Assessment of different seedling production techniques of E*uterpe edulis*

Leonardo Lima Pereira Regnier[1]*, Maria Luiza Faria Salatino[1]

[1]Departamento de botânica -Instituto de Biociências- Universidade de São Paulo - R. do Matão, 187 - Butantã, São Paulo - SP, 05508-09036036-900, Brazil
*regnier@alumni.usp.br

ABSTRACT

Euterpe edulis is an endangered species with high importance ecologically and economically. Seedling production seems to be one of the most important alternatives to population recovery. Besides that, the knowledge of seedling production methods' influence over germination is very restricted. Thus, this study aimed to evaluate the effects of parent populations, germination conditions, and the substrate to commercial seedling production of *E. edulis*. Nine thousand seven hundred and thirteen seeds were distributed between the heated water and control, greenhouse and open-field treatments. The parent population presented high differences between most of the germination indexes. Influencing the germination rate, mean germination time and germination speed, but not affecting synchrony and uncertainty indexes. Heated water treatment did not affect any of the studied indexes, presenting a close pattern of germination over time, indicating it is an appropriate method for seedling production. Greenhouse and open-field treatment presented variations at the same indexes affected in the parent population analysis. The most profitable method for *E. edulis* seed germination was the greenhouse production method, which provided the best indexes results.

Keywords: Palmiteiro; Forestry seeds; Seed development; Arecaceae; Germination

INTRODUCTION

Euterpe edulis Mart. also known as *Palmiteiro*, is one of the most representative and explored species from the Tropical Atlantic Rainforest [1]–[3], and also considered one of the most important [4]. Its slim, cylindrical and straight stipe with a great green sheath is easily recognizable [1]. This species presents low and slow germination due to the mechanical structure of the seeds, which are recalcitrant, been not resistant to dry and/or temperature reduction. Those features contribute to its long-term life cycle [5], [6]. Seedling growth seems to be limited by light exposure in natural conditions [7]. Adult individuals only reach sexual maturity between six and nine-years-old. These characteristics also hamper the natural regeneration of its populations.

This species also plays a relevant ecologic role as a food source to a great number of animals like birds, mammals, and insects [6], [8]. These interactions contribute to the reduced number of viable seeds that stay disposable to germinate and recover

the population. An even fewer number of germinated seeds reaches one-year-old [6].

The economic relevance of this species is its uses as the heart of palm (youngest part of the stipe, apical meristem, and young leaves) source. Heart of palm from this species presents high economic value, being an important product in the Brazilian and international markets [3], [4], [8], [9]. Besides that, this species does not present tillers from the stipe basis [4]. Thus, the harvesting of the heart of palm from *E. edulis* implicates in the individual's death. Furthermore, the illegal harvesting of this species heart of palm has been focusing on individuals at least 2 meters high. This criterion not only affects young individuals but also those in the reproductive phase (between six- and nine-years-old individuals), which is totally incompatible with this species slow life cycle, leading to a reduction of the natural populations and to extinction [1], [4], [9].

E. edulis is also a key-species to ecologic studies concerning the Atlantic rainforest [9]. Other authors have demonstrated that programs seeking *in situ* recovery must focus on seed banks' maintenance and replanting techniques to effective forestry management [2], [5]. Thus, scientific research development in seedling production and ecophysiology is essential to guide recovery programs and/or commercial production [4], [5], [7], [10].

The demand for reproduction, quality, seed conservation, and seedling production technologies has been growing due to recovery programs and the recent inclusion of *E. edulis* species in landscaping projects [11]. Seedling production is also pointed out as the main method for the management of the natural populations of *E. edulis* [4], [12]. However, this knowledge is still very restricted. Only recently two basic aspects, the removal of the mesocarp and light exposure influences on germination, were clarified [13]. The lack of technologies and knowledge in the exploration of native forestry resources [8] associated with natural habitats destruction are the main causes that still have been taken the native species to decline [8], [9].

In general, the parent plants seem to influence the seedling quality and the germination rate of its seeds [12], [14]. Most recently, to *E. edulis*, a great diversity has been recognized and better explored [9], [15]–[17]. Besides that, the comprehension of how those differences between populations could affect germination parameters was not adequately known [9]. This aspect could provide important information in seedling production since the choice of a progenitor population used as seeds sources could affect seedling production [12], [14].

The mechanical structure of this species seeds apparently hinders water penetration, generating great variations between seed germination rate [6]. One possible method to overcome this is by using hot water [18], [19], which also makes the removal of fruit pulp easier [5]. However, this method could reduce seed viability, reducing germination. Previous studies concerning the impact of hot water treatment on minerals bioaccessibility have already been conducted [20]. Nevertheless, possible germinations effects of this technique have not been extensively recognized in the literature.

In order to promote the germination, on the commercial seedling production context, there are two main processes, open-field germination, where seeds are directly exposed to environmental condition as sunlight and temperature; or germination at greenhouse, where seeds are exposed to greater temperature and moisture but less sunlight than the production area [21], [22]. Those different techniques could also impact seed germination and seedlings persistence [23], aspects that have not been explored regarding *E. edulis* yet.

Thereby, due to the high ecologic and economic importance associated with the great demand for reproductive techniques of this species. This study aimed to evaluate the effects of parent populations, heated water processing, and the open-field

production approach to commercial seedling production of *E. edulis*.

MATERIAL AND METHODS

This study was conducted at the Harry Blossfeld plant nursery of São Paulo, situated in Cotia (23°36'30.0"S 46°50'48.9"W). According to Köppen's climate classification, the study region presents Cwa, the altitude tropical climate [24]. Featuring concentered rains during summer, dry winter, and the highest mean temperature above 22°C.

Plant material was collected in the west region of São Paulo city, in July and August of 2018. Population I is 24 km distant from populations II and III and these last two were 1 km distant from each other. Fruits were gathered directly from the treetop and fallen fruits were also collected. All material was kept in open plastic bags at room temperature up to 2 days, during the processing stage.

The study was divided into three steps. First, seeking to evaluate parent population influence, about one thousand seeds from each of the three different populations were evenly distributed between two repetitions of the standard treatment [25]. This treatment consisted of exocarp and mesocarp removal with a sieve and running tap water. Seeds were planted in white trays containing vermiculite as a substrate and kept at greenhouse with white plastic covering and a fogging watering system with periodic activation every 35 minutes.

The second part consisted of six thousand seeds, from population I, divided between two replicas of the 2 treatments. The control treatment used was the same as the standard treatment used in the first part of the study. While in the second treatment all fruits were previously submerged in the heated water at 80 ºC, seeking to facilitate the exocarp and mesocarp removal with sieve and tap water. In sequence, seeds were planted in an open-field plant bed containing vermiculite as substrate without any kind of covering, and irrigation two times daily.

Seeking to evaluate the seed development in open-fields, 1 000 fruits from the same population, submitted to the standard treatment and planted as described in the second part were sowed in plant beds containing vermiculite as substrate without any kind of covering and irrigation two times daily, 8 am and 4 pm.

Plant emergence was recorded 134 days after seeding, with measurements nearly every 7 days.

The evaluation consisted of the use of indexes as mean germination time, or mean length of incubation time [26], and the standard deviation calculated as proposed by Haberlandt in 1875 [27]. All the other indexes were calculated as presented in Lozano-Isla *et al.* [28].

All data were compiled to the Excel component of Microsoft Corporation Office pack and represented through graphics and tables. Most usual germination indexes were obtained through GerminaQuant software [28]. All the statistical tests were executed with individual data of the replicas and performed using the GerminaQuant, based on R software [29]. The results were tested by ANOVA and in sequence submitted to the Tukey test adopting a critical p-value of 1% ($\alpha < 0.01$).

RESULTS AND DISCUSSION

Parent population

The parent population seems to influence the Germination proportion (GRP), Mean germination rate (MGR) and Germination speed (GSP) (Fig. 1A, 1B, 1D). While Uncertainty (UNC) and Synchrony (SYN) indexes were not different (Fig. 1E, 1F). Population I and II presented the greatest germination rates (91.3% and 78.57%) and were consistently different from population III (22.9%). Mean germination rate index (MGR) indicates, at this study, the frequency

of germinations, as the mean germinations/day. Thus, it is the reciprocal information of mean germination time (MGT) [26].

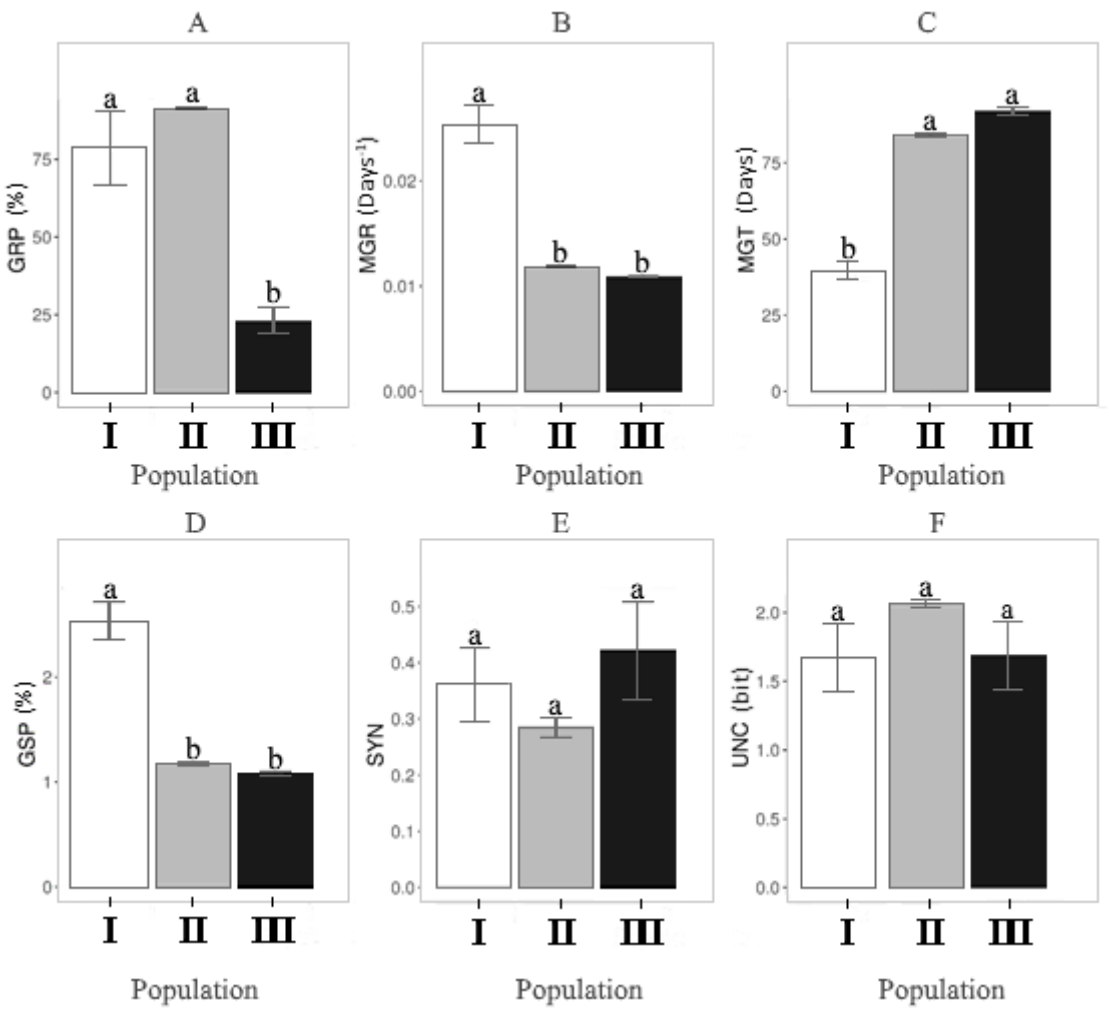

Figure 1. *E. edulis* germination indexes according to seed population origin (**I**, **II**, **III**). **A** - Germination Proportion (GRP), **B -** Mean germination rate (MGR), C - Mean germination time (MGT), **D** - Germination speed (GSP), **E** - Synchronization index (SYN) and **F** - Uncertainty index (UNC). Lowercase letters present the statistical differences adopting p<0.01. Error bars present the standard error.

Mean germination time (MGT) or mean length of germination time, demonstrate the required time to one seed of some species to germinate [26], [30]. It is an important index insofar as it demonstrates the mean expected time to most seeds of a seed lot to germinate. Thereby, it is possible to estimate the necessary time for some specific culture to properly grow. The data between the populations are significantly different (Fig. 1). Only populations II and III presented a similar mean germination rate (84.36 and 91.9 respectively). Population I reached stabilization about half the time obtained by the other populations, and also greater germination speed in this study (Fig. 1D). Those are a valuable aspect of a seedling production context. Thus, the population I have the most profitable offspring, seeking better production.

Mean germination time is associated with the general characteristics of the seed species, the seed lot quality, and environmental conditions [5], [31]. The unbridled harvesting of adult individuals that led

E. edulis natural population decline, related to the reproduction between related individuals could have been leading some populations to less vigorous offspring, a process called endogamy depression [12] what directly affects seed quality [2], [14]. This species presents aggregated spatial distribution of seedlings, favoring intraspecific competition and greater vulnerability to diseases and plagues [32]. These features, associated with the short distance gene flow [14] could result in high endogamy, reducing seed diversity and quality. This process impacts directly the seed harvesting approach seeking conservation projects [14] since that great variation between seed lots hamper commercial seedling production [12]. Studied parental populations had considerable variations on germination rate, and also germination speed indexes (Fig. 1). Indicating that choosing some population offspring not only influences the exploitation of a seed lot, based on the germination proportion but also on the time required for seedling emergence. Thus, the process of choosing a parental population to seedling production seems to be a very important aspect to be considered.

The germination rates of the studied parent populations provided significant differences between population III and the others (Fig. 1A). Cumulative germination course also reveals the discrepant performance of this population compared to the others (Fig. 2). Germination of *E. edulis* seems to differ highly due to genetic variations between fruits, even when they are harvested at the same time and present the same development stages [12]. An important aspect of genetic variability in the reproductive system [14]. It is estimated that the gene flow between individuals of *E. edulis* is 56 meters using DNA markers [14], which favors genetic isolation between populations, and high variations between them [9]. However, natural population decline seems to has been leading to endogamy depression. Parent plants age seems also to influence the germination indexes [3], [6] and the reproductive season [2]. Germination presented in other studies comprehends a range from 16% up to 52% at 12 days after sowing [6] and from 44% to 73% between 100 and 150 days after seeding [12], [33]. This present study data indicates a higher germination proportion to the population I and II than those previously mentioned values found by those authors (Fig. 2). Besides that, none of the studied populations reached at least 16% of germination during the first 12 days after sowing, as mentioned by Bovi [6]. However, these discrepancies are usual between non-domesticated species [14] due to genetic variations, and also the environmental conditions related to the locations were the studies are conducted.

It is important to notice that the germination pattern was very different between the populations (Fig. 2). Though populations I and II reach relatively close germination results at the end of the study period, their trajectory until reach those results were not equal. Most recent studies have been shown that, in general, diversity between this species populations remains high considering fragmentation, habitat destruction, and selective exploration [9]. Our results also presented a great diversity of germination responses between the populations. Corroborating this information, since conspicuous

variations between germination indexes were observed, when comparing populations of *E. edulis*.

In commercial seedling production, understanding factors that could impact seed lot quality is very important, especially to understanding the suboptimal results [34]. These study data indicate that choosing the parental population as a seed lot provider could drastically modify seed lot quality, being a crucial aspect in a seedling production context.

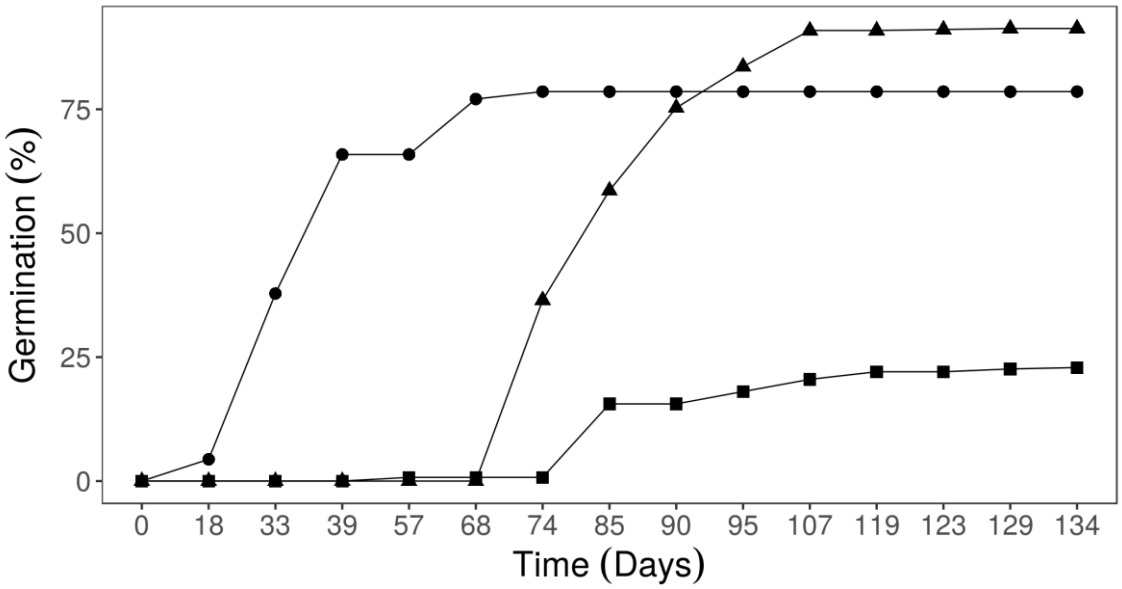

Figure 2. Cumulative germination of *E. edulis* according to seed origin. Population I (●), Population II (▲), and Population III (■).

Non-cumulative germination pattern indicates that, in general, germinations are concentrated at some specific period, when most of the seeds geminate synchronically (Fig. 3). Besides that, the values of the germination rate reached by each population vary. The population III pattern is similar to other populations but delayed and with lower values (Fig. 3). Non-cumulative germination analysis could provide some important information. In this study, seeds from different populations seem to exhibit a similar pattern, with relatively concentrated germinations at some specific time, diverging only by when this higher emergence period happens. This fact explains why the uncertainty and synchrony indexes were similar between the studied populations (Fig. 1). Since they presented a very similar distribution pattern but with contrasts about the required time to reach great germination occurrence. The different parent populations seem to provide variations on when the germination occurs and the relative distribution of the germination all over the time.

Although it is important to notice the long-term germinations presented by this species. Previously, other authors have already mentioned that those greater variations on the required time to seedling emergence is an adaptive strategy required to maintain a proper seed bank [12]. An essential implication on ecologic dynamics because if all the seeds from a single cohort present unsynchronized and long-term

emergence, this single reproduction event could provide new recruits to the population during very long periods, even with dramatic reductions in the parental population. However, these features hamper seedling production [35], because this context only favors faster and synchronized germinations.

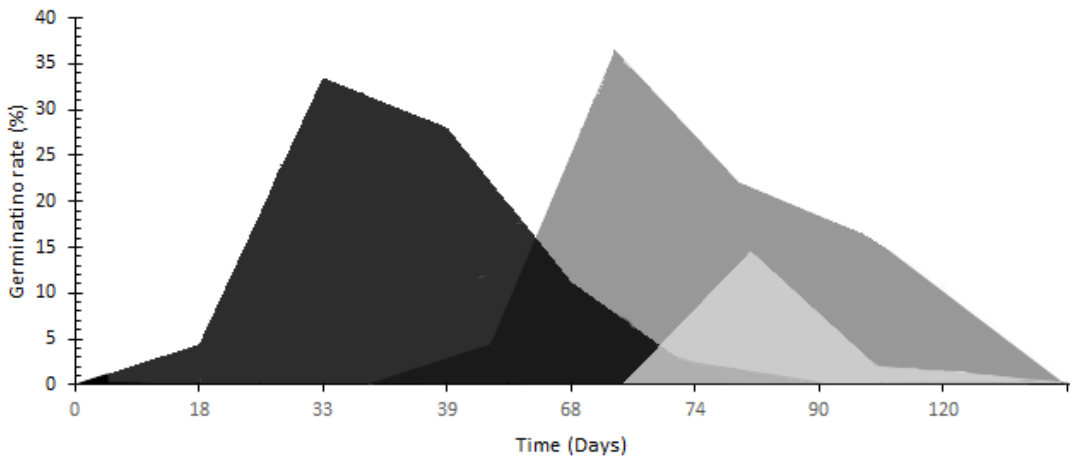

Figure 3. Non-cumulative germination of *E. edulis* according to seed origin. Population I (■), Population II (■), and Population III (■).

Heated water treatment

There was no difference between the control and heated water treatment. All the analyzed indexes did not present significant variations. It seems that using heated water only provides a slight reduction in germination proportion and mean germination time (Fig 4). In general, heated water promotes mechanical ruptures that could easily lead water to permeate [36]. However, high temperatures could also kill the embryos, reducing seed viability [5]. Our results show that any of the studied indexes related to the time required to germination, speed or germination rate was substantially affected by this method at this study parameters. Indicating that there is no significant reduction of seed viability when exposed to this temperature. Although, if *E. edulis* presents the mechanical structure that hinders water penetration as mentioned by other authors [6], heated water at 80°C is not providing its rupture. Better anatomic and germination studies are required to clarify the seed structure and provide methods to better germination rates of *E. edulis*. Cursi & Cicero [5] found that *E. edulis* fruits, when immersed during 20 minutes on water at 40 °C, presented higher germination. While exposure to 55 °C water before processing seems to be harmful to embryos. Our results are conflicting with this information. Although, the consistent variations observed to germination indexes provided by the populations' origin, and environmental conditions adopted during the experiment could also be responsible for this kind of discrepancies between studies.

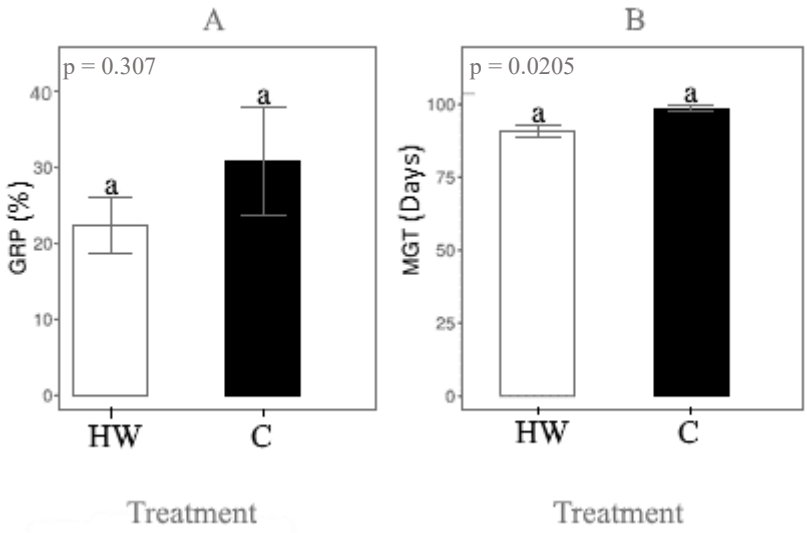

Figure 4. *E. edulis* germination indexes according to seed processing treatments: Heated water (HW) and control (C). **A** - Germination Proportion (GRP), **B** - Mean germination time (MGT). Lowercase letters present the statistical differences adopting p<0.01. Error bars present the standard error.

Cumulative germination pattern was also very similar between the treatments (Figure 5) emphasizing that this procedure does not substantially modify seed germination. Besides that, adopting a heated water procedure makes the removal of fruit external parts easier, and it seems to not substantially affect the seedling production seeking commercial proposes, which was also mentioned in previous studies [5]. Thereby, this kind of procedure could be used on the seedling production context, without major harmful effects.

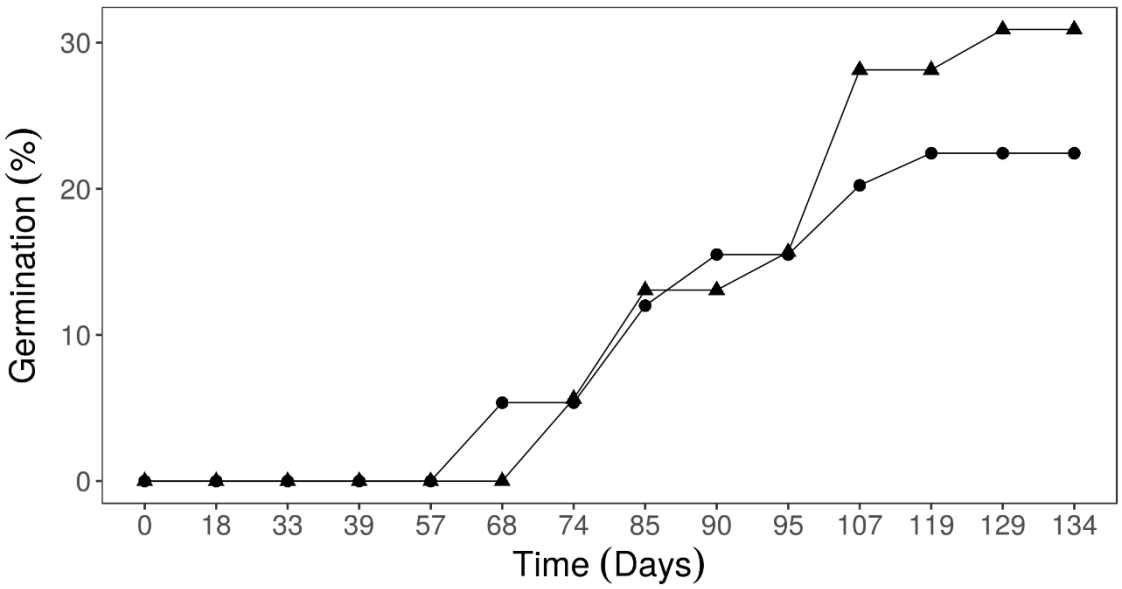

Figure 5. Cumulative germination of *E. edulis* according to seed processing. Heated water (●) and Control group (▲).

Open-field and greenhouse production process

Analyzing the production process of open field and greenhouse some variations were significant to all the analyzed parameters except for uncertainty and synchronization index (Fig. 6). The greenhouse process provided better results at all indexes, the required time to germination and stabilization were faster, and the germination rate was significantly greater, providing better exploitation of a seed lot. The greenhouse production process has been recognized to notably optimize plant production and reduce the limits of growth [37]. The results also emphasize that the greenhouse promotes faster and better germination of *E. edulis* seeds. Greenhouses usually promote a stable temperature, controlled moisture, and less susceptibility to the environmental conditions. This group of features favors seedling emergence. Contrasting with open field, the greenhouse promoted both better germination rate and reduced time required to germination, favorable aspects seeking commercial seedling production.

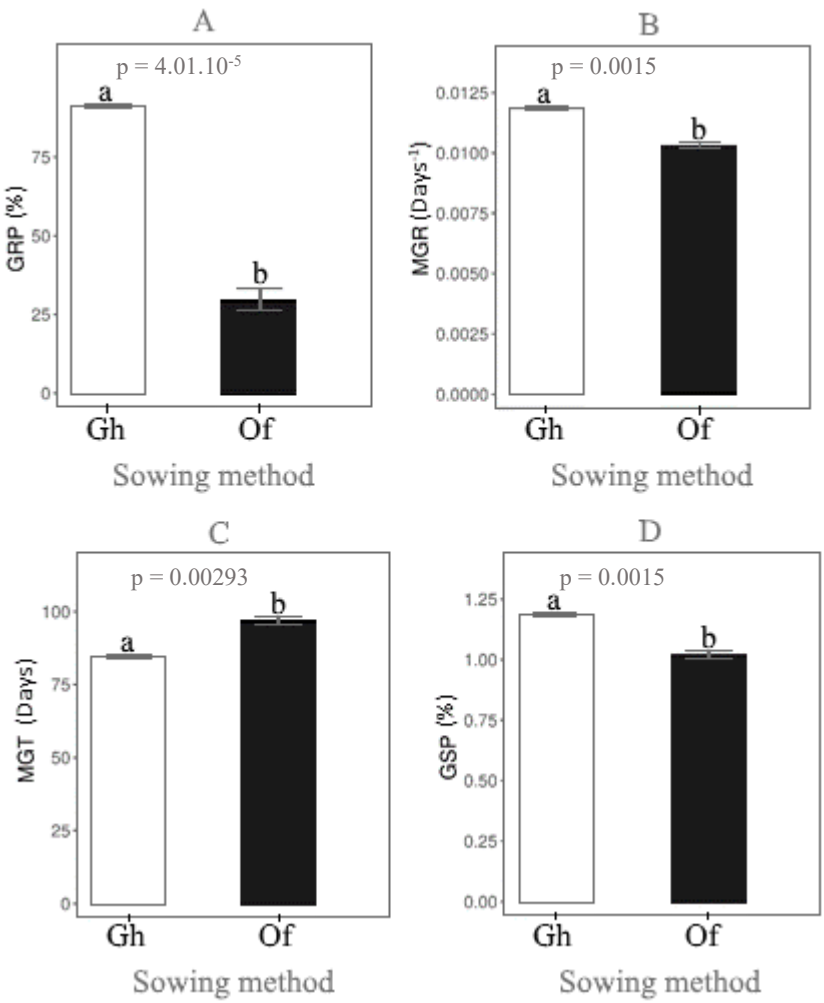

Figure 6. *E. edulis* germination indexes according to different sowing methods, greenhouse (Gh) and Open-field (Of). **A** - Germination Proportion (GRP), **B -** Mean germination rate (MGR), **C** - Mean germination time (MGT), **D** - Germination speed (GSP). Lowercase letters present the statistical differences adopting $p<0.01$. Error bars present the standard error mean.

The cumulative seed germination pattern was substantially distinct (Fig. 7). It is already recognized that controlled conditions favor the better expression of the germinative capacity of the seed lots, while in the open field the attack of animals, and also microorganisms, reduce the viability of seeds affecting the germination and seedling development [2], [38]. Open-field also propitiates higher sunlight exposure and less water provision. Water availability seems to be a crucial aspect during *E. edulis* seed development. Earlier life stages are the least tolerant to water deficit, and the resistance seems to gradually increase with age [10]. Thus, the lesser water provision in open-field treatment could be the main factor to reduce germination performance. In an ecological approach, the open-field treatment also provides environmental conditions relatively closer to natural or anthropic disturbed areas [23]. Providing a set of environmental conditions that not favor seedlings establishment. Reduced performance of germination on open-field technique has also been reported to

other species from Fabaceae [23]. Besides that, some authors affirm that even when this species is more exposed to environmental conditions, *E. edulis* plays the role of pioneer species, being the first mesophytic species to conquer disturbed environments [8].

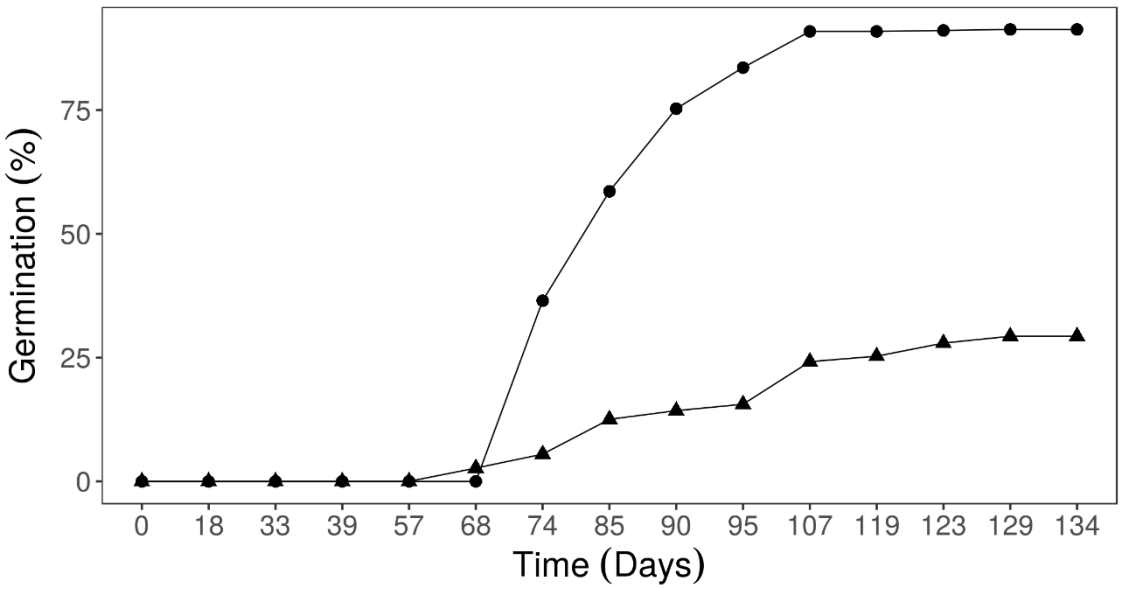

Figure 7. Cumulative germination of *E. edulis* according to the seed production process. Greenhouse (●) and Open-field (▲) production methods.

Even though the open-field production method does not provide the best environment for germination, this method is adopted to some crops because it requires less electricity and labor when compared to the greenhouse method [39], and facilities maintenance is cheaper than greenhouses, reducing the production cost [23]. Sometimes this practice is also used to recover degraded areas [38]. However, the cost-benefit ratio needs proper evaluations for further studies.

CONCLUSION

The different parent populations seem to influence the germination rate, mean time to germination and germination speed, but not affecting the uncertainty and synchrony indexes.

Heated water at 80°C did not affect germination indexes, indicating it is a feasible method for seedling production. The most profitable method to *E. edulis* seed germination was the greenhouse production method since it provided the best results, of germination, speed and time required to germination.

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