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Seed germination protocols for six dune species from the Tyrrhenian coast (central Italy)

Abstract

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Successful germination protocols for the following six dune species are presented: *Centaurea sphaerocephala* subsp. *sphaerocephala*, *Jacobaea maritima* subsp. *maritima*, *Matthiola sinuata*, *Medicago marina, Pancratium maritimum*, and *Silene canescens*. Seeds were collected in sand dunes along the Tyrrhenian coasts in south Tuscany and north Latium (central Italy). The germination ability was tested at the Tuscia Germplasm Bank (BGT) at six constant temperatures (from 5 to 30°C), under both light (with a 12/12h photoperiod) and total dark. Our results show a high germination ability and germination rate for the tested species.

Key words: coastal dunes, Latium, photoinhibition, psammophytes, Tuscany.

Mediterranean coastal dunes are dynamic and threatened environments, particularly vulnerable being subjected to the strong impact of human activities and in need of conservation actions. Research focused on the assessment of germination requirements of dune species are carried out at the Tuscia Germplasm Bank where over one hundred seed accessions of thirty species collected in Central Italy dunes are long term preserved. Here we present successful germination protocols for six species occurring in the Tyrrhenian sandy dunes: *Centaurea sphaerocephala* L. subsp. *sphaerocephala*, *Medicago marina* L., *Pancratium maritimum* L., and new data for Italy of *Jacobaea maritima* (L.) Pelser & Meijden subsp. *maritima*, *Matthiola sinuata* (L.) W.T.Aiton, and *Silene canescens* Ten.

15. *Centaurea sphaerocephala* L. subsp. ***sphaerocephala*** (*Asteraceae*) (Fig. 1a)

Accession data

It: Latium. Tarquinia (Viterbo), S. Agostino (WGS84: 42.173710°N, 11.738198°E), duna costiera, 3 m a.s.l., 4 Sept 2017, *S. Magrini & D. Barreca* (BGT-A-43017, Tuscia Germplasm Bank).

Germination data
Pre-treatments: sterilization with a solution of 5% sodium hypochlorite + Tween 20 for 5 minutes, followed by 3 rinses in sterile distilled water.

Germination medium: 1% agar.

Sample size: 100 achenes for each test (20 × 5 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
100%	constant 25°C	0/24h	1.0	2.4	7.7	2.9
100%	constant 25°C	12/12h	1.0	3.4	9.0	3.9
100%	constant 20°C	12/12h	1.0	1.8	7.7	3.3
100%	constant 20°C	0/24h	1.0	3.5	8.7	3.9
96.5%	constant 15°C	0/24h	4.0	3.7	9.7	4.9
95.0%	constant 15°C	12/12h	4.0	3.8	8.7	4.9
91.7%	constant 30°C	12/12h	1.0	2.6	11.7	4.1
90.0%	constant 30°C	0/24h	1.0	2.4	12.0	3.5
88.0%	constant 10°C	0/24h	5.0	7.1	17.0	7.6

Observations
Centaurea sphaerocephala is a perennial species of foredunes and fixed dunes, quite common in Central and South Italy (Acosta & Ercole 2015). High germination (> 72%) was obtained at a wide range of temperatures (10-30°C), independently from the applied light/dark conditions. Lower germination percentages have been reported for this species, 79% by Menini & al. (2014) for Tuscany (Italy) and 82% by Royal Botanic Gardens Kew (2019), both at 20°C under an 8/16h photoperiod.

16. *Jacobaea maritima* (L.) Pelser & Meijden subsp. ***maritima*** (*Asteraceae*) (Fig. 1b)

Accession data
It: Tuscany. Grosseto (Grosseto), loc. Collelungo, Marina di Alberese (WGS84: 42.638073°N, 11.067104°E), duna costiera, 3 m a.s.l., 30 Jun 2017, *S. Magrini & D. Barreca* (BGT-A-41717, Tuscia Germplasm Bank).

Germination data
Pre-treatments: sterilization with a solution of 5% sodium hypochlorite + Tween 20 for 5 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 1% agar.

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

Germination	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
81.5%	constant 15°C	12/12h	4.3	6.6	21.0	8.4

Observations
Jacobaea maritima subsp. *maritima* is a perennial dune species with a West Mediterranean distribution. Here we report the first germination data for this species for Italy. Germination percentages higher than 70% were obtained only at 15-25°C in the light, with the lowest percentages at 30°C in the dark (14.6%) and at 5°C in the light (18.9%). Light-promoted seed germination highlighted by Van der Meijden & Van der Waals-Kooi (1979) for a Dutch population of *Senecio jacobaea* L. can be confirmed in this species only at temperatures higher than 15°C, while photoinhibition was found at 10°C and 5°C.

17. *Matthiola sinuata* (L.) W.T.Aiton (*Brassicaceae*) (Fig. 1c)

Accession data
It: Latium. Tarquinia (Viterbo), S. Agostino (WGS84: 42.173365°N, 11.738726°E), duna costiera, 2 m a.s.l., 4 Sept 2017, *S. Magrini & D. Barreca* (BGT-A-43117, Tuscia Germplasm Bank).
It: Tuscany. Castiglione della Pescaia (Grosseto), loc. Roccamare, presso il fosso Tonfone (WGS84: 42.773051°N, 10.823768°E), duna costiera, 1 m a.s.l., 31 Aug 2014, *S. Magrini* (BGT-A-31114, Tuscia Germplasm Bank).

Germination data
Pre-treatments: sterilization with a solution of 5% sodium hypochlorite + Tween 20 for 5 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 1% agar.

Sample size: 100 seeds for each test (20 × 5 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]	Accession code
90.7%	constant 25°C	12/12h	2.0	3.6	12.0	3.8	BGT-A-43117
86.7%	constant 25°C	12/12h	3.0	5.3	15.0	7.6	BGT-A-31114
85.0%	constant 25°C	0/24h	1.7	3.6	17.3	4.3	BGT-A-31114

Observations

Matthiola sinuata is a short-lived (biennial) species typical of the sand beach drift-lines, occurring in Italy mainly along the Tyrrhenian coasts (Acosta & Ercole 2015). Here we report the first germination data for this species for Italy. Germination tests using six constant temperature regimes (5-30°C) showed that 25°C is the optimum temperature for both the accessions, the same temperature reported also by Pérez-García & al. (2007) for Spain. High germination (> 70%) was obtained only at warmer temperatures (20-30°C), independently from the applied photoperiod, unlike Royal Botanic Gardens Kew (2019) which reported the highest percentage (90%) at 11°C and at 25°C a lower percentage (78%) than those reported here.

Strong photoinhibition has been found in other dune species of the family *Brassicaceae*, like *Matthiola tricuspidata* (L.) R.Br., *Cakile maritima* Scop., and *Malcolmia littorea* (L.) R.Br. (Thanos & al. 1991, 1994; De Vitis & al. 2014, 2018; Carta & al. 2017). On the other hand, our results highlighted a strong photoinhibition of seed germination in *M. sinuata* exclusively at temperatures below 10°C and only a weak effect at 15-20°C.

18. *Medicago marina* L. (*Fabaceae*) (Fig. 1d)

Accession data

It: Tuscany. Grosseto (Grosseto), loc. Rosmarina, Marina di Grosseto (WGS84: 42.726583°N, 10.969202°E), duna costiera, 4 m a.s.l., 24 Jul 2016, *S. Magrini* (BGT-A-39416, Tuscia Germplasm Bank).

Germination data

Pre-treatments: manual scarification by sandpaper; washing with sterile distilled water + Tween 20 for 5 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 1% agar.

Sample size: 100 seeds for each test (20 × 5 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
100%	constant 20°C	12/12h	5.0	5.9	20.3	9.6
100%	constant 30°C	0/24h	1.7	8.1	43.7	20.5
100%	constant 5°C	0/24h	10.0	16.9	44.7	21.1
97.6%	constant 5°C	12/12h	13.3	16.3	67.3	24.6
95.2%	constant 30°C	12/12h	3.3	6.8	28.0	10.7
92.6%	constant 10°C	12/12h	8.3	11.1	41.0	18.8
90.5%	constant 20°C	0/24h	2.0	1.9	39.3	6.9
88.1%	constant 10°C	0/24h	5.0	5.6	18.0	13.3
88.1%	constant 15°C	0/24h	2.3	2.8	39.0	7.2
87.5%	constant 15°C	12/12h	5.0	5.5	25.0	9.0
86.1%	constant 25°C	0/24h	1.0	2.0	7.0	3.3
83.9%	constant 25°C	12/12h	3.0	4.9	36.3	11.4

Observations

Medicago marina is a perennial species characteristic of the embryonic shifting dunes, common along the Italian coasts (Acosta & Ercole 2015). As for many other genera of Fabaceae, *Medicago* seeds are characterized by a physical dormancy due to the impermeable coat. In accordance with Scippa & al. (2011), mechanical scarification by sandpaper improved seed germination, with high percentages (≥ 83.9%) at all the tested conditions (six constant temperatures from 5 to 30°C, both in the light and in the dark). In particular, the fastest germination was recorded at 25°C in darkness (T_1 = 1 day, T_{50} = 2 days and a total of 7 days for the maximum germination), even if with a lower germination percentage than reported, under the same conditions, by Scippa & al. (2011) for seeds from Adriatic coastal dunes (south Italy). The slowest germination was recorded at 5°C under light condition.

19. *Pancratium maritimum* L. (*Amaryllidaceae*) (Fig. 1e)

Accession data

It: Latium. Tarquinia (Viterbo), loc. Saline (WGS84: 42.210943°N, 11.708386°E),

duna costiera, 2 m a.s.l., 25 Sept 2016, *A. Caldelli & S. Magrini* (BGT-A-39816, Tuscia Germplasm Bank).

Germination data

Pre-treatments: sterilization with a solution of 5% sodium hypochlorite + Tween 20 for 5 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 1% agar.

Sample size: 60 seeds for each test (20 × 3 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
97.8%	constant 20°C	0/24h	12.0	15.3	23.7	14.9
97.8%	constant 15°C	12/12h	20.3	36.0	55.3	35.7
88.9%	constant 15°C	0/24h	24.3	32.3	58.3	31.9
82.2%	constant 30°C	0/24h	14.3	23.9	63.3	27.7

Observations

Pancratium maritimum is a species of coastal dunes, with a wide distribution along the Italian sandy coasts even if overcollection, urbanization, and tourism put serious threats to the species, resulting in a significant decrease of its populations (De Castro & al. 2012; Giovino & al. 2015). To date, these are the best germination data for this species for Latium. Our germination tests defined an optimal temperature range of 15–20°C, in accordance with data previously reported for Sardinia (95-98% at 15-20°C, in the dark; Bacchetta & al. 2007). Moreover, at all the temperatures in the dark (except 20°C), higher germination percentages have been recorded than those reported by Balestri & Cinelli (2004) for Tuscany (53% vs. 0% at 5°C, 80% vs. 25% at 10°C, 89% vs. 50% at 15°C, and 82% vs. 25% at 30°C) and by De Lillis & al. (2004) for Latium (60% with a longer T_1 at 22/15°C with a 14/10h photoperiod).

Our results, however, highlighted the ability of *P. maritimum* seeds to germinate in a wide range of temperatures (5-30°C) both in the dark (53-98%) and in the light (7-98%). Moreover, we observed a strong photoinhibition of seed germination exclusively at 5°C, with seeds weakly photoinhibited at T > 20°C, in accordance with Carta & al. (2017), and with no inhibition at 10-15°C.

20. *Silene canescens* Ten. (*Caryophyllaceae*) (Fig. 1f)

Accession data

It: Latium. Montalto di Castro (Viterbo), Montalto Marina (WGS84: 42.321758°N, 11.585935°E), duna costiera, 3 m a.s.l., 12 May 2016, *S. Magrini & D. Barreca* (BGT-A-36516, Tuscia Germplasm Bank).

Germination data

Pre-treatments: sterilization with a solution of 5% sodium hypochlorite + Tween 20 for 5 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 1% agar.

Sample size: 100 seeds for each test (20 × 5 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T_1 [d]	T_{50} [d]	T_{max} [d]	MTG [d]
100%	constant 20°C	12/12h	1.3	1.3	6.0	2.4
98.3%	constant 20°C	0/24h	1.0	1.3	11.3	2.3
98.3%	constant 25°C	0/24h	1.0	0.5	5.3	1.4
96.7%	constant 25°C	12/12h	1.0	0.7	3.3	1.4
95.0%	constant 15°C	12/12h	1.0	1.4	16.3	2.7
93.3%	constant 15°C	0/24h	1.0	0.7	9.3	1.7
91.7%	constant 10°C	0/24h	6.0	5.6	15.0	6.7
90.2%	constant 30°C	0/24h	1.0	0.5	3.0	1.1
90.0%	constant 30°C	12/12h	1.0	0.6	10.3	1.9

Observations

Silene canescens (= *S. colorata* Poir.) is an annual Mediterranean species of foredunes and fixed dunes, quite common along the Italian sandy coasts (Acosta & Ercole 2015). Here we report the first germination data for this species for Italy. Successful and fast ger-

mination (> 90%, T_{50} = 0.5-1.4) was obtained at a wide range of constant temperatures (15-30°C), in accordance with Royal Botanic Gardens Kew (2019), with germination percentages much higher than those reported for Portugal: 0% at 15-20°C and 28% at 25°C, under light condition (Marques & al. 2007). The lowest germination percentages were recorded at 5°C, where strong photoinhibition of seed germination was observed (Carta & al. 2017).

Fig. 1. Germinated seeds of: **a,** *Centaurea sphaerocephala* subsp. *sphaerocephala*; **b,** *Jacobaea maritima* subsp. *maritima*; **c,** *Matthiola sinuata*; **d,** *Medicago marina*; **e,** *Pancratium maritimum*; **f,** *Silene canescens*.

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