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Seed germination reports for coastal sand dune species from Sicily

Abstract

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This study investigated seed germination in nine psammophilous species occurring in Sicilian sand dunes, some of which, such as *Muscari gussonei*, *Launaea resedifolia* and *Pancreatium maritimum*, are endemic and/or rare and very scattered in the area. Different germination protocols were tested for one or more populations and the best germination results per species are provided, reporting experimental conditions and specific comments on the germination behaviour.

Key words: germination protocols, Mediterranean flora, psammophytes.

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

Accession data

- Si:** Gela (Caltanissetta), Contrada Roccazzelle (WGS84: 37.093372°N, 14.165187°E), 5 m a.s.l., 08 Jun 2010, *A. Lantieri* & *R. Galesi* (BGS-CT 32AL/NV/07, Banca del Germoplasma delle Specie Spontanee, Università di Catania).
- Si:** Sampieri (Ragusa), loc. Pineta (WGS84: 36.720606°N, 14.741661°E), 2 m a.s.l., 11 Jul 2011, *A. Lantieri* & *R. Galesi* (BGS-CT 01AL/RG/11, Banca del Germoplasma delle Specie Spontanee, Università di Catania).

Germination data

Pre-treatments: Manual removal of pappus. Disinfection with a 1% sodium hypochlorite (NaClO) water solution for 5 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 3 sheets of sterilized filter paper (Whatman 40), imbibed with 3 ml of sterilized distilled water.

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

Observations

Germination tests carried out at different constant temperatures (10°C to 25°C) indicated 20°C as optimal germination temperature, though significant interspecific variation was detected, with highly different germination rates between the two investigated populations. Samples from Roccazzelle, very close to the town of Gela, showed the highest germination

| Germination | Thermoperiod | Photoperiod [light/dark] | T ₁ [d] | T ₅₀ [d] | T _{max} [d] | MTG [d] | Accession code |
|-------------|---------------|-----------------------------|--------------------|---------------------|----------------------|---------|----------------------|
| 97.0% | constant 20°C | 12/12h | 2.0 | 3.4 | 22.0 | 5.0 | BGS-CT 32AL/NV/07 |
| 83.0% | constant 20°C | 12/12h | 2.0 | 4.3 | 31.0 | 7.9 | BGS-CT 01AL/RG/11 |

percentages, all exceeding 87% (88% at 10°C and 15°C, 97% at 20°C, 87% at 25°C), while seed germination values ranged from 57% (10°C) to 81% (25°C) and 83% (20°C) in the other accession from Sampieri beach. Increase in temperature enhanced the germination speed by over 70% in the first accession (T₅₀ = 6.8 at 10°C vs T₅₀ = 1.8 at 25°C) and over 55% in the second one (T₅₀ = 8.8 at 10°C vs T₅₀ = 4 at 25°C). Temperature also affected the germination rate out of the optimal level of 20°C, with lower values at both minor and major temperature for the two populations. Royal Botanic Gardens Kew (2019) reports for the species 82 % germination under 20°C and light 8/16 photoperiod.

3. *Cyperus capitatus* Vand. (*Cyperaceae*) (Fig. 1b)

Accession data

Si: Gela (Caltanissetta), Contrada Roccazzelle (WGS84: 37.093372°N, 14.165187°E), 5 m a.s.l., 08 Jun 2010, *A. Lantieri & R. Galesi* (BGS-CT 07AL/RG/10, Banca del Germoplasma delle Specie Spontanee, Università di Catania).

Germination data

Pre-treatments: Disinfection with a 1% sodium hypochlorite (NaClO) water solution for 5 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 3 sheets of sterilized filter paper (Whatman 40), imbibed with 3 ml of sterilized distilled water.

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

Observations

Based on Redondo-Gómez & al. (2011), *C. capitatus* seeds from SW Spain exhibit their greatest germination values at salinity levels between 0 and 1% under a 16 h light/8 h dark photoperiod and 25/15°C thermoperiod. Salt concentration > 1% in the substrate completely inhibited seed germination. Germination results, here firstly given for Sicilian plants, agree with these data since seeds achieved the highest germination percentage in distilled water, as well as in 0.1 M NaCl water solution though with much longer germination time. Conversely, increasing NaCl concentration (0.2M, 0.3M, 0.5M) in the germination medium strongly affected seed germination up to total inhibition (G = 0%) at the higher salinity conditions. Seed germination under light conditions was just a bit lower than in full darkness, indicating that seeds are not affected by photoinhibition and light

| Germination | Thermoperiod | Photoperiod [light/dark] | T ₁ [d] | T ₅₀ [d] | T _{max} [d] | MTG [d] |
|----------------------|--------------|-----------------------------|--------------------|---------------------|----------------------|---------|
| 100% | 25/15°C | 0/24h | 7.0 | 7.7 | 22.0 | 10.3 |
| 96.7% | 25/15°C | 12/12h | 13.0 | 15.3 | 28.0 | 16.7 |
| 96.7% ⁽¹⁾ | 25/15°C | 0/24h | 8.0 | 13.5 | 31.0 | 15.3 |
| 91.7% ⁽¹⁾ | 25/15°C | 12/12h | 16.0 | 25.9 | 35.0 | 27.2 |

⁽¹⁾ 0.1 M NaCl concentration in the germination medium.

only reduces the germination rate ($P_i < 0.1$, Carta & al. 2017). Alternating temperature also was a determinant factor for germination success, as germination tests carried out on this accession under constant temperatures (10°C, 15°C, 20°C, 25°C) showed very low germination values, ranging from 0% to 17%, except for 25°C with 57%. Constant temperatures appeared to increase photoinhibition since germination values in light-incubated seeds (12/12h) did not exceed 15%, with photoinhibition index (P_i) ranging between 0.7 and 1 that indicates strong inhibitory effects of light (Carta & al. 2017). Contrarily, Royal Botanic Gardens Kew (2019) reports 80% of germination at a constant temperature of 31°C and 12/12 h photoperiod, though only 20 seeds were tested.

4. *Launaea fragilis* (Asso) Pau (*Asteraceae*) (Fig. 1c)

Accession data

Si: Sampieri (Ragusa), loc. Pineta (WGS84: 36.720606°N, 14.741661°E), 2 m a.s.l., 11 Jul 2011, *A. Lantieri* & *R. Galesi* (BGS-CT 02AL/RG/11, Banca del Germoplasma delle Specie Spontanee, Università di Catania).

Germination data

Pre-treatments: Manual removal of pappus. Disinfection with a 1% sodium hypochlorite (NaClO) water solution for 5 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 3 sheets of sterilized filter paper (Whatman 40), imbibed with 3 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] |
|-------------|---------------|-----------------------------|--------------------|---------------------|----------------------|---------|
| 99.0% | constant 25°C | 12/12h | 2.0 | 3.6 | 15.0 | 4.9 |
| 98.0% | constant 15°C | 12/12h | 2.0 | 11.2 | 31.0 | 12.4 |

Observations

This species is a suffruticous chamaephyte with a Saharo-Sindian distribution area, which in Italy only occurs in the sand dunes of the southern and south-eastern Sicily, where it is rather rare and grows in the *Ammophila arenaria* mobile dune community. This is the first germination report for the species. Germination tests carried out on fresh seeds provided successful germination results also at 20°C (90%), while only 70% of germination was reached at 10°C.

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

Accession data

Si: Sampieri (Ragusa), loc. Pineta (WGS84: 36.720606°N, 14.741661°E), 2 m a.s.l., 11 Jul 2011, *A. Lantieri* & *R. Galesi* (BGS-CT 03AL/RG/11, Banca del Germoplasma delle Specie Spontanee, Università di Catania).

Germination data

Pre-treatments: Disinfection with a 1% sodium hypochlorite (NaClO) water solution for 5 minutes followed by 3 rinses in sterile distilled water. Mechanical scarification by sandpaper for 5 minutes. Imbibition in distilled water for 24 h.

Germination medium: 3 sheets of sterilized filter paper (Whatman 40) imbibed with 3 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] |
|-------------|---------------|-----------------------------|--------------------|---------------------|----------------------|---------|
| 98.0% | constant 10°C | 12/12h | 3.0 | 4.4 | 22.0 | 6.1 |
| 98.0% | constant 15°C | 12/12h | 2.0 | 2.7 | 22.0 | 4.4 |
| 98.0% | constant 20°C | 12/12h | 1.0 | 2.1 | 13.0 | 3.1 |

Observations

The examined Sicilian population of *Medicago marina* revealed an optimal temperature range of 10–20°C, which agree with Royal Botanic Gardens Kew (2019), where 100% germination was reported at both 15°C and 20°C with 12/12 photoperiod. Lower germination percentage (89%) was achieved at 25°C with the same pre-treatments. Seeds resulted positively photoblastic, because germination tests under full darkness condition provided a bit lower results, with seed germination percentages ranging from 87% to 90% at the same temperature regimes as the light-incubated seeds. Again, increase in temperature (25°C) led to lower seed germination percentage (50%), which is in contrast with the 98% germination obtained by Scippa & al. (2011) for plants from the Molise coastal dunes (Central-south Italy) under the same conditions. Ballesteros & al. (2015) reported a germination percentage of 95% for dark-incubated seeds at 20°C, but previously chemically scarified with sulfuric acid for 20 minutes.

6. *Muscari gussonei* (Parl.) Nyman (*Asparagaceae*) (Fig. 1e)

Accession data

Si: Gela (Caltanissetta), Contrada Roccazzelle (WGS84: 37.095833°N, 14.161388°E), 3 m a.s.l., 08 Jun 2010, *A. Lantieri* & *R. Galesi* (BGS-CT 06AL/RG/10, Banca del Germoplasma delle Specie Spontanee, Università di Catania).

Germination data

Pre-treatments: Disinfection with a 1% sodium hypochlorite (NaClO) water solution for 5 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 3 sheets of sterilized filter paper (Whatman 40) imbibed with 3 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] |
|-------------|---------------|-----------------------------|--------------------|---------------------|----------------------|---------|
| 100% | constant 10°C | 0/24h | 11.0 | 13.4 | 35.0 | 15.4 |
| 98.0% | constant 15°C | 12/12h | 12.0 | 13.7 | 36.0 | 14.9 |

Observations

Muscari gussonei is an Endangered endemic species (Orsenigo & al. 2018) which occurs in small fragmented populations along the south-western coast of Sicily. Seed germination resulted negatively affected by temperature since it decreased with increasing

temperature (84% at 20°C, 0% at 25°C). No statistically significant effect of light was instead detected among germination percentages, which were high and similar for both light- and dark-incubated seeds under 10°C (100%, in accordance with Royal Botanic Gardens Kew 2019) and 15°C (97%).

7. *Ononis variegata* L. (*Fabaceae*) (Fig. 1f)

Accession data

- Si:** Gela (Caltanissetta), Contrada Roccazzelle (WGS84: 37.093372°N, 14.165187°E), 5 m a.s.l., 23 Jul 2007, *A. Lantieri* & *R. Galesi* (BGS-CT 32AL/NV/07, Banca del Germoplasma delle Specie Spontanee, Università di Catania).
- Si:** Riserva Naturale Orientata Oasi del Simeto (Catania), foce del fiume Simeto (WGS84: 37.407266°N, 15.091696°E), 1 m a.s.l., 02 Jul 2010, *A. Lantieri* & *R. Galesi* (BGS-CT 11AL/RG/10, Banca del Germoplasma delle Specie Spontanee, Università di Catania).

Germination data

Pre-treatments: Disinfection with a 1% sodium hypochlorite (NaClO) water solution for 5 minutes followed by 3 rinses in sterile distilled water. Mechanical scarification by sandpaper for 5 minutes. Imbibition in distilled water for 24 h.

Germination medium: 3 sheets of sterilized filter paper (Whatman 40), imbibed with 3 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] |
|-------------|---------------|--------------------------|--------------------|---------------------|----------------------|---------|
| 100% | constant 10°C | 12/12h | 2.0 | 2.3 | 8.0 | 3.3 |
| 100% | constant 15°C | 12/12h | 1.0 | 2.1 | 7.0 | 3.2 |
| 99.0% | constant 20°C | 12/12h | 1.0 | 1.3 | 12.0 | 2.0 |

Observations

The species shows a physical dormancy linked to its hard, impermeable seed coat, which needs scarification pre-treatments. The examined Sicilian populations of *Ononis variegata* revealed a wide range of optimal germination temperature from 10°C to 25°C, with maximum germination percentages very close to 100%. These results to some extent agree with existing data from Royal Botanic Gardens Kew (2019). Germination tests carried out under 24h dark photoperiod provided germination rates similar to those of light-incubated seeds

(98-100%), but with faster germination speed ($T_{50} = 1-1.1$ d, MTG = 2.1-2.2 d). The population from the coastal area of Simeto River showed a very similar germination behaviour, i.e. 97-99% under 12/12h photoperiod and 97-100% under 24h dark photoperiod, at constant temperature regimes from 10°C to 25°C, but germination speed was much faster with lower values of T_{50} and MTG.

8. *Pancratium maritimum* L. (Amaryllidaceae) (Fig. 1g)

Accession data

- Si:** Marsala (Trapani), Isola Grande dello Stagnone (WGS84: 37.903868°N, 12.455125°E), 1 m a.s.l., 02 Oct 2004, *S. Pasta & L. Scuderi* (BGS-CT 087G5/04, Banca del Germoplasma delle Specie Spontanee, Università di Catania).
- Si:** Gela (Caltanissetta), Spiaggia di Macchitella (WGS84: 37.076439°N, 14.210627°E), 6 m a.s.l., 26 Sept 2004, *Lantieri, Sciandrello & Visalli* (BGS-CT 052G1/04, Banca del Germoplasma delle Specie Spontanee, Università di Catania).
- Si:** Riserva Naturale Orientata Oasi del Simeto (Catania), foce del fiume Simeto (WGS84: 37.407266°N, 15.091696°E), 28 Sept 2005, *Restuccia & Visalli* (BGS-CT 060AR/NV/05, Banca del Germoplasma delle Specie Spontanee, Università di Catania).

Germination data

Pre-treatments: Disinfection with a 1% sodium hypochlorite (NaClO) water solution for 5 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 3 sheets of sterilized filter paper (Whatman 40), imbibed with 3 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] | Accession code |
|-------------|---------------|--------------------------|-----------|--------------|---------------|---------|--------------------|
| 98.0% | constant 20°C | 12/12h | 1.0 | 6.7 | 14.0 | 7.2 | BGS-CT 087G5/04 |
| 98.0% | constant 20°C | 12/12h | 5.0 | 10.0 | 25.0 | 11.0 | BGS-CT 052G1/04 |
| 98.0% | constant 20°C | 12/12h | 3.0 | 7.7 | 18.0 | 9.0 | BGS-CT 060AR/NV/05 |

Observations

Three different populations of *P. maritimum* from Sicily were investigated, coming from 3 opposite areas, W Sicily (Isola Grande), S Sicily (Gela) and E Sicily (Simeto river), which are characterized by the same mean annual temperature (around 17.5°C), but by dif-

ferent mean annual rainfall and relative humidity (448.6 mm/77.5%, 354.2 mm/75.6%, and 547.2 mm/69.9%, respectively). However, each accession had its optimal germination response (98%) at a constant temperature of 20°C with 12/12 photoperiod. High levels of germination, all exceeding 92%, were also obtained at other constant temperatures: 92-95% at 10°C, 93-96% at 15°C, 92-97% at 25°C. Increasing temperatures in general enhanced seed germination speed (T_{50} of c. 25d at 10°C, c. 17d at 15°C, and c. 10d at 15°C). Seeds from all populations resulted positively photoblastic, because germination tests under full darkness provided very low germination rates at each thermoperiod, not exceeding 50%, which is in contrast with germination results from Sardinian and Tuscan populations (Bacchetta & al. 2007; Balestri & Cinelli 2004).

9. *Plantago macrorrhiza* Poir. (*Plantaginaceae*) (Fig. 1h)

Accession data

Si: Sampieri (Ragusa), loc. Pineta (WGS84: 36.720606°N, 14.741661°E), 2 m a.s.l., 11 Jul 2011, *A. Lantieri* & *R. Galesi* (BGS-CT 04AL/RG/11, Banca del Germoplasma delle Specie Spontanee, Università di Catania).

Germination data

Pre-treatments: Disinfection with a 1% sodium hypochlorite (NaClO) water solution for 5 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 3 sheets of sterilized filter paper (Whatman 40), imbibed with 3 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] |
|-------------|---------------|--------------------------|-----------|--------------|---------------|---------|
| 100% | constant 10°C | 12/12h | 2.0 | 3.7 | 17.0 | 5.2 |
| 100% | constant 15°C | 12/12h | 1.0 | 2.3 | 9.0 | 2.9 |
| 100% | constant 20°C | 12/12h | 1.0 | 2.3 | 6.0 | 2.6 |

Observations

The species shows a moderate sensitivity to temperature and salinity. Temperature regimes over 25°C tended to induce seed dormancy, with delay in both germination time and final germination percentage (94% at 25°C, 50% at 30°C); a 50% germination is also reported at 25°C and 12/12 h photoperiod by Royal Botanic Gardens Kew (2019). These results are in accordance with those given by Luciani & al. (2001), who studied germina-

tion behaviour of this species related to temperature, salinity and after-ripening time, assessing an optimal germinability (98%) at 15°C (full darkness and distilled water), and a seed dormancy induction with both rising temperature (> 20°C) and salinity (> 0.1 M NaCl), but basically overcome by longer after-ripening time.

10. *Thinopyrum junceum* (L.) Á. Löve (*Poaceae*) (Fig. 1i)

Accession data

Si: Riserva Naturale Orientata Oasi del Simeto (Catania), foce del fiume Simeto (WGS84: 37.407266°N, 15.091696°E), 2 m a.s.l., 09 Aug 2010, *A. Lantieri & R. Galesi* (BGS-CT 07AL/RG/10, Banca del Germoplasma delle Specie Spontanee, Università di Catania).

Germination data

Pre-treatments: Disinfection with a 1% sodium hypochlorite (NaClO) water solution for 5 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 3 sheets of sterilized filter paper (Whatman 40), imbibed with 3ml 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] |
|-------------|--------------|-----------------------------|--------------------|---------------------|----------------------|---------|
| 78.3% | 25/15°C | 12/12h | 6.0 | 7.9 | 23.0 | 9.8 |
| 73.3% | 25/15°C | 0/24h | 6.0 | 4.9 | 16.0 | 6.8 |

Observations

Results of germination tests less than 80% were due to the persistency of embedded seeds which did not show coat rupture and radicle protrusion. Since these seeds included a vital embryo, mechanical strengths by the pericarp are plausible (Mavroeiidi 2015). Tests performed with the addition of NaCl water solutions to the germination medium revealed that seed germination of Sicilian plants is negatively affected by salinity. In fact, while low NaCl concentration (0.1 M) did not alter significantly the germination rate ($G = 72-75\%$), increasing salt quantities in the germination medium progressively delayed germination, increased MTG and reduced final germination (65% with 0.2 M NaCl, 52% with 0.3 M, and 30% with 0.5M). Light had no significant effects on final germination; tests carried out under 12/12h photoperiod provided comparable results to full darkness (from 78.3% without salt to 72% with 0.1 M NaCl or less with higher salt concentrations). Ballesteros & al. (2015) reported about 90% of germination with a 12/12 h photoperiod and 20°C constant



Fig. 1. Germinating seeds of: **a**, *Centaurea sphaerocephala*; **b**, *Cyperus capitatus*; **c**, *Launaea fragilis*; **d**, *Medicago marina*; **e**, *Muscari gussonei*; **f**, *Ononis variegata*; **g**, *Pancratium maritimum*; **h**, *Plantago macrorrhiza*; **i**, *Thinopyron junceum*.

temperature. However, our tests carried out in the same condition provided a final germination percentage of 53%, while it reached 67% under full darkness. Other constant temperatures (15°C and 25°C) yielded germination percentages not higher than 60%, with lower values in the presence of light. Mavroeidi (2015) obtained high germination values in plants from Crete at constant temperature (94.7% at 10°C, 100% at 15°C and 98.7 at 20°C) under full darkness; in these experiments, however, seeds were previously scarified (dispersal unit removed) and this clearly produced their higher and faster germination, because germination at 15°C decreased to 69% in seeds with pericarp.

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