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# Morphology, germination, and storage behaviour in seeds of Tuscan populations of *Fritillaria montana* (Liliaceae), a rare perennial geophyte in Italy

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**Abstract:** *Fritillaria montana* Hoppe ex W.D.J.Koch (Liliaceae) is a rare perennial geophyte growing in south-eastern Europe. Although it occurs in most of the Italian territory, it is very rare and is included in the Red List of threatened plant species of most of the regions in which it grows; in Tuscany (central Italy), it is listed as Critically Endangered. The aim of our study was to investigate the seed size, weight, and morphology; the seed moisture and oil contents; the seed germination conditions; and the effect of different storage treatments on final germination success in *F. montana*. Seeds were collected from the only Tuscan population that set fruit in 2008, and they were then subjected to morphometric, moisture, and oil content measurements; germination tests; and different storage conditions. Morphometric measurements and shape index evidenced the flat and drop-like shape of the seeds, which have a moisture content of 14.62% and an oil content of 1.09%. They need a period of cold stratification to break dormancy and germinate, with a final percentage of 77.5%. The germination percentage after storage at 5 °C was 70%, while after storage in a drying room it was 55.2%. The differences between the final germination percentage of fresh seeds and treated seeds were only weakly significant. In the short time allotted for the trial, seeds of *F. montana* showed a good tolerance to drying and a potential predisposition to ex situ conservation techniques.

**Key words:** Ex situ conservation, dormancy, germination percentage, *Fritillaria*, seeds

## Introduction

The genus *Fritillaria* L. belongs to the family Liliaceae, subfamily Lilioideae, tribe Lilieae (Peruzzi et al., 2009). It includes 130 species (Rix, 2001) of bulbous perennial plants, distributed in all temperate regions of the northern hemisphere and adapted to many different environmental conditions, from Mediterranean areas to the mountains of northern Japan and Alaska. Despite the wide diffusion of this genus, information about its reproductive biology (Mancuso & Peruzzi, 2010) and seed characteristics is still partial, available in the literature for only a limited number of *Fritillaria* species. The Kew Gardens Seed Information Database (http://data.kew.org/sid/) is one of the most important data sources, reporting seed data for about 20 species of this genus. Unfortunately, germination data are not available for all species.

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*Fritillaria montana* Hoppe ex W.D.J.Koch is 1 of 4 species growing spontaneously in Italy. It was long confused with at least 5 different taxa (Kamari, 1991; Tomović et al., 2007; Bartolucci et al., 2009), like, for instance, *F. orientalis* Adams (Rix, 1980) or *F. tenella* M.Bieb. (Pignatti, 1982). The species is a nonclonal geophyte growing at 300-1800 m a.s.l., native to south-eastern Europe. The plant produces one or rarely more green-purplish, campanulate, and nodding flowers. Like many species of the genus, it is cultivated as an ornamental, such that it was sometimes intensively harvested (Marossy, 2007). Although it occurs in most of the Italian territory, it is very rare and is included in the Red List of threatened plant species of most of the regions in which it grows (Conti et al., 1997). In Tuscany, where *F. montana* is currently present in 7 extremely restricted populations with few reproductive individuals (Peruzzi et al., 2008), it is listed as Critically Endangered (Conti et al., 1997), although a proposal to downgrade it to Near Threatened was made by Peruzzi et al. (2008).

Liliaceae members produce seeds with linear, underdeveloped embryos (Martin, 1946; Baskin & Baskin, 2001). They never show physical dormancy (Baskin & Baskin, 2000), but rather morphological or morphophysiological dormancy, including the subtypes defined by Baskin and Baskin (2004), irrespective of the phylogenetic position within the clade (Kondo et al., 2006). Within the family, temperate species of the genera *Erythronium* L. and *Cardiocrinum* (Endl.) Lindl., and the alpine *Fritillaria tubiformis* Gren. & Godr. subsp. *moggridgei* (Boiss. & Reut. ex Planch.) Rix, germinate in the range of 5-15 °C (Kondo et al., 2002; Kondo et al., 2006; Carasso et al., 2011).

The purpose of this study was to sample seeds from each of these populations in Tuscany and to investigate their characteristics for the first time, in order to evaluate the possibility of planning ex situ management strategies. During the fruiting season in 2008, despite accurate searches, we could find fruits in just 1 of the 7 populations. This could indicate that the populations experience difficult reproduction, probably due to the sensitivity of the species to particular ecological conditions or disturbance factors in the locations we investigated (Peruzzi et al., 2008), and it raised the importance of this work for future conservational intents. Thus, the aim of our study was to investigate the seed size, weight, and morphology; the seed moisture and oil contents; the seed germination conditions; and the effect of different storage treatments on the final germination success in *F. montana*.

## Materials and methods

### Study species

*Fritillaria montana* is a perennial nonclonal bulbous geophyte belonging to the family Liliaceae. It is native to south-eastern Europe and grows on rocky slopes or in arid meadows between 300 and 1800 m a.s.l. (Pignatti, 1982). Plants show a stem, 16-40 cm tall, with linear opposite or alternate leaves and generally only 1 hermaphroditic flower with a campanulate, tessellate, green and purplish-brown perygonium (Pignatti, 1982; Bartolucci et al., 2009).

### Seed collection

All seeds used in this study were collected at the end of May 2008 from the *F. montana* population in Piazza al Serchio (province of Lucca; 44°11′N, 10°18′E) in north-western Tuscany, where it occupies a very small area of about 80 $m^2$ at the base of a basaltic rock in the proximity of the village, at an altitude of 520 m a.s.l. (Garbari & Betti, 2005; Peruzzi et al., 2008). We collected 9 capsules from 9 randomly selected individuals and allowed them to ripen in the laboratory for 10 days, exposed to the air. Only 9 fruits were collected from the 34 counted, to avoid compromising the reproductive season of the population. Seeds were subsequently manually extracted from dried fruits and counted.

### Seed morphometric measurements, fresh weight, and moisture and oil contents

After extraction, 50 seeds were randomly selected and the length, width, and thickness were individually measured to the nearest 0.01 mm using a manual calliper; the seed shape index (SI) was then calculated according to the methods of Thompson et al. (1993) as the variance of the 3 main dimensions after normalisation. To calculate the mean fresh weight, 5 lots of 5 seeds were randomly selected and weighed using an analytical balance with an accuracy of 0.01 mg. The same lots were subsequently reweighed with

the same balance after oven drying for 24 h at 105 °C to measure the dry weight, and again after 24 h at 130 °C to measure the ultra-dry weight. Moisture contents (MC) and oil contents (OC) were calculated with the following formulae:

% MC = (fresh weight – dry weight/fresh weight) × 100,

% OC = (dry weight – ultra-dry weight/dry weight) × 100.

**Seed germination tests**

Before germination tests, a cut test (Gosling, 2004) was performed on 20 randomly selected fresh seeds to calculate the percentage of viable seeds. This figure was then factored to correct the final germination percentages (Gosling, 2004). For germination tests, fresh seeds were placed on 1% (w/v) water agar in petri dishes of 60 mm in diameter. Tests were begun on 1 July 2008, and 50 seeds were randomly selected for each experimental condition and placed in 5 petri dishes (10 seeds per dish). Two tests were run without presowing treatments: 5 dishes (50 seeds) were incubated in the dark at 15 °C, and 5 dishes were wrapped in aluminium foil and then placed in the laboratory and exposed to the daily temperature fluctuations in July (Pisa, Italy): average temperature = 23.9 °C, average low = 17.7 °C, average high = 30.1 °C. In the third test, seeds placed on 1% (w/v) water agar in petri dishes were given a presowing treatment (60 days at 5 °C in a refrigerator) and were then transferred at 15 °C in the dark. During the germination period, observations of seeds were made in dim light for about 15 days at 2-day intervals and the emerged radicles were counted when they were ≥2 mm long.

**Effect of storage treatments on germination**

On 1 July 2008, at the same time as the beginning of the germination tests on fresh seeds, the remaining seeds were divided into 2 lots and exposed to different storage treatments. The first lot of seeds was stored at 5 °C in a refrigerator, and the second was stored in a controlled drying room at 15 °C and 15% relative humidity. After 5 months, based on the results of the germination tests conducted with fresh seeds, we randomly selected 50 seeds for each of the 2 storage treatments, placed them on 1% (w/v) water agar in petri dishes (10 seeds for each 60-mm-diameter petri dish), and tested them for germination with the presowing treatment: 60 days of cold stratification at 5 °C and then 15 days at 15 °C. Germination testing started on 2 February 2009, and observations of seeds were made following the same criteria as described previously.

**Data analysis**

All numerical data, including seed size, percentages of germination, and moisture and oil content, were calculated as the mean ± standard error of the mean (SEM), while the correlation of seed dimensions was calculated using the Pearson correlation coefficient and assuming a normal distribution for continuous variables with N = 50. To assess the effect of different presowing and storage treatments on final germination values, we used Student's t-test (df = 8) and the chi-squared test (2 × 3 contingency table, df = 2) based on the total number of germinated seeds per treatment; nonviable seeds, as estimated by a cut test (see above), were excluded from the calculations. All statistical tests were performed with R software (R Development Core Team, 2007).

## Results

**Seed morphometric measurements, fresh weight, and moisture and oil contents**

Seeds of *Fritillaria montana*, flat and drop-like (Figure 1), had a length of 5.67 ± 0.10 mm, width of 4.47 ± 0.08 mm, and thickness of 0.31 ± 0.01 mm. Length and width were positively correlated ($P < 0.01$, 2-tailed; Pearson coefficient = 0.837), while there was no significant correlation between these 2 dimensions and thickness. The seed had a shape index of 0.247.

Figure 1. Seeds of *Fritillaria montana*. Bar = 1 mm.

The mean fresh weight for a lot of seeds was 10.50 ± 0.17 mg, corresponding to 2.1 ± 0.03 mg for single seeds. Moisture content was 14.63 ± 0.28%, while oil content was 1.09 ± 0.16%. Weight loss after drying at 105 °C (1.54 ± 0.04 mg) and drying at 130 °C (0.11 ± 0.02 mg) was comparable among the 5 lots of seeds (Figure 2).

**Seed germination tests**

The cut test showed that 4 out of 20 seeds, or 20%, were not viable. In both germination tests without presowing treatments (the first at 15 °C, the other subjected to the daily temperature fluctuation as specified in Materials and Methods), no seed germinated after 30 days, while the germination of seeds subjected to cold stratification reached 77.50 ± 12.75% after the stratification period. The difference was statistically significant (Student's t-test, df = 8, $P < 0.001$). Moreover, we observed that most seeds (67.50 ± 17.05%) germinated during the 2-month stratification at 5 °C.

**Effect of storage treatments on germination**

Seeds from the lot stored in a refrigerator at 5 °C showed a germination percentage of 70.00 ± 9.35%, while seeds from the lot stored in a drying room at 15 °C and 15% relative humidity had 52.20 ± 7.29% germination; both germination tests were run after a 2-month cold stratification (Figure 3). Similar to fresh seeds, albeit in different percentages, a few roots emerged during the stratification: 20.00 ± 7.50% in dried seeds and 2.50 ± 2.50% in refrigerator-stored seeds. Transfer to the temperature regime set for germination effectively promoted root emergence. The differences in final germination among fresh, dried, and refrigerator-stored seeds were weakly significant (chi-square = 5.925, df = 2, P = 0.0516).

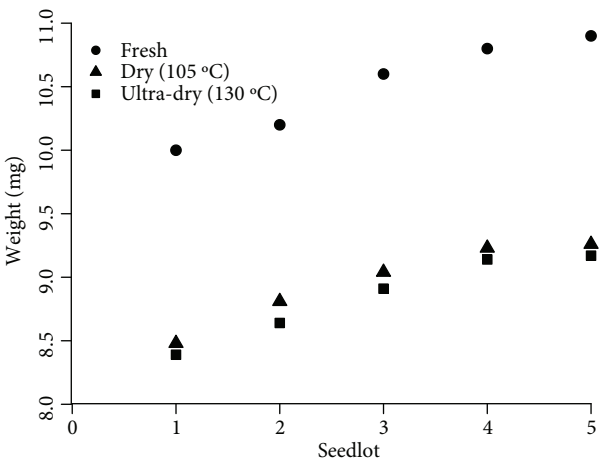


Figure 2. Weight of the same 5 seed lots of fresh (circles), dry (+24 h at 105 °C, squares), and ultra-dry (+24 h at 130 °C, triangles) seeds. Each seed lot was composed of 5 seeds

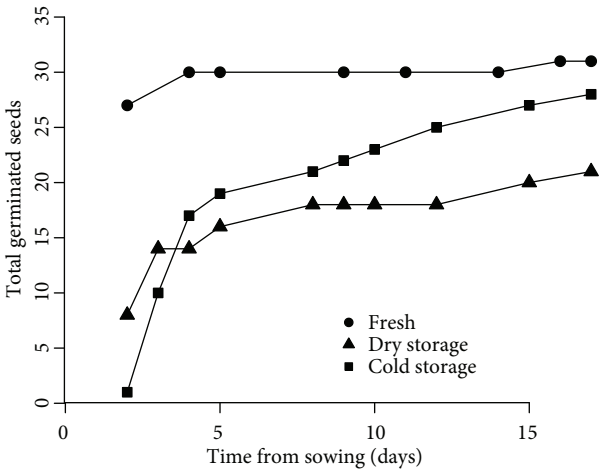


Figure 3. Germination percentages per day of observation at 15 °C in the dark after 60 days of cold stratification. Circles = fresh seeds, squares = dried seeds (5 months at 15 °C and 15% relative humidity), triangles = refrigerator-stored seeds (5 months at 5 °C).

## Discussion

Seed size and shape can be related to the rate of burial and can influence the formation of a persistent seed bank in the soil (Thompson et al., 1993, 1998, 2001; Funes et al., 1999), although their importance during the burial process is not unanimously accepted (Leishman & Westoby, 1998; Moles et al., 2000). The method proposed by Thompson et al. (1993) was also tested to distinguish species in the Italian flora contributing to the long-term soil seed bank from those of the short-term and transient seed bank (Cerabolini et al., 2003). Seeds of *F. montana*, which have a flattened shape (SI = 0.247) and a mean weight of 2.1 mg, comparable to that of most congeneric species (http://data.kew.org/sid/), would be short-persistent or transient in the soil seed bank. Although persistence in soil has still not been investigated in

this species, the prediction is supported by data about the related species *F. meleagris*, which indeed forms a short-term seed bank in the soil (Thompson et al., 1997).

Seed oil content is representative of the common presence of an oil endosperm in the family Liliaceae (Tamura, 1998). Although this peculiarity might indicate that it produces seeds of the intermediate type, as suggested for seeds of *F. meleagris* (http://data.kew.org/sid/), *F. montana* exhibits orthodox storage behaviour, at least under the tested conditions. As stated in Materials and Methods, the low production of seeds in situ limited the number of seeds available for germination tests; it would be interesting to run the tests with more numerous samples to check whether the effect of the treatments remained statistically weak or not. Results of the germination tests clearly showed that the seeds need a period of cold stratification to break dormancy, and this is supported by the temperature to which the species is subjected in natural conditions after seed dispersal, i.e. a relatively warm summer followed by a cool autumn and cold winter in a mountainous environment. The germination requirements are in line with those of other *Fritillaria* species (Chen et al., 1993. Nevertheless, seed germination during stratification and presumably in the last days, considering the small size of the emerged roots, indicates that less than 60 days is probably sufficient to break dormancy, as in the seeds of *F. meleagris* (http://data.kew.org/sid/) and of *F. persica*.

The period of cold stratification required for germination suggests that seeds of *F. montana* show a physiological, nondeep dormancy, according to the classification system of Baskin and Baskin (2004). However, a high proportion of fresh seeds are capable of germinating at 5 °C during cold stratification, while only a low proportion of dried and refrigerator-stored seeds retain this ability. This may suggest that cold stratification removes dormancy more effectively in fresh seeds, thus widening the temperature range for germination. Furthermore, as members of the family Liliaceae have linear, underdeveloped embryos (Martin, 1946; Baskin & Baskin, 2001), morphophysiological dormancy cannot be ruled out, and indeed is reported for a number of Liliaceae members (Baskin & Baskin, 2001; Finch-Savage & Leubner-Metzger, 2006), including *Fritillaria* species (Kondo et al, 2006). However, *Fritillaria tubiformis* subsp. *moggridgei*, endemic to the south-western Alps, shows only a morphological dormancy (Carasso et al., 2011).

Germination tests on fresh seeds indicated a good germination power (77.5 ± 12.75%) weakly affected by storage at 5 °C in a refrigerator or at 15 °C with 15% relative humidity in a drying room, at least in the short period allotted for this treatment and with the low numbers of seeds available for the tests. Although long-term tests and more numerous samples are needed to confirm this finding, it could still represent a positive signal for the application of commonly used techniques for ex situ seed conservation involving desiccation treatments (ENSCONET, 2009). Considering the rarity of the species, its low reproductive rates, its low ability to form a persistent soil seed bank, and its tolerance to desiccation treatments, ex situ conservation is an appropriate choice to contribute to the long-term conservation of *Fritillaria montana*.

Further research will be carried out to confirm the findings on dormancy-breaking requirements and storage behaviour in light of their implications for the ex situ conservation programmes for this rare species in Italy.

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