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# Seed germination reports for Policy species in the central Apennines

**Abstract**

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The germination ability of four policy species from the Central Apennine were studied: *Astragalus aquilanus*, *Iris marsica*, *Jacobaea vulgaris* subsp. *gotlandica*, *Klasea lycopifolia*. Mature seeds were collected in the field, within an elevation range of 365–1932 m a.s.l. Different experimental conditions were tested and the best methods with optimal germination results are provided and compared for each species. First germination records are given for the investigated taxa.

*Key words*: Habitat Directive, *Astragalus aquilanus, Iris marsica, Jacobaea vulgaris* subsp. *gotlandica, Klasea lycopifolia.*

## Introduction

The studied species are target species of the LIFE 15 NAT/IT/946 Project "FLORANET Safeguard and valorization of the plant species of EU interest in the Natural Parks of the Abruzzo Apennine", relating to action C.4 "Reproduction from seed", which is focused on Directive plant species occurring in the Abruzzo protected areas such as *Adonis distorta* Ten., *Androsace mathildae* Levier, *Astragalus aquilanus* Anzal., *Cypripedium calceolus* L., *Iris marsica* I. Ricci & Colas., *Jacobaea vulgaris* subsp. *gotlandica* (Neuman) B. Nord., and *Klasea lycopifolia* (Vill.) Á. Löve & D. Löve. The work was carried out in the Maiella Seed Bank – Lama dei Peligni (CH), Italy.

The germination ecology of these target species had never been investigated so far. The experimental design was set up starting from the available literature data and germination protocols already defined for allied taxonomic units (Ellis & al. 1985; Royal Botanic Gardens Kew 2021), to identify the optimal temperature ranges and any required pre-treatments (ISTA 2012; Baskin & Baskin 2014).

Currently, fulfilling results on germination have been obtained only for 4 out of the 7 species investigated during the LIFE project. *Adonis distorta* showed a deep morpho-physiological dormancy which resulted in low final germination percentages at the conditions tested so far. Similar results occurred in *Androsace mathildae*, which revealed physiological seed dormancy and final germination percentages not exceeding 50%. Studies on the asymbiotic germination of *Cypripedium calceolus* have been carried out in a specialized laboratory for orchid

germination at the Tuscia Germplasm Bank (Tuscia University). Unfortunately, the number of germinated seeds was not enough to develop a germination protocol, only the seeds from immature capsules have geminated. Since there are no literature data on the germination behaviour and propagation of these species, to date the activity is to be considered experimental.

**50. *Astragalus aquilanus*** Anzal. (*Fabaceae*) (Fig. 1a)

**Accession data**

**It:** Abruzzo. Casoli (Chieti), loc. Piano la Roma (WGS84: 42.137766°N, 14.258008°E), pascolo xerofitico, 365 m a.s.l., 27 Jul 2016, *V. Di Cecco* (MSB LIFE16A03, Maiella Seed Bank).

**It:** Abruzzo. L'Aquila (L'Aquila), loc. Monteluco di Roio (WGS84: 42.339791°N, 13.374163°E), bordo strada in pascolo xerofitico, 984 m a.s.l., 16 Sep 2018, *V. Di Cecco* (MSB LIFE18A22, Maiella Seed Bank).

**It:** Abruzzo. Gioia dei Marsi (L'Aquila), loc. Casali d'Aschi (WGS84: 41.963614°N, 13.702078°E), bordo strada sterrata in pascolo xerofitico, 950 m a.s.l., 29 Jul 2020, *L. Vitale & E. Trella* (MSB LIFE20A01, Maiella Seed Bank).

**Germination data**

*Pre-treatments*: scarification with 1) 180 grit abrasive paper for 1 minute; 2) 98% sulfuric acid for 20 min.; 3) seed piercing with a small hypodermic needle. Soaking in water for 24h, sterilization with a solution of 3% sodium hypochlorite plus Tween 20 for 1 minute followed by 3 rinses in sterile distilled water.

*Germination medium*: 1% agar, pH 5.75.

*Sample size*: 80 seeds for each test (20 × 4 replicates).

| Germination | Thermoperiod | Photoperiod [light/dark] | $T_1$ [d] | $T_{50}$ [d] | $T_{max}$ [d] | MTG [d] | Accession code |
|---|---|---|---|---|---|---|---|
| **100%**[1] | constant 20°C | 12/12h | 1.8 | 4.7 | 11.8 | 5.3 | 19A02 |
| **96.0%**[2] | constant 20°C | 12/12h | 2.0 | 2.6 | 6.8 | 3.6 | 18A22 |
| **98.8%**[3] | constant 20°C | 12/12h | 2.0 | 5.0 | 12.0 | 6.2 | 20A01 |

**Observations**

Physical dormancy (integument impermeability, PY) was detected in many species of *Astragalus* (Miklas & al. 1987; Patanè & Gresta 2006; Eisvand & al. 2006; Keshtkar & al. 2008; Salmeri & Castrogiovanni 2020). Ten different germination protocols were tested for *A. aquilanus* at the temperature of 20°C, both in full darkness and 12/12h photoperiod, with no seed scarification or after chemical scarification with $H_2SO_4$ for 5 and 20 minutes, mechanical scarification with 180 grit sandpaper, and seed piercing. The viability of the non-germinated seeds at the end of each test was estimated by the cutting test. No statistically significant differences among the investigated populations were detected for all the tested conditions.

*Astragalus aquilan*us also revealed a physical dormancy, as all scarification methods proved to be helpful in interrupting seed dormancy and reaching final germination percentages close to 100%. Conversely, only low germination values (23.75% in light at 20°C) were reached in a long time without seed scarification.

**51. *Iris marsica*** I. Ricci & Colas. (*Iridaceae*) (Fig. 1b)

**Accession data**

**It:** Abruzzo. Palena (Chieti), loc. Colle di Valle Caprara (WGS84: 41.926410°N, 14.084681°E), radura in faggeta, 1541 m a.s.l., 20 Jul 2016, *G. Ciaschetti & M. Di Cecco* (MSB LIFE16A11, Maiella Seed Bank).

**Germination data**

*Pre-treatments*: sterilization with a solution of 3% sodium hypochlorite plus Tween 20 for 5 minutes followed by 3 rinses in sterile distilled water. Vernalization at 5°C for 60 days.

*Germination medium*: 1% agar, pH 5.75.

*Sample size*: 80 seeds for each test (20 × 4 replicates).

| Germination | Thermoperiod | Photoperiod [light/dark] | $T_1$ [d] | $T_{50}$ [d] | $T_{max}$ [d] | MTG [d] |
|---|---|---|---|---|---|---|
| **82.9%** | constant 20°C | 12/12h | 14.8 | 26.3 | 43.8 | 28.1 |

**Observations**

Many *Iris* species have morphophysiological dormancy (Curtis & Brumback 1986; Xu & al. 2003; Diboll 2004), therefore germination tests on this species were carried out based on such information. The following protocols were applied: incubation at 20°C with a 12/12 h photoperiod after 60 days of vernalization, which provided the highest germination values, at 20°C under total darkness (10.5%), 20°C with 12/12h photoperiod (34.2%), 15° C with a 12/12 h photoperiod after 60 days of vernalization (55.0%), alternating temperature 20/10°C with 12/12h photoperiod and imbibition in 800 ppm $GA_3$ (34.7%), 20/10°C with 12/12h photoperiod (59.4%). Germination was generally quite slow and took place staggered over time (MTG from 28.1 to 80.5), but better results were obtained after a period of vernalization at 5°C.

**52. *Jacobaea vulgaris*** subsp. ***gotlandica*** (Neuman) B. Nord. (*Asteraceae*) (Fig. 1c)

**Accession data**

**It:** Abruzzo. Rocca di Mezzo (L'Aquila), loc. Colle del Nibbio (WGS84: 42.199106°N,

13.468466°E), pascolo in cresta, 1900 m a.s.l., 13 Sep 2018, *V. Di Cecco & L. Di Martino* (MSB LIFE18A17, Maiella Seed Bank).

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

*Germination medium*: 1% agar, pH 5.75.

*Sample size*: 80 seeds for each test (20 × 4 replicates).

| Germination | Thermoperiod | Photoperiod [light/dark] | $T_1$ [d] | $T_{50}$ [d] | $T_{max}$ [d] | MTG [d] |
|---|---|---|---|---|---|---|
| **94.9%** | constant 20°C | 12/12h | 1.5 | 2.5 | 5.0 | 3.3 |
| **80.8%** | constant 20°C | 0/24h | - | - | - | - |

**Observations**
Based on Van der Meijden & Van der Waals-Kooi (1979), *J. vulgaris* does not show any seed dormancy, thus no particular method for dormancy-breaking had to be applied to favour germination. Excellent results were obtained at 20°C with a 12/12h photoperiod. Germinations under full darkness were only detected at the end of the test. The other tests have shown a germination percentage of 77.7% at 5°C under total darkness and 60.6% at 20/10°C with a 12/12h photoperiod (60.6%).

**53. *Klasea lycopifolia*** (Vill.) Á. Löve & D. Löve (*Asteraceae*) (Fig. 1d)

**Accession data**

**It:** Abruzzo. Rocca di Mezzo (L'Aquila), loc. Altopiano delle Rocche (WGS84: 42.221142°N, 13.526929°E), prato sfalciato 1276 m a.s.l., 16 Aug 2018, *V. Di Cecco* (MSB LIFE18A10, Maiella Seed Bank).

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

*Germination medium*: 1% agar, pH 5.75.

*Sample size*: 80 seeds (20 × 4 replicates).

| Germination | Thermoperiod | Photoperiod [light/dark] | $T_1$ [d] | $T_{50}$ [d] | $T_{max}$ [d] | MTG [d] |
|---|---|---|---|---|---|---|
| **88.4%** | constant 20°C | 12/12h | 5.8 | 11.4 | 21.5 | 12.3 |

## Observations

There is no information about seed dormancy for this species. According to Finch-Savage & Leubner-Metzger (2006), in the *Asteraceae,* both physiological dormancy and lack of dormancy can occur. A recent work by Budisavljević & al. (2021) has given 62.6% of germination for *Klasea lycopifolia* at 23°C, after a cold stratification (5°C) for 12 weeks. We obtained the highest final germination percentage at 20°C and 12/12 h photoperiod without preventive stratification (88.4%). Tests were also performed at 20°C in full darkness (40.0%), with a 12/12 h photoperiod after a vernalization period of 60 days (72.5%), and at alternating temperature 20/10°C with a 12/12 h photoperiod, but with lower results (31.6%).

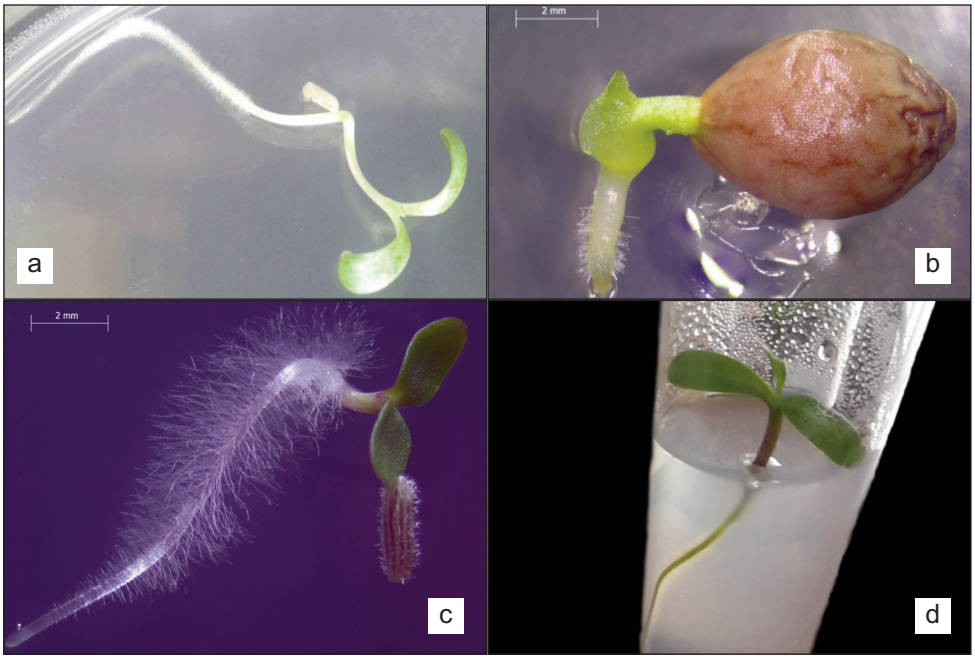


Fig. 4. Germinated seeds of: a, *Astragalus aquilanus*; b, *Iris marsica*; c, *Jacobaea vulgaris* subsp. *gotlandica*; d, *Klasea lycopifolia*.

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