Assessment of the germination potential of *Myrtus communis* (L.) based on seed size

Lahouaria Mounia Mansouri, Abdenour Kheloufi✉, Rabah Belatreche

Department of Ecology and Environment, University of Batna 2, Batna 05078, Algeria

✉ a.kheloufi@univ-batna2.dz

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Abstract

The woody fodder species *Myrtus communis* (L.) is commonly found in the forested areas of northeastern Algeria, thus helping to alleviate the goat husbandry shortage during the summer. This study aims to conserve the species and counteract its declining populations due to various biotic and abiotic factors. A specific objective of this study is to assess the correlation between seed size and germination vigor in *M. communis*, as well as emergence of seedlings. A sample of 15 *M. communis* individuals was selected, and 500 fruits were randomly collected in the vegetation of Jijel National Park (Jijel, northeast Algeria). One month after post-ripening, when fruit moisture content reached 25.2%, seeds were manually extracted from the fruits and categorized into three sizes (large, medium, and small). Then, 100 leaves, 100 intact fruits, and 100 seeds from each size category were measured and weighed. Various morphological characteristics were recorded, including leaf and fruit length and width, pulp weight, seed number, pulp-to-seed ratio, seed size, and moisture content. Seed germination and initial seedling growth were monitored weekly. Seeds of *M. communis* ranged in weight from 0.03 to 0.18 grams. In comparison with medium (1.5%) and small (0%) seeds, large seeds showed significantly higher germination rates (93%) after three weeks of sowing. Moreover, seedlings originating from large seeds grew vigorously, reaching a length of 10.9 cm. According to our findings, seed size in *M. communis* can affect seed germination and high-quality seedling establishment.

Keywords

Bioconservation; Germination behavior; Myrtaceae; Seed biometry; Reforestation

Contents

1 Introduction

Shrubs are an important source of feed, particularly for goats. Indeed, the use of forest ranges serves as a viable alternative during the dry period of the year when the herbaceous layer fails to provide sufficient sustenance. *Myrtus communis* L. (known as common myrtle) is among the most prevalent species in the shrubbery of these grazing areas in northeastern of Algeria. According to Tibaoui et al. (2020), myrtle leaves have been shown to replace up to 87% of sheep feed, yielding advantages in body weight, carcass characteristics, and meat quality. *M. communis* belongs to the significant Myrtaceae family. It is an evergreen shrub primarily found in the Mediterranean region and southwestern Europe. It has been observed at altitudes of up to 1000 m a.s.l. (Browicz 1980; Bouzabata et al. 2016). In Algeria, native populations of *M. communis* are found in the Tell region and coastal areas. It thrives in distributed populations in rocky and sandy areas where underground water sources exist (Migliore et al. 2012). This species has been recommended as a pioneer plant due to its high tolerance to drought, pests, and pathogens (Kordali et al. 2016). Additionally, it retains its leaves during harsh winters and can endure hot summers. Individuals of *M. communis* play a crucial role in agro-silvo-pastoral design as well as soil fertility conservation and improvement in arid and semi-arid regions. Its uses include livestock feed, wood provision, culinary seasoning, and essential oil extraction. It is traditionally employed as an antiseptic, disinfectant, and hypoglycemic agent (Sisay and Gashaw 2017). According to literature, myrtle essential oils possess antibacterial, antifungal, antioxidant, and antimutagenic properties. The chemical composition of myrtle essential oil has been extensively studied in various countries (Rasooli et al. 2002; Mahmoudvand et al. 2016; Kaya et al. 2020).

Myrtles typically propagate in summer from partially mature cuttings, but there are challenges associated with asexual propagation during hot and dry summers (Abd El Hameed 2018). On the other hand, the abundance of seeds produced by this plant makes seed propagation an important alternative. Germination control mechanisms are crucial in nature as they contribute to natural survival and species dissemination. Many seeds face germination problems, with seed propagation being affected by seed coat dormancy, leading to limited growth potential. Kheloufi et al. (2018, 2019) observed that seeds of many tree species in arid and semi-arid areas exhibit slow germination under optimal conditions, primarily due to their water-impermeable seed coats. Different pretreatments have been employed by various researchers to enhance seed germination of different common myrtle species (Khosh-Khui and Bassiri 1976; Benvenuti and Macchia 2001; Nadi et al. 2012). However, no study has specifically aimed to investigate the effect of seed size on *M. communis* germination. Indeed, as we will demonstrate in this study, *M. communis* fruit contains multiple seeds of varying sizes.

Ripe fruits of *M. communis* typically appear around mid-November and may persist on plants until mid-February. The primary animals contributing to seed dispersal include birds and goats, although carnivorous mammals such as foxes and weasels have been reported to consume the fruits and disperse intact seeds away from the parent plant (Aronne and Russo 1997). These fruits contain seeds with considerable differences in size. Within a species, variations in seed size reflect plant adaptive value through correlations between seed size and predation, germination, emergence, and seedling survival (Traveset et al. 2001).

In northeastern Algeria, the areas occupied by *M. communis* shrubs were approximately 80 000 hectares in 1990 (DGF 2010). This area has been decreasing due to poor management of spontaneous populations, scarcity of natural regenerations, overgrazing, forest fires, and clearing resulting from excessive local exploitation (Mebirouk-Boudechiche et al. 2014). Inadequate management of the germination process has so far delayed the further use of *M. communis* shrubs as fodder, medicinal species, and as effective ground cover to protect areas disposed to erosion and potential hydrogeological risks, while contributing to the richness of forest biodiversity. All these observations drive us to better understand the multiplication technique of this native species in order to preserve it better for future generations, whether for animal or human use. In this study, we addressed a seed germination model based on seed size under experimental conditions.

2 Materials and methods

2.1 Sampling site and fruit collection

Fruits of *Myrtus communis* were harvested on October 25, 2020, from a population of 15 shrubs in Taza National Park (Jijel, Algeria). Taza National Park was established in 1984, covering a total area of 3 807 ha. It is located in northeastern Algeria, extending between the geographic coordinates of 36°35' and 36°48' N and between 5°29' and 5°40' E. The park consists of mountainous areas with relatively low altitudes, with its highest point being Mount Koudiet El Kern at an altitude of 1 121 m. The study area is characterized by high relative air humidity (80%), which facilitates the establishment and maintenance of a relatively diverse flora (Bounar et al. 2016).

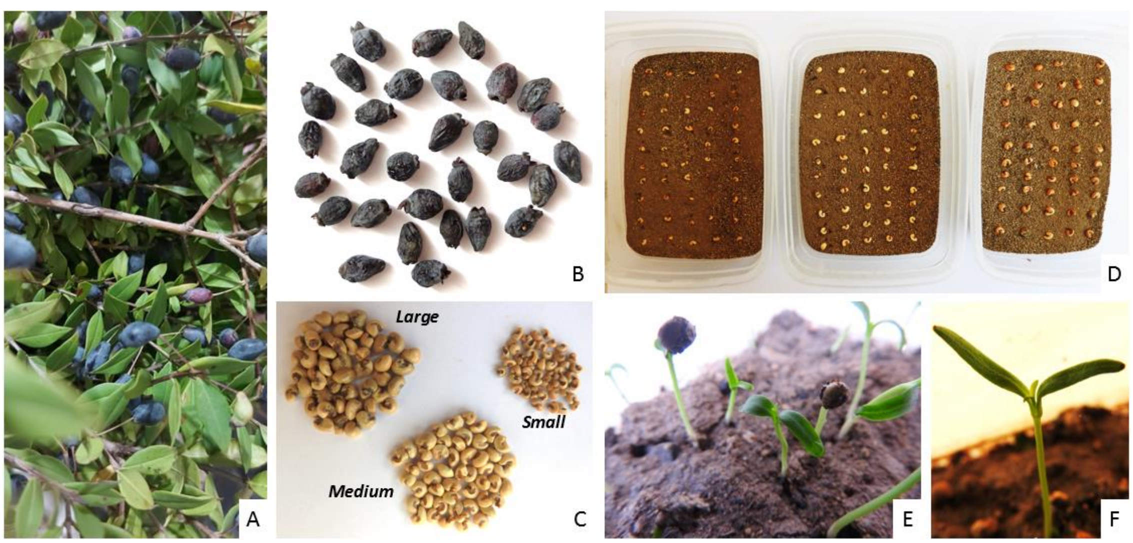

Figure 1. Photographic illustrations of *Myrtus communis*. A: Ripe fruits, B: Ripe fruits at 25.2% humidity, C: Separation by seed size, D: Experimental sowing setup, E-F: Two-week-old seedlings.

One month later, when post-ripening was completed and the fruit moisture content had reached 25.2%, the seeds were manually separated from intact fruits (showing no signs of predation or other damages) and carefully sorted into three size classes (large, medium, and small), (Figure 1). A sample of 100 leaves, 100 intact fruits, and 100 seeds from each size group was measured and weighed. The following morphological characteristics were recorded: length, width of leaves and fruits; pulp weight; number of seeds per fruit; pulp-to-seed ratio; length, width, and moisture content of seeds for each size group. Seed moisture content was determined after drying at 130°C for 2 hours (ISTA 2005).

2.2 Seed germination and seedling growth

A total of 4 replicates of 50 seeds from each seed group were sown in a semi-transparent plastic container (17 cm length x 12 cm height x 10 cm width) between two layers of moist substrate at 15% humidity and a temperature of 25-27 °C and 16 hours of photoperiod (Benvenuti and Macchia 2001) (Figure 1). This experimental design was replicated 3 times because seed germination and seedling length were evaluated weekly over a period of 3 weeks. Culture containers were monitored weekly to ensure that water saturation was maintained at 15% (ISTA 2005). Seed sorting was meticulously performed to better divide the samples according to size. The culture substrate was obtained from the sampling area, characterized as alkaline soil (pH 7.81), non-saline (EC = 231 µS/cm). This soil is highly calcareous (total limestone content 51.2%) and rich in organic matter (8.21%). The soil was previously sieved to 2 mm to obtain a homogeneous substrate.

The experiments were set up in a completely randomized design. Analysis of variance with one factor was performed using version 9.0 of the SAS (Statistical Analysis System) software (2002). Mean separation was conducted using the Tukey test for multiple comparisons, and the statistical significance threshold was set at 5%.

3 Results and discussion

3.1 Morphological characteristics

Myrtle berries are oblong-ellipsoidal fruits, bluish-black in color, with a long pedicel. They consist of a fleshy pericarp and snail-shaped seeds (Figure 1). The *M. communis* shrubs used in our fruit sampling exhibit excellent vigor, with an average height of 2 m and a width of 1.80 m. Common myrtle is a woody-stemmed shrub that remains green throughout the year. Its growth is slow, and it can reach a longevity of 300 years (Franceschini 2016).

Common myrtle has persistent, opposite leaves. Its leaves are leathery, shiny, oval to lanceolate in shape, measuring between 2.93 and 3.80 cm in length and 1.12 to 1.57 cm in width (Table 1). Each leaf possesses numerous secretory cavities, located on the leaf epidermis. The average number of fruits per shoot is five. The average number of seeds per fruit is seven. The average seed biomass per fruit is 0.09 g, and the average pulp-to-seed ratio is 1.35. The fresh fruit biomass (0.21 g), length (1.27 cm), and width (0.83 cm) present average values (Table 1).

According to the result of the analysis of variance and the Tukey test, a significant effect ($p<0.001$) exists among the three seed classes for all studied variables (Table 2). *M. communis* seeds possess a thick seed coat (Figure 1). According to Table 2, *M. communis* seeds can be classified into 3 groups (based on the length, width, and weight of each seed), namely large seeds, medium seeds, and small seeds. In the same fruit, only 1-2 size classes can be found, never all three at the same time. The moisture content in small seeds is the highest (16.4%), followed by large seeds (6.12%) and medium seeds (4.83%). The average weight of a large seed is around 0.18 g, followed by medium seeds and small seeds with average weights of 0.11 g and 0.03 g, respectively.

Table 1. Morphological characteristics of leaves and berries of *Myrtus communis* (Fruits with 25.2% moisture) (n=100).

Variables	Means	Min-Max
Leaf length (cm)	3.26	2.93-3.80
Leaf width (cm)	1.33	1.12-1.57
Number of fruits per shoot	5.20	3-7
Fruit weight (g)	0.21	0.15-0.29
Fruit length (cm)	1.27	1.11-1.46
Fruit width (cm)	0.83	0.72-0.97
Number of seeds per fruit	6.80	2-12
Pulp weight per fruit (g)	0.11	0.06-0.16
Total seed weight per fruit (g)	0.09	0.06-0.15
Pulp-to-seed weight ratio per fruit	1.35	0.81-1.88

Table 2. Morphological characteristics of the three seed classes of *Myrtus communis* (n=100).

Variables	Seed size classes			*P*
	Large	Medium	Small	
Seed length (mm)				
Average	4.36^a	3.62^b	2.62^c	<0.001
Min-Max	(3.78-5.19)	(3.19-3.72)	(2.32-2.95)	
Seed width (mm)				
Average	3.51^a	2.91^b	1.89^c	<0.001
Min-Max	(3.17-3.96)	(2.54-3.18)	(1.62-2.14)	
Average seed weight (g)	0.18^a	0.11^b	0.03^c	<0.001
Moisture content (%)	6.12^b	4.83^c	16.4^a	<0.001
Weight of 1000 seeds (g)	18.9^a	12.1^b	3.31^c	<0.001

^{abc}Means with the same letters are not significantly different at p < 0.05 (Tukey Test)

3.2 Germination and seedling emergence

The results of the ANOVA showed a significant effect of seed size on germination percentage and seedling length (p<0.001) over the three periods of the experiment (Table 3). The highest germination rate (93%) was obtained from large seeds, while the lowest rate (1.5%) was obtained from medium-sized seeds (Table 3). No germination was observed from the smallest seeds throughout the three weeks of the experiment (Table 3). Germination rate varied significantly depending on seed size. In this case, larger seeds should have more available nutritional reserves to support the germination process. Large seeds exceeded 50% germination by the second week of the experiment, reaching 67.5%.

Our results are in accordance with several other research findings. For example, the germination rate in *Cryptocarya alba* was significantly higher with increasing seed size (Chacon et al. 1998). Additionally, the results of Domic et al. (2020) showed that larger seeds of *Polylepis tomentella* are more likely to germinate than medium and small seeds. Germination rates of several species in the genus *Frankenia* were higher in species with large seeds than in species with small seeds (Easton and Kleindorfer 2008). Mtambalika et al. (2014) recommended that, for the production of high-quality *Afzelia quanzensis* seedlings in nurseries, large seeds are preferable. Tumpa et al. (2021) concluded that the origin and size of seeds in *Castanea sativa* have a significant impact on seed germination and seedling growth and are important factors to consider when selecting seeds.

Table 3. Average values of germination percentage and total seedling length in *M. communis* for each size class at three observation periods.

Variables	Size classes	Time after sowing (weeks)		
		1	2	3
Germination percentage (%)	Large	26[a]	67.5[a]	93[a]
	Medium	0[b]	1[b]	1.5[b]
	Small	0[b]	0[b]	0[b]
	F-value	202***	74.9***	3294***
Seedling length (cm)	Large	2,45[a]	6.95[a]	10.9[a]
	Medium	0[b]	0.15[b]	0.45[b]
	Small	0[b]	0[b]	0[b]
	F-value	176***	1330***	937***

[abc]Means with the same letters are not significantly different at p<0.05 (Tukey Test). ***: significant at 0.01%.

According to Table 3, seed size affected the initial growth of seedlings. Seedling length was significantly different (p<0.001) up to the third week and reached 10.9 cm for large seeds, increasing by an average of 4 cm each week. Seed size in *M. communis* consequently affects seedling emergence. The study suggested that large seeds of *M. communis* possess large cotyledons that play a significant role in inducing the germination process considerably. This is likely due to their reliance on cotyledon nutrient reserves for physiological and biochemical initiation of germination. According to the works of Heydecker (1972) and Ellis (1992), larger seeds of a plant species generally produce more vigorous seedlings than smaller seeds. However, germination is not always greater from larger seeds (Foster and Janson, 1985; Finch-Savage, 2020). Furthermore, a decrease in seed weight may be disadvantageous, as smaller seeds, as discussed above, are often associated with lack of germination, and the very few seedlings obtained from medium seeds could reduce seedling establishment and reproductive success chances (Khurana and Singh 2001). It has been demonstrated that seed size variation has several important ecological implications. This can affect seed germination, seedling emergence, seedling establishment, growth rates, plant size, plant survival, and reproductive capacity (Huang et al. 2016; Rubio de Casas et al. 2017; Perea et al. 2020). Variation in seed size and biomass has significant ecological importance in population establishment and maintenance, particularly in variable environments, which in turn plays a crucial role in the success of the development of a given species (Fenner et al. 2005).

4 Conclusions

The germination technique illustrated in this study can yield very satisfactory germination rates for propagating *Myrtus communis* through seeding. Seed sorting in *M. communis* could be carried out by removing seeds less than 4 mm in length. Larger seeds could be favored under natural conditions, especially in scenarios of intense competition following forest fires. We suggest using fully ripe fruits as seed sources, ideally those from late October, and using the same soil as the mother plant as a growing substrate to enhance seed germination. Nursery operations for *M. communis* seedling production should also consider seed origin and local environmental conditions in reforestation programs.

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