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Effect of the seed maturation stage and pre-germination treatments on emergence of *Erythrina crista-galli* L.

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ABSTRACT – The main goals of this study were to verify the existence of integumentary seed dormancy in *Erythrina crista-galli* seeds from mature pods and evaluate the seedling emergence from newly collected seeds in two stages of physiological maturation. Methods of overcoming dormancy were tested by comparing seedling emergence rate, mean emergence time and emergence index, with treatments: TC – control; T1– sanded and soaked in water for 48 h; T2 – sanded and soaked in water for 24 h; T3 – soaked in water without heating at the initial temperature of 60 °C, until reaching room temperature; T4 – only sanded; T5 – immature seeds. The results indicate that: i) newly collected seeds from mature pods do not require treatment to overcome dormancy; ii) immature seeds have germination similar to newly collected mature seeds.

Keywords: cockspur coral tree, Fabaceae, physical dormancy

RESUMO – Efeito do estágio de maturação e tratamentos pré-germinativos na emergência de *Erythrina crista-galli* L. Os objetivos deste trabalho foram verificar a existência de dormência tegumentar em sementes maduras de *Erythrina crista-galli* e avaliar a emergência de plântulas de sementes recém-coletadas, em dois estágios de maturação fisiológica. Foram testados métodos de superação de dormência, comparando-se taxa de emergência, tempo médio de emergência e índice médio de emergência das sementes, sendo os tratamentos: TC – controle; T1 – lixadas e embebidas em água por 48 h; T2 – lixadas e embebidas em água por 24 h; T3 – embebidas em água, fora do aquecimento, à temperatura inicial de 60 °C até atingir temperatura ambiente; T4 – apenas lixadas e T5 – sementes coletadas de legumes imaturos. Os resultados indicam: i) sementes recém-coletadas de vagens maduras não necessitam de tratamento para superação de dormência; ii) sementes imaturas apresentam germinação similar as maduras recém coletadas.

Palavras-chave: corticeira-do-banhado, dormência física, Fabaceae

INTRODUCTION

Erythrina crista-galli L. (Fabaceae, Papilionoideae), popularly known as cockspur coral tree, is a tree species which occurs from Maranhão State to Rio Grande do Sul State, being more recorded in the Brazilian South region (Martins 2019). Its distribution area also extends through Bolivia, Paraguay, Argentina and Uruguay (Mello *et al.* 2019). The species contributes to important environmental services, hosting several epiphytes species, and also attracting insects and hummingbirds due to their colorful flowering which give it an ornamental aspect (Gratieri-Sossella *et al.* 2008). It is also of great value for medicine and ecological restoration (Mello *et al.* 2019). In Rio Grande do Sul State, the tree-cutting of this species is prohibited pursuant to Article 33 of State Law number 9.519/92 (Rio Grande do Sul 1992).

Due to the diverse assigned uses and environmental services that the plant offers, there is particular interest in its reproduction, which can occur through either asexually or sexually means (Gratieri-Sossella *et al.* 2008, Mello *et al.* 2016). Seed breeding is the most recommended when it is used for ecological restoration, seeking to collect as many local matrices as possible in order to sustain

genetically stable populations (Sebbenn 2002). The species presents a phenological unevenness in the reproductive phase, occurring concomitantly during the fruiting, pods in different maturation stages (Gratieri-Sossella *et al.* 2008, Mello *et al.* 2013, Mello *et al.* 2016). Irregularity in the fruits maturation in a species in different populations or even in an individual is understood as a strategy to guarantee greater dispersion time and to reduce damage by predation (Willson & Traveset 2000, Aquino *et al*. 2006).

The presence of integumentary dormancy is also commonly reported for the *E. crista-galli* (Silva *et al*. 2006, Mello *et al*. 2016). This type of dormancy is related to the impermeability of the integument or pericarp, which impedes the water absorption and gas exchange (Fowler & Bianchetti 2000), making the germination processes difficult, which in practice means lower germinability and/or germination speed. This is a common condition for seeds from species of the Fabaceae family (Silva *et al*. 2006). Unlike primary dormancy, which occurs during development in the mother plant, secondary dormancy is induced after dispersion when the seed is not submitted to favorable environmental conditions for germination, such as in the case of integumentary dormancy (Cardoso 2009). However, this type of dormancy can develop in the fast dehydration phase which occurs at the end of maturation, still in the mother plant (Baskin & Baskin 1998). Some pre-germination treatments used for *E. crista-galli* are chemical scarification (H_2SO_4), mechanical scarification and thermic scarification (hot water) (Silva *et al.* 2006; Mello *et al*. 2016).

The maturation stage in which the seeds are collected is also important for the germination process. Some characteristics can be used to define the most appropriate time for collection, known as maturation indexes, namely: color, size, moisture and dry mass of fruits and seeds (Piña-Rodrigues & Aguiar 1993). However, the most pratical aspects used for collecting seeds is the coloring of fruits and seeds (Aquino *et al*. 2006). In dehiscent fruits such as *E. crista-galli*, identifying the right moment for the collection is even more important because the seeds can be lost during the spontaneous opening of the fruits (Piña-Rodrigues & Aguiar 1993).

The main of this study was to verify the existence of seed coat dormancy in seeds from mature pods and evaluate the seedling emergence from newly collected seeds in two stages of physiological maturation.

MATERIAL AND METHODS

Location, collection procedure and processing

Fruits were collected from eight *E. crista-galli* individuals at two different maturation stages in the location of Banhado da Marambaia (31°47'30"S and 52°18'57"W), Rio Grande municipality, Rio Grande do Sul State. The local vegetation is characterized by the predominance of wetlands, moist fields and swamp forests, which form typical vegetation mosaics for the pioneer formations in the Pampa Biome (Cordeiro & Hasenack 2009). The climate of the region is defined as Cfa type, humid temperate climate with hot summers, and no dry season (Alvares *et al*. 2013).

The evaluation of fruit and seed color according to the Munsell color chart for vegetable tissues was adopted as the criteria for distinguishing maturation stages (Wilde & Voigt 1977) and fruit dehiscence (Lazarotto *et al*. 2011, Mello *et al.* 2013) considering the following: i) immature: green-colored seeds (5GY 7/8) extracted from green pods (5GY 6/10); ii) mature: seeds with brown color (5YR 3/4) extracted from pods beginning dehiscence, dark brown (5YR 3/2). Fruits were collected at different maturation conditions in all mother trees of the same population in order to avoid noise through the addition of intrapopulation variability in the germination analysis. Respecting a minimal distance between mother trees of 50 m (Sena 2008). The pods were homogenized for both maturation stages, and the seeds were extracted manually. Only intact seeds were selected by visual evaluation, discarding the shriveled or those damaged by insects.

The experiment was conducted in a greenhouse at the Cascata Experimental Station (Embrapa Clima Temperado), Pelotas, Rio Grande do Sul State. Sowing was performed two days after collection, in 55 cm³ containers and commercial substrate (Turfa Fértil®) with manual irrigation performed twice a day.

Experimental design

After homogenization and pods processing, the intact seeds and the shriveled/damaged seeds were quantified for the mature collected fruits. The number of seeds per pod was estimated from the average of 100 pods (harvested mature). Next, 600 intact seeds were randomly selected to evaluate seed emergence, which were divided into five treatments: TC – control; T1– sanded (seeds subjected to abrasion with 60-grit sandpaper, on the opposite side of the hilum) and soaked in water for 48 h; T2 – sanded and soaked in water for 24 h; T3 – soaked in water outside of heating at the initial temperature of 60 °C until reaching ambient temperature; T4 – only sanded; and T5 – immature seeds (Fig. 1). The evaluations were performed every five days for 31 days when the emergence stabilized, and consisted in counting emerged seedlings from visualizing the epicotyl. The experimental design was completely randomized, with four replicates of 25 seeds per treatment.

Emergence measures

In addition to the emergence percentage (E%), the following measures were calculated in order to evaluate the kinetics of this process: the Emergence Speed Index: $ESI = E_1/N_1 + E_2/N_2 + ... + E_i/N_j$; in which: E_i = number of germinated seeds and N_j = number of days after sowing (Maguire 1962), and the Mean Emergence Time: $MET = \sum n_i * t_i/\sum n_i$; in which: t_i = time between the beginning of the experiment and the i-th evaluation; n_i = number of seeds which emerged between the evaluation interval of the seeds t_i (Borghetti & Ferreira 2004).

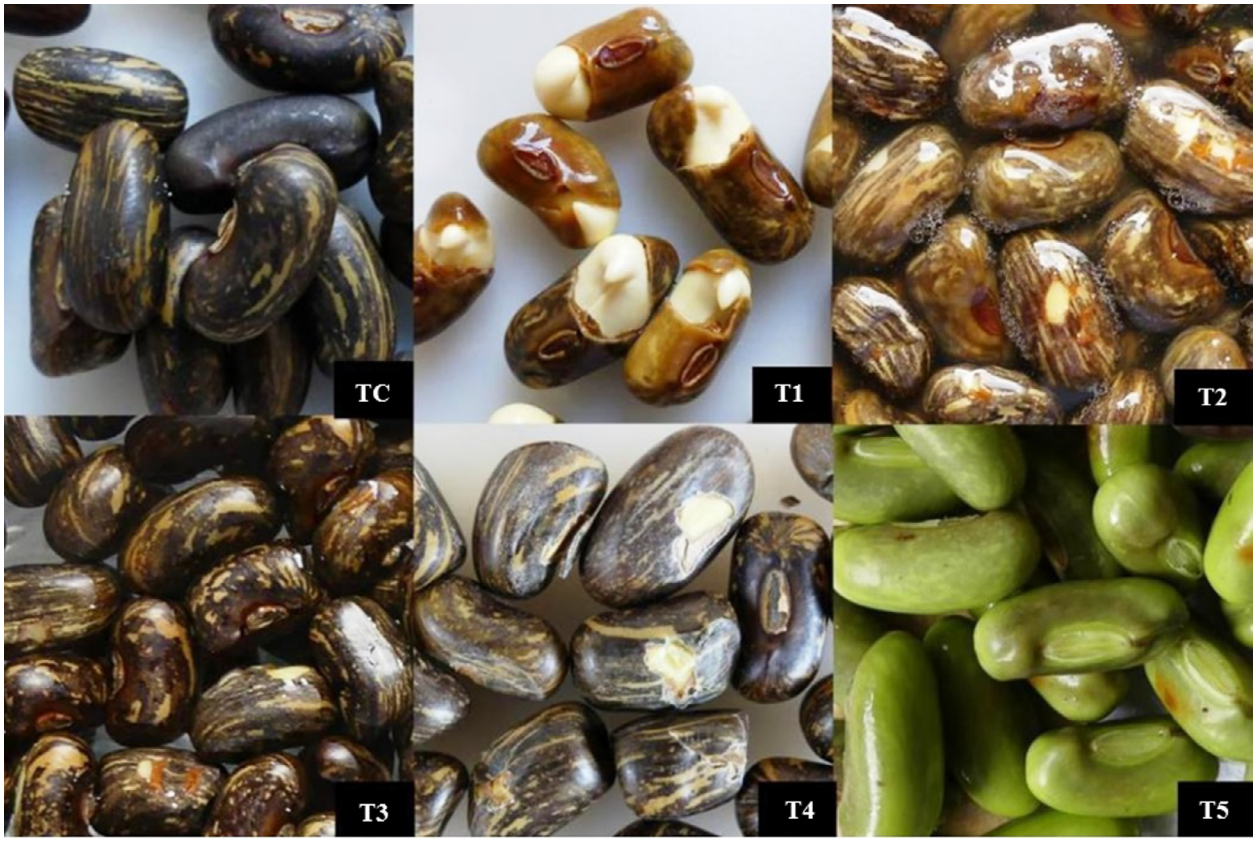

Fig. 1 TC, T1-T5. *Erythrina crista-galli*. Seeds in different maturation stages and submitted to pre-germination treatments. **TC.** control; **T1.** sanded (seeds subjected to abrasion with 60-grit sandpaper, on the opposite side of the hilum) and soaked in water for 48 h; **T2.** sanded and soaked in water for 24 h; **T3.** soaked in water outside of heating at the initial temperature of 60 °C until reaching ambient temperature; **T4.** only sanded; **T5.** immature seeds.

Statistical analysis

Data were analyzed using the R statistical software (R Core Team 2016), with significance level (α) ≤ 0.05. The residual normality (Shapiro-Wilk test) (Zar 2013) and the homogeneity of variances (Barlett's test) were previously evaluated (Zar 2013). The E% and MET variables did not present normality (W = 0.8465; p = 0.001 [E%] and W = 0.898; p = 0.02 [MET]) and homogeneous variance (K^2 = 11.862; d.f. = 5; p = 0.04 [E%] and K^2 = 20.123; d.f. = 5; p = 0.001 [MET]). The emergence data remained non-normal even after follow-up changes. For this case, we chose to use the non-parametric Kruskal-Wallis test (also called the one-way ANOVA on ranks) (Santana & Ranal 2004), followed by the Nemenyi post-hoc test for medians (Pohlert 2016).

The MET variable reached normality after the logarithmic transformation (Ln), but did not reach variance homogeneity (K^2 = 13.833, d.f. = 5, p = 0.0167), a fact shared by the ESI variable, which presented normality (W = 0.969; p = 0.654), but also did not present homogeneous variance (K^2 = 15.936; d.f. = 5; p = 0.007). In both cases, we used one factor analyses of variance (ANOVA) with a Tukey post-hoc test for multiple comparisons (Santana & Ranal 2004), even though it violated one of the four basic assumptions of ANOVA (homoscedasticity), since the data follow the other assumptions (independence, normality and additivity) (Zar 2013). Finally, the medians of the E% variable are presented, followed by their respective inter-quartiles intervals (QRI = Q1 – Q3, where Q1 = 1st quartile and Q3 = 3rd quartile), while the mean of the ESI and MET variables are presented followed by their respective standard deviations.

RESULTS AND DISCUSSION

The pods presented one to 18 seeds (mean ± standard deviation: 5.77 ± 3.32, n = 100 pods). Of the total seeds obtained (n = 2.188), 38% were shriveled/damaged by insect, showing a higher number of seeds/fruit and better quality compared with other studies. Lazarotto *et al.* (2011) obtained an average of 14 seeds/fruit, with four of these seeds/fruit having completed their maturation without damage (28.5%) and 10 seeds/fruit (71.4%) were aborted. Mello *et al*. (2016) obtained 4.35 seeds/fruit (n = 800), in which 53.85% of the total number of seeds obtained (n = 3.484) were shriveled or damaged.

Erythrina crista-galli seeds soaked in water with an initial temperature of 60 °C (T3) presented higher seedling emergence rate (median = 84 seeds, QRI = 4; Fig. 2), followed by the treatments: control (TC; median = 82

seeds, QRI = 17); immature seeds (T5; median = 80 seeds, QRI = 2); seeds only sanded (T4; median = 70, QRI = 8); seeds sanded and soaked in water for 48 h (T1; median = 30 seeds, QRI = 24) and seeds sanded and soaked in water for 24 h (T2; median = 12 seeds, QRI = 8).

Seedling emergence among treatments was statistically significant (H = 18.285; d.f. = 5; $p = 0.002$). The Nemenyi multiple comparisons post-hoc test indicated that T2 differed significantly from T3 ($p = 0.010$), from T5 ($p = 0.043$) and from TC ($p = 0.032$), while there was no significant difference for the other treatments and comparisons ($p \geq 0.05$). Immature seeds without pre-germination treatments (T5) had similar germination behavior to newly collected mature seeds (T3 and T4), or not (TC), to pre-germination treatments.

The emergence speed index (ESI) varied significantly between treatments (Table 1), with the lowest T2 (3.64 ± 1.42) and the highest T5 (16.25 ± 2.15). For this variable, the treatments TC, T3, T4 and T5 showed a significant difference in relation to T2. The mean emergence time (MET) also varied significantly between treatments (Table 1). The lowest was reported for T1 (4.73 ± 1.06) and the highest for T3 (10.96 ± 0.94), with treatments T1 and T2 significantly different from TC and T3.

The effect of different periods and storage methods as well as different treatments for dormancy breaking in *E. crista-galli* germination were evaluated by Silva *et al.* (2006). Seeds stored for three months in a refrigerator and without treatment for dormancy breaking obtained 6.7% of germination, with the best result (G% = 59) among the treatments being that obtained by immersion in sulfuric acid. When comparing the aforementioned study with the results presented here, it is notable that the treatment with water at 66 °C outside the heating was considered ineffective by the authors (G% = 14), whereas a very similar treatment in the present study (water at 60 °C outside of heating – T3) was the one that presented the best emergence result, but did not differ from the others. Storage possibly triggered the seed coat dormancy process in the aforementioned study.

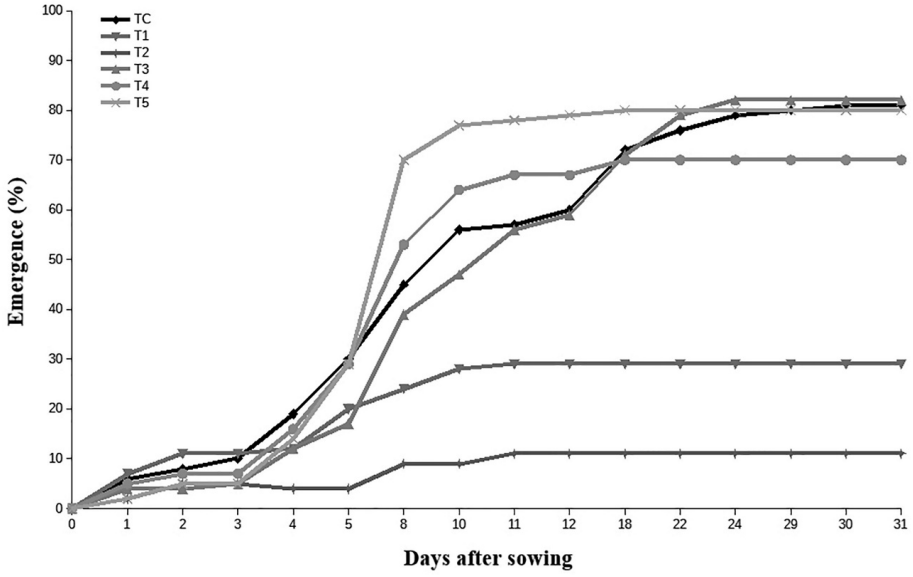

Fig. 2 TC, T1-T5. Seedling emergency (E%) in relation to the number of days after sowing (DAS). **TC.** control; **T1.** sanded and soaked in water for 48 h; **T2.** sanded and soaked in water for 24 h; **T3.** soaked in water outside of heating at the initial temperature of 60 °C until reaching ambient temperature; **T4.** only sanded; **T5.** immature seeds.

Table 1. Mean ± standard deviation for the ESI and MET variables. Equivalent letters mean that treatments do not differ statistically (one factor ANOVA, with Tukey test for multiple comparisons of means, $p \leq 0.05$). TC – control; T1– sanded and soaked in water for 48 h; T2 – sanded and soaked in water for 24 h; T3 – soaked in water outside of heating at the initial temperature of 60 °C until reaching ambient temperature; T4 – only sanded; and T5 – immature seeds.

Treatment	ESI	MET
TC	16.08 ± 8.52a	10.69 ± 4.66a
T1	10.45 ± 1.66ab	4.73 ± 1.06 b
T2	3.64 ± 1.42b	5.92 ± 1.50b
T3	13.18 ± 1.32a	10.96 ± 0.94a
T4	16.02 ± 3.96a	6.89 ± 1.21ab
T5	16.25 ± 2.15a	6.98 ± 0.26ab

ESI = Emergence Speed Index; MET = Mean Emergence Time.

Silva *et al.* (2006) also tested methods of overcoming dormancy for seeds stored for 27 months in a cold room and again the chemical scarification presented the best results (95% germinability), while the "control" (immersion in water at room temperature) reached 12.5% germinability. Furthermore, these authors obtained 5% germination (control treatment) to 95% (chemical scarification) for seeds stored in paper bags for four months without temperature control. Mello *et al*. (2016) also obtained better results (G% = 70) with chemical treatment (H_2SO_4), although the difference in treatment with mechanical scarification was not significant. The seeds that did not receive any treatment presented 10% germination, and the authors did not mention anything regarding the storage period and form. The data of these studies, when compared with the present research, show results much lower for the control treatments. Apparently, the manifestation of dormancy is related to time and storage conditions.

The data presented here show that newly collected mature seeds have not yet developed tegumentary hardness which is capable of inhibiting germination, since mature seeds not submitted to pre-germination treatments (TC) presented similar germination to other treatments, including immature seeds. This suggests that seed coat dormancy in *E. crista-galli* is developed after dispersion rather than in the rapid maturation phase. For Lazarotto *et al*. (2011), the seed coat dormancy in this species begins to manifest starting from ten weeks after anthesis, associated to the low water content found in the seed, thereby resulting in low germination for the material not submitted to pre-germination treatments. In general, other *Erythrina* species present seed coat dormancy, which can be overcome by applying pre-germination treatments (Matheus *et al*. 2010).

In the present experiment, the material sown 48 h after collection did not present a significant difference between the control treatment and those which presented the best results, including the immature seeds. The low emergence registered in the treatments with mechanical scarification and subsequent soaking in water at room temperature suggest that these were harmful, although T1 presented seeds with germination signs (tegument rupture and primary root emission) when still undergoing treatment (Fig. 1).

In some cases, seeds obtained from immature fruits or those collected in initial maturation stages do not present good germination results (Aquino *et al*. 2006; Godefroid *et al*. 2010); however, with some exceptions such as *Alibertia edulis* (Rich.) A. Rich. for which seeds obtained from "green fruits" presented higher germination than those extracted from "mature fruits". Nevertheless, seeds extracted from "green fruits" after 11 months of storage presented 19% germination, versus 88% in seeds of "mature fruits" stored for 13 months (Ferronato *et al*. 1997). Takahashi *et al*. (2006) affirm that immature seeds can present better germination results, because in addition to not having acquired integument impermeability, they will have been less exposed to attacks from animals and microorganisms. However, the authors recommend that these collected seeds should be sown immediately after collection to avoid damage and germination loss. They also report that anticipation in the collection of seeds to obtain a superior germination rate is only valid for seeds that acquire a certain integument impermeability when they are mature (secondary dormancy), otherwise immaturity will prevent normal germination.

Other factors may influence germination / emergence rates, in addition to those related to conditions and storage time, such as genetic, environmental factors and their possible interactions (Sert et al. 2009). Rego *et al*. (2005) evaluated the effect of genetic variability on the germination of *Albizia lebbeck* (L.) Benth. and found an expressive genetic control over the germination of the species. On the other hand, environmental conditions under which the plant is subjected during seed production may interfere with the establishment of physical dormancy (Molizane *et al*. 2018; Penfield & MacGregor, 2017). Germination of *Erythrina speciosa* seeds from the same population followed for six years showed strong interannual variation (Molizane *et al*. 2018). The authors attribute this oscillation in germination over the years to different degrees of dormancy, correlating it to environmental conditions (i.e. relative humidity) during seed formation.

Erythrina crista-galli seeds newly collected from mature pods in dehiscence do not require treatments to overcome the seed coat dormancy, at least 48 h after collection. Seeds from immature pods sown as soon as harvested normally germinate. Therefore, pre-germination treatments for this species are unnecessary and possibly harmful. It is recommended future researches with seeds obtained in the same population and at the same time be tested immediately after collection and after different storage periods and conditions, as well as seeds from different populations of *E. crista-galli* to evaluate genetic and environmental aspects.

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