Effect of temperature on seed germination of two *Callistemon* species (Myrtaceae)

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Abstract

The genus *Callistemon* belongs to the family Myrtaceae and comprises 34 species. They are woody aromatic trees or shrubs that are widely distributed in the wet tropics. According to the scientific literature, research on seed germination of this genus was rarely carried out. The germination requirements of *Callistemon citrinus* and *Callistemon linearis* were studied under controlled conditions in the laboratory. Seeds of both species were tested for germination at constant temperatures of 25°C, 30°C, 40°C, and at room temperature (23 ± 2°C) coupled with total darkness. Seeds were sown in Petri dishes (0.8% agar water) for 25 days of incubation. The kinetics of germination was determined according to four closely related parameters *viz.* final germination percentage (FGP), mean germination time (MGT), coefficient of velocity of germination (CVG) and time to 50% germination (T50). The room temperature of 23 ± 2 °C was found optimally suitable for both species, with 80% FGP and 11.1 days MGT for *C. citrinus*, and only 21.7% FGP and 12.4 days MGT were recorded for *C. linearis*. Furthermore, significant decrease in FGP was observed in both species at 25°C, 30°C and 40°C of temperature. The analysis also revealed that day 12-15 after seed sowing is suitable for final counts. Due to its very low FGP, seeds of *C. linearis* need an additional study to determine their viability.

Keywords

Agroforestry; Bottlebrush; *C. citrinus*; *C. linearis*; Myrtaceae; Seed germination

Contents

1 Introduction

Modern medicinal products and herbal remedies have great potential for the production of antimicrobial drugs and research in this area has recently increased substantially (Dutra et al. 2016). The *Callistemon* genus belongs to the Myrtaceae family and includes 34 species endemic to Australia. Renowned for its rich red bottlebrush-like flowers, it has been planted as an ornamental shrub in other countries with warm to hot climates (Harrison 2009). They are woody aromatic trees or shrubs (0.5 m to 7 m in height) that are widely distributed in the wet tropics, especially Australia, South America and tropical Asia, but are now spread all over the world (Oyedeji et al. 2009). *Callistemon* species have attractive narrow foliage and white paper bark. The leaves are in lanceolate arrangement (3-6 mm wide and 40-70 mm long) and very aromatic. The flowers are borne in spikes approximately 40-150 mm long with prominent red stamens (Perry and Roy 1982; Spencer and Lumley 1991). *Callistemon* has closely clustered small fruits or capsules along the stem, generating an infructescence that looks like bead bracelets on the bark, which lasts for years (Sutter 2010).

Among other uses, *Callistemon* species are used for forestry, essential oil production, farm windbreak planting, degraded-land restoring and ornamental horticulture (Maria et al. 2015). Callistemon are also used in environmental management as weed control and as bioindicators (Haque et al. 2012). *Callistemon citrinus* (Curtis) Skeels is the most commonly grown member of the *Callistemon* genus. It is a handy medium shrub to a large tree (5-7 m height) (Shaha and Salunkhe 2014). The bright red flower spikes of *C. citrinus* are very rich in nectar and attract many birds (Wrigley and Fagg 1993). *Callistemon linearis* (Schrad. & J. C. Wendl.) Colvill ex Sweet is a small tree or shrub with pendulous foliage, although some forms are more pendulous than others. In its natural habitat, it reaches a height of about 4 m, but is usually smaller in cultivation, particularly in temperate areas (Harrison 2009). *C. citrinus* and *C. linearis* have previously been reported for phytochemical, antimicrobial and antifungal activity (Jazet et al. 2009).

Establishment from seeds is an especially critical phase in the life cycle of plants. Seed germination behavior of *Callistemon* shrubs have rarely been studied although they form populations that may be an important resource for reforestation (Brown and Whelan 1998). The aim of the present study was, firstly, to investigate some morphological characteristics of the seeds (length, width and weight), secondly, to evaluate the effect of different temperatures and to observe the germination curves in order to determine the optimal condition to enhance and homogenize germination.

2 Materials and methods

2.1 Collection, characteristic and origin of seeds

Small woody fruits containing thousands of tiny seeds of *C. citrinus* and *C. linearis* were collected directly from a total of 5 shrubs per species and stored in paper bags on November 2019. The unopened fruits should be incubated (Memmert UM200 oven) in a paper bag at 40 °C for 48 hours until a great number of fine seeds were released (Figure 1). For each species, the seed sample was obtained by mixing the seeds to minimize inter-genetic variation (Kheloufi et al. 2018).

Seeds were selected properly, described, photographed (using a Coolpix Nikon P610, 16 megapixels) and then stored in a bottle glass at 4°C for one month. The length and the width of 50 seeds for each species were measured using Digimizer Image Analysis Software (version 5.4.3, MedCalc, Ostend, Belgium). A scale bar was added.

After processing, seeds were reserved for germination test. Seed provenances and morphological characteristics are shown on Table 1.

Table 1. Seed provenances and morphological characteristics.

		Callistemon citrinus	*Callistemon linearis*
Synonyms (GRIN 2020)		*Metrosideros citrine* Curtis	*Callistemon rigidus R. Br.*
Common names		Crimson bottlebrush	Stiff bottlebrush
	Color	Yellow orange	Dark brown
Seeds	Length (mm)	1.15 ± 0.11	1.17 ± 0.14
(n=50)	Width (mm)	0.53 ± 0.07	0.25 ± 0.05
	1000-seed weight (mg)	112.2	98.4
Region from Algeria		Municipal Park of Batna	Municipal Park of Oran
Geographical coordinates		35°32'58.81"N; 6°10'36.29"E	35°41'20.63"N; 0°38'47.98"W
Altitude (m)		1040	113

Figure 1. Overview of fruit and seeds of *Callistemon citrinus* (A, B) and *Callistemon linearis* (C, D).

2.2 Germination test in Petri dishes

The research experiment was carried out at the Laboratory of the Department of Ecology and Environment, University of Batna 2, Algeria. The experiment was performed in January 2020 at three constant temperatures of 25°C, 30°C, 40°C, and at room temperature (23 ± 2°C). To determine the effect of temperature during seed germination of *C. citrinus* and *C. linearis*, a total of four replicates of 50 seeds were disinfected with 1% sodium hypochlorite for one minute, rinsed with distilled water and immediately sown on 0.8% (water agar) in 9 cm Petri dishes under aseptic conditions. Seeds were incubated simultaneously for 15 days in the dark under three continuous temperature regimes (25, 30 and 40°C) and room temperature (23 ± 2°C). Seed germination was under dark conditions because we were only interested in studying the impact of temperature on seed germination, ignoring the influence of other factors, such as light. Germination counts were performed daily to determine germination kinetics. Seeds were considering germinating only when 2 mm radicles emerged. The parameters evaluated were as follows:

$$\text{FGP (\%)} = \frac{\sum ni}{N} \times 100,$$

where *FGP* is the final germination percentage, *ni* is the number of seeds germinated on the last day of testing, and *N* is the total number of seeds incubated per test (Côme 1970). However, FGP only reflects the final percentage of germination attained and provides no picture of the speed or uniformity of germination.

$$\text{MGT (days)} = \frac{\sum(ti.ni)}{\sum ni},$$

where *MGT* is the mean germination time, *ti* is the number of days since the start of the test, *ni* is the number of germinated seeds recorded at time *ti*, and *Σni* is the total number of germinated seeds (Orchard 1977).

$$\text{CVG (days)} = \frac{\sum \text{Ni}}{\sum NiTi} \text{ x } 100,$$

where *CVG* is the coefficient of velocity of germination, *Ni* is the number of seeds germinated each day, *Ti* is the number of days from sowing corresponding to *N* (Jones and Sanders 1987). The coefficient of velocity of germination gives an indication of the rapidity of germination. It increases when the number of germinated seeds increases and the time required for germination decreases. Theoretically, the highest CVG possible is 100, which is the case if all seeds germinated on the first day (Jones and Sanders 1987).

$$\text{T50 (days)} = t1 + \left(\frac{0.5-\text{n1}}{\sum NiTi}\right) \text{x } 100,$$

where *T50* (days) is the time up to 50% germination, *n1* is the cumulative percentage of germinated seeds which value is closest to 50% by a lower value, *n2* is the cumulative percentage of germinated seeds which value is the closer to 50% by higher value, *t1* is the time necessary for the germination of *n1* of seeds, *t2* is the time necessary for the germination of *n2* of seeds (Côme 1970).

2.3 Statistical analysis

The experiments were conducted with four replicates of 50 seeds (n=4) and the results were expressed as mean ± standard deviation (SD). All the data were subjected to one-way and two-way analysis of variance (ANOVA) and Duncan's multiple range test (P< 0.05) using SAS Version 9.0 (Statistical Analysis System) (2002) software.

3 Results and discussion

The effect of temperature on the germination kinetic of *Callistemon citrinus* and *Callistemon linearis* seeds is illustrated on Figure 3. Figure 3 indicates three stages; a first period of latency due to the imbibition, a second exponential period where germination is accelerated and a stationary phase follows. The differential behavior of both species of *Callistemon* indicated that the seed germination potential was significantly (P<0.0001) influenced by temperature. Indeed, there was a significant effect of temperature (P<0.0001), species (P<0.0001) and their correlation (Temperature × Species) on FGP, MGT, CVG and T50. Temperature may affect the percentage and rate of germination by affecting dormancy loss and the germination process itself (Murdoch and Ellis 2000).

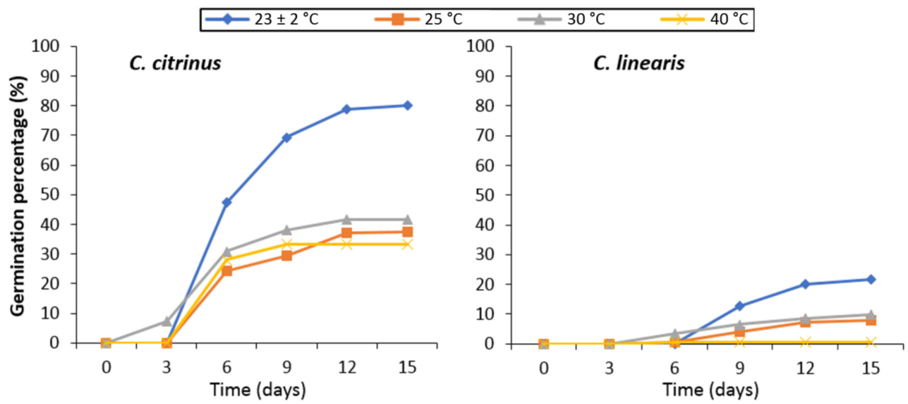

Figure 2. Effect of temperature on seed germination of two species of the genus *Callistemon* (*C. citrinus* and *C. linearis*) in Petri dishes (0.8% Agar water) for 15 days of incubation.

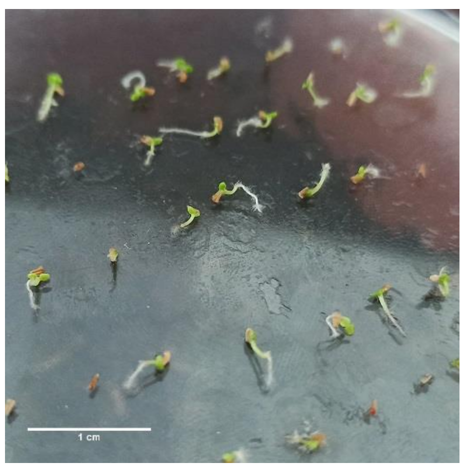

Figure 3. Seedling emergence of *Callistemon citrinus* after 15 days of incubation at room temperature (23 ± 2°C).

Temperature regimes and number of days to count have affected seed germination of both *Callistemon* species (Figure 2). The percentage of germination and days to germination varied according to temperature. For *C. citrinus*, the maximum seed germination was found on 15th day at room temperature, and on 12th day at 25°C, 30°C and 40°C. The mean percentage of seed germination over temperatures ranged from 33.3% (40°C) to 80% (room temperature 23 ± 2°C) (Figure 2, Table 2). For *C. linearis*, the maximum seed germination was found on 15th day at room temperature, 25°C and 30°C, and on 12th day at 40°C. The mean percentage of seed germination over temperatures in *C. linearis* was very low and ranged from 0.67% (40°C) to 21.7% (23 ± 2°C), (Figure 2, Table 2). Figure 3 shows an overview of seedling emergence of *Callistemon citrinus* after 15 days of incubation at room temperature (23 ± 2°C).

The primary environmental factors that influence germination in all species are temperature and water supply, influencing both the rate and the final percentage of germination (Bradford 1995; Finch-Savage and Leubner-Metzger 2006). A quantitative analysis of germination sensitivity to temperature would enable better prediction of the potential impacts of temperature fluctuations on this crucial stage in the plant's life cycle (Dürr et al. 2015). Characterization of the threshold values for germination can thus define the limits to the thermal environment a species will tolerate (Roberts and Ellis 1989; Bradford 2002; Cochrane 2020).

The differences in germination speed due to temperature, as shown by the position and slope of the curves in Figure 2, were more or less as might be expected. While the first seeds germinated at 23 ± 2 °C as rapidly, a minor slowing at 25 °C and 30 °C and a pronounced pause at 40 °C and room temperature was observed. Nevertheless, the fact that most of the seeds remaining at the end of the experiment were still apparently sound indicates that some factor was operating to prevent their germination.

Table 2. Final germination percentage (FGP), mean germination time (MGT), Coefficient of velocity of germination (CVG) and time to 50% germination (T50) for two species of the genus *Callistemon* exposed to different temperature (n=4).

Species	Temperature	FGP (%)	MGT (days)	CVG (%)	T50 (days)
C. citrinus	RT (23 ± 2°C)	80.0 ± 4.32[a]	11,1 ± 0.19[a]	90.1 ± 0.15[b]	9.80 ± 0.21[a]
	25°C	37.3 ± 7.71[b]	11,0 ± 0.12[a]	90.4 ± 0.10[b]	9.86 ± 0.16[a]
	30°C	41.5 ± 5.45[b]	10,5 ± 0.02[b]	95.3 ± 0.02[a]	9,24 ± 0.04[b]
	40°C	33.3 ± 7.36[b]	10,6 ± 0.14[b]	93.8 ± 0.12[a]	9.21 ± 0.17[b]
C. linearis	RT (23 ± 2°C)	21.7 ± 5.82[a]	12,4 ± 0.18[a]	80.1 ± 0.12[c]	11.1 ± 0.24[a]
	25°C	8.00 ± 1.63[b]	12,5 ± 0.71[a]	80.2 ± 0.47[c]	11.1 ± 0.68[a]
	30°C	10.0 ± 1.63[b]	11,6 ± 0.19[b]	86.0 ± 0.14[b]	10.4 ± 0.26[b]
	40°C	0.67 ± 0.94[c]	10,5 ± 0.04[c]	95.2 ± 0.07[a]	9.00 ± 0.09[c]

RT- room temperature. For each species, the same alphabet along the column indicates no significance difference (Duncan Multiple Range Test).

According to Table 2, seeds of both *Callistemon* species exhibit variable behaviors ($P < 0.0001$) at various temperature degrees studied at several parameters *viz.* the final germination percentage (FGP), and the mean germination time (MGT), the coefficient of velocity of germination (CVG) and time to 50% germination (T50). In Petri dishes, these parameters were measured over an incubation period of 15 days. These results showed temperature effect, which played a very important role in germination activity induction. Indeed, room temperature of (23 ± 2°C) improved *C. citrinus* seeds by higher germination rate as indicated by lower MGT (11.1 days) and T50 (9.80 days) with higher FGP (80%) and CVG (90.1%) (Table 2). On the other hand, the lowest germination was recorded in the seeds of *C. linearis* which recorded also at the room temperature condition a higher FGP (21.7%) and CVG (80.1%) with a lower MGT (12.4 days) and T50 (11.1 days), (Table 2).

Warm temperatures during spring increase seed metabolism and promote the biochemical reactions necessary for germination of nondormant seeds and can alleviate seed dormancy in some species. Some weeds only require the temperature to be above a minimum to germinate, whereas others require, in addition, daily temperature fluctuation (Baskin and Baskin 1998). As an energy source, temperature affects the germination rate, whereas as a signal, temperature can determine when dormancy ceases or germination is induced (Finkelstein et al. 2008). The germination temperature requirements of a nondormant seed are not necessarily the same as those for alleviating the dormancy of a highly dormant seed (Baskin and Baskin 2004; Finch-Savage and Leubner-Metzger 2006; Allen et al. 2018). Consequently, studies that distinguish between the effects of temperature on dormancy and on germination could provide more accurate and realistic information about seed biology.

4 Conclusion

Temperature affected time to germinate and germination percent in *C. citrinus* and *C. linearis*. Both studied species showed maximum germination at room temperature (23 ± 2°C). However, the final germination percentage of *C. citrinus* was higher (80%) compared to *C. linearis* (21.7%) after 15-day period. This temperature appears to be the actual optimum for seed germination and the establishment of seedlings. The seeds of *C. citrinus* are non-dormant and do not require any pre-treatment to germinate. Those of *C. linearis* need further studies to evaluate their viability and dormancy. These results could be suggested as a contribution for a seedling production protocol in nurseries or for other agroforestry studies.

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