Flora 249 (2018) 16–23

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.com/locate/flora

Germination and morpho-phenological traits of *Allium melananthum*, a rare species from south-eastern Spain

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ARTICLE INFO

Edited by Hermann Heilmeier

Keywords:
Bulb
Gibberellic acid
Life cycle
Seed dormancy
Stratification
In-situ conservation

ABSTRACT

The germination and life cycle of the rare species *Allium melananthum*, endemic to southeast Spain, were investigated. One alternating and four constant temperature regimes and two light conditions were tested. Gibberellic acid and cold or warm stratification were used as dormancy breaking treatments. The results showed that light can inhibit seed germination, although the light inhibitory effects varied with temperature. In continuous darkness, seed germination was hardly affected by temperature, but in alternating light/darkness it was sensitive to extreme temperatures of 25 and 10 °C, while mild temperatures of 15–20 °C permitted successful germination. However, a dormant stage was observed in a small fraction of seeds, which was only overcome by warm stratification. By contrast, cold stratification induced strong secondary dormancy. This germination pattern shows that primary and secondary dormancy, temperature and light play pivotal roles as components of the germination-timing strategies of *A. melananthum.* As regards its life cycle, the juvenile phase in a low proportion of individuals (0.93%) lasted 2 years from seeding, while 56.7% of individuals were capable of producing bulblets in the third year. We suggest that at least two growing seasons post seeding are necessary in the nursery to obtain suitable material for the introduction or reinforcement of *A. melananthum* populations in their natural habitat.

1. Introduction

The genus *Allium* L. (Amaryllidaceae) comprises more than 700 species that differ in many morphological characters, life form (bulbs or rhizomes) and habitat type (Hanelt et al., 1992). The most recent intrageneric classification divides the genus *Allium* into 15 subgenera and 72 sections (Friesen et al., 2006), *Allium* being the largest subgenus. Members of this subgenus are commonly found in the Mediterranean area, Asia Minor and Central Asia, and include a number of cultivated species, e.g., *A. sativum* (garlic) and *A. ampeloprasum* (leek) and ornamentals, e.g., *A. atroviolaceum* and *A. sphaerocephalon* (Kamenetsky and Rabinowitch, 2006). Among the wild species of this subgenus, *Allium melananthum* Coincy, which is endemic to south-eastern Spain (Govaerts, 1995; Mathew, 1996), is regarded as Near Threatened because it has a restricted and fragmented distribution with isolated subpopulations, is rare throughout most of its range and is considered threatened in some areas (Draper Munt, 2013). It was classified as Rare in the 1997 IUCN Red List of Threatened Plants (Walter and Gillett, 1998). In Murcia province (SE Spain) this species is considered Vulnerable (BORM, 2003). *Allium melananthum* is a bulbous plant, which grows up to 1.25 m in height, with bulbs of 0.8–2 cm diameter, ovoid to spherical in shape with a membranous outer tunic. The bulblets are yellowish-grey, and located in the external part of the parent bulb (stalked or sessile but not enclosed within sheath). The leaves are linear and hollow, and the stem is wrapped up to half of its length. The spathe opens in two parts up to 1.5 cm in length. Inflorescences are spherical, many-flowered and dense, without bulbils. The fruit is a globose capsule, about 3 mm long, with seeds of up to 3.5 mm long (Castroviejo et al., 1993). This species is found naturally in the provinces of Almeria, Murcia and Alicante (south-eastern Spain). There is also a disjunct population in the Sierra de Mijas (Malaga, southern Spain). It grows in rocky and sandy places from sea level to 400 m. It is also found in drylands with thyme bushes and in steppe vegetation (Mathew, 1996).

Both rare and threatened plant species are considered to be of the highest priority in the ambit of biodiversity conservation (Schemske et al., 1994) because of their restricted distribution, reduced ecological amplitude and vulnerability to genetic drift, inbreeding depression and stochastic environmental phenomena. According to Falk et al. (1996), there is a pressing need to improve knowledge of their biology, especially in the areas of population and reproductive biology, genetics and

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https://doi.org/10.1016/j.flora.2018.09.005
Received 13 April 2018; Received in revised form 27 August 2018; Accepted 7 September 2018
Available online 11 September 2018
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ecology, as a step towards preventing their extinction. In this sense, knowledge of the influence of environmental factors on germination is useful to ensure success in the conservation and management of these species, as such knowledge will provide a better understanding of their phenology in order to predict the most favorable periods for seedling establishment in the field and facilitate the provision of nursery plants from seed (Lentz and Johnson, 1998).

Although vegetative reproduction through the formation of daughter bulbs occurs regularly in *Allium* species, a capacity for mixed sexual and asexual reproduction has been observed in some of these species (Fialová et al., 2014; Ronsheim and Bever, 2000); in some cases, even, sexual reproduction prevails over vegetative reproduction in the natural populations (Kovács, 2007; Zammouri et al., 2008). However, in some *Allium* species seed germination involves problems, among them a low germination percentage and velocity as well as the slow growth of seedlings (Etemadi et al., 2011). Many studies can be found on the germination of crop species such as *A. cepa* or *A. ampeloprasum*, but only a few on wild alliums. Specht and Keller (1997) studied the temperature requirements for seed germination of 94 wild *Allium* species, finding a good relationship between geographical origin and temperature dependency. These authors showed that germination of most studied taxa within the subgenus *Allium*, to which *A. melananthum* belongs, took place at 16 °C within a period of 30 days, while for the rest of the studied subgenera of the genus *Allium*, there were significant differences in the temperature requirements for seed germination with respect to both final germination percentages and time which was necessary for germination. In addition to temperature, light is also an important micro-environmental factor for the timing of seed germination for many species (Baskin and Baskin, 1989). However, the role of light in controlling germination has barely been investigated in *Allium* species.

Moreover, seed dormancy has been identified in some wild *Allium* species (Dashti et al., 2012; Ebrahimi et al., 2014; Phillips, 2010), making it necessary to study the dormancy breaking requirements for seeds of these species. Dormancy is an adaptive trait that allows plants to synchronize germination so that it occurs in the most advantageous season for seedling survival. In geophytic species, such as those of the genus *Allium*, although they have a unique life-form in which perennial buds residing in the subterranean storage organs allow plants to persist through unfavourable seasons by entering a dormant phase, the seed generated in the reproductive growth phase must be able to avoid germinating until conditions exist that offer a higher chance of seedling survival (Phillips, 2010). Both bulb and seed dormancy characteristics are specific to environmental factors such as temperature, photoperiod and soil moisture of the natural habitat in which the particular geophytic species is found. Therefore, suitable conditions for the relief of dormancy are often those that prevail in the habitat of a species or population during the unfavourable season. Seed dormancy is often released upon exposure to prolonged cold temperatures similar to those found during winter in the natural habitat of the mother plants, although some species require warm temperatures to break dormancy (Phillips, 2010), the latter being a common adaptation in perennial plants occupying environments where there tends to be predictably long and dry summer period (Schütz et al., 2002), as occurs in semi-arid Mediterranean climates of south-eastern Spain. The application of exogenous plant growth regulators can also affect germination. Gibberellins have been shown to eliminate chilling requirements of some seeds and increase their germination (El-Refaey and El-Dengawy, 2005). The occurrence of seed dormancy can become a significant obstacle in cases of endangered and rare taxa, preventing the possibility of producing a large number of seedlings rapidly for reintroduction in the native populations (Moura and Silva, 2010). In the case of *A. melananthum*, there is no information about its germinative behaviour, so studies are needed to determine its seed germination requirements in order to obtain rapid and uniform germination and high germination percentages.

On the other hand, each *Allium* species is characterized by a specific morphology, life cycle and bulb periodicity as a result of its adaptation to the environment (Kamenetsky and Guttermans, 2000). In the case of *A. melananthum*, there is no information on its morphological and phenological traits, aspects that are of great interest for obtaining plants for both the successful reintroduction and reinforcement of populations in the wild. It is known that the life cycle and morphological traits of the storage organs vary significantly *in Allium* species, and that they can be grouped into several biomorphological groups that serve for both increasing our theoretical knowledge of the different *Allium* taxa incorporated in each of them and for practical purposes (Kamenetsky and Rabinowitch, 2006). So, it is to be expected that *A. melananthum* will show morphological and phenological characteristics similar to other *Allium* taxa of the bulbous group to which it belongs. In this biomorphological group, when bulbing is complete, the plant usually enters a dormancy period to survive the harsh winter and summer environmental conditions prevailing in the natural habitat of the mother plant, while sprouting recommences either in the autumn or in the spring. In bulbous species of Mediterranean origin, where mild winter conditions are favourable for plant growth and development, but where summers are dry and hot, summer dormancy is common (Kamenetsky, 1994). The juvenile phase lasts 2–5 years and post-juvenile plants flower in spring. When propagated from seed, all *Allium* plants undergo a reproductively incompetent juvenile phase, before they can respond to environmental induction and bloom. The juvenile phase has a limited duration and ends when the plant reaches a certain physiological age and/or a critical mass, which allows plants to shift to the reproductive stage.

Therefore, the aims of this study were (a) to determine the optimal conditions (temperature and light) for seed germination of this species, (b) to test the effects of several dormancy breaking treatments on seed germination, and (c) to increase our knowledge of the life cycle of this species by studying bulb development after growing from seeds, as well as the vegetative and reproductive (sexual and/or asexual) stages of plant development through monitoring vegetative growth, flowering and bulblet production during a 3-yr study.

2. Materials and methods

2.1. Seed collection

The study was carried out with seeds of *A. melananthum* collected from the population at La Azohia (Cartagena, Murcia; 37° 33′8″N; 1° 10′22″W), a coastal area with a semi-arid Mediterranean climate characterized by irregular rainfall and a severe, dry summer period. Annual mean precipitation is around 300 mm, most of which falls in autumn, and the mean annual temperature is 17 °C. August is the warmest month, with an average temperature of 24.9 °C and a maximum of 42 °C. The coldest month is January, with an average temperature of 10.6 °C and the minimum always > 0 °C. All the seeds collected were from mature fruits of many individuals belonging to this wild population with a healthy and vigorous appearance, so that high seed viability was assumed. All seeds were collected in June 2011 and taken to the laboratory, where they were divided into four lots. Two lots of fresh seeds (15 days after harvest) were cold or warm stratified for 2 months (see 2.2 Germination experiments), and the rest of the lots were placed in paper bags and stored at room temperature (20 °C) for 2 months until the germination tests began in September 2011. Thus, the age of the seeds was the same in all germination tests. Before the experiments, the seeds were disinfected with 15% NaOCl for 5 min.

2.2. Germination experiments

2.2.1. Experiment 1: effect of light and temperature regime on seed germination

For this experiment, one of the lots of seeds stored at room

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