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Seed germination of three provenances of *Pinus brutia* (Ten.) as influenced by stratification, temperature and water stress

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Abstract: *Seeds from three provenances of Pinus brutia were stratified for 0 or 45 d (days) at 4±1 ºC and then germinated at 15 ºC or 20 ºC on filter paper saturated with polyethylene glycol solutions to provide water potentials of 0, -0.2 and -0.4 MPa (mega Pascal). Regardless of stratification, germination was lower at 15 ºC than 20 ºC for seeds of all provenances. Stratification significantly increased germination percentage at all water potentials regardless of the germination temperatures. Lowering the water potential to –0.4 MPa reduced germination for all three provenances in unstratified and stratified seeds when averaged for two temperatures, but reaction to the increased water stress was different among the provenances. Combined over two temperatures, stratified or unstratified seeds from the highest elevation (Cehennemdere) had the lowest germination performance at all water potentials, and stratified and nonstratified seeds from a coastal elevation (Silifke) had the highest germination parameters at the lowest water potential (-0.4 MPa). It might be concluded that seed germination and resistance to water stress vary according to provenance and stratification.*

Key words: *Dormancy, Pretreatment, Provenance, Seed germination, Water potential*

Introduction

Pinus brutia Ten. is well adapted to the Mediterranean type climate and occurs abundantly throughout the Eastern Mediterranean area (Quezel, 1979; Panetsos, 1981). It grows mainly from sea level up to 1400 m in the Mediterranean part of Turkey and sparsely distributed along the Black Sea coastal area from sea level up to 600 m. *P. brutia* usually grows in pure stands and occupies 3.6 million ha of forest land, which constitutes about 20% of the total forest areas in Turkey, and it is valuable for its timber products as well as for soil stabilization and wildlife habitats (Neyisci, 1987; Boydak, 2004). Within its altitudinal and horizontal distribution range, *P. brutia* exhibits considerable variation in various form and growth characteristics (Isik, 1986; Dangasuk and Panetsos, 2004). This tree can be used for afforestation of degraded areas because of its drought resistance. The use of appropriate seed sources for the specific areas affects reforestation success. Thus, the investigation of the adaptive mechanisms regarding seed germination and seedling establishment are of great importance to conservation and regeneration of the Mediterranean pine ecosystems.

Soil water supply is an important environmental factor controlling seed germination (Kramer and Kozlowsky, 1979). Seed germination depend on the extent of reduction in water potential and gets delayed if the water potential is reduced. One technique for studying the effects of water stress on germination is to stimulate stress conditions using artificial solutions to provide variable osmotic potentials (Larson and Shubert, 1969 ; Thanos and Skordilis, 1987 and Falleri, 1994). Investigations of the effect of water stress on seed germination of some tree seeds demonstrated that significant differences existed among seed sources with regard to germination ability under water stress (Falleri, 1994; Dirik, 2002; Tilki, 2005) and *P. brutia* provenances did not show homogeneity in their germination response towards temperature and water stress (Calamassi, 1982; Boydak *et al*., 2003).

Stratification, consisting of moist chilling for a few to several weeks, is a commonly used dormancy breaking treatment in temperate zone species, and the degree of dormancy may be expected to show some variation related to climate of origin (Bradbeer, 1988; Leadem, 1996; Tilki, 2004; Tilki and Cicek, 2005). *Pinus brutia* exhibit considerable seed dormancy and significant interactions were observed between latitude and stratification requirement in the seeds (Shafiq and Omer, 1969; Skordilis and Thanos, 1995; Thanos, 2000).

The present study investigates the effects of various factors on germination of the seeds from three provenances of *P. brutia*. These factors comprise temperature, stratification, and water stress.

Materials and Methods

Seeds of *P. brutia* were collected by the Forest Tree Seeds and Tree Breeding Research Directorate from three different altitudes in the East Mediterranean part of Turkey: Silifke (latitude 36° 13' N, longitude 33° 42' W, altitude 100 m), Gulnar (latitude 36° 14' N, longitude 33° 15' W and altitude 600 m) and Cehennemdere (latitude 37° 05' N, longitude 34° 33' W and altitude 1000 m). Seeds were stored at 4±1 ºC in darkness until used.

For cold moist stratification treatments seeds were soaked in water for 24 hr at room temperature and then mixed

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with moistened sand and were maintained at a temperature of 4±1 ºC in the dark for 45 day.

Germination tests were performed with 4 replicates of 50 seeds in glass petri dishes (diameter 11 cm) with two layers of filter paper, moistened with distilled water or polyethylene glycol solution. Water potential of the germination substrates (0, -0.2 and –0.4 MPa) were determined using polyethylene glycol (PEG–6000) solution, prepared as described by Michel and Kaufmann (1973). Filter papers and solutions were changed every 3rd day to keep the water potential steady during the germination period. Before the germination tests, damaged and insect infected seeds were discarded, and the empty ones were eliminated using the floated method (Boydak *et al*., 2003; Dangasuk and Ponetsos, 2004). Experiments were carried out in a temperature controlled chamber, where the temperature was kept constant within ±1ºC. Petri dishes were kept in an incubator at a 15 or 20 ºC with a 12 hr photoperiod and illuminance of 1000 lux.

Germination was counted every day for 28 days and classified as normal or abnormal according to the ISTA (1993) rules. The criterion for germination was radicle protrusion; seeds exhibiting abnormal germination were excluded from germination counts. Results were expressed as germination percentage (GP) and germination value (GV). GP was the percentage of seeds that had germinated at the end of the test. GV was computed as $GV=(\sum DGS/N) x GP x 10$, where DGS is the daily germination rate computes by dividing GP by the number of days since the beginning of the test, and N is the number of DGS that were calculated during the test (Djavanshir and Pourbeik, 1976).

The experimental design was a split to split plot with temperatures as the main plots, stratification treatments as the subplots, and provenances and water stress as the sub to sub plots. Data were subjected to analysis of variance procedures. Percentage data were analyzed following arcsine transformation, and mean separation tests were performed by least significant difference (LSD) procedures at $p<0.05$.

Results and Discussion

Stratification, temperature and water stress significantly affected seed germination of *P. brutia*, but response to these factors varied among the seeds of three provenances. Regardless of stratification, germination was lower at 15 ºC than 20 ºC for seeds of all provenances (Tables 1-3). The Cehennemdere provenance exhibited the lowest GPs and GVs at both temperatures and all water potentials, and the Silifke provenance had the highest germination parameters at 0 and -0.2 MPa at 15 ºC. At 20 ºC the Silifke and Gulnar provenances had the highest GP at 0 MPa. GPs of the Silifke provenance exceeded the Gulnar provenance when germinated at –0.2 and –0.4 MPa. GVs of the Silifke provenance were the highest at all water potentials, followed by the Gulnar provenance at 20 ºC.

Table - 1: Influence of stratification, temperature and water potential on germination percentages of *P. brutia* seeds combined over three provenances

Pretreatment	Water stress (MPa)		
	0	-0.2	-0.4
		15 ºC	
Unstratified	43.2a	28.6a	9.1a
Stratified	71.8b	66.5b	42.1a
		20 ºC	
Unstratified	72.3a	66.8a	59.0a
Stratified	81.4a	81.8b	80.2b

Mean separation in a row by LSD, $p<0.05$

Table - 2: Influence of water stress and provenances on germination percentages of *P. brutia* seeds combined over two stratification treatments

Provenances	Water stress (MPa)		
	0	-0.2	-0.4
		15 ºC	
Silifke	78.7a	68.7a	44.8a
Gülnar	63.2b	57.7b	37.2a
Cehennemdere	33.2c	17.5c	4.0b
		20 ºC	
Silifke	90.5a	93.0a	90.2a
Gülnar	85.2a	84.2b	77.5b
Cehennemdere	52.2b	44.7c	31.7c

Mean separation within columns by LSD, $p<0.05$

Table - 3: Influence of water stress and provenances on germination values of *P. brutia* seeds combined over stratification treatments

Provenances	Water stress (MPa)		
	0	-0.2	-0.4
		15 ºC	
Silifke	24.6a	16.2a	5.4a
Gulnar	18.3b	11.7b	4.0a
Cehennemdere	3.4c	1.1c	0.0b
		20 ºC	
Silifke	41.1a	36.1a	27.6a
Gulnar	34.8b	26.5b	18.6b
Cehennemdere	14.0c	8.2c	4.3c

Mean separation within columns by LSD, $p<0.05$

In all three provenances of *P. brutia* seeds investigated here, 20 ºC was clearly the optimal temperature, in agreement with the Mediterranean character of the species. At relatively low temperature of 15 ºC, low germination was obtained both in the present and in earlier studies (Skordilis and Thanos, 1995; Dirik *et al*., 1999).

Table - 4: Influence of water stress and stratification on germination percentages of *P. brutia* seeds combined over two germination temperatures

Pretreatment	Provenances								
	Silifke			Gulnar			Cehennemdere		
	Water potential (MPa)								
	0	-0.2	-0.4	0	-0.2	-0.4	0	-0.2	-0.4
Control	81.7a	73.2b	54.2c	63.0a	55.0b	38.7c	28.5a	15.0b	9.2c
Stratification	87.5a	88.5a	80.7b	85.5a	87.0a	76.0b	57.0a	47.2b	26.5c

Mean separation in a row by LSD, $p<0.05$

Table - 5: Influence of water stress and stratification on germination values of *P. brutia* seeds combined over two germination temperatures

Pretreatment	Provenances								
	Silifke			Gulnar			Cehennemdere		
	Water potential (MPa)								
	0	-0.2	-0.4	0	-0.2	-0.4	0	-0.2	-0.4
Control	29.5a	21.9b	8.4c	21.3a	8.9b	3.8c	4.9a	1.8b	1.0c
Stratification	36.2a	30.3b	24.5c	31.8a	29.4a	18.8b	12.4a	7.6b	3.3c

Mean separation in a row by LSD, $p<0.05$

When germination data were combined over all provenances, seed stratification was required to maximize germination at both temperatures and all water potentials (Table 1). Stratification also increased germination parameters significantly in each provenance at all water potentials averaged over two temperatures (Table 4). GP and GV were the lowest in stratified or nonstratified seeds of the Cehennemdere provenance (Tables 4 and 5).

Regardless of stratification, water potential significantly affected GPs under two temperatures combined over all provenances (Table 1). Germination of *P. brutia* seeds was the lowest at -0.4 MPa and did not exceed 10% for nonstratified seeds germinated under -0.4 MPa at 15 ºC and 60% at 20 ºC. Regardless of the germination temperature, stratification significantly increased GP at the lowest water stress as well as control and –0.2 MPa, and GP was the highest at 0 MPa at 15 ºC in stratified and nonstratified seeds but it was the highest in 0 and –0.2 MPa at 20 ºC. GPs were the lowest at –0.4 MPa regardless of stratification at both temperatures.

Stratification has been found to have a beneficial effect on subsequent germination performance in *P. brutia* seeds (Shafiq and Omer, 1969; Isik, 1986). The present results showed that 45 d of stratification significantly increased GP and GV of all provenances both at two temperatures and at three water potentials. Skordilis and Thanos (1995) found differences in the stratification requirements among the three *P. brutia* seed provenances from different regions of Greece. In the present study, germination parameters of stratified or nonstratified seeds showed significant differences among provenances, and the Cehennemdere provenance from the highest elevation had the lowest germination parameters at each water potential regardless of stratification when averaged over two temperatures.

Reaction to the increased water stress was different among provenances (Tables 4 and 5). Nonstratified seeds from the Cehennemdere provenance germinated at 0 MPa did not exceed 30% germination combined over temperatures. However, germination increased with stratification to 57%. But, germination of nonstratified Silifke seeds exceeds 80% at 0 MPa although stratification increased germination to 87.5%. In stratified and nonstratified seeds germination parameters were the highest in Silifke provenance and the lowest in Cehennemdere provenance at the lowest water stress (–0.4 MPa) when combined over temperatures.

Lowering water potential to –0.4 MPa reduced germination for all three provenances in nonstratifed seeds, but reaction to the increased water stress was different among the three provenances. This result agrees with the findings of Falusi and Calamassi (1982) and Boydak *et al.* (2003) that lowering water potential decreases germination parameters in *P. brutia*. Decreasing the water potential also reduced the germination in some other conifers: *Pinus ponderosa* (Djavanshir and Reid, 1975), *Pinus pinaster* (Falleri, 1994), *Cedrus libani* (Dirik, 2002) and *Pinus sylvestris* (Tilki, 2005). In the present study, in nonstratified seeds, the Silifke provenance ranked the highest at all water potentials when averaged over two temperatures, and in stratified seeds Silifke and Gülnar ranked the highest at all water potentials. The seeds from the highest elevation (Cehennemdere provenance) had the lowest germination parameters regardless of stratification at all water potentials. According to Isik (1986) stratified seeds of *P. brutia* from high elevation sources had lower germination in nursery than those from other elevations. A very low GP in seeds of Cehennemdere provenance may be an opportunistic adaptive strategy in high elevation population to take advantage of intermittent summer rains at high elevations. While in the coastal zones where summer droughts prevail, favorable conditions for seed germination are often lacking in summer. Provenance Cehennemdere from the highest elevation was also the most susceptible to high water stress and its GVs was the lowest at –0.4 MPa. This might be due to the higher precipitation in the high elevation than in the coastal zone. Boydak *et al.* (2003) found that provenances of *P. brutia* from drought areas had the higher GPs at low water potentials compared to the provenances from rainy areas. In the present research, greater germination in the coastal zone provenance (Silifke) regardless of stratification at osmotic potentials up to – 0.4 MPa could be related to its better adaptation to water deficits than the other provenances. Although stratification significantly increased germination parameters of stratified seeds of Cehennemdere provenance, germination was still low at all water potentials regardless of germination temperatures. Calamassi *et al*. (1980) found that resistance to drought appears to be loosely correlated to the geographic characters of the *P. brutia*; better correlation appears in several provenances with the climatic conditions, especially with the length of the summer drought. This intraspecific variation agrees with the experimental data reported here.

Seed germination and resistance to water stress appear to vary according to provenance and stratification, and deserve further study, particularly study of stratification duration and provenances representing its extensive geographic range including different elevations.

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