ADVANCES IN FORESTRY SCIENCE

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Physiological quality of *Moquiniastrum polymorphum* seeds processed with a general seed blower

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Original Article

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Keywords:
Gochnatia polymorpha
Germination
Temperature
Imbibition curve

Palavras-chave:
Gochnatia polymorpha
Germinação
Temperatura
Curva de embebição

Received in

Accepted on

Published in
2022/12/31

DOI:
http://dx.doi.org/10.34062/afs.v9i4.11950

ABSTRACT: This study evaluates the physiological quality of *Moquiniastrum polymorphum* seeds, processed by a General seed blower, based on their germinability at different temperatures and on the imbibition curve, aiming to support future studies of *M. polymorphum* seeds processed by a General seed blower. Seeds were characterized regarding moisture, purity, germinability and thousand seed weight. The experiment was carried out in a completely randomized design (CRD). To characterize the triphasic process of germination, the imbibition curve during seed germination was modelled using 2 samples of seeds weighing 0.05 g. Physiological seed quality was assessed using a thermogradient table at 15, 20, 25, 30, 35 and 40 °C (with constant light supply) and by calculating the germination speed index (GSI), along with the time to reach 50% germination (T_{50}) and uniformity of germination (U_{75-25}). Each temperature treatment included 4 replications of 50 seeds each. It was found that the optimum temperature for germination was 30 °C with constant light supply, resulting in higher means of normal seedlings, GSI, T_{50} and U_{75-25}. Moreover, the imbibition curve at 30 °C and constant light supply exhibited a triphasic pattern common to seed germination. In conclusion, the new findings on the germinative potential of *M. polymorphum* seeds processed by a General seed blower uncovered here can be applied in the methodology of future studies of *M. polymorphum* or used as recommendations for species of the same genus.

Qualidade fisiológica de sementes de *Moquiniastrum polymorphum* beneficiadas com o soprador tipo general

RESUMO: Visando fornecer informações que sirvam de base para estudos científicos de *Moquiniastrum polymorphum*, o objetivo do presente estudo foi avaliar a qualidade fisiológica de sementes da espécie por meio da germinação em diferentes temperaturas e pela curva de embebição, utilizando sementes beneficiadas por meio do equipamento soprador tipo General. O lote de sementes beneficiadas foi caracterizado pelos testes de pureza, umidade, germinação e peso de mil sementes. O experimento foi conduzido em delineamento inteiramente casualizado (DIC). Para a avaliação do padrão trifásico da germinação, foi determinada a curva de embebição das sementes, por meio de duas repetições de 0,05 g de sementes. A avaliação da qualidade fisiológica das sementes foi realizada pelo teste de germinação conduzido por quatro repetições de 50 sementes, em mesa termogradiente nas temperaturas de 15, 20, 25, 30, 35 e 40 °C (com luz constante) e pelos índices de vigor IVG (índice de velocidade de germinação), T_{50} (tempo para atingir 50% de germinação) e U_{75-25} (uniformidade de germinação). Por meio dos resultados, constatou-se que a melhor condição para germinação foi resultante na temperatura de 30 °C, destacando-se as maiores médias na emissão de plântulas normais e pelos valores do IVG, T_{50} e U_{75-25}, sendo observado o padrão trifásico durante a curva de embebição. Em conclusão, o presente estudo apresentou novas descobertas sobre o potencial germinativo de *M. polymorphum* através de sementes processadas pelo soprador de sementes do tipo General, e essas informações podem ser utilizadas para estudos posteriores sobre a espécie ou como recomendação para espécies do mesmo gênero.

Introduction

Moquiniastrum polymorphum (Less.) G. Sancho, formerly known as *Gochnatia polymorpha*, is a forest species belonging to the Asteraceae family, which includes species with suitable characteristics for the initial composition of reforestation of degraded areas, such as anemochoric dispersion, fire resistance and regrowth capability. *M. polymorphum* is found in both primary and secondary forest successions within the Brazilian Neotropical savanna (Cerrado) and within the Open and Mixed Ombrophilous Forest, mostly in sites with lack of nutrients, especially sandy soils (Machado et al. 2015; Faria et al. 2017). Due to its good resistance to adverse conditions, with a density of 0.76 g cm³, *M. polymorphum* is mainly used as wood for small rural buildings and fencing. The species, however, also carries pharmacological, ornamental and environmental value (Pessatto et al. 2017; Strapasson et al. 2017; Corrêa et al. 2018; Brandes et al. 2019). For example, because of its phenotypic plasticity, it is successfully used in the restoration and rehabilitation of degraded forests (Rossatto and Kolb 2010; Ribeiro and Kolb 2016).

The main challenge preventing a wider use of *M. polymorphum* is the unknown cause for its low germinability, ranging from 9 to 50% (Faria et al. 2017; Machado et al. 2016). Faria et al. (2019) suggested that the low germinability of *M. polymorphum* is related to the high number of empty and malformed seeds dispersed by the species. The authors argue for the use of a General seed blower to obtain the largest number of viable seeds, thus increasing the quality of the seed lots. That is enabled by the different openings for air flow in the seed blower, allowing the separation of the light fraction from the heavy fraction without compromising seed quality. Seeds in the light fraction generally have low germination, comprised mainly of impurities and unfilled seeds, while seeds in the heavy fraction are filled and formed with an embryo (Shibata et al., 2016). The use of a seed blower has been proved successful separating filled and unfilled seeds of *Elephantopus mollis* (Felipe et al., 2019), *Vernonanthura discolor* (Grzybowski et al., 2019), *Panicum maximum* (Silva et al., 2019), and *Eremanthus erythropappus* (Feitosa et al., 2009).

Thus, the study of germination traits of forest species is a major area of interest within the field of silviculture and sustainable management, and a key aspect of seed germination is temperature regime. Temperature is directly linked to water absorption and biochemical reactions and, consequently, regulates germination speed and uniformity (Alves et al. 2016; Ramos et al. 2018; Fogliatto et al. 2020). Seed germination only occurs within certain temperature range, in which there is an optimum temperature for the process to occur with maximum efficiency. An appropriate thermal range for the germination of many tropical and subtropical species is between 20 and 30 ºC, and Brazilian specimens between 20 and 35 °C (Brancalion et al. 2010; Marcos-Filho 2015). The temperature range suitable for germination tends to be wider the wider the specimen’s geographic distribution (Liu et al. 2017; Han et al. 2018).

Given the importance of temperature regulating seed germination and seedling production and that there is no data reported on cardinal temperatures for *M. polymorphum* seeds, studies regarding the physical and physiological quality of *M. polymorphum* seeds are still necessary. Therefore, the present study aimed to improve the understanding on the germinative potential of *M. polymorphum* to support future studies of the species. To achieve that, the physiological quality of *M. polymorphum* seeds was assessed based on their germinability at different temperatures and on the imbibition curve, using seeds processed by a General seed blower.

Material and Methods

Seed collection and processing

The *M. polymorphum* seeds used in this study were collected from approximately 20 selected trees, located in Penápolis, São Paulo, Brazil; commercialized by the company Seeds Caiçara (São Paulo - SP, Brasil), during December 2014. The seeds were stored in semipermeable bags at 5 ºC and 60% relative humidity up to the time of the experiment. The experiment was conducted in the Seeds Laboratory of the Federal University of Lavras, in Lavras, MG, Brazil. We considered the dispersion structures known as cypsela as seeds. The seeds were sundried for 2 days for the removal of anemocoric dispersion materials and then shook and sieved. In order to best homogenize the seed lot and obtain the largest number of filled seeds, we processed the seeds using a General seed blower (DeLeo Ltda, Porto Alegre - RS, Brazil) in opening setting 5 for 30 seconds according to recommendations in Faria et al. (2019).

Characterization of seeds

The *M. polymorphum* seeds were characterized regarding moisture, purity, germination and thousand seed weight, according to the Brazilian guidelines to analyze seeds (Brasil, 2009).

We used 2 samples weighing 1 g each to calculate moisture and purity contents, reported in percentage of moisture and of pure seeds; 50 pure seeds replicated 4 times were tested for germination, reported in average percent germination of normal seedlings; and 100 pure seeds replicated 8 times to calculate thousand seed weight , reported in grams of seeds. An analytical scale of 0.0001 g accuracy was used to weigh seed samples.

To measure seed moisture, the samples were overdried at 105 ± 3 ºC for 24h and then cooled with desiccator containing silica gel. Moisture content was determined by the average of the replicates calculated through the equation:

$$MC(\%) = \frac{w - d}{w - T} \times 100 \quad (1)$$

Where: MC = moisture content (%); w = wet weight (g); d = dry weight (g); T = recipient weight (g).

Because the Brazilian guidelines to analyze seeds (Brasil, 2009) do not provide information on cypsela dispersal structures, pure seeds were determined according to the definition of achene, since achenes are the closest dispersal elements to cypsela. We measured seed viability through their germinability. Seeds were washed in sodium hypochlorite at 2% for 2 minutes and rinsed with distilled water. Then, they were incubated in germination boxes (acrylic gerbox) at 30 °C with constant light supply (20W fluorescent lamps). Blotting paper sheets moistened with distilled water (two and a half the weight of the paper) were used as substratum. To calculate the thousand seed weight , we averaged the weight of 100 seeds replicated 8 times.

Determination of imbibition curve

To characterize the triphasic process of germination of *M. polymorphum* seeds, we calculated the imbibition curve during germination in a completely randomized design (CRD) using 2 samples of seeds weighing 0.05 g, incubated in germination boxes (acrylic gerbox) at 30 °C and constant light supply (20W fluorescent lamps). The seeds were kept on 3 sheets of blotting paper moistened with distilled water. Every 3 hours, the seeds were weighed until we detected imbibition phases I, II and III.

The effect of temperature on seed germination

The effect of temperature on seed germination (radicle emergence) was studied on processed seeds, which achieved after processing 90% purity and 75% germinability (Table 1). To guarantee asepsis, the seeds were submerged for two minutes in sodium hypochlorite solution (2%) and then rinsed in distilled water. Subsequently, the seeds were distributed in Petri dishes on 3 sheets of paper saturated with water. To evaluate the temperature effect, we set a thermogradient table (Seed Processing Holland) at 6 different temperatures (15, 20, 25, 30, 35 and 40 °C) with constant light supply (20W fluorescent lamps). The experiment was conducted under a CRD design, where each temperature treatment included 4 replications of 50 seeds each. The percentages of root emergence, normal seedlings, abnormal seedlings, dead seeds and hard seeds were computed for 26 days. Were considered as normal seedlings those displaying uniform and proportional growth of aerial and root meristems, as well as main root colored white, with short and thin secondary roots and main stem colored light green with emission of the first pair of dark green leaflets. Seeds that did not germinate by the 26th day were opened with a scalpel to verify if they were dead, hard or unfilled (classified as dead).

The speed of germination (GSI) was calculated by the seedling germination speed index (Maguire 1962):

$$GSI = \sum Gn \times Nn \quad (2)$$

Where: GSI = germination speed index; G_n = number of normal seedlings computed in the nth count; N_n = number of days from the nth count from sowing.

Additionally, we calculated both the time to reach 50% germination (T_{50}) and uniformity of germination ($U_{75\text{-}25}$), the time interval between 25% and 75% of viable seeds to germinate, using the Germinator software (Joosen et al. 2010).

We estimated the germinability over time for each tested temperature according to logistic curves, following the equation:

$$Y = \frac{a}{1+b^{*}e^{-c^{*}X}} \quad (3)$$

Where: Y = germinability (%); X = time to reach germination in days.

We used Curve Expert software 1.4 to fit and select the best models.

Statistical analysis

To evaluate the effect of temperature on the germination parameters, we conducted analysis of variance (ANOVA). When a temperature effect was detected, we compared means with Tukey's post hoc tests (P < 0.05). Data was checked for normality (Shapiro-Wilk test, P > 0.05) and homogeneity of variance (Hartley test, P > 0.05). When assumptions of normality were not met, we used box-cox transformation. Statistical analyses were performed in R 3.5.2 with the *ExpDes* package (Ferreira et al. 2014).

Results and discussion

In the present study, using the General seed blower (DeLeo Ltda, Porto Alegre - RS, Brazil) in opening setting 5 for 30 seconds, according to recommended in Faria et al. (2019) to process seeds of *M. polymorphum*, allowed us to correctly separate the light fraction from the heavy fraction, without compromising seed quality. Additionally, processing the seeds lots with the seed blower significantly increased germination (Table 1), ultimately reaching 80% of radicle emergence and 75% of normal seedlings at 30 °C (Table 2).

According to Carvalho and Nakagawa (2012), denser seeds are the result of better nurturing, which makes them surpass late formed seeds regarding density. Several studies have shown the efficiency of the seed blower in the separation of filled and unfilled seeds, as for the *Elephantopus mollis* (Felipe et al. 2019), *Vernonanthura discolor* (Grzybowski et al. 2019) and *Panicum maximum* (Silva et al. 2019). The efficiency of using a seed blower was also verified in seeds of Asteraceae trees. In studies by Tonetti et al. (2006) and Feitosa et al. (2009) on *Eremanthus erythropappus*, and by Davide et al. (2011) on *Eremanthus incanus* (Less.), seed lots processed by a seed blower, compared to seed lots not processed by a seed blower, comprised the highest number of filled seeds, due to the efficient removal of unfilled and malformed seeds, and therefore increasing the percentage of seed germination.

In this study, average purity, germinability, and 1000-seed weight all significantly improved after *M. polymorphum* seeds were processed with the General seed blower (Table 1). Prior to processing, the seed lot had on average 43.5% purity, with most impurities in the form of leaf fragments and dispersion structure, while after processing, average purity reached 90%. Initial germinability was on average 13%, whereas after processing, it increased to 75%. Average 1000-seed weight was initially around 0.4 g, increasing to 0.9 g after processing. Seed moisture was not significantly different before and after processing with the seed blower (Table 1). Due to improvements reached in average seed purity, moisture and 1000-seed weight, germination analyses were carried out on seed lots processed by the General seed blower.

With regard to studies of the germination of *M. polymorphum*, the imbibition curve of seeds at 30 °C under constant light supply displayed a triphasic pattern, as described in Bewley et al., 2013 (Figure 1). What can be clearly seen in this chart is the rapid increase in seed weight during the first 6 hours of imbibition. This behavior is characteristic of the first phase of imbibition, indicating a rapid transfer of water from substrate to seed due to the difference between water potentials between them (Marcos-Filho, 2015). The average seed weight in this phase was approximately 0.11 g.

According to Carvalho e Nakagawa (2012), the cotyledonary seeds end phase I and enter phase II when water contents between 35 and 40% are reached. The duration of each phase depends on the inherent properties of seeds and environmental conditions (Evencio et al. 2011; Araujo et al. 2014). In this study, the second phase of imbibition was marked by the consistent decrease in water absorption, as described in Bewley et al. (2013), which persisted during the next 180 hours, with seed weight stabilizing at 0.14 g. For most orthodox seeds, this new mass stabilization indicates the beginning of the stationary phase of the imbibition curve, when the metabolic activities of the seeds are expected to resume (Finkelstein, 2004).

The third phase of imbibition, in which the seed starts to absorb water intensively (Bewley et al. 2013), was characterized by the beginning of the growth of the embryonic axis, given the radicle emergence. During this phase, the seed weight increased significantly, indicating greater growth of the embryonic axis and of the essential structures of the *M. polymorphum* seedling (Figure 1).

Table 1. Average purity, moisture, germination, and 1000-seed weight of *Moquiniastrum polymorphum* seeds before and after processing by the General seed blower.

Seeds	**Purity**	**Moisture**	**Germinability**	**1000-seed weight**
		%		**(g)**
Before processing	43.5 b	8.00 a	13 b	0.4350 b
After processing	90.0 a	7.35 a	75 a	0.8642 a

Different letters within columns indicate significant pairwise differences at $P < 0.05$.

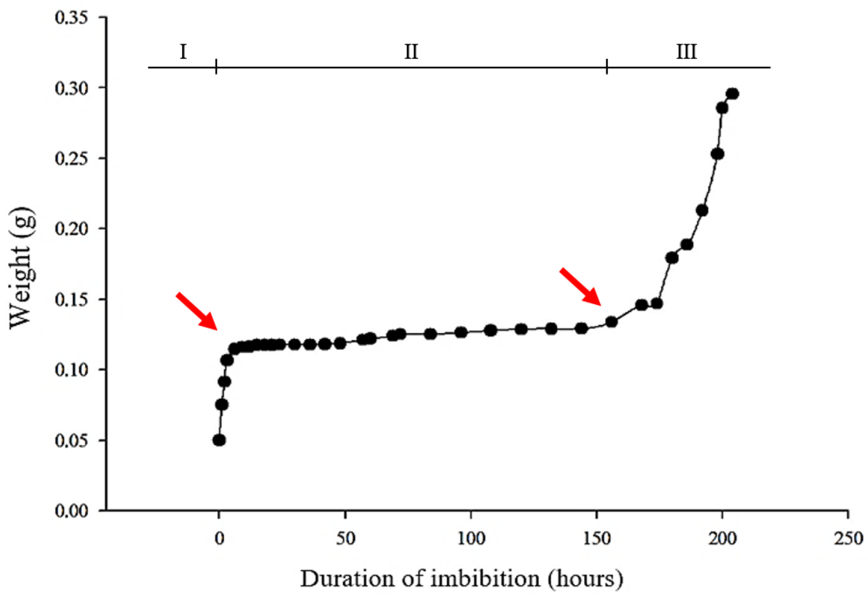

Figure 1. Imbibition curve of *Moquiniastrum polymorphum* seeds at 30 ºC and constant light supply. I = first phase of imbibition; II = second phase of imbibition; III = third phase of imbibition. Arrow = change between phases of imbibition.

When evaluating the influence of temperature on the germination of *M. polymorphum* seeds, we detected significant differences between the temperatures tested for all criteria used (Table 2 and Table 3). Although the temperatures tested were within the cardinal temperatures for germination of *M. polymorphum* tree species, since germination occurred under all temperatures, 30 °C was detected as optimal for germination, while temperatures above 35 °C started to limit seed germination (Table 2). Temperature affects the metabolic processes responsible for the seed germination of tree species, being highly related to the ecological characteristics of the species (Liu et al. 2017). Temperature may affect the dynamics of water absorption, the speed of biochemical reactions and the physiological processes of germination (Marcos-Filho 2015).

The highest statistical averages in root emergence of *M. polymorphum* seeds occurred within the temperature range 15 – 30 ºC, while the highest percentage of normal seedlings was observed within 20 – 30 ºC (Table 2). These results are similar to those found for several tropical and subtropical species, which report the maximum germinability at temperatures between 20 and 35 ºC (Brancalion et al. 2010; Marcos-Filho 2015). For instance, Brancalion et al. (2010) determined optimal germination for species from the Cerrado and Atlantic Forest biomes at 25 °C, and species from the Amazon biome at 30 °C. Some authors suggest the optimal germination of *M. polymorphum* to occur between 15 and 25 ºC under constant light supply, while temperatures below 10 °C and above 30 °C would limit germination (Ribeiro and Kolb 2016). However, smaller temperature ranges (15 °C – 20 °C) have been reported (Machado et al. 2016), even though the highest germinative percentage reached was as low as 11.5%. Despite the low germinative potential frequently reported for this species, the reason for such has not been determined by the authors.

Regarding the effect of temperature on seed germination of *Moquiniastrum* genus, Ribeiro and Kolb (2016) reported 20 and 25 ºC (or alternated between 25 and 30 °C) as optimal temperatures for *M. barrosii* and *M. polymorphum*. However, *M. polymorphum* seeds germinated under a wider range of temperatures: 10 – 30 °C in the light and 10 – 25 °C in the dark, a feature that may indicate ecological advantage. In our study, we found a similar optimal temperature, with the highest mean germination between 15 and 30 °C. The novelty of our study was to determine, in addition to the GSI and average germination of normal seedlings, the T_{50} and U_{75-25}.

According to results shown in Table 3, all studied variables indicated the optimal germination temperature to be 30 °C. Assuming that the optimal temperature for germination is the result of the physiological adaptation of the seeds to the environmental conditions of the places where the species occurs or where the species is cultivated, there may be a direct relationship between this temperature and the biome where the seeds were produced. Furthermore, ecological characteristics of the species, such as the successional group, may have a role in defining the temperature that most stimulates the germination process (Brancalion et al. 2010). Conversely, metabolic changes during exposure of seeds to low or high temperatures may be related to enzyme activity. The enzymes superoxide dismutase and catalase have little or no activity at temperatures below 15 °C and above 40 °C. Both enzymes act to eliminate free radicals, preventing damage to cell membranes and consequently to germination (Flores et al. 2014; Lin et al. 2019). In this study, the number of abnormal seedlings varied between 2 and 71%, with seeds subjected to 15 °C in the upper range, being significantly higher than the others (Table 2). Abnormal seedlings germinated at 15 °C were malformed, displaying deformations both in the root system and in the emergence of the leaflets.

Despite the high germinability achieved using the seed blower, unfilled and malformed seeds accounted as dead seeds ranged from 13 to 26% (Table 2). Moreover, we observed a significant increase in the number of hard seeds at 35 and 40 ºC, of 30 and 73%, respectively (Table 2). We only verified seed viability after exposure to varying temperature at the lowest resulting radicle emergence and normal seedlings. The higher the temperature, the more efficient and faster the germination process will be, as long as it does not exceed the maximum temperature determined for each species. Below the optimal temperature, the speed of germination is reduced, which can also lead to a reduction in the percentage of germination (Carvalho and Nakagawa 2012). Rix et al. (2011) showed that temperatures above 30 °C delayed the beginning of germination of some forest species, decreasing the percentage of germination and causing high seed mortality. In some cases, the authors also reported decrease in the development of normal seedlings. Exposure to high temperatures during germination affects membrane permeability, alters the speed and water absorption of seeds, favouring the disintegration of protein structures (Lin et al. 2016; Pereira et al. 2016).

The average T_{50} and U_{75-25} of radicle emergence were highest at 15 and 40 °C, hampering germination as these temperatures are both close to the lower and upper limit for germination of *M. polymorphum* seeds. The calculated GSI for normal seedlings was significantly higher at 30 ºC, combined with lower T_{50} and U_{75-25} of normal seedlings at the same temperature, thus indicating a higher speed of the germination process (Table 3). The emergence of normal seedlings started from the fifth day, reaching the maximum value at 22 days, which was the highest at 30 °C (Figure 2).

Table 2. Average percent germination of emergence of radicle, normal seedlings, abnormal seedlings, dead seeds and hard seeds of *Moquiniastrum polymorphum* under different temperatures.

Temperatures	**Criteria (%)**				
	Radicle emergence	**Normal seedlings**	**Abnormal seedlings**	**Dead seeds**	**Hard seeds**
15 °C	70 a	0 c	70 a	26 a	4 c
20 °C	74 a	53 b	21 b	21 ab	5 c
25 °C	71 a	58 ab	13 b	26 a	3 c
30 °C	80 a	75 a	5 b	13 b	7 c
35 °C	44 b	42 b	2 b	26 a	30 b
40 °C	14 c	0 c	14 b	13 b	73 a

Different letters within columns indicate significant pairwise differences at $P < 0.05$.

Table 3. Mean values of T_{50} and U_{75-25} for radicle emergence and normal seedlings, and speed of germination for normal seedlings during germination of *Moquiniastrum polymorphum* seeds under different temperatures.

Temperatures	**Criteria**				
	T_{50} Radicle emergence (days)	**U_{75-25} Radicle emergence (days)**	**T_{50} Normal seedlings (days)**	**U_{75-25} Normal seedlings (days)**	**GSI Normal seedlings**
15 °C	12.43 b	6.52 b	-	-	0.00 c
20 °C	7.38 c	1.26 c	12.78 a	6.50 a	4.01 b
25 °C	7.67 c	1.91 c	12.84 a	7.67 a	5.55 b
30 °C	7.46 c	2.44 c	10.79 b	3.44 b	9.47 a
35 °C	8.86 c	4.65 bc	13.19 a	6.35 a	3.62 b
40 °C	16.00 a	9.30 a	-	-	0.00 c

Where: T_{50} = 50% germination, U_{75-25} = uniformity of germination, GSI = speed of germination. Different letters within columns indicate significant pairwise differences at $P < 0.05$.

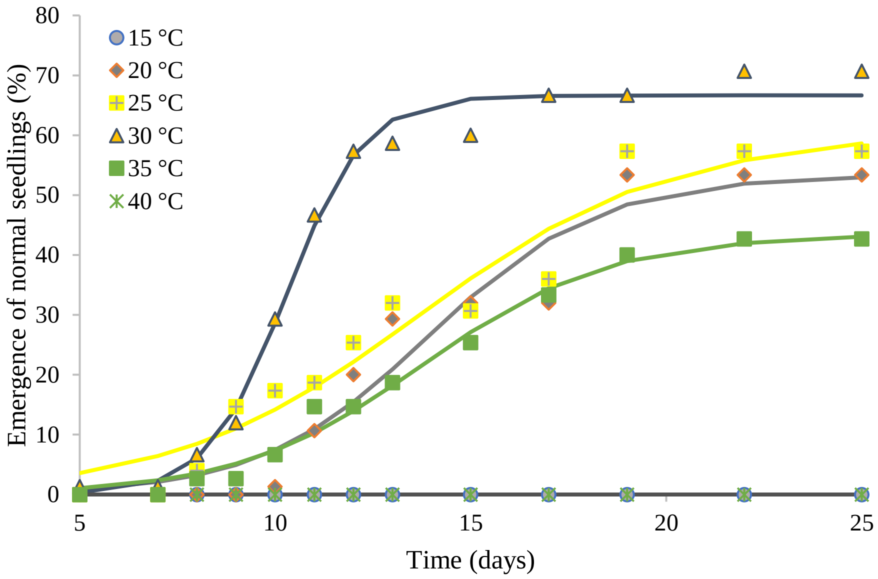

Figure 2. Germinability (radicle emergence of normal seedlings) of *M. polymorphum* seeds under different temperatures over time.

In conclusion, this study has identified that, under our experimental conditions, the optimum temperature for the germination of *M. polymorphum* seeds is 30 °C with constant light, producing an imbibition curve with a triphasic pattern common to seed germination. This study provides new findings on the germinative potential of *M. polymorphum* using seeds processed by the General type seed blower, which can be used for to improve the physiological quality of *M. polymorphum* seeds.

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