Seed Germination and Essential Oil of *Lavandula luisieri* from Central Eastern Portugal

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Abstract

***Lavandula luisieri* (Rozeira) Rivas-Martínez is an endemic plant from the Iberian Peninsula which belongs to the *Lamiaceae* family. *L.luisieri, Genista falcata* Brot. and *Lavandula pedunculata* (Miller) Cav. ssp. *sampaiana* (Rozeira) Franco can be found together in some regions of Central Eastern Portugal where old vegetation of meso-mediterranean communities occurs.**

Relevant aspects of *L. luisieri* species such as 1 - two new important products in its essential oil; 2 - the importance of its honey; 3 – this crop improvement; 4 - the ornamental interest in its use in Mediterranean gardens; and 5 - the difficulty of its seeds germination; are the main aspects of our study on seed germination and the identification of the essential oils compounds.

Seed germination of four populations collected in different locations in Central Eastern Portugal and of two different maturation dates were compared.

Seed germination experiments in laboratory (40 and 75 days after the harvest) were carried out using a constant temperature (25°C) and an alternating regime (8/18°C) with a photoperiod of 8 hours and another with a photoperiod of 16 hours. The results show significant differences in the seed germination proceeding of the four populations.

Some components identified in essential oils were irregular monoterpenoids with a cyclopentanic structure unique in the plant kingdom.

INTRODUCTION

Lavandula luisieri (Rozeira) Rivas-Martínez (Franco, 1984) or *Lavandula stoechas* L. subsp. *luisieri* (Rozeira) Rozeira (Tutin et al., 1972), commonly known in Portugal as "rosmaninho" or "rosmaninha" is an endemic plant from the Iberian peninsula. It is an interesting species, which could be an alternative crop in Beira Interior region, in Central Eastern Portugal, due to the recent discovery of two new products in its essential oil. Moreover, ornamental interest in Mediterranean gardens also contributes to the sustainability of the region agriculture.

The awareness of the environmental conditions required for seed germination is a relevant factor since there is little or no information about this matter concerning wild and endemic plants.

Variation in germination behaviour which occurs among different populations within the same species of Mediterranean Lamiaceae has been reported (Baskin and Baskin, 1998; Pérez-Garcia et al., 2003).

The nature of volatile components of *L. luisieri* differentiates it from other related species such as *L. stoechas* subsp.*stoechas*. The latter is very similar to *L. luisieri* when observed in its natural habitat.

Garcia-Vallejo et al. (1994) found that *L. luisieri* oil has an atypical composition characterised by irregular monoterpenoids, (1) trans-α- necrodol and (2) trans-α-necrodyl acetate. More recently Sanz and Garcia-Vallejo (2004) identified (3) cis-α-necrodyl acetate in addition to 1 and 2.

Baldovini et al. (2005) were the first to evaluate the antibacterial activity of its essential oil. The highest dilution (1/20) inhibited visible growth in bacteria after 18 hours and in fungi after 48 hours at 35°-37°C.

The aim of this work was to test the behaviour in seed germination of four populations from the Central Eastern Portugal. Altitude, latitude and longitude may influence their behaviour. Seed storage, constant versus alternate temperature and photoperiod, are relevant factors that were studied. We also tested if the origin of the populations influences the composition of the volatile compounds of essential oils.

MATERIALS AND METHODS

Seeds, inflorescences and leaves were collected in four different locations in the Beira Interior region, during spring and summer 2005 (Table 1).

Bulk collections of seeds were taken from approximately 20 randomly selected plants of each population.

Seed samples were cleaned manually, kept in glass tubes, and stored in a dry place in a laboratory at 22°-28°C until germination tests were carried out. Table 2 illustrates the conditions of temperature and light of the trials and seed storage time.

Seeds were placed in glass Petri dishes of 9 cm diameter above one sheet of filter paper moistened with 2.5ml of distilled water and four replicates of 100 seeds were used in each trial (I.S.T.A., 2002).

Treatments I, II and III took place in incubators under controlled conditions of temperature (precision±1°C) and light provided by one cool white fluorescence lamp "Osrame" L23W/21. Treatment IV took place in a laboratory room.

Seeds showing radical emergence were recorded and removed from the Petri dishes every day. The rate of germination was evaluated after 21 (Treatment II) and 30 days (Treatment I, III and IV).

An analysis of variance was used to compare the results of germination for each trial (SPSSWIN, version 12.0). One-Way factorials ANOVA and Scheffe test were performed for each population and for each treatment.

The analysis of essential oil from both leaves and inflorescences in both fresh and dry material from the four populations was carried out at the Department of Chemistry of Beira Interior University, Portugal using Mass Gas Cromatography (GC-MS).The GC-MS was conducted using Fision 8000 device with an ionic flame detector and a capilar column DBI with 30m of length and 0.25mm of diameter with a stationary phase of poli-dimetil-siloxeno and a mobile phase of helium. A mass spectrograph Fision VH Trio 1000, with an electronic impact of 70 (eV) was connected to the device; it was also connected to a computer system, which allowed the comparison with a range of specters. The injector and the detector temperature was 250°C. The helium fluxe was of 1.6ml/min.

The oil of *L. luisieri* leaves and flowers was obtained by separated hidrodestilation using 100 g of both fresh and dry material for 2 hours. Flowers were separated manually, just before dstillation.

RESULTS

The analysis of the germination parameters considered for each location (germination rate; germination mean time; and latent time) suggests Treatment II as having the best germination velocity. Latent time was generally reduced with the conservation time (Table 3).

Alternate temperatures had a positive effect on germination mean time, demonstrated in Table 3, as this parameter (alternate temperature) reduces the coefficient of velocity (CV). CV increases as more seeds germinate and with shorter germination time. We can notice significant differences between geographical origins in Treatments I and IV. The best results were obtained in Treatment II that simulates autumnal conditions. This indicates that alternate temperature and photoperiodic of 8 hours, are the best conditions for this species, irrespective of its geographical origin.

There are significant differences between Treatments I and IV as far as the location parameter is concerned. In both cases Mata shows the worse results (Table 4).

The chemical analyses indicate that the oil percentage was higher in the leaves when the extraction was done in fresh material (mean fresh leaves = 0.36%; mean fresh inflorescence= 0.20%; mean dry leaves= 0.07%; mean dry inflorescence= 0.08%). Camphor was only present in inflorescences; on the other hand, necrodol was more frequent in the leaves, and in fresh material. Necrodol (α-Necrodol) was first discovered by Eisner et al. (1986) as a constituent of a defensive spray against South America carrion beetle. The present study can show that fresh leaves of *L. luisieri* have this constituent (Table 5).

DISCUSSION

According to Cabello et al. (1998) *L. luisieri* is photoblastic positive, therefore only light conditions were used in our experiments.

In this study we found that *L. luisieri* can be reproduced by sexual propagation and it did not show post-harvest dormancy after 40 and 75 days. In contrast to the results Cabello et al. (1998) obtained, this species did not need stratification and the final percentages of germination are higher than 81% in all treatments after 2.5 month.

Every treatment showed good results in the first year, which may indicate a high adaptability to germinate irrespective of temperature and photoperiod.

Samples from different origins presented a high level of essential oil on fresh material, according to Sanz and Garcia-Vallejo (2004). The access (location) to Vila Velha de Ródão (VVR) always presented higher levels (mL of oil per 100g of fresh material) either when the material was distilled fresh or dry. The fresh inflorescences showed 0.27 mL and the leaves 0.5ml, whereas the dry inflorescences showed 0.09ml and the dry leaves showed 0.08m these results can be connected with the soil and climatic conditions, as the location VVR has a lower altitude and higher temperature (about 2 to 3 degrees Celsius) in the flowering time, than other places. Therefore, the fenchone levels of all the analyzed material are always higher when compared with other locations (Casal da Fraga e Mata). By contrast, only the inflorescences show necrodol, whereas this compost is only present in fresh leaves in other places.

The preservation and production of this plant can be extremely important in burnt areas, since wildfires have been a terrible disaster in lasts decades in Portugal. It restores endemic populations and it can be used in organic farming as an alternative to traditional agriculture.

More trials will be carried out in the coming years in order to study the influence of conservation time in *L. luisieri* germination.

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Tables

Table 1. Location of *Lavandula luisieri* seeds in Central Eastern Portugal in June, 9, 2005.

	Geometric coordenates		
Location	Latitude (°N)	Longitude (°W)	Altitude (m)
CF	40°02' 51.484''	7°34' 50,008''	627
M	39°53' 29.691''	7°19' 26,329''	258
P	40°12' 06.741''	7°06' 22,,085	558
VVR	39°40' 35.550''	7°38' 02,126''	128

CF- Casal da Fraga; M- Mata; P- Penamacor, VVR- Vila Velha de Ródão.

Table 2. Controlled conditions temperature, light and storage time

Treatment	Test conditions		Conservation time (days)
	Temperature (°C)	Photoperiod (Hours)	
I	25°	8	40
II	18/8°	8	40
III	25°	8	75
IV	25°	16	75

Table 3. Germination parameters of L. luiseri.

Location	Treatment	Germination (%)	Germination mean time (days)	Latent time (days)
CF	I	93	12.9	5
	II	91	6.8	5
	III	93	8.9	3
	IV	94	11.6	4
M	I	81	21.2	10
	II	93	6.5	5
	III	81	15	5
	IV	82	16.4	5
P	I	88	18.9	6
	II	94	7.6	6
	III	88	11.8	3
	IV	95	13.9	4
VVR	I	94	15.5	5
	II	92	6.7	5
	III	85	11.8	3
	IV	91	13.1	4

CF- Casal da Fraga; M- Mata; P- Penamacor, VVR- Vila Velha de Ródão.

Table 4. Germination rate (%) of L. luiseri for each location.

Location	Treatment I	Treatment II	Treatment III	Treatment IV
CF	97.5 ab	90.50 a	92.50 a	92.5 b
M	82.5 a	92.50 a	81.25 a	82.25 a
P	88.00 ab	94.25 a	87.50 a	94.50 b
VVR	93.75 b	92.25 a	85.25 a	90.75 ab

CF- Casal da Fraga; M- Mata; P- Penamacor, VVR- Vila Velha de Ródão. The values of each column followed by the same letter are not significantly different at the 0.05 level according to the Scheffé test.

Table 5. Mass Gas Cromatography of L. luisieri (%).

Compounds	Fresh inflorescences			Dry inflorescences		Fresh leaves			Dry leaves		
	CF	M	VVR	CF	M	CF	M	VVR	CF	M	VVR
1,8- cineole									5		
fenchone		13.2	36.7		18			10	5	10.6	19.2
linalool		5.5	6.2		4	4	5.3	3		4.5	
camphor	9.7	4.5		9.3	6						
necrodol			5			5.3	11.4				
trans-necrodyl acetate	27.1	24.6	10.2	9.4	8	49.9	47.9		9	34.7	20

CF- Casal da Fraga; M- Mata; P- Penamacor, VVR- Vila Velha de Ródão.