

RESEARCH PAPER

Seed traits and germination behaviour of four Sardinian populations of *Helichrysum microphyllum* subsp. *tyrrhenicum* (Asteraceae) along an altitudinal gradient

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Keywords

Dry after-ripening; endemic plant; germination rate; intraspecific variability; native plant species; seed mass; seed size.

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Editor

H. Pritchard

Received: 4 April 2018; Accepted: 14 August 2018

doi:10.1111/plb.12903

ABSTRACT

- *Helichrysum microphyllum* subsp. *tyrrhenicum* (Asteraceae) is an endemic taxon of Sardinia and Corsica, where it grows at different altitudes. The objective of this study was to investigate the seed traits and germination behaviour of four Sardinian populations of this taxon located at different altitudes.
- Seed traits were evaluated, and germination tests were carried out by incubating seeds at a range of constant (5–30 °C) and alternating (25/10 °C) temperatures. The dry after-ripening (DAR) pre-treatment was also applied by storing seed in dry conditions for 3 months at 25 °C. Seed traits and germination behaviour data were statistically analysed to identify if there was a correlation with altitude.
- Differences in seed size, area and mass among populations were recorded, however, no relationship was found with altitude. High germination percentages were obtained in all populations, both in untreated and DAR seeds, and were positively affected by alternating temperatures. The final germination percentage and time required to reach 50% final germination (T_{50}) showed no relationship with altitude.
- The differences in seed traits and germination detected among the studied populations of *H. microphyllum* subsp. *tyrrhenicum* were not correlated with altitude. This study provides new and important knowledge for this taxon. *H. microphyllum* subsp. *tyrrhenicum* is characterised by high germination percentages and low T_{50} values and does not seem to require any dormancy-breaking treatment. This species represents a high-potential native plant species that should be considered within environmental management plans.

INTRODUCTION

Under a Mediterranean climate, characterised by considerable unpredictability of temperature and precipitation, completion of seed germination must occur at the most appropriate time (Thanos *et al.* 1995; Doussi & Thanos 2002). For example, the long periods of drought during summer and/or the cold period during winter impose severe environmental stresses that limit plant growth and subsequently compromise survival (Medrano *et al.* 2009; Porceddu *et al.* 2017a). Knowledge of factors controlling the timing of germination enhances planning for the effective management and propagation of threatened and/or economically important plant taxa (Cuena-Lombrana *et al.* 2016). Thus, in this context, promoting the use of native plants in habitat restoration and in the plant production sector becomes even more relevant in order, for example, to contribute to long-term environmental protection (Ballesteros *et al.* 2015; De Vitis *et al.* 2017; Ladouceur *et al.* 2017).

Seed mass, seed size, seed dispersal, dormancy mechanisms and seed germination requirements are some of the morphological and physiological traits that underpin the ability of a seedling to emerge, grow and establish (Giles 1990; Cochrane *et al.* 2015). Seed source variations in natural populations have

often been observed in seed morphology, seed mass and seed germinability. These differences may be due to the environmental conditions to which the mother plant is exposed during seed maturation, in combination with genetic and physiological factors which play an important role in determining the plant potential seed quality (*e.g.* Vakshasya *et al.* 1992; Ginwal *et al.* 2005; Luzuriaga *et al.* 2006).

It is well known that the requirements for seed germination are specific for each species and depend on plant distribution, habitat and phylogeny (Finch-Savage & Leubner-Metzger 2006; Baskin & Baskin 2014). Indeed, each taxon is characterised by a temperature range over which germination is possible (Black *et al.* 2006), sometimes responding differently to constant or alternating temperature regimes (*e.g.* in *Clematis vitalba* seeds; Picciau *et al.* 2017), and may be linked to the climatic and ecological conditions to which the species is adapted and has grown (Probert 2000; Liu *et al.* 2013). Other factors may also affect the germination requirements of a species, *e.g.* light and water, as well as the amount of time during which seeds are exposed to dry after-ripening (DAR) (Finch-Savage *et al.* 2007). This last condition may be considered as a natural mechanism that can control dormancy and germination in dry climates and it is commonly reproduced in the laboratory to release seed dormancy (Bewley 1997; Probert 2000). DAR

is required by many plants and in particular by *Asteraceae* species (Schütz 1999). This treatment acts to increase the final germination percentage, widening the range of germination temperatures and decreasing the rate of germination processes (Bewley 1997; Finch-Savage *et al.* 2007).

Germination behaviour can vary widely within a single species from one population to another (*e.g.* Pérez-García *et al.* 2003, 2006; Baskin & Baskin 2014). Several works have reported that intraspecific variations in germination requirements among populations are related to altitude, local climate and habitat, as well as to differences in the post-dispersal environment (*e.g.* Holm 1994; Giménez-Benavides *et al.* 2005; Mondoni *et al.* 2008; Mattana *et al.* 2012). Since the germination percentage and rate could be influenced by the altitude at which seeds are collected, an estimate of germination capacity (both in terms of germination percentage and rate) is important in order to determine the reproductive efficiency of a species which might, therefore, vary with altitude (Vera 1997). Within this context, Vera (1997) reported a positive effect of altitude on seed germination percentage and rate of two heathland plants from north Spain. In particular, seeds of *Calluna vulgaris* and *Erica cinerea* from the highest altitude (2090 and 1200 m a.s.l., respectively) germinated faster and to higher percentages than those from the lowland, reaching average germination of 88% and 51%, respectively. In agreement, Giménez-Benavides & Milla (2013) found higher germination percentages for seeds collected from higher altitudes in two *Saxifraga* species endemic to the north of Spain. Conversely, no relationship was found between the germination responses and geographic location (including altitude) in populations of *Sarracenia purpurea* (Ellison 2001). Considering the lack of concordance between germination characteristics and altitude, further comparative studies on seed germination behaviour along an altitudinal gradient are needed. For example, in Sardinia (Italy), a typical species that grows over a wide altitudinal range is *Helichrysum microphyllum* (Willd.) Camb. subsp. *tyrrhenicum* Bacch., Brullo & Giusso (hereafter *H. m. tyrrhenicum*). This taxon is endemic to Sardinia and Corsica, where it occurs on different substrates and is common in stony places, arid grasslands, garrigues and maquis, from sea level up to about 1500 m a.s.l. (Bacchetta *et al.* 2003; Angiolini *et al.* 2005). Previous studies on related species showed their potential use in environmental restoration of metal-contaminated areas (*e.g.* Bini *et al.* 2016). Information on the germination behaviour of seeds from different populations is necessary for selecting, for example, the most suitable seed population to use in differing ecological restoration projects (Zhang *et al.* 2017). Although a recent study reported high

germination percentages (>65%) in fresh and dry after-ripened (DAR) seeds of a Sardinian population of *H. m. tyrrhenicum* (Picciau 2016), so far, no research has investigated thoroughly the germination ecophysiology of this species by comparing responses among populations. Therefore, the aims of this work were to: (i) evaluate the germination response to different temperatures and the effect of DAR on seed germination of four populations of *H. m. tyrrhenicum*, (ii) verify if seed size, area and mass showed differences among populations, and (iii) investigate the existence of a correlation of germination responses and seed traits with altitude.

MATERIAL AND METHODS

Seed lot details

During summer 2015, ripe seeds of four natural Sardinian populations of *H. m. tyrrhenicum* were randomly collected at optimum maturity from approximately 50 mother plants per population (Table 1). Subsequently, the accessions were cleaned, quantified, selected and processed at the Sardinian Germplasm Bank (BG-SAR), and the seeds were stored at room conditions for 2 weeks at 20 °C and 40% relative humidity before experiments were started (Porceddu *et al.* 2017b).

Seed size and mass

The seed of *H. m. tyrrhenicum* is an achene, obovate and elongated longitudinally. Major diameter (*i.e.* the longest axis of the seed), minor diameter (*i.e.* the shortest axis of the seed) and area (*i.e.* area inside the polygon defined by the perimeter) were evaluated by measuring 30 seeds per population, using the image analysis software ImageJ 1.5 (National Institutes of Health, Bethesda, MA, USA). Images were acquired using a Zeiss SteREO Discovery.V8, with an objective Achromat S 0.63 ×, FWD 107 mm (Carl Zeiss MicroImaging, Jena, Germany) at 1.25 × magnification, coupled to a Canon (Power Shot G11) digital camera.

Seed mass for each population was calculated (at 20 °C and 40% relative humidity) by weighing three replicates of 100 seeds each using an electronic analytical balance (Gibertini, Crystal 100 Model; Milan, Italy).

Germination tests

For each population, four replicates of 30 seeds were sown on the surface of 1% water agar in 60-mm diameter plastic Petri

Table 1. Seed lot details.

code	population	coordinates	altitude (m a.s.l.)	substrate	collection date
HMT1	Rio Oridda, Villacidro (VS)	39°24' N 08°34' E	414	Alluvial deposits	16/07/2015
HMT2	Genna Selole, Baunei (OG)	40°05' N 09°35' E	700	Limestones	20/07/2015
HMT3	Genn'e Impi, Gonnosfanadiga (VS)	39°26' N 08°37' E	1034	Granites	05/08/2015
HMT4	Brunco Spina, Fonni (NU)	40°02' N 09°17' E	1540	Metamorphic	23/08/2015

dishes and incubated in growth chambers (Sanyo MLR-351; SANYO Electric, Osaka, Japan) each equipped with white fluorescent lamps (FL40SS.W/37 70–10 $\mu\text{mol m}^{-2}\text{s}^{-1}$) under a 12 h/12 h photoperiod at a range of constant germination temperatures (5, 10, 15, 20, 25 and 30 °C) and under an alternating temperature regime (25/10 °C) for a maximum of 3 months. In the alternating temperature regime, the higher temperature coincided with the 12-h light period. The temperature conditions used under the alternating regime correspond to the mean diurnal temperature fluctuations present during and after seed dispersal (late July/August–September) in Sardinia. The effect of the DAR pre-treatment was evaluated by storing seeds at 25 °C for 3 months inside a sealed glass container with colour-changing silica gel at a ratio seed/silica gel of 1:1 which ensure a relative humidity $\leq 5\%$. At the end of DAR, seeds were incubated under a 12 h/12 h photoperiod at the above-listed temperatures.

All germination tests, including the DAR treatment, started 2 weeks after seed collection.

Germination, defined as visible radicle emergence (>1 mm), was recorded three times per week. At the end of the germination tests, when no additional germination occurred for two consecutive weeks, the viability of the remaining seeds was evaluated through a cut test.

Data analysis

The final germination percentages were calculated as mean of the four replicates (\pm SD) on the basis of the total number of filled seeds. Furthermore, the germination rate (T_{50}) was determined as the time (in days) required to reach 50% germination.

Generalised linear models (GLMs) were used to: (i) compare seed size, area and seed mass, (ii) evaluate the effect of treatment, population and their interaction on final germination percentages, as well as to (iii) assess the effect of treatment, population and temperature on T_{50} . Significant differences were then analysed with a *post-hoc* pair-wise comparisons *t*-test (with Bonferroni adjustment). GLMs with a log link function and quasi-Poisson error structure were used to analyse the seed size, area and mass, a GLM with a logit link function and quasi-binomial error structure was used to analyse the germination percentages, while *F* tests with an empirical scale parameter instead of chi-squared on the subsequent ANOVA were used in order to overcome residual overdispersion (Crawley 2007).

Correlation and linear regression analyses were performed to investigate the possible relationship between altitude and seed size, seed mass, final germination percentages and T_{50} values. Statistical analyses were carried out using R version 2.14.1 (R Development Core Team 2014).

RESULTS

Seed size and mass

The GLM highlighted that the population factor had a significant effect on each measurement ($P < 0.05$). The *post-hoc* test showed a statistically significant difference ($P < 0.05$) for HMT2 with respect to all other three populations, both for major and minor diameters, area and mass of the seeds; there were no statistically significant differences among HMT1, HMT3 and

HMT4 ($P > 0.05$; Fig. 1). More specifically, the highest values for each parameter were for the seeds of HMT2, with a major diameter of 0.88 ± 0.08 mm (Fig. 1A) and a minor diameter of 0.46 ± 0.05 mm (Fig. 1B), an area of 0.32 ± 0.05 mm² (Fig. 1C) and a mass (expressed as mg per 100 seeds) of 9.5 ± 0.1 mg (Fig. 1D). The HMT1, HMT3 and HMT4 populations had, on average, a major diameter of 0.73 ± 0.08 mm (Fig. 1A), a minor diameter of 0.37 ± 0.04 mm (Fig. 1B), an area of 0.21 ± 0.04 mm² (Fig. 1C) and a mass of 7.1 ± 0.1 mg (Fig. 1D).

The relationship between the four seed traits measured and altitude is also shown in Fig. 1. The major and minor diameters (Fig. 1A, B), as well as the seed area and mass (Fig. 1C, D), did not significantly vary with altitude ($P > 0.05$).

Seed germination

The GLM confirmed that the ‘population’ factor had a significant effect on seed germination ($P < 0.05$; Table 2), while no differences were detected for the ‘treatment’ factor ($P > 0.05$; Table 2), or for the two-way interaction ‘treatment \times population’ ($P > 0.05$; Table 2). In the test with fresh seeds, the highest germination percentage at 5 °C ($73.80 \pm 10.46\%$) was for HMT3; at the same temperature, the final germination percentages slightly increased after DAR pre-treatment in all populations (Fig. 2); however, in HMT2 and HMT4, both in fresh and DAR-treated seeds, germination did not reach 50%. In general, at 10, 15, 20, 25 and 30 °C germination was $>50\%$ in all populations, except for DAR-treated seeds of HMT1 at 10 and 30 °C and HMT4 at 30 °C (both populations showed a similar trend of germination, ca. 37%; Fig. 2). High germination ($>65\%$) was achieved in all seed lots under the alternating temperature regime, both for untreated and DAR-treated seeds (Fig. 2).

The *post-hoc* test on the ‘population’ factor showed a statistically significant difference ($P < 0.05$) for HMT3 with respect to HMT1, HMT2 and HMT4, also by combining the treatment response data set, highlighting the absence of statistical differences ($P > 0.05$) among HMT1, HMT2 and HMT4. No significant relationship ($P > 0.05$) was found between germination percentage and altitude, both for untreated ($r^2 = 0.0006$, $P = 0.9694$; Fig. 3) and DAR seeds ($r^2 = 0.0003$; $P = 0.9388$; Fig. 3).

Germination rate

The GLM highlighted a statistically significant effect of ‘population’, ‘treatment’ and ‘temperature’ factors on T_{50} ($P < 0.001$; Table 3). In general, the untreated and DAR-treated seeds of all tested populations reached T_{50} values within 30 days, except for seeds tested at 30 °C, which required > 30 days to reach this value (Fig. 4). The T_{50} values could not be calculated for untreated seeds of HMT2 and HMT4 tested at 5 °C, DAR-treated seeds of HMT1 incubated at 10 and 30 °C and HMT4 at 30 °C, since 50% germination was not reached. Under the alternating temperature regime, both in untreated and DAR-treated seeds, T_{50} was achieved within 30 days in all populations.

The relationship between T_{50} and altitude is shown in Fig. 5. Although time appears to decrease with altitude, no significant relationship ($P > 0.05$) was found between the two (Fig. 5).

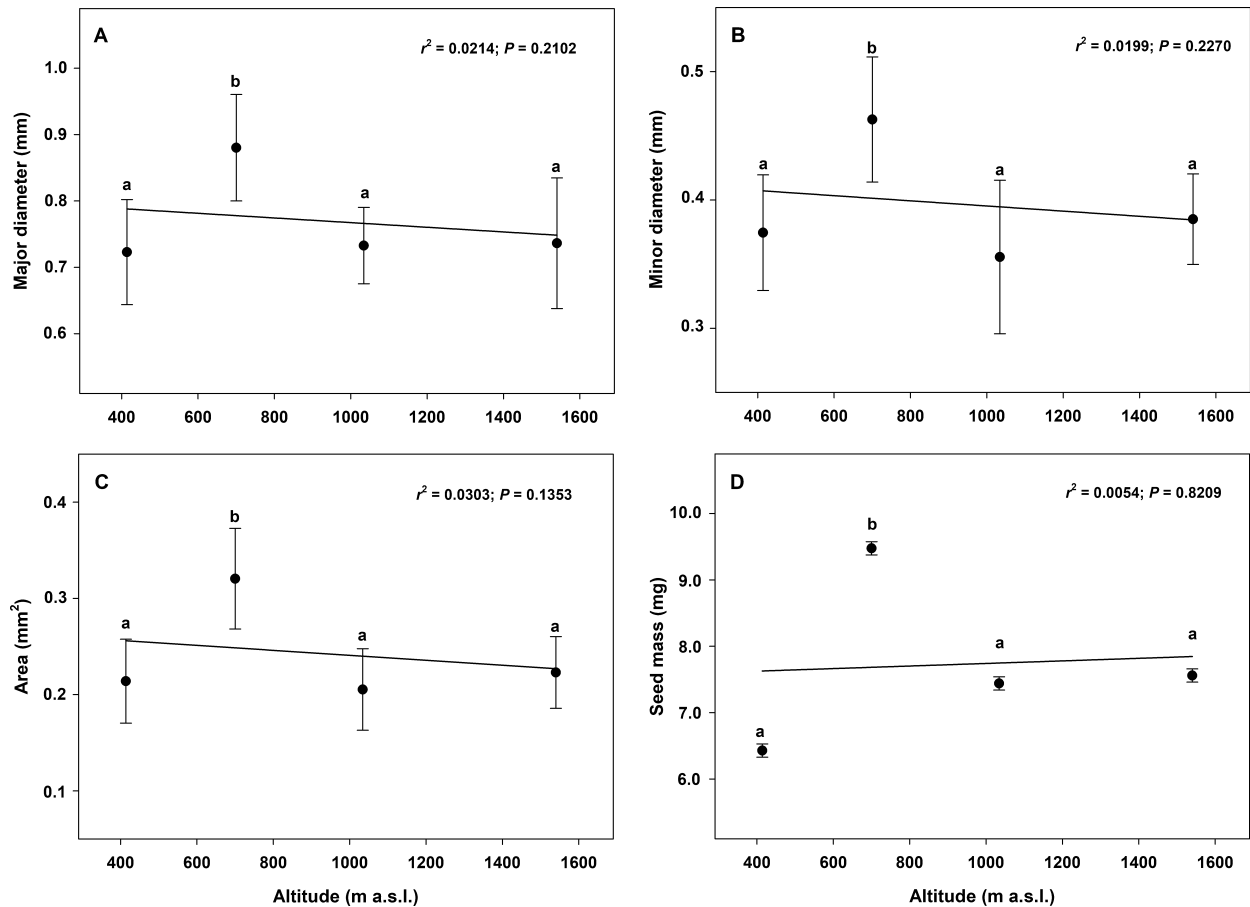


Fig. 1. A: Major diameter (i.e. the longest axis of the seed), B: minor diameter (i.e. the shortest axis of the seed), C: area (i.e. area inside the polygon defined by the perimeter) and D: seed mass of 100 seeds (mg) measured for seeds of each population of *H. m. tyrrhenicum* and their relationship to seed source altitude (m a.s.l.). Data for populations at 414, 700, 1034 and 1540 m a.s.l. correspond to HMT1, HMT2, HMT3 and HMT4, respectively (see Table 1). Values with the same letter are not different at $P > 0.05$ by *post-hoc* pair-wise *t*-test comparisons.

Table 2. GLM results for the effect on final germination percentages of the following factors: ‘treatment’ (untreated; DAR, dry after-ripening), ‘population’ (HMT1, 414 m a.s.l.; HMT2, 700 m a.s.l.; HMT3, 1034 m a.s.l.; HMT4, 1540 m a.s.l.) and their interaction. *** $P < 0.001$.

	df	deviance	resid. df	resid. dev.	F	P
Null			223	5426.1		
Treatment	1	18.19	222	5407.9	0.967	0.3265
Population	3	1155.69	219	4252.2	20.482	1.018 ^{e-11} ***
Treatment × population	3	19.11	216	4233.1	0.339	0.7974

DISCUSSION

The seed size variation may represent an advantageous adaptation in randomly varying environments (Giles 1990). Variation in seed traits has been observed in many species among populations, among plants within populations and within single plants (e.g. Pitelka *et al.* 1983; Stanton 1984; Antonovics & Schmitt 1986; Wolf *et al.* 1986; Giles 1990). Pluess *et al.* (2005) reported that seed mass increased with altitude among related

species, but not among populations of individual species. The seed traits of *H. m. tyrrhenicum* analysed in this work did not seem to be influenced by altitude, as also found with the correlation analysis. The intraspecific differences found in this study could be explained as a response to particularly favourable climate and environmental conditions of the collection site (probably related to temperature/humidity) or as a manifestation of natural genetic differences that are most likely present among the four populations.

Seeds of the *Asteraceae* were classified as either non-dormant (ND) or physiologically dormant (PD; Finch-Savage & Leubner-Metzger 2006). In ND seeds root emergence occurs in a few days (usually within about 4 weeks), while in seeds with PD germination takes more than 30 days, and seeds require a dormancy-breaking treatment (e.g. DAR, cold/warm stratification, etc.) to become ND (Baskin & Baskin 2014). Several studies confirm that seed germination (as well as dormancy release) can be improved by storing seeds in dry conditions at high temperatures (e.g. Bewley 1997; Kucera *et al.* 2005; Finch-Savage *et al.* 2007; Karlsson & Milberg 2007). In particular, within the *Asteraceae*, germination of *Helichrysum* spp. has been widely studied. For example, Willis & Groves (1991) found that seeds of *H. apiculatum* require a short period of exposure to high temperature to overcome innate dormancy.

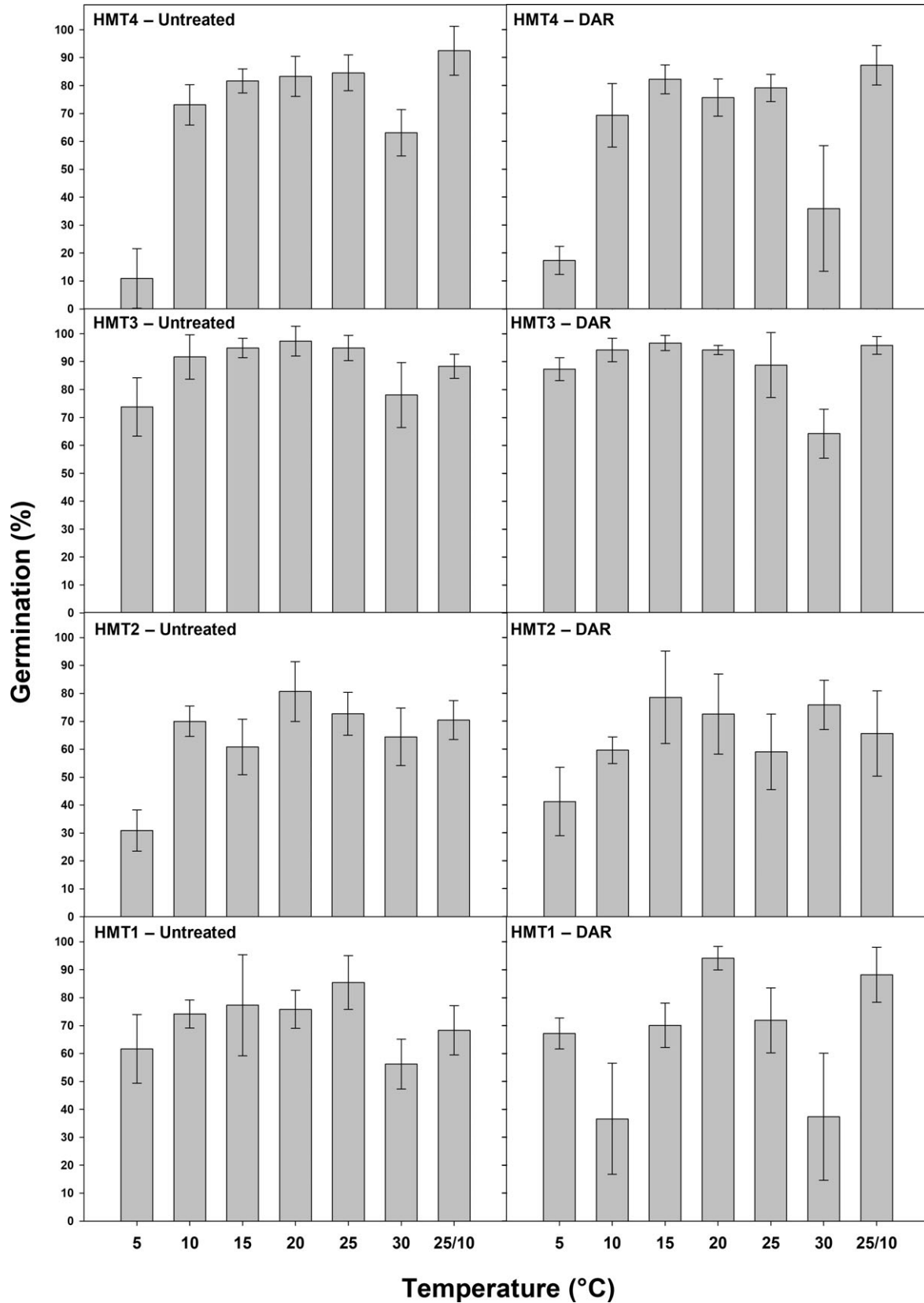


Fig. 2. Final germination percentages of *H. m. tyrrhenicum* achieved at the end of the germination tests for untreated and dry after-ripened (DAR, 25 °C for 3 months in dry conditions) seeds. Data are mean of the four replicates (\pm SD). HMT1, HMT2, HMT3 and HMT4 correspond to populations at 414, 700, 1034 and 1540 m a.s.l., respectively.

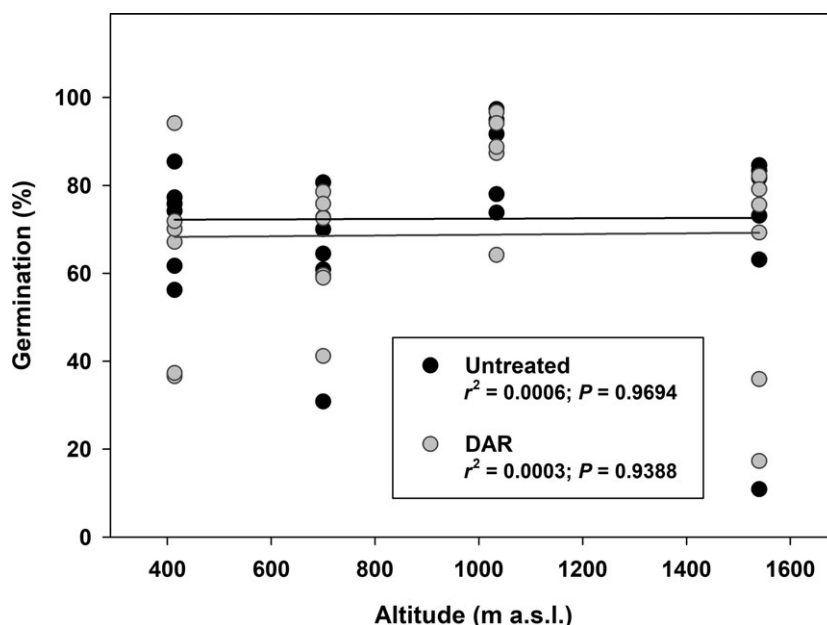


Fig. 3. Relationship between final germination percentages (achieved at the end of untreated and dry after-ripening 'DAR' tests) of *H. m. tyrrhenicum* seeds and seed source altitude (m a.s.l.).

Table 3. GLM results for the effect on T_{50} of the following factors: 'treatment' (untreated; DAR, dry after-ripening), 'population' (HMT1, 414 m a.s.l.; HMT2, 700 m a.s.l.; HMT3, 1034 m a.s.l.; HMT4, 1540 m a.s.l.) and 'temperature' (5, 10, 15, 20, 25, 30 and 25/10 °C). *** $P < 0.001$.

	df	deviance	resid. df	resid. dev.	<i>F</i>	<i>P</i>
Null			191	1849.2		
Treatment	1	29.77	190	1819.4	11.738	0.0008***
Population	3	176.78	187	1642.6	23.234	9.052e ⁻¹³ ***
Temperature	6	1235.44	181	407.2	81.184	<2.2e ⁻¹⁶ ***

In this study, we observed that application of the DAR treatment, compared to untreated seeds of each population, did not substantially enhance the seed germination percentage or the rate. Thus, based on our findings, we consider that seeds of *H. m. tyrrhenicum* did not show a dormancy state; indeed, high germination was detected for both untreated and DAR-treated seeds in each population, and in general germination occurred within about 30 days. However, we noted that most remaining viable non-germinated seeds were in batches subjected to 5 or 30 °C, so caution should be exercised in affirming the non-dormancy state for this taxon. Further studies will be needed to understand if the viable non-germinated seeds of *H. m. tyrrhenicum* require treatments to break any residual dormancy or if the germination percentage in this taxon could have some limit temperatures (*sensu* Soltani *et al.* 2017).

Regardless of the treatment, the highest germination percentages were at 15 and 20 °C. This is not surprising, since many studies reported that, under a Mediterranean climate, seed germination occurs at 15–20 °C, which ensures that seeds complete germination during the rainy period in autumn, thus allowing seedlings to avoid arid conditions during summer (*e.g.* Thanos *et al.* 1995; Doussi & Thanos 2002; Galmés *et al.* 2006; Luna *et al.* 2008; Kadis & Georghiou 2010). In the field, *H. m. tyrrhenicum* seed may be subjected to short dry after-ripening in the mother plant before natural dispersion and,

consequently, may benefit from this natural treatment in promoting germination of this taxon. Hence, considering that the timing of the phenological phases may depend on environmental characteristics of the collection area at a specific time, seed collecting should be carried out, as in this study, when seeds reach optimal maturity stage, therefore ensuring maximum viability. Moreover, in agreement with the Mediterranean character of the species, the highest rate response (*e.g.* T_{50} value) was at 20 °C, in about 4–5 days from sowing, both for untreated (HMT2) and DAR-treated (HMT4) seeds. This tendency was also noticed in the two other studied populations, which exhibited most rapid germination rates between 15 and 20 °C, regardless of the treatment. In addition, the results suggested that *H. m. tyrrhenicum* may have both autumn and spring germination behaviour.

We observed that the fluctuating temperature regime had a positive effect on seed germination of *H. m. tyrrhenicum*. In particular, seeds from the HMT4 population reached the highest germination percentage under fluctuating temperatures (both for untreated and DAR seeds) compared to those tested at constant temperature, suggesting that this taxon will likely establish in climate areas with large day/night differences in temperature. Furthermore, seeds of all populations of *H. m. tyrrhenicum* showed a better germination response under a fluctuating temperature regime than at 5 and 30 °C. These findings are in agreement with several studies, which reported a positive effect of a fluctuating temperature regime on seed germination percentage (*e.g.* Schütz & Rave 1999; Liu *et al.* 2013; Picciau *et al.* 2017). This germination behaviour represents an adaptive mechanism to the natural environment of a species with small seeds, as observed in *H. m. tyrrhenicum* (in terms of seed size and mass). Probert (2000) reported that small-seeded species mainly germinate close to the soil surface and are therefore more exposed to thermal changes between day and night. Moreover, light acts as a depth-sensing mechanism, avoiding possible fatal germination of small seeds buried too deeply in the soil (Plummer & Bell 1995; Milberg *et al.* 2000; Schütz *et al.* 2002).

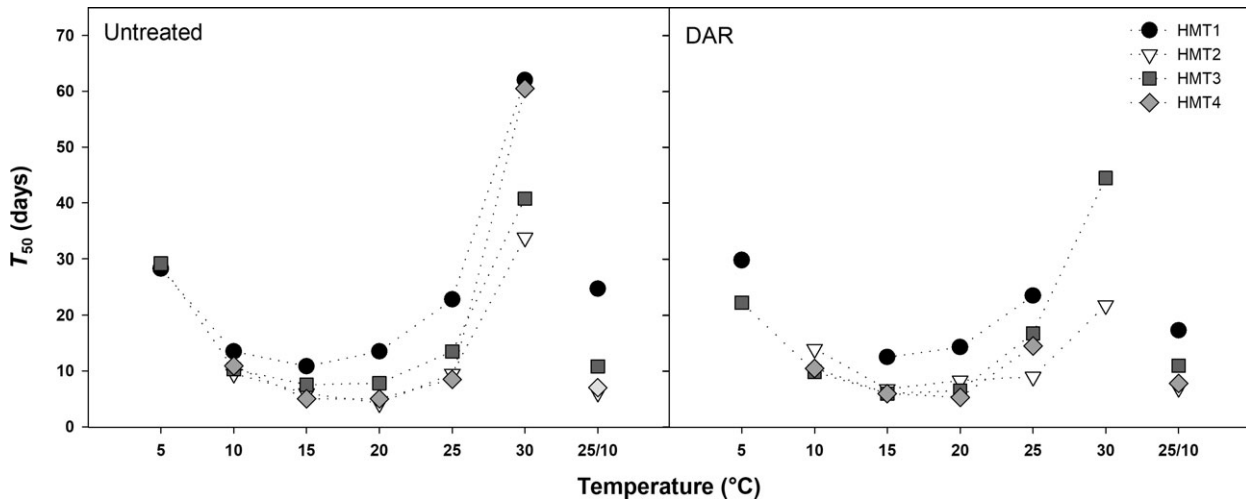


Fig. 4. Time to reach 50% germination (T_{50}) for untreated and dry after-ripening (DAR, 3 months at 25 °C under dry conditions) tests. Data are mean of replicates which reached 50% germination. HMT1, HMT2, HMT3 and HMT4 correspond to populations at 414, 700, 1034 and 1540 m a.s.l., respectively.

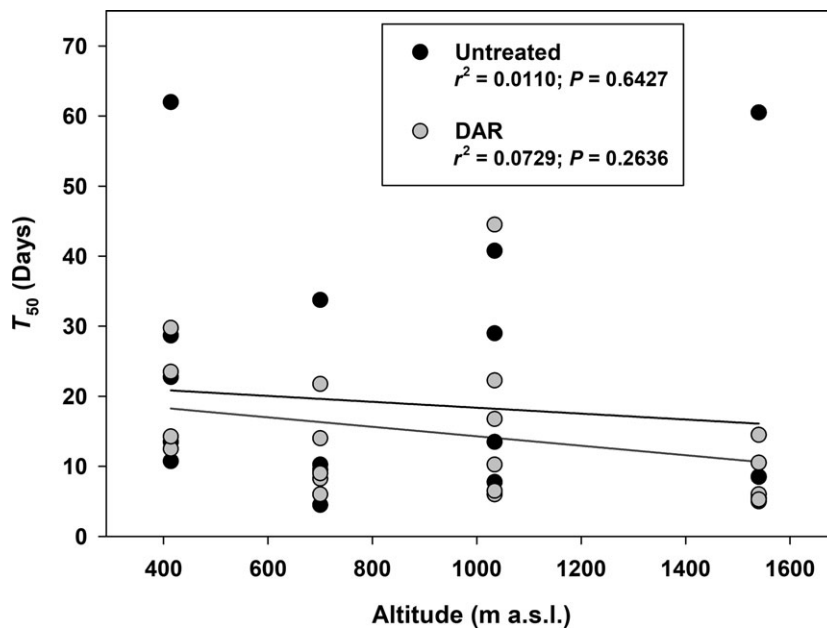


Fig. 5. Relationship between time in days to reach 50% germination (T_{50}) for untreated and dry after-ripening (DAR) tests and altitude (m a.s.l.) of each population of *H. m. tyrrhenicum*.

Several environmental factors (e.g. light, moisture, temperature, orientation and altitude) can cause differences in the germination response among populations of the same species (Fenner 1991; Guterman 1992; Holm 1994; Vera 1997; Cuenalombraña *et al.* 2016). These differences can be interpreted as one of the most important survival strategies for species growing in unpredictable environmental conditions (Guterman 1994; Kigel 1995). In particular, correlations between intraspecific differences in seed germination and altitude have been reported by several authors (e.g. Vera 1997; Cavieres & Arroyo 2000; Fernández-Pascual *et al.* 2013). However, in this work, we did not find a germination trend related to altitude, therefore our seeds seem not to depend on this factor. This behaviour was also detected in wild populations of *Capsicum annuum* L. from northwest Mexico, in which the differences in germination patterns did not correlate with climatic variables

(including altitude) of the collection sites (Hernández-Verdugo *et al.* 2001).

In addition, the results of this work indicate that the differences in germination among populations had no relationship with differences detected in seed traits. Many authors have reported the existence of a relationship between seed germination and seed size/mass, and observed that seed traits affected the time and the probability of germination, as well as seedling survival (e.g. Cideciyan & Malloch 1982; Stanton 1984; Jakobsson & Eriksson 2000; Bonito *et al.* 2011; Frattaroli *et al.* 2013; Lönnberg & Eriksson 2013), but other authors reported that seed germination was not influenced by seed mass (e.g. Walder & Erschbamer 2015). We suggest that the differences detected in *H. m. tyrrhenicum* are related to biotic/abiotic factors not examined in this study, such as the ecological conditions of the site, substrate or the occurrence of particular climatic

conditions during seed ripening. Nevertheless, the differences detected in the germination behaviour of *H. m. tyrrhenicum* are in agreement with Pérez-García *et al.* (2006), who reported that the origin of seed lots must always be taken into account when defining the germination requirements. On the basis of the provenance-specific patterns of the germination behaviour, the use of native seeds is recommended when planning environmental management activities, since they are better adapted to local habitat conditions (e.g. Keller & Kollmann 1999; Bischoff *et al.* 2006, 2010). Further studies are nevertheless necessary (such as those of Zhang *et al.* 2017) to evaluate if the seed germination characteristics found in *H. m. tyrrhenicum* are species-specific and constant from year to year, or if there is an appreciable variability over time.

In conclusion, this work highlights the existence of differences in seed size, mass and germination of *H. m. tyrrhenicum*

that are not correlated with source altitude. The extrapolation of germination requirements of a species should be conducted with caution and, whenever possible, through a comparative approach among populations. Since *H. m. tyrrhenicum* has high germination percentages, low T_{50} values and does not seem to require specific dormancy-breaking treatments, it represents a suitable potential native plant species for management plans, such as environmental restoration in scrub formations of the Mediterranean area, and such use might contribute to long-term environmental protection.

ACKNOWLEDGEMENTS

The authors thank Dr. Sarigu Marco for help with seed trait analysis. We are grateful to the anonymous reviewers, who provided valuable suggestions to improve this manuscript.

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