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# Seed germination of the endemic *Armeria helodes* (*Plumbaginaceae*) in Italy

**Abstract**

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*Armeria helodes* is a narrow endemic species of Friuli Venezia Giulia (Italy), with a restricted and fragmented range of small populations in remnants of lowland alkaline fens (habitat present in Annex I of the EU Habitats Directive). It is listed as Critically Endangered in the IUCN Red List, nevertheless, best practices for germplasm conservation/germination are still not studied. At maturity, achenes are held inside chaffy bracts (calyx) and their effects on germination are unknown. Therefore, we performed a germination test comparison between fruits embedded in the calyx or free from it, also applying two dormancy-breaking pretreatments (i.e. $KNO_3$, GA3). Approximately 26% of the inspected fruits enclosed inside the calyx were empty or had a small size and coat darkening. The optimal germination rate (60%) was reached when chaffy calyx was removed either before the test or during storage. Dormancy-breaking pretreatments did not increase the germination capacity. Our results represent the first report on the Italian *A. helodes* germination ability.

*Key words*: endangered species, alkaline fens, germplasm conservation, Friuli Venezia Giulia.

## Introduction

*Armeria helodes* F.Martini & Poldini (*Plumbaginaceae*) is a geographically and ecologically isolated neo-endemic entity of the Italian peninsula, as it is exclusively found in a small fragmented range, in few populations located in the low plain of Friuli Venezia Giulia, growing in wetland habitats characterized by fresh marshy soil (Martini & Poldini 1987; Martini 1987; Conti & al. 2005; Arrigoni 2015; Bartolucci & al. 2018). Although all populations are located in protected areas, the number of mature individuals is declining because of soil drainage and nutrients enrichments due to surrounding agricultural activities. Therefore, *A. helodes* was recently listed as Critically Endangered on the national Red List (Foggi & Abeli 2010; Rossi & al. 2015). It is also included in Annex II of the Habitats

Directive (Council Directive 92/43/EEC) as a plant of community interest whose conservation requires the designation of Special Areas of Conservation (SAC). For successful *ex situ* conservation and restoration actions, detailed knowledge about the seed germination ability of this endangered fen species is of utmost importance. An appropriate germplasm collection in *Armeria* species is limited by the low seed production (from 3 to 50% of seeds collected from the calyxes analyzed), due to non-fertilized ovaries because of poor pollen provision (Baker & al. 1994; Tauleigne-Gomes & Lefèvre 2005; Mouga & al. 2021). Seed germination of *A. helodes* is also expected to undertake a "warm germination strategy" (Thompson & Grime 1983) and to possess non-deep physiological dormancy (Baskin & Baskin 2014), as reported for other species from base-rich fens (Fernández-Pascual 2016).

## Materials and methods

### *Accession data*

*Armeria helodes* F. Martini & Poldini (*Plumbaginaceae*)

**It:** Friuli Venezia Giulia. Talmassons (Udine), Risorgive di Flambro (WGS84: 45.917902N°, 13.080120°E) in the protected area Risorgive dello Stella (SAC: IT 3320026), alkaline fens, 21-22 m a.s.l., 30 Jun 2021, *M. Tomasella* (accession id AE_F_2021_1, ITA368 Banca del Germoplasma Autoctono Vegetale del F.V.G. (BaGAV) Germplasm Bank).

Fruits were collected in June 2021 from natural populations in the protected regional area of Risorgive di Flambro. The collected mature dry fruits of *A. helodes* (achenes held inside their papery chaff) were separated into two different sub-groups, one left inside and the other removed from the calyx. The test aimed at understanding whether germination in *A. helodes* could be influenced by the presence of the covering structure, as reported in the Seed Information Database (Royal Botanic Gardens Kew 2022) for *A. alpina* (DC.) Willd. During calyx removal operation, visual inspection has allowed us to determine a large proportion of dead diaspores (50% on average), as most of the calyxes were empty or presented achenes with distinguishable morphological alterations as small size or a dark-brown coat (Fig. 1). The abnormal fruits in this sub-group were then discarded since they could affect germination results. The two distinct sub-groups were then stored at 2°C at the BaGAV Germplasm Bank of Region Friuli Venezia Giulia for 5 months until the beginning of the germination test.

### *Germination test*

Seven different trials on the accession germination parameters were performed:
1) achenes stored for 5 months within the calyx,
2) achenes as the latter, with the calyx removed just before the germination test,
3) achenes stored for 5 months without calyx,
4) achenes stored for 5 months without calyx, pre-treated with 1,000 ppm GA3 for 24h,

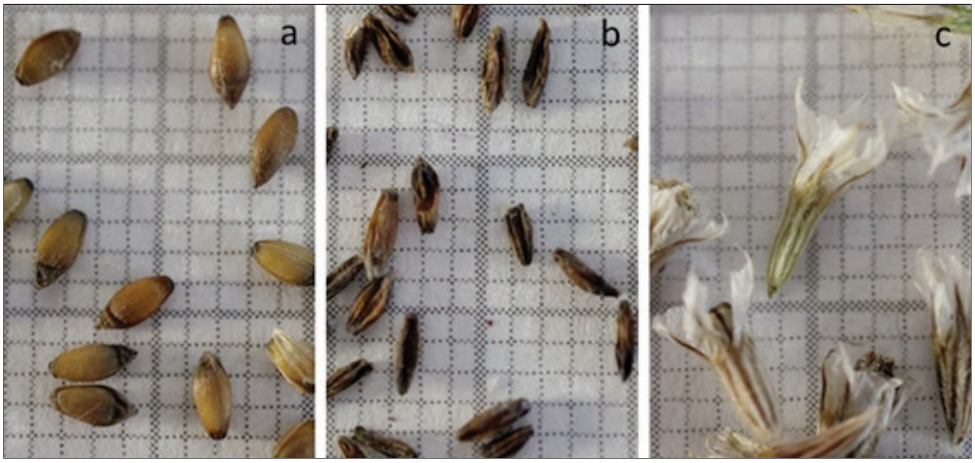


Fig. 1. Fruits of *Armeria helodes*: **a)** normal indehiscent single-seeded achenes; **b)** brown small-sized abnormal achenes not included in the germination test; **c)** chaffy calyx holding single-seeded achene. Pictures on 1 mm graph paper (M. Vuerich).

5) achenes stored for 5 months without calyx, pre-treated with 0.2% KNO3 for 24 h,
6) calyxes,
7) achenes stored for 5 months without calyx, both pre-treated with 2% $KNO_3$ for 24 h.

*Pre-treatment:* sterilization with a solution of 5% sodium hypochlorite for 10 minutes followed by 3 rinses in sterile ultrapure water.

*Germination data:* germination tests were carried out in parallel at the Department of Agricultural Food, Animal and Environmental Sciences, University of Udine and at the Centro Nazionale Carabinieri Biodiversità (CNCB – Peri, Verona) with an alternating temperature regime of 25/15°C and 12/12h of light/dark photoperiod for the first five trials and 20/30°C and 8/16h light/dark for the latter.

*Germination medium*: 0.8% agar (University of Udine) and sterile Whatman paper (CNCB - Peri) imbibed with 5 ml of sterile ultrapure water.

*Sample size*: 50 achenes or calyxes for each test (10 × 5 replicates) (University of Udine) and 100 achenes or calyxes (25 × 4 replicates) (CNCB – Peri).

**Results**

Here we report the first published germination reports for *A. helodes* in Italy (Table 1). Despite this endemic species has been already the object of conservation and some effective restoration actions by the LIFE project “FRIULI FENS” (LIFE06

NAT/IT/000060) carried out in the Special Areas of Conservation (SAC) present in the lower plan of Friuli Venezia Giulia (Mosanghini & al. 2022), no published reports on optimal germination potential of *A. helodes* are available to the best of our knowledge.

The results showed that the removal of the calyx was successful to increase the germination percentage in *A. helodes*, reaching 60 and 58% in fruits removed from the calyx before sowing or storage, respectively. Regarding the germination speed, the elimination of calyx before fruit storage or just before the germination test did not lead to major differences ($T_1$ = 1.4 days for both, $T_{50}$ = 2.50 and 2.58 days, and $T_{max}$ = 5.40 and 4 days, respectively). In contrast, the covering of chaffy structure negatively affected the final germination percentage (8.0%), with $T_1$ = 12.5 days, $T_{50}$ = 13 days and $T_{max}$ = 14 days, also due to a limited number of normal achenes found, suitable for reproducibility of germination test and restoration action occurring in the whole accession. It was observed that 26.0 ± 13.4 % of non-germinating fruits contained in the calyx were empty or non-fertilized ovaries and that the percentage of the viability of the total analyzed calyxes (germinated and alive non-germinated fruits inside calyxes) reached only 34.0 ± 13.4%.

Table 1. Results of the germination tests of *Armeria helodes* with calyx removal and different pre-treatments.

| **University of Udine** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Germination** | **Chaffy calyx removal** | **Pre-treatment** | **Thermoperiod** | **Photoperiod [light/dark]** | **$T_1$ [d]** | **$T_{50}$ [d]** | **$T_{max}$ [d]** | **MTG [d]** |
| **60.0%** | yes, before test | no | 15/25°C | 12/12h | 1.4 | 2.50 | 5.4 | 3.07 |
| **58.0%** | yes, before storage | no | 15/25°C | 12/12h | 1.4 | 2.58 | 4.0 | 2.95 |
| **42.0%** | yes, before storage | 1000 ppm GA3 | 15/25°C | 12/12h | 2.0 | 2.73 | 5.2 | 3.40 |
| **36.0%** | yes, before storage | 0.2% $KNO_3$ | 15/25°C | 12/12h | 1.8 | 2.34 | 3.8 | 2.88 |
| **8.0%** | no | no | 15/25°C | 12/12h | 12.5 | 13.00 | 14.0 | 13.33 |
| **CNCB – Peri** | | | | | | | | |
| **Germination** | **Chaffy calyx removal** | **Pre-treatment** | **Thermoperiod** | **Photoperiod [light/dark]** | **$T_1$ [d]** | **$T_{50}$ [d]** | **$T_{max}$ [d]** | **MTG [d]** |
| **60.0%** | yes, before storage | 2% $KNO_3$ | 20/30°C | 8/16h | 1.5 | 2.4 | - | 3.1 |
| **3.0%** | no | 2% $KNO_3$ | 20/30°C | 8/16h | 14.0 | - | 14.0 | 14.0 |

## Discussion

Regarding the influence of temperature regimes, *A. helodes* exhibited comparable seed germination traits under the two conditions tested in the different laboratories. This feature might suggest for *A. helodes* a "warm germination strategy" (Thompson & Grime 1983) already described for species of lowland alkaline fen grasslands, which germinate at warmer temperatures in autumn and subsequently acquire the ability to germinate towards colder temperatures in the short or long term (Fernández-Pascual & al. 2013; Fernández-Pascual 2016).

When comparing germination ability in the more closely related species, such as *A. alpina* (Martini & Poldini 1987; Pignatti & al. 2017), it could be observed that percentages ranged from 59 to 80% in light 8/16 at 15°C for *A. alpina,* where the removal of the covering structure from fruits increased the germination rate (Royal Botanic Gardens Kew 2022). For *A. maritima* and *A. alpina,* a dormancy-breaking pre-treatment with 2,000 ppm $KNO_3$ (Ellis & al. 1985) or 250 ppm GA3 (Mondoni & al. 2011) were also recommended. However, in our trial, fruit or calyx priming in 2% $KNO_3$ did not change the germination rate, whereas 1,000 ppm of GA3 or 0.2% $KNO_3$ even lowered the germination rate to 42 and 36%, respectively, suggesting that *A. helodes* seeds are not physiologically dormant.

The large proportion of dead or aborted seeds per total fruits could be ascribed to a specific reproductive vulnerability of *A. helodes* to unfavourable climatic (drought episodes or heat waves during the second half of June, Meteo FVG Report 2021) or habitat conditions during either pollination or seed ripening. This feature, associated with an intermediate level of germination capacity of seeds, could restrain a successful restoration program for this species.

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