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## Seed germination reports of four rare species of the Western Alps

### Abstract

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This study describes germination protocols of four Western Alps endemics. The species studied are: *Berardia lanuginosa*, *Potentilla valderia*, *Silene cordifolia*, and *Tephrosieris balbisiana*. Seeds were collected in the Maritime Alps (Cuneo, Italy) and conserved for a few days in the herbarium chamber or in the drying room of the seed bank before submitting to germination test. The germination tests were carried out in the Piedmont Germplasm Bank. Our results show a high germination response for all the analyzed species.

*Key words:* alpine species, *Berardia lanuginosa*, endemic, *Potentilla valderia*, *Silene cordifolia*, *Tephrosieris balbisiana*.

### Introduction

The Maritime and Ligurian Alps are one of the most important centres of flora diversification of the Mediterranean basin (Casazza & al. 2016). This part of the Alpine chain represented an important refuge for plants during the last glacial maximum, and this is the reason why it hosts a large number of endemic species.

This study investigated the germination response of *Berardia lanuginosa* (Lam.) Fiori & Paol., *Potentilla valderia* L., *Silene cordifolia* All., and *Tephrosieris balbisiana* (DC.) Holub. These endemic species show a distribution limited to the Western Alps, with the only exception of *T. balbisiana* which has a disjoint distribution with a few populations on the Northern Apennines.

Seeds were collected in the field at dispersal and immediately transferred to the Piedmont Germplasm Bank (Centro Regionale per la Biodiversità Vegetale, Chiusa di Pesio, CN) where germination tests were carried out. Our results showed high germination percentages in all the species studied, which are presently conserved at the germplasm bank.

**24. *Berardia lanuginosa*** (Lam.) Fiori & Paol. (*Asteraceae*) (Fig. 1)

### Accession data

**It:** Argentera (Cuneo), loc. Gias Colombart, Vallone di Colombart (WGS84: 44.360433°N, 6.916624°E), calcareous screes, 2350 m a.s.l., 28 Sept 2013, *V. Gosmar* (NA/13/1000, Piedmont Germplasm Bank).

**Germination data**

*Pre-treatments:* Manual detachment of the pappus from the seeds. Seed sterilization in a bleach solution (5% of chlorine) for 2 minutes followed by several rinses in sterilized distilled water. Weak scarification of the seed coat with a scalpel in sterile conditions.

*Germination medium:* agar 1%.

*Sample size:* 60 seeds (20 × 3 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T <sub>1</sub> [d]	T <sub>50</sub> [d]	T <sub>max</sub> [d]	MTG [d]
91.4%	constant 10°C	0/24h	9.0	12.4	32.0	17.2

**Observations**

*Berardia lanuginosa* is an endemic geophyte of the South-western Alps (Casazza & al. 2016; 2017) distributed along the boundary of the Italian and French border (Noble & Diadema 2011). The plant grows exclusively on high-altitude calcareous screes (*Berardietum lanuginosii*) (Corine biotopes 61.2322) with a major distribution on the

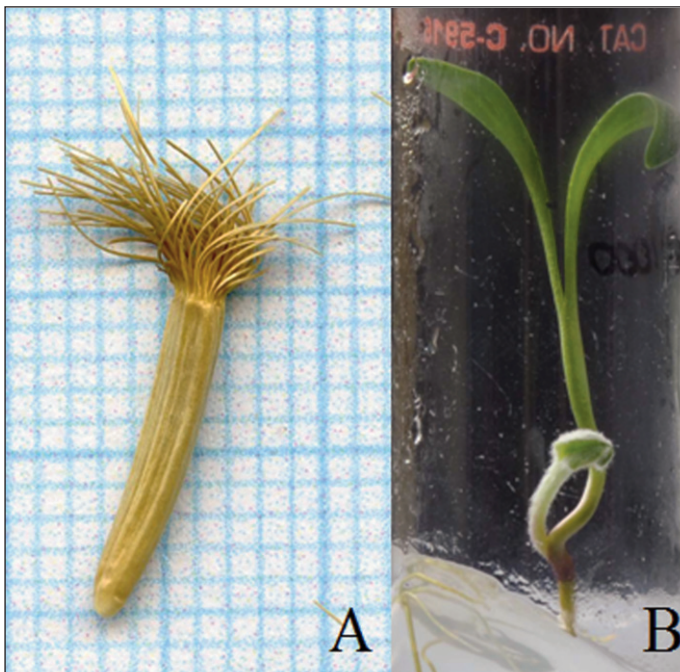


Fig. 1. An achene (A) and a seedling (B) of *Berardia lanuginosa* (photo by V. Gosmar/M. Mucciarelli, 2013).

Maritime, Cottian and Graian Alps and a secondary centre within the Ligurian Alps. The monospecific genus *Berardia* is a paleo-endemic of the alpine flora (Ozenda 2009).

Seeds used for this study were collected in the field at the end of September 2013 and stored for a few days at 15-20°C and 15-20% RH in the drying room of the Piedmont Germplasm Bank.

During the last 15 years, several tests have been carried out on this species in our seed bank, using not only fresh or dry seeds but also germplasm previously frozen at -20°C or conserved in the fridge at 4°C. Among the various methods adopted so far, the results presented in this study refer to the treatment with the highest final germination percentage (91%).

## 25. *Potentilla valderia* L. (*Rosaceae*) (Fig. 2)

### Accession data

**It:** Vinadio (Cuneo), loc. S. Anna di Vinadio (WGS84: 44.260131°N, 7.121159°E), acidophilous alpine grasslands, 1670 m a.s.l., 24 Aug 2017, *B. Gallino* (NA/17/1996, Piedmont Germplasm Bank).

### Germination data

*Pre-treatments:* Seed sterilization in a bleach solution (4.5% of chlorine) for 5 minutes followed by several rinses in sterilized distilled water. Cold stratification at 4°C for 60 days.

*Germination medium:* agar 1%.

*Sample size:* 100 seeds (50 × 2 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T <sub>1</sub> [d]	T <sub>50</sub> [d]	T <sub>max</sub> [d]	MTG [d]
82.0%	alternate 20/10°C	12/12h	7.0	4.7	17.0	8.9

### Observations

*Potentilla valderia* is a strict orophyte endemic to the South-western Alps (Argentera-Mercantour Massif) with distribution at the boundary of the Italian and French border (Noble & Diadema 2011). It is a thermophile and xerophile species which preferably develops on acidophilous alpine grasslands (*Festucion variae*, Corine biotopes 36.33; Biondi & al. 2010), which are typical for this species in the Maritime Alps (*Festuco-Potentilletum valderiae*) (Noble & Diadema 2011).

Seeds used for this study were collected in the field during summer 2017 and were conserved for a few days in the herbarium cold room (10°C and 30% RH).

*P. valderia* seed germination was optimal at 20/10°C reaching a value of 82% in the presence of a 12h light/12h dark photoperiod. No germination data are currently available via the RBG Kew's Seed Information Database (Royal Botanic Gardens Kew,

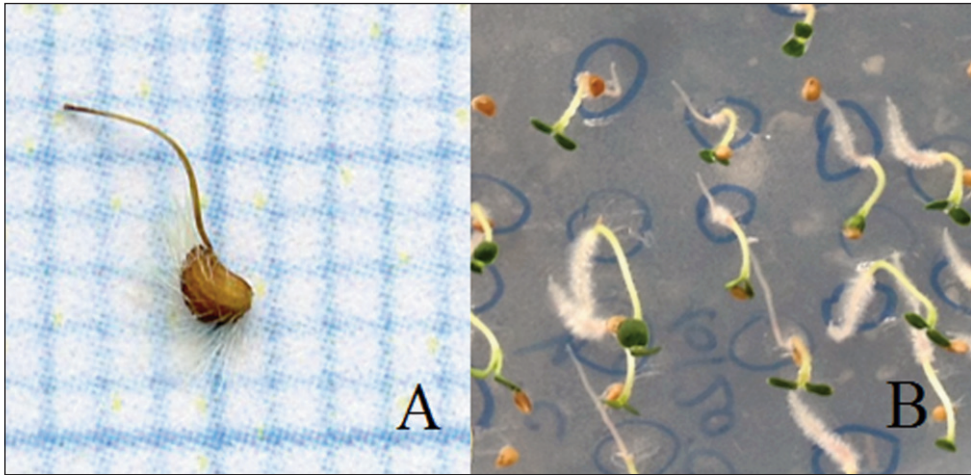


Fig. 2. An achene (A) and several seedlings (B) of *Potentilla valderia* growing on agar 1% (photo by V. Carasso, 2017).

2020) or in ENSCOBASE (2016). Therefore, as far as we know, this is the first report about the germination of this species.

**26. *Silene cordifolia* All. (Caryophyllaceae) (Fig. 3)**

**Accession data**

**It:** Vinadio (Cuneo), loc. Vallone S. Anna di Vinadio (WGS84: 44.260131°N, 7.121159°E), siliceous cliffs, 1670 m a.s.l., 24 Aug 2017, *B. Gallino* (NA/17/1992, Piedmont Germplasm Bank).

**Germination data**

*Pre-treatments:* Cold stratification at 4°C for 60 days.

*Germination medium:* 1% agar.

*Sample size:* 100 seeds (25 × 4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T <sub>1</sub> [d]	T <sub>50</sub> [d]	T <sub>max</sub> [d]	MTG [d]
97.0%	constant 15°C	0/24h	7.0	9.2	20.0	12.4

**Observations**

*Silene cordifolia* is an orophyte endemic to the Argentera-Mercantour Massif with distribution at the boundary of the Italian and French border (Noble & Diadema 2011). It is a tertiary

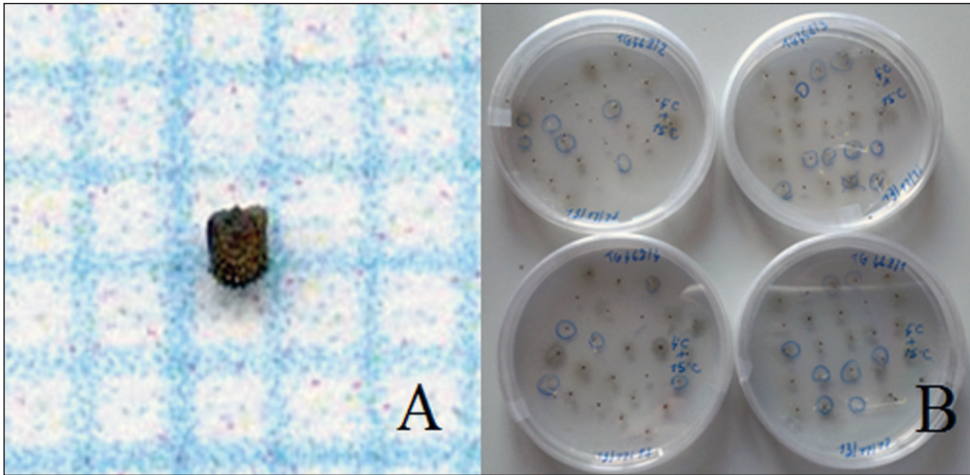


Fig. 3. A seed (A) and seedlings (B) of *Silene cordifolia* (photo by V. Carasso, 2017)

relict of the ancient flora that was present in this area before the onset of the Mediterranean climate regime and subsequent Quaternary glaciations. The plant grows in narrow crevices of the siliceous cliffs between 700 and 3000 m of altitude (Casazza & al. 2016).

The seeds used for this study were collected in the field during summer 2019 and were conserved for a few days in the herbarium cold room (10°C and 30% RH) of the Piedmont Germplasm Bank.

*S. cordifolia* seed germination was optimal (97%) at 15°C and light insensitive. No germination data are currently available via the RBG Kew's Seed Information Database (Royal Botanic Gardens Kew, 2020) or in ENSCOBASE (2016). Therefore, as far as we know, this is the first report about the germination of this species.

## 27. *Tephrosieris balbisiana* (DC.) Holub (*Asteraceae*) (Fig. 4)

### Accession data

**It:** Valdieri (Cuneo), loc. Pian della Casa, Vallone Gesso della Valletta (WGS84: 44.161267°N, 7.276504°E), meso-hygrophile tall herb communities, 1790 m a.s.l., 13 Jul 2017, B. Gallino & V. Carasso (NA/17/1931, Piedmont Germplasm Bank).

### Germination data

*Pre-treatments:* Manual detachment of the pappus from the seeds. Cold stratification at 4°C for 60 days. Manual scarification of the seed coat with a scalpel.

*Germination medium:* 1% agar.

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

Germination	Thermoperiod	Photoperiod [light/dark]	T <sub>1</sub> [d]	T <sub>50</sub> [d]	T <sub>max</sub> [d]	MTG [d]
98.0%	constant 25°C	12/12h	6.0	3.1	9.5	6.2
92.0%	constant 25°C	12/12h	5.0	6.9	24.0	10.2
89.0%	constant 25°C	0/24h	6.0	5.2	25.2	10.9
86.0%	constant 25°C	0/24h	8.7	17.0	52.7	24.3
85.0%	constant 25°C	0/24h	5.0	8.2	33.5	13.5

**Observations**

*Tephrosieris balbisiana* belongs to the alpine and subalpine meso-hygrophile tall herb communities at elevations as high as 2100 m a.s.l. The plant has a disjoint distribution with the main centre on the Mercantour-Argentera Massif at the boundary between the French Department of Alpes-Maritimes, where most of the populations lie and the Ligurian and Maritime Alps (Italy). Further ranges are the Cottian Alps north to Turin, the Ligurian



Fig. 4. Twenty days old seedlings of *T. balbisiana* incubated at 25°C and 12h light/12h dark photoperiod (photo by V. Carasso, 2017)

Apennines north to Genoa (Pignatti 1982; Bartolucci & al. 2018) and in Emilia Romagna (Bonafede & al. 2013).

Germination tests were carried out with seeds collected in the field during the summer 2017. Considering the high level of environmental humidity at the time of collecting, seeds were maintained ten days at 15-20°C and 15-20% (RH) in the drying room of the Piedmont Germplasm Bank. Our experimental design consisted of five different treatments to check the effects of light vs. dark incubation in combination with cold stratification and manual scarification.

Although the final germinations were >80% for all the treatments, germination values were slightly higher (98%) when all the pre-treatments were applied to 12h light incubation. To our knowledge, no reports are presently available in the literature on the germination requirements of this species.

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