RESEARCH PAPER

Light and temperature requirements for germination in the Mediterranean shrub Lavandula stoechas (Lamiaceae)

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ABSTRACT

- Available studies are far from giving a complete figure for the germination requirements of many Mediterranean Basin species.
- In this study, we investigated the germination properties of *Lavandula stoechas* L. (Lamiaceae) in response to different light and temperature regimes. We performed comprehensive experiments to test the effect of fixed and alternating temperatures, dark versus light conditions, and thermodormancy or thermoinhibition on several germination properties in three populations of L. *stoechas* from southwestern Turkey.
- Germination patterns showed a substantial variation among populations. Germination percentage was higher in the light conditions than in the dark at most temperatures, and alternating temperatures substituted light for the germination. The requirement of *L. stoechas* seeds to light for germination gradually increased through lower and higher temperatures than 15 °C. High temperature (30 °C) reduced the germination percentage to zero. However, in the presence of light, seeds were able to germinate after transferring to the optimum temperature (thermoinhibition), but this was not the case in darkness (thermodormancy). Seed germination in *L. stoechas* was stimulated by light, suggesting that germination has a phytochrome-mediated response.
- Our study makes an inference to the fire-environment interactions in the germination of Mediterranean seeder species by showing that both light and alternating temperatures stimulate germination in *L. stoechas*, a species well-known with its fire-related germination. Comprehensive germination studies are required for a better understanding of the early life-stage adaptations of plants to Mediterranean conditions, and for developing more robust strategies for conservation and ecosystem restoration.

INTRODUCTION

Germination is a critical stage in the life cycle of plants. In different regions of the world, species show different germination responses to environmental factors, such as temperature, moisture and light intensity or duration (Baskin & Baskin 2014). Although the germination requirements of seeds of some species have narrow temperature ranges, many species have broader temperature requirements for germination (van Rooyen & de Villiers 2004). Similarly, species can be stimulated or inhibited by light and by different photoperiod conditions (van Rooyen & de Villiers 2004; Carta *et al.* 2017). Moreover, germination patterns can be more complex, as some species have different responses to different light and temperature combinations, and exposure to different light or temperature conditions may change sensitivity of seeds to various germination cues (*e.g.* Tavşanoğlu *et al.* 2017).

In fire-prone areas, many plant species cannot resprout and consequently depend solely on seed germination for persistence after fire (Pausas *et al.* 2004). Fire-prone Mediterranean Basin ecosystems host species in which seeds must be able to germinate after fires, including several annual species (Çatav *et al.* 2018), but also obligate seeder shrubs (Moreira *et al.* 2010).

Germination patterns of Mediterranean obligate seeder species have been studied regarding heat shock- or smoke-stimulated germination (Moreira *et al.* 2010; Kazancı & Tavşanoğlu 2019, among others). In these studies, however, since the main goal is to understand the germination response to fire, most experiments have been designed to include several fire-related signals but only one incubation temperature or light treatment (*e.g.* Moreira *et al.* 2010; Çatav *et al.* 2018; Kazancı & Tavşanoğlu 2019). For these species, therefore, comprehensive germination experiments that consider the complexity of dormancy to reveal a complete understanding of germination requirements are lacking (Moreira & Pausas 2018).

Seed dormancy is an adaptive mechanism among plants, evolved to control the timing of germination in unpredictable environments (Ren & Abbott 1991). Dormancy can be imposed by environmental cues, such as temperature or photoperiod, or intrinsic mechanisms, such as dormancy cycling (Baskin & Baskin 1985). Seed dormancy allows plants to establish a persistent soil seed bank which increases the germination potential under favourable conditions for seedling establishment. Of the plant species in Mediterranean-type ecosystems, seeds of 35% and 9% have physiological and physical dormancy, respectively (Baskin & Baskin 2014). In the

Mediterranean Basin, many plant species in low altitude zones, where frequent fires occur, have physical or physiological dormancy (Moreira *et al.* 2010; Kazancı & Tavşanoğlu 2019), whereas seeds of most species in the high mountain zone can germinate immediately, without any treatment (Giménez-Benavides *et al.* 2005).

Lavandula stoechas L., an endemic Mediterranean plant, is a non-resprouter shrub that regenerates by seed after fires (Moreira *et al.* 2012). The species is one of the main components of many species-rich shrubland communities in the Mediterranean Basin (Herrera 1997; Moreira *et al.* 2012). Previous studies that have tested the germination properties of *L. stoechas* suggest that the seeds can germinate at different incubation temperatures between 10 °C and 25 °C under light or dark conditions (Cabello *et al.* 1998; Perez-Garcia *et al.* 2003; Moreira *et al.* 2012; Çatav *et al.* 2014; Chamorro & Moreno 2019). However, the effect of alternating temperatures on germination is not known in *L. stoechas*. There is also substantial variation in seed dormancy among *L. stoechas* populations (Keeley & Baer-Keeley 1999; Moreira *et al.* 2010) and partial (Perez-Garcia *et al.* 2003; Moreira *et al.* 2012; Çatav *et al.* 2014; Kazancı & Tavşanoğlu 2019) or no (Maher *et al.* 2000; Papafotiou *et al.* 2000; Perez-Garcia *et al.* 2003; Çatav *et al.* 2012; Chamorro & Moreno 2019) dormancy have been reported. On the other hand, to date, only a few studies have directly compared the germination properties of different *L. stoechas* populations (Perez-Garcia *et al.* 2003; Moreira *et al.* 2012). These studies suggest that seed weight (Perez-Garcia *et al.* 2003) or fire regime (Moreira *et al.* 2012) are responsible for the variability in germination among populations. Several studies on fire-related germination of *L. stoechas* indicate that germination is enhanced by heat shock (Moreira *et al.* 2010; Moreira *et al.* 2012; Kazancı & Tavşanoğlu 2019) and smoke (Moreira *et al.* 2010; Moreira *et al.* 2012; Çatav *et al.* 2014) treatments. Considering the results of the previous studies on germination, Baskin & Baskin (2014) concluded that *L. stoechas* seeds have physiological dormancy. Although some germination properties of *L. stoechas* seeds have been tested, our knowledge on the germination patterns of *L. stoechas* in response to various temperature and light regimes is still incomplete. Moreover, although one study found no germination at >30 °C in *L. stoechas* (Maher *et al.* 2000), it is not clear whether the lack of germination at high temperatures is a result of thermo-inhibition or thermo-dormancy. Therefore, available studies are inconclusive and far from providing complete data on the germination requirements of *L. stoechas*.

In this study, we aimed to disentangle the germination requirements of *L. stoechas* in relation to temperature and light by conducting a comprehensive germination experiment, and also to understand dormancy status in *L. stoechas*. Our experiments comprised various temperature regimes, including fixed (5–30 °C) and alternating (5/15–15/30 °C) temperatures, and light and dark conditions. We performed an additional experiment to understand the dormancy response of the species to high (30 °C) temperatures (*i.e.* thermo-dormancy *versus* thermo-inhibition). Finally, since previous studies showed that there is substantial variation in germination patterns among populations of *L. stoechas*, we included three populations to test whether germination response to different temperature and light conditions and dormancy status show among-population variations in *L. stoechas*.

MATERIAL AND METHODS

Seed collection and subsequent preparation

Clusters of ripe nutlets (seeds) of *L. stoechas* were collected from three natural habitats in Muğla Province, southwest Turkey, during the time of dispersal in July 2018 (Table 1). These sites (*i.e.* populations) were in various post-fire regeneration states of burned *Pinus brutia* Ten. (Turkish red pine) forests. The climate is typical Mediterranean, with a hot and dry summer and mild and rainy winter, with a pronounced dry period (Table 1). Fruit clusters were collected from more than 20 randomly selected individual plants in each population and transferred to the laboratory in paper envelopes in a container in darkness. Fruit clusters were stored in paper envelopes under dark and dry conditions for 5 days, after which seeds were manually extracted in the laboratory. Healthy-looking, intact and mature (black and brown colour) seeds were separated and mixed for each population to create seed lots for germination tests.

Seed germination experiment

Seed germination was tested in climate cabinets (NÜVE TK252 and TK600) at six constant temperatures of 5, 10, 15, 20, 25 and 30 °C, and at four alternating (light/dark) temperatures of 5/15, 10/20, 15/25 and 15/3°C. For light treatments alone, the photoperiod was set at 12 h/12 h light/dark (cool white fluorescent light). In the alternating temperature regime, the 12-h light period coincided with the highest temperature used. For constant dark treatments, the Petri dishes were wrapped with one layer of aluminium foil to create continuous darkness. Since only six climate cabinets were available, Petri dishes in each alternating temperature treatment were moved between cabinets set to the corresponding constant temperatures at the end of the 12-h photoperiod so that all experiments had the same time period.

The seeds collected from the field 10 days before the start of the experiment were used (*i.e.* seed were 10 days old). For each incubation temperature and population, four replicates of 25 seeds per treatment were placed on the surface of 0.7% agar with water in Petri dishes. Germinated seeds were checked daily, or every 2 days in some cases, then counted and removed from the Petri dishes. For samples germinated in dark conditions, germination was scored under a safe green light. Germination was recorded when the radicle was visible under a binocular

Table 1. Site characteristics where *Lavandula stoechas* fruits were collected. Geographic (Lat: latitude, Long: longitude, Alt: altitude), climate (T: annual mean temperature, P: mean annual total precipitation, P_d: mean total precipitation during the dry period [June–September]), habitat properties for each population (Pop).

| Pop | Geographic | | | Climatic | | | Habitat |
	Lat (°)	Long (°)	Alt (m)	T (°C)	P (mm)	P_d (mm)	Post-fire age (yrs)
P1	36.847	28.121	167	18.0	922	28	32
P2	36.827	28.056	15	18.5	895	24	11
P3	36.740	28.876	236	17.1	1003	34	˜30

microscope. Germination tests were considered complete when no further germination had occurred for seven consecutive days. Consequently, since germination rate differed in different incubation conditions: the germination experiments continued for 20–55 days, depending on treatment. At the end of germination experiments, non-germinated seeds were cut to determine viability (white, healthy embryo, no embryo or rotten embryo) using the cut test. Empty seeds were removed from data used to calculate germination and from statistical analyses.

An additional experiment was conducted to identify the thermo-inhibition or thermo-dormancy status of seeds at 30 °C using seeds of the P2 population. In this experiment, the seeds collected from the field 20 days before the start of the experiment (*i.e.* seed 20 days old) were placed on the surface of 0.7% agar with water in Petri dishes and subjected to 30 °C for 1, 2, 3, 5, 9, 12 and 15 days in continuous darkness. During this high-temperature application, the Petri dishes were wrapped with one layer of aluminium foil. Then, they were transferred to 15°C at two conditions of dark and 12:12 h light:dark for 13 days. Subsequently, the germinated seeds were counted.

Data analysis

The relationship between cumulative germination percentage and time after incubation of seeds was described using a logistic model with the NLIN procedure in SAS (Ghaderi-Far *et al.* 2012):

$$y = \frac{G_{max}}{\left(1 + \left(\frac{x}{D_{50max}}\right)^b\right)},$$

where y is total germination (%) at time x, G_{max} is maximum germination (%), D_{50} is time to 50% of maximum germination, and b indicates the slope of the curve at D_{50}. As maximum germination percentage differed among the tested temperatures and among populations, all treatments were compared at a lower percentage (Soltani *et al.* 2015). In this study, the germination percentage was equal to or > 30% in most treatments. Therefore, the germination rate (*i.e.* speed) was calculated based on the 30th percentile of the total seed population (GR = 1/t$_{30}$), estimated through interpolation after fitting the logistic model to the data. Note that germination rate was not estimated when a replicate had < 30% germination and the rate was then estimated for another replicate that has > 30% germination.

To quantify the response of germination to temperature and also determine the cardinal temperatures for germination, a Segmented model was used (Soltani *et al.* 2006):

$$y = \frac{\left(\frac{(T - T_b)}{(T_o - T_b)}\right)}{f_o} \rightarrow if \rightarrow T_b < T < T_o$$

$$y = \frac{\left(\frac{1 - (T - T_o)}{(T_c - T_o)}\right)}{f_o} \rightarrow if \rightarrow T_o < T < T_c$$

$$y = 0 \rightarrow if \rightarrow T \leq T_b \dots or \dots T \geq T_c,$$

where y is germination rate, T is test temperature; T_b, T_o and T_c are base temperature, optimum and ceiling temperatures, respectively (Soltani *et al.* 2006). Hence, T_b and T_c are the temperatures at which no germination occurs and T_o is the temperature for germination.

Relative germination in the light (RLG) was used to calculate the light requirement index for each temperature × population combination (Galindez et al. 2017):

$$RLG = G_L/(G_D + G_L),$$

where G_L and G_D are final germination percentage in light and dark conditions, respectively. RLG can have values from 0 to 1, indicating that germination occurs only in darkness or only in the light, respectively.

For the statistical analysis of germination data, final germination rates obtained in treatments and populations were compared using the analysis of deviance (generalized linear model, GLM). In this analysis, the germinated *versus* non-germinated seeds in each replicate were considered, and therefore a binomial error distribution was assumed (Moreira *et al.* 2010; Çatav *et al.* 2018). *Post-hoc* tests for GLM were performed by estimating marginal means for different temperature treatment combinations in each light treatment and population. Marginal means were estimated using the *emmeans* package in R (Lenth 2020). Additional generalized linear mixed model (GLMM) analyses were performed on germination data to understand the general germination response of the populations to temperature and light regimes. In this analysis, we considered temperature or light (or both) as fixed factors and population as random factor and assumed a binomial error distribution. Model results (χ^2 and *P*-values) were obtained with likelihood ratio tests. GLMM analyses were performed using the *glmer* function in the *lme4* package in R (Bates *et al.* 2015). RLG and germination rate data were analysed with a two- or three-way ANOVA, respectively. In RLG analyses, incubation temperature and population were considered as fixed factors, while for germination rate we included the light regime as an additional fixed factor besides incubation temperature and population. Statistical analyses were performed using R statistical software (https://www.R-project.org/).

RESULTS

The logistic model fitted well to the cumulative germination percentage data *versus* time ($R^2 > 0.98$ in all cases) for all treatments and populations (Figure S1). There was a remarkable difference among treatments in terms of germination percentage, germination rate and time to start of germination.

Germination percentage was higher in the light than the dark at most constant and alternating temperatures (Fig. 1, Tables S1, S2). However, germination response to light regime showed significant variation among studied populations (Table S3). At constant temperatures, maximum germination occurred at 15 °C in the dark (50–87%), whereas germination occurred at 10–25 °C in the light (71–99%). Although the difference in germination under dark and light conditions was lowest at 15 °C constant temperature and 5/15 °C alternating temperature in all populations ($\chi^2 < 60$, GLMM; Table S2, Fig. 1), differences were observed at other incubation temperatures ($\chi^2 > 88$, GLMM; Table S2, Fig. 1). At 5 °C, seeds germinated only under light conditions in all populations, and at 30 °C, no seeds germinated under light or dark conditions.
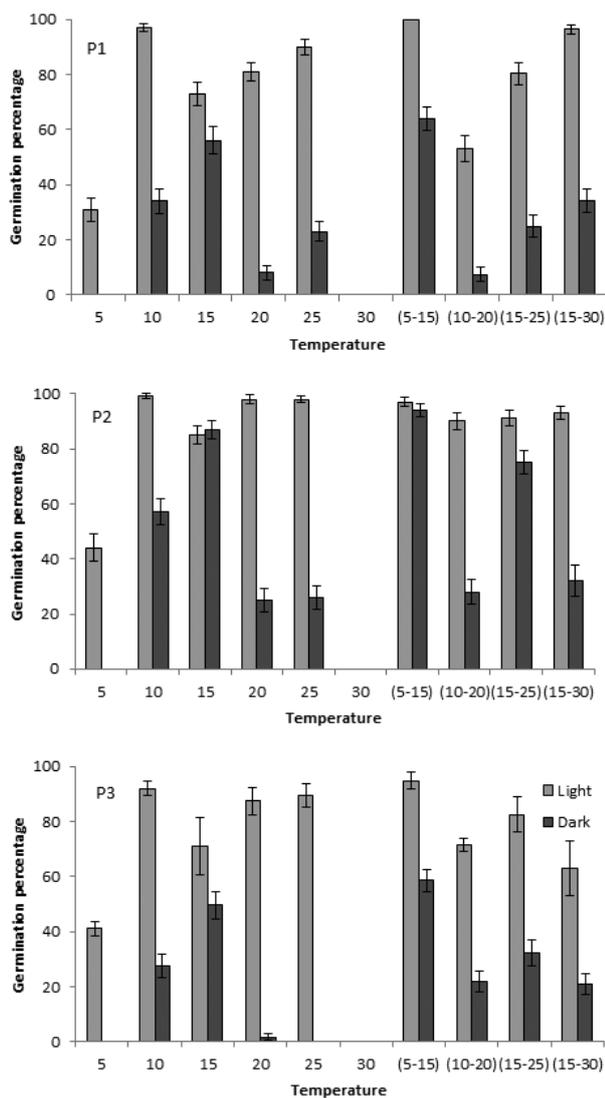
Fig. 1. Seed ginal germination (%) at constant (5, 10, 15, 20, 25 and 30 °C) and alternating (5/15, 10/20, 15/25 and 15/30 °C) incubation temperatures under light and dark conditions in different *L. stoechas* populations (P1, P2 and P3). Vertical bars represent SE of mean based on binomial distribution.

Alternating temperatures improved germination when the average temperature was compared to the corresponding constant temperature under dark conditions in all populations, except for 15/30 °C (Fig. 1, Tables S4, S5, S6). Hence, in many cases, significantly higher germination percentages were obtained at 10/20 °C and 15/25 °C than at 15 °C and 20 °C constant temperatures (Tables S4, S5, S6). The alternating temperature of 5/15 °C gave significantly higher germination than 10 °C constant temperature only in the P2 population (Tables S4, S5, S6). However, this was not the case in the light, where no difference in germination was found between alternating temperatures and corresponding constant temperatures (Tables S4, S5, S6).

Relative light germination (RLG) also exhibited variations among populations and treatments (Fig. 2, Table S7). At constant temperatures, the lowest RLG was at 15 °C in all
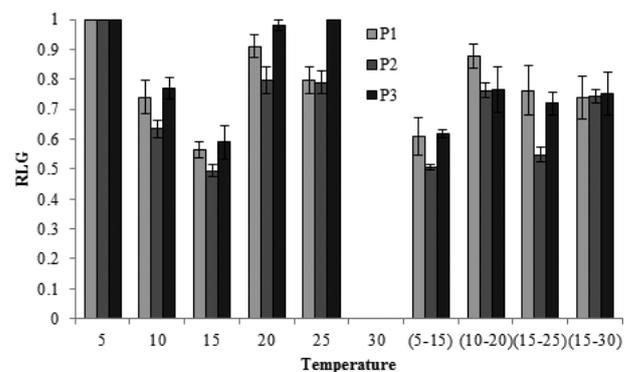
Fig. 2. Relative germination in the light (RLG) at constant (5, 10, 15, 20, 25 and 30° C) and alternating (5/15, 10/20, 15/25 and 15/30 °C) temperatures in different *L. stoechas* populations (P1, P2 and P3). Vertical bars represent SE of mean.

populations but increased significantly at higher and lower temperatures. At alternating temperatures, the lowest RLG was at 5/15 °C (Fig. 2).

The germination rate showed differences among treatments and populations (Table S8). In the light, germination was higher than in darkness at all incubation temperatures (Fig. 3, Table S8).

The estimates of cardinal temperatures for seed germination of *L. stoechas* populations using the Segmented model (Fig. 3) at constant and alternating temperatures are given in Table 2. At constant temperatures, the base temperature (T_b) was not significantly different under light or dark conditions. However, T_b varied among different *L. stoechas* populations at alternating temperatures: P3 population had a lower T_b than the other two populations. Under dark conditions, T_b could not be estimated for the P1 population due to lack of enough points to fit a Segmented model. T_b at alternating temperatures was lower than at constant temperatures under both dark and light conditions in all populations. For alternating temperatures, the optimum temperature (T_o) under light and dark conditions was 20 °C according to the Segmented model. In contrast, T_o was significantly different under light and dark conditions at constant temperatures (Table 2). T_o was higher in the light than the dark, except for the P3 population. The ceiling temperature (T_c) was also not significantly different among all conditions and populations (Table 2).

Since germination did not occur at 30 °C, we investigated in more detail how long *L. stoechas* seeds require at this high temperature to induce dormancy (Fig. 4). Under dark conditions, seed germination was not influenced by high temperature during the first ˜2.7 days, and seeds germinated to almost 99%. After that time, seeds started to enter thermo-dormancy at 30 °C. From this time point onwards, seed germination declined sharply until 40 days, at which no further seeds germinated (Fig. 4). Seeds exposed to 30 °C that were transferred to optimal temperature under light conditions could germinate (Fig. 4). Consequently, seeds did not enter thermo-dormancy at any time during treatments under light conditions.

DISCUSSION

Our results show that fresh mature seeds of *L. stoechas* had conditional dormancy because they germinated to high
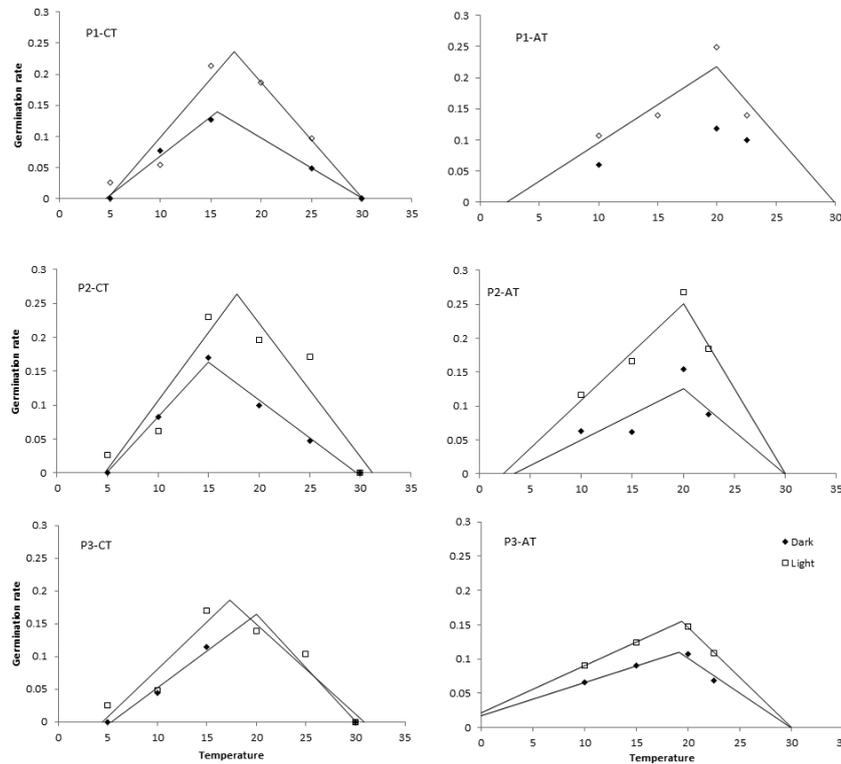
Fig. 3. Curve fitting of Segmented model to germination rate *versus* temperature under light and dark conditions for constant (CT) and alternating (AT) temperatures in different *L. stoechas* populations (P1, P2 and P3).

Table 2. Cardinal temperatures (°C; mean ± SE) under light and dark conditions for constant (CT) and alternating (AT) temperatures in different *L. stoechas* populations (P1, P2 and P3). T_b, T_o, and T_c are base, optimum and ceiling temperatures, respectively.

		T_b		T_o		T_c	
		Dark	Light	Dark	Light	Dark	Light
P1	CT	4.53 ± 0.86	4.76 ± 1.88	15.65 ± 1.81	17.34 ± 1.72	30.10 ± 1.96	30.12 ± 1.89
	AT	–	2.28 ± 6.78	–	20.00 ± 2.12	–	29.89 ± 1.98
P2	CT	4.93 ± 0.75	4.76 ± 2.64	14.96 ± 0.88	17.77 ± 2.45	29.60 ± 1.13	31.20 ± 3.11
	AT	3.47 ± 9.51	2.39 ± 3.12	20.0 ± 3.41	20.00 ± 1.00	29.94 ± 3.28	30.00 ± 0.95
P3	CT	5.30 ± 0.83	4.42 ± 2.33	20. 00 ± 1.45	17.34 ± 2.08	30.07 ± 0.67	30.88 ± 2.53
	AT	-3.57 ± 9.35	-3.15 ± 1.01	19.11 ± 1.44	19.42 ± 0.16	29.91 ± 0.94	30.04 ± 0.10

percentages at 10 to 25 °C in the light, and germination decreased at 5 and 30 °C and reached zero at 30 °C. These results are consistent with the findings of Maher *et al.* (2000) who found germination percentages of 76–96% and 37–92% between 10–25°C under light and dark conditions, respectively. Since seeds that imbibe water need 30 days or less for embryo growth and germination under favourable environmental conditions do not have physical or morphological dormancy (Baskin & Baskin 2014), the results suggest that *L. stoechas* seeds do not possess physical dormancy (PY), morphological dormancy (MD), morphophysiological dormancy (MPD) or combinational dormancy (PY+PD). Since after-ripening (dry storage at room temperatures for 2 years) increased germination of this plant in light and dark at several temperatures, and gibberellic acid (GA) improved germination percentage in light and dark (Ghaderi-Far *et al.* unpublished data), it is clear that

the slight dormancy exhibited by fresh seeds of *L. stoechas* is in the physiological dormancy class (PD).

The results also showed that fresh mature seeds of *L. stoechas* can germinate both in light and dark conditions at constant and alternating temperatures (Fig. 1). Moreover, germination in the light was higher than that in the dark. The germination response of seeds to light can be classified into three classes (Baskin & Baskin 2014; Carta *et al.* 2017). The first comprises seeds that only germinate in the dark (photo-inhibition), the second includes those that only germinate in the light (photo-stimulation), and the third is seeds that can germinate both in the dark and light. The third class can be divided into three further levels according to relative germination percentages in the light and dark (dark > light, light > dark and light = dark). Hence, our results suggest that *L. stoechas* can be classified to the second level (*i.e.* light>dark) of the third class among these
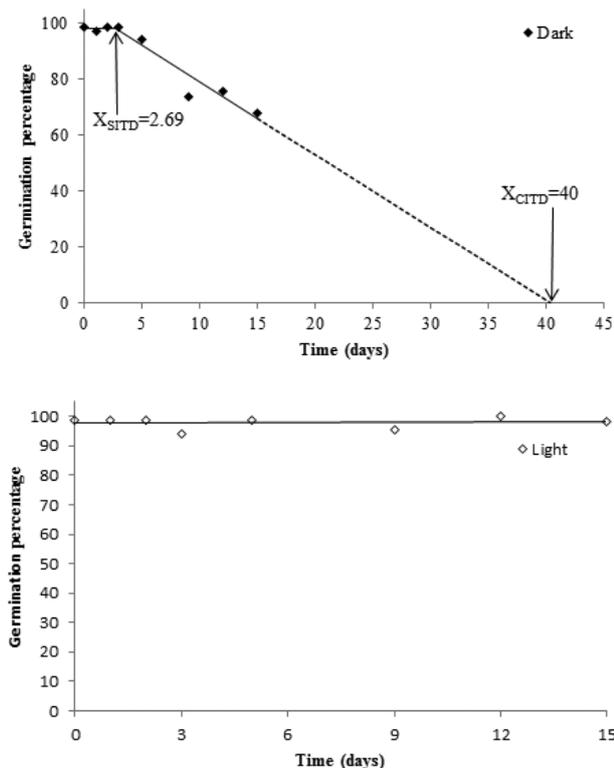
Fig. 4. Required time to induce thermo-dormancy in *L. stoechas* seeds (P2 population) in dark or light conditions.

categories. Hereupon, seeds of *L. stoechas* were stimulated by light, suggesting that seed germination has a phytochrome-mediated response.

Light and temperature had an interaction effect on the germination percentage, as RLG showed different trends at different incubation temperatures. While germination percentage was similar between light and dark conditions at 15 °C, the dependence of seeds on light increased with increasing and decreasing temperature. At alternating temperatures, the response to light depended on incubation temperature, and germination in light was higher than in dark, except for 5/15 °C with the lowest RLG (Fig. 2). However, the light dependence of seeds was lower at alternating than at constant temperatures, as RLG values were lower at alternating temperatures (Fig. 2). This suggests that alternating temperature increases the proportion of seeds germinating in the dark in comparison to the constant temperature, so alternating temperatures can substitute for light in *L. stoechas*. This germination pattern appears in many species worldwide (Probert 2000). Germination stimulation under fluctuating temperatures allows seeds to germinate in favourable conditions during the spring (Fenner & Thompson 2005). Moreover, alternating temperatures also act as depth sensers and in gap detection in plants (Thompson & Grime 1983), and therefore can substitute for light, which is another cue for gap detection. Observations on heat shock- or smoke-stimulated germination under dark conditions in *L. stoechas* (Moreira *et al.* 2010; Moreira *et al.* 2012; Çatav *et al.* 2014; Kazancı & Tavşanoğlu 2019) suggest that fire-related cues can also substitute for light in germination of this species. Since the distribution range of *L. stoechas* is restricted to fire-prone Mediterranean environments

(Moreira *et al.* 2012), fire plays a significant role in the life history of this species. Indeed, light, heat shock and smoke stimulate germination in Mediterranean fire-dependent species (Tavşanoğlu et al. 2017), and it seems *L. stoechas* also uses these signals as a germination cue.

Keeley & Baer-Keeley (1999) showed that germination of *L. stoechas* was similar in light and dark conditions at 18 °C and classified the seeds as light-neutral. Maher *et al.* (2000) stated that seeds of *L. stoechas* were unable to germinate at 5, 30 and 35 °C. Also, germination in dark conditions in their study was reduced by 2–5% compared to light at 10, 15 and 20 °C, while at 25 °C it was as low as 37%. Thus, germination percentage of *L. stoechas* at 5, 20 and 25 °C in the present study was higher than that obtained in Maher *et al.* (2000) (Fig. 1). These differences may be due to differences in environmental conditions prevailing during seed development and maturation, the local adaptation of populations or other unconsidered factors (Perez-Garcia *et al.* 2003).

Cardinal temperatures of germination varied between constant and alternating temperatures (Table 2). Although T_b at alternating temperature was lower than at constant temperatures in all populations, there was little difference between light and dark conditions. In field conditions, seeds are exposed to alternating temperatures on the soil surface, thus, *L. stoechas* seeds will germinate at lower temperatures. Also, these seeds have a higher optimal temperature (T_o) at alternating temperatures than at constant temperatures. Therefore, seeds of *L. stoechas* are capable of rapid germination in a broad range of temperatures under natural conditions.

Lavandula stoechas seeds did not germinate at 30 °C (Tc), and no germination was expected above this temperature. Papafotiou *et al.* (2000) observed that *L. stoechas* seeds sown in the October–March period had close to 100% germination, while seeds sown in April had only 5% germination and those sown in May did not germinate, suggesting deterrence of germination at higher temperatures in *L. stoechas*. Non-germination of *L. stoechas* seeds at 30 °C can be attributed to thermo-dormancy or thermo-inhibition (Hills & Van Staden 2003; Geshnizjani *et al.* 2018). Maher *et al.* (2000) did not observe any germination of *L. stoechas* at constant 30 and 35 °C and concluded this was a result of thermo-inhibition or thermo-dormancy. In the present study, seeds exposed to 30 °C for 1 to 15 days had different responses in light and dark after transfer to the optimum temperature (Fig. 4). In the presence of light, seeds of *L. stoechas* could germinate after transfer to the optimum temperature; in other words, thermo-inhibition was dominant. However, in the dark, during the first 2.7 days, seed germination was not influenced by high temperature, and thus seeds germinated to almost 98%, while seeds went into thermo-dormancy after 2.7 days of exposure to 30 °C. Therefore, the germination response of *L. stoechas* to high incubation temperatures seems more complex than previously thought. Different mechanisms have been proposed for the induction of dormancy in seeds at high temperatures, such as weakening of the endosperm, hormonal balance and changes in phytochrome (Saini *et al.* 1989; Chen & Bradford 2000; Nascimento *et al.* 2004; Leymarie *et al.* 2009). The limited capacity of *L. stoechas* seeds to germinate at high temperatures and also the low physiological dormancy of fresh seeds could be an advantage in warm and summer/dry fire-prone environments of Mediterranean habitats. Indeed, therm-odormancy and thermo-inhibition have been previously observed in Mediterranean plants

(Thanos *et al.* 1989; Cristaudo *et al.* 2019), and our results on *L. stoechas* widen our understanding of these phenomena. At the time of the dispersal of *L. stoechas* seeds, the combination of high summer soil temperatures and lack of water availability in the soil would be unfavourable for germination and seedling growth. Indeed, in summer soil temperatures can reach >40 °C for several hours in Mediterranean shrublands (Santana *et al.* 2013). Therefore, we conclude that the regeneration niche of *L. stoechas* is constituted by two distinct germination-delaying mechanisms: (1) accumulation of dormant seeds in the soil seed bank until the next fire occurs, and (2) delay in germination of non-dormant seeds until favourable environmental conditions. Such a dual bet-hedging strategy would have a selective advantage for *L. stoechas*, and possibly many other Mediterranean seeder species, by allowing seedling establishment in both fire and non-fire years. Further studies on this aspect in different species is promising and will allow us to obtain a more complete figure for germination dynamics in Mediterranean seeder species.

Although we have examined other general germination patterns, the germination response to different incubation temperatures in the studied *L. stoechas* populations showed some variation. To date, only two studies have directly compared germination among *L. stoechas* populations (Perez-Garcia *et al.* 2003; Moreira *et al.* 2012). Perez-Garcia *et al.* (2003) showed considerable variation in germination percentage among 17 populations in Spain, which appear to be slightly related to seed size. In their study, including six *L. stoechas* populations from Spain and Turkey, Moreira *et al.* (2012) found that although there was substantial variation in germination in control and heat-shock groups, smoke application removed this variation among populations. Since both studies were performed at only one incubation condition (15 and 20 °C, respectively, and a specific light condition), our results are the first to examine among-population variation of germination response in different incubation conditions (temperature and light) in *L. stoechas*. Although, it is difficult to correlate the results with environmental variables or other seed traits (*e.g.* seed size) since we only studied three populations, our results suggest that the variation in germination response to temperature is more apparent than to light regime in *L. stoechas*. Therefore, further studies are needed to test the association between local climate conditions and germination at different incubation temperatures in order to fully understand the reasons of germination variability in *L. stoechas*.

In conclusion, our study demonstrates that temperature and light have combinational effects on germination of *L. stoechas*, and that these interactions show significant variation among populations. Our study also infers the involvement of fire–environment interactions in the germination of Mediterranean seeder species by showing that both light and alternating temperatures stimulate germination of *L. stoechas*, a species well-known for its fire-related germination.

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AUTHOR CONTRIBUTIONS

F.G.F. and Ç.T. designed the study, Ç.T. collected seeds from the field, all authors performed the laboratory experiments, F.G.F. and Ç.T. analysed data and wrote the first draft of the manuscript, and all authors contributed to the final version of the manuscript.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Cumulative germination (%) versus time (days) graphs at constant (5, 10, 15, 20, and 25°C) and alternating (5/15, 10/20, 15/25, and 15/30°C) incubation temperatures under light and dark conditions in different *L. stoechas* populations (P1, P2, and P3). Curves are logistic models fitted ($R^2 > 0.98$) to each germination versus time data. Data points and germination curves were not shown when no additional germination occurs for seven consecutive days in any treatment at the end of the experiment.

Table S1. Summary of the analysis of deviance (GLM) for the effects of incubation temperature (*Temp*), light regime (*Light*), and origin population (*Pop*) on the germination probability. Results of GLMM analysis considering *Temp* and *Light* as fixed factors and *Pop* as the random factor are also presented. Degrees of freedom (*df*), deviance (for GLM), chi-square (for GLMM), and significance (*P* value) are given. Significant *P* values at α = 0.05 level are given in bold.

Table S2. Results of the analysis of deviance for the difference in germination probability between light regimes (dark vs. light) under different incubation temperatures (*Temp*) in different populations (degrees of freedom are 1). Results of overall GLMM analysis (*Overall*) considering temperature and population as the fixed and random factors, respectively, are also presented. Deviance (*Dev.*; for GLM), chi-square values (for GLMM), and significance (*P* value) are given. Significant *P* values at α = 0.05 level are given in bold.

Table S3. Results of the analysis of deviance for the difference in germination probability among incubation temperatures under different light regimes (dark and light) in different populations (degrees of freedom are 9). Results of overall GLMM analysis considering light regime and population as the fixed and random factors, respectively, are also presented. Deviance (for GLM), chi-square (for GLMM), and significance (*P* value) are given. Significant *P* values at α = 0.05 level are given in bold.

Table S4. Pairwise comparisons of germination probability between temperature treatments under light and dark conditions in population 1 (based on results of post-hoc tests for the analysis of deviance). Significant *P* values at α = 0.05 level are given in bold.

Table S5. Pairwise comparisons of germination probability between temperature treatments under light and dark

conditions in population 2 (based on results of post-hoc tests for the analysis of deviance). Significant P values at $\alpha = 0.05$ level are given in bold.

Table S6. Pairwise comparisons of germination probability between temperature treatments under light and dark conditions in population 3 (based on results of post-hoc tests for the analysis of deviance). Significant P values at $\alpha = 0.05$ level are given in bold.

Table S7. Summary of the analysis of variance for the effects of incubation temperature (*Temp*) and origin

population (*Pop*) on the relative light germination (RLG). Degrees of freedom (*df*), the sum of squares (*SS*), F value, and significance (*P* value) are given. Significant P values at $\alpha = 0.05$ level are given in bold.

Table S8. Summary of the analysis of variance for the effects of incubation temperature (*Temp*), light regime (*Light*), and origin population (*Pop*) on the germination rate ($1/t_{30}$). Degrees of freedom (*df*), the sum of squares (*SS*), F value, and significance (*P* value) are given. Significant P values at $\alpha = 0.05$ level are given in bold.

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