Mechanical scarification, seed origin, and substrate influence on germination of *Samanea tubulosa*

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ABSTRACT

Samanea tubulosa is an important species mainly to its uses in reforestation projects and as a wood and fruit resource. This species seed dormancy and other scarce germination information have been limiting the adequate seedling production process. Thus, this study tried to understand how seed origin, mechanical scarification, and substrate could influence the seedling production process. The population affected the time required to germination and germination speed, probably due to high genetic variability. While mechanical scarification did not present statistical differences to seed in natural conditions, indicating that seed dormancy could be variable. Substrate promoted significant differences at the germination rate. The population seems to affect but not to preclude seedling production. Mechanical scarification did not provided relevant enhance of germination. Vermiculite presented a significantly higher germination rate than the organic-based substrate.

Keywords: Forestry seeds, Fabaceae, Seedling production, Sete-cascas, Saman

1. Introduction

Samanea tubulosa (Benth.) Barneby & J. W. Grimes popularly known in Brazil as *Sete-cascas*, and *Saman* in Spanish America, is an arboreous and deciduous tree. This species is native of South America and presents high-tolerance to sunlight and the natural occurrence fires of Cerrado, the Brazilian savannah. Its characterized as a pioneer species, colonizing glades and open-fields [1]. Promoting this species common use in urban afforestation, and reforestation programs, especially in disturbed areas [2]–[5].

Fruits are the main resource of this species. They are usually employed as cattle food and, when empowered, is applied to goat and chicken feeding, due to its high protein and sugar content [1]. Fruits can be eaten by humans and are usually utilized in cachaça production, resulting in “Aguardente-de-saman”. The wood from this species is widely exploited, especially in the furniture industry and fences [6]. Alcohol

production, timber, and paper production are other recent uses to this species [1].

Seed dormancy is characterized by seeds that even when exposed to favorable environmental conditions, for instance, light exposure, moisture, oxygen, and temperature, they do not germinate [7], [8]. Seed dormancy is a usual problem in the seedling production of arborous trees and especially usual in the Fabaceae family [7], [9]–[11], being nocuous to seedling production process because fast and uniform germinations are required [10].

Understanding seed behavior from seeding until the seedling establishment is also important to the successful application of wild species in reforestation programs [12], [13]. Thus, recognizing techniques that could improve the seedling production process is important [10], [11]. Especially to *S. tubulosa* since it is one of the most indicated species to use at this kind of project and urban afforestation.

S. tubulosa high-level dormancy is already recognized as a result of its impermeable tegument [1], [6], [7]. In general, in the natural environment, temperature variations, acid soil, microorganism metabolism, and animal consumption of fruits are the main tegumentar dormancy overcoming methods [11]. Chemical scarification using acid compounds is the most usual pre-germinative procedure to overcome *S. tubulosa* seed dormancy. Besides that, this seed dormancy overcoming procedure is very expensive and imposes some practical obstacles. In general, those compounds are very difficult to manage when a large number of seeds are submitted to this process, present the risk of accidents [9], [11], or even generate wounds to seed embryonic tissues [7] promoting abnormalities in seedlings [14]. Alternative methods, such as hot water treatment are mentioned as not adequate to overcome *S. tubulosa* seed dormancy [10], [11].

Mechanical scarification is recommended by some authors [4], [7], [11], [15] as a viable alternative to chemical scarification. However, those authors recommend the manual scarification techniques with sandpaper and discourage the use of the rotary drum, due to the great damage this last technique could provide to the embryo. Oliveira et al. [10] also measured the impact of mechanical scarification of *S. tubulosa* with scissors. Besides that, those mentioned mechanical methods are infeasible on the seedling production context, which requires the processing of an extensive number of seeds without taking to much time.

In general, to forestry seeds, the parent plants seem to influence the seedling quality [16]–[18]. Affecting some germination parameters, as the total germination rate [19], the time required to germination, or even both [18]. This aspect is very important to be analyzed because in seedling production context the choice of a

progenitor population used as seeds sources could affect seedling production [16], [17].

The substrate is essential in seedling germination [11]. Conflicting information also could be found about the best germination substrate, while some authors point out that to *S. tubulosa* better germination indexes were obtained when sowed in organic-based substrate [20], others affirm that vermiculite is the best substrate to *S. tubulosa* production [11]. Although, proper comparison between germination on inert substrates as sand or vermiculite and in the organic-based substrate has not been extensively explored.

Thus, this study focused on understanding how the mechanical scarification, seed origin, and substrate could influence *Samanea tubulosa* germination.

2. MATERIAL & METHODS

This study was conducted at the Harry Blossfeld plant nursery of São Paulo, situated at the city of Cotia (23°36'30.0"S 46°50'48.9"W). According to Köppen's climate classification, the study area presents Cwa, an altitude tropical climate [21]. Featuring moderate and concentered rains during summer, dry winter [21], and the highest mean temperature above 22°C.

Plant material was collected in the city São Paulo between September and October of 2018 from two different populations about 125 km distant from each other. The harvesting method consisted of mature fruits gathered directly from the ground. The material was kept in open plastic bags at room temperature during 15 days, to recognize the emergence of insects and/or pathogens [17]. After this period, fruits were open and seeds removed, as indicated by Carvalho [1], using a rubber hammer.

Population influence on germination was measured using 630 seeds from population I and 126 from population II. These seeds, without treatment, were planted in 280 cm³ greenhouse tube, with an organic-based substrate composed by 2 liters of local horizon B soil, 8 liters of rice husk, 5 liters of Basaplant® forestry substrate, 46.5g of Yoorin K® potassium thermosetting fertilizer, and 46.5 g of gradual release fertilizer Osmocote Plus®.

Evaluation of seed processing method involved a sample of 252 scarified seeds and 630 seeds in a natural condition, planted in the previously mentioned organic-base substrate and greenhouse tubes. The mechanical scarification procedure consisted in the use of electronic emery until conspicuous seed tegument rupture of the opposite side of mycropile.

Seeking to test substrate influence, 252 scarified seeds were sowed in the same previously mentioned organic-based substrate and

the greenhouse tubes. While 1452 scarified seeds were seeded in white polypropylene trays containing vermiculite as the substrate.

After all the mentioned treatments, the material was kept at greenhouse with white plastic covering and a fogging watering system with periodic activation every 35 minutes. Plant emergence was recorded about 20 days after seeding, with measurements nearly every 7 days.

All data were compiled to the Excel component of the Microsoft Corporation Office pack. Most usual germination indexes were obtained through GerminaQuant software [22]. All the statistical tests were executed with the data of the repetitions, also using the GerminaQuant software, with ANOVA followed by the Tukey test adopting a critical p-value of 5% ($\alpha < 0.05$).

3. RESULTS AND DISCUSSION

Population origin

The population seems to influence the time required to *S. tubulosa* seed germination (Figure 1) since we observed only differences between the mean germination rate (Figure 1B) mean germination time (Figure 1C), and Germination speed (Figure 1D). Germination rate (Figure 1A) was not affected by the seed origin to the analyzed period. These same indexes were also affected when comparing the population influence to *Pterocarpus rohrii*, another species of the Fabaceae family [18].

Mean germination time (MGT) represents the mean required time to one seed of a seed lot germinate. In this study, population II presented the double value of germination speed (GSP), and its seeds take about 5 days to germinate. While in population I the mean time required to one seed germinate (MGT) comprehends 13 days, almost three times the obtained value of the population I. To seedling production process, differences of 7 days are relevant but not crucial [18]. Thus, the seedling production process could be affected by seed origin, but it seems that it is not substantially impaired to this influence. Similar results were found to *Hymenaea courbaril* [23], but opposite findings to *P. rohrii* [18], both members of the same family.

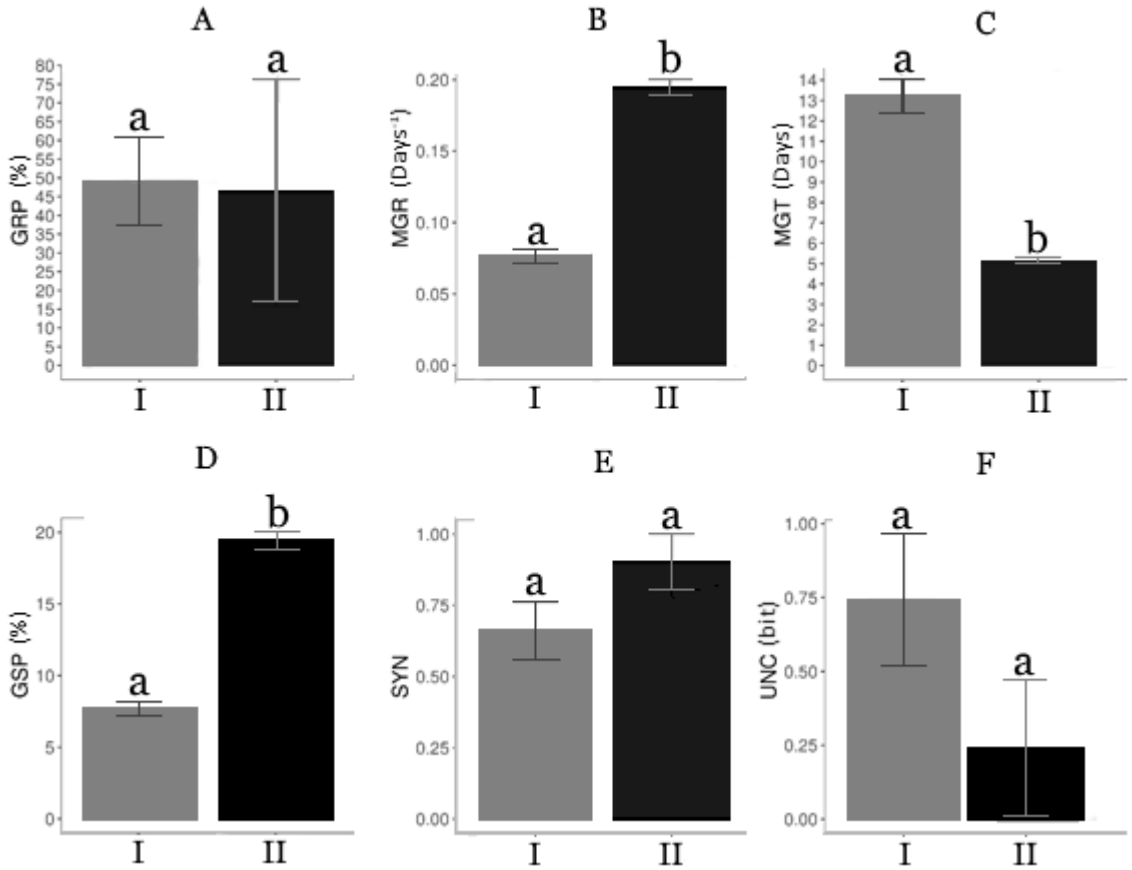

Figure 1. **A-** Germination Proportion (GRP), **B-** Mean germination rate (MGR), **C-** Mean germination time (MGT), **D-** Germination speed (GSP), **E-** Synchronization index (SYN) and **F-** Uncertainty index (UNC) according to seed origin: population I (I), population II (II). Lowercase letters present the statistical differences adopting $p<0.05$. Error bars present the standard error mean.

In general, variations between populations are expected to non-domesticated species [17]. Wild species present greater regional variations because they are under a continuous evolutionary process, and this could create great variations between populations [17], [18], [24]. Thereby, depending on the study sampling standardization, the results will be only regionally applicable [17].

Our results indicate that, to *S. tubulosa,* choosing a progenitor population seems to affect the time indexes, but not the germination proportion, faster germinations are better, reducing the time a seed lot occupies in the greenhouse or in the production area. Thus, choosing the adequate parent population could provide a slight reduction of the required time to a seed lot germination.

The main differences between the germination patterns were the seedling emergence (Figure 2). Both populations reached the same germination amount at the end of this experiment. Besides that, seeds from the population I

presented faster emergence (also indicated by the indexes). It is important to notice that a greater time of experiment possibly could provide different results. Shorter evaluations than 9 days could lead to the erroneous conclusion that population I seeds almost did not germinate. Therefore, as mentioned before, it is important to consider time during germination evaluations [18], [25].

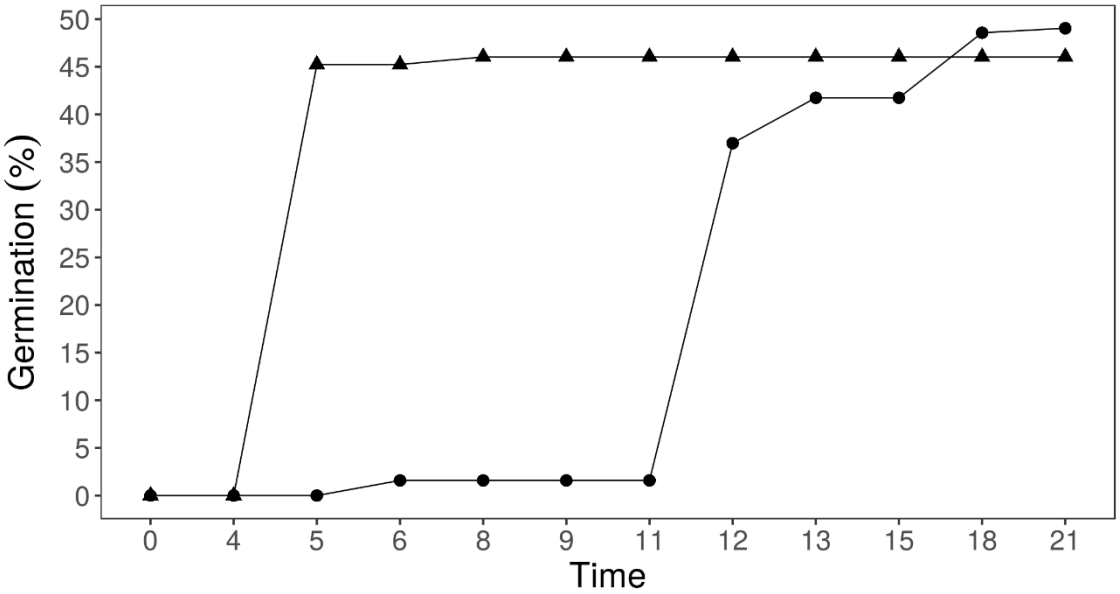

Figure 2. Cumulative germination of *S. tubulosa* according to seed origin. Population I (●), Population II (▲).

Seed processing method

Mechanical scarification with emery and seeds in natural conditions did not present significant differences to any of the studied indexes (Figure 3). Variations of germination were grater to non-processed seeds to germination proportion (Figure 1A), synchrony (Figure 1E) and uncertainty (Figure 1F) indexes. Indicating that when seeds are not scarified they present great variations between seed lots to those mentioned indexes. Great uniformity promoted by scarification is already recognized. Especially because during the imbibition phase water is the main agent to promote germination, as scarification facilitates water uptake, higher values of uniformity are obtained [26].

Other studies showed that *S. tubulosa* acid scarified seeds presented germination rates higher than 85% and seeds in natural condition reaches 55% [4]. These values are very discrepant from the data we obtained to mechanical scarified seeds. In general, both, scarified or natural condition

seeds, reached about 50% of germination. Although those authors promoted mechanical scarification using sandpaper, while in this study we promoted the scarification by emery. Martins et al. [9] mentioned that scarification with sandpaper efficiency is highly variable according to the person dexterity to execute the procedure. However, the genetic variations found to wild species, the evaluation time, of two months, and the controlled temperature on the mentioned study [4], could not be rejected as possible reasons to the discrepancies. It is already known that temperatures higher than 25°C promote better germination rates in *S. tubulosa* [7]. Thus, as those authors adopted 30°C during the experiment, this could explain the greater results than the present study.

Other authors have already mentioned that there is a slight increase, not always significant, on germination speed when seeds are mechanically scarified [4], [13]. We found a similar pattern, although without statistical significance.

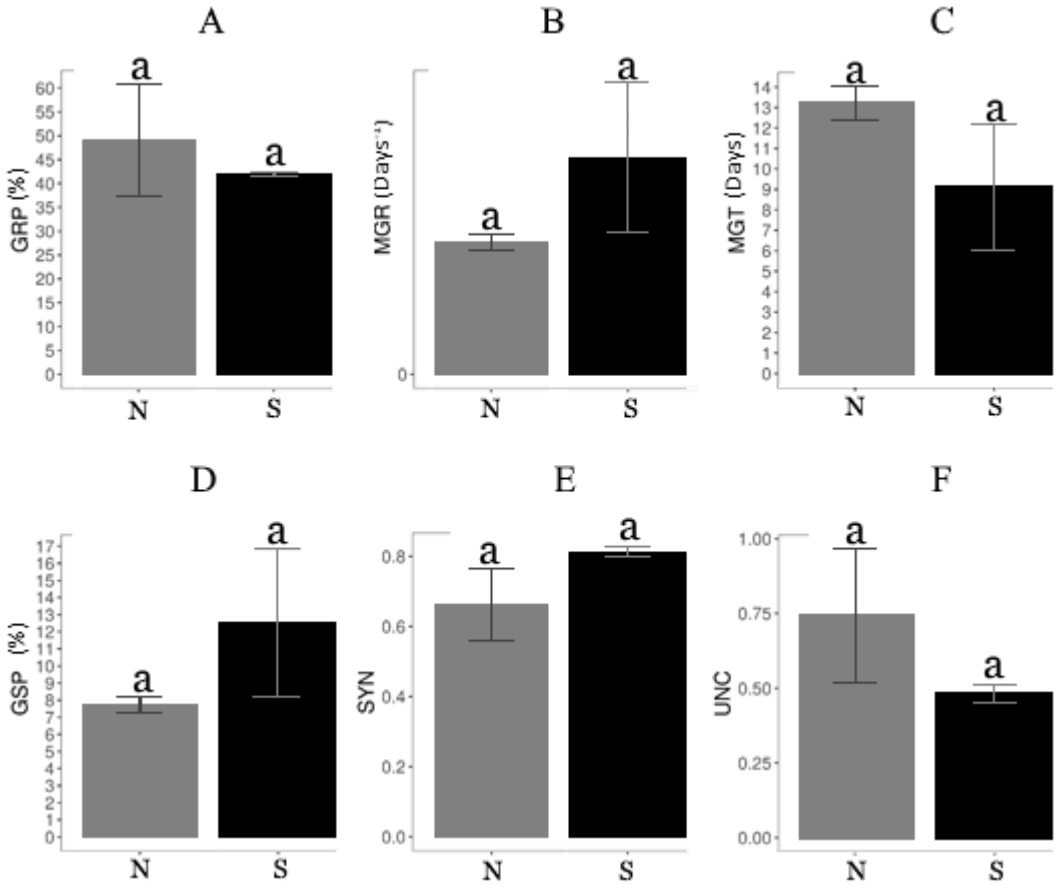

Figure 3. **A-** Germination Proportion (GRP), **B-** Mean germination rate (MGR), **C-** Mean germination time (MGT), **D-** Germination speed (GSP), **E-** Synchronization index (SYN) and **F-** Uncertainty index (UNC) according to seed processing method: Scarified (S), and non-processed seeds (N). Lowercase letters present the statistical differences adopting $p<0.05$. Error bars present a standard error mean.

Seed dormancy and germination are also affected by the genetic diversity of native species [17], [18]. In general, in the Fabaceae family, the dormancy level could vary between populations [27] or even in the same seed lot [28]. This effect could be associated with tegument thickness and waterproofing compounds, resulting in different degrees of seed dormancy [10], [13]. Thus, beyond the influence of the analysis period mentioned before, variations between seed dormancy could also interfere with germination.

Seeds in the natural condition presented a delayed emergence when compared to scarified seeds (Figure 4). Although, at the end of this study analysis germination pattern of the treatments had close values of germination rate. This general pattern was also obtained by Cruz et al. [28] to *H. intermedia*, another member of the Fabaceae family, that present seeds with different dormancy degrees [27]. Mechanical scarification also did not promote enhanced germination to *S. tubulosa* in the study conducted by Barbosa et al. [13].

Carvalho [1] indicates that scarified seeds emergence occurs between 14 and 42 days after sowing, reaching about 99% of germination. While non-processed seeds emergence comprehends between 40 and 90 days obtaining 2% of germination. In the present study, the results were very discrepant when compared to the data presented by this author. We found that scarified seeds presented the first emergencies on the fifth day, reaching about 49% of the germination rate (Figure 4). While seeds in natural conditions presented the first emergencies in the sixth day after sowing, with 42% of germination. Our results are closer to Oliveira et al. [10] data, that first emergence of non-processed seeds is about the tenth day, and scarified seeds three days after seeding. In general, great variations between seed lots and different climatic regions could be responsible for the discrepancies of some experimental data.

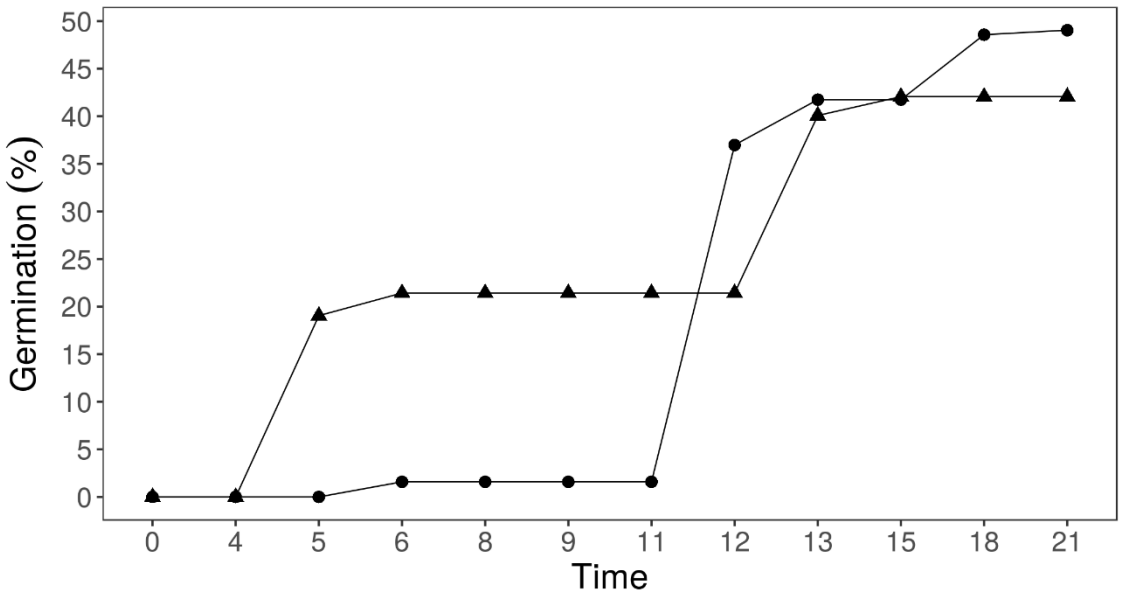

Figure 4. Cumulative germination of *S. tubulosa* according to seed processing method: seeds in natural conditions (●), and scarified seeds (▲).

Germination substrate

Substrate seems to influence only the germination proportion (Figure 5A). All the other studied indexes were not consistently affected. Vermiculite seems to be a better option since it presented a greater germination rate than the organic-based substrate. Firstly, Carvalho [1] found that *S. tubulosa* easily developed in the sand, indicating that probably inert substrates promote better germination. Sales [11] measured differences between inert substrates of sand and the vermiculite, and this last substrate had presented the best results. After that, Gonçalves [20] compared different compositions of organic-based substrates, but not measured the differences between vermiculite and his results. This present study emphasizes Carvalho [1] findings and the hypothesis that vermiculite is the best substrate to *S. tubulosa* germination, even when compared to the organic-based substrate.

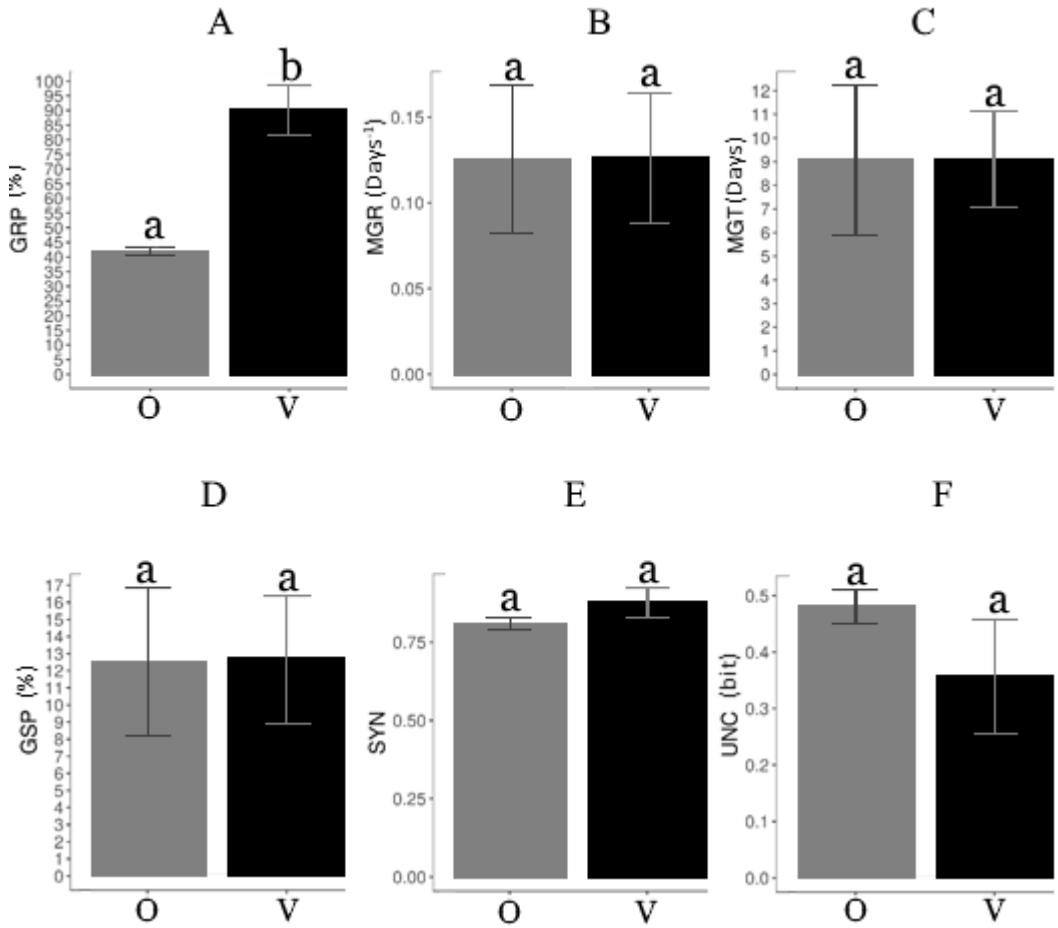

Figure 5. **A-** Germination Proportion (GRP), **B-** Mean germination rate (MGR), **C-** Mean germination time (MGT), **D-** Germination speed (GSP), **E-** Synchronization index (SYN) and **F-** Uncertainty index (UNC) according to the substrate: Organic-based (O), and vermiculite (V) substrate. Lowercase letters present the statistical differences adopting $p<0.05$. Error bars present the standard error mean.

Germination pattern seems to accumulate differences over time, being clear the split promoted by those methods at the end of the analyzed period (Figure 6). The substrate can influence germination since aeration, structure, water retention, pathogenic infestation and other aspects can be highly different [29], [30]. It is important to notice that the cumulative germination curves were very similar in shape, but the vermiculite promoted an upper germination proportion at the end of the study analysis.

In literature, the germination rate found to this species comprehends a range between 40 and 60% depending on the adopted temperature when scarified seeds are sowed in vermiculite [11]. At the end of the analyzed period, we found a mean of 90.26% germination, a greater value than the mentioned range of germination. Sales [11] measured germination 8 days after sowing, if we analyze this author

provided range with the germination obtained to the eighth day (Figure 6), we obtained a corresponding value of this mentioned range. Another important factor is the environmental conditions that could influence seed germination. This author used continuous light and constant temperature. This conditions could also be responsible for the difference between those mentioned values and this study data.

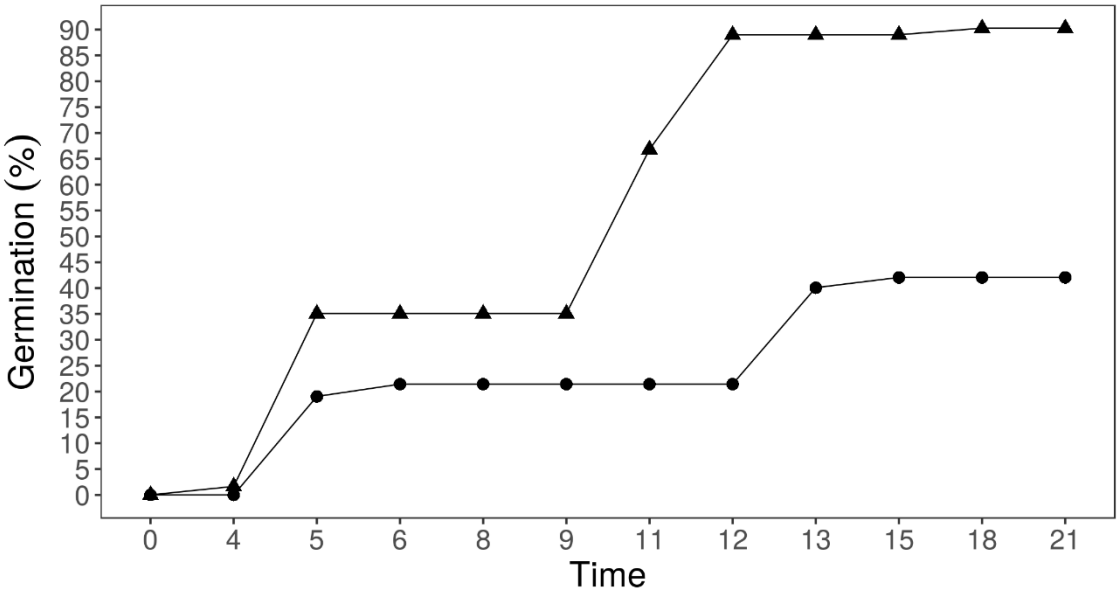

Figure 6. Cumulative germination of *S. tubulosa* according to the substrate: vermiculite (▲), and organic-based (●).

4. CONCLUSION

The parent population could affect the seedling production process, but this influence does not substantially preclude seedling production. The mechanical scarification procedure seems not to enhance seed germination at any of the studied indexes during the analysis period. Vermiculite promoted a better germination rate when compared to the organic-based substrate.

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