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## Seed germination protocols for three coastal dune plants in central Italy

### Abstract

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Here, successful germination protocols for the following three coastal dune species are presented: *Glaucium flavum*, *Helichrysum stoechas* and *Plantago coronopus*. It is the first report of germination data for these species in the Italian peninsula. Seeds were collected in coastal 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 constant temperatures (from 5 to 30°C), under both light (with a 12/12h photoperiod) and total darkness. Our results show a high germination ability and germination rate for all the tested species.

*Key words:* beach, Latium, psammophytes, Tuscany.

### Introduction

Mediterranean coastal dunes are dynamic environments, particularly vulnerable due to the strong impact of human activities and in need of conservation actions. The research focused on the assessment of germination requirements of dune species is carried out at the Tuscia Germplasm Bank where over 150 seed accessions of 35 species collected in the beaches and dunes along the Tyrrhenian coasts of central Italy are long-term preserved. Here we present successful germination protocols for some populations of the following species: *Glaucium flavum* Crantz, *Helichrysum stoechas* (L.) Moench, and *Plantago coronopus* L.

### 88. *Glaucium flavum* Crantz (*Papaveraceae*)

#### Accession data

**It:** Tuscany. Orbetello (Grosseto), Ansedonia (42.408195°N, 11.302421°E), beach, 1 m a.s.l., 21 Jul 2020, *S. Magrini* (BGT-A-67820, 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 <sub>i</sub> [d]	T <sub>50</sub> [d]	T <sub>max</sub> [d]	MTG [d]
98.3%	constant 10°C	0/24h	21.0	31.1	37.0	29.8
90.0%	constant 10°C	12/12h	30.0	39.4	40.0	39.2
80.0%	constant 15°C	12/12h	8.0	16.6	70.7	21.9

### Observations

*Glaucium flavum* is a short-lived perennial native to Europe, Northern Africa, Macaronesia and temperate zones in Western Asia, growing mainly on the seashore along the Italian coasts (Acosta & Ercole 2015). Here we report the first germination data for this species in the Italian peninsula. Germination tests were carried out using six constant temperature regimes (5–30°C) under both light and dark conditions.

The results showed high germination percentages ( $\geq 80\%$ ) in the range between 10°C and 15°C, with the maximal response at 10°C in both full darkness and light (98.3% and 90.0%, respectively), showing the characteristic Mediterranean-type germination (Thanos 1989).

The germination decreases with increasing temperature, with very low germination percentages recorded at 25–30°C (2% in the light and 5% in darkness) and no germination at 5°C. This species showed a very strict temperature-dependent germination, as highlighted also for Sicilian populations (Walter & al. 2020). The germination was quite slow, the fastest at 15°C in the light ( $T_1 = 8$  days and  $T_{50} = 16.6$  days).

Seed germination under light conditions was just a little lower than in full darkness, indicating that the seeds of this population are not affected by the strong photoinhibition reported for this and other sand beach species, like *Cakile maritima* Scop., *Matthiola sinuata* (L.) W.T.Aiton, and *Malcolmia littorea* (L.) R.Br. (Thanos & al. 1989, 1991; De Vitis & al. 2014, 2018; Carta & al. 2017; Magrini & al. 2019).

### 89. *Helichrysum stoechas* (L.) Moench (*Asteraceae*)

#### Accession data

**It:** Tuscany. Grosseto (Grosseto), Principina a Mare (WGS84: 42.690539°N, 10.997792°E), back dune, 3 m a.s.l., 28 Jun 2020, *S. Magrini* (BGT-A-64720, 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 <sub>1</sub> [d]	T <sub>50</sub> [d]	T <sub>max</sub> [d]	MTG [d]
90.1%	constant 15°C	0/24h	2.0	4.9	16.0	6.3
87.9%	constant 20°C	0/24h	2.0	2.1	13.8	4.2
87.8%	constant 25°C	0/24h	2.0	2.0	12.6	4.0
85.0%	constant 10°C	0/24h	5.4	10.3	24.8	11.3
84.9%	constant 25°C	12/12h	2.0	4.5	27.6	7.9
84.5%	constant 15°C	12/12h	2.0	2.7	30.0	10.9

### Observations

*Helichrysum stoechas* is widely spread along the Mediterranean coasts, common in the dune sclerophyllous scrubs along the Italian coasts (Acosta & Ercole 2015). Here we report the first germination data for this species in central Italy. Germination tests were carried out using eight constant temperature regimes (5–40°C) under both light and dark conditions.

High germination percentages ( $\geq 85\%$ ) were recorded at 10–25°C in the dark, with slightly lower values at 15°C and 25°C under a 12/12h photoperiod. Lower percentages ( $< 63\%$ ) were recorded at  $T \geq 30^\circ\text{C}$ , especially at 35°C in darkness (33.3%) and at the lower temperatures, 5°C under both light and dark conditions (3.3 and 12.9%, respectively) and 10°C with a light/dark photoperiod (28.8%). No germination was recorded exclusively at 40°C. Seeds germinated within two days at 15–25°C, with the fastest germination recorded at 20°C and 25°C in darkness ( $T_{50} = 2.1$  and 2.0 days; Mean Germination Rate - MGR = 0.216 and 0.203 day<sup>-1</sup>, respectively). The slowest germinations were recorded at 5°C under both light and total darkness ( $T_{50} = 28.2$  and 25.9 days, MGR = 0.035 and 0.037 day<sup>-1</sup>, respectively).

Our results obtained at 15°C with a 12/12h photoperiod are in accordance with Royal Botanic Gardens Kew (2022) and with Salmeri & Brullo (2021) which reported a similar germination percentage ( $G = 83\%$ , even if after a longer period, 63 days instead of 30, and 81%, respectively) for Sicilian populations. On the other hand, the germination percentage reported by Salmeri & Brullo (2021), at the same temperature but with a 0/24h photoperiod, is lower than our results ( $G = 82\%$  vs. 90.1%, respectively).

Strong photoinhibition was recorded exclusively at 5–10°C (PISG index = 0.68 and 0.53; Carta & al. 2017), while germination percentages under light conditions were slightly lower than in full darkness at the other temperatures.

### 90. *Plantago coronopus* L. (Plantaginaceae)

#### Accession data

**It:** Latium. Tarquinia (Viterbo), Sant’Agostino (WGS84: 42.173181°N, 11.738444°E), back dune, 3 m a.s.l., 6 Sep 2021, *L. Zucconi* (BGT-A-87321, Tuscia Germplasm Bank).

### Germination data

*Pre-treatments:* sterilization with a solution of 5% sodium hypochlorite 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 <sub>1</sub> [d]	T <sub>50</sub> [d]	T <sub>max</sub> [d]	MTG [d]
100.0%	constant 20°C	12/12h	1.0	1.0	6.4	2.9
99.1%	constant 10°C	0/24h	4.0	3.9	14.8	5.5
99.0%	constant 15°C	12/12h	1.0	3.3	4.2	3.3
97.9%	constant 15°C	0/24h	1.6	3.4	5.6	3.6
96.9%	constant 20°C	0/24h	1.0	1.0	4.6	1.4
96.9%	constant 25°C	12/12h	1.0	1.0	2.2	1.1
96.9%	constant 25°C	0/24h	1.0	1.0	2.0	1.4
94.4%	constant 10°C	12/12h	3.4	4.8	16.2	6.4
92.4%	constant 30°C	12/12h	1.0	1.0	4.0	1.6
91.8%	constant 30°C	0/24h	1.0	1.0	16.0	2.2

### Observations

*Plantago coronopus* is widely spread along the Mediterranean coasts. It is common in the back dunes along the Italian shorelines (Acosta & Ercole 2015). Here we report the first germination data for this species in Italy. Germination tests were carried out using six constant temperature regimes (5–30°C) under both light and dark conditions.

*P. coronopus* seeds exhibit a dormancy period but dormancy is gradually broken as post-maturation time increases (Luciani & al. 2001). Following the results in Luciani & al. (2001), seeds with a long after-ripening time (402 days) were used, obtaining good results. Our results highlighted the ability of *P. coronopus* seeds to germinate in a wide range of temperatures (5–30°C) both in the dark (29–99%) and in the light (25–100%). High germination percentages (≥ 91.8%) were recorded at all the tested temperatures under both light and dark conditions, except at 5°C where germination percentages lower than 30% were recorded. Our results are higher than those reported by Royal Botanic Gardens Kew (2022) at 15°C and 20°C in the light (G = 99% vs. 90% and G = 100% vs. 90%, respectively). We are not in accordance with the statement by Luciani & al. (2001) that at higher temperatures the germination of *P. coronopus* tends to decrease, becoming rather scarce at 30°C, since higher percentages (> 90%) were recorded in our test even at such temperature.

*P. coronopus* showed fast germination in the range of 15–30°C (T<sub>1</sub> = 1 day and T<sub>50</sub> = 1–3.4 days) with total germination in 2 days at 25°C in the light. The fastest germination was recorded in the 20–30°C range under both light and dark conditions (T<sub>50</sub> = 1 day) and the slowest at 5°C under light and dark conditions (T<sub>50</sub> = 21–22 days). Species like these are

defined as “very fast germinating species” (Parsons 2012), because seeds germinate in a very short period after the substrate is wetted, allowing a rapid response to favourable environmental conditions that increase their colonization ability, particularly important for species growing in stressful habitats like beaches and coastal dunes (Luciani & al. 2001; Liu & al. 2013).

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