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Germination Capacity of *Artemisia umbelliformis* Lam. Wild Populations and Agro-techniques to Enhance Seedling Production

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ABSTRACT

Artemisia umbelliformis Lam. is an alpine herbaceous species that grows in the wild and is mainly used for producing génépi liqueur. It has applications as an ingredient in several food products. Its propagation and cultivation have become the only way to ensure a sustainable supply, thus conserving its occurrence in natural habitats. However, low success rates in seed germination usually limit its propagation. Selected seeds and fine-tuned agro-techniques are priorities in nursery production systems. The current research explored seed germination in three wild *A. umbelliformis* populations from Valnontey, Soana, and Urtier in the Italian Alps. We evaluated seed germination ability as a description of final germination percentage (FGP), first germination time (FGT), halftime of germination (T_{50}), germination period (GPD), and mean germination time (MGT). Also, assessments of early seedling development appeared valuable. We used three germination substrates with various peat and perlite ratios (v:v), i.e., S1 (100:0), S2 (80:20), and S3 (60:40), either in combination with or without two generalist arbuscular mycorrhizal fungi (AMF), namely, *Rhizophagus intraradices* and *Funneliformis mosseae*. Results highlighted variations in germination capacity, indicating that the Valnontey population appeared superior to Soana and Urtier populations regarding FGP (34.0%, 7.2%, and 8.6%, respectively), FGT (8.7, 13.2, and 14.1 days, respectively), MGT (12.5, 16.5, and 17.1 days, respectively), and T_{50} (13.8, 17.4, and 16.8 days, respectively). Among the substrates, S3 allowed a higher average FGP value (22.9%) than the other substrates, i.e., in S1 and in S2. No AMF symbiosis occurred, suggesting room for future research into the pros and cons of applying bio-inoculants on génépi seeds and seedlings.

Introduction

The production and growth of medicinal and aromatic plants in mountainous areas are attracting an increasing amount of interest from a

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sustainable economic point of view. Medicinal plants can act as new sources of income for companies located in those areas. With many plants on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Rodrigues et al., 2006; Betts et al., 2020), domestication and cultivation are the best strategies to preserve natural populations. Moreover, cultivation allows for the production of plants with higher uniformity and quality standards. It entails efforts to guarantee a consistent and continuous supply, as many industries require it (Carlen et al., 2012). For alpine medicinal and aromatic plants, ecotype selection and optimal cultivation practices are essential to ensure maximum yield and income.

In the Alps, five perennial and aromatic *Artemisia* species(namely *A. eriantha* Ten., *A. genipi* Weber, *A. glacialis* L., *A. nivalis* Br.-Bl., and *A. umbelliformis* Lam.) have long assisted the process of flavoring an alcoholic beverage, génépi, thus giving it a bitter taste and alleged tonic properties (Rubiolo et al., 2009; Binet et al., 2011).

In Piedmont (Italy), Aosta Valley (Italy), Wallis (Switzerland), and Savoy (France), the production of liqueurs has been growing steadily since the 1960s. Nowadays, it is likely that several hundreds of kilograms of dried plants are processed into génépi every year (Vouillamoz et al., 2015). More recently, the interest in *Artemisia* species has spread to other food and beverage products, i.e., teas, syrups, candies, chocolates, cakes, jams, and mustards (Vouillamoz et al., 2015). However, the availability of these plants in the Alpine landscape is limited, primarily because collection, depending on the location, is either forbidden or unfeasible, with domestication achieved only very recently (Vouillamoz et al., 2015; Boggia et al., 2017). *Artemisia umbelliformis* (syn. *Artemisia mutellina* Vill., *Artemisia laxa* (L.) Fritsch), also called Alpine wormwood or white génépi, is a widespread and easy-to-cultivate plant. *Artemisia umbelliformis* turned out to be the only species suitable for large-scale domestication in the western Alps of Italy and breeding purposes, showing genotypes with erect growth and a relatively high yield potential compared to the other four alpine génépi species (Pieroni and Giusti, 2009; Carlen et al., 2012; Comino et al., 2015). The propagation and cultivation of *A. umbelliformis* have become the only way to secure a renewable supply chain for this plant. Propagation is generally by seed, characterized by a small yield capacity and limited by several constraints, primarily a low germination rate, inadequate longevity, and disease susceptibility.

Artemisia umbelliformis germination rate depends on ecotype, genotype, seed quality, and dormancy (Bewley, 1997; Mondoni et al., 2011; Comino et al., 2015). Gran Paradiso National Park (North West Italy) launched research activities on *A. umbelliformis* cultivation in the early 2000s, applying the strictest collection protocols and monitoring the effects of the collection in wild protected areas. Few studies in the available literature have considered germination capacity *in vitro* (Oliva Branas et al., 1997; Mondoni et al., 2011; Pace et al., 2020). Despite some experiences and projects (Rey and Slacanin, 1997; Bondaz et al., 2000; Rey et al., 2002), scientific research has been scanty on germination aptitude and cultivation of *A. umbelliformis* in protected or open field conditions. The limitations that have caused these drawbacks are their restricted geographic distribution, the difficulty in their identification, and a lack of cultivation protocols, e.g., optimal substrates and fertilization.

Beneficial microbes such as arbuscular mycorrhizal fungi (AMF) are a promising alternative to traditional agricultural techniques as a mean to maintain agro-ecosystem health and productivity (Berruti et al., 2015; Etesami et al., 2021; Gouda et al., 2018). Research has indicated that in colonized plants, AMF alleviates the detrimental effects of abiotic stresses in alpine habitat and induces nutritional benefits (Berruti et al., 2013; Caser et al., 2018, 2019; Stelluti et al., 2023, 2024). AMF application, particularly during seed germination, is an efficient and convenient tool for introducing beneficial microbes into the soil, the rhizosphere, and plant tissues (Ma et al., 2016). AMF symbiosis reportedly increased the percentage of seedling emergence. It protected and nourished seedlings in *Cymbopogon martinii* (Roxb.) Wats. var. Motia Burk (Pankaj et al., 2021) and in *Capsicum annuum* var. aviculare (Dierb.) D'Arcy & Eshbaugh (Rueda Puente et al., 2010) through mycorrhizal hyphae penetration and colonization of young radicles. However, the commercialization and implementation of bioinoculants in medicinal and aromatic plant cultivation still encounter limiting factors due to poor microbial survival and ineffective colonization of plant hosts (Ma et al., 2016; Victorino et al., 2021; Voyron et al., 2022).

Very few studies have reported the application of AMF in the cultivation of *A. umbelliformis*. Binet et al. (2011) indicated that inoculation of native AMF from alpine grassland on *A. umbelliformis* and *A. glacialis* under greenhouse conditions significantly increased Phosphorus concentration in shoots. Use the mycorrhizal

species *Trifolium pratense* as a companion plant improved mycorrhizal colonization of *A. umbelliformis* (Binet et al., 2011). To our knowledge, there has been no research on applying AMF to *A. umbelliformis* germinating seeds.

Specifically, this work investigated the feasibility of using a combination of two general and widely distributed AMF species (*Rhizophagus intraradices* and *Funneliformis mosseae*) for enhancing seed germination and early seedling development in three *A. umbelliformis* wild populations while testing three substrates as various peat and perlite mixtures.

Materials and Methods

Seed collection

In autumn 2018, seeds from three *A. umbelliformis* wild populations, namely Valnontey, Soana, and Urtier, were collected from different sites within the Gran Paradiso National Park (North West Italy) following proper protocols (ENSCONET, 2009) without affecting the reproduction and conservation of the species populations (Fig. 1). This geographic area hosts high-quality wild populations, as assessed by previous research on their phytochemical profile (Vouillamoz et al., 2015; Boggia et al., 2017). Seeds were manually cleaned and kept at 4 °C for four months.

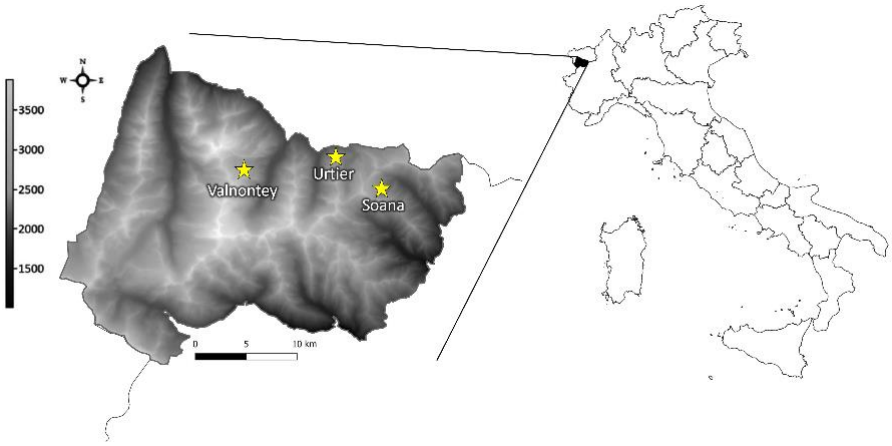

Fig. 1. Geographical localization of the three *A. umbelliformis* wild populations (Valnontey, Soana and Urtier).

Seed germination

The germination test was performed at the Department of Agricultural Forest and Food Sciences (DiSAFA) of the University of Torino (45°06′23.21″N Lat, 7°57′82.83″E Long; 300 m a. s. l.) in spring 2019. The seeds of each wild population were sown in plastic seed pot trays (three seeds per seed pot) that measured 5 cm in diameter and 5 cm in height, filled with 250 mL of germination substrate. Three germination substrates composed of different ratios of peat-based substrate (Flora-gard, Germany) and perlite (v:v). The following ratios were used: S1 (100:0: peat-based substrate: perlite), S2 (80:20: peat-based substrate: perlite), and S3 (60:40: peat-based substrate: perlite). Each germination substrate was evaluated in combination with a spore mix of *Rhizophagus intraradices* and *Funneliformis mosseae* (MycAgro Lab, France) (AMF + treatment) or carrier, composed of calcined clay, vermiculite, and zeolite (Carrier treatment, MycAgro Lab, France). Non-inoculated seeds were the controls (AMF- treatment). Ten grams of AMF inoculum or carrier were placed in the substrate directly under the seeds. A randomized block experiment was conducted with three replications (100 seeds each). Plastic seed pot trays were placed in an open tunnel, where they received regular watering for 35 days after treatment (DAT). Seeds were considered germinated when seedling structures became visible (Fig. 2). Non-germinated seeds were either dead or dysfunctional.

Thousand seed weight (TSW)

Based on the International Rules for Seed Testing Association (2019), seeds were sorted in 8 replicates of 100 seeds per wild population. For each replicate, weight was recorded in grams to three decimal places (Caser et al., 2022). The mean weight of 100 seeds was then used for calculating the weight of 1000 seeds (thousand seed weight, TSW). Thus, the number of seeds per gram was measured.

Germination indices

Data were collected daily until germination values became unchanged (ISTA, 2019). Five

germination indices were calculated: final germination percentage (FGP %), first germination time (FGT days), halftime of germination (T_{50} days), germination period (GPD days), and mean germination time (MGT days). The formulae for calculating the indices, explanations, and references are presented in Table 1.

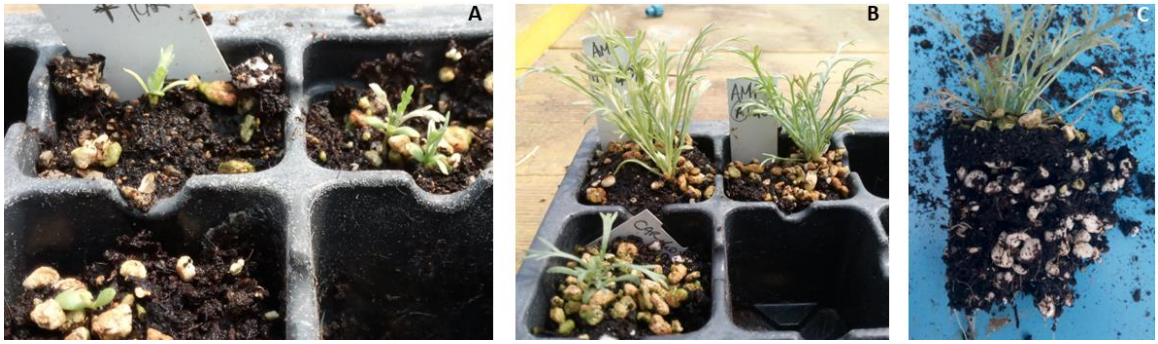

Fig. 2. Germination and early seedling development phases of *Artemisia umbelliformis*. Cotyledons (a), and true leaves (b) appearance and seedlings after 35 days after sowing (c).

Table 1. Germination indices and equations, with explanations and references.

Germination index	Equations	Explanation	Reference
Final germination percentage (FGP)	$FGP = 100 \times \frac{GN}{SN}$	GN = total number of germinated seeds. SN = total number of seeds tested.	Wang et al., 2009
First germination time (FGT)		Number of days from the beginning of the experiment to first germination.	Bu et al., 2007
Half time of germination (T_{50})		Number of days from the beginning of the experiment to the occurrence of 50% germination in the seed population.	Caser et al., 2014
Germination period (GPD)		Number of days from the beginning of the experiment to the maximum number of seeds germinated.	Kumar et al., 2011
Mean germination time (MGT)	$MGT = \frac{\Sigma(\text{NO } of\ germinated\ seeds \times days\ after\ sowing)}{Total\ \text{NO } of\ seeds\ germinated}$	Calculation is based on the daily count of normal seedlings until the final date of the germination test.	Caser et al., 2022

Germination performance was also assessed according to germination potential (GP), germination pattern (GPa), and germination response (GRe), as indicated by Caser et al. (2022). GP was either defined as high (FGP > 75%), moderate (FGP 74%- 20%), or low (FGP < 19%). The GPa was defined either as synchronous if 90% of seeds germinated within 15 days after FGT or asynchronous in all other cases. The GRe was defined as fast (MGT < 10 days), moderate (10 days < MGT < 20 days), or slow (MGT > 20 days).

Early seedling growth evaluation

At the end of the germination trial (35 DAT; Fig. 2C), we measured the height and diameter of each

seedling per treatment were used to calculate the growth index (GI, cm^3; $\boldsymbol{\Pi} \times \left\{\frac{[(\frac{D'+D''}{2}]}{2}\right\} \mathbf{2} \times \boldsymbol{H}$, where D′ is the widest width, D″ is the perpendicular width, and H is the height (Caser et al. 2017)).

AMF evaluation

At the end of the germination trial (35 DAT), the roots of six seedlings per treatment were harvested, cleared from the topsoil, cleaned, and stained to evaluate AMF colonization as reported by Caser et al. (2019). Briefly, roots were stained with 0.1% (w:v) cotton blue in 90% lactic acid overnight and de-stained two times, with water (2 h) and 90% lactic acid in deionized water 1:2 (v:v) (2 h). The roots were left in 90% lactic acid. The protocol was performed twice to ensure a better staining. The roots were cut into fragments of ~1 cm and placed on microscope slides (20 fragments per slide) for further analysis under the light microscope. Mycorrhiza population, AM fungal colonization intensity, and presence of arbuscules and coils in the whole root system were evaluated according to Trouvelot et al. (1986).

Statistical analysis

An arcsin transformation was performed on all data before statistical analysis to improve the homogeneity of the variance (Levene test; $p < 0.05$). Statistical analyses were done using SPSS Version 26.0 (IBM SPSS Statistic, Segrate, Italy). All analyzed data were checked for the normality of variance by using Shapiro–Wilks test ($p > 0.05$). For all parameters, mean differences were computed using a one-way and univariate ANOVA with Tukey's Bonferroni adjustment post-hoc test ($p \leq 0.05$).

Results

Seed characteristics

The thousand seed weight and number of seeds per gram ranged from 0.283 g and 3,533 seeds in Soana to 0.449 g and 2,227 seeds in Valnontey (Table 2). In addition to a heavier weight, Valnontey also showed the longest (2.61 mm) and widest (1.01 mm) seeds.

Table 2. Thousand seed weight (TSW) and number of seeds per gram of the studied *A. umbelliformis* wild populations.

Wild population	TSW (g)	Seeds g^{-1} (nr.)	Length (mm)	Width (mm)
Valnontey	0.449[a]	2,227	2.61[a]	1.01[a]
Soana	0.283[c]	3,533	2.22[b]	0.92[b]
Urtier	0.316[b]	3,161	1.99[b]	0.96[b]
p	***		**	**

Mean values showing the same letter are not statistically different at $p \leq 0.05$ according to the Tukey's Bonferroni adjustment post-hoc test. The statistical relevance is provided (** = $p < 0.01$; *** = $p < 0.001$).

Effect of wild population on germination

The three wild populations showed different germinations (Table 3A). Valnontey had significantly superior FGP than Soana and Urtier (FGP equal to 34.0%, 7.2%, and 8.6%, respectively). Regarding the germination potential (GP), Valnontey showed a moderate potential to germinate while the others showed a low potential. Regarding FGT, the fastest population to germinate was Valnontey, with a significantly lower value than Soana and Urtier (8.7, 13.2, and 14.1 days, respectively). A similar behavior was observed for both MGT and T_{50}, with significantly lower times for Valnontey, followed by Soana and Urtier (12.5, 16.5, and 17.1 days, and 13.8, 17.4 and 16.8 days, respectively). No significant differences occurred in GPD among the accessions. All wild populations showed a synchronous GPa and a moderate level of GRe.

Effect of substrate on germination and seedling growth

Seeds sown in the germination substrate with higher content of perlite (S3) resulted in higher FGP (22.9%) than the others (11.4% and 15.6% in S1 and S2, respectively) (Table 3B). S3 also induced a moderate ability to germinate, while the others had low germination potential. S3 caused the lowest values of FGT, MGT, and T_{50} parameters without significant differences with S1. No differences appeared in GPD. As for the population effect, GPa was synchronous, and GRe was moderate. At the end of the germination trial (35 DAT), the growth index of the obtained seedlings was measured, with data shown in Figure 3. Valnontey population showed superior data, significantly higher than the others. Regarding the substrate, S2 and S3 induced a significant increase in GI than S1.

Table 3. Effect of (A) wild population, (B) germination substrate (S1, 100:0 (v:v), peat:based substrate; S2, 80:20 (v:v), peat-based substrate:perlite; S3, 60:40 (v:v) peat-based substrate:perlite) and (C) AMF treatment (AMF, i.e. inoculum composed by a combination of *Rhizophagus intraradices* and *Funneliformis mosseae*. Carrier i.e., AMF substrate composed by calcined clay, vermiculite, and zeolite without inoculum. AMF i.e., not-inoculated seeds and without carrier) and their interaction on final seed germination percentage (FGP%), first germination time (FGT days), mean time of germination (MGT days), halftime of germination (T_{50} days) and germination period (GPD days) of the *A. umbelliformis* collected seeds. Germination potential (GP) was described as high (H, FGP > 75%), moderate (M, FGP 74%-20%) or low (L, FGP < 19%). Germination pattern (GPa) was either synchronous (S) or asynchronous (A). Germination response (GRe) was indicated as fast (F), moderate (M) or slow (S).

Wild population (A)	FGP	GP	FGT	GPa	MGT	GRe	T_{50}	GPD
Valnontey	34.0^{a}	M	8.7^{b}	S	12.5^{b}	M	13.8^{b}	22.3
Soana	7.2^{b}	L	13.2^{a}	S	16.5^{a}	M	17.4^{a}	23.1
Urtier	8.6^{b}	L	14.1^{a}	S	17.1^{a}	M	16.8^{a}	23.9
p	**		***		***		**	ns
Substrate (B)								
S1	11.4^{b}	L	10.3^{b}	S	14.2^{b}	M	15.1^{b}	20.8
S2	15.6^{b}	L	15.1^{a}	S	17.6^{a}	M	18.1^{a}	25.2
S3	22.9^{a}	M	9.9^{b}	S	13.6^{b}	M	14.3^{b}	22.7
p	**		***		***		**	ns
AMF Treatment (C)								
AMF+	16.2	L	11.4	S	15.3	M	15.5	22.4
Carrier	15.1	L	14.1	S	16.4	M	17.8	23.8
AMF-	18.5	L	11.5	S	14.7	M	15.4	23.4
p	ns		ns		ns		ns	ns
Interactions	*p*		*p*		*p*		*p*	*p*
A×B	**		**		***		**	ns
B×C	ns		ns		ns		ns	ns
A×C	ns		ns		ns		ns	ns
A×B×C	ns		ns		ns		ns	ns

Mean values showing the same letter are not statistically different at $p \leq 0.05$ according to the Tukey's Bonferroni adjustment post-hoc test. The statistical relevance is provided (** = $p < 0.01$; *** = $p < 0.001$; ns = not significant).

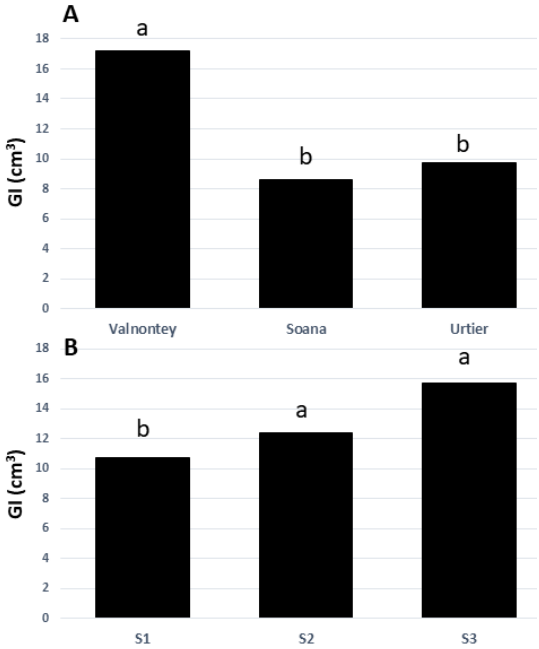

Fig. 3. Effect of (A) population and (B) germination substrate (S1, 100:0 (v:v), peat-based substrate; S2, 80:20 (v:v), peat-based substrate:perlite; S3, 60:40 (v:v) peat-based substrate:perlite) on the growth index (GI, cm3) of the *A. umbelliformis* seedlings at 35 DAT. Mean values showing the same letter are not statistically different at $p \leq 0.05$ according to Tukey's Bonferroni adjustment post-hoc test (ns = not significant).

Interaction between wild population and substrate on germination

Significant interactions between wild population and germination substrate were highlighted in FGP, FGT, MGT, and T_{50} parameters (Table 4).

Table 4. Influence of wild population and germination substrate (S1, 100:0 (v:v), peat-based substrate; S2, 80:20 (v:v), peat-based substrate:perlite; S3, 60:40 (v:v) peat-based substrate:perlite) on final seed germination percentage (FGP%), first germination time (FGT days), mean time of germination (MGT days) and halftime of germination (T_{50} days) of the *A. umbelliformis* seeds.

Wild population	Substrate	FGP	FGT	MGT	T_{50}
Valnontey	S1	18.6^{c}	9.0^{b}	13.8^{bcd}	15.8^{bc}
	S2	32.9^{b}	8.8^{b}	12.5^{cd}	13.2^{bc}
	S3	46.1^{a}	8.4^{b}	11.1^{d}	12.4^{c}
Soana	S1	8.2^{cd}	10.6^{b}	14.8^{bcd}	15.8^{bc}
	S2	4.2^{d}	16.9^{a}	18.1^{b}	18.5^{ab}
	S3	9.3^{cd}	11.8^{b}	16.5^{bc}	17.7^{abc}
Urtier	S1	7.9^{cd}	11.4^{b}	13.8^{bcd}	13.6^{bc}
	S2	8.7^{cd}	19.7^{a}	22.4^{a}	22.5^{a}
	S3	9.8^{cd}	9.6^{b}	13.1^{cd}	12.7^{c}
	p	***	***	***	***

Mean values showing the same letter are not statistically different at $p \leq 0.05$ according to Tukey's Bonferroni adjustment post-hoc test. The statistical relevance is provided (*** = $p < 0.001$).

Therefore, focusing on the differences between the populations according to the germination substrate, the results highlighted how S3 resulted in a significantly superior FGP and higher germination speed (i.e., FGT, MGT, and T_{50}) in Valnontey seeds (46.1%, 8.4 days, 11.1 days and 12.4 days, respectively) in comparison to the other combinations.

Effect of AMF treatment on germination

AMF application did not influence germination (Table 3C). AMF evaluation on young root fragments before transplanting seedlings (35 DAT) revealed no visible colonization structures.

Discussion

The successful cultivation of *A. umbelliformis* is determined largely by the germinability of the seeds. For commercial production, rapid and uniform germination from direct seeding in cultivation substrate or field is needed. Germination tests should provide reliable information about the horticultural values of a given batch of seeds, which are also decisive criteria in case of any question of liability occurring in the seed business. Unlike other cultivated plants, there is little pertinent information about the seed germination and seedling emergence of *A. umbelliformis* (Oliva Branas et al., 1997; Mondoni et al., 2011; Pace et al., 2020). Germination is an irreversible process and must be timed to occur when the environment is favorable for subsequent seedling establishment (Tudela-Isanta et al., 2018; Caser et al., 2022). Germination timing is controlled both by environmental and morphological cues such as seed weight (Domic et al., 2020). In this study, the *A. umbelliformis* wild population seed weight and dimensions greatly differed with Valnontey seeds as the heaviest, the longest, and the widest. Different sizes of seeds having different levels of starch and other food storage may be one factor that affects the expression of germination and early seedling growth. Therefore, as reported here, larger seeds are generally associated with higher germination potential, healthier seedlings, and overall higher rates of viability as reported by different authors (Kotowski, 1926; Ranal and Garcia de Santana, 2006; Benard and Toft, 2007; Domic et al., 2020; Ge et al., 2020; Veselá et al., 2021).

The final germination percentage is one of the most important characteristics in seeds to consider when adopting the most effective germination protocol. Seed germination of wild alpine species can be naturally low and variable, and some seed ecological traits can determine obstacles (Aiello et al., 2017; Ladoucer et al., 2018; Caser et al., 2022). Similarly to our work, Oliva Branas et al. (1997) reported variability among wild populations of *A. umbelliformis*, showing a wide germination rate *in vitro*, ranging from 40% to 88%. Other parameters, such as rapid and uniform germination are essential when selecting species as putative candidates for establishing a horticultural productive system. These data are valuable not only for cultivation and seed propagation but to change the cultivation system from transplanting to direct

sowing (Aiello et al., 2014). Therefore, the half-time of germination (T_{50}) and germination period (GPD) are also important features because a population with a good germination percentage, a rapid onset of germination, and low T_{50} could be very effective. Our results are generally in line with Carlen et al. (2012) about fast germination (about 90% of seeds germinated after ten days) of *A. umbelliformis* selections in an experiment conducted in petri dishes..

Regarding the most suitable propagation and cultivation techniques, the effect of the type of substrate was significant. The ratio of peat to perlite influenced germination. As the concentration of perlite increased, the substrate turned out to be favorable for both germination and seedling development. This finding is in agreement to what previously observed by other authors (Carlen et al., 2012; Vouillamoz et al., 2015) whom highlighted that, Carlen et al. (2012) and Vouillamoz et al. (2015) highlighted that, under open field conditions, *A. umbelliformis* seeds require drained and porose soils to improve germination and growth, mimicking the soil where plants naturally germinate and develop at high altitudes. Similarly, in other *Artemisia* species, Fascella et al. (2012) indicated that the best substrate for rooting and growth development of *Artemisia arborescens* L. cuttings was 50:50 v:v peat:perlite. Thus, a substrate characterized by reduced water retention, high aeration capacity, and ultra-light weight should be preferred. Overall the Valnontey population appears to be more promising than the others, especially when cultivated in a porose and draining substrate.

In this study, the use of AMF in the early stages of seedling development showed no effects, and no visible root colonization occurred, possibly due to the very short and thin roots of the young plants. The tested period was probably not sufficient for the establishment of symbiosis.

In fact, under greenhouse conditions, AMF colonization was only slightly developed in *A. umbelliformis* after three months in trap cultures that included several native AMF, including Glomus tenue, *Glomus intraradices*, *G. claroideum/ etunicatum* and *Acaulospora* species (Binet et al., 2011). These authors also reported low AMF colonization in other *Artemisia* species, such as *A. glacialis* and *A. genipi.* Low or null colonization was also found by Read and Haselwandter (1981), with 10% of AM infection by *Rhizophagus tenuis* in *A. umbelliformis* and no AMF in *A. genipi* in the Tyrolean Central Alps in Austria. Plant species range from non-mycorrhizal to obligate, through facultative, dependency on mycorrhizal associations in some environmental settings (Johnson et al., 1997).

Thus, our results indicated that *A. umbelliformis* may be a low mycorrhiza-dependent species, as reported for other alpine plants like *Carex curvula* All., *Primula minima* L., *Festuca halleri* All., *Antennaria dioica* (L.) Gaertn. and *Campanula scheuchzeri* Vill. (Read and Haselwandter, 1981; Trappe, 1988).

However, Zubek and Błaszkowski (2009) and Huo et al. (2021) found that wild-collected *Artemisia* species from Poland and China are considered Arum-type AMF dependent and exhibit high biomass responses to certain AMF species present in natural soils (Lindsey, 1984; Smith and Smith, 1997; Shan et al., 2017). Therefore, the possibilities and advantages of bioinoculant application for improving génépi production require further research.

Conclusions

Alpine plants such as *A. umbelliformis* are particularly vulnerable to current threats to plant diversity, such as land management changes, tourism, and climate change. Moreover, the indiscriminate picking of these wild plants has diminished their already rare presence almost everywhere, and their harvesting is strictly regulated or forbidden in Switzerland and Italy, creating an urgent need to develop production systems.Information provided in this study represents an useful source for the implementation of propagation and cultivation of *A. umbelliformis*. Overall, the germination rate and the onset of germination are the main points to meet the needs of the nursery industry.

Among the studied *A. umbelliformis* wild populations, Valnontey could represent a new validated entrant for further studies, thus deepening the yield specifics and chemistry properties. Regarding the substrate, a mixture of 60% peat-based substrate and 40% perlite could be preferred for nursery propagation as it improved plant performances and seedling development. Future research can consider adding more inorganic substrate to the culture medium. The absence of AMF colonization roots during the early seedlings' development suggests that *A. umbelliformis* could be a low mycorrhiza-dependent species during the early seedling development phase.

However, AMF species could require more time for detection in the roots. Detecting wild AMF species associated with specific populations of *A. umbelliformis* would help to understand the AMF biodiversity of individuals, populations, and communities of *A. umbelliformis* plants to

determine the best AMF inoculum for further experiments.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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