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Effect of temperature, light and storage on seed germination of *Salvia plebeia* R.Br., *Leonurus japonicus* Houtt., *Mosla scabra* (Thunb.) C.Y.Wu & H.W.Li and *Perilla frutescens* (L.) Britton

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ABSTRACT

Understanding seed storage and germination ecophysiology is important for cultivation and for *ex situ* conservation of economically important species. Here we investigated the germination of four economically important plant species from the subtropical area of southeast China in relation to different environmental parameters. The seeds were subjected to different alternating temperatures (5/10 °C, 10/20 °C, 20/30 °C, 25/35 °C and 35/40 °C) and photoperiods (12 h light/12 h darkness and 24 h darkness). We also tested the effects of dry-storage on germination of selected species. The germination of all the species varied significantly with the temperature of incubation. The germination of *L. japonicus* and *S. plebeia* was highest under warm conditions, at 25/35 °C and 20/30 °C, respectively. However, seeds of *M. scabra* and *P. frutescens* showed the highest germination at 10/20 °C and the lowest at 35/40 °C, respectively. Exposure to 12 h of light enhanced the germination in all species (germination reached between 19 % and 87 %). Seed germination percentages of all the species were significantly enhanced by storage time, although the extent was species-specific. This study established a successful approach for optimizing seed germination of different species of Lamiaceae that could be utilize for their large scale production.

1. Introduction

The Lamiaceae family is the sixth largest and most widely distributed plant family with 236 genera and 7000 species worldwide (Harley et al., 2004). China is well known for its high diversity of Lamiaceae with 1000 species grouped in 100 genera (Li and Hedge, 1994). Many Lamiaceae species are economically important and widely used for different purposes such as medicine, cuisine, perfumery and ornaments (Agostini et al., 2009; Mamadalieva et al., 2021; Zhao et al., 2021). However, most of these species are not produced to fulfill the demand but rather they are collected from wild areas and frequently subjected to over exploitation. *Salvia plebeia*, R.Br., *Leonurus japonicus* Houtt., *Mosla scabra* (Thunb.) C.Y.Wu & H.W.Li and *Perilla frutescens* (L.) Britton are members of the Lamiaceae family and are commonly used in Traditional Chinese Medicine (TCM) for treating various ailments. These species' bioactive compounds and pharmacological properties are well known (Table 1). Due to their medicinal value, these species are subjected to over exploitation in their natural habitat. However, knowledge of seed germination response of these species to various environmental conditions is still scarce.

Unsustainable exploitation of economically important species is considered to be one of the main threats to biodiversity (Millenium Ecosystem Assessment, 2005). Little progress has been done so far to encourage the sustainable utilization of economically important species (Secretariat of the Convention on Biological Diversity, 2011; Williams et al., 2014). However, cultivation is proposed as a sustainable alternative for exploitation of wild populations that will not only help conservation by reducing the pressure on wild populations but also will increase the income of local communities (Nogueira and Nogueira-Filho, 2011; Sarasan et al., 2011). Propagation by seeds is one of the most cost effective, convenient and promising method for large scale cultivation (Chen et al., 2016). However, information about the seed germination requirements for most of the economically important Lamiaceae species still remains unexplored making their cultivation very challenging.

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Understanding the role of environmental factors is key for achieving maximum germination. Environmental factors such as temperature and light play an important role in regulating seed dormancy and germination. Therefore, understanding the effect of those factors on seed germination is extremely important in order to determine the best time for seed sowing as well as the seed sowing depth in the soil. Optimum temperature requirements for seed germination are species-specific and each species has their temperature range (low and high) within which they can germinate and below and above of these temperatures, germination is completely inhibited (Finch-Savage and Leubner-Metzger, 2006). Similarly, photoperiod contributes to determining when and where germination will take place (Fenner and Thompson, 2005), and this response is also species-specific (e.g., some species require only light or darkness to germinate, whereas others germinate equally well in light and darkness; Baskin and Baskin, 2014). Moreover, the interaction between light and temperature can modify the sensitivity of seeds to both factors (i.e., temperature and light) (Baskin and Baskin, 2014). Therefore, understanding the germination response to temperature and light is important to develop efficient propagation protocols.

Generally, the freshly mature seeds are either dormant or non-dormant depending on species (Russell, 2011; Baskin and Baskin, 2014). Non-deep physiological dormancy is prevalent in the family Lamiaceae and this type of dormancy is reported to be broken during dry storage at room temperature (i.e. after-ripening) (Baskin and Baskin, 2014, 2020; Holdsworth et al., 2008). Previous studies reported that temperatures between 15 and 20 °C are optimal for storing seeds (Liu et al., 2011; Mbofung et al., 2013; Bhatt et al., 2020). However, some studies reported that storing seeds at field conditions can enhance the after-ripening process and consequently enhance the germination due to wide fluctuation in weather conditions in nature, especially due to temporal changes in temperature (Commander et al., 2009; Baskin and Baskin, 2014).

In the present study, laboratory experiments were conducted to obtain eco-physiological information on seed germination of selected economically important species of family Lamiaceae (*S. plebeia*, *L. japonicus*, *M. scabra* and *P. frutescens*). The specific objectives of this study were to: (i) determine the dormancy status of freshly collected seeds of the selected species, (ii) determine the effect of wide range of temperature and light conditions and their interaction on germination and (iii) determine the effect of seed storage on dormancy alleviation.

2. Materials and methods

2.1. Seed collection

Mature seeds of *S. plebeia*, *L. japonicus*, *M. scabra* and *P. frutescens* were collected from wild populations at the time of their natural dispersal during 2020 (Table 2). For each species, seeds were collected from 25 to 30 randomly chosen plants to represent the genetic diversity of the population. After collection, seeds were cleaned and divided in to two batches. One batch was immediately tested for germination within a week after collection (hereafter fresh seeds). The other batch were stored in a nylon bag (mesh size 0.2 mm) and placed in a room on the soil surface until February 2021 (hereafter stored seeds) and were kept in room (without controlling temperature) in order to mimic the field conditions. These stored seeds were retrieved at the end of February and tested for germination. To keep track of the environmental conditions of stored seeds, the minimum/maximum air temperature and relative humidity were recorded using a Thermo-Hygrometer (Electronic Temperature Instrument Ltd, UK) (Fig. 1).

2.2. Seed morphological traits determination

A Stereo Microscope (Nikon SMZ800N) fitted with a microscope camera IMG-SC600C was used to examine seed shape, dimensions (length, width and height) and color. From each species 15 seeds were examined, attaching them ventrally to filter-paper using double-sided sticky tape.

Seed mass was determined at the time of seed collection from three 100-seed replicates per species, using a Sartorius electronic balance (Sartorius Co., Goettingen, Germany).

2.3. Water imbibition

Water imbibition was assessed by recording the mass of three 25-seed replicates before and after placing them for 24 h at room temperature (22 ± 2 °C) in 9-cm-diameter Petri dishes containing two sheets of Whatman No. 1 filter paper moistened with 10 mL of distilled water. The water uptake by seeds was calculated using the formula (Water

Table 1
Ethno- medicinal and pharmacological uses of selected species.

Species	Traditional uses	Main bioactive compounds	Pharmacological uses	References
S. plebeia	Nephritis, cough, hepatitis, diarrhea, hemorrhoids, rheumatoid arthritis and tumors	Diterpenoids, flavonoids, lignans and sesquiterpenoids	Antioxidant, anti-proliferative, antiviral, anti-inflammatory and anti-obesity.	Wang et al. (2018);Ma et al. (2017); Bang et al., 2017;Zou et al. (2018);Akram et al. (2015);Choi et al. (2016);Liang et al. (2020);Choi et al. (2015);Jin et al. (2008);Jung et al. (2009)
L. japonicus	Regulate menstrual disturbance, dysmenorrhea, amenorrhea, blood stasis, and postpartum hemorrhage, as well as activating blood circulation, diuretics and dispelling edema	Monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids, steroids, alkaloids, flavonoids, and phenylpropanoids	Vasorelaxant coagulant, cytotoxic, angiogenic, antibacterial, anti-platelet aggregative, and an effect on the uterine smooth muscle	Peng (2011); Chinese Pharmacopoeia Commission, 2015;Peng et al. (2013); Xiong et al. (2015a), (2015b);Zhou et al. (2015);He et al. (2018);Xiong et al. (2015a), (2015b);Xiong et al. (2015a), (2015b);Zhou et al. (2015);Peng et al. (2017);Liu et al. (2018)
M. scabra	colds, fever, cough, chronic bronchitis and pneumonia	Apigenin, acacetin, 5-hydroxy-6,7-dimethoxyflavone, 5-hydroxy-7,8-dimethoxyflavone, andamanicin, magnosalin and ursolic acid	antibacterial, antioxidant, anti-tumor, anti-inflammatory and antiviral activity as well as immunity-modulation	Wang et al. (2000);Yu et al. (2010); Osawa et al. (1990);Li et al. (2010); Mazzio and Soliman (2010);Yu et al. (2010))
P. frutescens	Asthma, depression-related disease, anxiety, influenza, cough, chronic bronchitis, vomiting, tumors, allergies, intoxication, fever, headache, stuffy nose, constipation, abdominal pain and indigestion	Phenolic acids, flavonoids, anthocyanins, triterpenes, phytosterols, fatty acids, tocopherols, and policosanols	Antioxidant, antibacterial, antifungal, Anti-Allergic, Anti-Depressive, Anti-inflammatory, antitumor and anti-HIV-1	Ueda et al. (2002);Takano et al. (2004); Ahmed (2019); Wei-Wei et al. (2014); Yamamoto and Ogawa (2002);Choi et al. (2010);Inouye et al. (2006);Shin et al. (2000);Heo et al. (2011);Chen et al. (2015);Huang et al. (2014);Wang et al. (2018);Lin et al. (2007);Cho et al. (2011); Yamasaki et al. (1998); Yi et al. (2013); Zhou et al. (2014)

Table 2
Lamiaceae species habit, seed collection time and population ubication and habitat.

Species	Code	Collection time	Place	Latitude	Longitude	Altitude (m asl)	Habit	Habitat
S. plebeia	SP1	May	Wucheng	29°8′11.27″	115°58′51.89″	21.12	Annual	Open area
L. japonicus	LJ	June	Saiyang	29°32′18.53″	115°53′25.77″	107.14	Annual	Streamside
M. scabra	MS	October	Guling	29°37′41.38″	116°1′35.206″	467.98	Annual	Open area
P. frutescens	PF	November	Guling	29°34′35.40″	115°59′35.52″	1133.07	Annual	Open area

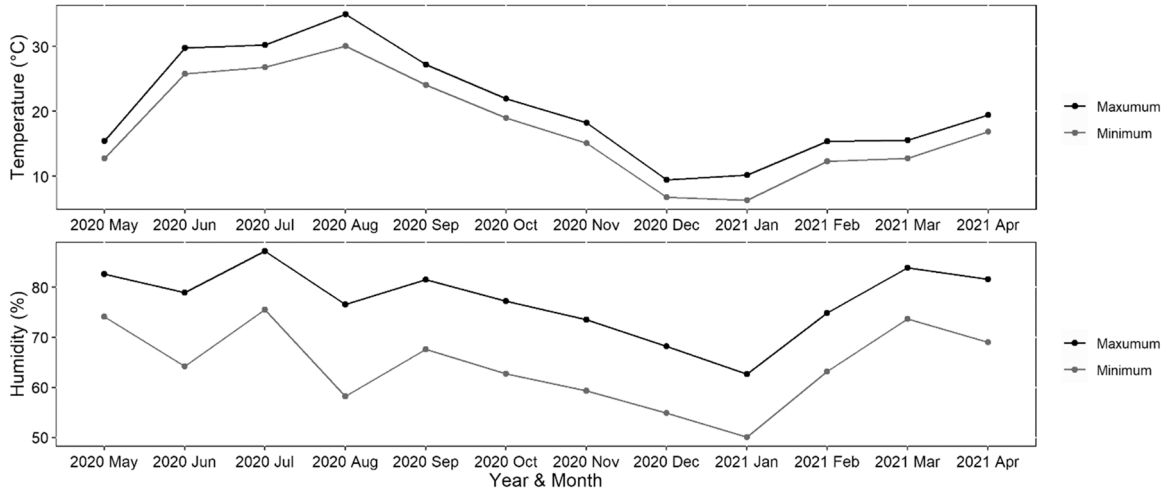

Fig. 1. Maximum and minimum temperature and relative humidity during seed storage.

absorption (%) = [(W2 − W1)/W1] × 100), where, W2 is the mass of the seeds after imbibition during a given time interval, in this case 24 h, and W1 is the initial seed mass (Baskin et al., 2004).

2.4. Effect of temperature and light on seed germination

The seeds were surface sterilized in a solution of 0.5 % sodium hypochlorite for 1 min, subsequently washed thrice with deionized water to avoid fungus attack. To determine the effect of temperature and light, seed germination was conducted in incubators (Kesheng incubators, Model- DRX-800 C- LED, China) set at five different alternating temperature regimes (5/10 °C, 10/20 °C, 20/30 °C, 25/35 °C and 35/40 °C) in either 24- h darkness (hereafter dark treatment) and 12 h light/12 h darkness (hereafter light treatment). In all temperature regimes the higher temperature coincided with the hours of light. Incubators were fitted with cool-white fluorescent tubes (60 μmol photons $M^{-2}S^{-1}$). The temperature regimes were chosen to stimulate the average temperature at different months (i.e., 5/10 °C - December to February, 10/20 °C- March- April and October - November, 20/30 °C - May, June and September, 25/35 °C - July and August) at the seed collection area while the higher temperature (35/40 °C) were used to investigate the ability of seeds to germinate under high temperatures.

Seeds were sown in 9-cm petri dishes containing three disks of Whatman No. 1 filter paper moistened with 10 mL of distilled water and placed in incubators. The petri dishes for the dark treatment were wrapped in two layers of aluminum foil. Four replicates of 25 seeds each were used for each treatment. The seeds were considered to be germinated with the emergence of the radicle (≥2 mm). Germinated seeds were counted and removed daily for a 30 days period. However, seeds incubated in the dark were checked only at the end of the test.

2.5. Effect of storage on germination

The stored seeds were tested for germination at the end of April 2021. During this time, we used the best two temperature conditions for seed germination for each species based on the results of the experiment using fresh seeds. All the other experimental conditions were the same as the ones mentioned above. At this time, ungerminated seeds were evaluated for viability by dissection and examination under a binocular microscope. Seeds with a white embryo were counted as viable, and those with a turgid/brown or absent embryo non-viable (data not shown).

2.6. Data analysis

Morphological traits (length, width and height) were analyzed as a function of the species identity with generalized linear models (GLM) with Gaussian error structure. The germination of freshly collected seeds was analyzed as a function of the light conditions and the temperature of incubation and the germination of stored seeds was evaluated as a function of the temperature (the best two temperatures of incubation selected from the results of the trial using freshly collected seeds) and the light treatments, in both cases GLM with binomial error structure were used. The mean germination time (MGT) was calculated as: MGT = ΣDN/ΣN; where D is the number of days that passed from the date of sowing and N is the number of seeds germinated on day D (Ellis and Roberts, 1981). The MGT was calculated for freshly collected and stored seeds incubated in the light. In both trials the MGT was evaluated as a function of the temperature of incubation using generalized linear models with Gaussian error structure. For all the variables analyzed, first the full model was fitted with all the factors and interactions, after which, the models were simplified, by dropping first the least significant interaction, and then the least significant individual factor at each step. The comparison between models was based on the scaled deviance for the Gaussian data and on the likelihood ratio test (LRT) for the binomial data, until all of the remaining terms were significant (Zuur et al., 2009). All the analysis were done with R (version 3.5.2 R Core Team, 2018).

3. Results

3.1. Morphological traits

All the morphological traits showed a significant variation among species (Table 3). *Leonurus japonicus* exhibited the higher length and width, *P. frutescens* the higher height and *S. plebeia* the lower length, width and height (Fig. 2 and Table S1). *Leonurus japonicus* also showed the higher difference between morphological traits (difference between length, width and height, Fig. 2). Additionally, *L. japonicus* exhibited the higher seed mass (Table S1).

3.2. Water imbibition

Seeds of all the species increased their weight after imbibed in water for 24 h. However, the degree of mass increase was species-specific (Fig. 3 and Table S2). The species that most increased their mass were *L. japonicus* (50.8 %) and *S. plebeia* (54.5 %) while *M. scabra* and *P. frutescens* only increased their mass in 27.8 % and 31.0 %, respectively.

3.3. Effect of temperature and light

The germination of all the species was significantly affected by the temperature and light conditions and their interaction except the germination of *M. scabra* that was only affected by the individual factors (Table 4). The highest germination of *S. plebeia* was recorded under 20/30 °C and light conditions (87 %), while the highest germination of *L. japonicus* was recorded at 25/35 °C and light conditions (28 %, Fig. 4). The lower germination of *S. plebeia* (0 %) was recorded under light conditions and 5/10 °C and the lower germination of *L. japonicus* was recorded under light conditions and complete darkness at 5/10 °C (0 %, Fig. 4). Conversely, the highest germination of *P. frutescens* was recorded at 10/20 °C and light conditions (64 %) and lowest at 35/40 °C and complete darkness (0 %, Fig. 4).

The germination of all the species varied significantly with the temperature of incubation (Table 4 and Table S3). The germination of *L. japonicus* and *S. plebeia* was highest under warm conditions, at 25/35 °C and 20/30 °C, respectively and the lower germination of both species was recorded at 5/10 °C. The germination of *M. scabra* and *P. frutescens* was highest at 10/20 °C and lowest at 35/40 °C. However, the germination of *M. scabra* was also lowest at 5/10 °C (Fig. 5).

The germination of all the species was significantly higher under light conditions compared with the complete darkness treatment (Table 4). However, the difference of germination between the two light conditions was species specific (Fig. 5). *Salvia plebeian* exhibited the higher difference between treatments, the germination of this species was 63.4 % under light conditions and only 5.8 % under complete darkness. The lower difference between treatments were recorded in *L. japonicus*, the germination of this species was of 15.8 % under light conditions and of 7.6 % under complete darkness (Fig. 5).

The Mean Germination Time (MGT) of all the species varied with the temperature of incubation (Table 5). The MGT of *L. japonicus*, *M. scabra* and *P. frutescens* was higher under colder conditions, 10/20 °C for the first two species and 5/10 for *P. frutescens*. The lower MGT of these species was recorded under warmer conditions, at 25/35 °C for *L. japonicus* and *P. frutescens* and 20/30 °C for *M. scabra*. The faster and

Table 3
Seed length, width and height as a function of species identity.

	Factor	Scaled dev.	Pr (>Chi)
Length	Species	193.5	< 2.2e-16 ***
Width	Species	110.44	< 2.2e-16 ***
Height	Species	107.36	< 2.2e-16 ***

Signifiance codes: n.s.: P > 0.05; * P < 0.05. ** P < 0.01. *** P < 0.001.

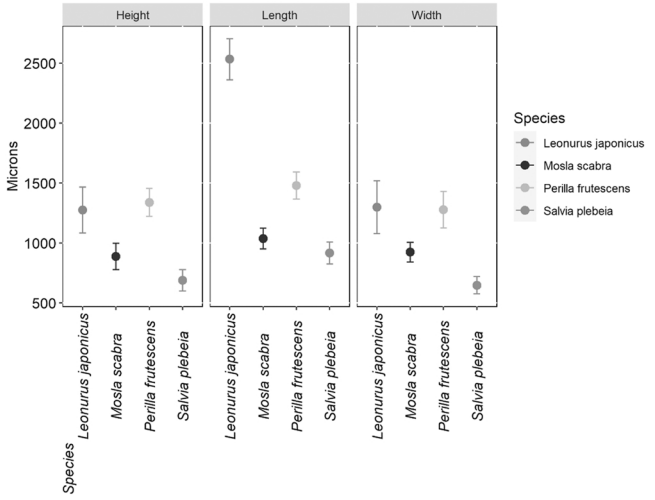

Fig. 2. Seed height, length and width of *L. japonicus, M. scabra, P. frutescens* and *S. plebeia* Error bars indicate standard deviations.

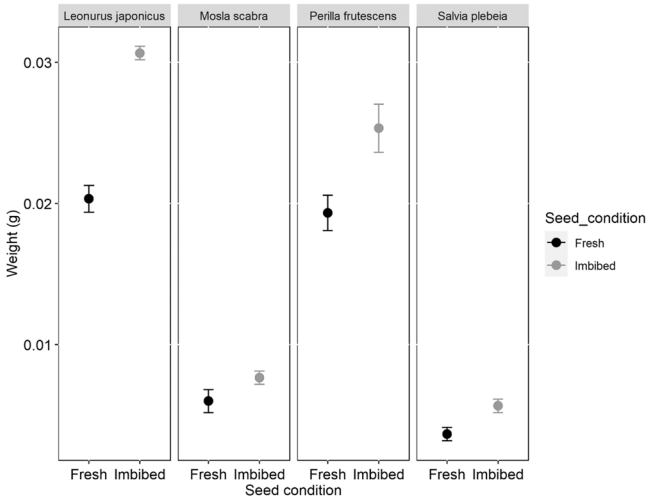

Fig. 3. Seed weight before (fresh) and after imbibition in water for 24 h. Error bars indicate standard deviations.

Table 4
Effect of temperature and light of incubation on the germination of L. japonicus, M. scabra, P. frutescens and S. plebeian.

Germination trial	Factor	LRT	Pr (>Chi)
L. japonicus	Temperature	91.508	< 2.2e-16 ***
	Light condition	17.958	2.259e-05 ***
	Temperature*Light condition	14.839	0.005048 **
M. scabra	Temperature	67.116	9.217e-14 ***
	Light condition	4.857	0.02754 *
	Temperature*Light condition	0.8017	0.9382
P. frutescens	Temperature	192.276	< 2.2e-16 ***
	Light condition	74.319	< 2.2e-16 ***
	Temperature*Light condition	13.542	0.008908 **
S. plebeia	Temperature	239.58	< 2.2e-16 ***
	Light condition	479.14	< 2.2e-16 ***
	Temperature*Light condition	29.369	6.579e-06 ***

Signifiance codes: n.s.: P > 0.05; * P < 0.05. ** P < 0.01. *** P < 0.001.

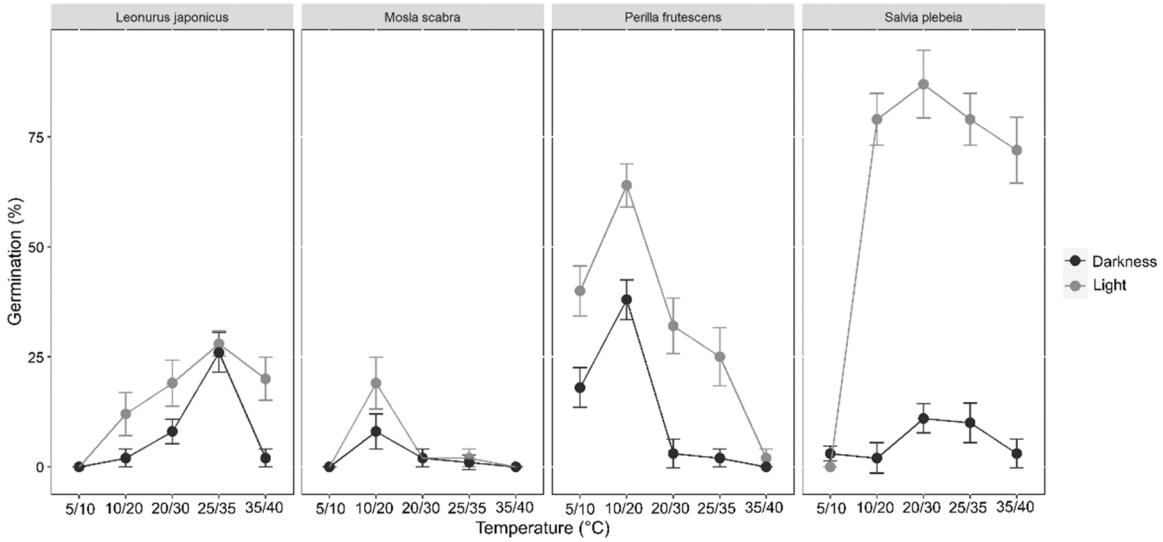

Fig. 4. Germination of *L. japonicus, M. scabra, P. frutescens* and *S. plebeia* as a function of the light conditions and the temperature of incubation. Error bars indicate standard errors.

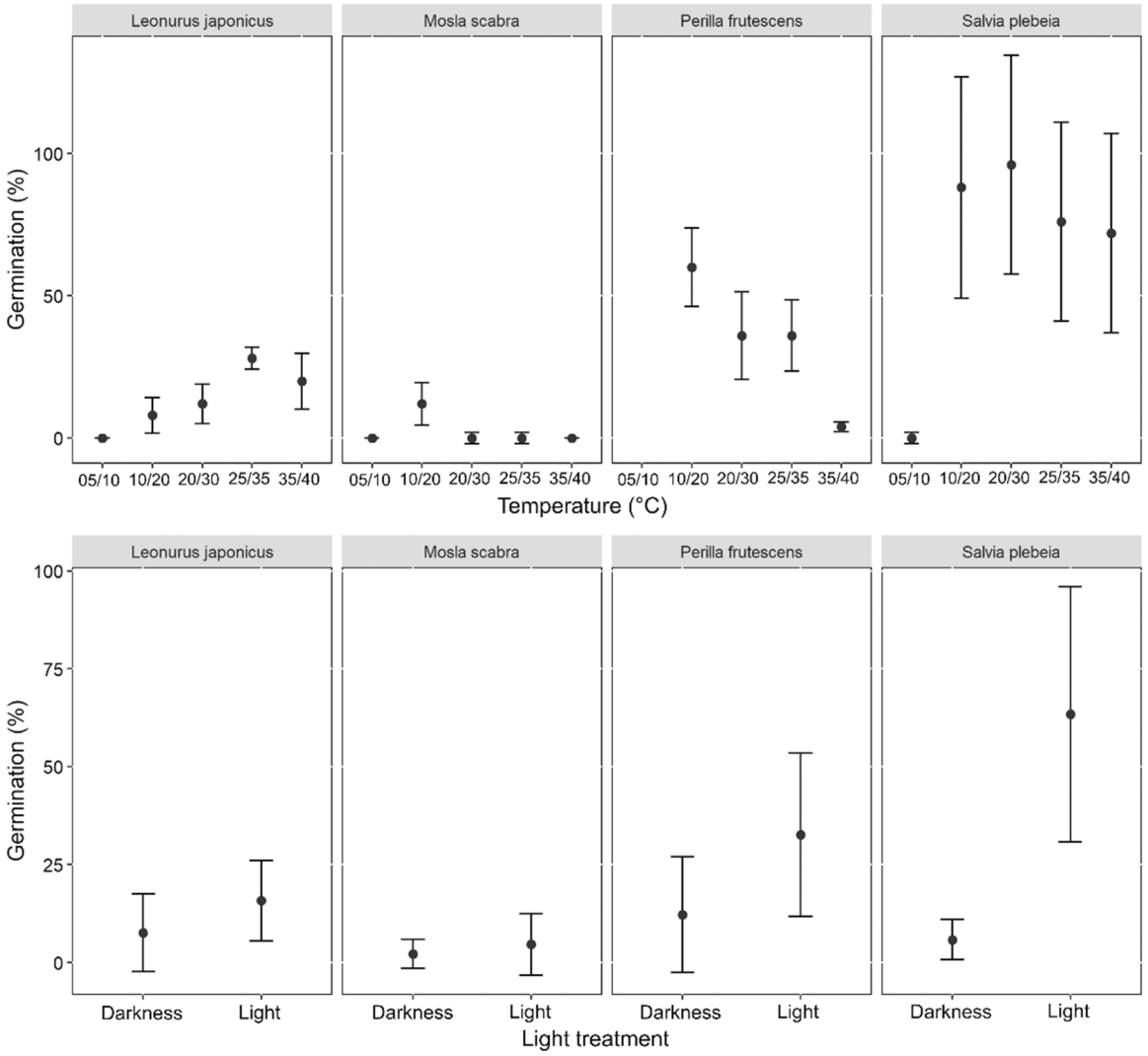

Fig. 5. Germination of *L. japonicus, M. scabra, P. frutescens* and *S. plebeia* as a function of the temperature and the light conditions as individual factors. Error bars indicate standard errors.

Table 5
Mean Germination Time as a function of the temperature of incubation.

Species	Factor	scaled dev.	Pr (>Chi)
S. plebeian	Temperature	68.431	9.252e-15 ***
L. japonicas	Temperature	15.077	0.001752 **
M. scabra	Temperature	18.4	0.000101 ***
P. frutescens	Temperature	23.002	0.0001265 ***

Signifiance codes: n.s.: P > 0.05; * P < 0.05. ** P < 0.01. *** P < 0.001.

slower germination of *S. plebeia* were recorded at 35/40 and 25/35 °C, respectively (Table S5).

3.4. Effect of storage on germination

The interaction between light conditions and temperature of incubation only influenced significantly the germination after storage of *M. scabra* (Table 5; Fig. 6 and Table S4). The temperature of incubation, as single factor, only affected the germination of *M. scabra* (Table 4). *M. scabra* germinated significantly more under warmer conditions (20/30 °C) compared with colder conditions (10/20 °C) (Fig. 7). The germination of *S. plebeia, M. scabra* and *P. frutescens* was significantly higher when the seeds were incubated under light conditions compared with the germination recorded under complete darkness (Table 6 and Fig. 7).

The MGT of stored seeds of *M. scabra, P. frutscens* and *S. plebeia* varied as a function of the temperature of incubation (Table 7). The MGT of *M. scabra* and *P. frutscens* was significantly higher under 20/30 °C compared to the 10/20 °C treatment while *S. plebeia* germinated significantly faster at 25/35 °C compared with the 20/30 °C treatment (Table S6).

4. Discussion

Knowledge of seed dormancy and germination is essential not only for understanding plant reproduction in nature but also can be used for developing effective cultivation methods that will not only reduce the pressure on wild population but also assist in conservation actions and contribute to increase local people livelihood (Sarasan et al., 2011; Phondani et al., 2015). Our results evidence that there are variations in seed morphological traits and germination requirements among species, even though they belong to the same family. Generally, seed morphological parameters (i.e., length, width, height, mass, etc.) influence the seed imbibition ability, seed moisture content and seed germination percentage (Balkaya and Odabas, 2002; Wyllie-Echeverria et al., 2003; Mandal et al., 2008). We detected a considerable variation in seed trait (mass, length, width and height) among species likely because these traits are under strong genetic control (Moles et al., 2005). Moreover, variation in seed traits among different species may affect various aspects species' performance including seed dispersal ability, seedling emergence and competitive abilities (Chilpa-Galván et al., 2018).

We observed a significant variation in water imbibition capacity among species, this variability could be attributed to differences in seed coat structure/morphology that affect seed coat permeability. *Salvia plebeia* had the lowest seed mass, but showed maximum increase in seed mass after imbibition. The higher water imbibition of *S. plebeia* could be linked to the production of mucilage by seeds after hydration (personal observation). These results are consistent with previous studies, who reported that mucilage facilitate water uptake and retention (Bhatt et al., 2016, 2019). Besides the ability of the studied species to imbibe in water is indicative of seeds not having physical dormancy.

Freshly collected seeds of *S. plebeia* showed the highest germination (up to 87.0%) followed by *P. frutescens* (up to 64%) indicating that these species have little and intermediate levels of dormancy respectively. Whereas, poor germination of freshly collected seeds *of L. japonicus* and *M. scabra* indicate the presence of high level of innate dormancy. On the basis of our results, we can confirm that although, seeds of all the study species have physiological dormancy at the time of maturation they exhibit different levels of dormancy and this variation in dormancy could be important to determine germination timing, seedbank dynamic and reduce the competition for resources (i.e., nutrients and water) at seedling stage (Baskin and Baskin, 2014; Yi et al., 2019). Moreover, despite that previous studies reported that family Lamiaceae have, in general, non-deep physiological dormancy (Baskin and Baskin, 2014), interspecific variation of physiological dormancy among freshly collected seeds is fairly common (Baskin and Baskin, 2014).

In our study, the species responded differently to the tested temperature regimes. Freshly mature seeds of *S. plebeia* germinate well in all the tested temperatures except at low temperature (5/10 °C). These results indicate that seed can emerge in nature throughout the year since availability of moisture is not a constraint in the study area, except in winter. However, in order to achieve maximum germination, seeds of *S. plebeia* should be sown in either May to June or in September when temperature is around 20/30 °C. Seeds of *L. japonicus* should be sown during July- August, when temperature is around 25/35 °C to obtain maximum germination. Whereas, *M. scabra* and *P. frutescens* seeds should be sown either during March - April or during October-November, when temperature is around 10/20 °C. These results reveal species-specific temperature requirement for optimal germination, in line with previous studies (see Cochrane, 2016; Bhatt et al., 2019; Park et al., 2019). In the present study, only *P. frutescens* seeds were able to germinate at low temperature (5/10 °C) although their germination percentage were lower at this temperature regime than at 10/20 °C. The ability of this species to germinate at low temperature could be linked to climatic conditions of the seed collection site (this species' seeds were collected at a higher altitude than the other species i.e. 1133.07 m asl). Previous studies also reported that the temperature range for seed germination is usually determined by the thermal conditions of their habitat (Probert, 2000). Germination of *S. plebeia*, *L. japonicus* and *M. scabra* were severely inhibited by low temperature, as these species'

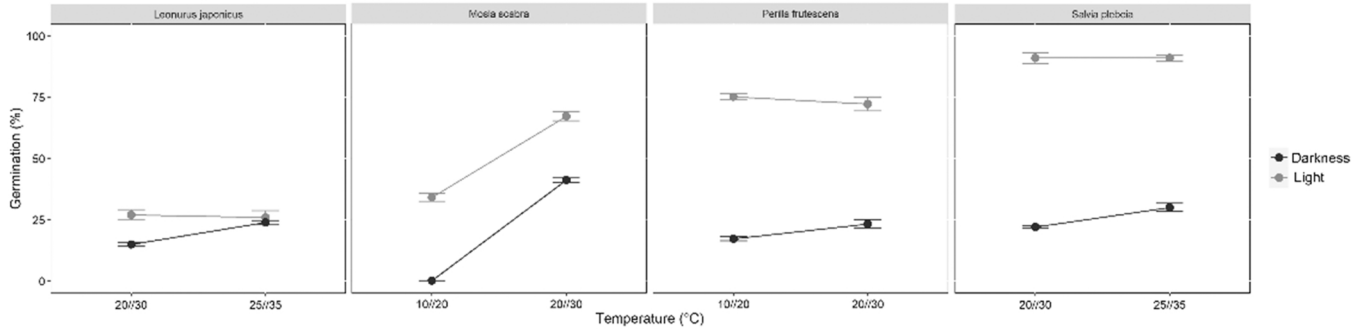

Fig. 6. Germination after storage of *L. japonicus, M. scabra, P. frutescens* and *S. plebeia* as a function of the light conditions and the temperature of incubation. Error bars indicate standard errors.

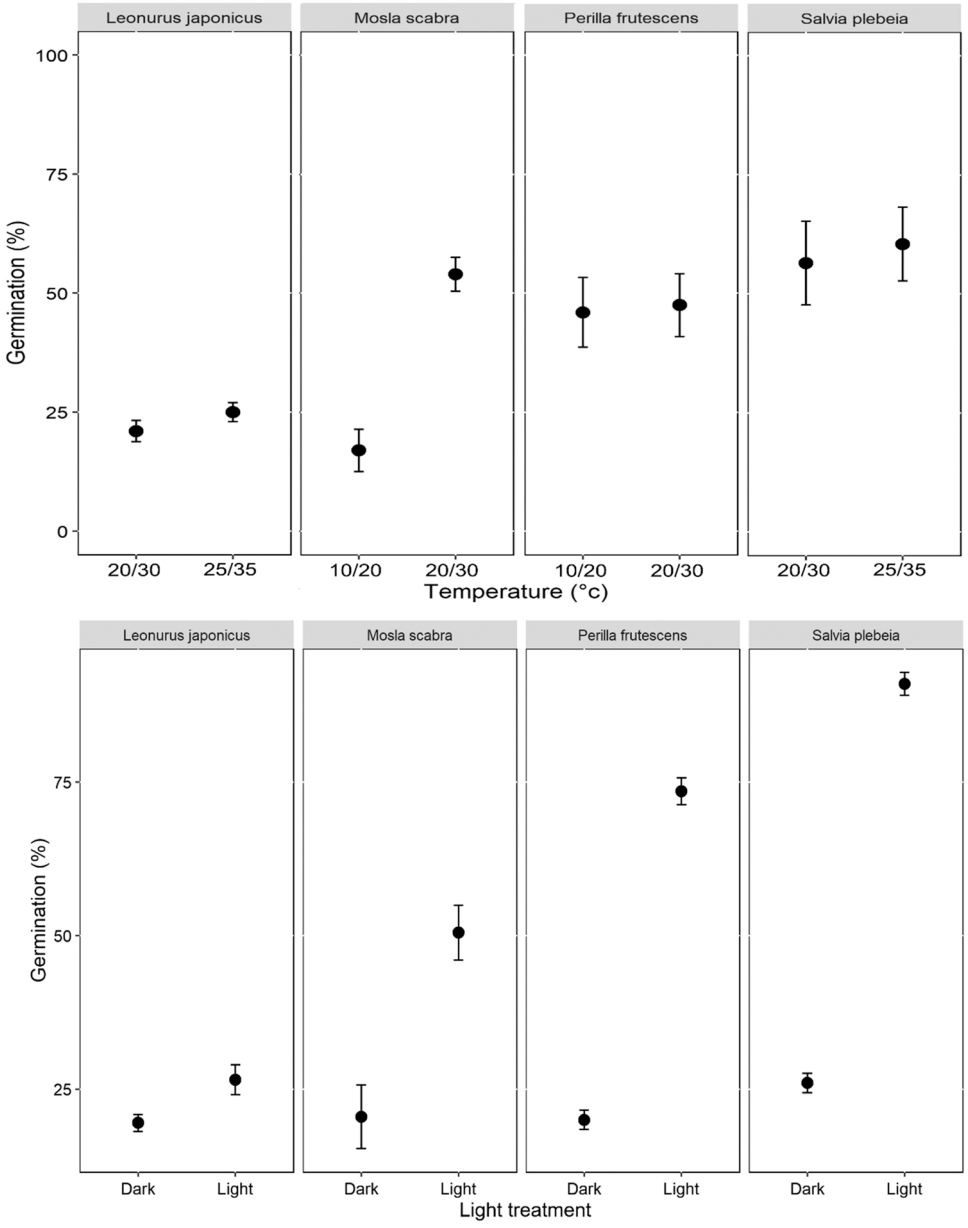

Fig. 7. Germination of *L. japonicus, M. scabra, P. frutescens* and *S. plebeia* after storage as a function of the temperature and the light conditions as individual factors. Error bars indicate standard errors.

Table 6
Effect of temperature and light of incubation on the germination after storage of S. plebeian, L. japonicus, M. scabra and P. frutescens.

Germination storage	Factor	LRT	Pr (>Chi)
L. japonicas	Temperature	0.01514	0.90206
	Light condition	2.77542	0.09572.
	Temperature*Light condition	1.1293	0.2879
M. scabra	Temperature	69.898	< 2.2e-16 ***
	Light condition	48.089	4.074e-12 ***
	Temperature*Light condition	19.829	8.47e-06 ***
P. frutescens	Temperature	0.127	0.7217
	Light condition	121.413	< 2e-16 ***
	Temperature*Light condition	1.2327	0.2669
S. plebeian	Temperature	1.169	0.2797
	Light condition	193.173	< 2e-16 ***
	Temperature*Light condition	0.49954	0.4797

Signifiance codes: n.s.: P > 0.05; * P < 0.05. ** P < 0.01. *** P < 0.001.

Table 7
Mean Germination Time of seeds stored as a function of the temperature of incubation.

Species	Factor	scaled dev.	Pr (>Chi)
L. japonicas	Temperature	0.74937	0.3867
M. scabra	Temperature	12.205	0.0004766 ***
P. frutescens	Temperature	18.314	1.873e-05 ***
S. plebeian	Temperature	20.742	5.255e-06 ***

Signifiance codes: n.s.: P > 0.05; * P < 0.05. ** P < 0.01. *** P < 0.001.

seeds were collected from lower altitudes and germination under warmer conditions could be an adaptive strategy because if these species germinate in winter (December to February), their seedling may not be able to survive due to extreme cold and frost. These results further confirm that thermal germination behavior may be affected by the maternal environment conditions. In the present study, *S. plebeia* seeds were able to germinate better at higher temperature (35/40 °C) as

compared to other species indicating that this species has ability to germinate at the warmer end of the temperature range. Therefore, the chances of *S. plebeia* to survive in climate warming scenario will be high.

In general, seeds of all the studied species germinated better in light conditions as compared to complete darkness in all the tested temperatures, indicating that all the species are light sensitive and therefore they will germinate better if they remain on or near the soil surface. The light dependency of small seeds for germination is well known because they have limited resources and therefore, they need to photosynthesize soon after germination (Milberg et al., 2000; Pearson et al., 2002). The smaller difference between the germination percentage under light conditions and complete darkness of *L. japonicus* seed could be attributed to higher seed mass of this species. Moreover, *L. japonicus* seeds germinate equally well in light and darkness at optimum temperature (25/35 °C), but above and below the optimum temperature, germination was significantly inhibited under complete darkness. These results indicate that interactions between temperature and light play an important role conditioning species germination and that seeds might require light at a certain temperature but not at others (Pons, 1992).

Lower temperatures (5/10 and 10/20 °C) increase the MGT, whereas, higher temperatures reduced the MGT. The higher MGT at lower temperature could be attributed to the reduction or inhibition of enzymatic and metabolic activity (Kamaha and Magure, 1992; Thygerson et al., 2002), whereas, higher temperatures can speed up the chemical reactions and consequently increase the germination speed (Balkaya, 2004). The degree of variation in MGT according to temperature was species-specific and confirms that each species has its own temperature requirement related to germination speed.

In the present study, germination increased after storage at dry conditions at room temperature, which further confirm that these species have physiological dormancy and therefore they require after-ripening before they can germinate (see also Holdsworth et al., 2008; Bhatt et al., 2019). However, the after-ripening requirements seems to be vary depending on the species. Among the tested species, *L. japonicus* showed the lowest germination even after storage, indicating that seeds might have deep physiological dormancy and therefore they may require more time to alleviate the dormancy. Germination percentage after storage was significantly enhanced under light condition for all the studied species. Additionally, seeds of most of the species also germinated better under dark after storage compared to fresh seeds. This indicates that sensitivity of seeds to light for germination is changed during the storage. Temperature influences the MGT of the stored seeds, which endorse that temperature play an important role in affecting MGT. High germination percentage of seed stored at room temperature indicates that seeds of these species can be stored at room temperature without substantial loss of seed viability. However, further studies toned to be done at different time intervals in order to know how long the stored seeds can maintain the viability.

5. Conclusions

Our study has significant implications for successful germination which is not only important for large scale cultivation, but also a step forward for sustainable utilization and conservation of the studied plant species by reducing their extraction from wild populations. Seeds of all the studied species have physiological dormancy and they can be stored at room temperature in order to break their dormancy without affecting their viability.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jarmap.2022.100402.

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