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REGULAR PAPER

Inter- and intra-specific variability in seed dormancy loss and germination requirements in the *Lavatera triloba* aggregate (Malvaceae)

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Background and aims – The genus *Lavatera* is comprised of perennial and annual species that grow in different ecosystems such as coastal cliffs, plains, endorheic lagoons and ditches. In this study, we compared the seed germination ecology of three phylogenetically related taxa: *L. agrigentina*, *L. triloba* subsp. *pallescens* and *L. triloba* subsp. *triloba*, all of which belong to the *L. triloba* aggregate (section *Glandulosae*) and grow on chalky plains, limestone cliffs and endorheic lagoons with clayey sediments.
Methods – For each taxon, the effects of seed scarification and dry after-ripening (90 days at 25°C) on seed dormancy loss and of light and temperature (constant 5 to 25°C and alternating 25/10°C) on seed germination were evaluated.
Key results – Scarification allowed water imbibition and subsequent seed germination, indicating physical dormancy (PY) for all three investigated taxa. In addition, dry after-ripening positively affected seed germination in *L. agrigentina*, thus, seeds of this species have combinational dormancy [physical (PY) + physiological (PD)]. Light did not affect the final germination in any of the taxa. The germination response to incubation temperatures varied among the taxa, suggesting ecological adaptations, with the highest germination in the range of 10–20°C for *L. agrigentina* and *L. triloba* and 5–15°C for *L. pallescens*. Inter-population variability in final germination was detected for both *L. agrigentina* and *L. triloba* subsp. *triloba* and in germination rate only for *L. agrigentina*.
Conclusions – The three *Lavatera* taxa differed in their germination requirements, and these could be related to the habitat/ecology of each taxon. Our results suggest that seeds of the three taxa can germinate in the field from autumn to spring, when conditions are favourable for seedling establishment in the Mediterranean climate.

Key words – Combinational dormancy, dry after-ripening, germination, Mediterranean vascular flora, physical dormancy, physiological dormancy, scarification.

INTRODUCTION

Successful germination is crucial in the life cycle of terrestrial Angiosperms and dormancy is an innate seed property that defines the environmental conditions in which the seed is able to germinate (Baskin & Baskin 1998). Seed dormancy is defined as an intrinsic block to the completion of germination of a viable seed under unfavorable conditions for germination (Finch-Savage & Leubner-Metzger 2006). Seed dormancy caused by a physiological inhibiting mechanism, is called physiological dormancy (PD; Baskin & Baskin 1998). A water-impermeable seed coat can be cause of a physical dormancy (PY), which develops during the maturation drying stage of the seed (Van Staden et al. 1989) and the breaking of PY involves disruption or dislodgement of small opening(s) (water gap) in a morpho-anatomically specialised area in seeds or fruits, causing the seeds/fruits to become permeable (Gama-Arachchige et al. 2013). The water-gap region is a morpho-anatomically specialised area, which differs from the rest of the seed or fruit coat. The location, anatomy, morphology and origin of water-gaps can differ between and even within families (Gama-Arachchige et al. 2013). In addition, combinational dormancy can be observed when PY is associated with PD (PY + PD; Baskin & Baskin 1998), as reported for species belonging to the Anacardiaceae, Fabaceae and Malvaceae (Fenner & Thompson 2005, Dunn 2011).

Several environmental factors, including light, moisture, temperature and soil composition, can determine differences in germination behaviour and cause specific adaptations even in phylogenetically related species (Ellison 2001). Various researchers have found that among congeneric species, seed germination patterns can have a direct impact on the level of rarity of the species, thus, influencing their distribution and reflecting different ecological adaptations (Ramírez-Padilla & Valverde 2005). Several studies have highlighted the presence of intra-specific variation (inter-population variability) in germination and dormancy (e.g. Andersson & Milberg 1998, Baskin & Baskin 1998, Keller & Kollman 1999), which can be due to environmental differences (Cruz et al. 2003), genetic variation, or a combination of both factors (Degreef et al. 2002).

Seed drying at warm temperatures (dry after-ripening) is a natural mechanism that controls dormancy in dry climates (Finch-Savage et al. 2007). A period of usually several months of dry storage at room temperature of freshly harvested, mature seeds, is a common method used in laboratories to mimic this mechanism and release physiological seed dormancy (Bewley 1997).The dry after-ripening in Mediterranean habitats has an important ecological value and its reproduction under laboratory conditions allows to simulate the dry summer season, typical of the Mediterranean climate (Baskin & Baskin 1998, Santo et al. 2014a, 2014b).

In the Mediterranean area, several species of Malvaceae occur both in coastal and inland habitats. Here, the genus *Lavatera* comprises perennial and annual species growing in different ecosystems (e.g. coastal cliffs, plains, endorheic lagoons, ditches) and on various substrates (limestones, clays, saline sediments; Fernandes 1968, Bacchetta et al. 2011). The *Lavatera triloba* aggregate is a monophyletic group of perennial herbs or sub-shrubs that is endemic to the Western Mediterranean region (Escobar García et al. 2009). *Lavatera* species are reported to have orthodox seeds (Royal Botanic Gardens of Kew 2008) with an axil-folded embryo and firm-fleshy endosperm (Martin 1946). Finch-Savage & Leubner-Metzger (2006) reported seeds of the Malvaceae to be non-dormant (ND), physically dormant (PY) or with a combinational dormancy (PY + PD). These findings are in agreement with those of Gama-Arachchige et al. (2013), who recently reported that this family has a complex 'type III' water-gap, in which narrow linear or circular openings are occluded by plug-like structures usually formed by water-impermeable sclerenchyma cells. Concerning the three species in this study, seed germination data are available only for *Lavatera triloba* subsp. *triloba*: for this taxon, high germination (c. 85%) is reported when seeds were chipped with a scalpel and incubated at 15 and 20°C, in the light (8 h irradiance per day; Royal Botanic Gardens of Kew 2008).

However, for none of the three species, factorial germination experiments have been carried out to determine the key factors that stimulate germination and no data exist concerning inter-population variability in seed germination.

The aim of this study was to compare the seed ecology of three phylogenetically related *Lavatera* taxa by: (1) characterising their seed dormancy; (2) identifying their germination requirements in terms of light and temperature and (3) investigating their inter-population variability in seed dormancy and germination requirements.

MATERIAL AND METHODS

Study species

The three taxa investigated in this study were *Lavatera agrigentina* Tineo, *L. triloba* L. subsp. *pallescens* (Moris) Nyman and *L. triloba* L. subsp. *triloba*, all of which belong to the section *Glandulosae* R. Fern. and to the *Lavatera triloba* aggregate (Escobar García et al. 2010).

Lavatera agrigentina Tineo is a nanophanerophyte, 30–200 cm tall (Escobar García et al. 2010). Flowering occurs from April to May. Fruits are schizocarps, with 15–23 mericarps fused together, which ripen from late May to late July (Escobar García et al. 2010). It grows in open habitats on clayey-chalky sediments at 200–750 m a.s.l. This taxon is distributed in southern Sicily (Bacchetta et al. 2011) and occurs mainly in arid grassland dominated by *Lygeum spartum* L., on steep slopes of badlands and sometimes in the shrubby vegetation of the *Pegano-Salsoletea* class (Brullo et al. 2010, 2013).

Lavatera triloba subsp. *pallescens* (hereafter *L. pallescens*) is a nanophanerophyte, (50–)70–150 cm tall (Escobar García et al. 2010). Flowering occurs from late April to late June. Fruits are schizocarps with 10–20 mericarps fused together, which ripen from late May to late July (Fenu et al. 2010). This taxon actually occurs in only one population in southwestern Sardinia, on limestone cliffs from 20 to 48 m offshore (Fenu et al. 2010).

Lavatera triloba subsp. *triloba* (hereafter *L. triloba*) is a nanophanerophyte, 30–200 cm tall (Escobar García et al. 2010). Flowering occurs from May to late June. Fruits are schizocarps, with 12–16 mericarps fused together, which ripen from late June to late August. This taxon is distributed on the Iberian Peninsula and southern Sardinia, growing in open habitats on clayey saline sediments, often subruderal, and can be locally abundant around endorheic lagoons (Escobar García et al. 2010).

Seed-lot details

Mericarps (hereafter seeds) of the three taxa were collected in one to three different natural populations per species (table 1) at the time of natural dispersal. Seeds were separated from the fruit and collected by hand. Mean seed mass (± 1 SD) for each population was calculated by weighing 10 replicates of 20 seeds each (table 1).

Imbibition tests

To detect the presence of water-impermeable teguments, i.e. the physical component of seed dormancy and the potential need for seed scarification for germination, three replicates of 50 seeds each with scarified (i.e. manually chipped with a scalpel) or intact seeds from one seed lot for each taxon (table 1), were soaked for 120 hours in distilled water, then blotted dry and subsequently incubated in a growth chamber (SANYO MLR-351) at a constant temperature of 20°C. Dur-

Table 1 – Population data and seed-lot details for the three *Lavatera* taxa.
In the column "Experimental trials", the different experiments carried out for each seed lot are reported (IMB = Imbibition test; L = Light; T = Temperature; DAR = Dry after-ripening).

Taxon	Population	Locality	Coordinates (WGS84, UTM)	Substrate	Mean altitude (m a.s.l)	N° of sampled individuals	Date of collecting	Mean seed mass (mg ± SD)	Experimental trials
L. agrigentina	La1	Agìra (EN) E Sicily	37°33'N 14°32'E	Clays	232	10	07 Jul. 2010	8.69 ± 0.10	T
L. agrigentina	La2	Ássoro (EN) E Sicily	37°37'N 14°25'E	Chalky clays	530	18	07 Jul. 2010	8.66 ± 0.01	IMB, L, T, DAR
L. pallescens	Lp1	Buggerru (CI) SW Sardinia	39°24'N 08°24'E	Limestone cliffs	19	37	22 Jul. 2010	3.53 ± 0.07	IMB, L, T, DAR
L. triloba	Lt1	Elmas (CA) SW Sardinia	38°16'N 08°01'E	Clayey saline sediments	0.5	70	19 Jul. 2010	6.35 ± 0.02	T
L. triloba	Lt2	Pula (CA) SW Sardinia	38°59'N 08°59'E	Clayey saline sediments	2	20	24 Jul. 2010	5.66 ± 0.03	T
L. triloba	Lt3	Domus de Maria (CA) SW Sardinia	38°54'N 08°52'E	Clayey saline sediments	0.5	25	24 Jul. 2010	6.65 ± 0.02	IMB, L, T, DAR

ing incubation seeds were weighed every hour for the first 12 h, and then every 24 h for a total of 120 h to detect seed mass increase as a measure for the start of germination.

Germination tests

A preliminary test was carried out to evaluate the effect of light on seed germination for seeds from one seed lot for each taxon (table 1). Seeds were sown on 1% water agar substrate, which provided a solid, non-sterile medium for germination, in 90-mm-diameter plastic Petri-dishes. Three replicates of 20 seeds from one population for each species were incubated in the light (12 h irradiance per day) and in the dark, in growth chambers (SANYO MLR-351) at 15°C. This temperature was chosen as it was the best germination condition reported for *L. triloba* seeds (Royal Botanic Gardens of Kew 2008). Darkness was achieved by wrapping dishes with two layers of aluminum foil. The criterion for germination was visible radical protrusion. Seeds incubated in the light were scored daily and germinated seeds were discarded, whereas seeds incubated in the dark were scored only at the end of the test, to avoid any exposure to irradiance (Baskin et al. 2006). When no additional germination occurred in the light for two consecutive weeks, tests were stopped both in the light and in the dark and the viability of any remaining seeds was checked by a cut test (Santo et al. 2014c).

To evaluate the effect of temperature, three replicates of 20 seeds each from all seed lots (table 1) were incubated in a range of constant temperatures (5, 10, 15, 20 and 25°C) and in an alternating temperature regime (25/10°C) in the light (12 h irradiance per day). In the alternating temperature regime, the higher temperature period coincided with the light period (Baskin et al. 2006).

To test the effect of a three-month dry after-ripening (DAR) period, a sub-lot of freshly collected seeds from one seed lot of each taxon (table 1) was placed in a dry room (15°C and 15% relative humidity). The progress of drying was monitored by measuring the water activity (aw) using a hygrometer Hygropalm Aw1 (Rotronic), equipped with the AW-DIO probe. When seeds reached aw = 0.18, they were closed in a sealed transparent polyethylene envelope, together with two microbags containing silica gel (0.5 g each) within a hermetic 2,000-mL glass jar with 100 g of granular brown silica gel (diameter 2–5 mm), to maintain a low level of humidity (Santo et al. 2014b). The jar was then incubated at 25°C in a growth chamber and after three months, seeds were sown in Petri-dishes and tested in the light as described above.

Data analysis

Water uptake was calculated following Hidayati et al. (2001) in relation to the seed mass:
$\%Ws = [(Wi - Wd) / Wd] \times 100$, where Ws = increase in mass of seed, Wi = mass of seed after a given interval of imbibition, and Wd = seed mass at the beginning.

The final germination was calculated as the mean of the three replicates (± 1 standard deviation). The rate of germination was estimated by using a modified Timson's index of germination velocity (TI; Khan & Ungar 1984): $TI = \sum G/t$, where G is the percentage seed germination at two-day intervals and t is the total germination period. Using this index, a higher value indicates more rapid germination. When ANOVA assumptions were satisfied for arcsin-transformed germination percentages and Log_{10}-transformed TI, one- or two-way ANOVAs, with subsequent Fisher's Least Significant Difference (LSD) *post hoc* test, were carried out to evaluate

Table 2 – Seed mass increase (%) during the imbibition tests for intact and scarified seeds of *Lavatera agrigentina* (La2), *L. pallescens* (Lp1) and *L. triloba* (Lt3).

Taxon	Seed treatment	Time (hours)														
		1	2	3	4	5	6	7	8	9	10	11	12	24	48	72	96	120
L. agrigentina	intact	4.1 ± 2.6	5.9 ± 1.8	6.3 ± 1.1	6.7 ± 0.6	8.5 ± 4.9	8.7 ± 2.4	6.1 ± 1.3	9.1 ± 0.8	9.1 ± 1.3	7.9 ± 1.3	7.9 ± 1.2	8.1 ± 1.4	9.3 ± 3.8	10.7 ± 3.4	11.1 ± 0.7	11.6 ± 2.4	12.2 ± 3.7
	scarified	23.0 ± 0.4	77.3 ± 3.1	92.6 ± 2.1	96.0 ± 6.7	98.8 ± 2.8	105.1 ± 3.0	104.7 ± 2.9	104.1 ± 1.3	105.1 ± 1.6	105.3 ± 0.6	105.2 ± 2.7	102.3 ± 5.3	106.7 ± 2.5	110.2 ± 1.4	111.2 ± 0.4	111.9 ± 1.8	108.2 ± 4.1
L. pallescens	intact	4.3 ± 0.5	4.2 ± 1.0	3.8 ± 0.8	5.4 ± 0.9	3.3 ± 1.2	4.5 ± 1.0	5.3 ± 0.9	5.9 ± 1.2	6.9 ± 0.8	6.3 ± 1.5	6.1 ± 1.7	6.1 ± 1.8	7.9 ± 2.5	10.5 ± 2.1	12.1 ± 1.4	14.2 ± 3.7	13.2 ± 3.3
	scarified	69.1 ± 2.1	80.9 ± 1.0	91.2 ± 3.2	97.4 ± 2.4	98.8 ± 1.3	98.4 ± 0.7	99.9 ± 2.8	100.8 ± 2.0	99.5 ± 2.7	100.2 ± 2.2	100.5 ± 2.7	100.9 ± 2.7	103.3 ± 3.0	109.7 ± 1.2	110.2 ± 0.5	111.1 ± 1.4	112.7 ± 2.1
L. triloba	intact	4.5 ± 2.0	4.8 ± 1.9	5.7 ± 1.7	5.6 ± 1.6	5.7 ± 1.5	6.4 ± 1.0	7.1 ± 2.6	6.4 ± 0.7	6.3 ± 0.6	6.4 ± 0.3	6.6 ± 0.4	6.4 ± 0.4	6.5 ± 0.5	7.3 ± 1.0	7.9 ± 2.1	8.9 ± 0.3	10.8 ± 2.2
	scarified	54.6 ± 4.5	81.8 ± 1.3	88.6 ± 0.8	91.9 ± 0.4	95.4 ± 0.9	98.4 ± 2.1	97.8 ± 5.1	100.5 ± 3.8	102.0 ± 4.2	101.2 ± 3.6	101.4 ± 3.6	101.5 ± 3.6	104.8 ± 3.6	106.6 ± 4.8	107.9 ± 1.6	109.3 ± 6.0	108.6 ± 5.8

the effect of temperature, light, population and pretreatment. The TI values were calculated both for fresh and dry after-ripened (hereafter DAR) seeds, only for seeds germinated in the light. When the assumptions of ANOVA were not satisfied, the non-parametric Kruskal-Wallis test, followed by a Mann-Whitney *U*-test, was carried out. All statistical analysis were carried out using the software Statistica 7.0 for Windows (Statsoft Release 7).

RESULTS

Imbibition tests

The seed mass of intact seeds of all three study species did not increase by more than 15%, even after 120 h, but that of scarified seeds increased by c. 23, 70 and 54%, for *L. agrigentina*, *L. pallescens* and *L. triloba*, respectively, in the first hour (fig. 1). Scarified seeds of *L. agrigentina* and *L. triloba* reached a maximum increase in mass (c. 110%) after 90 h, whereas those of *L. pallescens* reached a maximum after 120 h (table 2). Moreover, scarified seeds of all three taxa germinated during the imbibition test starting at 9 h of incubation for *L. pallescens*, 48 h for *L. agrigentina* and 120 h for *L. triloba* (fig. 1) whereas intact seeds never germinated during the test. Therefore, all subsequent germination tests were conducted using scarified seeds only.

Effect of light and temperature on seed germination

For all three taxa, one-way ANOVA showed a non-significant ($P > 0.05$) effect of light on seed germination (table 3). Fresh seeds of *L. agrigentina*, *L. pallescens* and *L. triloba* incubated in light and in darkness germinated at c. 63% and 60%, 88% and 78%, 80% and 93%, respectively (table 3).

For fresh seeds of *L. agrigentina,* two-way ANOVA on the final germination showed a highly significant effect of temperature (T = $P < 0.001$), population origin (Pop = $P < 0.001$) and their interaction (T × Pop = $P < 0.001$; table 4A). Seeds of this taxon showed the highest final germination at low temperatures (i.e. 10–15°C), whereas at higher temperatures (i.e. 25°C), germination decreased significantly (table 5). The alternating temperature regime (25/10°C) did not promote germination. Significant differences ($P < 0.05$)

Table 3 – Final germination at 15°C in the light (12/12) and dark (0/24) for the three *Lavatera* taxa.

One-way ANOVA was conducted for each species to detect the effect of light (L) on seed germination. A Fisher's LSD *post hoc* test was conducted to identify significant differences at $P < 0.05$. Data are the mean (± 1 SD) of three replicates. Values with the same letters are not different at $P < 0.05$. Population codes are the same as in table 1.

Taxon	Germination (%)	
	Light (12/12)	Dark (0/24)
Lavatera agrigentina (La2)	63.0 ± 7.6 [a]	60.0 ± 10.0 [a]
Lavatera pallescens (Lp1)	88.0 ± 12.0 [a]	78.3 ± 7.6 [a]
Lavatera triloba (Lt3)	80.0 ± 10.0 [a]	93.3 ± 5.8 [a]

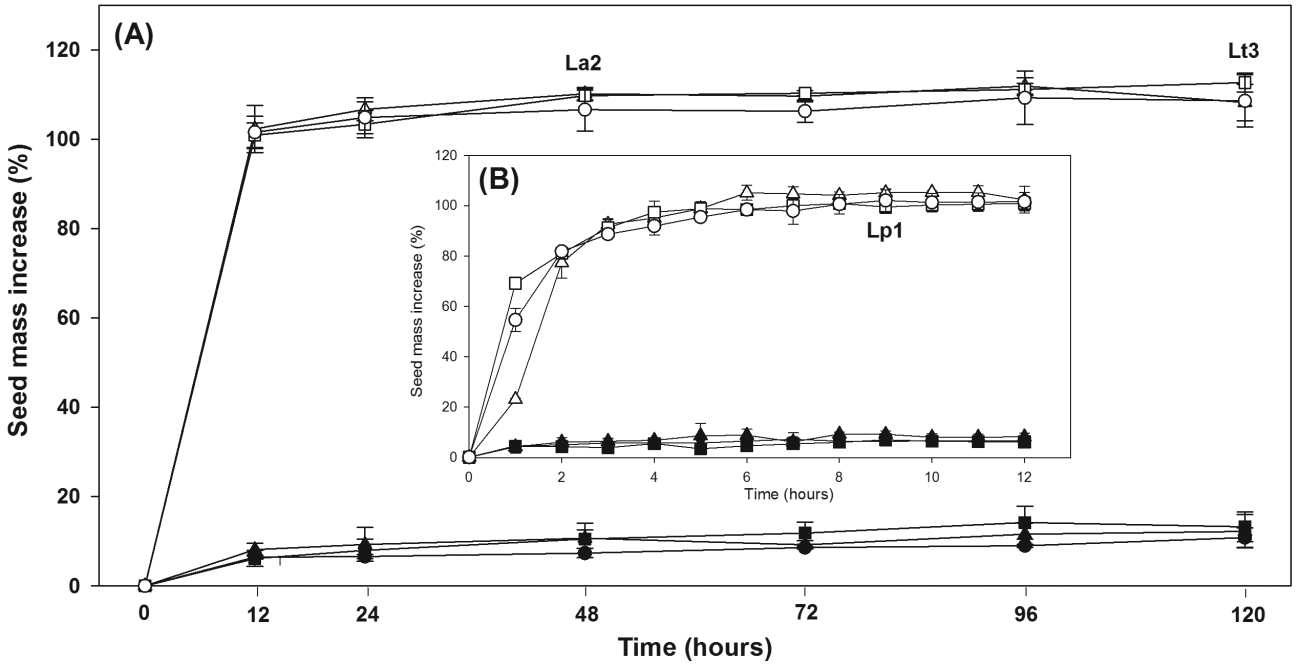

Figure 1 – Imbibition curves for the three *Lavatera* taxa: (A) total duration of the experiment (120 h) and (B) detail of the first 12 h. Symbols indicate: ▲ = *Lavatera agrigentina* (La2), ■ = *L. pallescens* (Lp1) and ● = *L. triloba* (Lt3). Black symbols indicate intact seeds; white symbols indicate scarified seeds. See table 1 for the explanation of population codes. The positions of the codes of each seed lot highlight when seeds of the different taxa began to germinate during the imbibition test.

among the two populations (La1 and La2) were observed at 20°C and 25/10°C, with the higher values detected for La2 at both temperatures (table 5). The two-way ANOVA conducted on *L. agrigentina* for germination rate (TI) showed a highly significant effect ($P < 0.001$) for temperature (T), population (Pop) and their interaction (T × Pop; table 4B). Germination rate (TI) was highest at low temperatures of 10 and 15°C (TI values up to 7.9), although velocity significantly decreased ($P < 0.05$) at 5°C (TI < 1.3) and 25°C (TI < 1.5). The TI values differed significantly ($P < 0.05$) between populations at temperatures higher than 10°C, with higher TI values for La2 than for La1 (table 5).

For *L. pallescens* seeds, one-way ANOVA showed a highly significant effect of temperature (T) on final germination ($P < 0.001$) and germination rate ($P < 0.001$; table 4A and 4B). The highest germination rate was observed at 5–15°C (c. 90%), and the lowest at 20°C (c. 67%; table 5). The alternating temperature regime (25/10°C) did not promote germination. The significantly highest ($P < 0.05$) germination rate was detected at 10°C (TI of c. 6.5; table 5).

For *L. triloba*, two-way ANOVA showed a highly significant effect ($P < 0.001$) on seed germination for temperature (T) and population (Pop), but not for their interaction (T × Pop = $P > 0.05$; table 4A). Independently from the population origin, seeds of this taxon showed the highest final germination at 15–20°C, and the lowest at 5°C. Seeds of *L. triloba* germinated from c. 35% (Lt1) to c. 80% (Lt2 and Lt3) within the temperature range (i.e. 15–20°C), whereas germination significantly ($P < 0.05$) decreased (< 70% for all populations; table 5) at the highest constant temperature of 25°C. The lowest germination rate was at 5°C (c. 20% for Lt1 and c. 50% for Lt2 and Lt3; table 5). The alternating temperature regime (25/10°C) did not promote germination for any of the populations (table 5). Significant differences ($P < 0.05$) were detected among populations, and Lt1 showed a lower germination rate than Lt2 and Lt3, at all temperatures (table 5). The two-way ANOVA conducted for germination rate showed a highly significant effect ($P < 0.001$) for temperature (T), but not for population (Pop) ($P > 0.05$) and a highly significant ($P < 0.001$) effect of their interaction (T × Pop; table 4B). The highest germination rate was observed at 20°C (TI values up to 9.2) and the lowest at 5°C (TI < 1; table 5).

Effect of dry after-ripening (DAR)

Lavatera agrigentina – There was a highly significant effect ($P < 0.001$) of temperature (T) and pretreatment (Pre), and a significant effect (T × Pre = $P < 0.05$) of their interaction on germination rate (table 6A). The final germination of DAR seeds was significantly higher ($P < 0.05$) than that of fresh seeds (fig. 2). For DAR seeds, germination was significantly ($P < 0.05$) lower at 25°C (< 70%) than at the other temperatures (c. 95%; fig. 2). The two-way ANOVA showed a highly significant effect ($P < 0.001$) on germination rate (TI), temperature (T) and pretreatment (Pre), as well as their interaction (T × Pre) (table 6B). In particular, the germination rate of DAR seeds (c. 8 and 12, respectively) was significantly higher ($P < 0.05$) at 10 and 15°C, compared to that of fresh seeds at the same temperatures (c. 2.7 and 8, respectively; fig. 2). For DAR seeds, more rapid germination occurred at 15°C (TI of c. 12) than at any other temperature.

Lavatera pallescens – Pretreatment (Pre) did not significantly ($P > 0.05$) influence seed germination, whereas tempera-

Table 4 – Effect of temperature (T), population (Pop), and their interaction (T × Pop) on: (A) final germination, and: (B) on germination rate (TI) of fresh seeds for the three *Lavatera* taxa.
P values were considered not significantly ($P > 0.05$, ns), significantly ($P < 0.05$, *) or highly significantly ($P < 0.001$, ***) different, by two-way ANOVA; SS = Sum of squares; DF = Degrees of freedom; MS = Mean square; F = Fisher variable; P = *P* value).

A

Taxon	Factor	SS	DF	MS	F	P
Lavatera agrigentina	Temperature (T)	6363.72	5	1272.74	18.5839	***
	Population (Pop)	912.45	1	912.45	13.3231	***
	T × Pop	1544.84	5	308.97	4.5114	***
	Error	1643.67	24	68.49		
Lavatera pallescens	Temperature (T)	1956.9	5	391.4	7.226	***
	Error	650.0	12	54.2		
Lavatera triloba	Temperature (T)	1659.5	5	331.9	5.831	***
	Population (Pop)	6747.4	2	3373.7	59.268	***
	T × Pop	358.8	10	35.9	0.630	ns
	Error	2049.2	36	56.9		

B

Taxon	Factor	SS	DF	MS	F	P
Lavatera agrigentina	Temperature (T)	2.504727	5	0.500945	26.13780	***
	Population (Pop)	0.774132	1	0.774132	40.39185	***
	T × Pop	0.764942	5	0.152988	7.98246	***
	Error	0.421642	22	0.019166		
Lavatera pallescens	Temperature (T)	0.332817	5	0.066563	41.038	***
	Error	0.019464	12	0.001622		
Lavatera triloba	Temperature (T)	6.610982	5	1.322196	94.3537	***
	Population (Pop)	0.073299	2	0.036649	2.6153	ns
	T × Pop	1.803592	10	0.180359	12.8707	***
	Error	0.504475	36	0.014013		

ture (T) and the interaction (T × Pre) had a highly significant ($P < 0.001$) and a significant effect ($P < 0.05$), respectively (table 6A). The germination rate of DAR seeds was significantly higher at 20°C ($P < 0.05$) compared to that of fresh seeds (fig. 2). For DAR seeds, a higher final germination was observed at temperatures below 20°C (c. 95% for all temperatures), whereas at 25°C and 25/10°C germination was significantly ($P < 0.05$) lower (c. 70%). The two-way ANOVA for germination rate (TI) showed a significant effect of pretreatment (Pre = $P < 0.05$), a highly significant effect of temperature (T = $P < 0.001$) and a significant effect for their interaction (T × Pre = $P < 0.05$) (table 6B). A significantly higher ($P < 0.05$) germination rate for DAR seeds was detected only at 20 and 25°C (c. 4.2 and 4.0, respectively) compared to that of fresh seeds (c. 3.0 and 2.8, respectively; fig. 2). For DAR seeds, the highest germination velocity was found at 10°C (TI of c. 6.5) whereas the lowest was observed at 25/10°C (c. 3; fig. 2).

Lavatera triloba – Temperature (T) caused a highly significant ($P < 0.001$) increase in final seed germination, whereas pretreatment (Pre = $P > 0.05$) and the interaction between temperature and pretreatment did not (T × Pre = $P > 0.05$; table 6A). At all the tested temperatures, the DAR pretreatment did not improve final germination compared to that of fresh seeds ($P > 0.05$). For DAR seeds, the highest final germination occurred between 10 and 20°C (c. 90%), whereas the lowest was observed at 5°C, 25°C and 25/10°C (c. 60%, for all; fig. 2).

The two-way ANOVA for germination rate (TI) showed a non-significant effect of pretreatment (Pre = $P > 0.05$), but was highly significant ($P < 0.001$) both for temperature (T) and for the interaction between temperature and pretreatment (T × Pre) (table 6B). A significantly higher ($P < 0.05$) germination rate for DAR seeds was detected only at 5 and 25/10°C (c. 0.8 for both; fig. 2) with respect to fresh seeds (c.1.3 for both; fig. 2). For DAR seeds, the highest germination velocity was found at 20°C (TI of c. 7.5) and the lowest at 5°C and 25/10°C (TI of c. 1.4).

DISCUSSION

Scarification greatly improved water uptake in all the three investigated taxa, highlighting the presence of a physical component of dormancy (PY), as previously reported for

other *Lavatera* species (Baskin et al. 2000, Royal Botanic Gardens of Kew 2008). In the wild, various abiotic and biotic factors can produce seed scarification (Baskin & Baskin 1998). Daily temperature fluctuations and heat from fires are considered to be the two most important factors in the breaking of physical dormancy (Baskin & Baskin 1989, 1998). These factors are more intense at the soil surface than below it, therefore, seeds at or near the surface have a greater probability of germinating than those that are buried by several centimeters (Baskin & Baskin 1989). In the Mediterranean climate, which is characterised by unpredictable precipita-

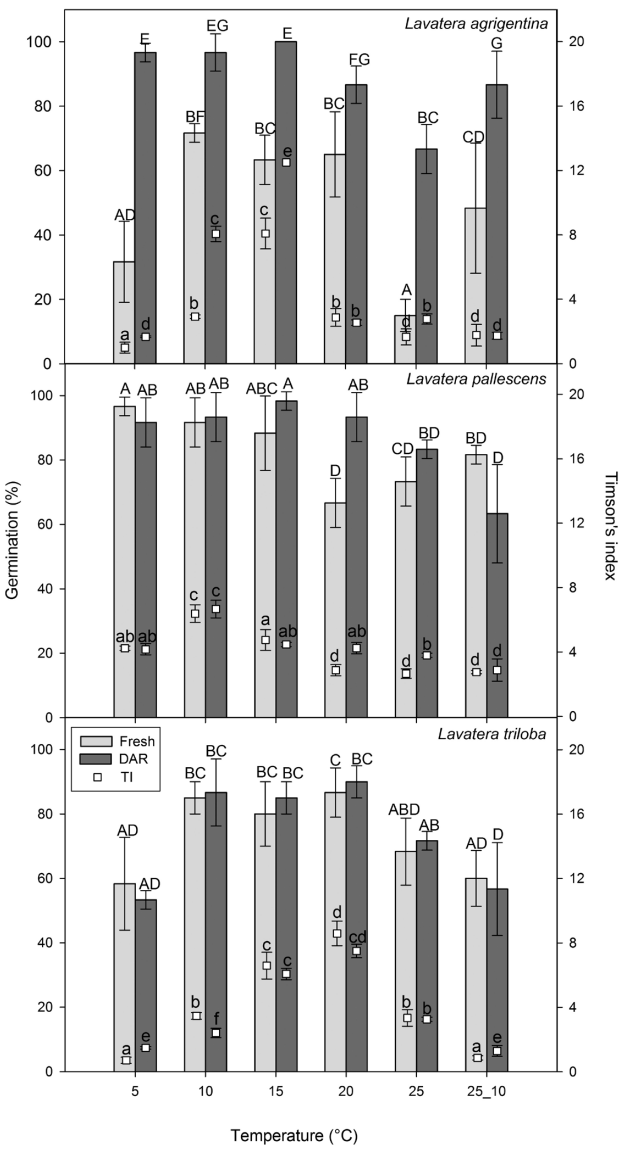

Figure 2 – Final germination (bars) and Timson's index values (TI) (squares) in each temperature regime for fresh and dry after-ripened (DAR) seeds incubated in the light of *Lavatera agrigentina* (La2), *L. pallescens* (Lp1) and *L. triloba* (Lt3). Bars and squares with the same letters are not significantly different at $P < 0.05$ (two-way ANOVA followed by Fisher's Least Significant Difference (LSD) *post hoc* test). Data are the means (± 1 SD) of three replicates. See table 1 for the explanation of population codes.

Table 5 – Final germination and Timson's index values (TI) for different populations of the three *Lavatera* taxa.
A two-way ANOVA was conducted for final germination and TI of seeds belonging to populations of the same species to detect the effect of temperature (T), population (Pop) and their interaction (T × Pop), whereas a one-way ANOVA was conducted for the single *L. pallescens* population to evaluate the effect of temperature (T) on germination and TI. A Fisher's LSD *post hoc* test was conducted to identify significant differences at $P < 0.05$ between different temperature regimes. Data are the mean (± 1 SD) of three replicates. Values with the same letters are not different at $P < 0.05$. Capital letters in columns relate to the same temperature, whereas lower-case letters in rows refer to the same population.

Taxon	Population	Parameter	Temperature (°C)					
			5	10	15	20	25	25/10
L. agrigentina	La1	Germination (%)	50.0 ± 5.0^{abA}	63.3 ± 15.3^{bA}	55.0 ± 17.3^{abA}	35.0 ± 17.3^{aA}	5.0 ± 5.0^{cA}	6.7 ± 5.8^{cA}
		TI	1.3 ± 0.1^{acA}	2.4 ± 0.6^{bA}	2.3 ± 0.7^{bcA}	1.3 ± 0.7^{aA}	0.3 ± 0.3^{dA}	0.2 ± 0.2^{dA}
	La2	Germination (%)	31.7 ± 12.6^{adA}	71.7 ± 2.9^{bA}	63.3 ± 7.7^{bcA}	65.0 ± 13.2^{bcB}	15.0 ± 5.0^{dA}	48.3 ± 20.2^{acB}
		TI	0.8 ± 0.3^{aA}	2.7 ± 0.1^{bA}	7.9 ± 0.9^{cB}	2.7 ± 0.5^{bB}	1.5 ± 0.5^{dB}	1.6 ± 0.7^{dB}
L. pallescens	Lp1	Germination (%)	96.7 ± 2.9^{a}	91.7 ± 7.7^{ab}	88.0 ± 11.5^{ab}	67.0 ± 7.7^{c}	73.0 ± 7.7^{cd}	81.7 ± 2.9^{bd}
		TI	4.4 ± 0.1^{a}	6.5 ± 0.5^{b}	4.9 ± 0.6^{a}	3.0 ± 0.3^{c}	2.8 ± 0.3^{c}	2.9 ± 0.1^{c}
L. triloba	Lt1	Germination (%)	21.7 ± 10.4^{aA}	35.0 ± 5.0^{abA}	40.0 ± 13.2^{abA}	45.0 ± 13.2^{bA}	21.7 ± 10.4^{aA}	31.7 ± 2.9^{abA}
		TI	0.6 ± 0.5^{aA}	0.8 ± 0.1^{bA}	4.0 ± 1.3^{cA}	5.6 ± 1.6^{cA}	5.4 ± 2.6^{cA}	3.9 ± 0.4^{cA}
	Lt2	Germination (%)	46.6 ± 20.2^{aB}	75.0 ± 5.0^{bB}	76.6 ± 15.5^{bB}	73.3 ± 11.5^{bB}	65.0 ± 15.0^{abB}	75.0 ± 8.7^{bB}
		TI	0.7 ± 0.3^{aAB}	1.9 ± 0.1^{bB}	5.5 ± 0.9^{cAB}	9.2 ± 1.4^{dB}	1.4 ± 0.3^{bB}	2.1 ± 0.2^{bB}
	Lt3	Germination (%)	58.3 ± 14.4^{aB}	85.0 ± 5.0^{bcdB}	80.0 ± 10.0^{bcdB}	87.0 ± 7.7^{bB}	68.0 ± 10.0^{adB}	60.0 ± 8.7^{acB}
		TI	0.8 ± 0.2^{aB}	3.5 ± 0.2^{bC}	6.7 ± 0.8^{cB}	8.7 ± 0.8^{cB}	3.4 ± 0.5^{bA}	1.0 ± 0.1^{aC}

Table 6 – Effect of temperature (T), dry after-ripening (DAR) pretreatment (Pre), and their interaction (T × Pre) on: (A) final germination, and: (B) on germination rate (TI) for the three *Lavatera* taxa.
P values were considered not significantly ($P > 0.05$, ns), significantly ($P < 0.05$, *) or highly significantly ($P < 0.001$ ***) different, by two-way ANOVA; SS = Sum of squares; DF = Degrees of freedom; MS = Mean square; F = Fisher variable; P = *P* value.

A

Taxon	Factor	SS	DF	MS	F	*P*
Lavatera agrigentina	Temperature (T)	4247.6	5	849.5	14.891	***
	Pretreatment (Pre)	9758.2	1	9758.2	171.053	***
	T × Pre	1338.9	5	267.8	4.694	*
	Error	1369.2	24	57.0		
Lavatera pallescens	Temperature (T)	2361.9	5	472.4	6.248	***
	Pretreatment (Pre)	218.4	1	218.4	2.889	ns
	T × Pre	1219.2	5	243.8	3.225	*
	Error	1814.5	24	75.6		
Lavatera triloba	Temperature (T)	5666.7	5	1133.3	14.703	***
	Pretreatment (Pre)	11.1	1	11.1	0.144	ns
	T × Pre	172.2	5	34.4	0.447	ns
	Error	1850.0	24	77.1		

B

Taxon	Factor	SS	DF	MS	F	*P*
Lavatera agrigentina	Temperature (T)	3.502955	5	0.700591	70.3301	***
	Pretreatment (Pre)	0.431849	1	0.431849	43.3520	***
	T × Pre	0.237762	5	0.047552	4.7736	***
	Error	0.239075	24	0.009961		
Lavatera pallescens	Temperature (T)	0.49966	5	0.09993	48.738	***
	Pretreatment (Pre)	0.01088	1	0.01088	5.306	*
	T × Pre	0.05100	5	0.01002	4.887	*
	Error	0.04921	24	0.00205		
Lavatera triloba	Temperature (T)	4.118135	5	0.823627	229.629	***
	Pretreatment (Pre)	0.003839	1	0.003839	1.070	ns
	T × Pre	0.192216	5	0.038443	10.718	***
	Error	0.086083	24	0.003587		

tions (Thanos et al. 1995), seeds with PY might receive an ecological advantage and germinate only when environmental conditions are favourable for seedling establishment (Baskin & Baskin 1989, 1998). Breaking of PY involves the formation of a small opening(s) (water gap) in a morpho-anatomically specialised area in seeds or fruits, known as the water-gap complex (Gama-Arachchige et al. 2013). For the Malvaceae family, Gama-Arachchige et al. (2013) reported a 'type III' water-gap complex, in which narrow linear or circular openings are occluded by plug-like structures, which are usually formed by water-impermeable sclerenchyma cells. A similar water-gap complex might be responsible for the presence of PY in the investigated taxa.

For *L. agrigentina,* the dry after-ripening pretreatment greatly promoted germination at all temperatures, highlighting the presence of a physiological component to seed dormancy (PD). In particular, the pretreatment widened the germination range both at low and high temperatures, leading to a type 3 non-deep PD (*sensu* Baskin & Baskin 1998, 2004) and, therefore to a combinational dormancy (PY + PD; Baskin & Baskin 1998, 2004).

Thanos et al. (1991, 1995) found that the germination of seeds of several Mediterranean coastal species that lack PY is photo-inhibited, highlighting the presence of a surface-avoiding mechanism that enables seeds to avoid germinating under the harsh conditions of the soil surface. However, seeds of all three investigated *Lavatera* taxa, did not show this type of surface-avoidance mechanism and their germination was not photo-inhibited. In a survey study of 271 species, Grime et al. (1981) found that the incidence of light-dependence decreased with increasing seed size. Species with seeds that weighed less than 0.1 mg were largely light-requiring. These findings were consistent with our results. The seed mass of the investigated species was greater than 3

mg (see table 1) and the final germination of each taxon was independent of light.

The three taxa differed in their germination requirements, reflecting the conditions of their different ecosystems of origin. *Lavatera agrigentina* showed a germination optimum (final germination and rate) in the 10–20°C range, with lower and slower germination at lower (5°C) and higher (25°C) temperatures. This pattern suggests that seed germination in the field might occur in the autumn following seed dispersal, when due to rainfall (Brullo et al. 1996, Minissale et al. 2011), seeds have the highest water availability, which is a highly limiting factor in the arid grasslands where this taxon occurs (Brullo et al. 2010).

Lavatera pallescens, showed a highest final germination in the 5–15°C range and the highest germination rate at 10°C. These findings suggest a natural germination during winter after seed dispersal. However, high germination rates (c. 60%) were observed at temperatures > 20°C, which indicates that germination might also occur in early autumn, soon after dispersal.

Similar to *L. agrigentina*, *L. triloba* showed an optimum final germination in the 10–20°C range, with a maximum germination rate at 20°C. *Lavatera triloba* seeds might therefore also germinate after dispersal from autumn onwards. The high germination at 20°C might indicate the ability of seeds of this taxon to germinate additionally in late spring, when the presence of water is not a limiting factor in its native ecosystem (endorheic lagoons).

The germination of *L. pallescens* seeds appeared to be the most similar to that of Mediterranean coastal species, although a significant decrease in germination at high temperatures was not recorded. Therefore, with a preferential germination during cold months, *L. pallescens*, might receive an ecological advantage, due to a longer period of seedling growth during winter and spring, under the harsh conditions of coastal cliffs.

The germination of *L. agrigentina* and *L. triloba* differed from the typical germination of Mediterranean species, for which an increase of germination at low temperatures (5–15°C) and a decrease at high temperatures are widely recognised traits (Thanos et al. 1989, 1995). In addition, for *L. agrigentina*, PD of seeds was released after a three-month dry after-ripening period (25°C).

Inter-population variability in seed germination was detected for both *L. agrigentina* and *L. triloba*, even between considerably close populations (e.g. only c. 9 km between La1 and La2). Although the general responses to temperature were similar among the tested populations, germination behaviour can vary greatly within a single species from one population to another, or from year to year, as a function of environmental factors (Fenner 1991, Gutterman 1992), during seed maturation (Meyer et al. 1989) or among individuals (Urbanska & Schütz 1986). According to Gutterman (1994) and Kigel (1995), the variability in germination characteristics can be interpreted as one of the most important survival strategies for species growing under unpredictable environmental conditions.

In conclusion, our results highlight the presence of combinational dormancy in *L. agrigentina* and only of physical dormancy in *L. pallescens* and *L. triloba*. The three investigated taxa differ in their germination ecology, with each demonstrating ecosystem-related germination requirements. The findings of this study for all three taxa, suggest that their germination occurs in the field from autumn to spring, although with species-specific requirements, representing an advantageous ecological adaptation for seedling establishment, towards the unpredictable Mediterranean rainfall pattern (Thanos et al. 1989, 1991).

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