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Effects of heat shock on seed germination of Turkish red pine (*Pinus brutia*)

Efectos de choque térmico sobre la germinación de semillas de pino rojo de Turquía (*Pinus brutia*)

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SUMMARY

Fire plays an important ecological role in Mediterranean-type ecosystems. Many Mediterranean plant species exhibit enhanced germination capacity when exposed to heat. In the present study, the effect of high temperatures and exposure times on germination of Turkish red pine (*Pinus brutia*) was analyzed in order to reveal the response of seeds to fire and the implications on species regeneration. Seeds were heated to a range of temperatures (from 75 to 170 ºC) and exposure times (from 30 seconds to 30 minutes) similar to those obtained in surface soil layers during natural fires. In total, twenty treatments were performed. Seed germination percentages and germination values were calculated for each treatment. The results of ANOVA performed for germination values showed that temperature had a significant effect. Germination percentages increased in thermal treatments at 70 ºC for 10, 15, 20, 25, and 30 min; at 105 ºC for 10, 15, 20, and 25 min, and at 130 ºC for 1 and 2 min. The increase in both temperature and exposure times generally decreased the germination percentage especially at 130 °C and over in comparison with the control. Maximum germination percentage was reached in the treatment at 130 ºC for 1 min while the minimum germination was reached at 130 ºC for 10 min.

Key words: fire, heat treatment, high temperature, Mediterranean, soil seed bank.

RESUMEN

El fuego juega un importante papel ecológico en los ecosistemas de tipo mediterráneo. Muchas especies de plantas del Mediterráneo exhiben una capacidad mejorada de germinación cuando se exponen al calor. En el presente estudio, el efecto de altas temperaturas y los tiempos de exposición sobre la germinación de pino rojo de Turquía (*Pinus brutia*) fue analizado con el fin de revelar la respuesta de las semillas al fuego y las implicaciones en la regeneración de la especie. Las semillas se calentaron en un rango de temperaturas (75 a 170 ºC), con tiempos de exposición (30 segundos a 30 minutos) similares a los obtenidos en las capas superficiales del suelo durante los incendios naturales. En total se realizaron veinte tratamientos. Se calcularon los porcentajes de germinación de la semilla y los valores de germinación para cada tratamiento. Los resultados de ANOVA realizados para los valores de germinación, mostraron que la temperatura tuvo un efecto significativo. Los porcentajes de germinación aumentaron en los tratamientos térmicos a 70 ºC durante 10, 15, 20, 25 y 30 min; a 105 ºC durante 10, 15, 20 y 25 min, y a 130 ºC durante 1 y 2 min. El aumento de la temperatura y los tiempos de exposición generalmente disminuyeron el porcentaje de germinación, especialmente a 130 °C y superior en comparación con los controles. El porcentaje máximo de germinación se alcanzó en el tratamiento a 130 ºC durante 1 minuto, mientras se llegó a la germinación mínima a 130 ºC durante 10 min.

Palabras clave: fuego, tratamiento térmico, alta temperatura, mediterráneo, banco de semillas del suelo.

INTRODUCTION

Turkish red pine is one of the main tree species used in reforestation activities in the Mediterranean region with Aleppo pine. *Pinus brutia* Ten. is a tree species that is well adapted to forest fires with its remarkably short juvenile phase, cone characteristics, seed ecology and physiology, seed dispersal period, bark thickness and resin content, etc. (Boydak *et al.* 2006). Several studies carried out in the Mediterranean basin give information about the post-fire regeneration in *P. brutia* and *Pinus halepensis* Mill. ecosystems and their fire-adapted characteristics. Trabaud (2000) explains that pine forest vegetation in Mediterranean ecosystems is more flammable than the pine itself. Easy flammability of *P. brutia* and *P. halepensis* and their post-fire regeneration are not considered to be a contradiction but a balance of fire-adapted ecosystems. However, *P. brutia* can regenerate with and without fire (Boydak *et al.* 2006).

Canopy seed banks (*P. brutia* and *P. halepensis*) or soil seed banks (Cistaceae and Leguminosae) are the sources of seeds for post-fire regeneration in Mediterranean ecosystems. Closed cones (canopy seed bank) are stimulated to open and disperse the seed by fire or heat. Heat also overcomes the dormancy of *P. brutia* and stimulates germination by softening the seed coats of hard seeded plants (Cistaceae and Leguminosae) (Thanos 1999). The expansion of Cistaceae and Leguminosae populations is often limited to the post-fire conditions (Arianoutsou and Thanos 1996, Thanos 1999, Arianoutsou and Ne'eman 2000). The results of the research conducted in Samos Island, Greece in 1982 revealed that *P. brutia, Cistus* species and Leguminosae species had an important role in post-fire regeneration (Thanos *et al.* 1989) and the total average density of *P. brutia* seedlings in six and ten years following the fire was the same (0.15 per m²) (Thanos and Marcou 1993). After a forest fire (in 1989) at a 60-year old *P. brutia* forest (Thasos Island, Greece), on average 2-6 pine seedlings per m² were recorded by the end of the recruitment period (May 1990) and the density was stabilized at about 0.6-2 seedlings m^{-2} in five years (Spanos *et al.* 2000).

The aim of the present study is to investigate the effect of high temperature and exposure times on *P. brutia* seeds in order to reveal the response of seeds lying on soil, in surface soil layers and in the scorched cones after forest fires. Thus, to understand whether they may stay alive and contribute to post fire regeneration or not. In relation to the aim, the hypothesis of the study is that different heat shock treatments have no effect on germination percentage and germination value of the seeds of *P. brutia*.

METHODS

Seed material. Cones were collected by the Turkish Ministry of Forest and Water Affairs, Forest Tree Seeds and Tree Breeding Research Directorate from the natural *P. brutia* seed stand of 66 years of age situated in Marmaris-Çetibeli (37° 00' 17'' N latitude, 28° 19' 42'' E longitude, altitude 60 m). After their extraction from the cones, seeds were put in partially sealed small glass jars and put in a refrigerator at +5 ºC till the tests.

Heat-shock treatments and germination tests. Seeds were heated for different periods of time to simulate the effects of fire. Considering fire behavior, a set of treatment temperatures (75-105-130-150-170 ºC) and exposure times (30 sec, 1-2-3-7-10-15-20-25-30 min) were selected. After the treatments, germination tests were carried out at alternating temperatures from 20 to 30 °C in the Jacobsen apparatus. Seeds were exposed to 20 ºC for 16 hours and to 30 °C for 8 hours. During the treatment period at 30 °C, seeds were also subjected to about 1,400 lux fluorescent light. Four replicates of 100 seeds were used per treatment. Seeds were considered as germinated when radicles grew more than 2 mm long from the seed and were removed. Seeds were checked every two days for 28 days.

Statistical analyses. Germination data were expressed as germination percentage (GP) and germination value (GV). GP is the percentage of the seeds that germinated at the end of the test and GV was calculated according to the following equation (Djavanshir and Pourbeik 1976):

$$GV = \frac{\sum DGS}{N} \times \frac{N_{gs}}{100} \times 10 \qquad [1]$$

Where, GV = germination value, DGS = daily germination speed, N = frequency or the number of DGS that are calculated during the test, N_{gs} = number of germinated seeds, 10 is the constant value.

In order to find out the normal distribution of residuals and homogeneity of variances prior to the analyses of variance, the germination percentages were transformed using arcsine transformation. The significance of the fixed effects was tested with the *F* test. The following linear models were used for the analysis:

$$y_{ij} = \mu + T_i + e_{ij} \qquad [2]$$

Where, y_{ij} = observation of j-th germination of the i-th treatment, μ = overall mean, T_i = effect of treatment, and e_{ij} is the error;

$$y_{ijk} = \mu + K_i + F_j + KF_{ij} + e_{ijk} \qquad [3]$$

Where, y_{ijk} = observation of k-th germination of the i-th exposure time of the i-th temperature, μ = overall mean, K_i = effect of temperature, F_j = effect of exposure time, KF_{ij} = interaction effect of temperature-exposure time and e_{ij} = error.

Following the ANOVA, when significant effects were detected, Duncan's multiple range test was used to determine significant differences between the means ($P < 0.05$)

RESULTS

The ANOVA applied to the germination data showed significant differences among the treatments for both germination percentages and germination values ($P < 0.0001$) (table 1). The analyses of variances of germination values also indicated significant effects of temperature (tables 2 and 3). Maximum germination percentage (70.3 %) was reached during the treatment at 130 ºC for 1 min while the minimum germination (4 %) was reached at 130 ºC for 10 min (figure 1 and table 4).

In general, the germination of *P. brutia* seeds was not significantly influenced by temperatures from 75 to 105 ºC. On the other hand, a negative influence on germination was observed when temperatures were higher than 130 ºC and exposures times increased (figures 2 and 3). At the temperature of 130 ºC and above, *P. brutia* seeds were more sensitive to the exposure time than to temperatures (figure 4).

Table 1. ANOVA results regarding the effects of heat shock (treatment) on germination percentage and germination value in *Pinus brutia* seeds.

Resultados del ANDEVA de los efectos de choque térmico (tratamiento) en el porcentaje de germinación y el valor de germinación en las semillas de *Pinus brutia.*

Source	df	Germination percentage			Germination value		
		MS	F	*P*-value	MS	F	*P*-value
Treatments	20	460.68	33.95	< 0.001	132.36	30.50	< 0.001
Error	63	13.57			4.34		

Table 2. Effect of heat shocks of 75 ºC-10, 15, 20, 25, 30 min, 105 ºC-10, 15, 20, 25 min on the germination of *Pinus brutia* seeds.

Efecto de los choques de calor 75 ºC - 10, 15, 20, 25, 30 min, 105 ºC-10, 15, 20, 25 min sobre la germinación de semillas de *Pinus brutia*.

Source	df	Germination percentage			Germination value		
		MS	F	*P*-value	MS	F	*P*-value
Temperature	1	7.26	0.96	0.335	39.06	13.66	0.001
Exposure time	4	6.45	0.85	0.503	3.37	1.18	0.341
Temperature X Exposure times	4	0.38	0.05	0.995	3.47	1.21	0.326
Error	30	7.55			2.86		

Table 3. Effect of heat shocks of 150 ºC-1, 2 min, 170 ºC-1, 2 min on the germination of *Pinus brutia* seeds.

Efecto de los choques de calor 150 ºC-1, 2 min, 170 ºC-1, 2 min sobre la germinación de semillas de *Pinus brutia*.

Source	df	Germination percentage			Germination value		
		MS	F	*P*-value	MS	F	*P*-value
Temperature	1	100.00	5.10	0.037	74.56	11.89	0.003
Exposure time	2	21.70	1.11	0.352	18.31	2.92	0.080
Temperature X Exposure time	2	95.53	4.87	0.020	14.93	2.38	0.121
Error	18	19.60			6.27		

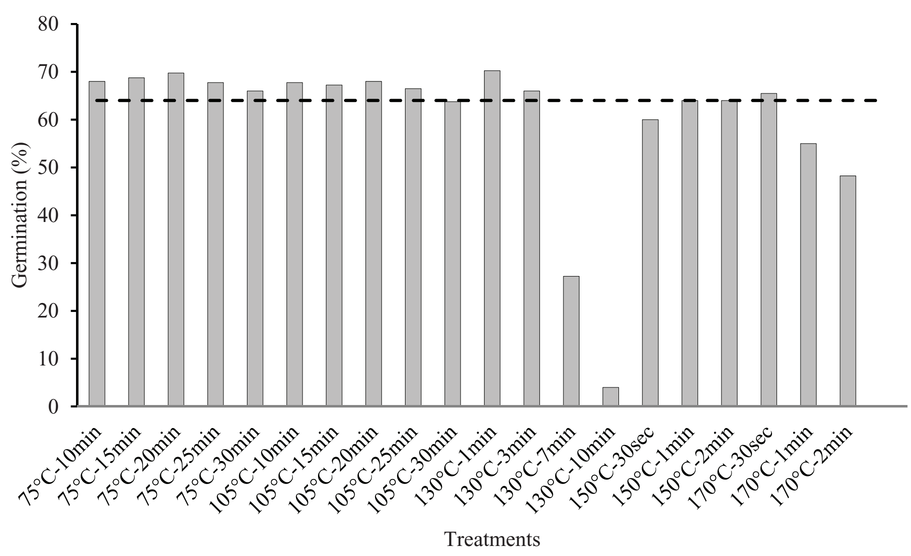

Figure 1. Germination response of *Pinus brutia* seeds after heat shock treatments of different intensity and exposure times (dashed line represents control).

Respuesta de germinación de semillas de *Pinus brutia* después de los tratamientos de choque térmico de diferente intensidad y tiempos de exposición (la línea discontinua representa el control).

Table 4. Effect of heat shocks (75 ºC-170 ºC) on the germination of *Pinus brutia* seeds.

Efecto de los choques de calor (75 ºC-170 ºC) sobre la germinación de semillas de *Pinus brutia*.

	Treatments	Germination percentage	Germination value
1	75 ºC-10 min	68.0 a	14.7 ab
2	75 ºC-15 min	68.8 a	15.8 ab
3	75 ºC-20 min	69.8 a	16.4 a
4	75 ºC-25 min	67.8 a	15.6 ab
5	75 ºC-30 min	66.0 a	14.8 ab
6	105 ºC-10 min	67.8 a	14.7 ab
7	105 ºC-15 min	67.3 a	14.1 ab
8	105 ºC-20 min	68.0 a	12.5 b
9	105 ºC-25 min	66.5 a	12.7 ab
10	105 ºC-30 min	63.8 ab	11.3 bc
11	130 ºC-1 min	70.3 a	16.9 a
12	130 ºC-3 min	66.0 a	15.9 ab
13	130 ºC-7 min	27.3 d	2.1 f
14	130 ºC-10 min	4.0 e	0.1 g
15	150 ºC-30 s	60.0 ab	12.5 b
16	150 ºC-1 min	64.0 ab	13.2 ab
17	150 ºC-2 min	64.0 a	12.1 bc
18	170 ºC-30 s	65.5 a	11.9 bc
19	170 ºC-1 min	55.0 bc	9.1 dc
20	170 ºC-2 min	48.3 c	6.3 de
21	Control	64.0 ab	13.8 de

Means in the same column followed by the same lowercase letter are not significantly different ($P < 0.05$).
Medias en la misma columna seguidas por la misma letra minúscula no son significativamente diferentes ($P < 0,05$).

The Duncan's test performed on the germination results (GP) of the *P. brutia* seeds revealed six treatment groups (table 4). The first group consists of the treatments of 75 ºC-10, 15, 20, 25, 30 min, 105 ºC-10, 15, 20, 25 min, 130 ºC-1, 3 min, 150 ºC-2 min, 170 ºC-30 s and the second group includes 105 ºC-30 min, 150 ºC-30 sec, 150 ºC-1 min, and the control. The other treatments (170 ºC-1 min, 170 ºC-2 min, 130 ºC-7 min, 130 ºC-10 min) were grouped individually. But at 150 ºC-1 min and 2 min, more than 64 % of germination was achieved. Besides, more than 55 % and 48 % of germination were also achieved at 170 ºC-1 min and 2 min, respectively (table 4).

DISCUSSION

The most effective disturbance in *P. brutia* forests in the Mediterranean ecosystem is natural or man-made fire. Studies point out that *P. brutia* forests are exposed to high-intensity and low-intensity fires almost every 25 years and every nine years, respectively, in Doyran-Antalya, Turkey (Neyişçi 1986, 1993).

In the present study, *P. brutia* seeds exposed to heat in an oven for 30 minutes at 105 ºC and two minutes at 150 ºC showed as high germination percentages as the control groups. Under different heat shocks, twelve out of twenty treatments showed better germination results compared to the control groups. Germination declined in three treatments (130 ºC-7, 10 min, and 170 ºC- 2 min). In one of these treatments (130 ºC-10 min), only 4 % of the seeds survived. Moreover, in two cases (170 ºC-1 min and 150 ºC-30 s), germination percentages were also lower than that of the control, but germination percentages were higher than 55 % and 60 %, respectively.

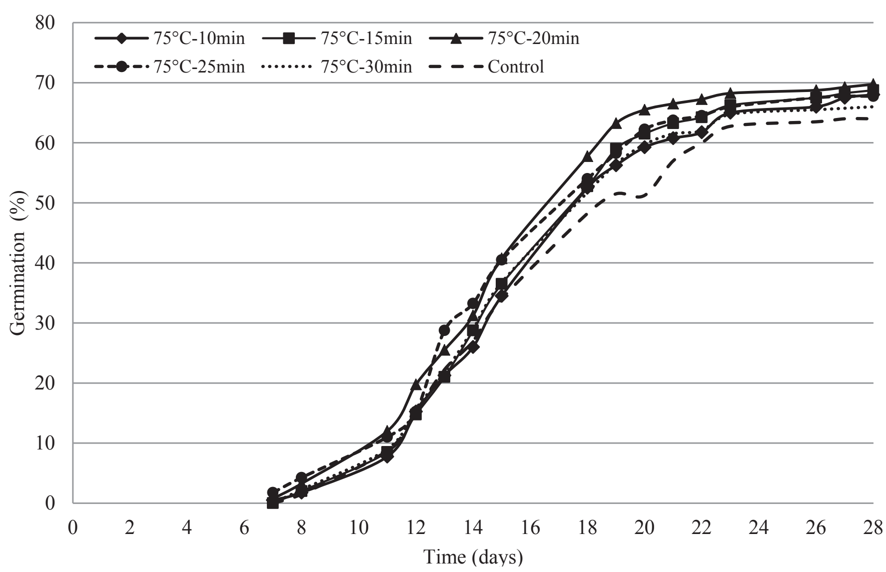

Figure 2. Dynamics of *Pinus brutia* seed germination after heat shock treatments at 75 ºC in different exposure times.

Dinámica de la germinación de las semillas de *Pinus brutia* después de los tratamientos de choque térmico, a temperatura 75 ºC a diferentes tiempos de exposición.

Similar to our results, heated seeds of *P. brutia* (125 ºC for 20 minutes and 150 ºC for 10 minutes) could maintain their viability. According to the results, there were no significant differences between 40 ºC and 110 ºC. Although a sharp decline was recorded in germination rapidly after 110ºC (Neyişçi and Cengiz 1985). Besides, higher germinations were obtained from the *P. brutia* seeds that were heated at 75 ºC (with 5, 10, 15 and 20 minutes) than from the control, and germination percentages were closer to the control at 100 ºC with the same exposure times (Cetin 2010).

On the other hand, in a *P. brutia* ecosystem, fire heat at 250 ºC on the soil surface decreases to 66 ºC, 46 ºC and 40 ºC

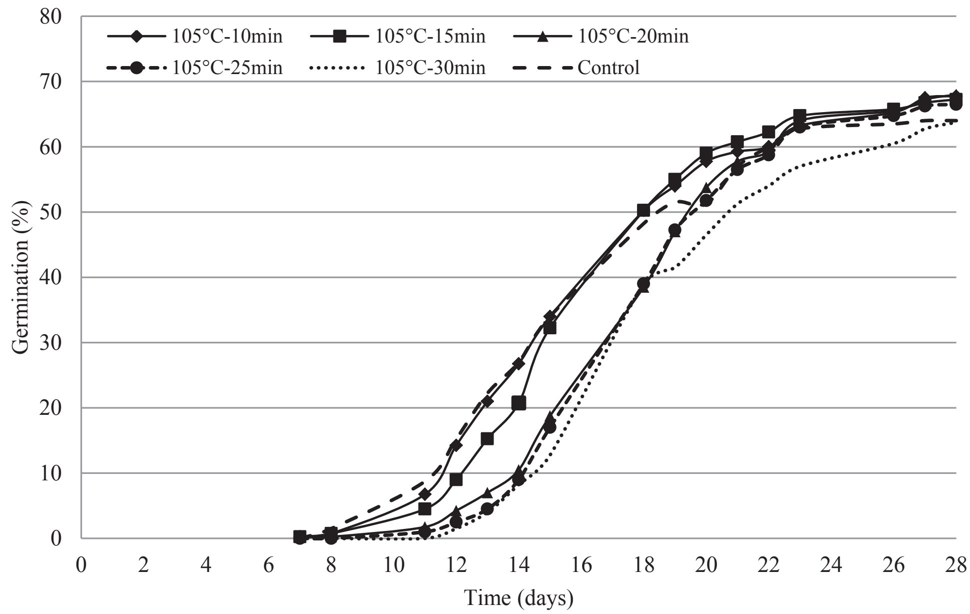

Figure 3. Dynamics of *Pinus brutia* seed germination after heat shock treatments at 105 ºC in different exposure times.

Dinámica de la germinación de las semillas de *Pinus brutia* después de los tratamientos de choque térmico, a temperatura 105 ºC a diferentes tiempos de exposición.

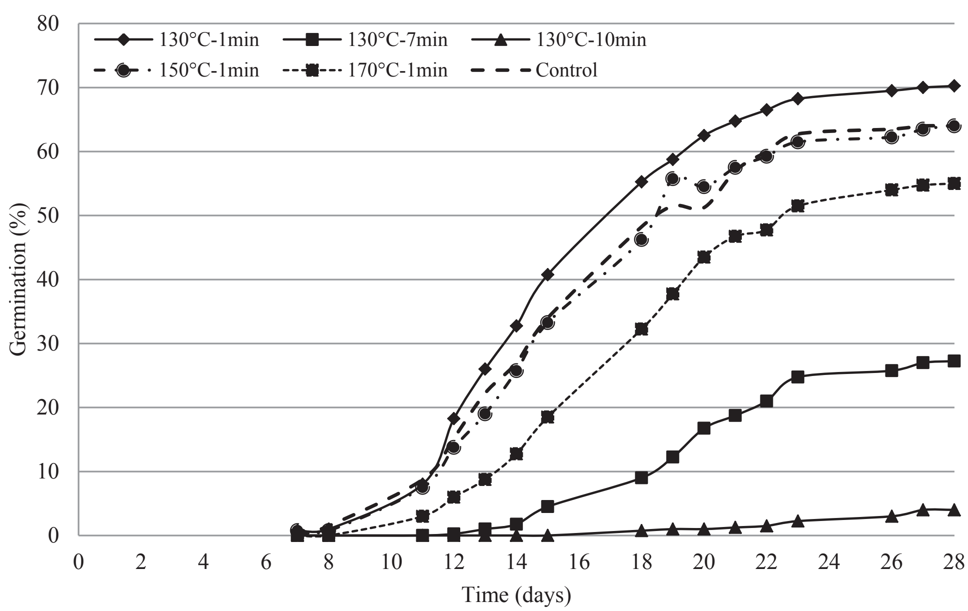

Figure 4. Dynamics of *Pinus brutia* seed germination after treatment of varying heats (130-150-170 ºC) and exposure times.

Dinámica de la germinación de las semillas de *Pinus brutia* después del tratamiento con diferentes grados de calor (130-150-170 ºC) y tiempos de exposición.

in the soil depths of 2.5 cm, 5 cm and 10 cm, respectively (Neyişçi 1989). Moreover, in *P. brutia* forests, post-fire regeneration was achieved by lying scorched cone bearing branches on soil (Eron and Sarıgül 1992). Seeds can remain viable if cones are exposed to a heat range of 367 to 632 ºC for a very short time (Neyişçi and Cengiz 1985). Wright (1931) explained that the oven heat between 65 ºC and 120 ºC could reach the embryo in four minutes. Seed coat thickness of *P. brutia* which constituted a significant fraction of seed weight was high (55.5 %) (Thanos 2000) and this also contributed to the preservation of the seed viability in *P. brutia*. After the fire in Samos Island (Greece) in 1983, those seeds which were collected from the surface of the burned soil were found to be highly viable (Thanos *et al.* 1989). Considering these findings we can conclude that some of the *P. brutia* seeds lying on soil, in surface soil layers and in the scorched cones may survive and contribute to post fire regeneration. Besides, *P. brutia* disperses seeds throughout the whole year (Ürgenç *et al.* 1989, Boydak 2004). This could also be accepted as another silvicultural advantage of the survival of *P. brutia* after fire in the Mediterranean ecosystem.

Thousands of hectares of even-aged old and pure *P. brutia* forests at different locations in the Mediterranean Region of Turkey could be considered as an indirect evidence for the establishment of these forests after fire in addition to the other evidences (Boydak *et al.* 2006).

Different effects of heat-shock on seed germination of other species have also been demonstrated. For example, Reyes and Trabaud (2009) stated that heat in moderate doses enhanced germination in *Cistus monspeliensis* L., *Melica ciliate* L., *Bituminaria bituminosa* L. C. H. Stirt., *Coronilla glauca* L., *Argyrolobium zanonii*, *Emerus major* Mill., *Spartium junceum* L. But high temperature (150 ºC) inhibited germination of all these species. Núñez and Calvo (2000) also observed that high temperature did not increase the germination in *Pinus halepensis* and *Pinus sylvestris*. Silveira and Overbeck (2013) tested the seeds of Fabaceae species and the seeds were exposed to different heat treatments (exposure to 60° and 80 °C for 5 minutes, to 100 °C for two minutes). The effect of heat-shock treatments (60, 80, 100, 120 and 140 °C for five min) on the germination of 21 herbaceous species in Central Anatolian steppe was evaluated and it is suggested that the seeds of plant species in Central Anatolian steppes are resistant to low-intensity surface fires (Tavsanoglu *et al*. 2015). Ribeiro *et al.* (2013) investigated the effects of heat shocks on seed germination of Brazilian savanna species and only one species presented accelerated germination due to heat treatments. Seeds of the dominant forest species in Chaco were subjected to different experimental heat shock treatments (Jaureguiberry and Díaz 2015). It was found that seed germination of the vast majority of the species studied tolerated low and medium temperature heat-shock treatments, suggesting that these seeds are able to survive moderate intensity fires.

CONCLUSIONS

In conclusion, the germination of the *P. brutia* seeds was not affected by low and moderate heat shock. Taking into consideration the above mentioned research results, we may conclude that in *P. brutia,* during the fires at certain degrees, most seeds in the cones and some seeds on the soil or in the scorched cones may continue to their viability and contribute to regeneration. Thus, under natural and/or man caused forest fire conditions, the abundance post-fire regeneration of *P. brutia* in the Mediterranean basin can be attributed to its seed characteristics either in closed cones (crown seed bank) and seeds on the soil or in the scorched cones which are not affected by low or moderate heat shock. Moreover, during the natural regeneration activities, application of low or moderate intensity prescribed fire can increase success of regeneration.

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