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Effects Of Drought Stress on Germination in Fourteen Provenances of *Pinus Brutia* Ten. Seeds in Turkey

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A B S T R A C T

Pinus brutia Ten., Red pine, known to be tough drought resistant pine specie, could effectively be used for afforestation of disturbed areas. It is of great interest for the afforestation in arid zones. Appropriate seed sources for the specific areas guarantees reforestation success. Away from its native areas *Pinus brutia* Ten. is planted for its ornamental value and timber production purposes. Selection of drought resistant provenances can very well increase the survival success. In this study, the effects of water potential on germination were studied in fourteen provenances of *Pinus brutia* Ten. from Turkey. Water potentials between 0 and -8 bars were obtained using polyethylene glycol 6000 (PEG-6000) solutions. Seeds were kept for 35 day at 20 ± 0.5°C. A decrease in water potential produced a marked reduction in germination percentage and germination value. As a result, significant variations between the provenances were found. It was determined that, under a -8 bar water stress, Isparta-Bucak and Mersin-Silifke, respectively corresponding to 58% and 57% of the control group, were the least water stress affected provenances.

Introduction

The plants used in planting studies in rural-arid areas, where ecological problems are plenty can be protected from drought effects by the addition of organic materials, watering and consistent care. The plants suffer later wither because these applications cannot be performed in many areas, consequently planting works fail because no good growth can be attained. Recently, water matter and aridity have started becoming a big threat for the plants of problematic areas due to global warming and significant air temperature increases in the summer. One of the solutions of these problems is to use plants surviving on minute amounts of water (Pulatkan and Var, 2010). Plant to be utilized in landscape projects are expected to withstand drought, this objective is the most important criteria affecting the plants selection in such studies (Yılmaz and Yılmaz, 2009).

Utilization of natural plant species in landscape projects require effective watering as well as plant medicine use and fertilization, so maintenance costs can drastically be reduced. Furthermore, the natural species acclimatizing the environmental conditions can endure plant diseases and pests better, so along with economical benefits, ecological benefits in terms of environment and nature protection can be acquired. Besides, consumer demands in recent years are turning to the selection of natural species (Atik and Karagüzel, 2007). Thus, such species must be prioritized in landscape projects.

Pinus brutia Ten. is well adapted to the Mediterranean type climate and spreads 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 (Tilki and Dirik, 2007).*Pinus brutia Ten* 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; it is valuable for its timber products as well as for soil stabilization and wildlife habitats (Neyisci, 1987; Boydak, 2004; Tilki and Dirik, 2007).

Pinus brutia Ten. is also well suited to landscape projects. It is extensively used in school yards (Şişman and Gültürk, 2011), in parks (Ekici and Sarıbaş, 2006; Acar et al., 2007), in amelioration works of abandoned mining sites (Akpınar, 2005) and is recommended in such projects.

Pinus brutia Ten. 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 (Tilki and Dirik, 2007).

Away from its native areas, *Pinus brutia Ten.* is planted for ornament and timber in the Mediterranean south to Israel, west to Spain and east to Pakistan, and in Australia, New Zealand, South Africa, California and Arizona. It is of great interest for the afforestation of arid zones (Frankis, 2004).

That the endurance of the very same specie towards drought differs drastically among provenances is indicated in many studies. In order to increase the success of afforestation in dry regions, it is very imperative to use the most drought resistant provenances of such species.

Various methods are utilized to determine the drought tolerance of different species and provenances, one of which is to apply different concentrations of PEG on seeds. (Afzali et al., 2006; Hamayun et al., 2010; Shafeeq, et al., 2006; Ahmad et al., 2009; Mujtaba et al., 2007). Polyethylene (PEG) induced water stress has successfully been used to screen drought tolerance in many plant species (Raziuddin et al., 2010).

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; Boydak et al., 2003)

In this study, the effects of water stress were examined in 14 provenances of *Pinus brutia* Ten. 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 *Pinus brutia* Ten. seeds from different provenances.

Materials and Methods

Seeds were collected from 14 natural stands of *Pinus brutia* Ten. 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. The locations of seed stands are shown in Table 1.

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 with water solutions. Four 50 seed replicates for each lot and for each experimental condition were used, culminating to a total of 280 replicates (4 replicates [each has 50 seeds]* 5 water potential levels *14 provenances = 280). Filter papers and solutions were changed every 3 day 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. Germination counts were performed daily for 35 days and germination was considered to have occurred if a radicle protruded 2 mm from the seed coat (Boydak et al., 2003). Seeds with abnormal radicles were excluded from the germination counts.

Cumulative (germinated seed/total germination percentage) germination percentage, (GP%) was evaluated daily and the final value was obtained after 35 days. 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. Data were subjected to multi-way analysis of variance and Duncan test.

Mean Germination values (GV) were also 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 = (SDGS/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 (Boydak et al., 2003).

Table 1 The locations of seed stands

Pop. No	Region	Local Region	Altitude (m)
1	Muğla	Marmaris	60
2	Mersin	Silifke	100
3	Mersin	Bozyazı	250
4	Isparta	Bucak	350
5	K.Maraş	Antakya	385
6	Mersin	Anamur	500
7	Mersin	Bozyazı	500
8	Mersin	Gülnar	650
9	Antalya	Gündoğmuş	650
10	Isparta	Sütçüler	650
11	Adana	Pos	735
12	Denizli	Acıpayam	850
13	Mersin	Erdemli	900
14	Mersin	Anamur	925

Results

Analysis of variance showed highly significant differences among both provenances and water potentials (Table 2).

According to Table 2, there were significant differences among provenances and applications for all characters (Germination Percentage, Relative Germination Percentage and Germination Value). Average values and results of Duncan test for applications and provenances are shown in Table 3 for germination percentage.

Table 2 Results of variance analysis

		Aplications					Provenance				
		SS	df	MS	F	Sig.	SS	df	MS	F	Sig.
GP	Between Groups	45252	4	11313	77,324	.000	27865	13	2144	9,895	,000
	Within Groups	40235	275	146			57621	266	217		
	Total	85486	279				85486	279			
GP (Rel.)	Between Groups	129915	4	32479	248,873	.000	18835	13	1449	2,622	,002
	Within Groups	35888	275	131			146968	266	553		
	Total	165802	279				165803	279			
GV	Between Groups	13514	4	3379	133,424	.000	3528	13	271	4,260	,000
	Within Groups	6964	275	25			16950	266	64		
	Total	20478	279				20478	279			

SS= Sum of Squares, MS= Mean Square

Table 3 Effects of water potential on germination percentage of *Pinus brutia* Ten. seeds from 14 provenances.

Provenance	Water potential (bars)					
	0	2	4	6	8	Average
1	44	42.5	35	30.5	18.5	34.1 ef
2	80	65.5	60	50.5	45.5	60.3 ab
3	68	53	48.5	40	33	48.5 cd
4	76	80	59	48.5	44	61.5 a
5	49	36.5	24.5	16	11.5	27.5 f
6	67	36.5	34	25.5	19.5	36.5 ef
7	54	42	42	32.5	22	38.5 e
8	64	53	37.5	29	24.5	41.6 de
9	58	41	38.5	34	9	36.1 ef
10	76	56.5	53.5	47	26	51.8 bc
11	44	38.5	36.5	25	16.5	32.1 ef
12	45	40	36	24.5	21	33.3 ef
13	62	45.5	35	24.5	21	37.6 ef
14	52	63.5	47.5	26	18.5	41.5 de
Average	59.9 a	49.6 b	42 c	32.4 d	23.6 e	

As followed through Table 3, the germination percentage is decreased with the increasing water stress and it, being around 59.9% in control group, decreases to 23.6% at a -8 bar water stress. As for the provenance point of view, it was observed that number “4” had the highest germination percentage of 61.5%, while number “5” had the least germination percentage of 27.5%. However, as opposed to average germination percentage, the purpose of the study is to determine the hardiest drought resistant provenance; the amount of water stress related germination reduction becomes important. When the control group is taken into account as 100%, the values attained in germination percentage and the results of Duncan test applied to such values can be followed through Table 4.

Based on the findings of Table 4, water germination percentage reduces depending upon the stress level and at around -8 bar water stress, it gets down to about 38.7% of the total germination percentage. As for provenances, the most intensive reduction occurred in the provenance, “9”. In this provenance also, at around -8 bar water stress, it gets down to about 16% of the total germination percentage; the provenances, “4” and “6” follow this one with 23% and 29%, respectively.

Under a -8 bar water stress, Isparta-Bucak, provenance “4”, and Mersin-Silifke, provenance “2”, respectively corresponding to 58% and 57% of the control group, were the least water stress affected provenances. Although under a -2 bar water stress, in provenances “4” and “14”, the germination percentage registered higher compared to that of the control group, this is thought to be an exception. In general, the more the water stress increases, the less the germination percentage gets.

The effects of water potential on germination value (GV, %) of seeds from 14 provenances of *Pinus brutia* Ten. are shown in Table 5.

As examined through Table 5, on the basis of water stress, while the germination value is about 21.6; as the water stress increases, this particular value reduces and, at around -6 bar water stress, gets down to 3.7, and at around -8 bar water stress, gets further down to 2.3. According to the results of Duncan test, while the levels of -6 and -8 bar are placed in the same homogenous group, the rest is placed in different groups. On the other hand, when the provenances are taken into account, the provenance “4”, being 34.7 in control group and dropping to 15.7 at -8 bar and the provenance “2” being 35.7 in control group and dropping to 15.2 at -8 bar, could be regarded as the most water stress tolerant provenances. Besides, the provenance “9”, being 22.4 in GV control group and dropping to 0.25 at -8 bar and the provenance “5” being 12.5 in control group and dropping to 0.45 at -8 bar, are determined as the least water stress tolerant provenances.

Discussion

Drought tolerance screening related to Polyethylene (PEG) induced water stress has successfully been performed in many agricultural plant species such as *Matricaria chamomilla* (Afzali et al., 2006), Soybean (Hamayun et al., 2010), *Helianthus annuus* (Ahmad et al., 2009), *Triticum aestivum* (Raziuddin et al., 2010; Mujtaba et al., 2007; Shafeeq, et al., 2006), sugarcane (Errabi et al., 2006; Errabi et al., 2007), rice (Ahmad et al., 2007; Liu et al., 2007; Lefèvre et al., 2001), cowpea (Costa et al., 2007), alfalfa (Safarnejad, 2008), lentil (Yupsanis et al., 2001), maize (Ashraf et al., 2007) and halophyte species i.e. *Sevium portulacastrum* (Slama et al., 2007), *Cantaurea ragusina* (Radić et al., 2005; Radić et al., 2006), *Suaeda salsa* and *Kalanchoe claigremontiana* (Kefu et al., 2003).

In this study, the seeds of *Pinus brutia* Ten. from 14 regions were germinated under water stress. Decreasing the water potential in the substrate decreased germination, indicating that water stress inhibits germination. Germination percentage was adversely affected when moisture stress increased, while it was reduced at -8 bars by more than half. This result agrees well with the findings of Boydak et al., (2003) and Falusi and Calamassi (1982). Similar trends have also been observed in some other conifer species; lowering the water potential to -8 bars reduced the germination of *Pinus nigra* (Buyurukcu, 2011), *Pinus pinaster* (Falleri, 1994), *Pinus contorta* and *Picea engelmanii* by approximately 50% (Kaufman & 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%.

In contrast, Thanos and Skordilis (1987) reported that *Pinus brutia* Ten. 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 *Pinus 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 *Pinus brutia* Ten. is well adapted to the Mediterranean-type climate and is a drought-resistance species with respect to several physiological characteristics (Boydak et al., 2003).

In another study, water priming with aerated solutions of polyethylene glycol improved both final germination and the speed of germination in *Pinus brutia* Ten. (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 for *Pinus brutia* Ten. (Calamassi et al., 1980; Falusi and Calamassi, 1982), *Pinus nigra* (Buyurukcu, 2011; Calikoglu, 2002), *Pinus elderica* and *Pinus halepensis* (Calamassi et al., 1980; Falusi et al., 1983; Thanos and Skordilis, 1987), *Pinus sylvestris* (Tilki, 2005), *Pinus taeda* (Dunalp and Barnett, 1984) and *Pinus ponderosa* (Djavanshir and Reid, 1975).

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Table 4 Effects of water potential on relative cumulative germination percentage of *Pinus brutia* Ten. seeds from 14 provenances.

Provenance	Water potential (bars)					
	0	2	4	6	8	Average
1	100	97	80	69	42	77.6 ab
2	100	82	75	63	57	75.4 ab
3	100	78	71	59	49	71.4 abc
4	100	105	78	64	58	81.0 a
5	100	74	50	33	23	56.0 c
6	100	54	51	38	29	54.4 c
7	100	78	78	60	41	71.4 abc
8	100	83	59	45	38	65.0 abc
9	100	71	66	59	16	62.4 bc
10	100	74	70	62	34	68.0 abc
11	100	88	83	57	38	73.2 ab
12	100	89	80	54	47	74.0 ab
13	100	73	56	40	34	60.6 bc
14	100	122	91	50	36	79.8 a
Average	100 a	83.4 b	70.6 c	53.8 d	38.7 e	

Table 5 Effects of water potential on germination value (GV, %) of seeds from 14 provenances of *Pinus brutia* Ten.

Provenance	Water potential (bars)					
	0	2	4	6	8	Average
1	10.5	4.55	3.8	2.6	0.9	4.5 de
2	35.7	12.95	11.4	7.55	7.15	15.2 ab
3	26.6	8.1	6.95	4.35	3.85	10.0 bcd
4	34.7	19.6	9.85	7	7.15	15.7 a
5	12.5	3.8	1.9	0.8	0.45	3.9 e
6	28.5	3.35	3.25	1.6	1.05	7.6 cde
7	16.8	4.95	7.2	3.3	2.25	6.9 cde
8	27.9	8.65	5	3.05	1.35	9.2 cde
9	22.4	5.2	4	3.35	0.25	7.0 cde
10	32.3	8.1	7.9	7.45	2.3	11.6 abc
11	11.2	4.25	4.15	2.55	1.05	4.6 de
12	11.7	10.8	4.3	2.3	2.05	6.2 cde
13	19.9	5.25	2.7	1.85	1.2	6.2 cde
14	12	13.8	8.25	3.75	1.45	7.9 cde
Average	21.6 a	8.1 b	5.8 c	3.7 d	2.3 d	

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