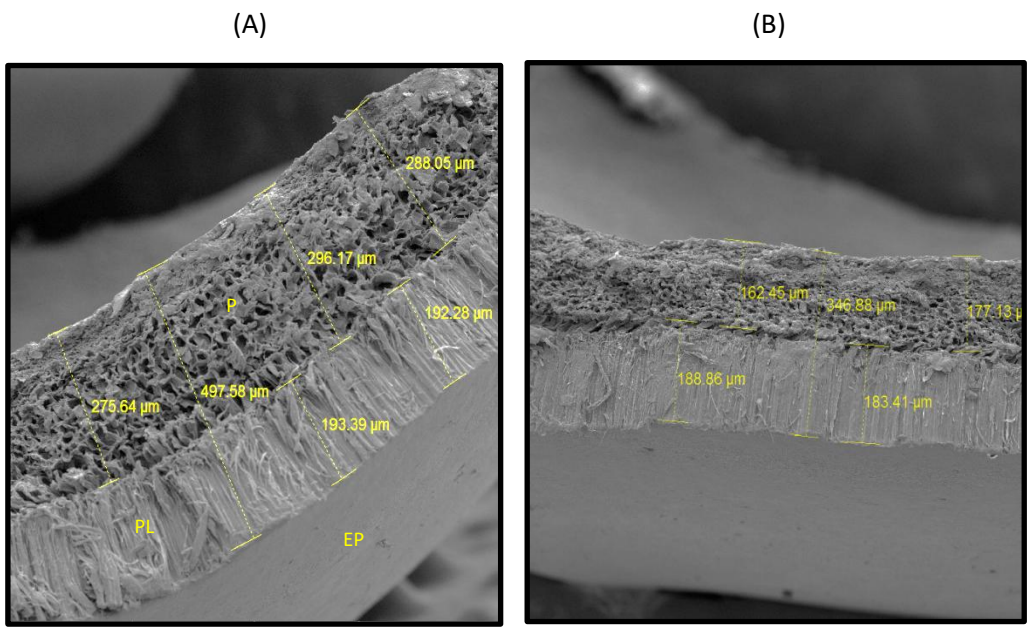


Figure 4. Electron micrographs of cross-sectional sections of the seed coat of reddish (A) and orangish (B) seeds of *Erythrina velutina* Willd. (EP = epidermis; PL = palisade layer; P = parenchyma cell layer).

obtained for palisade layer thickness were similar between the two colors, with an average of 195 µm for reddish seeds and 185 µm for orangish seeds. In turn, the hypodermal parenchyma cell layer thickness showed greater variation, with an average of 290 µm for reddish seeds and 160 µm for orangish seeds. Seed coat permeability in heteromorphic seeds is related to seed coat thickness, which is usually lower in seeds with lower intensity of dormancy (Liu et al., 2018).

Regarding the data obtained in the first germination count, it is possible to verify that for both colors, scarified seeds were statistically superior to intact seeds. However, intact orangish seeds already had more than 50% of germination, while reddish seeds showed an average lower than 30% (Figure 5A).

Germination results, obtained at 14 days, also showed significant differences as a function of seed coat color. However, intact orangish seeds showed germination similar to that of scarified seeds, *i.e.*, for seeds of this color there is no need to overcome dormancy. However, reddish seeds showed different behaviors, with scarified seeds being statistically superior to intact ones (Figure 5B). At the end of the germination test, a higher percentage of hard seeds was also found in reddish seeds compared to orangish ones, corroborating the results found in the first germination

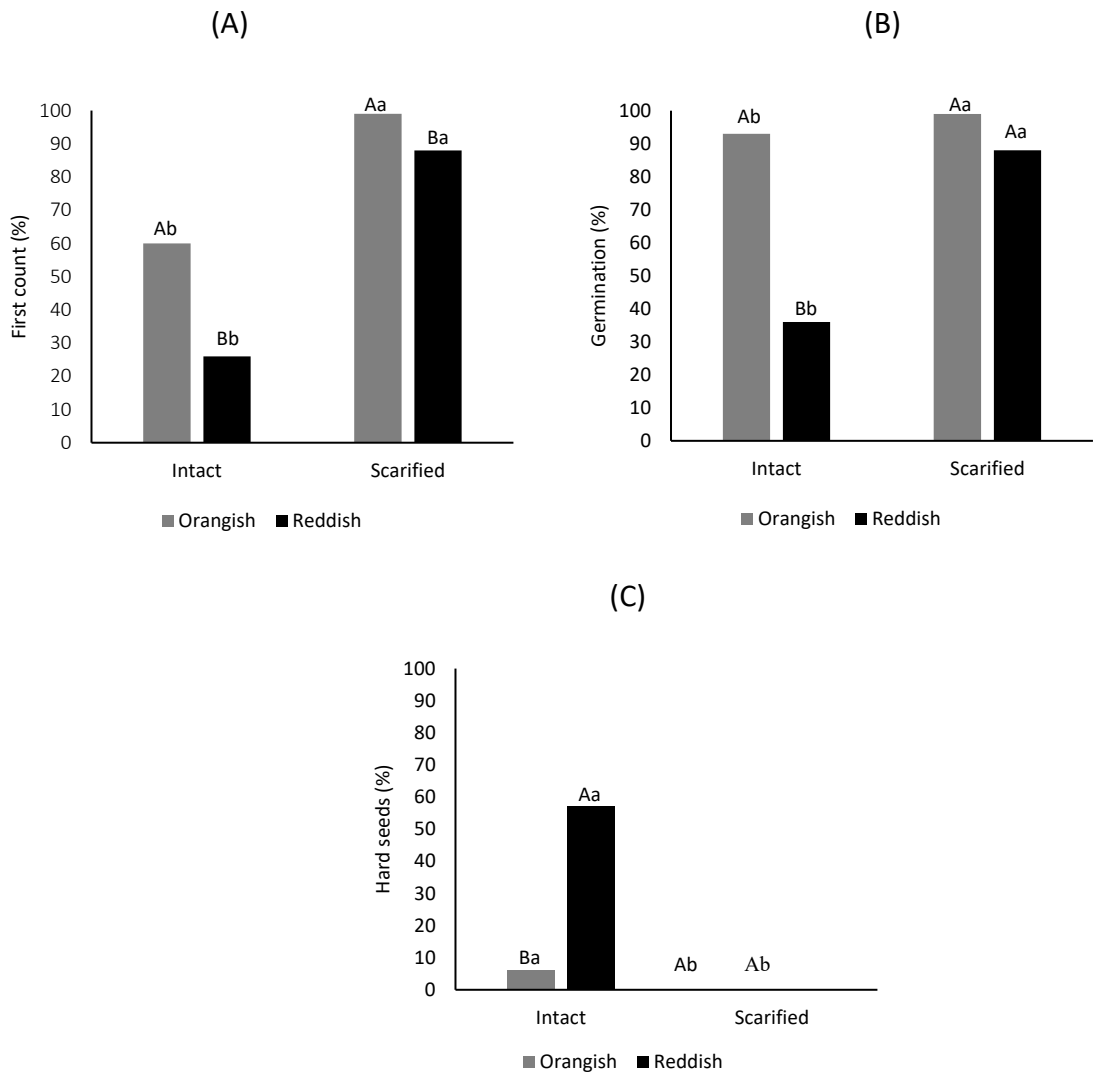


Figure 5. Means of the first count (A), germination (B) and hard seeds (C) of *Erythrina velutina* Willd. as a function of scarification (intact and scarified) and seed coat color (orangish and reddish). Uppercase letters compare bars of different colors in each condition of scarification, and lowercase letters compare bars with the same color between conditions of scarification.

count (Figure 5C). These results indicate a higher intensity of dormancy in reddish seeds; therefore, for a faster and more uniform germination of reddish seeds, dormancy must be broken before planting.

Shoot and root lengths had higher means in seedlings grown from scarified orangish seeds compared to intact ones, but in reddish seeds there was no statistical difference between scarified and non-scarified seeds (Figures 6A and 6B).

In relation to shoot and root dry mass, the means for seedlings grown from orangish seeds were statistically superior to those grown from reddish seeds, for both intact and scarified seeds (Figures 6C and 6D). However, it was observed that scarified seeds resulted in seedlings with greater growth of dry mass accumulation. For seeds that were subjected to scarification, there was greater growth of root, shoot and length of seedlings. This result reinforces the hypothesis that the scarification treatment not only favors the processes that depend on germination, but will also promote greater seedling growth, allowing easier establishment in the environment (Silva et al., 2016a).

Regardless of the method of overcoming dormancy, orangish seeds showed higher vigor. The difference in seed color is possibly related to the maturation process; reddish seeds reach physiological maturity first than orangish ones, thus losing more dry matter and their seed coat becomes harder. Although the moisture content of orangish seeds had already decreased, they were closer to the point of post-physiological maturity, showing greater accumulation of

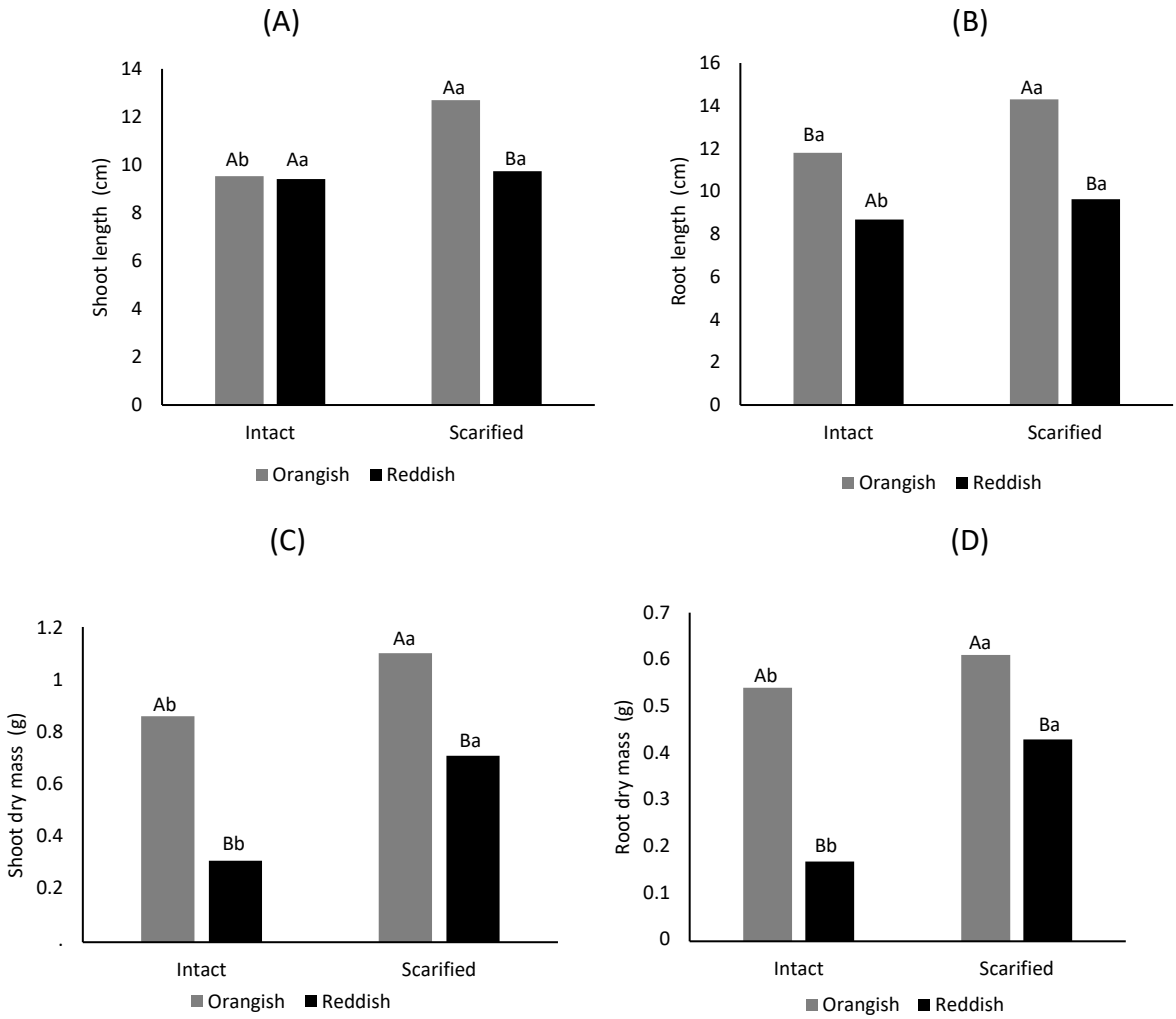


Figure 6. Means of shoot length (A), root length (B), shoot dry mass (C) and root dry mass (D) of *Erythrina velutina* Willd. as a function of scarification (intact and scarified) and seed coat color (orangish and reddish). Uppercase letters compare bars of different colors in each condition of scarification, and lowercase letters compare bars with the same color between conditions of scarification.

dry matter for having been "stored" for a shorter time in the field. According to Santos et al. (2022), throughout the development and maturation of the seed coat, there is a reduction in its permeability, that is, older seeds have lower seed coat permeability.

In other plant species, it was also possible to verify the influence of seed coat color on seed performance, such as in *Crotalaria ochroleuca* L., for which the red color resulted in lower germination and germination speed index compared to those with pink, light beige and gray seed coats (Silva et al., 2016b). Also, when evaluating the moment of dormancy onset during maturation in *Peltophorum dubium* (Spreng) Taub. seeds, Muller et al. (2016) found that darker seeds had higher dormancy intensity and lower physiological potential compared to lighter seeds.

In heteromorphic seeds of *Medicago denticulata*, Sethi et al. (2020) concluded that brownish seeds do not have seed coat dormancy, while cream-colored seeds have seed coat-imposed dormancy. *Erythrina speciosa* Andrews seeds show great differences in their maturation process depending on the place and time in which they are formed, both in dry matter accumulation and in germination and dormancy (Ribeiro et al., 2024).

The results obtained in this study provide new information on the reproductive biology of *E*. *velutina*, especially in relation to germination behavior and vigor according to seed coat color.

CONCLUSIONS

Seed coat color in *E*. *velutina* seeds does not influence physical characteristics, but orangish seeds have lower dormancy intensity and greater physiological potential than reddish ones.

AUTHORS' CONTRIBUTION

Hannah Lanier de Oliveira contributed with: Conceptualization, Methodology, Investigation, Data Curation and Visualization; Clarisse Pereira Benedito contributed with: Conceptualization, Methodology, Formal analysis, Writing - review & editing; Giovanna Dias de Sousa contributed with: Methodology and Investigation; Pablo Ferreira da Silva contributed with: Writing - review & editing; Salvador Barros Torres contributed with: Supervision, Resources, Writing - review & editing, Daise Feitoza da Rocha contributed with: Writing - review & editing.

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