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Effects of Water Stress on Germination in Six Provenances of *Pinus brutia* Seeds from Different Bioclimatic Zones in Turkey

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Abstract: The effects of water potential on germination were studied in six provenances of *Pinus brutia* from different bioclimatic zones in Turkey. Water potentials between 0 and –8 bars were obtained using polyethylene glycol 6000 (PEG-6000) solutions. Seeds were kept for 28 d in light (12 h photoperiod at 1000 lux) and at 20 ± 0.5 °C. A decrease in water potential produced a marked reduction in germination percentage, germination speed and germination value. Significant variations between the provenances were found. The provenances of humid regions were the most susceptible to high moisture stress, and their germination value and germination percentage showed significant reductions at –6 bars. These differences in germination ability of provenances might be attributed to intraspecific variations resulting from the effects of natural selection.

Key Words: *Pinus brutia*, germination, polyethylene glycol, water potential

Türkiye'nin Farklı Biyoklimatik Zonlarından Altı Kızılçam (*Pinus brutia*) Orijinine Ait Tohumların Çimlenmesi Üzerine Su Stresinin Etkileri

Özet: Bu çalışmada, Türkiye'nin farklı biyo-iklim bölgelerine ait altı *Pinus brutia* orijininin çimlenmesi üzerine ozmotik stresin etkisi belirlenmeye çalışılmıştır. PEG-6000 eriyiği kullanılarak 0 ile -8 bar arasında stres düzeyleri oluşturulmuştur. Tohumların çimlendirilmesi 20 ± 0.5°C sıcaklıkta 12 saat ışık (1000 lüks) altında 28 gün esas alınarak yapılmıştır. Ozmotik potansiyelin azalması ile çimlenme yüzdesi, çimlenme hızı ve çimlenme değeri bütün orijinlerde önemli oranda azalmakla birlikte orijinlerin tutumu arasında önemli farklılıklar görülmektedir. Yağışlı bölgelerden elde edilen orijinler yüksek strese karşı en fazla duyarlılığı göstermekte, az yağışlı bölgeleri temsil eden orijinlere ait tohumlar ise özellikle yüksek stres derecesinde (-6 bar) daha fazla oranda çimlenebilmektedirler. Orijinlerin ozmotik strese karşı gösterdikleri bu farklılığın bir doğal seleksiyon etmeni olarak kuraklığın etkisiyle oluşmuş bir intraspesifik varyasyondan ortaya çıktığı ifade edilebilir.

Anahtar Sözcükler: Kızılçam, Çimlenme, Polietilen glikol, su potansiyeli

Introduction

Pinus brutia Tenore, Calabrian pine, is found largely in the Eastern Mediterranean region, and primarily in Turkey (85%) (Nahal, 1984). The natural distribution of *P. brutia* in Turkey indicates that it is well adapted to the Mediterranean-type climate; it is found in the thermomediterranean, mesomediterranean and submediterranean zones (Quezel, 1979).

P. brutia is one of the most commercially important forest species in Turkey, where it is dominant on about 3 million ha (Usta, 1990). It is a medium-lived, intolerant to moderately tolerant tree with rapid juvenile growth. It is fast growing and thus has a short rotation. This species can be managed as even-aged natural stands, or can be regenerated artificially and managed in plantations. Clearcutting with direct seeding is used on most sites in Turkey including natural fire affected areas (Boydak, 1993). Thus, the seed germination and survival of different provenances are of great importance for natural or artificial regeneration. In addition, the intraspecific variations of drought tolerance can be remarkable.

The climate over most of the *P. brutia* range is semi-humid with hot summers and mild winters. Average annual rainfall varies from 500 mm to 1500 mm (Atalay, 1993; Atalay et al. 1998).

A large number of studies have been carried out on the effects of water stress on the germination of forest tree species including *P. brutia*, and most species and

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provenances have shown a different sensitivity to water stress as regards germination and subsequent root growth (Bonner and Farmer, 1966; Barnett, 1969; Djavanshir and Reid, 1975; Kaufmann and Eckard, 1977; Calamassi et al., 1980; Falusi and Calamassi, 1982; Falusi et al., 1983; Dunalp and Barnett, 1984; Thanos and Skordillis, 1987; Falleri, 1994; Lopez et al., 2000). However, in none of the studies was the selection of provenances at the beginning of the research based on a bioclimatic classification.

Soil water supply is an important environmental factor controlling seed germination (Kramer and Kozlowski, 1979). If the water potential is reduced, seed germination will be delayed or prevented depending on the extent of its reduction (Hegarty, 1978). One technique for studying the effect of water stress on germination is to simulate stress conditions using artificial solutions to provide variable water potentials (Larson and Shubert, 1969; Sharma, 1973; Falusi et al., 1983). In the present work, the effects of water stress were examined in six provenances of *P. brutia* using PEG solutions with water potentials ranging from 0 to –8 bars. The purpose of this study was to evaluate the influence of water stress on germination and to determine whether there was a significant intraspecific variation in drought tolerance between provenances of *P. brutia* seeds from different bioclimatic zones.

Materials and Methods

Seeds were collected in 1998 from six natural stands of *P. brutia* by the Forest Trees and Seeds Improvement Division, Ministry of Forestry, Turkey. The seeds were extracted, cleaned and stored in a dark and cool place at 4 °C until used in 2000.

The climatic conditions prevailing in each of the six natural stands were considered when selecting locations in Turkey (Figure 1 and Table 1). Thus, the Emberger bioclimatic classification method developed for Turkey by Akman and Daget (1971) was used (Figure 2).

Before the germination tests, damaged and insect-infected seeds were discarded, and the empty ones were eliminated using the floating method in distilled water. The water potential of the germination substrates (0, -2, -4, -6 and -8 bars) was determined using PEG-6000 solution, prepared as described by Michel and Kaufman (1973).

Germination tests were performed in 11-cm-diameter glass petri dishes on two layers of filter paper saturated

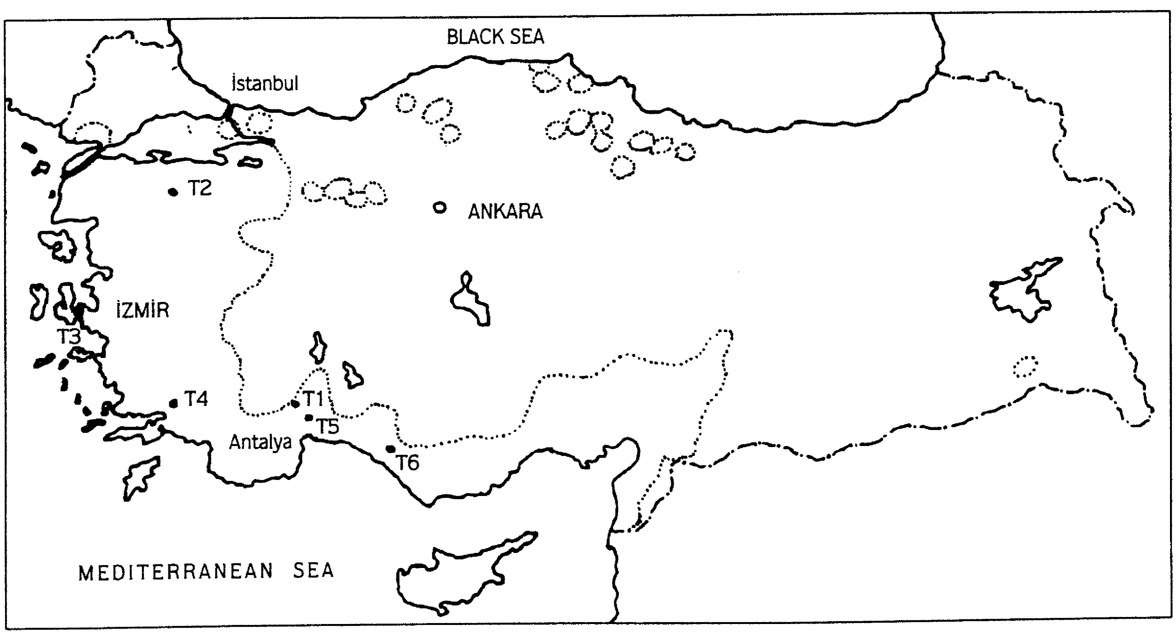

Figure 1. Location of *P. brutia* provenances studied: T1 (Bucak), T2 (Mustafa Kemalpaşa), T3 (Urla), T4 (Yaras), T5 (Duzlercami) and T6 (Kargi) (adapted from Saatçioğlu, 1976).

Table 1. List of *P. brutia* provenances.

Provenances	Latitude (North)	Longitude	Altitude (m)	Mean precip. (mm)	Mean temp. (°C)	Precip.-temp. index (Q)	Bioclimatic zone
T1 Bucak	37° 24'	30° 37' E	800	712	14.1	74.7	Low rain-cold
T2 M. Kemalpasa	39° 58'	28° 40' E	250	702	14.6	86.3	Low rain-cool
T3 Urla	38° 14'	26° 36' E	150	695	17.6	87.5	Low rain-warm
T4 Yaras	37° 06'	28° 32' E	750	1209	15.0	133.5	High rain-cool
T5 Duzlercamı	36° 59'	30° 33' E	275	1089	18.7	135.7	High rain-warm
T6 Kargı	36° 36'	31° 57' E	350	1080	18.1	155.8	High rain-hot

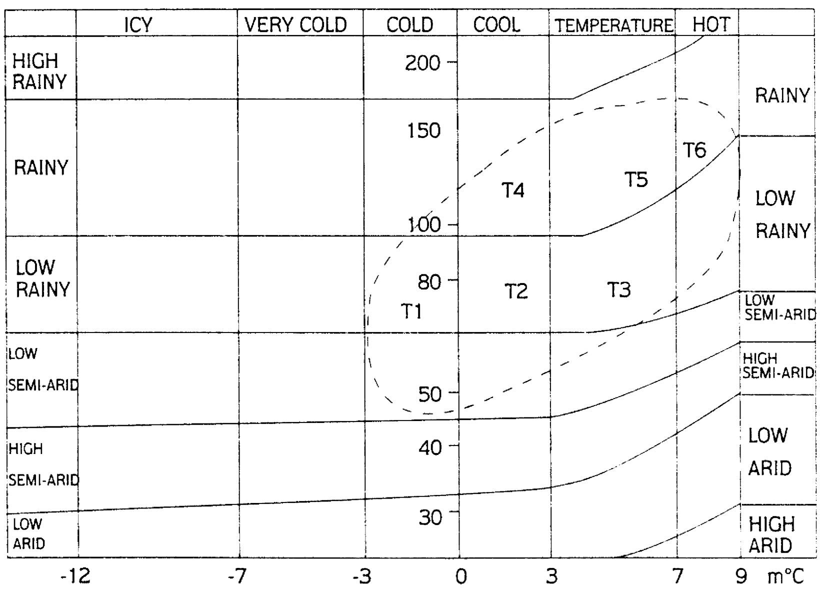

Figure 2. Location of *P. brutia* provenances on Emberger's Bioclimatic Diagram. Dotted lines show the natural distribution of *P. brutia* in Turkey (adapted from Akman and Daged, 1971).

with water solutions. Four 50-seed replicates for each lot and for each experimental condition were used, for a total of 120 replicates (4 replicates [each has 50 seeds]* 5 water potential levels *6 provenances = 120). Filter papers and solutions were changed every 3 d in order to keep the water potential steady during the whole test period. Experiments were carried out in a temperature-controlled growth chamber at 20 ± 0.5 °C with a photoperiod of 12 h and illuminance of 1000 lux.

Germination counts were performed daily for 28 d and germination was considered to have occurred if the radicle protruded 2 mm from the seed coat. Seeds with abnormal radicles were excluded from the germination counts (Edwards and Wang, 1995). Cumulative germination percentage, (GP%) was evaluated daily and the final value was obtained after 28 d. Then all cumulative germination percentages of the provenances at every stress level were transformed to relative cumulative germination percentages by considering the control germinations (0 bars) to be 100. These percentages were transformed to arc sin $P^{1/2}$ for statistical analysis. The results were analysed by factorial analysis and the means were compared with Duncan's test. Mean germination time (MGT) and germination value (GV), the parameters of germination speed, were also calculated for suplementary explanations. MGT, day was computed as follows: $MGT = \Sigma n_i d_i / n$, where n is the total number of germinated seeds during the germination test, n_i is the number of germinated seeds on day d_i and i is the number of days during the germination period (between 0 and 28 days) (Yousheng and Sziklai, 1985). In the present study, GV, day was calculated by the formula of Djavanshir and Pourbeik (1976) because it is believed to give a more reliable estimate of subsequent survival for the genus *Pinus*. Thus, GV was computed as follows: GV = (ΣDGS/N) x GP x 10, where DGS is daily germination speed, which is computed by dividing the cumulative germination percentage by the number of days since the beginning of the test. N is the number of DGS calculated during the test.

Results

Analysis of variance showed highly significant differences among both provenances and water potentials. The interaction between provenances and water stress was not significant (Table 2).

Although lowering the water potential to -8 bars reduced germination for all provenances by more than 50%, the reaction to increased water stress differed among the six provenances: T1 seems to be the most tolerant, with a threshold between -6 and -8 bars, and T5 the least tolerant, with a tolerance threshold between -4 and -6 bars. Considerable tolerance was also observed in T2, with a threshold between -4 and -6 bars (Table 3).

According to Duncan's test, T1 and T2 were combined in an independent group as the most drought resistant provenances. T2 was also included in another group with T4 and T6, while T6 with T3 were in another different group. T5 was the least drought tolerant provenance. On the other hand, there were no significant differences between 0, -2 and –4 bar stress levels according to the germination percentages of different provenances, while –6 and –8 bar stress levels differed separately (Table 4).

Table 2. Results from the ANOVA on relative cumulative percentage germination.

Source of variation	df	MS	F	P
Provenances (A)	5	60.80	4.79	0.001
Water potentials (B)	4	1067.48	84.11	0.001
Replicate (C)	3	48.21	3.80	0.05
Interaction A x B	20	16.42	1.29	
Interaction A x C	15	13.87	1.09	
Interaction B x C	12	11.45	0.90	
Residual	60	12.69		
Total	119			

Table 3. Effects of water potential on cumulative germination percentage of *Pinus brutia* seeds from six provenances.

Provenances	Water potential (bars)					
	0	-2	-4	-6	-8	Average
T1	74.0	96.0	75.0	70.0	31.0	69.2
T2	91.0	90.5	92.0	66.5	40.5	76.1
T3	90.5	93.0	79.0	47.5	32.0	68.4
T4	62.5	66.5	66.5	41.5	12.0	49.8
T5	94.5	91.0	84.5	46.0	15.0	66.2
T6	93.5	95.5	86.5	61.5	20.5	71.5
Average	84.3	88.7	80.6	55.5	25.2	

Table 4. Effects of water potential on relative cumulative germination percentage of *Pinus brutia* seeds from six provenances.

Provenances	Water potential (bars)					
	0	-2	-4	-6	-8	Average
T1	100	129.7	101.3	94.6	41.9	93.5 a[1]
T2	100	99.5	101.1	73.1	44.5	83.6 ab
T4	100	106.4	106.4	66.4	19.2	80.0 b
T6	100	100.5	95.1	67.6	22.5	77.0 bc
T3	100	102.8	87.3	52.5	34.8	75.5 bc
T5	100	90.5	86.8	48.4	15.8	68.3 c
Average[2]	100 a	104.9 a	96.3 a	67.1 b	29.8 c	

1 Values in the column with the same initial(s) are not significantly different ($P < 0.001$).

2 Values in the row with the same capital initial are not significantly different ($P < 0.001$).

Relative cumulative percentage germination was significantly reduced between –4 and –6 bars for all provenances (Table 4). Germination of three provenances (T4, T5 and T6) from humid regions decreased from 100% to around 20%, while the other provenances (T1, T2 and T3) continued to germinate more than 34% at the lowest water potential (-8 bars).

MGT differed in the six provenances and was markedly influenced by water potential (Table 5). Even a stress of -2 bars caused a significant increase in MGT (decrease in germination rate) in five provenances (T2, T3, T4, T5 and T6), but in T1 the tolerance threshold was between -4 and -6 bars. Provenance T4 was the most susceptible to high moisture stress since its germination rate was the lowest (highest MGT values), -8 bars.

Table 5. Effects of water potential on mean germination time (MGT, days) of seeds from six provenances of *Pinus brutia*. See Materials and Methods for calculation of MGT.

Provenances	Water potential (bars)					
	0	-2	-4	-6	-8	Average
T1	16.58	15.45	16.58	21.88	23.23	18.74
T2	10.18	11.66	14.64	18.94	21.86	15.46
T3	9.92	11.67	14.69	20.04	23.61	15.98
T4	12.81	15.74	17.60	21.09	30.25	19.50
T5	11.78	16.24	17.21	21.70	23.86	18.16
T6	12.75	14.88	17.34	22.46	24.12	18.31
Average	12.74	14.27	16.34	21.02	24.49	

Table 6. Effects of water potential on germination value (GV, %) of seeds from six provenances of *Pinus brutia*. See Materials and Methods for calculation of GV.

Provenances	Water potential (bars)					
	0	-2	-4	-6	-8	Average
T1	21.99	30.20	16.32	9.06	1.86	15.89
T2	41.44	37.97	28.54	12.03	3.77	24.75
T3	40.37	38.92	23.87	5.06	1.84	22.01
T4	15.72	14.36	11.37	3.07	0.30	8.96
T5	37.71	24.81	18.17	3.71	0.40	16.96
T6	33.81	31.26	21.25	6.63	0.56	18.70
Average	31.84	29.59	19.92	6.59	1.45	

GVs of all provenances were affected at -4 bars (Table 6). Each further increment in water stress produced additional significant decreases in GVs. The GVs for T1, T2 and T3 were higher than those for the other provenances at the lowest water potential (-8 bars), and the lowest tolerance again occurred in T4, T5 and T6.

Discussion

The seeds of *P. brutia* from the six regions used in this work germinate under water stress without any prechilling treatment. Decreasing the water potential in the substrate decreased germination, indicating that water stress inhibits germination.

In this study, germination percentage was adversely affected when moisture stress reached –6 bars, while it was reduced at –8 bars by more than half. This result agrees well with the findings of Falusi and Calamassi (1982), who reported that a decrease in germination capacity in *P. brutia* seed occurred at –4 bars. Similar trends have also been observed in some other conifers; lowering the water potential to –8 bars reduced the germination of *P. pinaster* (Falleri, 1994), *Pinus contorta* and *Picea engelmanii* by approximately 50% (Kaufman and Eckard, 1977). Falusi et al. (1983) observed in *Pinus halepensis* that a reduction of the water potential of the germination substrate even to –2 bars lowers germination percentages considerably, while at –8 bars germination was lowered to approximately 25%. Djavanshir and Reid (1975) reported that a marked reduction of germination capacity occurred at –4 bars in *Pinus ponderosa* seeds. Similar trends were also reported for the seeds of *Pinus palustris* and *Pinus elliotti* (Barnett, 1969). In contrast, Thanos and Skordilis (1987) reported that *P. brutia* seeds exhibited water inhibition of germination at values lower than –10 bars, and the absolute values of the water potential required for 50% inhibition of germination in *P. halepensis* were between -14.6 and -19.5 bars at both 15 °C and 20 °C.

The results of this research support the idea that *P. brutia* is well adapted to the Mediterranean-type climate and is a drought-resistance species with respect to several physiological characteristics (Abido, 1983; Grunwald and Schiller, 1988; Dirik, 1994). The drought resistance of *P. brutia* has already been shown in the germination phase.

Drought is a major obstacle to the regeneration of *P. brutia* throughout its natural range. At low elevations, a portion of the seeds dispersed during the dry summer and early autumn germinate after autumn precipitation although the soil does not have enough moisture content. Autumn germination is important in natural regeneration and in broadcast seeding in the thermomediterranean zone (Boydak, 1993). These characteristics of *P. brutia* might be attributed to the fact that the cumulative germination percentage was not adversely affected when moisture stress reached –6 bars; lowering the water potential to –4 bars even increased the germination percentage in some provenances (Table 3). However, GV and MGT were not affected in the same way. In another study, water priming with aerated solutions of polyethylene glycol improved both final germination and the speed of germination in *P. brutia* (Dirik et al., 1999).

The response of germination to water stress differed among the six provenances. This intraspecific variation agrees with the experimental data reported previously for *P. brutia* (Calamassi et al., 1980; Falusi and Calamassi, 1982), *Pinus elderica* and *P. halepensis* (Calamassi et al., 1980; Falusi et al., 1983; Thanos and Skordilis, 1987), *P. taeda* (Dunalp and Barnett, 1984) and *Cedrus libani* (Dirik, 2000).

According to the results, T1 and T2 were the most stress-tolerant provenances on a water stress gradient. T4 and T6 followed these provenances. However, when we take into account the lowest water stress level (-8 bars), T1, T2 and T3, which have precipitation-temperature indexes (Q) below 100, have higher germination capacities than the other seed provenances. In addition, the relative germination capacities of T1 and T2 were highest at –6 and –8 bars. Climatic differences among the provenances might account for this behaviour; i.e. the higher amount of rain in the area of T4, T5 and T6 and the lower coefficient of precipitation-temperature index (Q) of T1, T2 and T3.

GVs decreased with decreasing water potential, an observation consistent with studies on *P. elderica* (Djavanshir and Reid, 1975), *P. halepensis* (Falusi et al., 1983), and *P. pinaster* (Falleri, 1994). As with germination percentages, the GVs for T1, T2 and T3 were higher than those for the other provenances at the lowest moisture stress (-8 bars).

MGT differed among the six provenances, and the differences were markedly increased with decreasing water potential. Even moderate water stresses (-2 bars) caused an increase in MGT. Especially at lower values of water potential, the germination rate significantly decreased. Moderate water stress seems to have a stronger effect on germination capacity than higher water stress. These results are consistent with those of other studies on the genus *Pinus* (Falusi and Calamassi, 1982; Falusi et al., 1983; Falleri, 1994) and *Liquidambar* (Bonner and Farmer, 1966). However, at the lowest bars (-8 bars), there were no significant differences among the provenances in terms of germination rate.

The higher germination percentage and value in T1, T2 and T3 (Q < 100) at the lowest water potential (-8 bars) may be related to their better adaptation to water deficits compared to the other three provenances (Q > 100). Furthermore, differences under water stress also show that provenances are characterised by a significantly different tolerance to drought.

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