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Seed yield and germination characteristics of wild accessions of *Arnica montana* L. from Trentino (Italy)





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ABSTRACT

Two *Arnica montana* L. wild accessions, collected from natural sites (Monte Bondone and Malga Juribello) of the province of Trento (Italy), were compared in an experimental field to investigate their seed yield and germination characteristics. From second to fourth year of cultivation the two accessions showed a mean thousand seed weight of 1.69, 1.54 and 1.40 g, a mean germination capacity of 91.6%, 81.4% and 81%, a mean germination time of 9.2, 9.3 and 8.8 days and a mean missing plants percentage of 9.4%, 9.4% and 12.5%, respectively. The seed yield of the accession from Malga Juribello was, with 10 gm^{-2} , higher than the accession from Monte Bondone (8.5 g m⁻²).

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1. Introduction

Arnica montana L. (Asteraceae) is an herbaceous perennial plant growing in mountainous meadows, pastures and heaths from South Norway and Latvia to South Portugal, North Apennines and South Carpathians (Tutin et al., 1976). In Italy, it is distributed on dry meadows and pastures of the Alps, always in acid soil, from 500 to 2200 m of altitude, and from Piedmont to Friuli-Venezia Giulia region (Pignatti, 1982).

Its flowers have been used for centuries to treat blunt injuries and rheumatic disorders, as tinctures, ointments or gels for external use (Merfort, 2010).

This species is subjected to protection measures in different European countries and in some of them the species is listed as vulnerable (Bosnia-Herzegovina, Croatia, Slovenia, Germany and Lithuania), critically endangered (Luxembourg), near threatened (Sweden) and extinct in the wild (Hungary) (Falniowski et al., 2011). Nevertheless most

http://dx.doi.org/10.1016/j.jarmap.2014.03.001 2214-7861/© 2014 Elsevier GmbH. All rights reserved. of arnica flowers commercialized are collected from Romanian and Spanish wild populations (Schmitt and Kathe, 2007).

Arnica cultivation is necessary because of the more stringent rules to protect the species in those countries where wild collecting has been practiced, of the labour cost and also of the increased demand from the industry. Different arnica cultivation trials have been carried out in various European countries such as Italy (Bezzi and Ghidini, 1989; Seeber et al., 1997), Germany (Weyel, 1989; Bomme et al., 1995a,b; Bomme, 1999), Switzerland (Delabays and Mange, 1991), Finland (Galambosi et al., 1998; Galambosi, 2004). "Arbo" (Jelitto, Schwarmstedt, Germany) is so far the only commercialized cultivar. Data about seed germination capacity and thousand seed weight (TSW) (Dachler and Pelzmann, 1999; Galambosi et al., 1998; Seed Information Database, 2013) can be found, while we found no data about seed vield and mean germination time (MGT). These data are important not only for cultivation of flowers and seed propagation, but in order to change the cultivation system in the next future from transplanting to direct sowing. The aim of this work was to investigate germination characteristics and seed

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yield of arnica wild accessions collected from two characteristic pasture areas of the province of Trento (Italy). Particularly the Monte Bondone is an harvesting site of hay which is used fresh for healthy treatments called "hay baths" and Malga Juribello is an important hut managed by the Trentino livestock breeders federation with both productive and educational purposes.

2. Materials and methods

Two wild accessions of arnica, collected at Monte Bondone (1543 m a.s.l., geographic coordinates: 46°00'41" N and 11°02′57″ E) and at Malga Juribello (1859 m a.s.l., geographic coordinates: 46°18′50″ N and 11°46′31″ E), both within province of Trento-Italy, were compared in a field trial at Mala of Sant'Orsola-Trento (990 m a.s.l.), oriented to south (151°), during the years 2009–2012. The climate of the zone is oceanic temperate (Eccel and Saibanti, 2005). Thermo-pluviometric data were taken from a weather station in S. Orsola (792 m a.s.l.), near the field trial, from 2000 to 2012 (only data available), that is: 5.8 °C the annual average minimum temperature, 15.3 °C the annual average maximum temperatures and 1088 mm the annual average rainfall. During the three years, temperatures were within or just slightly out of this range, while in 2010 rainfall was exceptionally high (Table 1) (Anon, 2013, http://meteo.iasma.it).

The physical and chemical soil characteristics of experimental plot are reported in Table 2.

The seeds collected in 2008 were sown (02/03/2009) in plastic boxes containing a substrate made of neutral sphagnum peat (Manna flor, Manna, Andriano-Italy) and sand (about 66% and 33% in volume, respectively) and kept in a heated greenhouse (6 °C as a minimum temperature). The plantlets were transplanted in 72-cell plastic trays filled with the same substrate on 8th April 2009. The trial was established on 04/06/2009 according to a completely randomized design with 4 replications. The basic plot (2 m²) was made of 2 rows of 2 m and the stand density was of 10 plants m⁻² (50 cm × 20 cm). Weed control was carried out manually. Plants were irrigated as needed and no fertilizer was used.

Each year, before to start the seeds harvest, the living plants were counted on each single plot, with the aim to calculate the missing plants percentage. The flower heads

| Table 2 | | | |
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| Physical and chemical analysis of the soil. |
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|---|

| Sand (%) | 68.3 |
|---|-------|
| Silt (%) | 27.7 |
| Clay (%) | 4.0 |
| pH (in H ₂ O) | 6.0 |
| Total CaCO ₃ (%) (gas-volum. meth.) | <0.5 |
| Active CaCO ₃ (%) (extrac. ammon. oxalate/titrat.) | <0.5 |
| Organic matter (%) (oxidat. bicromate/titrat.) | 5.2 |
| Total N (%) (Kjeldahl meth.) | 0.3 |
| P_2O_5 (ppm) (Olsen meth.) | 33.0 |
| K ₂ O (ppm) (extrac. in ammon. acet./ICP-OES) | 155.0 |
| | |

at the fruit stage (pappuses completely developed and with visible seeds) (Fig. 1) were harvested by hand 2 times at week, from the end of June and the first half of July in 2010, 2011 and 2012. In 2009 the plants did not bloom. After a first cleaning to remove the residues of the pappuses and other floral parts, seeds were cleaned by the use of sieves and a winnower set at a low air flow. Particularly we used a seed blower equipped with two glass tubes, two drawers (7.7 and 3.7 cm of diameter) where the seeds were put and a ring (range from 0 to 140) to adjust the air flow. We chose the larger drawer and we set the ring at 20 ca. to have a proper low air flow. Moreover we followed the same procedure on all seed lots of the accessions in the different years of harvest. Seeds were then kept in paper bags at 4 °C, until germination tests (Fig. 2).

The germination tests (four replications of 100 seeds each in 9 cm diameter plastic Petri dishes) were carried out in a thermostatic chamber provided with cool white fluorescent tubes (OSRAM TL40W/33) at constant temperature $(20 \pm 0.5 \,^{\circ}\text{C})$ and light $(8 \text{ h} \text{ at } 32 \,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1})$ photosynthetic photon-flux density) and for 30 days. It was chosen a constant temperature of 20 °C because this value is best for most plants and frequently adopted (ISTA, 2006). Similar temperatures were utilized also by Weyel (1989) (18-20 °C), Galambosi et al. (1998) (18-22 °C) and by SID's laboratory (15, 20 and 25 °C), but in this last case, agar was used as medium. Determination of TSW and germination tests started 3-4 months after seed harvest of the same year. Sterilized sand was chosen as the growing medium, because in this medium the seedlings with not developed radicles (radicles with just a brown apex after the collar) were much rarer compared to the filter paper. The substrate was moistened with demineralized water and Petri dishes were

Table 1

| Months/years January | 2010/11/12 – Min. temp. (°C) | | 2010/11/12 – Max. temp. (°C) | | 2010/11/12 – Rain (mm) | | | | |
|-------------------------|------------------------------|------|------------------------------|------|------------------------|------|--------|--------|--------|
| | -3.7 | -2.3 | -2.5 | 2.1 | 4.2 | 5.9 | 34.8 | 20.8 | 18.6 |
| February | -2.4 | -0.2 | -4.4 | 4.8 | 8.3 | 5.7 | 56.6 | 61.4 | 13.4 |
| March | 0.4 | 1.5 | 3.7 | 9.6 | 11.5 | 16.1 | 74.2 | 79.2 | 32 |
| April | 4.4 | 6.8 | 4.2 | 15.6 | 19.1 | 13.4 | 32 | 26.6 | 176 |
| May | 8.6 | 9.1 | 8.1 | 17.6 | 22.3 | 20 | 159.8 | 151 | 113.2 |
| June | 12.6 | 12.4 | 13.5 | 23.2 | 22.4 | 24.3 | 118.2 | 214.8 | 77.2 |
| July | 15.2 | 12.7 | 14 | 27.3 | 24.5 | 25.9 | 106.8 | 93.2 | 184.6 |
| August | 13.3 | 14.6 | 15.4 | 23.8 | 27.4 | 27.9 | 261.8 | 105.2 | 56.6 |
| September | 9.4 | 12.7 | 10.9 | 18.8 | 24.3 | 19.9 | 215 | 117.8 | 129.6 |
| October | 4.8 | 5.8 | 6.6 | 13 | 16.5 | 15.2 | 185.2 | 133 | 171.2 |
| November | 1.9 | 2.3 | 3.1 | 7.6 | 10.9 | 9.3 | 225.8 | 69.4 | 262.4 |
| December | -4.1 | -1 | -2.7 | 1.5 | 6.3 | 3.4 | 161 | 32.6 | 38.4 |
| Average | 5.0 | 6.2 | 5.8 | 13.7 | 16.5 | 15.6 | 1631.2 | 1105.0 | 1273.2 |

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