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Pros and cons of using a standard protocol to test germination of alpine species

Vera Margreiter · Konrad Pagitz · Christian Berg · Patrick Schwager · Brigitta Erschbamer

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Abstract Storing seeds in seed banks is an effective way to preserve plant diversity and conserve species. An essential step towards a valuable conservation is the validation of germination. This study presents a germination screening of seeds from 255 species of the European Eastern Alps, which were to be stored at the Millennium Seed Bank (Kew, UK). The final germination percentage (FGP) was determined using a standard protocol in the laboratory. Species were classified according to species rarity, plant community, occurrence at elevation belts, bedrock types, as well as CSR strategies, and further, seed mass was examined. We could not find statistically significant differences of FGP within these classes, but 74.9% of all tested species germinated using the standard protocol, and half of them had FGP ≥ 20.1–100%. A treatment with gibberellic acid enhanced the germination in half of the species to which this treatment was applied. Common families in alpine regions, i.e. Asteraceae, Poaceae and Saxifragaceae were highlighted in terms of their germination behaviour. The results provide an evaluation of the application of standard protocols to a broad Alpine species pool on the one hand, and on the other hand, provide ecological insights of the species tested. Germination is not only one of the most important events of the reproductive cycle of plants but could also be a key feature in species' responses to changing environmental conditions.

Keywords Seed bank · Eastern alps · Plant community · Rarity · Seed mass · Strategy

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V. Margreiter (✉) · K. Pagitz · B. Erschbamer
Department of Botany, University of Innsbruck, Sternwartestr. 15, 6020 Innsbruck, Austria
e-mail: vera.margreiter4@gmail.com

K. Pagitz
e-mail: konrad.pagitz@uibk.ac.at

B. Erschbamer
e-mail: brigitta.erschbamer@uibk.ac.at

C. Berg · P. Schwager
Institute of Biology, Department of Plant Science, University of Graz, Holteigasse 6, 6020 Graz, Austria
e-mail: christian.berg@uni-graz.at

P. Schwager
e-mail: p.schwager@gmx.at

Introduction

Seed germination is one of the essential processes in a plants´ life cycle (Harper 1977; Fenner and Thompson 2005). It is highly influenced by individual species´ requirements (Baskin and Baskin 2014),

characteristics of species' niches (Schwienbacher et al. 2012; Rosbakh and Poschlod 2015; Jiménez-Alfaro et al. 2016; Tudela-Isanta et al. 2018a, b), and the environmental conditions during seed development (Donohue et al. 2010; Bernareggi et al. 2016; Fernández-Pascual et al. 2017). With increasing temperatures in the Alps (Gobiet et al. 2014), knowledge on germination traits have become highly important. Generally, for common species, germination may be enhanced by increased temperatures (Milbau et al. 2009; Mondoni et al. 2012; Rosbakh and Poschlod 2015; Orsenigo et al. 2015). However, specific germination requirements (Graae et al. 2008; Shevtsova et al. 2009) and local adaptation (Kawecki and Ebert 2004; Mondoni et al. 2009) are bottlenecks for the growth of plant populations in a changing environment.

Alpine plant species require relatively high temperatures for germination (Bliss 1958; Amen 1966; Billings and Mooney 1968; Cavieres and Arroyo 2000; Giménez-Benavides et al. 2005; Graae et al. 2008; Schwienbacher et al. 2011; Mondoni et al. 2012; Baskin and Baskin 2014), whereas species below treeline use a broader temperature range (Walder and Erschbamer 2015; Fernández-Pascual et al. 2017). If so, increasing temperatures in the course of climate warming could be beneficial for both, alpine species and upward-migrating lowland species, in contrast to habitat specialists (Casazza et al. 2014) and species with specific dormancy strategies (Mondoni et al. 2012, 2018). Field studies on that matter were hardly performed and lab studies lack a comprehensive screening of species from different elevations and plant communities in the Alps (Mondoni et al. 2011, 2012; Isselin-Nondedeu and Bédécarrats 2013; Orsenigo et al. 2015; Walder and Erschbamer 2015; Tudela-Isanta et al. 2018a, b). Here, a broad germination project can close this gap.

The germination temperature can be regarded as a proxy for the germination niche of a species ('regeneration niche' according to Grubb 1977), which represents the ability of the species to adapt to environmental conditions (Rosbakh and Poschlod 2015; Jiménez-Alfaro et al. 2016). To investigate optimal seed germination temperatures, several temperature regimes (Mondoni et al. 2012) and seed pretreatments (Fernández-Pascual et al. 2017) are needed. In cases where seeds, time or working space is limited, a standard protocol with only one temperature regime can be used. This procedure is practiced, for example, when testing the germination ability of seed lots that are to be stored in ex situ seed banks. It remains questionable whether such standard protocols can provide reasonable data on the germination ability of species and whether the results allow an ecological interpretation.

One factor that hinders the success of germination tests is seed dormancy (Baskin and Baskin 2004, 2014; Finch-Savage and Leubner-Metzger 2006). It was found that physiological dormancy occurs frequently in alpine species (Schwienbacher et al. 2011). Here, a distinction must be made between non-deep, intermediate and deep physiological dormancy (Baskin and Baskin 2004). Non-deep physiological dormancy can be broken by dry-cold storage (Wang et al. 2010). This is done by storing the seeds in paper bags in a refrigerator, which is a very simple method of releasing dormancy. In contrast, breaking dormancy in seeds with medium or low physiological dormancy requires more effort, e.g. via stratification. In this method, the seeds are stored under wet-cold conditions for a certain period of time prior to a germination test (Baskin and Baskin 2004, 2014). Furthermore, physical, or morphological dormancy and all combinations can occur. Physical dormancy is a typical feature of hard-coated seeds, such as of Fabaceae. To release this type of dormancy, the seeds can be scarified so that water can penetrate through the damaged seed coat. Morphological dormancy occurs in seeds with immature embryos. These seeds require a post-ripening phase and often some additional treatments, such as a series of cold and warm treatments or vice versa, to break the dormancy (Baskin and Baskin 2014). A generally simple and timesaving method is treatment with gibberellic acid (GA_3). This method is recommended by ENSCONET (2009a; b) when a standard protocol is used or when the type of dormancy is unknown.

Among seed traits, seed mass seems to influence germination (Schwienbacher and Erschbamer 2001; Bu et al. 2007; Erschbamer et al. 2010; Liu et al. 2013), whereby large seeds are considered to have a superior germination and survival. However, contradicting results are found in the literature, noting only weak or no influence of seed mass on germination (Vera 1997; Eriksson 1999; Schwienbacher et al. 2012; Walder and Erschbamer 2015).

The CSR strategy concept of Grime (1979) classifies the life strategies of plants as stress tolerant (S), competitive (C), ruderal (R) and intermediate strategists (e.g. CSR). Ruderal strategists do not have specific requirements for germination, while stress tolerant species may be less flexible and more demanding. In alpine environments, stress tolerant species represent the most common strategy group (Caccianiga et al. 2006).

This study presents the results of a broad-based germination screening of 255 Alpine plant species from the European Eastern Alps, including species from lowlands (dry grasslands, montane meadows), subalpine dwarf-shrub heaths, forest understorey species, species of wet habitats, tall herbs, species of alpine grasslands, rock crevices and scree sites. Seeds were collected by two partners of the "Alpine Seed Conservation and Research Network" (https://www.alpineseedconservation.eu/). The network was founded as a partner of the Millennium Seed Bank Partnership of the Royal Botanic Gardens, Kew (UK). One goal of this institution is to "store seeds of 25% of the world's flora by 2020" (Müller et al. 2017, https://www.kew.org/science/). Contemporaneously, the study of germination behaviour is one of the main tasks of current seed ecology research, with the aim to evaluate the needs of species, local adaptation and the suitability of a species for ex situ conservation (Müller et al. 2017).

Having access to various species from different habitats and elevations, the study aimed to test the germination success of these different species under a standard protocol. Only one day/night temperature regime was used to evaluate if such an approach delivers appropriate germination data for an ex situ conservation. We were interested in finding out whether the results of our standard protocol provide evidence for adaptive mechanisms of species distribution along elevation gradients and habitats, and whether conclusions can be drawn about functional effects that influence the final germination percentage (FGP). Based on this focus, we addressed the following questions: (a) Does germination differ between common, scattered, rare and endemic species? (b) Does the FGP differ between species from different plant communities and (c) from different elevation belts (e.g. alpine, subalpine and montane/colline)? (d) Are there differences between species from siliceous and calcareous bedrock? (e) Is it possible to assign a germination pattern to the CSR strategy types sensu Grime (1979)? (f) Is there a correlation between FGP and seed mass? (g) Does treatment with gibberellic acid (GA_3) enhance FGP? (h) Do important families have a consistent germination pattern among their genera/species? Answers of these questions improve the interpretation of germination data from standard protocols in terms of ecological significance and can also be useful when predicting species responses to global warming.

Material and methods

Seed collection

Seeds were collected in the European Eastern Alps with two centres in Austria, one in the western part (Tyrol) and one in the south-eastern part (Styria). The test species (Table 1) were selected in agreement with the Millennium Seed Bank (MSB), representing 255 characteristic Alpine taxa from different plant communities, endemic/rare species, and species that were not already stored in the MSB. Nomenclature of the species and affiliation to families follow Fischer et al. (2008). The seed collection was carried out in the summers of 2016, 2017 and 2018. In all three years, an above-average number of warm days combined with above-average periods of sunshine were recorded in Austria by the research institute ZAMG (https://www.zamg.ac.at). The summer of 2016 was a warm and wet summer, the summer of 2017 was wet in mountain areas, while the summer of 2018 was recorded as a summer with above-average dry periods (ZAMG 2016, 2017, 2018). Collections were done in elevations from 600 m above sea level (a.s.l.) to 2700 m a.s.l. with a central focus at the subalpine and alpine elevation belt (1900–2400 m a.s.l.). According to international standards (ENSCONET 2009a), collections were performed at the time of natural dispersal. The major fraction of the cleaned seeds was sent to the MSB. A small fraction of each collection was stored dry in paper bags in a refrigerator at + 4 °C (average relative humidity of a refrigerator ~ 40%) until the germination tests started. The storage period lasted between 1 and 7 months. Fruits such as achenes, legumes or caryopses were treated as seeds.

Table 1 Test species in alphabetical order, their family affiliation and rarity classification following Fischer et al. (2008)

Species	Family	Rarity	Community	T	R	CSR	Seed mass	FGP (%)	SE FGP	GA FGP (%)	SE GA
Achillea atrata	Asteraceae	Sca	ROSC	1	5	css	0.242	92.80	3.44		
*Achillea clavennae**	Asteraceae	Sca	GRASS	1.5	5	css	0.447	70.40	3.71		
*Achillea clusiana**	Asteraceae	end	ROSC	1.5	5	css	0.326	81.60	6.76		
Achillea moschata	Asteraceae	Com	ROSC	1	2	css	0.220	51.72	5.59		
Aconitum napellus	Ranunculaceae	Com	TALL	3	3	ccs	3.100	0.00	0.00	1.80	1.11
Aconitum variegatum	Ranunculaceae	Com	TALL	3	5	ccs	0.843	0.00	0.00	0.00	0.00
*Adenostyles alliariae**	Asteraceae	Com	TALL	2	3	ccs	1.258	45.60	5.60		
Agrostis alpina	Poaceae	Sca	GRASS	1.5	4	crs	0.277	26.40	4.12		
*Agrostis rupestris**	Poaceae	Sca	GRASS	1	2	crs	0.108	60.80	1.50		
*Allium schoenoprasum**	Alliaceae	Sca	GRASS	2.5	4	crs	0.990	56.80	6.97		
Androsace lactea	Primulaceae	Sca	GRASS	2.5	5	crs	1.013	0.00	0.00	15.58	4.66
Androsace obtusifolia	Primulaceae	Com	GRASS	1.5	2	css	0.730	0.00	0.00	0.80	0.80
Antennaria carpatica	Asteraceae	Sca	GRASS	1.5	3	crs	0.080	20.80	5.85		
Antennaria dioica	Asteraceae	Sca	SHFO	2	2	crs	0.047	86.40	3.25		
Anthericum ramosum	Anthericaceae	Sca	DRYM	3.5	4	crs	4.790	0.00	0.00	0.00	0.00
Anthyllis vulneraria ssp. *alpicola*	Fabaceae	Com	GRASS	1.5	5	crs	2.366	12.80	1.50	0.00	0.00
Aposeris foetida	Asteraceae	Sca	SHFO	2.5	4	ccs	2.170	1.60	1.55	64.24	3.28
Arabidopsis halleri	Brassicaceae	Sca	MEAD	2.5	2	crs	0.160	77.60	7.96		
*Arabis ciliata**	Brassicaceae	Sca	ROSC	2.5	4	crs	0.111	92.80	5.43		
Arabis stellulata	Brassicaceae	Sca	ROSC	1.5	5	rss	0.234	4.74	2.31	58.80	4.96
*Arenaria marschlinsii**	Brassicaceae	Rar	WET	1	3	rss	n.a	95.20	1.50		
*Armeria alpina**	Plumbaginaceae	Sca	ROSC	1	2	css	2.206	18.40	3.71		
Arnica montana	Asteraceae	Com	MEAD	2	3	crs	1.670	82.40	5.00		
*Artemisia mutellina**	Asteraceae	Sca	ROSC	1	3	rss	0.280	64.80	11.83		
Aster alpinus	Asteraceae	Sca	GRASS	2	3	crs	1.350	80.80	3.44		
Astragalus australis	Fabaceae	Rar	GRASS	1.5	4	ccs	3.110	15.20	2.33		
Athamantha cretensis	Apiaceae	Sca	ROSC	2	5	css	2.150	1.60	0.98		
*Atocion rupestre**	Caryophyllaceae	Com	ROSC	2.5	2	css	0.074	43.20	6.50		
Bartsia alpina	Orobanchaceae	Com	GRASS	2	3	rss	0.425	0.00	0.00	59.38	3.25
Blysmus compressus	Poaceae	Sca	WET	3	4	crs	0.905	10.40	3.49	0.00	0.00
Bothriochloa ischaemum	Poaceae	Sca	DRYM	4.5	3	crs	0.600	42.40	0.98		
*Buphthalmum salicifolium**	Asteraceae	Com	DRYM	3	4	crs	0.700	61.60	3.25		
Calluna vulgaris	Ericaceae	Com	SHFO	2.5	1	css	0.030	52.80	5.43		
Campanula alpina	Campanulaceae	Rar	ROSC	2	2	css	0.333	0.80	0.00	58.13	3.94
*Campanula barbata**	Campanulaceae	Com	GRASS	2	2	crs	0.050	49.60	4.66		
*Campanula cochleariifolia**	Campanulaceae	Com	ROSC	2	5	css	0.045	7.00	2.41	34.73	7.62
Campanula pulla	Campanulaceae	end	GRASS	1.5	4	css	0.132	1.60	0.98	0.00	0.00
Campanula rapunculus	Campanulaceae	Rar	DRYM	4.5	3	crs	0.040	43.20	3.44		
Campanula scheuchzeri	Campanulaceae	Com	GRASS	1.5	3	crs	0.090	7.20	2.65	90.26	3.77
*Carduus personata**	Asteraceae	Sca	TALL	2.5	4	ccr	1.709	91.20	2.94		
Carex brachystachys	Cyperaceae	Sca	GRASS	2.5	5	css	0.496	0.00	0.00	0.00	0.00

Table 1 continued

Species	Family	Rarity	Community	T	R	CSR	Seed mass	FGP (%)	SE FGP	GA FGP (%)	SE GA
Carex curvula	Cyperaceae	Com	GRASS	1	2	ccs	2.120	0.00	0.00	0.00	0.00
Carex davalliana	Cyperaceae	Com	WET	3	4	css	0.839	8.00	3.58	42.14	11.85
Carex echinata	Cyperaceae	Com	WET	3	2	css	0.840	29.60	3.49		
*Carex ferruginea**	Cyperaceae	Com	GRASS	2	4	ccs	1.210	28.00	2.83		
Carex firma	Cyperaceae	Com	GRASS	1.5	5	css	0.770	53.60	3.25		
Carex frigida	Cyperaceae	Sca	WET	1.5	4	css	0.320	2.50	2.50	5.00	2.24
Carex nigra	Cyperaceae	Com	WET	2.5	2	css	0.760	0.00	0.00	0.00	0.00
Carex panicea	Cyperaceae	Com	WET	3	4	css	2.473	0.00	0.00	0.00	0.00
*Carex parviflora**	Cyperaceae	Sca	GRASS	1	5	crs	0.450	7.20	1.96	79.68	4.94
Carex rostrata	Cyperaceae	Sca	WET	3	2	css	1.500	17.60	6.01		
Carex vesicaria	Cyperaceae	Sca	WET	3.5	3	css	1.800	0.00	0.00	0.00	0.00
Carlina acaulis	Asteraceae	Com	DRYM	3	2	crs	3.400	100.00	0.00		
Centaurea pseudophrygia	Asteraceae	Sca	DRYM	2.5	3	ccs	n.a	38.40	3.49		
Centaurea stoebe	Asteraceae	Sca	DRYM	4.5	4	crs	1.404	71.20	5.57		
Centaurium erythraea	Gentianaceae	Sca	DRYM	3.5	3	rrs	0.010	92.80	2.33		
Cerastium carinthiacum	Caryophyllaceae	Sca	ROSC	1.5	5	css	0.200	32.80	4.63		
Cerastium eriophorum	Caryophyllaceae	Sca	GRASS	1.5	4	css	0.288	54.40	8.82		
Cervaria rivini	Apiaceae	Sca	TALL	4	4	ccs	4.020	0.80	0.80	28.10	6.00
Chaerophyllum aureum	Apiaceae	Sca	TALL	3	4	ccc	8.100	0.00	0.00	0.00	0.00
Chaerophyllum villarsii	Apiaceae	Sca	TALL	2	3	ccs	7.666	0.00	0.00	0.00	0.00
Cirsium carniolicum	Asteraceae	Rar	TALL	2.5	4	ccs	5.400	40.80	0.80		
Cirsium erisithales	Asteraceae	Rar	TALL	2.5	4	ccs	1.956	55.20	4.08		
Cirsium spinosissimum	Asteraceae	Sca	TALL	1.5	3	ccs	2.168	49.60	3.71		
Comarum palustre	Rosaceae	Rar	WET	3	2	ccs	0.320	1.60	1.60	6.50	1.57
Crepis alpestris	Asteraceae	Sca	MEAD	2	5	ccs	2.319	85.60	3.71		
Crepis aurea	Asteraceae	Com	MEAD	2	3	ccs	0.856	26.40	8.73		
*Crepis conyzifolia**	Asteraceae	Rar	MEAD	2	2	ccs	2.403	87.20	3.44		
Crepis jacquinii	Asteraceae	Sca	ROSC	1.5	5	css	0.897	92.00	2.83		
Crepis kerneri	Asteraceae	Sca	GRASS	1.5	5	css	n.a	86.40	0.98		
Crepis paludosa	Asteraceae	Sca	WET	3	3	ccs	0.652	59.20	4.27		
*Crepis pyrenaica**	Asteraceae	Sca	TALL	2.5	4	ccs	3.097	36.80	6.37		
Dactylorhiza incarnata	Orchidaceae	Sca	WET	3.5	4	crs	0.001	0.00	0.00	0.00	0.00
*Dianthus alpinus**	Caryophyllaceae	end	ROSC	1	5	css	0.580	47.20	6.12		
*Dianthus superbus**	Caryophyllaceae	Sca	WET	3.5	4	crs	0.583	68.00	5.06		
Dianthus sylvestris	Caryophyllaceae	Sca	ROSC	3	3	crs	0.850	48.00	4.38		
Drosera rotundifolia	Droseraceae	Com	WET	3	1	sss	0.020	0.00	0.00	12.00	3.00
Dryas octopetala	Rosaceae	Com	GRASS	1.5	5	crs	0.372	83.20	4.96		
*Epilobium alpestre**	Onagraceae	Com	TALL	2.5	4	ccs	0.267	96.80	1.50		
Epilobium anagallidifolium	Onagraceae	Sca	WET	1.5	3	css	0.060	92.00	2.53		
Epilobium hirsutum	Onagraceae	Sca	TALL	3.5	4	ccc	0.100	99.20	0.80		
*Epilobium nutans**	Onagraceae	Rar	WET	2	2	crs	0.075	48.00	7.38		
Epilobium palustre	Onagraceae	Sca	WET	3	3	crs	0.090	4.80	2.94	72.72	5.01

Table 1 continued

Species	Family	Rarity	Community	T	R	CSR	Seed mass	FGP (%)	SE FGP	GA FGP (%)	SE GA
*Epipactis palustris**	Orchidaceae	Rar	WET	3.5	4	crs	0.004	0.00	0.00	0.00	0.00
Erigeron atticus	Asteraceae	Rar	GRASS	2	2	crs	0.234	65.60	7.65		
*Erigeron glabratus**	Asteraceae	Com	GRASS	1.5	5	css	0.199	58.40	5.31		
*Erigeron uniflorus**	Asteraceae	Sca	GRASS	1	3	css	0.180	87.20	5.85		
Eriophorum latifolium	Cyperaceae	com	WET	3	4	css	0.654	0.80	0.80	1.67	1.67
Eriophorum vaginatum	Cyperaceae	rar	WET	2.5	1	css	0.577	83.20	7.42		
Erysimum rhaeticum	Brassicaceae	sca	GRASS	3	2	crs	n.a	76.00	6.07		
Euphrasia officinalis ssp. *picta*	Orobanchaceae	sca	GRASS	2	3	rrs	n.a	0.80	0.80	11.52	3.91
*Festuca picturata**	Poaceae	sca	GRASS	1.5	2	ccs	n.a	47.20	6.50		
Festuca pseudodura	Poaceae	com	GRASS	1.5	2	ccs	0.334	29.60	9.26		
Festuca varia	Poaceae	com	GRASS	1.5	2	ccs	1.150	10.40	4.12	27.14	12.33
Galium anisophyllon	Rubiaceae	com	GRASS	2	3	ccs	0.365	27.20	4.27		
Gentiana acaulis	Gentianaceae	sca	GRASS	1.5	2	css	0.452	0.00	0.00	38.70	2.59
Gentiana bavarica	Gentianaceae	sca	WET	1.5	3	css	0.140	0.00	0.00	52.40	8.63
Gentiana clusii	Gentianaceae	sca	GRASS	1.5	5	css	0.410	0.00	0.00	47.50	2.13
Gentiana lutea	Gentianaceae	rar	GRASS	2.5	4	ccs	1.500	0.80	0.80	57.14	7.23
Gentiana nivalis	Gentianaceae	sca	GRASS	1.5	3	css	0.081	0.00	0.00	21.56	4.64
Gentiana pannonica	Gentianaceae	sca	GRASS	2	3	ccs	0.460	33.60	4.12		
Gentiana pumila	Gentianaceae	sca	WET	1.5	5	css	0.167	0.00	0.00	70.66	2.23
Gentiana utriculosa	Gentianaceae	sca	WET	2.5	4	crs	0.060	0.00	0.00	77.60	6.01
*Gentianella aspera**	Gentianaceae	com	GRASS	2.5	4	crs	0.219	0.00	0.00	12.38	2.24
*Globularia cordifolia**	Globulariaceae	com	GRASS	2.5	5	css	0.530	32.80	3.44		
Globularia nudicaulis	Globulariaceae	rar	GRASS	2	4	ccs	0.720	15.20	3.88		
Gnaphalium sylvaticum	Asteraceae	sca	SHFO	3	2	crs	0.061	94.40	0.98		
Gypsophila repens	Caryophyllaceae	sca	ROSC	2	5	crs	0.710	88.86	2.35		
Hedysarum hedysaroides	Fabaceae	sca	SHFO	1.5	4	ccs	4.125	55.20	6.12		
Helianthemum nummularium	Cistaceae	sca	GRASS	4.5	4	ccs	1.100	1.60	1.60	2.40	0.98
Helictotrichon parlatorei	Poaceae	sca	GRASS	1.5	4	css	1.789	0.80	0.80	1.60	0.98
Heracleum austriacum	Apiaceae	sca	SHFO	2.5	4	crs	5.429	0.00	0.00	9.72	2.62
Hieracium hoppeanum	Asteraceae	sca	SHFO	2.5	2	crs	0.276	57.60	6.27		
Hieracium intybaceum	Asteraceae	sca	ROSC	2	2	css	0.647	7.20	2.33	68.80	3.88
Hieracium maculatum	Asteraceae	rar	DRYM	3.5	3	css	0.523	47.20	7.09		
Hieracium racemosum	Asteraceae	rar	SHFO	4.5	2	css	0.511	56.80	6.62		
Hieracium sphaerocephalum	Asteraceae	sca	GRASS	1.5	2	css	0.128	29.60	3.49		
Hieracium umbellatum	Asteraceae	sca	SHFO	4	2	css	0.530	64.80	9.75		
Hieracium villosum	Asteraceae	com	GRASS	2	5	css	0.445	0.80	0.80	63.36	4.85
Homogyne alpina	Asteraceae	com	SHFO	2	2	crs	0.946	30.40	7.86		
*Homogyne discolor**	Asteraceae	rar	GRASS	1.5	4	crs	0.891	13.89	4.00	66.00	2.00
Hornungia alpina	Brassicaceae	com	ROSC	1	4	rss	0.318	37.60	2.99		
*Hypochaeris uniflora**	Asteraceae	sca	MEAD	2	2	crs	3.800	60.80	8.14		
Juncus trifidus	Juncaceae	sca	GRASS	1.5	1	css	0.176	0.00	0.00	14.56	2.23

Table 1 continued

Species	Family	Rarity	Community	T	R	CSR	Seed mass	FGP (%)	SE FGP	GA FGP (%)	SE GA
Knautia maxima	Dipsacaceae	com	TALL	3	3	ccs	3.225	12.00	3.58	96.36	2.23
Kobresia myosuroides	Cyperaceae	sca	GRASS	1.5	3	ccs	0.655	0.80	0.80	19.68	4.01
*Kobresia simpliciuscula**	Cyperaceae	rar	WET	1.5	4	css	0.491	88.00	3.10		
Koeleria hirsuta	Poaceae	rar	ROSC	1.5	2	css	n.a	85.33	6.67		
Laserpitium halleri	Apiaceae	sca	GRASS	2	2	ccs	6.829	0.00	0.00	1.00	1.00
*Laserpitium krapfii**	Apiaceae	rar	SHFO	3	3	ccs	n.a	0.00	0.00	0.00	0.00
Laserpitium latifolium	Apiaceae	sca	SHFO	3	4	ccs	9.680	0.00	0.00	0.00	0.00
Leontopodium alpinum	Asteraceae	rar	GRASS	1.5	4	ccs	0.127	74.40	3.25		
Leucanthemopsis alpina	Asteraceae	com	GRASS	1	2	ccs	0.380	29.29	3.45		
Leucanthemum atratum	Asteraceae	end	GRASS	1.5	5	css	0.578	95.20	1.50		
Leucanthemum vulgare	Asteraceae	com	MEAD	3	3	crs	0.420	92.80	1.96		
Linaria alpina	Antirrhinaceae	com	ROSC	1.5	4	rrs	0.278	0.00	0.00	89.38	1.69
Linum alpinum	Linaceae	sca	GRASS	2	4	crs	1.883	52.00	3.10		
Luzula alpina	Juncaceae	sca	GRASS	1.5	2	crs	0.257	86.40	2.71		
*Luzula alpinopilosa**	Juncaceae	sca	GRASS	1	2	css	0.200	54.40	2.99		
*Luzula glabrata**	Juncaceae	sca	GRASS	1.5	4	css	0.281	6.40	3.49		
Luzula spicata	Juncaceae	sca	GRASS	1.5	2	crs	0.340	0.00	0.00	0.80	0.80
*Luzula sudetica**	Juncaceae	rar	WET	2	2	crs	0.373	67.20	4.63		
Menyanthes trifoliata	Menyanthaceae	rar	WET	3	3	css	2.400	0.00	0.00	15.20	2.65
*Minuartia austriaca**	Caryophyllaceae	sca	ROSC	1.5	5	css	0.290	0.80	0.80	0.76	0.80
Minuartia cherlerioides	Caryophyllaceae	rar	ROSC	1	5	css	0.061	10.40	6.76	82.24	5.66
Minuartia sedoides	Caryophyllaceae	sca	GRASS	1	3	css	0.258	7.20	2.65		
Moehringia ciliata	Caryophyllaceae	sca	ROSC	1	4	css	0.387	0.00	0.00	1.60	0.98
Molinia arundinacea	Poaceae	sca	SHFO	3.5	4	css	n.a	4.00	1.79	12.75	2.66
*Mutellina adonidifolia**	Apiaceae	com	SHFO	1.5	2	ccs	2.185	0.00	0.00	0.00	0.00
Noccaea crantzii	Brassicaceae	end	GRASS	2	5	crs	0.828	7.20	1.50	90.30	3.73
*Oreochloa disticha**	Poaceae	sca	GRASS	1	1	crs	0.373	20.00	4.90		
Orobanche flava	Orobanchaceae	sca	TALL	2.5	4	rss	0.006	0.00	0.00	0.00	0.00
Orobanche salviae	Orobanchaceae	rar	SHFO	3	4	rss	n.a	0.00	0.00	0.00	0.00
*Oxytropis montana**	Fabaceae	sca	GRASS	1.5	5	ccs	3.253	6.40	0.98	0.00	0.00
Pachypleurum mutellinoides	Apiaceae	rar	GRASS	1	3	crs	1.020	0.80	0.80	7.26	2.19
*Papaver alpinum**	Papaveraceae	sca	ROSC	1.5	4	sss	0.143	15.20	3.44	84.15	1.64
*Parnassia palustris**	Parnassiaceae	sca	WET	2	4	css	0.030	60.00	6.69		
Pedicularis palustris	Orobanchaceae	rar	WET	3	3	css	0.829	0.00	0.00	8.00	2.19
Pedicularis portenschlagii	Orobanchaceae	end	GRASS	1	3	css	0.646	1.60	0.98	81.28	3.76
Pedicularis recutita	Orobanchaceae	sca	TALL	2	4	css	0.820	0.00	0.00	57.76	4.22
Pedicularis rostratocapitata	Orobanchaceae	com	GRASS	1.5	5	css	1.055	0.00	0.00	53.50	3.32
Petasites paradoxus	Asteraceae	com	TALL	2	5	ccs	0.476	2.40	1.60		
*Peucedanum oreoselinum**	Apiaceae	sca	SHFO	4.5	3	ccs	4.520	56.80	6.37		
Peucedanum ostruthium	Apiaceae	sca	TALL	2	3	ccc	1.208	26.40	4.12		
Peucedanum verticillare	Apiaceae	rar	TALL	4	4	ccs	n.a	72.00	5.51		
Phleum hirsutum	Poaceae	sca	TALL	2	4	ccs	0.169	89.60	2.99		

Table 1 continued

Species	Family	Rarity	Community	T	R	CSR	Seed mass	FGP (%)	SE FGP	GA FGP (%)	SE GA
Phleum rhaeticum	Poaceae	com	TALL	2	3	ccs	0.464	67.13	5.34		
Phyteuma betonicifolium	Campanulaceae	sca	TALL	2	2	ccs	0.045	3.20	1.50	67.82	14.11
Phyteuma confusum	Campanulaceae	sca	GRASS	1.5	2	css	0.043	8.00	2.53	89.78	4.10
*Phyteuma globulariifolium**	Campanulaceae	sca	GRASS	1	2	css	0.052	3.20	1.50	81.23	4.61
Phyteuma hemisphaericum	Campanulaceae	com	GRASS	1.5	1	css	0.101	21.60	4.49		
*Phyteuma orbiculare**	Campanulaceae	sca	MEAD	2.5	4	css	0.215	0.00	0.00	74.97	1.64
Phyteuma ovatum	Campanulaceae	sca	TALL	2	3	ccs	0.180	0.00	0.00	84.68	4.56
*Pinguicula leptoceras**	Lentibulariaceae	rar	WET	1.5	3	sss	0.020	4.00	2.19	66.15	5.79
Plantago alpina	Plantaginaceae	rar	GRASS	1.5	2	css	0.600	42.40	3.71		
Poa alpina	Poaceae	com	GRASS	1.5	3	crs	0.354	48.80	7.74		
Poa chaixii	Poaceae	rar	TALL	2.5	2	ccs	0.503	5.60	2.71	95.00	2.74
Poa minor	Poaceae	sca	GRASS	1	5	css	0.299	84.80	6.37		
*Potentilla aurea**	Rosaceae	com	MEAD	1.5	2	ccs	0.368	35.20	3.88		
Potentilla brauneana	Rosaceae	rar	GRASS	1	5	css	0.615	0.80	0.80	46.96	4.43
*Potentilla clusiana**	Rosaceae	end	ROSC	1.5	5	css	0.373	25.60	5.74		
*Potentilla frigida**	Rosaceae	rar	GRASS	1	2	css	0.200	3.20	0.80	6.93	2.26
*Potentilla grandiflora**	Rosaceae	sca	GRASS	1.5	2	ccs	0.449	8.80	3.20	48.00	13.80
Primula auricula	Primulaceae	sca	GRASS	1.5	5	css	0.250	0.00	0.00	87.14	3.18
*Primula clusiana**	Primulaceae	end	GRASS	1.5	5	css	0.315	32.00	8.10		
Primula elatior	Primulaceae	com	MEAD	3	4	ccs	0.800	0.00	0.00	35.40	3.28
Primula farinosa	Primulaceae	rar	WET	2	4	css	0.060	5.60	2.71	97.60	1.60
Primula glutinosa	Primulaceae	sca	GRASS	1	2	css	0.121	5.60	1.60	16.00	8.00
*Primula minima**	Primulaceae	com	GRASS	1.5	2	sss	0.123	25.38	2.96		
*Pulsatilla alpina**	Ranunculaceae	sca	SHFO	2	4	crs	6.680	0.00	0.00	69.10	8.29
Pulsatilla alpina ssp. *Apiifolia**	Ranunculaceae	sca	SHFO	2	2	crs	n.a	0.00	0.00	63.00	13.00
Ranunculus alpestris	Ranunculaceae	com	GRASS	1.5	4	css	0.278	18.40	6.40		
Ranunculus montanus	Ranunculaceae	com	MEAD	2	4	ccs	1.547	0.00	0.00	54.76	2.72
*Rhododendron ferrugineum**	Ericaceae	com	SHFO	2	2	ccs	0.027	38.40	3.71		
*Rhododendron hirsutum**	Ericaceae	com	SHFO	2	4	ccs	0.029	38.40	4.31		
*Rhodothamnus chamaecistus**	Ericaceae	sca	SHFO	2	4	ccs	0.040	0.00	0.00	84.00	7.04
*Salix helvetica**	Salicaceae	sca	SHFO	1.5	2	ccs	n.a	0.00	0.00		
*Salix pentandra**	Salicaceae	rar	SHFO	2.5	3	ccc	0.096	91.20	2.94		
Saponaria pumila	Caryophyllaceae	com	GRASS	1	2	css	1.210	0.00	0.00	10.16	2.59
Saussurea pygmaea	Asteraceae	rar	GRASS	1	4	css	4.750	95.20	2.33		
Saxifraga adscendens	Saxifragaceae	rar	ROSC	1.5	4	rss	0.011	39.00	2.52		
Saxifraga aizoides	Saxifragaceae	com	WET	2	4	css	0.054	83.00	1.91		
Saxifraga androsacea	Saxifragaceae	sca	ROSC	1	4	sss	0.036	0.00	0.00	25.00	4.50
Saxifraga aspera	Saxifragaceae	sca	ROSC	2	2	css	0.015	0.00	0.00	0.00	0.00
Saxifraga biflora	Saxifragaceae	sca	ROSC	1	4	css	0.016	11.33	7.33	63.53	6.70
Saxifraga bryoides	Saxifragaceae	com	ROSC	1	2	css	0.042	0.00	0.00	2.17	2.17
Saxifraga burseriana	Saxifragaceae	rar	ROSC	2	5	sss	0.052	0.00	0.00	33.03	2.97

Table 1 continued

Species	Family	Rarity	Community	T	R	CSR	Seed mass	FGP (%)	SE FGP	GA FGP (%)	SE GA
Saxifraga caesia	Saxifragaceae	com	GRASS	1.5	5	sss	0.024	14.00	2.58	55.60	6.58
Saxifraga exarata	Saxifragaceae	rar	ROSC	1	2	sss	0.055	0.00	0.00	4.00	1.63
Saxifraga hostii	Saxifragaceae	end	GRASS	2	5	sss	0.055	0.00	0.00	12.87	4.04
Saxifraga moschata	Saxifragaceae	com	ROSC	1	4	sss	0.040	22.00	4.16		
Saxifraga mutata	Saxifragaceae	sca	ROSC	2.5	4	sss	0.030	38.00	7.75		
Saxifraga oppositifolia	Saxifragaceae	com	ROSC	1	4	css	0.014	0.00	0.00	29.33	3.71
Saxifraga paniculata	Saxifragaceae	com	ROSC	2	4	sss	0.040	0.00	0.00	19.35	2.89
Saxifraga rotundifolia	Saxifragaceae	sca	TALL	2.5	3	ccs	0.039	96.00	2.83		
Saxifraga rudolphiana	Saxifragaceae	end	ROSC	1	4	css	0.070	80.00	1.63		
Saxifraga sedoides	Saxifragaceae	sca	ROSC	1.5	5	css	0.077	0.00	0.00	15.13	1.85
Saxifraga seguieri	Saxifragaceae	rar	ROSC	1	2	css	0.037	1.00	1.00	47.36	4.37
Saxifraga squarrosa	Saxifragaceae	end	ROSC	2	5	sss	0.029	29.00	8.70		
Saxifraga stellaris	Saxifragaceae	com	WET	2	3	css	0.040	93.00	3.42		
Saxifraga tridactylites	Saxifragaceae	rar	DRYM	4.5	4	rrs	0.011	0.00	0.00	54.48	10.11
*Scabiosa lucida**	Dipsacaceae	sca	GRASS	1.5	4	crs	1.841	52.80	6.37		
Scorzonera humilis	Asteraceae	sca	WET	3.5	4	ccs	4.068	3.20	1.50		
Scorzoneroides crocea	Asteraceae	rar	MEAD	2	3	crs	2.198	51.20	4.27		
Scorzoneroides helvetica	Asteraceae	com	MEAD	1.5	2	crs	1.570	73.60	2.04		
Sedum album	Crassulaceae	com	ROSC	3	4	sss	0.040	72.00	5.51		
Senecio abrotanifolius ssp. *abrotanifolius**	Asteraceae	sca	SHFO	2	4	css	1.989	53.60	8.16		
Senecio abrotanifolius ssp. *tiroliensis*	Asteraceae	sca	SHFO	1.5	2	css	n.a	20.80	2.65		
Senecio doronicum	Asteraceae	sca	GRASS	1.5	4	css	1.800	13.60	4.49	65.34	6.80
Seseli libanotis	Apiaceae	sca	DRYM	3	4	ccs	1.309	8.80	3.20	28.80	2.94
Sesleria ovata	Poaceae	sca	ROSC	1	5	css	0.008	0.00	0.00		
Silene acaulis ssp. *exscapa*	Caryophyllaceae	sca	GRASS	1	2	css	0.419	32.00	6.57		
Silene acaulis ssp. *longiscapa**	Caryophyllaceae	com	GRASS	1	4	css	0.379	39.20	4.80		
Silene nutans	Caryophyllaceae	com	SHFO	3	3	ccs	0.368	69.60	6.01		
*Silene vulgaris**	Caryophyllaceae	com	MEAD	3	3	crs	1.535	81.60	9.17		
Soldanella alpina	Primulaceae	com	SHFO	2	3	css	0.240	83.20	1.50		
*Soldanella pusilla**	Primulaceae	com	GRASS	1.5	2	css	0.069	84.00	2.83		
*Solidago virgaurea**	Asteraceae	com	SHFO	3.5	3	crs	0.690	75.86	4.96		
*Tofieldia calyculata**	Tofieldiaceae	sca	WET	2.5	4	css	0.030	74.62	6.26		
*Tofieldia pusilla**	Tofieldiaceae	rar	WET	1.5	3	css	0.035	92.80	2.33		
Trichophorum alpinum	Cyperaceae	rar	WET	3	2	css	0.182	0.00	0.00	1.67	3.73
*Trichophorum cespitosum**	Cyperaceae	sca	WET	2.5	3	css	0.584	0.00	0.00	0.00	0.00
Trifolium badium	Fabaceae	sca	GRASS	2	4	crs	0.750	55.20	3.44		
*Triglochin palustris**	Juncaginaceae	sca	WET	2.5	4	sss	1.761	54.40	5.60		
Trollius europaeus	Ranunculaceae	sca	MEAD	2.5	3	ccs	0.810	0.80	0.80	41.50	2.01
*Valeriana celtica**	Valerianaceae	end	GRASS	1	2	css	0.675	0.00	0.00		
*Valeriana elongata**	Valerianaceae	end	ROSC	2	5	crs	n.a	9.60	2.40		

Table 1 continued

Species	Family	Rarity	Community	T	R	CSR	Seed mass	FGP (%)	SE FGP	GA FGP (%)	SE GA
Valeriana montana	Valerianaceae	com	ROSC	2	5	css	1.103	4.80	2.94	35.80	6.53
Valeriana supina	Valerianaceae	end	ROSC	1	5	css	1.232	3.20	1.50		
Valeriana tripteris	Valerianaceae	com	TALL	2.5	3	css	0.926	7.20	2.33	31.10	5.03
Veratrum album	Melanthiaceae	sca	TALL	2.5	4	ccs	7.060	0.00	0.00	3.08	1.28
Veronica alpina	Antirrhinaceae	com	GRASS	1.5	2	css	0.040	80.00	5.66		
Veronica aphylla	Antirrhinaceae	com	GRASS	1.5	5	css	0.099	0.00	0.00	3.20	2.33
*Veronica bellidioides**	Antirrhinaceae	sca	GRASS	1	1	css	0.110	0.80	0.80	68.46	4.63
Veronica fruticans	Antirrhinaceae	sca	GRASS	1.5	3	css	0.124	1.80	0.98	4.06	2.19

Community affiliation, temperature value (T), soil reaction value (R) and CSR strategy type were extracted from Landolt et al. (2010) and grouped as described in Material and Methods. Seed mass (mg/seed) was determined by seed collectors or extracted from data bases. Germination was tested in a growth cabinet using a standard protocol with one alternating temperature 25/15 °C or, if marked with asterisks (*) with 20/10 °C. Final germination percentage (FGP %) is calculated as germinated seeds from the initial number of seeds laid per species (see Supplementary Material Table S1). If FGP was below 15%, a treatment with gibberellic acid (GA) was applied

Elevation values (T) 1–1.5 = nival-alpin, 2–2.5 = subalpine, 3–4.5 = montane-colline. Bedrock values (R) 1 = acidic, 2–3 = acidic-neutral, 4–5 = neutral-alkaline. Stress tolerant (S) = SSS, CSS, RSS; competitive (C) = CCC, CCS, CCR; intermediate strategists CSR; ruderal (R) = RRR, RRS, RRC

com common, *sca* scattered, *rar* rare, *end* endemic, *ROSC* rocky habitats, scree sites, *GRASS* alpine grasslands above treeline, *WET* bogs, mires, wetlands, *SHFO* scrub heaths, understorey of forests, subalpine meadows, *TALL* tall forbs, *DRYM* dry meadows of low elevations, *MEAD* meadows of the colline and montane belt, *n.a.* not available, *SE* Standard error

Germination protocol

The germination tests were carried out at the laboratory in Innsbruck, following a standard protocol. Tests were done on Petri dishes filled with three layers of filter paper moistened with deionized water. In each Petri dish, 25 seeds were laid and five Petri dishes per species were prepared. Depending on seed availability, either the number of seeds laid per Petri dish or the number of Petri dishes was adjusted (total numbers of seed laid are given in Supplementary Material Table S1). The Petri dishes were then put in a growth cabinet (SANYO MLR-350H, Sanyo Electric Biomedical Co Ltd, Japan) under 16 h day (20,000 l×) and 8 h night (0 l×) regime with 60% relative air humidity. The maximum photosynthetic photon flux density in the growth cabinet was 180 µmol m^{-2} s^{-1}. Initially, a day/night temperature regime of 20/10 °C was used (see species with * in Table 1) according to former germination protocols for alpine species (Schwienbacher and Erschbamer 2001; Schwienbacher et al. 2012; Walder and Erschbamer 2015). Later, the setting was changed to a day/night temperature regime of 25/15 °C according to the protocol of ENSCONET (2009b). Due to former experiences with alpine species (Schwienbacher and Erschbamer 2001; Erschbamer and Pfattner 2002) and due to technical reasons, we did not stratify the seeds. The only exceptions were *Saxifraga* species; for them we applied cold-wet stratification in the fridge at + 4 °C for 12 weeks.

The number of germinated seeds was counted every 3–4 days and germinated seeds (radicle emergence at least half as long as the seed) were removed. Seeds infested with fungi were subjected to a pressure test using tweezers and, in the case of softness, removed from the Petri dish and counted as non-viable. Using the same method, non-germinated seeds were checked for vitality at the end of the test period and were counted as non-viable if not passing the test. One germination period lasted for 30 days. The final germination percentage (FGP) after 30 days was calculated as germinated seeds from the initial number of seeds laid, pooled from all Petri dishes, representing a mean per species.

Seeds of species with no or ≤ 15% germination were soaked in gibberellic acid (0.001 molar GA_3, 10 ml) for 3 days in the growth cabinet to stimulate

their germination, assuming non-deep physiological dormancy (Baskin and Baskin 2014). After 3 days, seeds were washed with deionized water and were again laid out on Petri dishes with three layers of filter paper in the growth cabinet. Final germination percentage after GA_3 treatment (GA FGP) was determined after another 30 days. For species of the families Cyperaceae and Apiaceae from the 2018 collection, parallel GA_3 treatment to the standard tests were performed due to the availability of enough seeds. For 10 species with zero or $\leq$ 15% FGP, GA_3 treatment was not possible due to scarcity of seeds. Numbers of seeds laid, germinated seeds and non-viable seeds are given in Supplementary Material Table S1.

Classifications

Similarly to Alsos et al. (2013), species with FGP $\leq$ 10.0% were classified as very weak germinators, FGP of 10.1–20.0% as low germinators, 20.1–50.0% as intermediate ones and those with 50.1–100% as species with high germination. Species rarity was extracted from Fischer et al. (2008) and the following classes were defined: com (common, species occurring very frequently in the Eastern Alps); sca (scattered, species not very frequent, but in some regions of the Eastern Alps they may be locally common); rar (rare); end (endemic for the North-Eastern or South Alps). A classification of the test species addressing their ecology was done using ecological indicator values (i.e. temperature (T), soil reaction (R), CSR strategy) and affiliation to plant communities provided by (Landolt et al. 2010). The test species were grouped in seven plant communities: ROSC = rocky habitats, scree sites; GRASS = alpine grasslands above treeline; WET = bogs, mires, wetlands; SHFO = scrub heaths, understorey of forests, subalpine meadows; TALL = tall forbs; DRYM = dry meadows of low elevations; MEAD = meadows of the colline and montane belt. Temperature values (T) act as a proxy for the elevation distribution of a species, they were grouped as follows: 1–1.5 = niv-alp (nival, upper alpine belt, lower alpine belt); 2–2.5 = subalpine (subalpine belt, upper montane belt); 3–4.5 = mon-coll (montane belt, colline belt). Ranges of soil reaction (R) represent the bedrock on which the species mostly occurs. These values were distinguished as: 1 = acidic (very acidic and acidic); 2–3 = aci-neu (weakly acidic, neutral to alkaline); 4–5 = neu-alk (alkaline). CSR strategies were grouped in S = stress tolerant (SSS, CSS, RSS); C = competitive (CCC, CCS, CCR); CSR = intermediate strategists; R = ruderal (RRR, RRS, RRC). Further, seed mass (mg/seed) was either determined by seed collectors, or extracted from the following databases: KEW Seed Information Database (SID), The LEDA Traitbase, BiolFlor Database, The VISTA Plant Trait Database, Ecological Flora of the British Isles.

Data analyses

We defined FGP $\geq$ 20.1% (i.e. species with intermediate and high germination) as successful proof of the functioning of the standard protocol. For species with FGP $\leq$ 20.0%, we assumed that the standard protocol did not work and therefore excluded these species from further analyses. In addition, the strategy type R was excluded from the analyses, because this group contained only one species with FGP $\geq$ 20.1% (*Centaurium erythraea*). Using this reduced dataset, FGP (species means) was analysed by a generalized linear model (GLM) with a quasi-binomial setting and a logit-link function. A binomial distribution is typically used for proportion data such as germinated seeds from seeds laid. The model showed an over-dispersion. Therefore, a quasi-binomial setting was used. This setting accounts for over-dispersion by means of a dispersion parameter that allows additional variance in the data and returns adjusted standard errors.

The full model included all factors, i.e. rarity (sca, com, rar, end), plant community (ROSC, GRASS, WET, SHFO, TALL, DRYM, MEAD), elevation belt (niv-alp, subalpine, mon-coll), bedrock type (acidic, aci-neu, neu-alk), strategy type (S, C, CSR) and seed mass. F-test was used to test the significance of the factors in the model. Post hoc tests with Bonferroni-Holm correction were applied to test for significances between the levels of the factors. The significance level was set to alpha 0.05; the model was verified via diagnostic residual plots. Because only one factor showed significance, the factors were ranked according to least significance and were successively excluded from the model to check the influence of the factors on the model. This procedure was done to prevent an over-fitting of the model, but as this did not lead to any changes in factor level differences

compared to the full model, the full model was chosen as the final one.

FGP for species from the families Asteraceae, Poaceae, and Saxifragaceae are visually shown to highlight the germination behaviour of these families.

The analyses were performed in R (version 3.6.1, R Core Team 2020) and RStudio (version 1.2.1568, RStudio 2016) with the MASS package (Venables and Ripley 2002). For data manipulation, we used the packages tidyverse (Wickham 2017) and reshape (Wickham 2018a), for descriptive statistics the package pastecs (Grosjean et al. 2018). For data visualization, we used the packages ggplot2 (Wickham and Winston 2019), gridExtra (Auguie and Antonov 2017) and scales (Wickham 2018b).

Results

In total, 255 species out of 39 families were tested. Regarding the germination classes, 74.9% germinated under the standard protocol, and 25.1% failed to germinate (Table 1, Fig. 1a). From the 255 species, 31.4% had a high FGP. Only one species among the tested (*Carlina acaulis*) showed 100% FGP. Intermediate germination was observed in 17.6%, low germination was noted for 6.3%, and 19.6% had a weak germination. Scattered species represented about half of the species (48.6%, Fig. 1b), being the largest group in this class. Species from alpine grasslands represented 35.7% of the species tested (Fig. 1c); 43.9% were classified as species from the niv-alp elevation belt (Fig. 1d). Of all species tested, only 2.8% were classified as species on very acidic bedrock (Fig. 1e), while the classes aci-neu and neu-alk contained 47.1% and 50.2% of species, respectively. Half of the species tested (50.6%) was classified as being stress tolerant (S, Fig. 1f), and only 1.6% represented the ruderal strategy type (R). Seed mass ranged from 0.001 to 9.68 mg/seed, with *Dactylorhiza incarnata* having the lightest seeds and *Laserpitium latifolium* the heaviest.

The mean FGP was analysed by a GLM using a dataset of 124 species, comprising species with FGP $\geq$ 20.1%. Strategy type was the only significant factor in the model (Table 2). However, post hoc tests did not reveal any significances between the strategy type factor levels. We could not find any statistically significant effects of rarity, plant community,

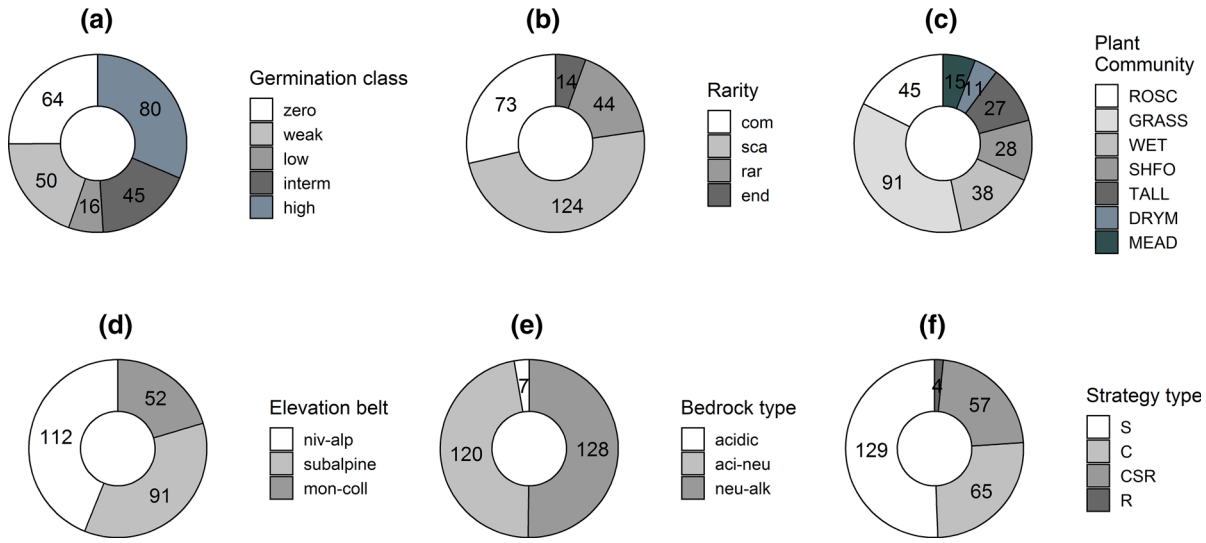

Fig. 1 Species tested using the standard protocol (total 255 species) and classifications made in the study. The graphs present the numbers of species per class: **a** Germination class defined as zero: FGP = 0.0%, weak: FGP $\leq$ 10.0%, low: FGP $\geq$ 10.1 $\leq$ 20.0%, intermediate: FGP $\geq$ 20.1 $\leq$ 50.0% and high: FGP $\geq$ 50.1–100.0%. **b** Rarity (*com* common, *scat* scattered, *rar* rare, *end* endemic). **c** Plant community (*ROSC* rocky habitats, scree sites, *GRASS* alpine grasslands above treeline, *WET* bogs, mires, wetlands, *SHFO* scrub heaths, understorey of forests, subalpine meadows, *TALL* tall forbs, *DRYM* dry meadows of low elevations, *MEAD* meadows of the colline and montane belt). **d** Elevation belt (nival, subalpine, montane-colline). **e** Bedrock type (acidic, acidic-neutral, acidic-alkaline), and **f** Strategy type *S* stress tolerant, *C* competitive, *CSR* intermediate, *R* ruderal strategy

Table 2 Analyses of deviance-table listing the effects of tested factors on species mean FGP, comprising species with FGP ≥ 20.1%

Factors	Df	Deviance	*F* value	*p* value	Label
Rarity	3	16.604	0.816	0.488	n.s
Plant community	6	56.765	1.395	0.224	n.s
Elevation belt	2	7.374	0.544	0.582	n.s
Bedrock type	2	15.817	1.166	0.316	n.s
Strategy type	3	42.312	3.119	0.049	*
Seed mass	1	3.201	0.472	0.494	n.s

A GLM with quasi-binomial error and logit-link function was used to analyse the data; F-test was used to test the significance of the factors in the model

Dispersion parameter in the GLM was taken to be 6.8

Df degrees of freedom; *n.s.* not statistically significant

Significance levels are labelled as. $p \sim 0.05$, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

elevation belt, bedrock type or seed mass on FGP. Consequently, post hoc tests on the single factors per class resulted in non-significant differences. Common and endemic species had the lowest mean FGP (55.4% and 55.8%, respectively), and rare species the highest (67.7%; Fig. 2a). Within plant communities (Fig. 2b), the lowest FGP was found in GRASS species (54.7%), the highest in the WET community class (72.5%). In contrast to this high FGP, the WET class had the highest proportion of non-germinating, weak and low germinating species (i.e. 23 out of 38 species, Fig. 1c and Fig. 2b). Species from the mon-coll elevation belt had the highest mean FGP (67.4%) followed by subalpine species (60.5%) and niv-alp species (58.5%; Fig. 2c). Species classified to the acidic bedrock type had the lowest FGP (52.5%), neu-alk the highest (62.5%, Fig. 2d). Regarding strategy types (Fig. 1e), C strategists had the lowest (56.7%) and intermediate strategists the highest FGP (65.2%, Fig. 2e). Seed mass (Fig. 2f) of species with FGP ≥ 20.1% ranged from 0.01 mg/seed (*Saxifraga adscendens*) to 5.40 mg/seed (*Cirsium carniolicum*).

A treatment with gibberellic acid (GA_3) was applied to 44.7% out of 255 species (Fig. 3). Germination was stimulated in half of them (50.8% out of 114 species), resulting in GA FGPs of 20.1–97.6%. The other half of the GA_3 tested species comprised 29.8%, classified as low and weak germinators, and 19.3% did not germinate (Fig. 3). Illustrations of GA FGP of the species according to the classifications can be found in the Supplementary Material Fig. S1.

The most abundant species collected were from the family Asteraceae (53 species, Fig. 4), whereby 86.8% had FGP ≥ 20.1–100%, and 13.2% had low and weak germination. None of the Asteraceae species was noted to have zero germination using the standard protocol. Poaceae species (17 species) germinated with a similar pattern to Asteraceae species (Fig. 5). 58.8% had high to intermediate germination, 35.3% had low and weak germination, and only one species did not germinate under the standard protocol (*Sesleria ovata*). Species among the family Saxifragaceae (21 species) were cold stratified before the germination test. Here, 38.1% had FGP ≥ 20.1%, and 47.6% did not germinate under the standard protocol (Fig. 6a). On 13 species a GA_3 treatment was applied (Fig. 6b), which enhanced the germination of 12 species, one did not germinate at all (*Saxifraga aspera*).

Discussion

In total, 74.9% of all investigated species germinated using the standard protocol, and among them, 49% had an intermediate to high FGP. If it is noted that half of the species germinate, this is a fairly good result, indicating that the collected seeds were in good condition and able to germinate without pre-treatment (except for the Saxifragaceae which were stratified). High-quality seeds are mandatory for ex situ seed banking. Alsos et al. (2013) suggested to consider the annual variation of seed production and to extend seed

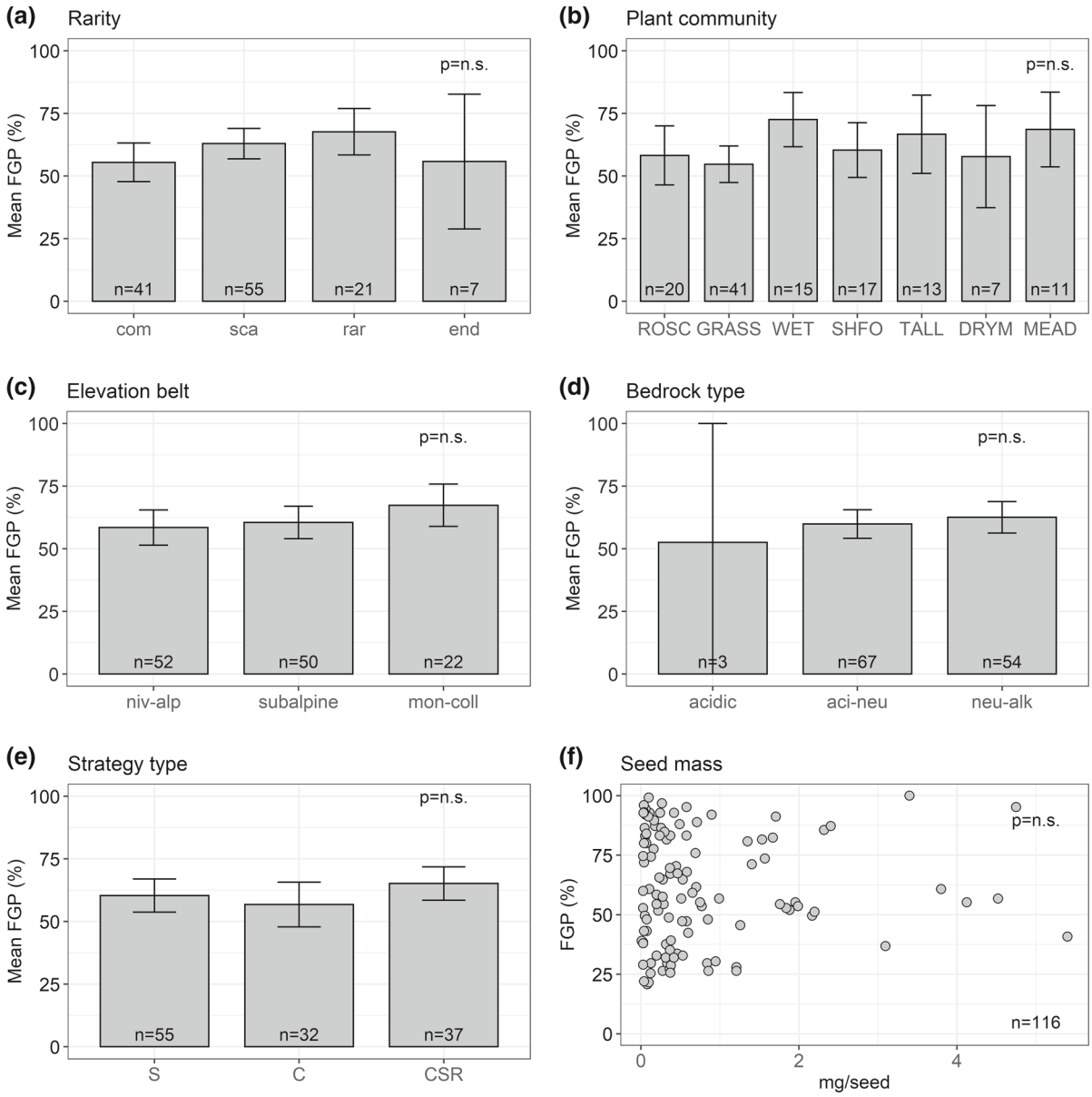

Fig. 2 Final germination percentage (FGP) of intermediate and high germinating species (in total 124 species), tested using the standard protocol and analysed via a GLM. Comparisons between the levels of the classes **a** Rarity, **b** Plant community, **c** Elevation belt, **d** Bedrock type and **f** Strategy type were made with Post hoc tests and Bonferroni-Holm correction. For abbreviations see Fig. 1 or Material and Methods. *n* number of species per class. In **e** Seed mass was not available for 8 species. Error bars show confidence intervals (95%). *p* indicates the *p* value label from the post hoc tests, *n.s.* not statistically significant

collections over several seasons to overcome possible insufficient germination from one season. This would further increase the genetic diversity of the stored seeds. From our field collections, we have learned that several rare and endemic species often produce seeds in small quantities and over such a long ripening period that the necessary amount of seeds for seed banking and germination trials cannot be obtained within one year. A complete ex situ seed bank of rare and endemic species seems therefore hardly possible. Nonetheless, seed banks make an important contribution to the conservation of these species, but strict

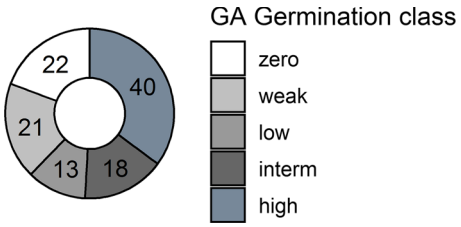

Fig. 3 Numbers of species tested with a gibberellic acid treatment (GA) using the standard protocol (total 114 species), and grouped in the germination classes zero: FGP = 0.0%, weak: FGP ≤ 10.0%, low: FGP ≥ 10.1 ≤ 20.0%, intermediate: FGP ≥ 20.1 ≤ 50.0% and high: FGP ≥ 50.1–100.0%

conservation measures in the field are indispensable, including the protection of entire plant communities.

The standard protocol used in this study with only one temperature regime, worked well in a high percentage of species, but 25.1% did not germinate. This amount of non-germinating species weakens an ecological interpretation of the results regarding germination success. It seems to be mandatory to perform germination trials at least under two (or preferably more) different temperature regimes to fully cover the germination niche of a species, or to identify unknown germination needs. In addition, seed pre-treatments, such as cold-wet stratification and scarification, would have been necessary for at least some of the species studied. For technical reasons, many species have been tested after a relatively long storage period (dry-cold). In some of these cases, storage may have induced seed dormancy (Baskin and Baskin 2014). Considering these limitations of the study, we must interpret our results with caution.

(a) A classification of the tested species into common, scattered, rare and endemic species did not reveal differences of FGP. Despite the uneven quantity of species within these classes, about 50% of the species in each class germinated, and about 50% did not germinate. This may indicate that widespread species and species with a small distribution range do not have different germination temperature requirements, or these results may indicate that there are differences. For ex situ seed banking, these results could mean a 50/50 chance of germination success for rare and endemic Alpine species, when testing under a single standard protocol. Further, this strongly suggests applying more temperature regimes if seeds are available. In any case, besides germination in the laboratory, field experiments to analyse recruitment success under different environmental conditions are necessary, to test the performance and population persistence of these groups (Margreiter et al. in review).

(b) Although germination traits are important drivers of community composition and species persistence (Donohue et al. 2010; Jiménez-Alfaro et al. 2016; Tudela-Isanta et al. 2018a, b), no clear effect of habitat on germination was found in our study. The highest germination was recorded for species from WET environments (although several *Carex* species did not germinate, Table 1) and from MEAD. The conditions for seed development might have been favourable in these habitats, whereas in other habitats the growing seasons are often too short (ROSC, GRASS) or unfavourable for a complete seed maturation (DRYM, TALL, SHFO). This is in line with the findings of Bu et al. (2007) who germinated seeds of alpine species at the eastern Tsinghai-Tibet plateau, China, and in other habitats, when used germination temperatures that did not reflect the distribution areas of the tested species (Schütz et al. 1997; Thompson et al. 1999).

(c) Under the temperature regime used, there was no significant pattern of FGP between species from high and low elevations, but we may spot a trend with nival-alpine-subalpine species having lower FGPs compared to montane-colline centred species. In situ, a low germination may be the bottleneck for alpine seedling establishment under climate change (Shevtsova et al. 2009; Graae et al. 2011), since a high mortality of alpine seedlings was evidenced by several authors (Marcante et al. 2009; Graae et al. 2011; Winkler et al. 2015; Milbau et al. 2017). A high germination percentage could mean an important pre-requisite for a successful invasion of species from lower to higher elevations. Thus, novel communities can arise (Alexander et al. 2015, 2018), and it is unknown how coexistence or competition will shape species turnovers and community compositions in the future.

(d) The effect of bedrock (soil reaction) on germination has been notably investigated in the field (Forbis 2003; Wenk and Dawson 2007; Isselin-

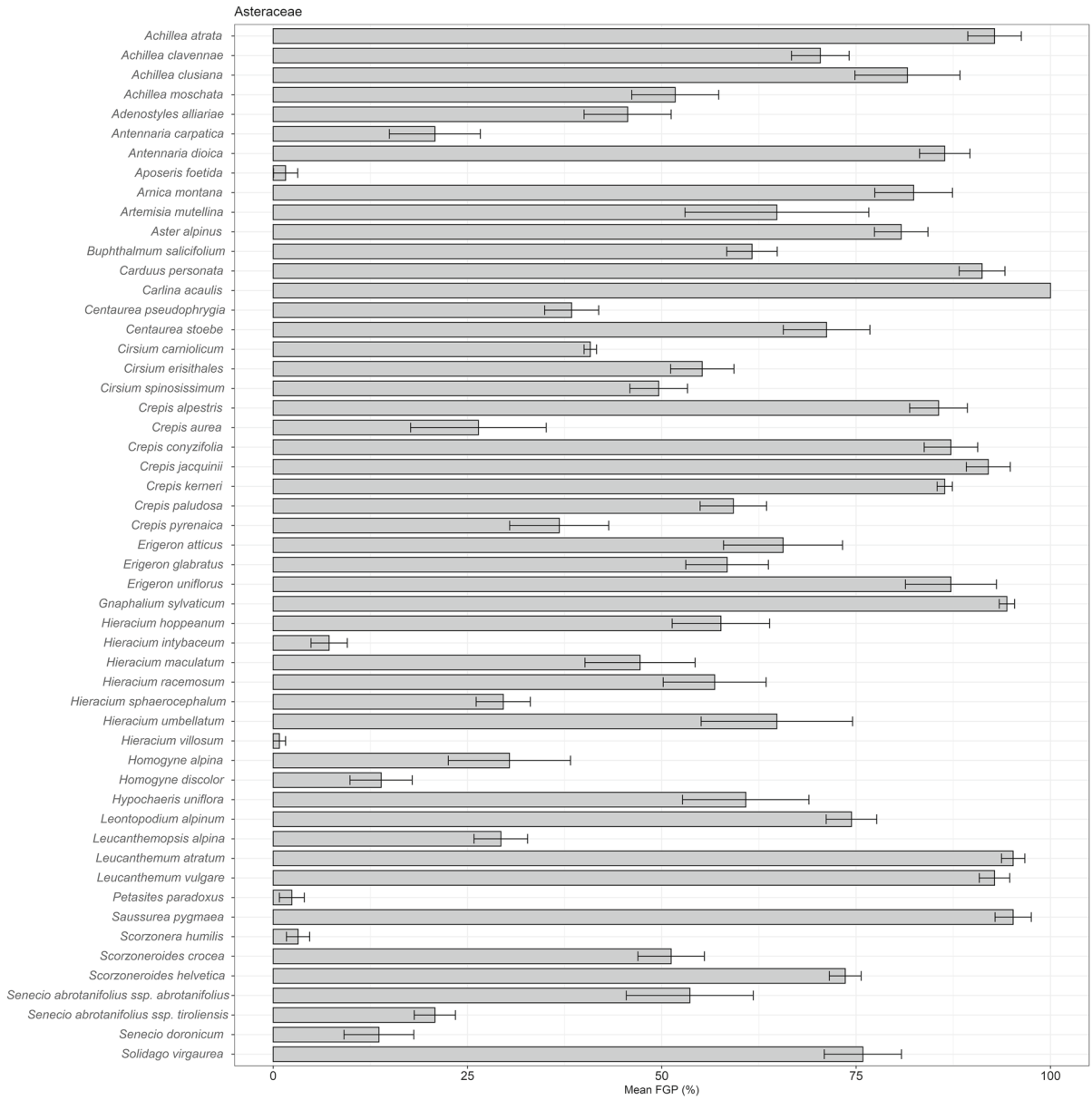

Fig. 4 Final germination percentages (FGP) of 53 species from the family Asteraceae, tested under the standard protocol. Error bars show standard errors

Nondedeu and Bédécarrats 2013), with the result, that soil chemistry was not related to FGP. Recent studies however (Fernández-Pascual et al. 2017; Tudela-Isanta et al. 2018a, b), reported contrasting results stating a consistent relation between germination strategy and bedrock type. Our results on FGP classified to three bedrock types, did not reveal any significant differences. However, we may note a trend of species from very acidic bedrock having a lower germination rate than species from slightly acidic to neutral soil reaction sites.

(e) Key parts of the CSR strategy concept (Grime 1979; Pierce et al. 2014) and the leaf-height-seed concept (LHS, Westoby 1998) are seed traits such as seed mass and volume, where seed mass is thought to be an indicator for seedling survival. Jiménez-Alfaro et al. (2016)

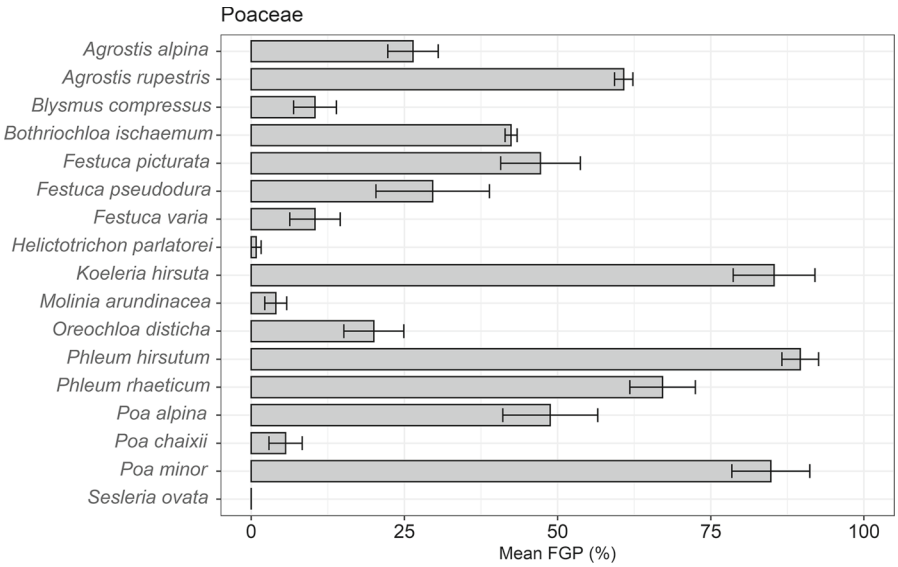

Fig. 5 Final germination percentage (FGP) of 17 species from the family Poaceae, tested under the standard protocol. Error bars show standard errors

mentioned that germination does hardly correlate with CSR or LHS traits. This can be supported by our results, as no pattern was found. Stress tolerant species built the biggest group in alpine environments (Caccianiga et al. 2006) as also seen by our collections, and ruderal species occur only in a small proportion.

(f) No correlation was found between seed mass and FGP of species with high and intermediate germination. This is in line with results of a glacier foreland study, where representative species from different successional stages were investigated (Schwienbacher et al. 2012). In contrast, seed mass was highly related to FGP of selected glacier foreland species, after a winter burial trial in the field (Schwienbacher and Erschbamer 2001). Liu et al. (2013) reported of significant responses to temperature fluctuations of small seeded species compared to larger seeded ones. Generally, seed mass is thought to be an indicator for seedling survival (Grubb 1977; Westoby 1998; Coomes and Grubb 2003), and to correlate with several species traits (Fenner and Thompson 2005; Moles and Westoby 2006). Further, seed mass has also been regarded as an indicator for the competitive ability of a species (Tilman 1994). Thus, seed mass might be an essential trait for seedling performance, but not for FGP per se.

(g) A treatment with GA_3 was applied to 44.7% of the investigated species. In half of these species, germination was stimulated. They can be classified as having non-deep and intermediate physiological dormancy (Baskin and Baskin 2004, 2014). For the other half, deep physiological dormancy must be assumed. According to Schwienbacher et al. (2011), breaking deep physiological dormancy requires a cold-wet stratification for more than four months and additional preconditions such as temperature fluctuations (Baskin and Baskin 2014). Several authors suggested that cold-wet stratification is necessary for alpine species to germinate (Cavieres and Arroyo 2000; Shimono and Kudo 2003; García-Fernández et al. 2015; Fernández-Pascual et al. 2017), because this simulates a winter period which is an essential factor for inducing germination in spring. Cavieres and Sierra-Almeida (2018) recently found that this stratification effect does not increase with elevation. For some alpine species, even negative effects of stratification occur (Giménez-Benavides and Milla 2013). Morphophysiological dormancy might have also occurred in the non-germinated species of our study, for instance in species from Apiaceae, Campanulaceae, Gentianaceae, and Ranunculaceae (Threadgill et al. 1981; Forbis and Diggle 2001; Baskin and Baskin 2005; Vandelook

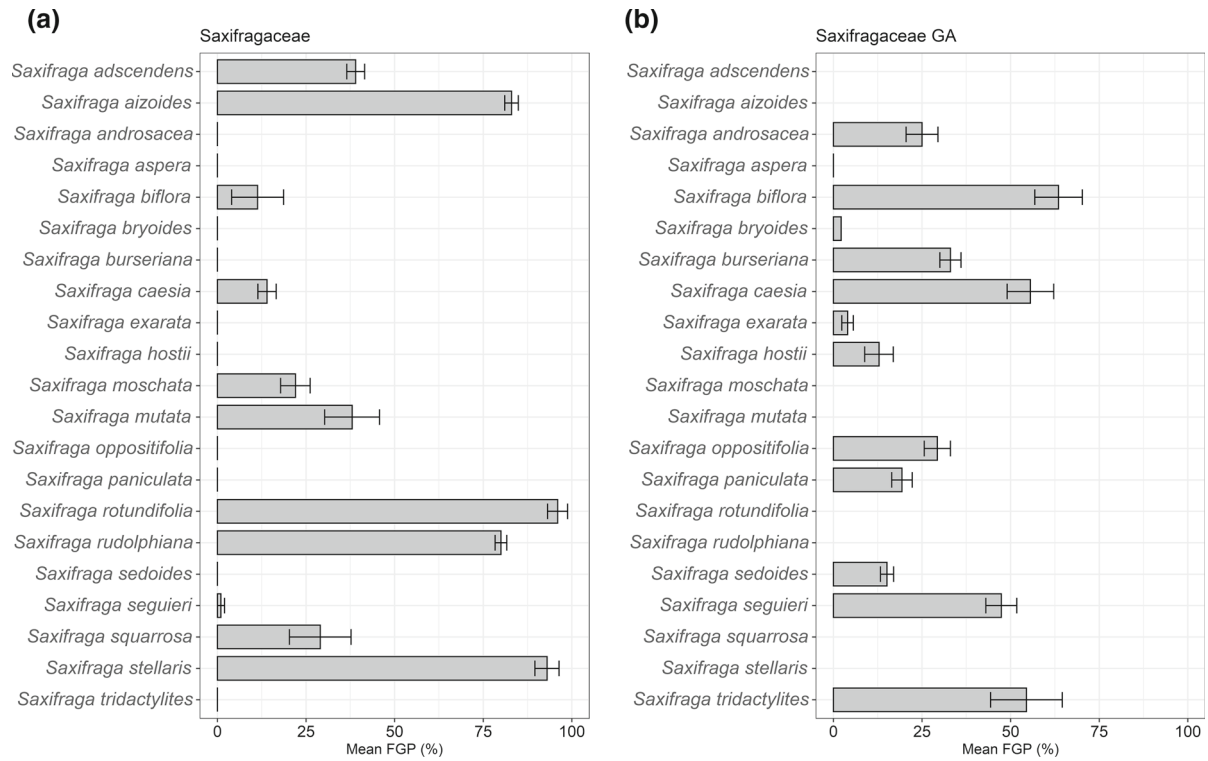

Fig. 6 **a** Final germination percentage (FGP) of 21 species from the family Saxifragaceae, tested under the standard protocol and **b** FGP after a treatment with gibberellic acid (GA). These species were cold-wet stratified before the germination test

et al. 2009). In particular, Ranunculaceae have rudimentary underdeveloped embryos (Finch-Savage and Leubner-Metzger 2006). *Noccaea crantzii* and *Pedicularis portenschlagii*, both endemic species, were stimulated by GA_3, however, for other endemic species (i.e. *Valeriana* species) we did not have enough seeds to perform the GA_3 treatment and it remains unknown if the dormancy of these species could have been broken by a GA_3 treatment. Further, some endemic species require a cold-wet stratification (i.e. 90 days for an endemic *Telekia*, Brusa et al. 2007; at least nine months for an endemic *Androsace*, Frattaroli et al. 2013), whereas for an endemic *Campanula*, 24 h seed imbibition and a specific germination medium is required (Frattaroli et al. 2013). These examples and our results illustrate that no generalisations for GA_3 treatment are possible, due to the individual germination response of endemic species. Moreover, even between individuals of the same population, dormancy may vary (Andersson and Milberg 1998). The loss of physiological dormancy may be closely related to the environment of the maternal habitat (Rosbakh and Poschlod 2015). Thus, it would be advisable to test a variety of temperature regimes for germination in combination with seed pre-treatments.

(h) Among the studied species with weak or zero germination, only few might have had physical dormancy. This mechanism is typical for Fabaceae and for sure, scarification might have been an efficient dormancy release for species from this family (Schütz 1988; Flüeler 2011; Schwienbacher et al. 2011). However, we did not consider such treatments in our screening project. Asteraceae and Poaceae are among the most widespread families in the Alps (Körner 2003). According to our results, most species of these two families germinated to a high extent using the standard protocol, i.e. at warm temperatures (20/10 °C or 25/15 °C). This means, higher temperatures in the field that occur due to

climate warming may be tolerated for germination without problems, which may lead to increases in seedling numbers, and may turn into increased seedling recruitment. This hypothesis is in line with several statements made already for Poaceae (Venn et al. 2014; Porro et al. 2019). In contrast, species from the family Saxifragaceae seemed to have problems with the conditions of the standard protocol, although Schwienbacher et al. (2011) recorded an optimum germination temperature of 20/10 °C for alpine *Saxifraga* species. Their germination might be restricted by different types of dormancy. However, after a stratification period of three month, some species did still not germinate, and several were stimulated only weakly by a GA_3 treatment. Giménez-Benavides and Milla (2013) even noted a negative effect of cold-wet stratification on germination success on two *Saxifraga* species from northern Spain. The only annual species among the collected, *S. tridactylites,* did not germinate under the standard protocol. It needs low temperatures of 5 °C and 10 °C for germination (Pemadasa and Lovell 1975) while high temperatures enforce dormancy. This is a nice example of germination temperature reflecting adaptation to habitat conditions of winter annuals.

Importance of findings for ex situ seed conservation and seedling recruitment in the field

In any classification in this study, about half of the species germinated up to intermediate and high extents using the standard protocol. All the species tested originated from the European Eastern Alps. It seems that most Alpine species require relatively high temperatures for germination, but that germination is restricted by different types of dormancy. For ex situ seed banking, an application of different dormancy release treatments is advisable when testing germination of Alpine species. However, for rare and endemic species, seeds for several parallel trials are often limited or not available. In these cases, standard protocols must be applied, and for Alpine species, a high temperature regime might be recommended.

Seedling recruitment is a highly risky developmental stage in nival-alpine environments (Stöcklin and Bäumler 1996; Erschbamer et al. 2008; Marcante et al. 2009; Erschbamer and Caccianiga 2017). Therefore, high germination rates are mandatory for alpine plants to persist or migrate. The same holds for upward-migrating species along elevation gradients. Climate warming may enhance germination as shown for arctic species (Alsos et al. 2013). However, according to niche models (Schwager and Berg 2019) habitat suitability will change remarkably, and several Alpine species might be suffering from habitat loss. Germination experiments in the laboratory are valuable, however, they do not substitute for observations in the field under real environmental conditions. Investigations of the reproductive output, seedling survival and recruitment, as well as the demographic behaviour of species should be focussed by future projects.

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Data availability The data are currently not on a repository. The datasets are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval The study did not cause any harm to the environment, animals, or plants. The study did not involve human participants and/or animals.

Informed Consent The publication in Plant Ecology has been approved by all co-authors.

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