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Germination protocols for seven Mediterranean taxa of the genus *Anthemis* (*Asteraceae*) from Sicily

Abstract

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In this study, we characterize the germination behaviour of seven closely related taxa within the genus *Anthemis*, with respect to temperature, light, and cypsela morph. The study taxa are *Anthemis aeolica, A. aetnensis, A. cretica* subsp. *messanensis, A. cupaniana, A. maritima* subsp. *maritima, A. parlatoreana,* and *A. pignattiorum,* all native or endemic to Sicily. The species selected are representative of both lowland and mountain habitats.
Seeds were collected from wild populations, at the time of natural dispersal. All of the species possess heteromorphic cypselas with different size, shape, surface ornamentation, and colour. For all of the taxa, two cypsela morphs were identified and tested separately: cypselas with thin and light-coloured pericarp and cypselas with thick and dark-coloured pericarp. Germination was tested in fresh and after-ripened cypselas. Seven constant and, only for *A. aeolica,* four alternating temperatures were applied both in light and total darkness. The best germination protocols for each seed accession are provided.
Fresh light-coloured cypselas reached optimal germination at temperatures ranging from 10 to 20°C. This seed morph both widened its thermal range for germination and increased the final germination percentage following one to three months of after-ripening, showing to possess non-deep physiological dormancy. Dark cypselas showed low germination under any temperature and light conditions, suggesting the presence of a deeper degree of physiological dormancy. However, the role of the pericarp has to be better assessed.

Key words: after-ripening, temperature, light, seed dormancy, cypsela heteromorphy.

Introduction

Anthemis is one of the largest genera within the tribe *Anthemideae*, including approximately 175 species worldwide (Oberprieler & al. 2009, 2022a). It has a circum-Mediterranean distribution, with a geographical range that encompasses most of Europe, the Mediterranean Basin, and part of South-West Asia, as well as North and East Africa

(Oberprieler & al. 2009). The genus *Anthemis* is characterized by a high degree of endemism. It separated from its closest relatives about 10-15 Ma (Oberprieler 2005), becoming a species-rich group. The high phenotypic plasticity of *Anthemis* species allowed these plants to colonize many habitat types. Indeed, the ancestors of this genus were adapted to a montane-humid climate, whereas younger lineages became more adapted to a dry and Mediterranean-like climate (Lo Presti & Oberprieler 2009).

In Sicily, the genus *Anthemis* is represented by 13 species (Oberprieler & al. 2022b), seven of which are the subject of this study. *Anthemis aeolica* Lojac. and *A. maritima* L. subsp. *maritima* are coastal species; *A. aetnensis* Spreng., *A. cupaniana* Tod. ex Nyman, and *A. cretica* L. subsp. *messanensis* (Brullo) Giardina & Raimondo are mountain species; *A. parlatoreana* Raimondo, Bajona, Spadaro & Di Gristina and *A. pignattiorum* Guarino, Raimondo & Domina instead grow at hilly elevations (300-700 m a.s.l.). All of them are endemic to Sicily except for *A. maritima* subsp. *maritima*, which has a Mediterranean distribution.

Differences in the biogeographical history, isolation, and adaptation to peculiar habitats could have influenced many of the life history traits in the present *Anthemis* species. The timing for seed germination and subsequent seedling establishment is a crucial trait for plant reproduction success and has to be finely tuned to the environmental conditions. This given, the germination behaviour of the study species may differ between montane and lowland taxa. Indeed, the establishment of seedlings should have to occur when sufficient water is available in the soil and temperature is favourable for their growth, avoiding the onset of summer drought in Mediterranean environments and winter frosts, especially for high mountain species.

Physiological dormancy has been reported for the seeds of many *Anthemis* species, such as *A. arvensis*, *A. cotula, A. chrysantha*, and *A. ruthenica* (Ellis & Ilnicki 1968; Martinkova & al. 1997; Aguado & al. 2011). Moreover, the presence of heteromorphic cypselas has been highlighted, with central diaspores (i.e., disc cypselas) and peripheral ones (i.e., ray cypselas) differing in their morphology, anatomy, dispersal, and germination behaviour. Cypsela heteromorphy has been found in the genus *Anthemis* itself (*A. ruthenica* and *A. chrysantha*), as well as in other *Asteraceae* taxa from highly seasonal environments within the genera *Bidens*, *Senecio*, *Picris*, and *Crepis* (McEvoy 1984; Corkidi & al. 1991, Baskin & Baskin 2014). Central cypselas are often less dormant and germinate over a wider range of temperatures, to higher percentages and at faster rates than peripheral ones (Brändel 2007). The pericarp is the structure that protects the true seed within cypsela, appearing to be primarily responsible for the heteromorphy and, in part, the cause of the different germination responses among diasporas (Brändel 2007; Aguado & al. 2011). For instance, a thick pericarp may slow down or, eventually, prevent the imbibition of embryos or impose on them a mechanical compulsion, so conditioning germination occurrence and/or its parameters.

All the species considered in this report possess heteromorphic cypselas: with thin and light-coloured pericarp and with thick and dark-coloured pericarp (Fig.1). Entire cypselas, hereafter seeds, were used as germination units. Seven constant temperatures (3, 5, 10, 15, 20, 25, 30, and 35°C) were tested for all the species and further four alternating temperatures (15/10, 20/10, 20/15, and 25/20°C) only for *A. aeolica*. All of the temperature treatments were tested both in light/dark (12/12 h photoperiod) and darkness conditions (0/24 h). Under alternating temperatures, the light period was associated with the higher temperature. Both fresh and afterripened seeds (T = 22 ± 2°C; RH = 50%; 1- to 12-month duration) were compared. Finally, tests

were repeated separately for the two identified cypsela morphs to account for possible differences in the germination behaviour due to the pericarp structure.

Our results identified and described the best germination protocols for each seed accession.

91. *Anthemis aeolica* Lojac. (*Asteraceae*) (Fig. 2a)

Accession data

Si: Lipari (Messina), Lisca Bianca - Panarea (WGS84: 38.63906°N, 15.114147°E), volcanic tuff, 7 m a.s.l., 16 Jul 2017, *S. Catara & A. Cristaudo* (SO.PRO.ME/CT/17/707, BGS-CT).

Si: Lipari (Messina), Lisca Bianca - Panarea (WGS84: 38.63906°N, 15.114147°E), volcanic tuff, 7 m a.s.l., 16 Jul 2020, *M. Castrogiovanni & C. Impelluso* (SiMaSeed/CT/20/669, BGS-CT).

Germination data

Pre-treatments: no treatment; *seeds submitted to a 3-months after-ripening period

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 6 ml of sterilized distilled water.

Sample size: 100 seeds (25 × 4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
99%	constant 5°C	12/12 h	9.0	11.7	19.0	12.6
99%	constant 15°C	12/12 h	4.0	4.8	8.0	5.5
98%	**alternating 15/10°C	12/12 h	5.0	5.0	8.0	5.6
97%	**alternating 20/10°C	12/12 h	4.0	4.4	8.0	4.9
97%	**alternating 20/15°C	12/12 h	3.0	3.9	11.0	4.6
93%	constant 10°C	12/12 h	5.0	6.6	9.0	7.0
93%	constant 25°C	12/12 h	2.0	5.2	16.0	6.8
93%	**alternating 25/20°C	12/12 h	3.0	4.0	10.0	5.0
91%	constant 20°C	12/12 h	4.0	4.5	10.0	5.1
88%	constant 3°C	12/12 h	20.0	23.4	30.0	24.4
84%	*constant 30°C	12/12 h	2.0	3.3	29.0	7.1

**Alternating temperatures were tested only in fresh seeds from the SO.PRO.ME/CT/17/707 accession

Observations

Anthemis aeolica is a narrow endemic species whose only known population occurs on the islet of Lisca Bianca, in the Aeolian Islands. Therefore, it is a species of great biogeographical and conservation concern. According to the IUCN (2012), this species must be considered Critically Endangered (CR) (Orsenigo & al. 2018). It grows on volcanic tuff and shares its habitat with many rupicolous and halophytic taxa, such as *Dianthus rupicola* subsp. *aeolicus*, *Hyoseris taurina*, *Limonium minutiflorum*, *Limbarda crithmoides*, and *Lotus cytisoides*.

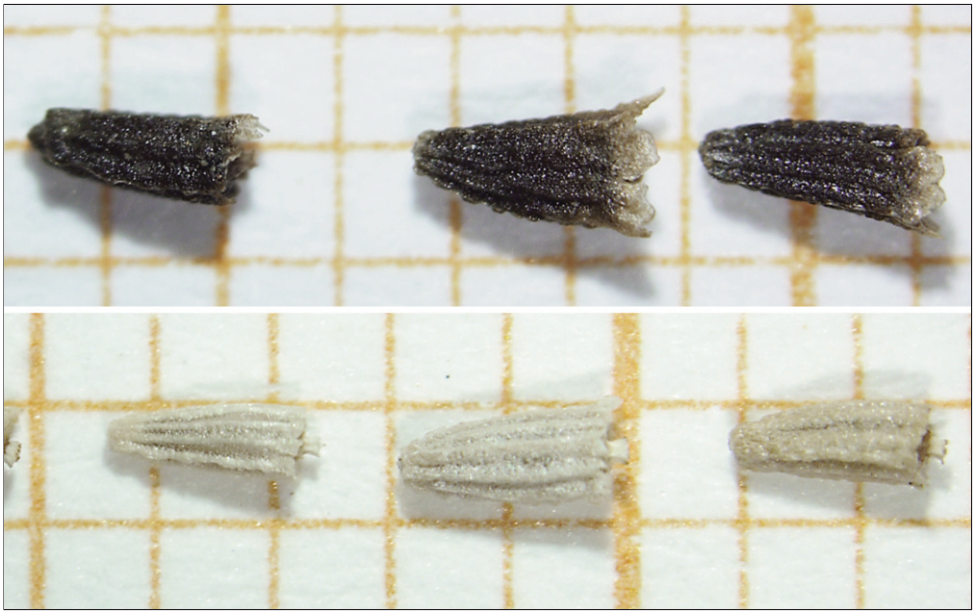

Fig. 1. Examples of a) dark and b) light-coloured cypselas from *Anthemis aeolica.*

A. aeolica produces dimorphic cypselas (Fig. 1), which showed opposite germination behaviour. Fresh light-coloured ones reached high germination percentages (> 90%) within a wide range of constant temperatures (5-25°C) and under alternating regimes in the presence of light. High germination values (> 80%) were also reached at 30°C, under light conditions, but only in 3-month aged seeds, indicating a positive physiological response to after-ripening. Conversely, dark cypselas displayed a deep dormancy level with low or no germination (3-8%) under all the conditions tested, favouring their inclusion in the soil seed bank. The fastest germination was obtained for light cypselas at 20 and 25°C constant regimes, under which almost all of the seeds germinated within three days. The lack of germination at very high temperatures in fresh light-coloured seeds suggests a protection strategy (i.e., thermo-dormancy or thermo-inhibition) against casual rainfalls during the summer (Hills & van Staden 2003; Cristaudo & al. 2019; Carruggio & al. 2021).

A generalized negative effect of darkness on seed germination was detected, making useful further experiments on more specific gap detection factors such as light quality and nitrate concentration (Fenner & Thompson 2005), which could be greatly important under high competition conditions like those experienced by *A. aeolica*.

92. *Anthemis aetnensis* Spreng. (*Asteraceae*) (Fig. 2b)

Accession data

Si: Nicolosi (Catania), Crateri Silvestri (WGS84: 37.69714°N, 15.01239°E), volcanic ash and lapilli fields, 1,787 m a.s.l., 20 Jun 2020, *A. Cristaudo & F. Carruggio* (SiMaSeed/CT/20/646, BGS-CT).

Germination data

Pre-treatments: no treatment.

Germination medium 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 6 ml of sterilized distilled water.

Sample size: 100 seeds (25 × 4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
100%	constant 20°C	12/12 h	3.0	4.9	15.0	6.1
97%	constant 15°C	12/12 h	3.0	5.7	18.0	6.6
97%	constant 25°C	12/12 h	3.0	5.9	18.0	6.9
96%	constant 10°C	12/12 h	5.0	7.4	18.0	8.7
87%	constant 30°C	12/12 h	3.0	9.2	22.0	10.2

Observations

A. aetnensis is an endemic species to Mount Etna. The plant forms small cushions on the high slopes of the volcano, sometimes together with *Astragalus siculus* Biv. According to the IUCN criteria (2012), *A. aetnensis* has been considered as Near Threatened (NT) (Orsenigo & al. 2018).

The germination of fresh light-coloured cypselas was promoted in the presence of light, in a wide temperature range (10-30°C). Fresh dark-coloured cypselas reached germination values of 50-70% at temperatures ranging from 20 to 30°C, only if exposed to light, confirming the presence of positive photoblastic seeds even in this species.

Germination range partially broadened towards higher temperature values in light-coloured cypselas following one year of after-ripening (60% of germination at 35°C in the light). Moreover, seeds from this morph were equally able to germinate in both the presence and absence of light after this period. Changes in germination requirements following an after-ripening treatment suggest that a physiological change occurred. These results show that the species is adapted to Mediterranean mountain ecosystems, avoiding germination at low temperatures when the risk of winter frost could affect seedling establishment (Picciau & al. 2019; Carruggio & al. 2020).

93. *Anthemis cretica* subsp. ***messanensis*** (Brullo) Giardina & Raimondo (*Asteraceae*) (Fig. 2c)

Accession data

Si: Messina (Messina), Antennamare (WGS84: 38.15373°N, 15.462297°E), semi-rupestrian habitats, 1,062 m a.s.l., 26 Jun 2019, *A. Cristaudo* (SiMaSeed/CT/19/379, BGS-CT).

Germination data

Pre-treatments: 12-month dry after-ripening at 22 ± 2°C and 50% RH.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 6 ml of sterilized distilled water.

Sample size: 100 seeds (25 × 4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
93%	constant 15°C	12/12 h	5.0	5.8	21.0	7.2
90%	constant 10°C	12/12 h	8.0	10.4	20.0	11.3
89%	constant 20°C	12/12 h	3.0	4.2	26.0	5.5
88%	constant 25°C	12/12 h	2.0	4.0	25.0	5.8
87%	constant 10°C	0/24 h	-	-	-	-
82%	constant 15°C	0/24 h	-	-	-	-

Observations

Anthemis cretica subsp. *messanensis* is a rare endemic species to Peloritani Mountains (NE Sicily) that grows in rocky habitats. A single population was known so far for Mt. Antennamare, whereas a new population was recently found close to Mt. Poverello (Fiumedinisi - Messina) (Tavilla & al. 2021), 12 km apart from the first one. The IUCN conservation status for *A. cretica* subsp. *messanensis* is Critically Endangered (CR) (Orsenigo & al. 2018).

Our tests highlighted a germination behaviour that deviates from that of the other investigated taxa. Indeed, fresh light-coloured cypselas showed a pronounced dormancy under all the temperatures tested, both in the presence and absence of light. Some germination was achieved at 5 and 10°C in the light but without exceeding 50% final germination. After a 3-month after-ripening period, dormancy began to be released, only in seeds exposed to light whereas it was maintained in seeds incubated in total darkness. Final germination values above 80% occurred one year after seed collection, at temperatures ranging from 10 to 25°C in the light and at 10 and 15°C in darkness. Germination slightly decreased at 20 and 25°C (approx. 70%) and was completely inhibited by higher temperatures in the dark. Seeds with dark cypselas had very low germination under all the temperatures and light treatments.

In conclusion, seeds of *A. cretica* subsp. *messanensis* possess physiological dormancy. Moreover, the small proportion of seeds able to germinate soon after natural dispersal only responds to low temperatures, such as in late autumn/winter plants. The preference for low-temperature germination, which is a more typical trait of Mediterranean coastal species rather than mountain species (Picciau & al. 2019), ensures that an adequate supply of water in the soil is available for seedlings at the time of their growth and establishment.

94. *Anthemis cupaniana* Tod. ex Nyman. (*Asteraceae*) (Fig. 2d)

Accession data

Si: Monreale (Palermo), Serra del Frassino (WGS84: 37.99833°N, 13.25041°E), calcareous rocky slopes, 1,060 m a.s.l., 22 Jul 2020, *F. Carruggio, M. Castrogiovanni*, *R. Galesi & C. Impelluso* (SiMaSeed/CT/20/684, BGS-CT).

Germination data

Pre-treatments: 2-month dry after-ripening at 22 ± 2°C and 50% RH.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 6 ml of sterilized distilled water.

Sample size: 100 seeds (25 × 4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
100%	constant 10°C	12/12 h	5.0	6.6	11.0	7.5
95%	constant 15°C	12/12 h	4.0	5.7	17.0	6.9
90%	constant 20°C	12/12 h	3.0	4.8	16.0	6.5
84%	constant 25°C	12/12 h	4.0	6.0	14.0	7.0

Observations

Anthemis cupaniana is a Sicilian endemic species, which occurs on the calcareous and dolomitic mountains of Central-Western Sicily from 500 to 1,600 m of altitude (Domina & al. 2015; Oberprieler & al. 2022a). It is a tetraploid species, probably originated by the union of two diploid species within the *A. pedunculata* group, from Northern Africa, and the *A. cretica* group, from Southern Europe and Southern-Western Asia (Oberprieler & al. 2022a). According to the IUCN criteria, *A. cupaniana* has been recognized as Near Threatened (NT) (Orsenigo & al. 2018).

The highest and fastest germination for seeds exposed to after-ripening for two months was recorded at temperatures ranging from 10 to 25°C, in the presence of light. The optimal conditions for germination were similar to those detected for *A. aetnensis*, a species close to *A. cupaniana* for both ecology and phylogeny.

Germination in darkness is lower under all the tested conditions (although 70% germination percentage was reached in the 10-20°C range). No germination was recorded in seeds from dark cypselas under all the conditions tested.

95. *Anthemis maritima* L. subsp. ***maritima*** (*Asteraceae*) (Fig. 2e)

Accession data

Si: Balestrate (Palermo), spiaggia Balestrate (WGS84: 38.04406°N, 12.988917°E), coastal dune, 3 m a.s.l., 21 Jul 2020, *F. Carruggio, M. Castrogiovanni & R. Galesi* (SiMaSeed/CT/20/673, BGS-CT).

Germination data

Pre-treatments: 3-month dry after-ripening at 22 ± 2°C and 50% RH.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 6 ml of sterilized distilled water.

Sample size: 100 seeds (25 × 4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
100%	constant 20°C	12/12 h	2.0	4.0	15.0	5.0
98%	constant 20°C	0/24h	-	-	-	-
97%	constant 15°C	0/24h	-	-	-	-
94%	constant 10°C	0/24h	-	-	-	-
94%	constant 25°C	0/24h	-	-	-	-
94%	constant 15°C	12/12 h	3.0	4.7	15.0	5.8
93%	constant 10°C	12/12 h	5.0	7.3	17.0	8.6
93%	constant 25°C	12/12 h	2.0	3.3	12.0	4.0
91%	constant 5°C	0/24h	-	-	-	-
80%	constant 30°C	12/12 h	2.0	3.2	22.0	5.0

Observations

Anthemis maritima is a perennial species, mainly distributed in the Western Mediterranean region and growing on sand dunes, which it contributes to build up (De Lillis & al. 2004).

Germination data for *A. maritima* subsp. *maritima* were already published by Porceddu & al. (2019), who reported a final germination percentage above 80% only for seeds incubated at 20°C in the presence of light.

The best germination for our accession was achieved after a three-month after-ripening period at temperatures ranging from 10 to 30°C in the presence of light and from 5 to 25°C in darkness. After a further six months, seeds incubated in the absence of light were able to germinate to high percentages even at 30°C. Seeds from dark cypselas did not exceed 50% germination under any temperature and light conditions after a two-month after-ripening period.

The lack of photoinhibition in this psammophilous species contradicts the general pattern known for plants with similar habitat preferences (Carta & al. 2017). Photoinhibition in sandy coastal habitats avoids seedling establishment in the proximity of soil surface where the risk of desiccation and mechanical damage from shifting sand is higher. However, *A. maritima* subsp. *maritima* is not the only psammophilous species indifferent to light. A similar germination behaviour has been reported also for *Dianthus morisianus* from Sardinia (Cogoni & al. 2012) and for other sand dune species occurring in Sicily (Salmeri & Trubia 2019).

96. *Anthemis parlatoreana* Raimondo, Bajona, Spadaro & Di Gristina (*Asteraceae*) (Fig. 2f)

Accession data

Si: Castellammare del Golfo (Trapani), M.te Inici (WGS84: 38.01929°N, 12.870433°E), rocky outcrops, 277 m a.s.l., 21 Jul 2020, *F. Carruggio, M. Castrogiovanni & R. Galesi* (SiMaSeed/CT/20/672, BGS-CT).

Germination data

Pre-treatments: 3-month dry after-ripening at 22 ± 2°C and 50% RH.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 6 ml of sterilized distilled water.

Sample size: 100 seeds (25 × 4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
100%	constant 20°C	12/12 h	2.0	1.71	5.0	2.3
99%	constant 3°C	12/12 h	12.0	15.4	21.0	16.0
99%	constant 5°C	12/12 h	9.0	9.7	14.0	10.4
99%	constant 10°C	12/12 h	4.0	4.0	8.0	4.6
99%	constant 15°C	12/12 h	2.0	2.53	5.0	3.1
98%	constant 25°C	12/12 h	1.0	1.62	6.0	2.2
98%	constant 20°C	0/24h	-	-	-	-
94%	constant 10°C	0/24h	-	-	-	-
94%	constant 15°C	0/24h	-	-	-	-
88%	constant 5°C	0/24h	-	-	-	-

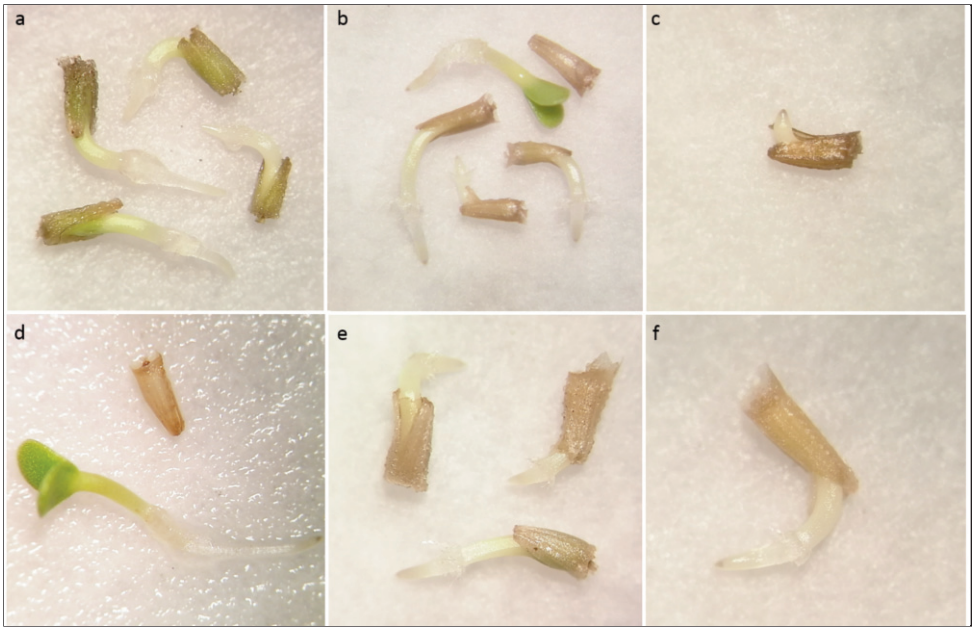

Fig. 2. Germinated seeds and seedlings: a) *Anthemis aeolica*; b) *A. aetnensis*; c) *A. cretica* subsp. *messanensis*; d) *A. cupaniana*; e) *A. maritima* subsp. *maritima*; f) *A. parlatoreana.*

Observations

Anthemis parlatoreana has been recently described as a new plant species by Raimondo & al. (2021). It is related to *A. pignattiorum* and *A. ismelia,* with which it shares similar ecological requirements, and to *A. cupaniana*, from which differs in having more thermophilous adaptive traits. *A. parlatoreana* is currently known only for Mount Inici, a limestone coastal relief in the province of Trapani (NW Sicily). It grows on rocky outcrops and screes, on N-NE facing slopes, at an altitude between 250 and 750 m. This report presents the first germination data for this species.

After 50 days of collection, seeds of *A. parlatoreana* achieved high germination percentages at temperatures ranging from 3 to 15°C, in the presence of light, and within a slightly narrower range in darkness (5-15°C). Seeds tested after three months of after-ripening expanded the thermal range for germination, reaching almost 100% even at 20 and 25°C in the presence of light and at 20°C in darkness.

Our results demonstrate that seeds of *A. parlatoreana* exhibit a non-deep physiological dormancy, with germination being regulated by conditional dormancy, quite similarly to *A. messanensis* and *A. pignattiorum* (Soltani & al. 2017; Carruggio & al. 2021). The ability to germinate at low-temperature values and the partial or full inhibition at higher ones (> 25°C) confirm a pattern that has been highlighted in other lowland Mediterranean species (Picciau & al. 2019).

As in the other study species, the dark cypselas are almost unable to germinate under all the conditions tested.

97. *Anthemis pignattiorum* Guarino, Raimondo & Domina (*Asteraceae*)

Accession data

Si: Avola (Siracusa), Cava Grande del Cassibile (WGS84: 36.96803°N, 15.093833°E), calcarenite cliffs, 461 m a.s.l., 09 Jun 2020, *F. Carruggio, M. Castrogiovanni & R. Galesi* (SiMaSeed/CT/20/641, BGS-CT).

Germination data

Pre-treatments: 3-month dry after-ripening at 22 ± 2°C and 50% RH.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 6 ml of sterilized distilled water.

Sample size: 40 seeds (20 × 2 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
100%	constant 10°C	12/12 h	5.0	6.1	9.0	6.9
100%	constant 15°C	0/24h	-	-	-	-
90%	constant 20°C	12/12 h	4.0	5.8	15.0	7.0
89%	constant 5°C	12/12 h	8.0	9.9	24.0	11.8
89%	constant 10°C	0/24h	-	-	-	-

85%	constant 15°C	12/12 h	3.0	4.9	9.0	5.6
85%	constant 30°C	12/12 h	6.0	14.9	30.0	14.9
85%	constant 20°C	0/24h	-	-	-	-
80%	constant 25°C	12/12 h	4.0	14.5	29.0	16.5

Observations

Anthemis pignattiorum is an endemic species to Hyblaean Mountains (SE Sicily). It has been recorded only at Cava Grande del Cassibile (Siracusa), where it grows on vertical calcarenite cliffs with northern exposure (Guarino & al. 2013). *A. pignattiorum* has been assessed as Endangered (EN), according to the IUCN criteria (Orsenigo & al. 2018). The germination trials were performed with only two replicates of 20 seeds each, due to the poor availability of viable seeds. Indeed, *A. pignattiorum* is a very rare species and grows on vertical and unreachable sites. In addition, the high relevance of pre-dispersal seed loss due to the incidence of larval infestation limits the number of available seeds. However, the data reported are the first germination records for this species.

Fresh seeds of *A. pignattiorum* (20 days) proved high germination capability over a narrow range of temperatures (5-15°C) in light conditions. The thermal range for germination enlarged with after-ripening, reaching an upper limit of 30°C after 90 days from collection. Such as in *A. parlatoreana* and *A. messanensis*, germination appeared regulated by conditional dormancy (Soltani & al. 2017).

Dark cypselas failed to achieve high germination percentages under all the conditions tested.

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