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Seed germination reports for five coastal and inland species from the Maltese Islands

Abstract

Buhagiar, J. A., Lamoliere, A. & Iannaccone, M.: Seed germination reports for coastal and inland garrigue species from the Maltese Islands [in Magrini, S. & Salmeri, C. (eds). Mediterranean Plant Germination reports – 3] Fl. Medit. 31: 283-290. 2021. http://dx.doi.org/10.7320/FlMedit31.283

This study investigated seed germination in five indigenous species of plants in the Maltese Islands, predominantly occurring in coastal garrigue habitats but also inland, namely *Drimia maritima*, *Periploca angustifolia*, *Asparagus aphyllus*, *Myrtus communis*, and *Pancratium maritimum.* Different germination protocols were tested for one or more populations, namely: four different temperature regimes - constant 15, 20 and 25°C as well as alternating 23.5/15°C; three different photoperiods, 12/12, 16/8 and 0/24 light/dark. The best germination results for each species are provided and additional notes on the species and germination behaviour are also given.

Keywords: germination protocols, Mediterranean flora, coastal and inland garrigue.

Introduction

Based on Sommier & Caruana Gatto (1915), Borg (1927), Haslam (1969), Haslam & al. (1977), Pignatti (1982), Lanfranco (1984, 1995), Lanfranco & Schembri (1986), Anderson & Schembri (1989), Schembri (1994, 1997), Savona-Ventura (2001), Casha (2015, 2020), the flora of the Maltese islands, including the autochthonous and allochthonous ferns and spermatophytes, amounts to about 1100 taxa, species and subspecies. Several taxa described in the past are thought to have become locally extinct though there is the occasional rediscovery, often in some very localised areas and in depleted numbers (Brullo & al. 2020). Studies using karyotyping and DNA barcoding are ongoing to clarify some taxonomic doubts.

With regards to seed germination studies of the indigenous plant populations very little is known. In this paper, we present seed germination results for five different indigenous species occurring in the Maltese Islands. The five species selected are indigenous elements of the Mediterranean flora from similar coastal and more inland habitats. Three of the species considered are coastal and more inland elements and include *Drimia maritima* (L.) Stearn, *Periploca angustifolia* Labill., and *Asparagus aphyllus* L. Another species, namely

Myrtus communis L., was selected for its unique characteristics, occurring in a sheltered valley close to the sea and having white to light purple fruits. The remaining species, namely *Pancratium maritimum* L.*,* occurs on sand dunes which in the Maltese Islands are severely limited.

54. *Drimia maritima* (L.) Stearn (*Asparagaceae*)

Accession data

Si(M): Armier Bay, Mellieha, Malta (WGS84: 35.994250°N, 14.365611°E), 11 m a.s.l., 19 Nov 2020, *J. A. Buhagiar, M. Iannaccone & A. Lamoliere* (BDUM/20/018, Seed bank of the Department of Biology, University of Malta).

Germination data
Pre-treatments: None.

Germination medium: Seeds were germinated on 2 sheets of sterilized filter paper (Whatman™ Grade 91), imbibed with 5 ml of sterilized distilled water.

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

Germination	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
97%	constant 15°C	0/24h	7	6.5	14	7.1
97%	constant 15°C	12/12h	7	6.6	18	7.4
93%	constant 20°C	16/8h	4	6.5	18	7.3
91%	alternating 23.5/15°C	16/8h	4	6.7	28	8.5
85%	alternating 23.5/15°C	0/24h	4	6.3	11	6.5
81%	constant 20°C	0/24h	4	6.5	25	8.3

Observations

Drimia maritima is a common element of the Maltese maritime garrigue landscape although it is also found inland. It is a geophyte, which is extremely well adapted to the Mediterranean climate, surviving the hot dry summer as semi-exposed dormant bulbs from March/April well into September. It is interesting in that it is the first flowering element towards the end of Summer (mid-to-late August) when the Maltese landscape is still very brown and dry, thereby avoiding competition for pollinators. This species tends to form sizable clumps by tillering.

Drimia maritima gave very good germination percentages at different temperatures and light/dark treatments with the highest percentage of 97% at 15°C. The maximum germination times varied between 11 and 28 days. Indeed, Marques & Draper (2012) report that fast and high germination rate is characteristic of several species of geophytes. The seeds take slightly longer to germinate at higher temperatures, and the germination percentage decreases to 77% at 25°C. It is also interesting that a diurnal cycle of 23.5/15°C combined

with a 16/8h light/dark cycle also gave a good germination percentage. Comparing the germination percentages with those reported by Royal Botanic Gardens Kew (2021), the highest germination percentage of over 95% were obtained at 5, 10 and 15°C as well as a 25/10°C alternating temperature using a 16/8 light/dark cycle. However, there is high variability between different tests and germination times reported which are much longer, a good many over 40 days and one up to 105 days.

55. *Periploca angustifolia* Labill. (*Apocynaceae*)

Accession data

Si(M): Wied Babu, Zurrieq, Malta, (WGS84: 35.821139°N, 14.459528°E), 70 m a.s.l, 04/06/2021, *J. A. Buhagiar, M. Iannaccone & A. Lamoliere* (BDUM/21/4, Seed bank of the Department of Biology, University of Malta).

Germination data
Pre-treatments: None.

Germination medium: Seeds were germinated on 2 sheets of sterilized filter paper (Whatman™ Grade 91), imbibed with 5 ml of sterilized distilled water.

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

Germination	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
90.0%	constant 15°C	0/24h	3	6.7	12	8.1
85.0%	alternating 23.5/15°C	0/24h	3	4.6	24	5.6

Observations

Periploca angustifolia is a common maritime garrigue element in the Maltese Islands with distribution being more common on the southwestern part of the coastline, where it forms a typical shrubby vegetation association described as *Periploco angustifoliae-Euphorbietum dendroidis* (Brullo & al. 2020). *P. angustifolia* gave the highest percentage germination at 15°C and 0/24h photoperiod. Interestingly, the germination percentage declined drastically to 60% in a 12/12h photoperiod regime for this temperature, showing photoinhibition. Higher temperatures also showed a decline in germination percentage ranging from 52 to 77% for 20°C and 25°C under light/dark conditions tested. The exception was for the diurnal cycle of 23.5/15°C combined with a 0/24h light/dark cycle which gave a germination percentage of 85%, though in the light the germination again declined to 69%. Noumi & al. (2010) reported the highest germination percentage for this species at 25°C though it must be noted that all tests were carried out in complete darkness. Abdellaoui & al. (2013) tested germination at 25°C in complete darkness for seeds collected and stored for different periods ranging from

0 to 15 years. Germination percentage was mostly below 70%, with only three- and seven-year storage being over 80%. The germination data in this report, therefore represent a notable improvement in the germination protocol for this species compared to previously published reports.

56. *Asparagus aphyllus* L. (*Asparagaceae*)

Accession data

Si(M): Wied Znubber, Birżebbuġa, Malta, (WGS84: 35.809167°N, 14.511278°E), March 2021, *J. A.Buhagiar, M. Iannaccone & A. Lamoliere* (BDUM/20/21, Seedbank of the Department of Biology, University of Malta).

Germination data

Pre-treatments: None.

Germination medium: Seeds were germinated on 2 sheets of sterilized filter paper (Whatman™ Grade 91), imbibed with 5 ml of sterilized distilled water.

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

Germination	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
90.0%	alternating 23.5/15°C	0/24h	17	26.4	49	28.6
85.0%	alternating 23.5/15°C	16/8h	17	25.7	49	28.1
84.0%	constant 15°C	0/24h	17	26.1	52	26.2
83.0%	constant 15°C	12/12h	19	28.3	61	31.4

Observations

Asparagus aphyllus is present in numerous habitats of the Maltese islands (Brullo & al. 2020). It has high germination percentages at two of the thermoperiods tested, namely 15 and 23.5/15°C with no significant change between total darkness and 12/12 or 16/8 photoperiods tested. The highest percentage of 90% was at an alternating thermoperiod of 23.5/15°C under full dark conditions, though a 16/8 photoperiod also gave good germination results (85%) indicating that seeds are not negatively affected by light. Significantly lower germination rates were obtained for the other thermoperiods with the lowest being at 25°C both in complete darkness and 16/8h light/dark conditions (23% and 17% respectively). No germination data is given in the RBG Kew's Seed Information Database (https://data.kew.org/sid/) and other sources do not elaborate on germination at different temperatures. Therefore, these results provide a germination protocol for this species, with direct application in ecological restoration, but also food production.

57. *Myrtus communis* L. (*Myrtaceae*)

Accession data

Si(M): Gnien Ingraw, Mellieha, Malta. (WGS84: 35.959278°N, 14.358333°E), 95 m a.s.l., 09 Mar 2021, *J. A. Buhagiar, M. Iannaccone & A. Lamoliere* (BDUM/21/17, Seedbank of the Department of Biology, University of Malta).

Germination data
Pre-treatments: None.

Germination medium: Seeds were germinated on 2 sheets of sterilized filter paper (Whatman™ Grade 91), imbibed with 5 ml of sterilized distilled water.

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

Germination	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
93.0%	constant 15°C	0/24h	3	14.0	28	15.3
93.0%	alternating 23.5/15°C	0/24h	7	10.0	19	11.0
93.0%	alternating 23.5/15°C	16/8h	7	10.8	26	12.0
92.0%	constant 15°C	12/12h	3	15.0	29	16.1
91.0%	constant 25°C	16/8h	3	7.0	24	8.4
89.0%	constant 20°C	16/8h	5	8.7	14	10.2
89.0%	constant 25°C	0/24h	3	5.7	10	6.6
88.0%	constant 20°C	0/24h	7	8.6	21	10.2

Observations

Myrtus communis is a species with high germination percentages. It does not seem to be dependent on any particular thermo- or photoperiod with all percentages being above 88% irrespective of germination in complete darkness or with a light/dark photoperiod. These results are consistent with previous results comparing blue and white morphs of *M. communis* from Mallorca (Traveset & al. 2001). For *M. communis* subsp. *communis*, the RBG Kew's Seed Information Database (Royal Botanic Gardens Kew 2021) gives comparable germination percentages from 83 to 93% for tested temperatures of 10, 15, 20 and 25°C using a 12/12h photoperiod. However, maximum germination times were much longer and range from 49 days at 25°C to 77 days for 10 and 15°C. This species does not qualify as threatened according to IUCN (Chadburn & Wilson 2018), however, this phenotype with its pale coloured and sweet fruits, but also since it is a small population restricted to few locations in the Maltese Islands, though a few specimens possibly derived from this population are present in some gardens.

58. *Pancratium maritimum* L. (*Amaryllidaceae*)

Accession data

Si(M): Armier Bay, Mellieha, Malta (WGS84 35.992333°N, 14.365722°E), 5 m a.s.l., 19

Nov 2020, *J. A. Buhagiar, M. Iannaccone & A. Lamoliere* (BDUM/20/13, Seed bank of the Department of Biology, University of Malta).

Germination data

Pre-treatments: None.

Germination medium: Seeds were germinated on 2 sheets of sterilized filter paper (Whatman™ Grade 91), imbibed with 5 ml of sterilized distilled water.

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

Germination	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
94.0%	alternating 23.5/15°C	16/8h	7	19.6	68	24.0
91.0%	alternating 23.5/15°C	0/24h	5	20.9	59	23.1
89.0%	constant 15°C	0/24h	3	20.4	49	22.6
89.0%	constant 15°C	12/12h	3	22.5	61	25.4
86.0%	constant 20°C	16/8h	5	22.8	59	24.3
86.0%	constant 25°C	0/24h	7	22.3	38	23.3
86.0%	constant 25°C	16/8h	3	21.8	61	23.8
80.0%	constant 20°C	0/24h	10	19.5	52	22.2

Observations

Pancratium maritimum is another species with high germination percentages. Like the previous species, it does not seem to be dependent on any particular thermo- or photoperiod with all percentages being above 80% irrespective of germination in complete darkness or with a light/dark photoperiod. However, an alternating day and night thermoperiod of 23.5/15°C and an 18/6h photoperiod gave the highest germination percentage, 94%, followed closely by 91% for a 0/24h period. Thus, light exposure does not appear to be a crucial requirement, though in two cases, a slightly higher percentage was obtained.

These results are consistent with germination trials carried out on populations from Egypt (Mohamed & al. 2018) and Italy (Magrini & al. 2019; Salmeri & Trubia 2019), though the latter found photoinhibition at 5°C. These results represent the first documented germination data reported for this species from the Maltese archipelago, where it has a very restricted distribution and population size. Royal Botanic Gardens Kew (2021) also gives comparable results both in terms of percentages and also maximum days for germination. Together with *Eryngium maritimum* and other species, it forms a scare association in the Maltese Islands, described as a *Cypero capitati-Agropyretum juncei* (Kuhnholtz-Lordat 1923; Brullo & al. 2020).

Conclusion

Through the present research, it was possible to successfully investigate seed germination for five coastal and inland garrigue species from the Maltese Islands. Germinated seeds were eventually grown on and used for habitat restoration. It is emphasised that pre-sowing sterilisation was purposely avoided in order to preserve as much as possible the seed-associated microbiome and thereby produce more environmentally adapted and resilient

seedlings. Further studies on seed conditioning, seed viability as well as fungal associations occurring during germination are envisaged.

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