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A CONTRIBUTION TO EX-SITU CONSERVATION OF MEDITERRANEAN THYMES: GERMINATION TRIALS

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ABSTRACT. *A contribution to ex-situ conservation of Mediterranean thymes: Germination trials*. The germination process was studied of the seeds of seven endemic or threatened Mediterranean thymes collected from natural populations of the SW Iberian Peninsula. Four replicates of 50 seeds of each population were induced to germinate by alternating temperatures (6 hours at 20°C, 18 hours at 30°C; 40-60% humidity). The accumulated percentage germination curves, the germinability (percentages), and the germination rate (in terms of the vigour index, *Iv*) were determined. The results were as follows: *Thymus caespititius,* germinabilities *null* or *low* and germination rates *slow* (0%-11%; Iv = 0-1.26); *Thymus mastichina*, germinabilities *moderate* or *high* and germination rates *fast* or *very fast* (59%-92%; Iv = 12.84-35.67); *Thymus praecox* subsp. *penyalarensis,* germinabilities *high* and germination rates *fast* (79%; Iv = 19.53); *Th. pulegioides,* germinabilities *low* or *moderate* and germination rates *slow* or *medium* (2%-41%; Iv = 0.33-7.58); *Thymus villosus* subsp. *lusitanicus,* germinabilities *moderate* and germination rates *medium* (35%; Iv = 9.00); *Thymus zygis* subsp. *sylvestris,* germinabilities *low*, *moderate,* or *high* and germination rates *slow, medium,* or *fast* (2%-68%; Iv = 0.52-19.36); and *Thymbra capitata,* germinabilities *high* and germination rates *fast* (92%; *Iv* = 19.07). There were both interspecific and interpopulational differences in germinative capacity. Cooling the seeds for 7 days at 10-12°C prior to sowing produced no significant alterations in the results. A relationship was observed between seed weight and germination percentage.

Key words. Germination, Iberian Peninsula, seed, *Thymus*, vigour.

RESUMEN. *Contribución a la conservación ex-situ de los tomillos mediterráneos: ensayos de germinación.* Se estudiaron los procesos de germinación de las semillas de siete tomillos endémicos o amenazados de la Región Mediterránea, recolectados en poblaciones silvestres del SO de la Península Ibérica. Se indujeron a germinar 4 réplicas de 50 semillas de cada población con alternancia de temperaturas (6 horas a 20ºC, 18 horas a 30ºC; humedad del 40-60%). Se determinaron curvas de porcentajes acumulados de germinación, germinabilidad (en porcentaje) y la velocidad de germinación (mediante el índice de vigor, *Iv*). Para *Thymus caespititius* las germinabilidades fueron *nulas* o *bajas* y la velocidad de germinación fue *lenta* (0%-11%; Iv = 0-1.26); *Thymus mastichina*, germinabilidades *moderadas* o *altas* y velocidades de germinación *rápidas* o *muy rápidas* (59%-92%; Iv = 12.84-35.67); *Thymus praecox* subsp. *penyalarensis,* germinabilidades *altas* y velocidades *rápidas* (79%; Iv = 19.53); *Th. pulegioides,* germinabilidades *bajas* o *moderadas* y velocidades *lentas* o *medias* (2%-41%; Iv = 0.33-7.58); *Thymus villosus* subsp. *lusitanicus,* germinabilidades *moderadas* y velocidades *medias* (35%; Iv = 9.00); *Thymus zygis* subsp. *sylvestris,* germinabilidades *lentas, moderadas* o *altas* y velocidades *lentas, medianas* o *rápidas*(2%-68%; Iv = 0.52-19.36); y *Thymbra capitata,* germinabilidades *altas* y velocidades *rápidas* (92%; *Iv* = 19.07). Se observaron diferencias interespecíficas e interpoblacionales en la capacidad germinativa. Un pretratamiento térmico (7 días a 10-12°C) no produjo alteración significativa en los resultados. También se observó relación entre el peso de las semillas y el porcentaje de germinación.

Palabras clave. Germinación, Península Ibérica, semilla, *Thymus*, vigor.

INTRODUCTION

The genus *Thymus* L., belonging to the Lamiaceae family, is very well represented in the Mediterranean Region with 114 species (91 counting only the binomial level), of which 37 are located in the Iberian Peninsula (Morales, 2000). *Thymbra capitata* (L.) Cav., which was once included in the Thymus genus, is also a member of the Lamiaceae family, and is a clearly circum-Mediterranean species. The taxa are biologically interesting in terms of their reproduction, taxonomy, ecology, and chorology, and also because of their applications. Information about their germinative capacity would help to understand them from all of these viewpoints.

Correct seed storage is necessary to maintain genetic resources for subsequent agricultural use or for *ex situ* conservation in seed banks (Kretschmer, 1989; Caixinhas *et al*., 1993; Pérez-García *et al*., 2006). In the context of the potential use of aromatic plants as alternative crops on marginal lands, the physiology of the germination of correctly stored seeds of thymes and other labiates has been studied by many workers (Thanos, 1993; Thano, Kadis and Skarou, 1995).

Most publications on this topic have focused on thymes that are grown for their economic importance as medicinal and culinary plants. A prime example is *Th. vulgaris* L. which is grown in Germany (Kretschmer, 1989), Japan (Ushitani, 1991; Takano *et al.*, 1993), Switzerland (Rey *et al*., 1993), and the US (Linhart, 1999). Biological studies of this species have dealt with its floral architecture, reproductive processes (Belhassen *et al*., 1987; Belhassen *et al*., 1989; Belhassen *et al*., 1990), and ecological biochemistry (Tarayre *et al*., 1995).

Published information on the germination conditions of some thymes can be found within the context of more broadly focused studies. Some examples are: the investigations of Erikson (1998) on *Th. serpyllum* L., of Morales (1986) in a taxonomic review of the genus at the level of the Iberian Peninsula, of Vokou and Margaris (1986), Thanos (1993) and Thanos, Kadis and Skarou (1995) who studied Greek populations of *Thymbra capitata*, and the recent publication of Pérez García (2003) on *Th. zygis* Loefl.ex L.and *Th. mastichina* (L.) L. amongst other labiates. The international ISTA (1999) guidelines also contribute interesting data in this sense.

The species studied in this investigation are plants of great interest biogeographically, since they are Iberian [*Th. mastichina, Th. zygis* subsp. *sylvestris* (Hoffmanns. and Link) Brot. ex Coutinho*, Th. villosus* subsp. *lusitanicus* (Boiss.) Coutinho] or Ibero-Macaronesian (*Th. caespititius* Brot.) endemics, Mediterranean plants of areas in regression (*Thymbra capitata*), or plants with a marked Atlantic character that only penetrate into the Mediterranean region in mountainous zones or in areas that are especially cool and where the summer drought is not harsh [*Th. praecox* subsp. *penyalarensis* (Pau) Rivas Martínez, Fernández González & Sánchez Mata and *Th. pulegioides* L.]. This chorological situation is the reason for the importance of the principal objective of the present work – to obtain new information about the germination conditions of the seeds of various taxa, and to evaluate the influence of the thermal factor on that process.

MATERIAL AND METHODS

Collection of the material

Nucules (henceforth termed seeds for the sake of simplicity) were collected from fruited individuals of *Thymus caespititius, Th. mastichina, Th. praecox* subsp. *penyalarensis, Th. pulegioides, Th. villosus* subsp. *lusitanicus, Th. zygis* subsp. *sylvestris,* and *Thymbra capitata* of the natural populations listed in Table 1.

The seeds were inspected under 20× stereomicroscopy, cleansing them of impurities and contaminating elements, and eliminating any suspected of being inviable. They were then stored in sealed and labeled paper sachets for 4-6 months in darkness at the laboratory's ambient temperature (22-28°C).

Germination trials

Germination was curried out under controlled conditions of temperature, relative humidity, and lighting. The values for these parameters were chosen in view of the available literature reports concerning the germination of these or closely related species (Vokou and Margaris, 1986; Kretschmer, 1989; Tarayre *et al*., 1995; Thanos, 1993; Thanos, Kadis and Skarou, 1995; ISTA 1999; Pérez-García, 2003).

The experiments were performed in a precision incubator (P SELECTA, Hot-Cold Model-S), in which the seeds were placed in 8.5 cm diameter Petri dishes on Albert filter paper soaked in distilled water. As many populations as possible were studied for each taxon, using 4 replicates of 50 seeds for each population as suggested by the ISTA (1999) guidelines. The duration of the experiments was taken to be 21 days for the same reason. Seeds with a radicle of at least 0.75 mm in length were considered to have germinated. Counts were made daily, removing any germinated seeds.

Control seeds (C) were subjected to cycles of alternating temperatures of 6 hours at 20°C and 18 hours at 30°C. Identical batches of seeds were subjected to a cooling pre-treatment (T) consisting of maintaining them at 10-12°C for 7 days. All the experiments were carried out in darkness and at a relative humidity of 40-60%.

Evaluation of the germination

The data were used to construct the corresponding germination curves, and to calculate the germination percentage and the vigour index (*Iv*). This last parameter, a measure of the germination rate, is calculated according to the expression: $Iv = (a/1 + b/2 + c/3 \ldots + z/n) \times 100/s$, where a, b, c, … z, are the number of seeds that germinate each day, n is the number of days that the experiment lasts, and s is the number of seeds sown (Jain and Saha, 1971).

From the germination percentages, the germinability was expressed in terms of the categories of Devesa et al. (1998). These are: germinability *null* (0%), *low* ($0 < \% < 30$), *moderate* ($30 \leq \% \leq 70$), *high* ($70 < \% < 100$), and *maximal* (100%).

The *Iv* were used to evaluate the germination rate in terms of the categories of Cabello *et al.* (1998): *slow* ($0 \leq Iv < 5$), *medium* ($5 \leq Iv \leq 11.11$). *fast* ($11.11 < Iv \leq 33.33$), and *very fast* ($33.33 < Iv \leq 100$).

Seed weights

Seed weights were determined by weighing two 100-seed replicates for each population, dividing each by 100, and then taking the average of the two replicates (tab. 1).

Statistical analyses

The statistical package SPSS 11.0 for Windows was used to evaluate the differences in germination percentages and vigour indices

Thymus caespititius

CÁCERES: Descargamaría. Fuente La Malena. 29TQE16. Heathes, junipers and oakwoods. Supramediterranean bioclimate. 5/08/2003 6/03. J. Blanco and D. García nº 6/03. $15x10^{-2}$ mg

CÁCERES: Robledillo de Gata. Puerto Viejo. 29TQE16. Heathes, junipers and oakwoods. Supramediterranean bioclimate 5/08/2003. J. Blanco and D. García. nº 7/03. $8.5x10^{-2}$mg

Thymus mastichina

BADAJOZ: Los Santos de Maimona. 29SQC25. Pine grove of *Pinus pinea* L. Mesomediterranean bioclimate.7/08/2002. J. Blanco and D. Martín. nº 9/02. $23.5x10^{-2}$ mg

BADAJOZ: Santo Domingo. 29SPC68. Dehesa (open evergreen-oak woodland). Mesomediterranean bioclimate. 12/08/2002. J. Blanco and D. Martín nº 11/02. $26.5x10^{-2}$ mg

BADAJOZ: Badajoz. Ctra. Campo Maior. 29SPD70. Mediterranean scrub on alcaline soil. Mesomediterranean bioclimate. 24/8/2002. J. Blanco. nº 15/02. 26.10^{-2} mg

CÁCERES: Cáceres. El Portanchito. 29SQD27. Edges of olives groves. Mesomediterranean bioclimate. 3/9/2002. J. Blanco and J. Pozo. nº 16/02. $25x10^{-2}$ mg

BADAJOZ: Alconera. 29SQC15. Dehesa (open evergreen-oak woodland) and open land on limenstone quarry. Mesomediterranean bioclimate. 6/09/2002. J. Blanco and D. Martín. nº 17/02. $25x10^{-2}$ mg

BADAJOZ: Villafranca de los Barros. 29SQC37. Thyme scrubs. Mesomediterranean bioclimate. 6/09/2002. J. Blanco and D. Martín. nº 18/02. $21x10^{-2}$ mg

CÁCERES: La Garganta. 30TTK66. Thyme and broom scrubs. Supramediterranean bioclimate .11/09/2002. J. Blanco and J. Pozo nº 21/02. $32x10^{-2}$ mg

CÁCERES: Aliseda. 29SPD96. Open cork-oak woodland. Mesomediterranean bioclimate. 3/09/2002. J. Blanco and J. Pozo. nº22/02. 21. $5x10^{-2}$ mg

BADAJOZ: Fuente del Maestre. Sierra de San Jorge.29SQC26. Scrub on alcaline soil. Mesomediterranean bioclimate. 7/08/2003. J.J. Barrantes and J. Blanco. nº 10/03. $24x10^{-2}$ mg

CÁCERES: Alía. . 30SUJ06. Dehesa (open evergreen-oak woodland). Mesomediterranean bioclimate. 8/08/2003. J.J. Barrantes and J. Blanco. nº11/03. $27x10^{-2}$ mg

BADAJOZ: Benquerencia de la Serena. 30STH88. Edges of olives groves and road. Mesomediterranean bioclimate. 13/08/2003. J.J. Barrantes and J. Blanco. nº 12/03. $24x10^{-2}$ mg

BADAJOZ: Bienvenida. 29SQC44. Olive grove. Mesomediterranean bioclimate. 13/08/2003. J. Blanco. nº13/03. $20x10^{-2}$ mg

CÁCERES: Descargamaría. Fuente La Malena. 29TQE16. Heathes, junipers and oakwoods. Supramediterranean bioclimate. 4/09/2003. J. Blanco and D. García nº 14/03. $20x10^{-2}$ mg

Thymus praecox* subsp. *penyalarensis

CÁCERES: El Calvitero. 30TTK. Oromediterranean bioclimate. 27/08/2003. J. Blanco and D. García. nº 15/03. $15x10^{-2}$ mg

Thymus pulegioides

CÁCERES: Piornal. 30STK54. Near to a stream. Supramediterranean bioclimate. 11/09/2002. J. Blanco and J. Pozo. nº 19/02. $10x10^{-2}$ mg

CÁCERES: La Garganta. 30TTK66. Oakwood and pine grove. Supramediterranean bioclimate. 11/09/2002. J. Blanco and J. Pozo. nº 20/02. 10x 10^{-2} mg

Thymus villosus* subsp. *lusitanicus

CÁCERES: Alía. 30SUJ16.Dehesa (open evergreen-oak woodland). Mesomediterranean bioclimate. 8/08/2003. J. Barrantes and J. Blanco. nº 16/03. $15x10^{-2}$ mg

Thymus zygis* subsp. *sylvestris

BADAJOZ: Los Santos de Maimona. 29SQC25. Pine grove of *Pinus pinea* L. Mesomediterranean bioclimate.7/08/2002. J. Blanco. nº 8/02. $22.5x10^{-2}$ mg

BADAJOZ: Guadajira. 29SPD90. Thyme srubs on the hillside. .Mesomediterranean bioclimate. 12/08/2002. J. Blanco and D. Martín. Nº10/02. $19.5x10^{-2}$ mg

BADAJOZ: La Albuera. 29SPC88. Tomillar sobre ladera. Mesomediterráneo. 14/08/2002. J. Blanco and J. Pozo. nº 12/02. 10.5x10^{-2} mg

BADAJOZ: Solana de los Barros. 29SQC18. Thyme scrub on the hillside T. Mesomediterranean bioclimate. 14/08/2002. J. Blanco. nº 13/02. 11.5x10^{-2} mg

BADAJOZ: Badajoz. Ctra. Campo Maior. 29SPD70. Mediterranean thyme scrubs on alcaline soils. Mesomediterranean bioclimate. 21/8/2002. J. Blanco. nº14/02.13x10^{-2} mg

BADAJOZ: Cabeza del Buey. 30SUH08. Hillside on slates. Mesomediterranean bioclimate. 6/06/2003.J.J. Barrantes and J. Blanco.8/03. 21.5x10^{-2} mg

CÁCERES: La Garganta. 30TTK66. Thyme and broom scrubs. Supramediterranean bioclimate. 7/08/2003. J.J. Barrantes and J. Blanco nº 9/03. 9.5x10^{-2} mg

Thymbra capitata

BADAJOZ: Villafranca de los Barros. 29SQC37. Edge of road and livestock farm. Mesomediterráneo. 6/09/2002. J. Blanco and D. Martín. nº 33/02. 30.5x10^{-2} mg

Table 1. Provenance of the study material, indicating province, locality, UTM coordinates, habitat type, climate (see . Rivas-Martínez, 1987; Tormo et al., 1995), collection date, legit, collection number and seed weight (see Material and Methods). Voucher specimens in the HSS Herbarium (Badajoz, Spain). *Procedencia del material estudiado, indicando provincia, localidad, coordenadas UTM, tipo de hábitat, clima (ver . Rivas-Martínez, 1987; Tormo et al., 1995), datos de recolección, legit, número de recolección y peso de semillas (ver Material y Métodos). Testimonios en el herbario HSS (Badajoz, España).*

(*Iv*). The Kruskal-Wallis and Mann-Whitney tests were used to test for significance in the differences between species and between populations of the same species. The Wilcoxon test was used to compare results between the control (C) and pre-treated (T) seeds.

The possible correlation between germination percentage and seed weight was evaluated using the Spearman test (Zar 1996).

RESULTS

Germination tests

With respect to the germination percentages (tab. 2), for *Th. praecox* subsp. *penyalarensis* the values were 79% in the control (C) and 88% in the pre-treated (T) seeds, i.e., *high* germinability according to the classification of Devesa, Ruiz and Rodríguez, (1998). *Th. villosus* subsp. *lusitanicus* reached 35% in the control (C) and 47% in the pre-treated (T) seeds, corresponding to *moderate* germinability in both cases. In *Th. caespititius*, the percentages were 0-11% in the control (C) and 1-8% in the pre-treated (T) seeds, implying *null* or *low* germinabilities. In *Th. pulegioides*, the percentages were 2-41% in the control (C) and 2-27% in the pre-treated (T) seeds, i.e., *low* or *moderate* germinabilities. In *Th. zygis* subsp. *sylvestris*, the percentages were 2-68% in the control (C) and 7-74% in the pre-treated (T) seeds, i.e., ranging from *low* through *moderate* to *high* germinabilities. In *Th. mastichina*, the percentages were 59-92% in the control (C) and 65-98% in the pre-treated (T) seeds, i.e., *moderate* and *high* germinabilities in both cases. Lastly, in *Thymbra capitata*, the percentages were 92% in the control (C) and 86% in the pre-treated (T) seeds, i.e., *high* germinability in both cases.

The values of the vigour index were as follows. *Thymus praecox* subsp. *penyalarensis*: 19.53 in the control (C) and 21.05 in the pre-treated (T) seeds, i.e., *fast* germination rates according to the classification of Cabello, Ruiz and Devesa (1998). *Th. villosus* subsp. *lusitanicus*: 9.00 in the control (C) and 11.00 in the pre-treated (T) seeds, i.e. *medium* germination rates. *Th. caespititius*: 0.00-1.26 in the control (C) and 0.17-1.26 in the pre-treated (T) seeds, i.e. *slow* germination rates. *Th. pulegioides*: 0.33-7.58 in the control (C) and 0.35-4.73 in the pre-treated (T) seeds, i.e., *slow* to *medium* germination rates. *Th. zygis*

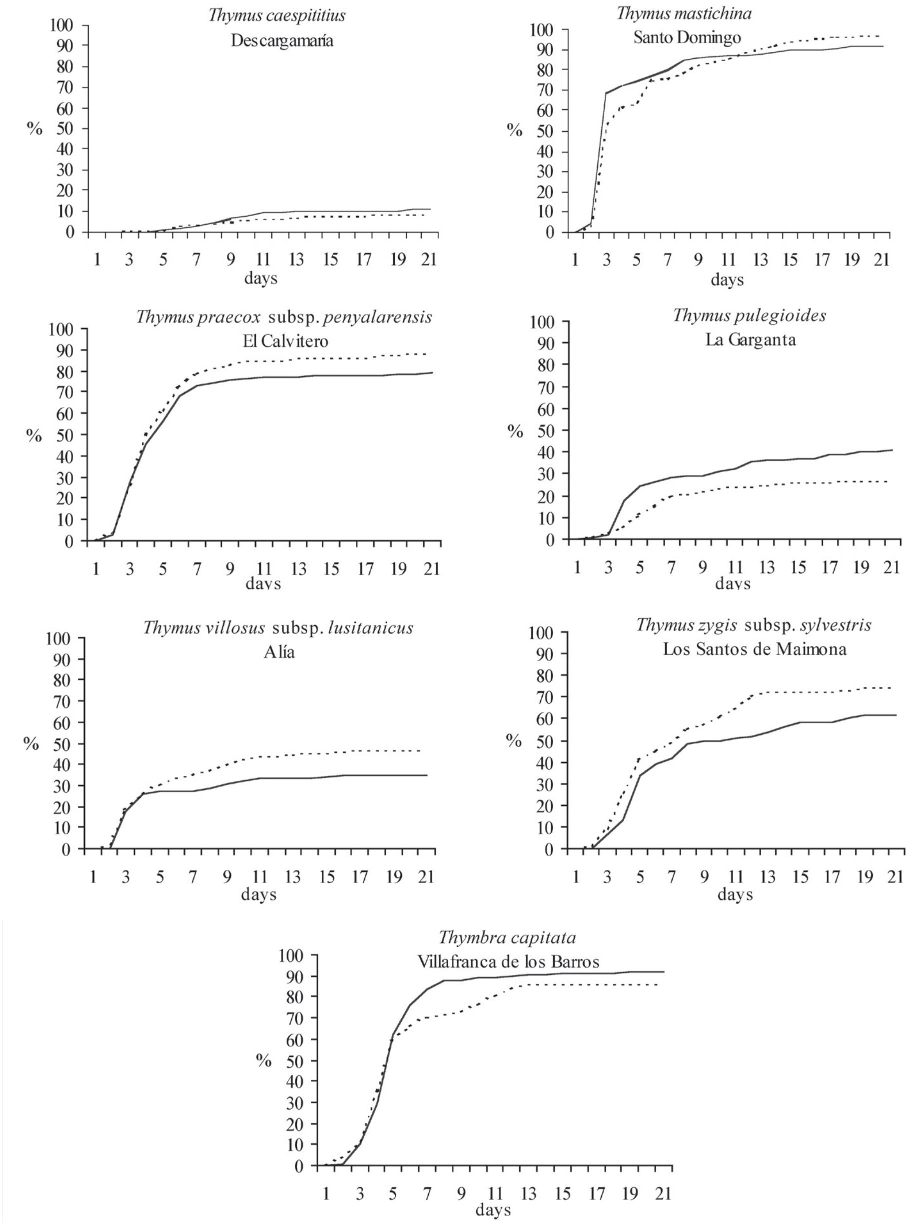

Figure 1. Cumulative germination percentages obtained over an experimental period of 21 days. For the taxa with more than one population available, that which attained the greatest percentage is shown. The values represented are the means of 4 replicates of the samples of control (C, continuous line) and pretreated (T, dashed line) seeds. *Porcentajes acumulados de germinación obtenidos a lo largo de un periodo experimental de 21 días. Para las especies de las que se dispuso de más de una población se presenta la que obtuvo mayores porcentajes. Los valores representados son el significado de las cuatro réplicas de las muestras control (C, línea continua) y pretratadas (T, línea discontinua).*

subsp. *sylvestris*: 0.52-19.36 in the control (C) and 1.14-16.63 in the pre-treated (T) seeds, i.e., ranging over all germination rate classes except *very fast*. *Th. mastichina*: 12.84-35.67 in the control (C) and 13.42-26.54 in the pre-treated (T) seeds, i.e., *fast* and *very fast* germination rates. Finally, *Thymbra capitata*: 19.07 in the control (C) and 18.28 in the pre-treated (T) seeds, i.e., *fast* germination rates in both cases.

The germination data were used to construct the corresponding percentage accumulated germination curves (fig. 1).

Percentage germination – Seed weight

Figures 2 and 3 were constructed from the seed weight for each population (tab. 1) and the results of the germination trials (tab. 2).

Statistical analysis

The Wilcoxon test showed no significant differences between the control (C) and the pre-treated (T) seeds either in the germination percentage (ns p = 0.508) or in the vigour index (ns p = 0.914).

With respect to interspecific differences, the Kruskal-Wallis test showed there were significant differences in the germination percentages for both the control (C) (*** p = 0.000) and the pre-treated (T) seeds (*** p = 0.000), and likewise in the vigour indices (*** p= 0.000 for C; *** p = 0.000 for T).

The application of the Mann-Whitney test (tab. 3) showed a grouping of the three species *Thymus mastichina, Th. praecox* subsp. *penyalarensis*, and *Thymbra capitata*, because there were no significant differences between them (ns, tab. 3). Similarities were also observed between the species *Th. pulegioides*, *Th. zygis* subsp. *sylvestris,* and *Th. villosus* subsp. *lusitanicus*. *Th. caespititius*, however, could only be related to *Th. pulegioides*. There were few differences between the control and the pre-treated seeds, although it was noteworthy that the control seeds of *Th. pulegioides* could not be included in the group formed with *Th. zygis* subsp. *sylvestris* and *Th. villosus* subsp. *lusitanicus*. There were also differences between the control (C) and pre-treatment (T) germination percentages for the population pairs *Thymbra capitata – Th. mastichina* and *Thymbra capitata – Th. praecox* subsp. *penyalarensis*.

Interpopulational comparisons were carried out for the species which were represented by several populations – *Th. caespititius*, *Th. pulegioides*, *Th. zygis* subsp. *sylvestris*, and *Th. mastichina*. The Kruskal-Wallis test was applied to the last two species, and the Mann-Whitney test to all four. The Kruskal-Wallis test showed significant differences in the germination percentage for both the control (C) (*Th. zygis* subsp. *sylvestris* *** p = 0.001; *Th. mastichina* *** p = 0.001) and the pre-treated (T) (*Th. zygis* subsp. *sylvestris* *** p = 0.001; *Th. mastichina* *** p = 0.000) seeds. The same was the case for the vigour index.

The results of the Mann-Whitney test show the pairs of populations that were significantly different from each other in both germination percentage and vigour index.

For *Th. caespititius*, the significant differences found in the control (C) (percentage * p= 0.029; Iv * p = 0.029) seeds were not present in the pre-treated (T) (percentage ns p = 0.200; Iv ns p = 0.200) seeds. There were statistically significant differences between the two populations of *Th. pulegioides* that were studied in both the control (C) (percentage * p = 0.029; Iv * p = 0.029) and the pre-treated (T) (percentage * p = 0.029; Iv * p = 0.029) seeds.

Table 4 gives the results for *Th. zygis* subsp. *sylvestris*. In general, they are the same in both the control (C) and the pre-treated (T) seeds, and also in both the germinability and the vigour index.

In these results, there appear to be groups of populations that do not differ significantly from each other, but do differ from the rest of the populations. It can be established that the

	Locality	C/T	Replicate 1		Replicate 2		Replicate 3		Replicate 4		Mean	
Thymbra capitata	Villafranca	C	100	(21.58)	94	(19.78)	90	(18.87)	84	(16.03)	92	(19.07)
		T	82	(17.80)	86	(16.26)	90	(19.44)	86	(19.62)	86	(18.28)
Thymus praecox subsp. ***penyalarensis***	El Calvitero	C	78	(20.28)	82	(19.70)	84	(18.88)	72	(19.28)	79	(19.53)
		T	76	(17.44)	94	(23.75)	84	(19.29)	98	(23.72)	88	(21.05)
Thymus villosus subsp. ***lusitanicus***	Alía	C	36	(9.25)	18	(4.89)	38	(9.71)	48	(12.23)	35	(9.00)
		T	46	(10.48)	44	(9.72)	48	(11.89)	48	(11.90)	47	(11.00)
Thymus caespititius	Descarga.	C	6	(0.66)	24	(3.06)	2	(0.18)	12	(1.14)	11	(1.26)
		T	12	(1.53)	14	(1.71)	6	(1.04)	0	(0.00)	8	(1.07)
	Robledillo	C	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)
		T	2	(0.33)	0	(0.00)	2	(0.33)	0	(0.00)	1	(0.17)
Thymus pulegioides	Piornal	C	2	(0.40)	4	(0.83)	0	(0.00)	2	(0.10)	2	(0.33)
		T	0	(0.00)	2	(0.40)	0	(0.00)	6	(1.00)	2	(0.35)
	La Garganta	C	50	(10.76)	26	(4.14)	34	(7.69)	52	(7.76)	41	(7.58)
		T	24	(5.21)	30	(4.71)	24	(4.62)	28	(4.39)	27	(4.73)
Thymus zygis subsp. ***sylvestris***	Los Santos	C	60	(10.48)	52	(8.42)	64	(12.57)	70	(12.61)	62	(11.02)
		T	72	(15.06)	58	(11.67)	78	(15.13)	88	(13.70)	74	(13.90)
	Guadajira	C	20	(5.66)	12	(4.50)	20	(6.83)	20	(7.00)	18	(6.00)
		T	22	(7.36)	42	(7.98)	44	(13.59)	30	(9.09)	35	(9.50)
	La Albuera	C	12	(3.30)	2	(0.40)	26	(4.56)	20	(4.15)	15	(3.10)
		T	6	(1.50)	2	(0.50)	32	(7.14)	18	(4.10)	15	(3.31)
	Solana	C	2	(0.67)	10	(1.69)	12	(1.70)	4	(0.69)	7	(1.18)
		T	12	(1.59)	4	(1.00)	8	(1.49)	4	(0.47)	7	(1.14)
	Cabeza Buey	C	68	(21.42)	70	(22.96)	72	(16.42)	60	(16.64)	68	(19.36)
		T	64	(17.89)	66	(18.71)	56	(13.10)	54	(16.83)	60	(16.63)
	Badajoz	C	12	(3.57)	10	(1.88)	58	(12.00)	32	(5.43)	28	(5.72)
		T	38	(8.45)	26	(4.63)	38	(7.04)	66	(12.74)	42	(8.22)
	La Garganta	C	2	(0.40)	4	(1.00)	2	(0.66)	0	(0.00)	2	(0.52)
		T	6	(3.00)	14	(3.88)	6	(1.73)	6	(2.12)	8	(2.68)
Thymus mastichina	Los Santos	C	80	(19.32)	82	(15.91)	80	(16.18)	78	(18.84)	80	(17.56)
		T	84	(22.12)	76	(23.41)	74	(21.33)	76	(19.76)	78	(21.65)
	S. Domingo	C	100	(28.72)	84	(23.28)	84	(23.86)	98	(32.21)	92	(27.02)
		T	98	(28.18)	98	(25.40)	96	(21.81)	98	(23.01)	98	(24.60)
	Badajoz	C	80	(24.41)	60	(14.55)	74	(19.33)	68	(17.84)	71	(18.84)
		T	62	(13.08)	74	(15.26)	66	(11.68)	78	(13.68)	70	(13.42)
	Portanchito	C	100	(29.82)	86	(22.39)	92	(25.18)	86	(26.88)	91	(26.07)
		T	90	(25.20)	84	(22.12)	88	(22.03)	84	(19.48)	87	(22.21)
	Alconera	C	82	(25.81)	88	(29.97)	72	(23.41)	92	(31.03)	84	(27.55)
		T	70	(18.94)	70	(20.29)	58	(16.53)	78	(22.12)	69	(19.47)
	Villafranca	C	76	(18.97)	76	(19.53)	88	(25.30)	84	(18.12)	81	(20.48)
		T	78	(17.54)	78	(16.29)	80	(16.62)	86	(21.47)	81	(17.98)
	La Garganta	C	70	(34.32)	62	(26.92)	82	(39.93)	84	(41.53)	75	(35.67)
		T	82	(28.61)	64	(21.26)	88	(27.24)	64	(20.60)	75	(24.43)
	Aliseda	C	62	(13.09)	56	(12.96)	54	(11.92)	64	(13.39)	59	(12.84)
		T	74	(18.50)	60	(13.74)	64	(16.38)	60	(14.81)	65	(15.86)
	F. Maestre	C	86	(22.63)	94	(21.39)	84	(21.24)	80	(17.98)	86	(20.81)
		T	88	(26.01)	92	(29.56)	84	(25.91)	78	(24.69)	86	(26.54)
	Alía	C	78	(13.11)	82	(15.72)	80	(10.30)	74	(15.05)	79	(13.54)
	Benquerencia	C	96	(25.38)	88	(23.44)	95	(25.94)	86	(18.83)	91	(23.40)
		T	92	(18.33)	92	(25.31)	80	(16.64)	92	(17.85)	89	(19.53)
	Bienvenida	C	84	(19.30)	76	(17.14)	74	(14.29)	74	(19.99)	77	(17.68)
		T	68	(17.10)	78	(18.50)	64	(13.20)	68	(13.16)	70	(15.49)
	Descarga.	C	80	(18.02)	72	(12.03)	90	(18.54)	78	(16.06)	80	(16.16)
		T	90	(22.29)	76	(16.44)	98	(25.10)	84	(20.95)	87	(21.20)

Table 2. Germination percentages and vigour indices (Iv, in parentheses) obtained for the different replicates of the trials with control (C) and pretreated (T) seeds. *Porcentajes de germinación e Índices de Vigor (Iv, entre paréntesis), obtenidos en las diferentes réplicas de los experimentos con semillas control (C) y pretratadas (T).*

Control (C)	***Thymus caespitius* *1***	***Thymus pulegioides* *2***	***Thymus zygis* *3***	***Thymus villosus* *4***	***Thymus mastichina* *5***	***Thymus praecox* *6***	***Thymbra capitata* *7***
1	-----						
2	ns 0.105 (ns 0.083)	-----					
3	** 0.005 (*** 0.000)	ns 0.358 (ns 0.236)	-----				
4	** 0.008 (** 0.004)	ns 0.368 (ns 0.073)	ns 0.457 (ns 0.254)	-----			
5	*** 0.000 (*** 0.000)	*** 0.000 (*** 0.000)	***0.000 (***0.000)	*** 0.000 (*** 0.000)	-----		
6	** 0.004 (** 0.004)	** 0.004 (** 0.004)	*** 0.000 (** 0.003)	* 0.029 (* 0.029)	ns 0.634 (ns 0.914)	-----	
7	** 0.004 (** 0.004)	** 0.004 (** 0.004)	*** 0.000 (** 0.004)	* 0.029 (* 0.029)	* 0.021 (ns 0.701)	* 0.029 (ns 0.886)	-----

Pretreat. (T)	***Thymus caespitius* 1**	***Thymus pulegioides* 2**	***Thymus zygis* 3**	***Thymus villosus* 4**	***Thymus mastichina* 5**	***Thymus praecox* 6**	***Thymbra capitata* 7**
1	-----						
2	Ns 0.195 (ns 0.195)	-----					
3	*** 0.000 (*** 0.000)	* 0.033 (* 0.013)	-----				
4	** 0.004 (** 0.004)	** 0.004 (** 0.004)	ns 0.279 (ns 0.332)	-----			
5	*** 0.000 (*** 0.000)	*** 0.000 (*** 0.000)	*** 0.000 (*** 0.000)	*** 0.000 (*** 0.000)	-----		
6	** 0.004 (** 0.004)	** 0.004 (** 0.004)	*** 0.000 (*** 0.000)	** 0.029 (** 0.029)	ns 0.153 (ns 0.529)	-----	
7	** 0.004 (** 0.004)	** 0.004 (** 0.004)	*** 0.000 (*** 0.001)	** 0.029 (** 0.029)	ns 0.173 (ns 0.470)	ns 0.886 (ns 0.486)	-----

Table 3. Mann-Whitney test significance levels for the comparison of the germination and vigour index (Iv, in parentheses) results between pairs of species, on samples of control (C) and pretreated (T) seeds: ns, not significant; *, $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. *Niveles de significación estadística del test de Mann-Whitney, para la comparación de los resultados de germinación e Indice de vigor (Iv, entre paréntesis), entre pares de especies, sobre muestras de semillas control (C) y pretratadas (T). ns, no significativo, *, p < 0.05, ** p < 0.01, *** p < 0.001.*

Control (C)	**Garganta 1**	**Solana 2**	**Albuera 3**	**Guadajira 4**	**Badajoz 5**	**Cab. Buey 6**	**Los Santos 7**
1	-----						
2	Ns 0.114 (ns0.114)	-----					
3	Ns 0.114 (ns 0.114)	ns 0.343 (ns 0.343)	-----				
4	* 0.029 (* 0.029)	* 0.029 (* 0.029)	ns 0.886 (ns 0.057)	-----			
5	* 0.029 (* 0.029)	ns 0.114 (* 0.029)	ns 0.486 (ns 0.486)	ns 0.886 (ns 0.486)	-----		
6	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	-----	
7	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	ns 0.057 (ns 0.114)	ns 0.343 (* 0.029)	-----

Pretreat. (T)	**Garganta 1**	**Solana 2**	**Albuera 3**	**Guadajira 4**	**Badajoz 5**	**Cab. Buey 6**	**Los Santos 7**
1	-----						
2	Ns 0.686 (* 0.029)	-----					
3	Ns 0.686 (ns 1.000)	ns 0.686 (ns 0.343)	-----				
4	* 0.029 (* 0.029)	* 0.029 (* 0.029)	ns 0.114 (* 0.029)	-----			
5	* 0.029 (* 0.029)	* 0.029 (* 0.029)	ns 0.057 (ns 0.114)	ns 0.886 (ns 0.486)	-----		
6	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (ns 0.057)	ns 0.200 (* 0.029)	-----	
7	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (ns 0.057)	ns 0.057 (ns 0.057)	ns 0.114 (ns 0.200)	-----

Table 4. Mann-Whitney test significance levels for the comparison of the germination and vigour index (Iv, in parentheses) results between pairs of populations of Th. zygis subsp. sylvestris, on samples of control (C) and pretreated (T) seeds: ns, not significant; *, $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. *Niveles de significación estadística del test de Mann-Whitney, para la comparación de los resultados de germinación e Indice de vigor (Iv, entre paréntesis), entre pares de poblaciones de Th. zygis subsp. sylvestris, sobre muestras de semillas control (C) y pretratadas (T). ns, no significativo, *, p < 0.05, ** p < 0.01, *** p < 0.001.*

germinations corresponding to La Albuera, Solana de los Barros, and La Garganta were significantly similar to each other, as were those of La Albuera, Guadajira, and Badajoz (n.s.), and of Guadajira, Badajoz, Cabeza del Buey, and Los Santos de Maimona.

For *Th. mastichina* (tabs. 5a and 5b), one observes that, in the controls (C), the pre-treated seeds (T), the germinability, and the germination rate, there were in most

Control (C)	Aliseda 1	Badajoz 2	Garganta 3	Bienvenida 4	Alía 5	S.Maimo. 6	Villafranca 7	Alconera 8	F.Maestre 9	Descarga. 10	Portanch. 11	Benquere. 12	S.Domingo 13
1	-------												
2	ns 0.114 (* 0.029)	------											
3	ns 0.057 (* 0.029)	ns 0.486 (* 0.029)	-------										
4	* 0.029 (* 0.029)	ns 0.343 (ns 0.886)	ns 0.686 (* 0.029)	-------									
5	* 0.029 (ns 0.486)	ns 0.200 (ns0.114)	ns 0.886 (* 0.029)	ns 0.686 (ns 0.114)	-------								
6	* 0.029 (* 0.029)	ns 0.114 (ns 0.686)	ns 0.886 (* 0.029)	ns 0.343 (ns 0.886)	ns 0.686 (* 0.029)	-------							
7	* 0.029 (* 0.029)	ns 0.114 (ns 0.486)	ns 0.343 (* 0.029)	ns 0.200 (ns 0.486)	ns 0.686 (* 0.029)	ns 1.000 (ns 0.200)	-------						
8	* 0.029 (* 0.029)	ns 0.114 (ns0.057)	ns 0.200 (ns 0.114)	ns 0.486 (* 0.029)	ns 0.343 (* 0.029)	ns 0.343 (* 0.029)	ns 0.686 (ns 0.057)	-------					
9	* 0.029 (* 0.029)	* 0.029 (ns 0.486)	ns 0.114 (* 0.029)	ns 0.057 (ns 0.114)	ns 0.057 (* 0.029)	ns 0.114 (ns 0.114)	ns 0.346 (ns 0.886)	ns 0.886 (* 0.029)	-------				
10	* 0.029 (ns 0.200)	ns 0.200 (ns 0.486)	ns 0.686 (* 0.029)	ns 0.686 (ns 0.486)	ns 1.000 (ns 0.200)	ns 0.686 (ns 0.486)	ns 1.000 (ns 0.057)	ns 0.486 (* 0.029)	ns 0.200 (ns 0.114)	-------			
11	* 0.029 (* 0.029)	* 0.029 (ns 0.057)	* 0.029 (ns 0.057)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	ns 0.114 (ns 0.114)	ns 0.343 (ns 0.486)	ns 0.343 (ns 0.057)	ns 0.114 (* 0.029)	-------		
12	* 0.029 (* 0.029)	* 0.029 (ns 0.200)	* 0.029 (* 0.029)	* 0.029 (ns 0.114)	* 0.029 (* 0.029)	* 0.029 (ns 0.114)	ns 0.057 (ns 0.343)	ns 0.343 (ns 0.343)	ns 0.200 (ns 0.200)	ns 0.114 (* 0.029)	ns 0.886 (ns 0.486)	-----	
13	* 0.029 (* 0.029)	* 0.029 (ns 0.114)	ns 0.057 (ns 0.114)	ns 0.057 (0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	ns 0.200 (ns 0.114)	ns 0.343 (ns 0.886)	ns 0.486 (* 0.029)	ns 0.114 (* 0.029)	ns 0.686 (ns 0.886)	ns 1.000 (ns 0.486)	-----

Table 5a. Mann-Whitney test significance levels for the comparison of the germination and vigour index (Iv, in parentheses) results between pairs of populations of Th. mastichina, on samples of control (C) and pretreated (T) seeds: ns, not significant; *, $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. *Niveles de significación estadística del test de Mann-Whitney, para la comparación de los resultados de germinación e Indice de vigor (Iv, entre paréntesis), entre pares de poblaciones de Th. mastichina, sobre muestras de semillas control (C). ns, no significativo, *, $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$*

Pretreat. (T)	Aliseda 1	Badajoz 2	Garganta 3	Bienvenida 4	Alía 5	S.Maimo. 6	Villafranca 7	Alconera 8	F.Maestre 9	Descarga. 10	Portanch. 11	Benquere. 12	S.Domingo 13
1	-------												
2	ns 0.200 (ns 0.114)	------											
3	ns 0.200 (* 0.029)	ns 0.686 (* 0.029)	-------										
4	ns 0.200 (ns 0.686)	ns 0.886 (ns 0.343)	ns 0.886 (* 0.029)	-------									
5	* 0.029 (ns 0.686)	ns 0.057 (ns 0.057)	ns 0.886 (* 0.029)	ns 0.057 (ns 0.686)	-------								
6	* 0.029 (* 0.029)	ns 0.200 (*0.029)	ns 0.886 (ns 0.686)	ns 0.200 (* 0.029)	ns 0.686 (* 0.029)	-------							
7	* 0.029 (ns 0.343)	ns 0.057 (* 0.029)	ns 0.886 (ns 0.114)	ns 0.057 (ns 0.486)	ns 0.886 (ns 0.343)	ns 0.200 (ns 0.114)	-------						
8	ns 0.686 (ns 0.057)	ns 0.886 (* 0.029)	ns 0.686 (ns 0.114)	ns 0.686 (ns 0.114)	ns 0.057 (ns 0.114)	ns 0.200 (ns 0.343)	ns 0.057 (ns 0.486)	-------					
9	* 0.029 (* 0.029)	* 0.029 (* 0.029)	ns 0.200 (ns 0.686)	* 0.029 (* 0.029)	ns 0.200 (* 0.029)	ns 0.057 (* 0.029)	ns 0.343 (* 0.029)	* 0.029 (* 0.029)	-------				
10	* 0.029 (ns 0.057)	ns 0.057 (* 0.029)	ns 0.200 (ns 0.486)	ns 0.057 (ns 0.114)	ns 0.200 (ns 0.114)	ns 0.114 (ns 1.000)	ns 0.486 (ns 0.343)	ns 0.057 (ns 0.486)	ns 0.886 (ns 0.057)	-------			
11	* 0.029 (* 0.029)	* 0.029 (* 0.029)	ns 0.114 (ns 0.686)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	ns 0.057 (ns 0.886)	ns 0.114 (ns 0.057)	* 0.029 (ns 0.343)	ns 0.886 (ns 0.057)	ns 0.886 (ns 0.886)	-------		
12	* 0.029 (ns 0.200)	* 0.029 (* 0.029)	ns 0.114 (ns 0.114)	* 0.029 (ns 0.343)	ns 0.114 (ns 0.200)	ns 0.057 (ns 0.343)	ns 0.057 (ns 0.343)	* 0.029 (ns 0.886)	ns 0.343 (ns 0.057)	ns 0.686 (ns 0.886)	ns 0.343 (ns 0.343)	-----	
13	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (ns 0.886)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (ns 0.200)	* 0.029 (* 0.029)	* 0.029 (ns 0.057)	* 0.029 (ns 0.343)	ns 0.114 (ns 0.200)	* 0.029 (ns 0.343)	* 0.029 (ns 0.114)	-----

Table 5b. Mann-Whitney test significance levels for the comparison of the germination and vigour index (Iv, in parentheses) results between pairs of populations of Th. mastichina, on samples of control (C) and pretreated (T) seeds: ns, not significant; *, $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. *Niveles de significación estadística del test de Mann-Whitney, para la comparación de los resultados de germinación e Indice de vigor (Iv, entre paréntesis), entre pares de poblaciones de Th. mastichina, sobre muestras de semillas pretratadas (T). ns, no significativo, *, p < 0.05, ** p < 0.01, *** p < 0.001*

cases no differences, or when there were they had a minimal significance level (* $p < 0.05$). Within that first overall impression of homogeneity, however, one can distinguish two sets of populations that stand out from the rest – Aliseda on the one hand, and the Santo Domingo, Benquerencia, and El Portanchito block on the other.

The Spearman test for the possible relationship between germination percentage and seed weight showed significant correlations (** correlation coefficient = 0.801 for C; ** correlation coefficient = 0.735 for T). This correlation was studied at the species level for *Th. zygis* subsp. *sylvestris* and *Th. mastichina*, for which there were many populations available. For *Th. zygis* subsp. *sylvestris*, the Pearson test showed significant correlations (** correlation coefficient = 0.893 for C; * correlation coefficient = 0.857 for T). There was no such correlation for *Th. mastichina*, however (correlation coefficient = 0.084 for C; correlation coefficient = 0.088 for T).

DISCUSSION

In germination biology, seeds collected in the wild present typical characteristics of dormancy, a wide range of states of maturity of the collected material, and a limited range of temperatures and conditions under which the seeds will germinate with rapidity (Finch-Savage, Gray and Dickson, 1991a). These are limiting factors for establishing in practice the cultivation of wild species, factors which have to be surmounted by techniques of seed technology (Finch-Savage, Gray and Dickson, 1991b) and optimization of the ranges of germination.

Information on the germination processes of aromatic plants of the Lamiaceae family could at best be considered as sparse in general (Thanos, 1993). With respect to the thymes, there have been studies for some of the species of the influence of such factors as: optimal germination temperatures (Thanos, Kadis and Skarou, 1995); optimal storage temperature (Kretschmer, 1989); light (Thanos, 1993); the inhibitory effect of essential oils (Thanos, 1993; Tarayre *et al*., 1995; Thanos, Kadis and Skarou, 1995); seed viability under extreme conditions such as fire (Belhassen *et al.,* 1989; Eriksson, 1998); and the possible influence of seed weight and environmental conditions on the final germination percentage (Pérez-García *et al*., 2003).

Slow germination is a typical strategy of Mediterranean plants. It protects them from the frequent dry spells at the beginning of the rainy season (Thanos, 1993). Indeed, those species that, because of their area of distribution, have a more northerly character, also present a faster germination rate and a wider range of germination conditions (Thanos and Doussi, 1995).

Another typical strategy of Mediterranean plants is for dormancy to be broken by low temperatures, such as occur in nature in the period prior to the germination of the seeds in spring. Thermal pre-treatment is thus often found to promote increased germination. In the case of the Lamiaceae family considered overall, there have been recommendations to pre-chill the seeds to improve germination behaviour (ISTA, 1999). In the present work, however, no such effect was found. Hence, at least with the species, populations, and conditions of our study, it can be stated that pre-chilling led to no statistically significant improvement in germination.

The germination of *Thymbra capitata* has been studied by Thanos (1993) and Thanos, Kadis and Skarou (1995) on material whose provenance was the island of Crete. In a first study, germination percentages were obtained of less than 30% at 20°C in darkness. In a second, light was found to have little effect on germination, and it was reported that the optimal germination temperature was 15°C, with very low germination percentages having been obtained at 25°C and 30°C. Other workers (Vokou and Margaris, 1986) studied a population from the vicinity of Athens. They found *high* germinabilities (73%) at 20°C in darkness.

Thymbra capitata is a circum-Mediterranean species. In Iberia, it is distributed over the southern half of the Peninsula. It inhabits all types of substrate, although it prefers base-rich soils. It is found from sea level to approximately 800 m altitude. The seeds of Extremadura provenance that were tested in the present work behaved differently from those described in the foregoing paragraph, and thereby indirectly reflect the genotypic variability of the species, although our results may be close to those of Vokou and Margaris (1986). In our case, this species presented the best germination results of the entire study, in both germination percentage (*high* germinabilities of up to 92%) and vigour index (*fast* germination rates, *Iv* of up to 19.07), for both the control and the pre-treated seeds. Hence, for material from Extremadura, the recommendable germination conditions could be taken to be those used in the present study.

Th. praecox subsp. *penyalarensis* is a taxon with a very restricted area of distribution – the Sistema Central of the Iberian Peninsula. It inhabits clearings in scrub and xerophile upland pastures. There appear to have been no previous studies approaching questions of its germination requirements, even though it is a very interesting element biogeographically as well as being a threatened species (D.O.E, 2001). The present study material gave *high* levels of germinability (79% C; 88% T) and a *fast* germination rate (19.53 C; 21.05 T), so that the recommendable germination conditions for this taxon could again be those applied in this work.

Th. villosus subsp. *lusitanicus* is an Iberian endemic that appears in disconnected areas of Estremadura and Beira Litoral (Portugal), Cáceres (the Sierras of Guadalupe and of Altamira), and Ciudad Real (Sierra de Río Frío). It inhabits acid substrates (quartzites, shales, sands, rañas), and very rarely calcareous soils. It is a sporadic plant of cleared scrubland, especially of mixed scrub or Cistus and Erica, and it also has chorological interest. There have been no previous studies of its germinative capacity. The present material showed *moderate* germinability (35% C; 47% T) and *medium* germination rates (9.00 C; 11.00 T). The treatment conditions applied here could in principle be recommended for this population.

Th. caespititius is another species that is interesting chorologically, being limited to the NW Iberian Peninsula, Madeira, and the Azores. It is a typical thyme of zones of Atlantic influence with wet climates. Its southern limit in the Peninsula is represented by the Cáceres and Salamanca populations, where it forms part of characteristic heathlands. It is found from sea level to 1200 m (Serra de Gerês, Portugal), and tolerates frosts poorly. There is no information in the literature on its germinative capacity. This taxon gave the worst results in the present study, with *null* or *low* germinabilities (0-11% C; 1-8% T) and *slow* germination rates (0.00 and 1.26 C; 0.17 and 1.26 T). Further trials are therefore required with other germination conditions to look for improvements in the development of its germination. Given the chorological and ecological characteristics of the wild populations, it would perhaps be advisable to try temperatures that are much cooler and more uniform.

Th. pulegioides has a wide distribution throughout Europe. It is another thyme that, to the best of our knowledge, has not previously been studied in terms of its germination. The present results again do not make it recommendable to apply this experiment's treatment conditions, since the observed germinabilities were *low* and *moderate* (2 and 41% C; 2 and 27% T), and the germination rates were *slow* to *medium* (0.33 and 7.58 C;

0.35 and 4.73 T). In view of its natural ecology (hygrophilous high-mountain meadows and mat-grass swards) and distribution area, lower temperatures might provide more successful germination.

Th. zygis subsp. *sylvestris* has an area of distribution restricted to the SW quadrant of the Iberian Peninsula. It inhabits alkaline soils by preference, although it can grow on acid substrates. It is often found on calcareous, loamy, and gypseous soils from the coasts of Portuguese Estremadura to altitudes of up to 1000 m in the centre of the Peninsula. The only data we know of concerning its germination behaviour correspond to Pérez-García *et al.* (2003) who studied the germination of 14 Spanish populations from Badajoz, Ciudad Real, and Cuenca under conditions of constant temperature (15°C) and alternating photoperiod (16h light: 8h dark). The populations probably belonged to *Th. zygis* subsp. *zygis* (the material from Cuenca) and *Th. zygis* subsp. *sylvestris* (the rest), although they were all indistinctly assigned the binomial *Th. zygis*. The ranges of the results were highly variable, which may partly have been because of the non-recognition of the sub-species categories.

In principle, while the treatment applied in the present experiments may be considered that of choice for this subspecies, it is necessary to distinguish the provenance of the material. In our results, there was a clear lack of overall homogeneity with respect to the collection date, the band of altitude of the geographical site, or the particular ecology of the population. For the material collected in 2000 from Badajoz province (Pérez-García *et al*., 2003), the germinabilities were quite variable – from 4% (the material from Usagre), through 43% (Zafra), to 77% (Solana de los Barros). Such a high degree of dispersion and disparity of the results may reflect the fact that this is a taxon of only recent phylogenetic diversification (Morales, 1986).

Variation in germination behaviour between different populations of the same species is a well-known phenomenon (Pérez-García *et al*., 1995; Baskin and Baskin, 1998; Meyer and Allen, 1999). It may be caused by genetic differences or by other complex factors such as the weather conditions during the formation of the flower or the ripening of the seeds (Meyer *et al*., 1989; Gutterman, 1992; Buchwald and Kitkowska, 2001). In the case of the species studied in the present work, there is hardly any published information in this respect (Perez-García *et al*., 2003).

Thymus mastichina is endemic to the Iberian Peninsula, where it is found in cleared scrubland, from sea level up to 1800 m. It grows preferentially on siliceous soils of loose, more or less sandy, texture, although it can also be found in mountain screes, gypseous loams, and even limestone boulder fields. Its germination was studied in the work of Pérez-García *et al.* (2003), under conditions of constant temperature (15°C) and alternating photoperiod (16h light: 8h dark), using 8 Iberian populations, 5 of which were from sites close to those of the present work (provinces of Badajoz and Cáceres). The results for the accumulated percentage germination, considering only these 5 Extremadura populations, varied between 65% and 95%. In our trials, the germination results were very good: germinabilities of 80-98% for the material from El Portanchito, Benquerencia, and Santo Domingo, and an average of above 70% for the remaining populations, in no case being below 59% (Aliseda).

With respect to a possible correlation between seed weight and germination percentage as is observed in other species (Vera, 1997; Baloch *et al*., 2001), although there was a correlation in overall terms, the separate study of the relationship for the two taxa *Th. mastichina* and *Th. zygis* subsp. *sylvestris* showed clearly different behaviour – the positive correlation between seed weight and germination percentage was maintained in *Th. zygis* subsp. *sylvestris*, but not in *Th.*

mastichina. Such distinction was not described in the work of Pérez-García *et al.* (2003).

In conclusion, the treatment that was applied here can be considered recommendable for *Thymbra capitata, Thymus praecox subsp. penyalarensis*, and *Th. mastichina* since mostly *high* germinabilities and *fast* germination rates were obtained; and not recommendable for *Th. caespititius* and *Th. pulegioides*, for which it will be necessary to test other conditions, since with the present trials only *low* germinabilities and *slow* germination rates were obtained. The results for *Th. villosus* subsp. *lusitanicus* and *Th. zygis* subsp. *sylvestris* were heterogeneous, so that the treatment can be proposed as recommendable in principle.

Nevertheless, because there exists not only interspecific but also interpopulational variability, it would in general be advisable to distinguish the provenance of the material.

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