







## Maturation of *Erythrina speciosa* Andrews seeds collected in the Southeast and South Regions of Brazil

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**ABSTRACT:** Analyzing unripe phases of orthodox seeds and the variations in traits depending on environmental conditions under which they were formed is a way of gaining knowledge on the differences and similarities between orthodox and recalcitrant seed behavior. From this perspective, in this study fruits of *Erythrina speciosa* Andrews were collected in Florianópolis (SC), Lajeado (RS) and São Paulo (SP) in August and November. Seeds were extracted and classified into six different maturation stages. Seeds were stored at 7 °C for a maximum of five days until the beginning of the experiments. Moisture content and dry matter content were measured through the oven method (103 ± 3 °C). Germination was analyzed at 25 °C and 70% of relative humidity, with evaluations being made every two days for 30 days. To induce dormancy, seeds were dried to 10% moisture content. We ran an analysis of variance (ANOVA) and Tukey's test at 5% as statistical analysis. We observed that unripe seeds showed a wide behavior variation depending on the collection period and site, for a same chronological age, reinforcing the idea that the seed behavior variation may be due to the maturation degree at the moment of seed dispersal.

**Index terms:** orthodox seeds, seed maturation, seed dormancy.

**RESUMO:** Uma das formas de se obter conhecimento sobre as diferenças e semelhanças entre os comportamentos das sementes ortodoxas e recalcitrantes durante o processo de maturação é analisando as fases imaturas das sementes ortodoxas e as variações em suas características, conforme as condições ambientais nas quais foram formadas. Diante disto, neste trabalho, os frutos de *Erythrina speciosa* Andrews foram coletados em Florianópolis (SC), Lajeado (RS) e São Paulo (SP) entre os meses de agosto e novembro. As sementes, extraídas, foram classificadas em seis estádios diferentes de maturação. Armazenadas a 7 °C, e os experimentos realizados iniciaram em até cinco dias. O teor de água e a massa seca foram medidos pelo método da estufa a 103 ± 3 °C. A germinação foi analisada a 25 °C e 70% de umidade relativa, com avaliações a cada dois dias por 30 dias. Para indução de dormência, as sementes foram secas até 10% de água. A análise estatística usou ANOVA e teste de Tukey a 5%. Observou-se que as sementes imaturas apresentaram grandes variações de comportamento em função de época e local de coleta, para uma mesma idade cronológica, reforçando a ideia de que variações no comportamento das sementes podem ser decorrentes do seu grau de maturação no momento em que são dispersas.

**Termos para indexação:** sementes ortodoxas, maturação de sementes, sementes dormentes.

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## INTRODUCTION

Brazil has one of the greatest diversities of plant species, requiring priority in initiatives for conserving its flora (Forzza et al., 2012). Conserving a species means not only ensuring its survival, but also maintaining its intra-specific genetic variability (Pilon et al., 2017; Oliveira et al., 2017; Monteiro et al., 2018). Seed conservation, in addition to being the most common form of *ex situ* conservation, since the seed is the natural propagation unit of most higher plant species, is the most economical, longest-lived, easiest to handle and the one that occupies the smallest physical spaces (Botanic Gardens Conservation International, 2001). However, some species produce seeds that are intolerant to desiccation and, therefore, cannot be stored for long periods; these are called recalcitrant.

Several studies have been carried out, such as those by Hossel et al. (2016), Bonjovani and Barbedo (2018) and Félix et al. (2017), among others, seeking to adapt the classic storage methodologies, that is, those applied to desiccation-tolerant seeds (called orthodox), to recalcitrant seeds, especially seeking to reduce moisture content to the minimum possible and reduce storage temperature to levels that are not lethal to the seeds, but with unpromising results. Such failure is mainly due to the fact that there is not enough knowledge on the characteristics of desiccation-sensitive seeds when compared to desiccation-tolerant seeds. Perhaps, the success of the development of appropriate methodologies for the storage of recalcitrant seeds depends on, first, understanding the differences between orthodox and recalcitrant seeds and then seeking the development of new technologies (Barbedo, 2018). One of the ways to understand the behavior of recalcitrant seeds and their differences compared to orthodox seeds, is to analyze the unripe phases of orthodox seeds, when they are still sensitive to desiccation. However, research on the behavior of orthodox seeds during unripe stages is also scarce, especially regarding the influence of the environment in which the seeds are formed.

There are still large gaps in knowledge about the differences and similarities between the behaviors of orthodox and recalcitrant seeds and not having this knowledge has been an obstacle to the development of new technologies for seed conservation (Barbedo, 2018; Barbedo, 2021). Thus, studies on these behaviors are increasingly necessary to understand the processes of seed physiology (Walters et al., 2013). Another important aspect for seed conservation is the acquisition and presence of dormancy, such as the mechanisms that prevent, or at least hinder, the imbibition of water, called physical dormancy, as verified in seeds of *Erythrina speciosa* Andrews (Molizane et al., 2018).

*E. speciosa* is a species native to Brazil, which has orthodox seeds with physical dormancy. In view of the lack of sufficient knowledge on the behavior of orthodox seeds in their unripe stages, especially when related to the environmental conditions under which the seed develops, in this study the aim was to evaluate the behavior of orthodox seeds of *E. speciosa* in their unripe phases, formed in different locations and times.

## MATERIAL AND METHODS

### *Plant material*

*E. speciosa* fruits were collected at three locations: Florianópolis, SC (27°36'14.0"S, 48°31'17.9"W), in August (SCI) and October (SCII); Lajeado, RS (29°25'48.56"S, 51°56'32.83"W), in October (RSI) and November (RSII); and in São Paulo, SP (23°32'39"S, 46°37'41"W), in October (SP).

The extraction of seeds from the green to yellowish fruits of *E. speciosa* collected in Lajeado was carried out at the Botany Laboratory of Univates; fruits of the collection in Florianópolis were packed in thermal bags and taken to the Seed Laboratory of the Institute of Environmental Research, in São Paulo, where their seeds were extracted, not exceeding 24 hours after collection; seeds collected in São Paulo were extracted in the same laboratory. The extractions from all collections were carried out by manually breaking the fruits and removing the seeds, which were separated into six stages of maturation (Figure 1), according to the classification of seed coat pigmentation from light green to brown, based on the separation carried out by Molizane et al. (2018) and Hell et al. (2019).



Figure 1. *Erythrina speciosa* Andrews seeds in their different collection stages (I-VI) with the color-based separation methodology used in the present study. Scale: 1cm.

All experiments started within a maximum of five days after extraction of the seeds from the fruit. During this period, the seeds were stored in BOD chamber at 7 °C.

#### *Initial physiological determinations*

Moisture content and dry matter content of the seeds were evaluated by the oven method at  $103 \pm 3$  °C, for 17 hours, in 4 replications of 5 seeds each (Brasil, 2009). Moisture content was calculated on a wet basis, being expressed as a percentage (%), and the dry matter content was expressed in  $\text{mg}\cdot\text{seed}^{-1}$  (Brasil, 2009).

The germination test, with non-scarified seeds of the six maturation stages described above, was carried out with 4 replications of 20 seeds of each stage in paper rolls, pre-moistened with a volume of water equivalent to 2.5 times the dry weight of the paper, incubated in a germination room at 25 °C, with relative humidity of 70% under constant light (Brasil, 2009). Germination evaluations were carried out every two days for 30 days. After this period, ungerminated seeds were manually scarified and placed again to germinate. Seeds with primary root length equal to or greater than 2 mm were considered germinated, and seedlings with root and shoot without defects and with proportional development were considered normal seedlings (NS).

#### *Induction of dormancy*

For the analysis of induction of seed coat dormancy, as described by Molizane et al. (2020), the seeds of all maturation stages from the different collections were dried until they reached 10% moisture content, using an oven with forced air circulation at 40 °C. After drying, germination was evaluated again, as previously described. Seeds not germinated after 30 days were manually scarified and placed again to germinate for more 30 days, in order to confirm that they were alive.

#### *Experimental design and statistical data treatment*

A completely randomized experimental design was used for all experiments. The data were statistically analyzed from a one-way ANOVA using R software version 4.1.3, at 5% probability level, and the means were compared with each other by Tukey test, also at 5% probability level.

## **RESULTS AND DISCUSSION**

The separation of the six stages of maturation found based on the morphological characteristics of the seeds resulted in variations in the physiological characteristics between the collection sites and, in some cases, even in the same site, but at different times (Figures 2 to 6). Moisture content, for example, had the greatest reduction between the V and

VI stages for seeds of SCI, SCII and RSI (Figures 2, 3 and 4), but between the IV and V stages for RSII and SP (Figures 5 and 6). For several authors, the advance of moisture content is one of the best diagnoses of the maturation stage of seeds, especially those with orthodox behavior (Marcos-Filho, 2015). On the other hand, morphological characteristics of fruits and seeds are used as criteria to define the appropriate time to harvest and obtain seeds with the highest quality (Borges et al., 2005; Vidigal et al., 2011; Araújo et al., 2023). From the results of the present study, we verified that these parameters can be used to estimate the evolution of the process, but there are variations according to the environmental conditions under which the seeds are formed. Therefore, the use of these characteristics to estimate the maturation stage of seeds and define the harvest moment may have its advantage in terms of speed and practicality, but it was evident from the results obtained that the correct definition of the maturation stage depends on a more cautious analysis, especially considering environmental variations, such as place and time.

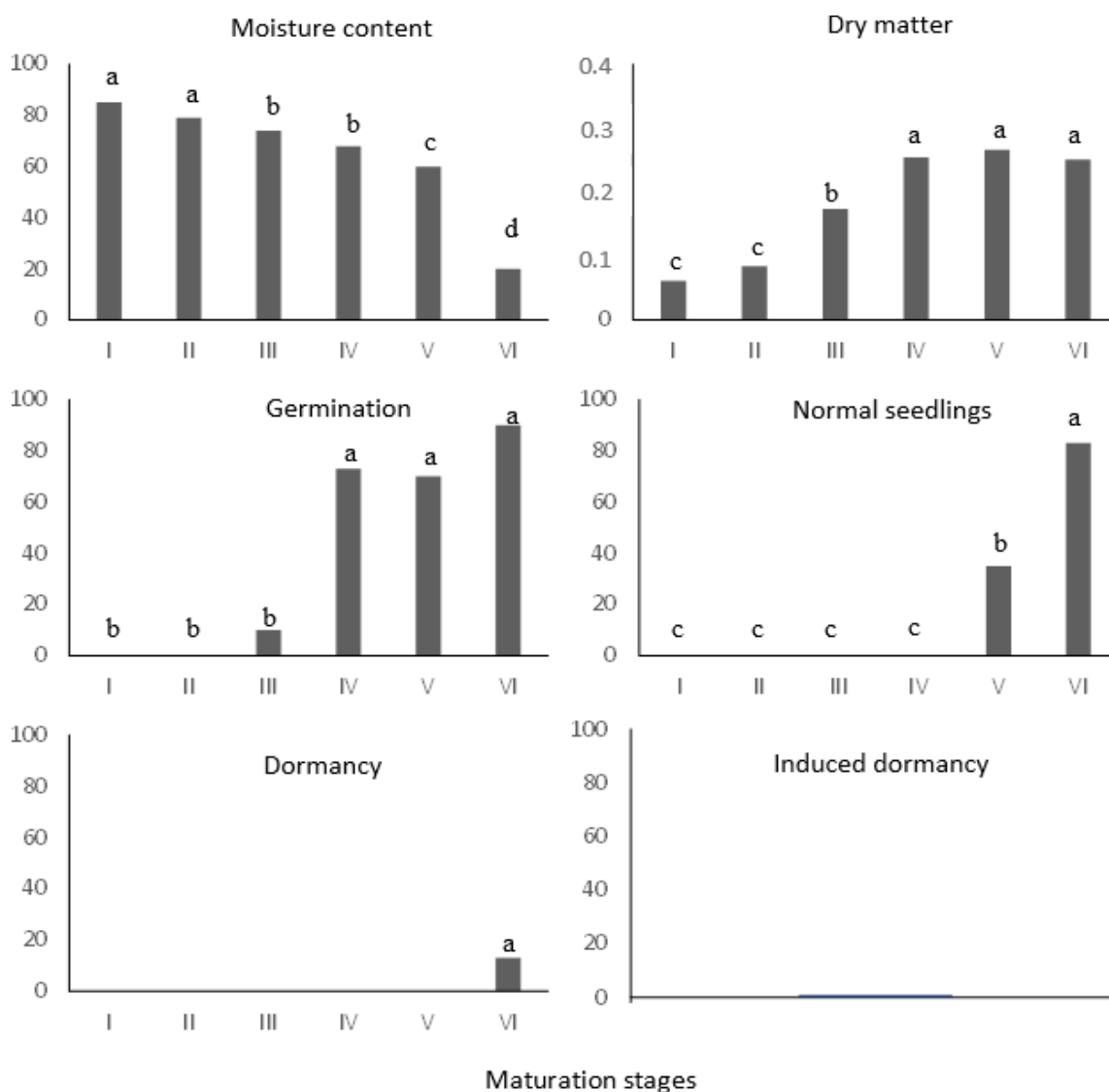


Figure 2. Moisture content (%), dry matter (mg.seed<sup>-1</sup>), germination (%), development of normal seedlings (%), dormancy (%) and induced dormancy (%) of *Erythrina speciosa* Andrews seeds, from stages I to VI, from the SCI collection. Equal letters for stages do not differ from each other by Tukey test at 5% probability level.

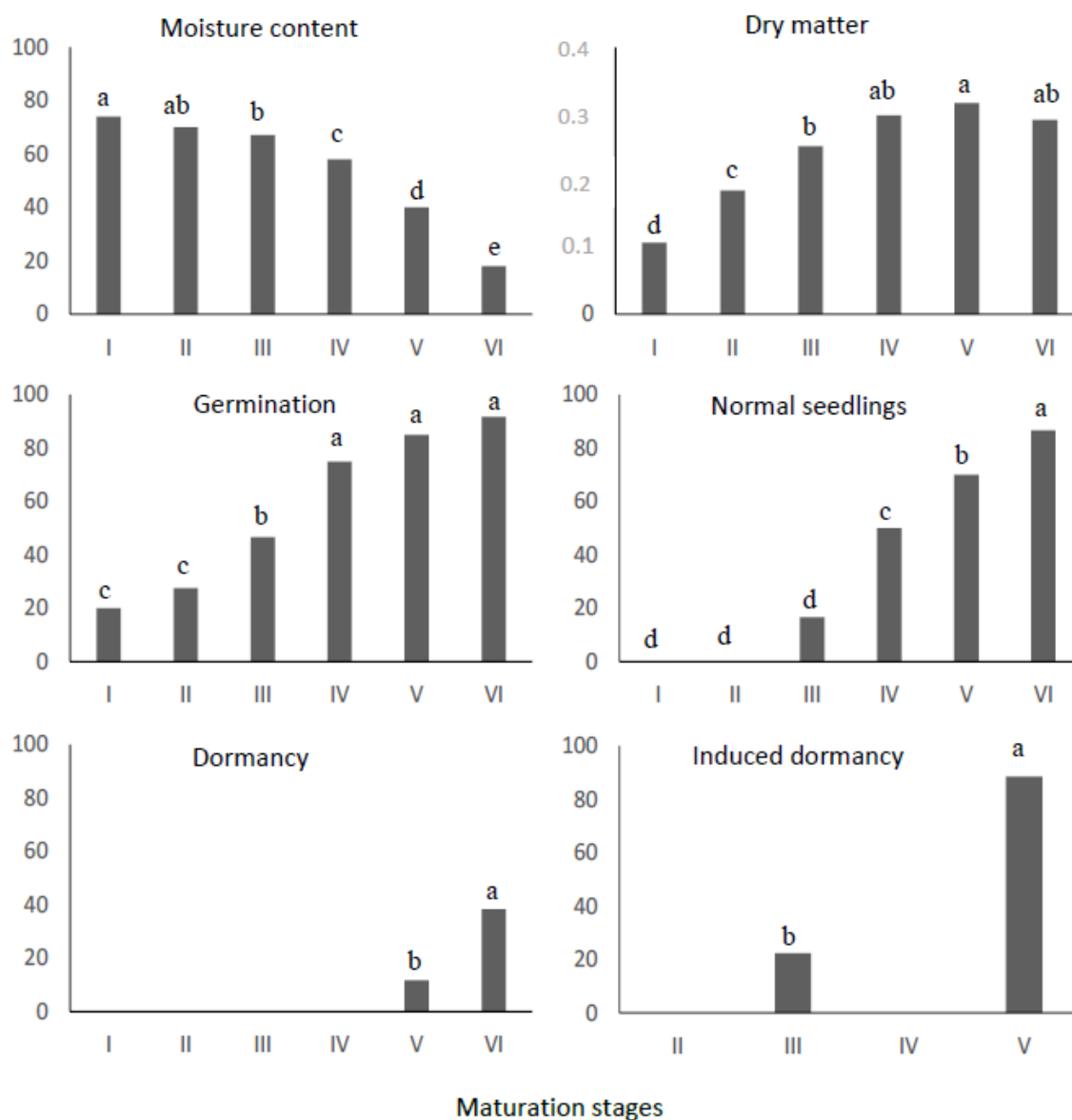


Figure 3. Moisture content (%), dry matter (mg.seed<sup>-1</sup>), germination (%), normal seedlings (%), dormancy (%) and induced dormancy (%) of *Erythrina speciosa* Andrews seeds, from stages I to VI, from the SC II collection. Equal letters for stages do not differ from each other by Tukey test at 5% probability level.

The influence of environmental conditions on the evolution of seed maturation, sometimes differently on each trait, was reported by Barbedo (2018). Indeed, the evolution of the moisture content itself was different under the different conditions. For example, the lowest values of moisture content up to stage IV were found in SCII seeds and, in the following stages, in SP seeds. SCII seeds from stage I had 74% moisture content, and SP seeds, from the same stage, 81%. In the end, in stage VI, these values dropped to 18% and 9%, respectively (Figures 3 and 6).

Another characteristic widely used in conjunction with moisture content to diagnose the maturation stage of seeds is dry matter content. Some authors even consider that the maximum accumulation of dry matter identifies the physiological maturity of seeds (Borges et al., 2005; Araujo and Barbedo, 2017). However, variation was also observed for this characteristic, especially between collection sites. As the maturation process progressed, the seeds increased the amount of dry matter for all locations and times, but the highest values were not always reached at the same stage. For example, in SCI, SCII and RSII, it was reached in stage IV (Figures 2, 3 and 5), while in RSI and SP it was reached in

stage III (Figures 4 and 6). In addition, the maximum value of dry matter was also different between the different sites and times, reaching differences close to twice as much between different sites, as in the comparison between RSII seeds ( $0.411 \text{ mg.seed}^{-1}$ , Figure 5) and SCI seeds ( $0.254 \text{ mg.seed}^{-1}$ , Figure 2), or even for the same location, at different times, as in the comparison between RSI and RSII (Figures 4 and 5, respectively).

Germination capacity, in turn, gradually increased throughout seed development for all locations and times (except RSII, for which it was not possible to obtain seeds of the more unripe stages - Figure 5). However, also for this characteristic the highest values did not occur in the same stage for the different places and times. For example, in SCI and SCII it was reached in stage IV (Figures 2 and 3), in RSI in stage VI (Figure 4) and in RSII and SP in stage III (Figures 5 and 6). It is curious to observe that, for SCII, this characteristic was already present from stage I (Figure 3), while for SCI, that is, in the same plants, but at a different time, the beginning of germination appeared only in stage III (Figure 2).

The percentage of seeds with capacity to develop normal seedlings was maximum in the last stage of maturation for all collections (Figures 2, 3, 5 and 6), except for RSI (Figure 4), but also showed variations in evolution throughout maturation for different locations and times. For example, in stage IV, SCI seeds had not yet developed this capacity (Figure 2), while for SCII (same plants, different times) more than 50% of the seeds already had this capacity (Figure 3).

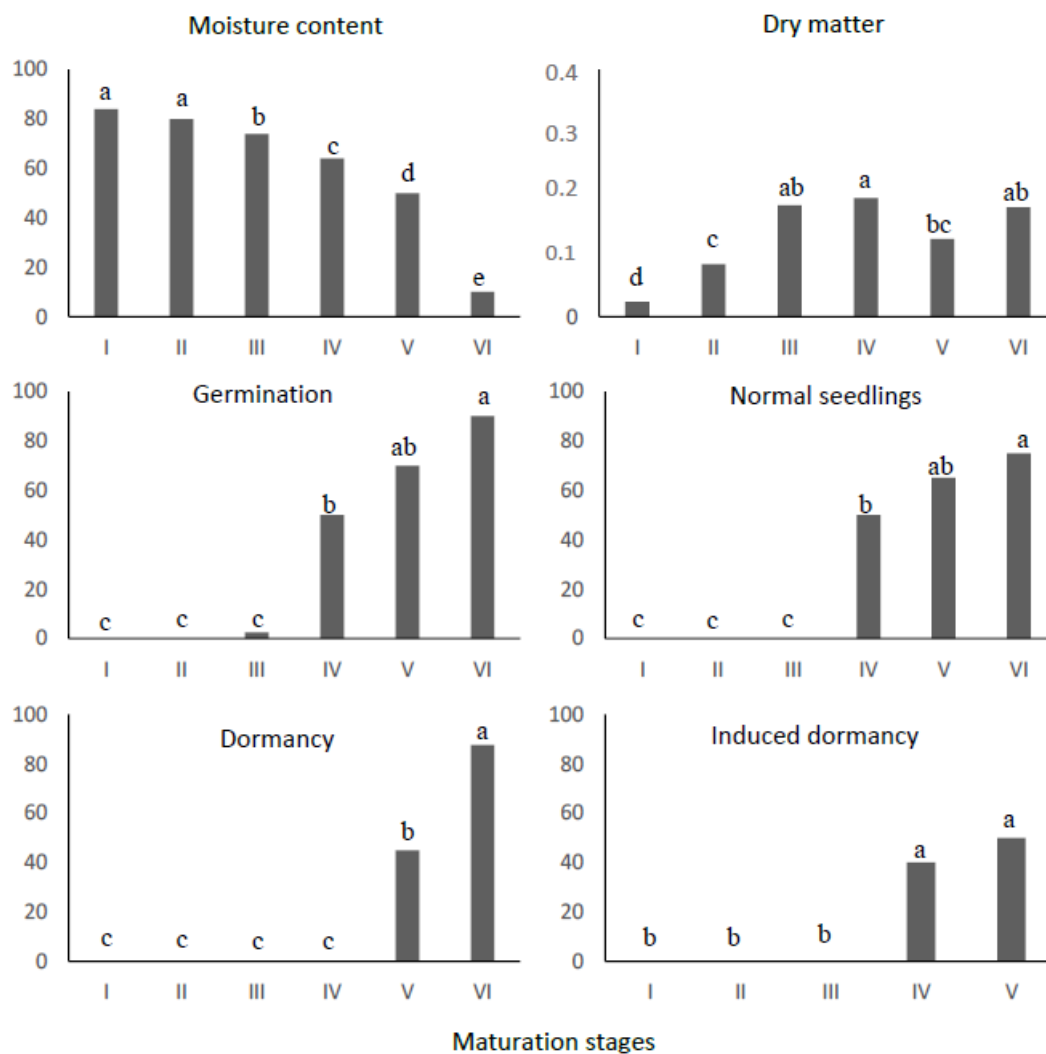


Figure 4. Moisture content (%), dry matter ( $\text{mg.seed}^{-1}$ ), germination (%), normal seedlings (%), dormancy (%) and induced dormancy (%) of *Erythrina speciosa* Andrews seeds, from stages I to VI, from the RSI collection. Equal letters for stages do not differ from each other by Tukey test at 5% probability level.

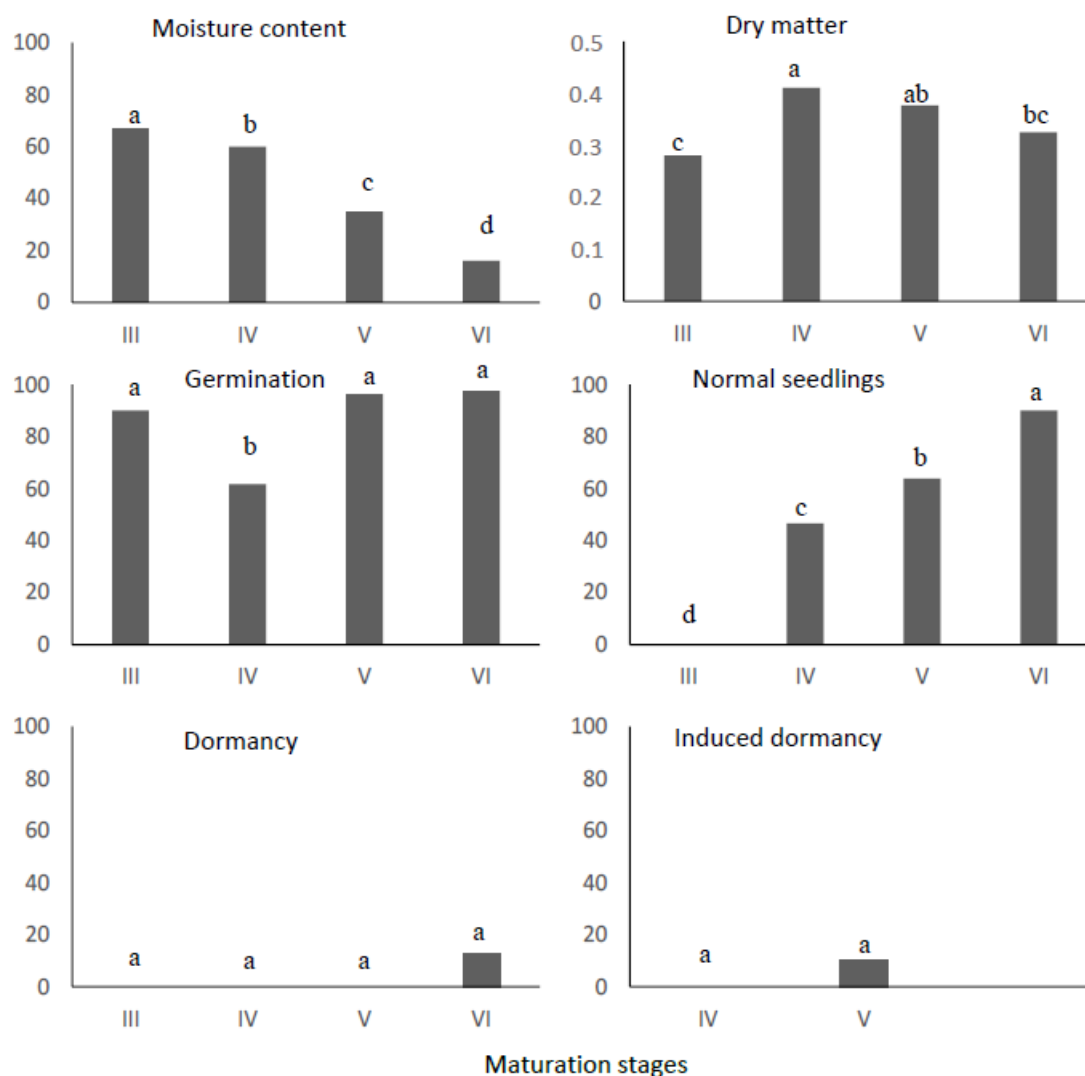


Figure 5. Moisture content (%), dry matter ( $\text{mg}\cdot\text{seed}^{-1}$ ), germination (%), normal seedlings (%), dormancy (%) and induced dormancy (%) of *Erythrina speciosa* Andrews seeds, from stages I to VI, from the RSII collection. Equal letters for stages do not differ from each other by Tukey test at 5% probability level.

Finally, both the emergence and the maximum percentage of dormancy in the seeds also showed variations. Taking RS as an example, in RSI dormancy appeared at stage V, reaching maximum value in VI, when more than 90% of the seeds were dormant (Figure 4), while in RSII (same plants, different times) dormancy practically did not appear (Figure 5). These differences, although to a lesser extent, were also observed between SCI and SCII (Figures 2 and 3).

Dormancy in *E. speciosa* seeds is strongly related to the reduction in moisture content (Molizane et al., 2018). It is possible, for example, to induce dormancy in unripe seeds by drying (Molizane et al., 2020). Therefore, for a correct evaluation of the dormancy degree of these seeds, it is essential to subject them to drying before the germination test. Indeed, in SCII seeds, drying increased the degree of dormancy in seeds in stage V (Figure 3), reaching more than 90% against less than 20% before drying. These seeds, before drying, had a very high moisture content. However, even this induction was dependent on place and time, as observed for RSII seeds of the same stage V, which did not show increased dormancy after drying (Figure 5). It is also important to note the values of dormant seeds of stage V of RSI, even with still high values of moisture content (Figure 4), as well as the almost non-existence of dormancy in seeds of stage VI of SCI and RSII, even with very low values of moisture content (Figures 2 and 5).

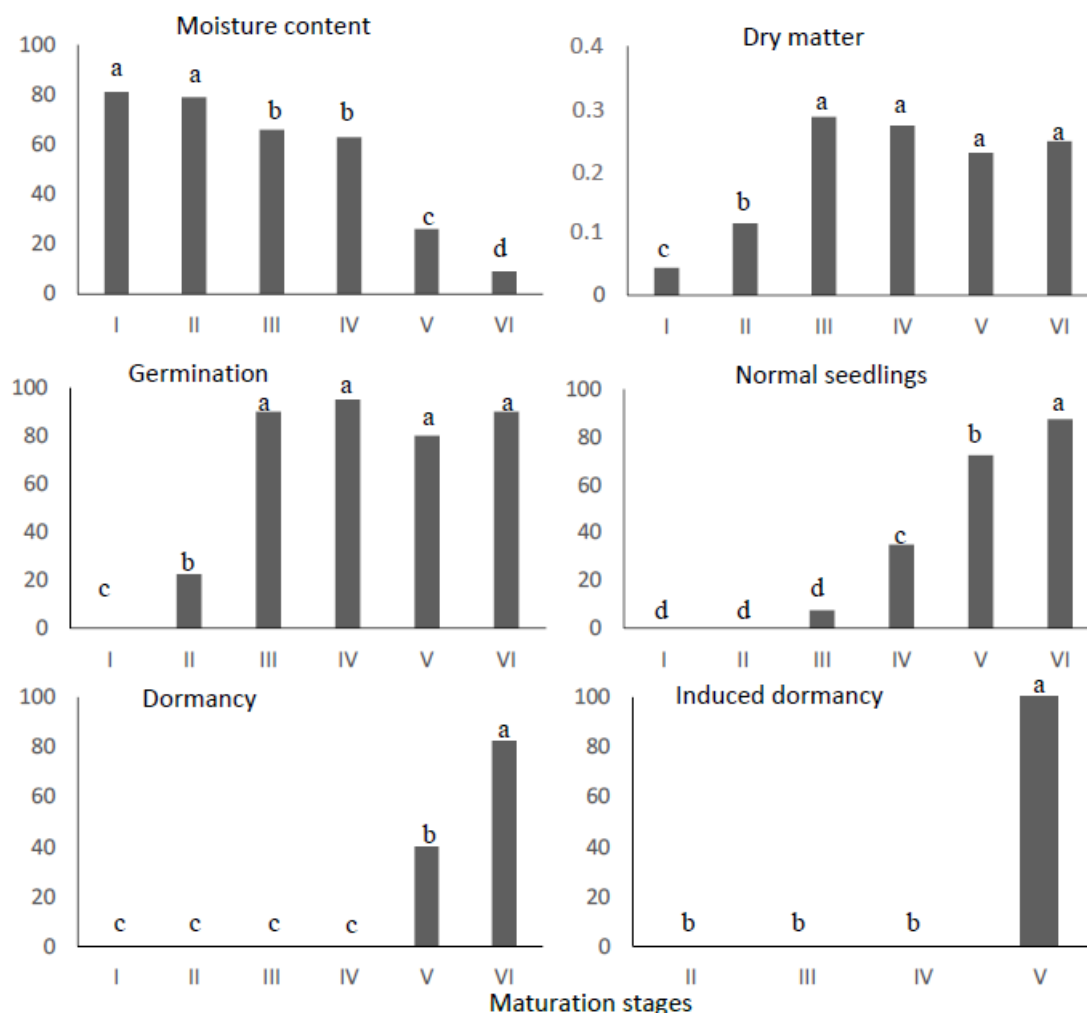


Figure 6. Moisture content (%), dry matter (mg.seed<sup>-1</sup>), germination (%), normal seedlings (%), dormancy (%) and induced dormancy (%) of *Erythrina speciosa* Andrews seeds, from stages I to VI, from the SP collection. Equal letters for stages do not differ from each other by Tukey test at 5% probability level.

Specifically, regarding the dormancy of *E. speciosa* seeds, it has already been shown that environmental conditions interfere not only with the degree of dormancy, but also with the sensitivity of the seeds to the dormancy breaking (Molizane et al., 2018). It has also been shown that, although this dormancy can be induced in unripe seeds by drying, the efficiency of this induction is also dependent on the conditions under which the seeds are formed (Molizane et al., 2020). The results of the present study confirm this influence of the environment not only on dormancy, but also on all other characteristics analyzed of the seeds, such as moisture content, dry matter content, germination and capacity to produce normal seedlings.

In all collection sites, despite these differences between the stages for the different conditions under which the seeds were formed, it was observed that *E. speciosa* seeds followed the classic model of orthodox seeds (Ali et al., 2022; Matilla, 2022), losing water up to low values (close to 10%) in the last stages, increasing the dry matter content progressively until reaching a maximum and also progressively increasing the percentage of seeds with the capacity to germinate and produce normal seedlings as they advanced in their maturation process.

According to Barbedo (2018), variations in the characteristics of seeds formed under different conditions can be interpreted under two-time scales. In the first, conditioned by the evolution of the species in the different environments, the responses are long-term, generating wide variations, leading to great differences in the behavior of the seeds of



different species. It can also lead to differences between different populations of the same species, in places with very different environmental conditions. In the second, conditioned by momentary and occasional climatic variations, the differences in seed characteristics are smaller and can occur even in those from the same plants, but at different times (Molizane et al., 2020). This seems to be the case of *E. speciosa* seeds, for which variations in all the characteristics analyzed occurred both between different populations and between seeds of the same plants, produced at different times. In some cases, it is even possible to notice greater proximity of behaviors between seeds from different locations than between seeds from the same plants produced at different times. For example, the emergence and gradual increase in the capacity of seeds to produce normal seedlings has a more similar behavior between RSI and SCII than between SCII and SCI seeds. Likewise, the presence and degree of dormancy in seeds have more similarity between SP and RSI than between RSI and RSII. Therefore, the differences in the behavior of seeds during their maturation may be not only due to their genetic characteristics, but also to the conditions of the environment in which they are formed.

## CONCLUSIONS

*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. Seeds from different regions, such as RSI and SCII, may have a more similar behavior to each other than those from the same region, at different times, such as SCII and SCI.

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