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EFFECTS OF WATER STRESS ON GERMINATION OF *PINUS NIGRA* ARNOLD. SEEDS

OSMAN TOPACOGLU[1*], HAKAN SEVIK[2] AND EROL AKKUZU[3]

[1] *Department of Silviculture, Faculty of Forestry, Kastamonu University, 37150, Kastamonu, Turkey*
[2] *Department of Environmental Engineering, Kastamonu University, Faculty of Engineering and Architecture, 37150, Kastamonu, Turkey*
[3] *Department of Forest Entomology and Conservation, Kastamonu University, Faculty of Forestry, Kastamonu, Turkey*
[*] *Corresponding author's email: otopacoglu@kastamonu.edu.tr*

Abstract

Climate change, global warming and the deterioration of related environmental conditions cause an important problem for forest tree species. For this reason, it is necessary to determine the response of trees to these conditions. The objective of this study was to investigate the effects of water stress on seed germination of fifteen *Pinus nigra* Arnold. provenances in Turkey. For this purpose, the water stresses between 0 and -8.0 bars were obtained using polyethylene glycol-6000 (PEG) solutions. Seeds were kept for 35 days at 20 ± 0.5°C. In this study, significant variations between the provenances were found. Ankara Uluhan (% 95, 08) and Isparta Tota (% 85, 41) provenances at -8.0 bars having the highest cumulative germination percentages were the most resistant provenances against the water stress. This study has shown that the water stress reduced the germination speed, germination percentage and germination value.

Key words: Black pine, *Pinus nigra* Arnold., Water stress, PEG.

Introduction

Plants exposed to environmental stress causes is an adverse effect on plants that prevents the normal functions of a biological system (Naz *et al*., 2015). Due to the increase of environmental stress factors and climatic problems over the last 100 years, degradation in natural ecosystems has reached to significant proportions As a result of this situation, serious crop losses, yield and quality reduction have occurred in agriculture and forestry (Guo *et al.*, 2012).

Climate change, global warming and related environmental degradation are mentioned as a major problem in forest tree species. Thus, determination of forest tree species response to changing conditions becomes points of interest (Perdiguero *et al.*, 2012). Plants have their own mechanisms to survive against various biotic and abiotic stress factors. However, these abilities can be insufficient due to rapidly changing ecological conditions, and especially sensitive species can face with the threat of extinction (Gou *et al.*, 2012).

Black pine (*Pinus nigra* Arnold.) is the most important species which can be spread to the steppe regions in Turkey. Away from its native areas Black pine is planted for its ornamental value and timber production purposes. It is extensively used in school yards (Kelkit, 2002; Ekici & Saribas, 2006; Gul *et al*., 2006; Sisman & Gulturk**,** 2011), university campus areas (Yilmaz & Kirzoglu, 1997; Ertekin & Corbaci, 2010), urban afforestation areas (Saribas & Kapuci, 2001; Kuter & Erdogan, 2010), amelioration works of abandoned mining sites (Akpinar, 2005; Cindik & Acar, 2010) and is recommended in such projects.

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 & Var, 2010). 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 (Atik & Karaguzel, 2007). 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 (Yilmaz & Yilmaz, 2009).

In this study, the effects of water stress were examined on 15 provenances of *P. nigra* using polyethylene glycol-6000 (PEG) solutions with water stresses ranging from 0 to -8.0 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 of *P. nigra* seeds from different provenances.

Materials and Methods

Study area and methods: Seeds were collected from 15 natural stands of *P. nigra* 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.

The water stress levels of the germination substrates (0, -2.0, -4.0, -6.0 and -8.0 bars) were determined using PEG solution, prepared as described by Michel and Kaufman (1973) and Boydak *et al.* (2003). For each water stress level, PEG solutions were prepared using 104.98 gr/lt at -2.0 bars, 164.30 gr/lt at -4.0 bars, 209.45 gr/lt at -6.0 bars, and 247.40 gr/lt at -8.0 bars.

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 300 replicates (4 replicates [each has 50 seeds]x 5 water stress levels x15 provenances = 300). Filter papers and solutions were changed every 3 day in order to keep the water stress 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 28 days and germination was considered to have occurred if a radicle protruded 2mm from the seed coat (Boydak *et al.*, 2003). Seeds with abnormal radicles were excluded from the germination counts.

Statistical analysis: Germination speed (GS) was calculated after 7 days. Germination percentage (GP) and cumulative germination percentage (CGP) were evaluated daily and the final value was obtained after 35 days. Then all CGP of the provenances at every water stress level were transformed to CGP 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 calculated by the formula of Djavanshir & Pourbeik (1976), to give 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 GS, which is computed by dividing CGP 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).

Results

Analysis of variance showed highly significant differences among both provenances (Table 2) and water stresses (Table 3). According to Table 2 and Table 3, there were significant differences among provenances and applications for all characters (GS, GP, GV, and CGP).

Average values and results of Duncan test for applications are shown in Table 4 for germination speed. As followed through Table 4, GS is decreased with increasing water stress and it, being around 64.73% in control group, decreases to 37.21% at a -8.0 bars water stress. As for the provenance point of view, it was observed that the number-9 (Uluhan provenance) had the highest GP of 80.93%, while the number-2 (Kalkim provenance) had the least GP of 13.80%.

Table 1. The locations of seed stands.

Pop. No.	Region	District	Unit	Coordinates	Altitude (m)
1	Canakkale	Yenice	Asar	39°51'N - 27°14'E	270
2	Canakkale	Kalkim	Kalkim	39°48'N - 27°12'E	550
3	Canakkale	Bayramic	Karakoy	39°50'N - 26°53'E	850
4	Bursa	Bursa	Bursa	40°10'N - 28°55'E	950
5	Kutahya	Tavsanli	Alabarda	39°53'N - 29°26'E	1050
6	Balikesir	Alacam	Golcuk	39°49'N - 28°55'E	1050
7	Kutahya	Simav	Kicir	39°14'N - 28°42'E	1100
8	Bursa	Inegol	Bogazova	39°97'N - 29°43'E	1200
9	Ankara	Nallihan	Uluhan	40°32'N - 31°26'E	1250
10	Bursa	Keles	Sorgun	39°53'N - 29°19'E	1350
11	Balikesir	Bigadic	Aktuzla	39°36'N - 28°56'E	1378
12	Kutahya	Domanic	Derecarsamba	39°80'N - 29°45'E	1400
13	Kutahya	Tavsanli	Balikoy	39°30'N - 29°05'E	1500
14	Denizli	Cal	Inceler	37°43'N - 29°34'E	1560
15	Isparta	Sutculer	Tota	37°52'N - 31°23'E	1600

Table 2. Summary of one-way ANOVAs showing the seed germination speed (GS), germination percentage (GP), germination value (GV), and cumulative germination percentage (CGP) of populations.

		Sum of squares	df	Mean square	F	Sig.
GS	Between groups	66768.214	14	4769.158	19.664	.000
	Within groups	32741.148	135	242.527		
	Total	99509.362	149			
GP	Between groups	80845.285	14	5774.663	43.309	.000
	Within groups	18000.470	135	133.337		
	Total	98845.755	149			
GV	Between groups	70620.166	14	5044.298	12.603	.000
	Within groups	54034.341	135	400.254		
	Total	124654.506	149			
CGP	Between groups	44277.684	14	3162.692	12.070	.000
	Within groups	74678.049	285	262.028		
	Total	118955.733	299			

Table 3. Summary of one-way ANOVAs showing the effect of water stress on seed germination speed (GS), germination percentage (GP), germination value (GV), and cumulative germination percentage (CGP).

		Sum of squares	df	Mean square	F	Sig.
GS	Between groups	10334.840	4	2583.710	4.201	.003
	Within groups	89174.521	145	614.997		
	Total	99509.362	149			
GP	Between groups	8653.001	4	2163.250	3.478	.010
	Within groups	90192.754	145	622.019		
	Total	98845.755	149			
GV	Between groups	12552.708	4	3138.177	4.059	.004
	Within groups	112101.798	145	773.116		
	Total	124654.506	149			
CGP	Between groups	35683.840	4	8920.960	31.603	.000
	Within groups	83271.893	295	282.278		
	Total	118955.733	299			

Table 4. Effects of water stress on germination speed of *Pinus nigra* seeds.

Pop. No.	Alt. (m)	Control	-2.0 Bar	-4.0 Bar	-6.0 Bar	-8.0 Bar	Average
1	270	41.29	23.10	23.27	19.54	18.67	25.17b
2	550	18.26	19.02	12.4	10.09	9.25	13.80a
3	850	54.90	27.02	14.93	15.56	7.16	23.91b
4	950	62.05	44.89	36.57	34.66	31.94	42.02c
5	1050	75.16	69.99	60.23	53.44	37.32	59.23d
6	1050	80.01	74.58	67.46	64.97	40.57	65.52d
7	1100	60.39	55.44	48.84	45.05	34.52	48.85c
8	1200	81.92	71.03	65.47	62.25	53.13	66.76d
9	1250	84.13	80.44	82.97	84.87	72.25	80.93e
10	1350	85.66	82.38	79.6	70.42	67.19	77.05de
11	1378	31.97	33.18	19.88	17.64	12.23	22.98ab
12	1400	76.37	73.68	70.81	57.25	54.09	66.44de
13	1500	46.81	30.49	22.51	19.78	12.65	26.45b
14	1560	88.77	80.81	76.76	70.17	53.19	73.94e
15	1600	83.20	82.37	75.74	79.22	54.00	74.91e
Average		**64.73**	**56.56**	**50.5**	**46.99**	**37.21**	**51.2**

Values with different lowercase letters within a column are significantly different (Duncan's Multiple Comparison Test, p<0.05)

Average values and results of Duncan test for applications are shown in Table 5 for GP. According to Table 5, GP is decreased with the increasing water stress and it, being around 77.65% in control group, decreases to 54.83% at a -8.0 bars water stress. As for the provenance point of view, it was observed that the number-9 (Uluhan provenance) had the highest GP of 93.42%, while the number-2 (Kalkim provenance) had the least GP of 23.76%. However, as opposed to average GP, the purpose of the study was 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 GP and the results of Duncan test applied to such values can be followed through Table 6. Based on the findings of Table 6, water GP reduces depending upon the water stress level and at around -8.0 bars water stress level, it gets down to about 66.98% of the total GP. As for provenances, the most intensive reduction occurred in the provenance -3. In this provenance also, at around -8.0 bars water stress, it gets down to about 16.29% of the total GP; the provenances, "2 (Kalkim)" and "13 (Balikoy)" follow this one with 46.94% and 50.39%, respectively. Under a -8.0 bars water stress, Uluhan provenance (9), and Tota provenance (15), respectively corresponding to 95.08 % and 85.41% of the control group, were the least water stress affected provenances. According to Table 6 and Fig. 1, the more the water stress increases, the less the GP gets.

Effects of water stress on GV (%) of seeds from 15 provenances of *P. nigra* are shown in Table 7. As examined through Table 7, on the basis of water stress, while the GV is about 54.42; as the water stress increases, this particular value reduces and, at around -6.0 bars water stress, gets down to 37.91, and at around -8.0 bar water stress, gets further down to 26.99. The Tota provenance (15), being 54.42 in control group and dropping to 40.2 at -8.0 bars and the Alabarda provenance (5) being 55.26 in control group and dropping to 45.35 at -8.0 bars, could be regarded as the most water stress tolerant provenances. Besides, the Karakoy provenance (3), being 31.5 in GV control group and dropping to 5.25 at -8.0 bars and the Bursa provenance (4) being 46.7 in control group and dropping to 10.8 at -8.0 bar, are determined as the least water stress tolerant provenances.

Table 5. Effects of water stress on germination percentage (GP) of *Pinus nigra* seeds.

Pop. No.	Alt. (m)	Control	-2.0 Bar	-4.0 Bar	-6.0 Bar	-8.0 Bar	Average
1	270	52.28	35.76	28.55	27.75	26.46	34.16b
2	550	31.91	25.46	23.93	22.5	14.98	23.76a
3	850	72.55	46.82	37.69	32.23	11.82	40.22b
4	950	73.81	59.1	57.06	48.38	47.32	57.13c
5	1050	84.78	81.28	77.39	61.17	59.30	72.78d
6	1050	97.71	96.83	95.56	96.11	78.84	93.01gh
7	1100	82	66.28	64.41	62.66	54.47	65.96d
8	1200	94.68	90.05	88.23	80.61	79.62	86.64efgh
9	1250	96.74	93.82	92.98	91.58	91.98	93.42h
10	1350	96.74	94.9	90.93	87.28	81.78	90.33fgh
11	1378	53.38	45.79	29.17	28.83	27.78	36.99b
12	1400	89.76	84.7	84.32	83.76	71.81	82.87ef
13	1500	59.58	53.18	56.53	51.4	30.02	50.14c
14	1560	89.84	84.86	85.96	74.53	70.22	81.08 e
15	1600	88.98	88.91	88.25	85.91	76.00	85.61 efg
Average		**77.65**	**69.85**	**66.73**	**62.31**	**54.83**	**66.27**

Values with different lowercase letters within a column are significantly different (Duncan's Multiple Comparison Test, $p<0.05$)

Table 6. Effects of water stress on cumulative germination percentage (CGP) of *Pinus nigra* seeds.

Pop. No.	Alt. (m)	Control	-2.0 Bar	-4.0 Bar	-6.0 Bar	-8.0 Bar	Average
1	270	100a	68.40de	54.61d	53.08de	50.61de	65.34fg
2	550	100a	79.79abc	74.99c	70.51cd	46.94e	74.45def
3	850	100a	64.53e	51.95d	44.42e	16.29f	55.44g
4	950	100a	80.07bcd	77.31c	65.55cd	64.11cde	77.41ed
5	1050	100a	95.87ab	91.28ab	72.15c	69.95bcd	85.85abcd
6	1050	100a	99.10a	97.80a	98.36a	80.69abc	95.19ab
7	1100	100a	80.83cde	78.55bc	76.41bc	66.43bcd	80.44cd
8	1200	100a	95.11ab	93.19ab	85.14abc	84.09ab	91.51abc
9	1250	100a	96.98a	96.11a	94.67a	95.08a	96.57a
10	1350	100a	98.10a	93.99a	90.22ab	84.54ab	93.37ab
11	1378	100a	85.78abc	54.65d	54.01de	52.04de	69.30ef
12	1400	100a	94.36ab	93.94a	93.32ab	80.00abc	92.32ab
13	1500	100a	89.26abc	94.88a	86.27abc	50.39de	84.16bcd
14	1560	100a	94.46ab	95.68a	82.96abc	78.16abc	90.25abc
15	1600	100a	99.92a	99.18a	96.55a	85.41ab	96.21a
Average		**100 a**	**88.17b**	**83.21bc**	**77.57c**	**66.98d**	

Values with different lowercase letters within a column are significantly different (Duncan's Multiple Comparison Test, $p<0.05$)

Table 7. Effects of water stress on germination value (GV) of *Pinus nigra* seeds.

Pop. No.	Alt. (m)	Control	-2.0 Bar	-4.0 Bar	-6.0 Bar	-8.0 Bar	Average
1	270	28.3	20.4	12.65	13.65	8.01	16.6ab
2	550	15.25	13.25	6.40	7.60	4.25	9.35a
3	850	31.50	18.75	12.45	12.70	5.25	16.13ab
4	950	46.7	36.2	26.95	23.50	10.80	28.83b
5	1050	55.26	52.55	51.70	46.50	45.35	50.27c
6	1050	98.75	83.75	78.90	73.90	48.75	76.81e
7	1100	74.30	68.30	56.45	49.45	31.00	55.9cd
8	1200	66.85	51.60	43.10	31.20	22.20	42.99c
9	1250	90.30	88.05	81.30	70.45	69.65	79.95e
10	1350	62.55	46.55	45.20	38.50	40.05	46.57c
11	1378	21.05	17.70	17.55	10.45	4.85	14.32a
12	1400	58.60	55.60	50.40	50.30	36.75	50.33c
13	1500	39.20	33.45	26.05	28.85	10.80	27.67b
14	1560	78.80	76.40	75.95	65.15	27.00	64.66d
15	1600	48.85	49.20	47.100	46.50	40.20	46.37c
Average			**54.42**	**47.45**	**42.14**	**37.91**	**26.99**

Values with different lowercase letters within a column are significantly different (Duncan's Multiple Comparison Test, $p<0.05$)

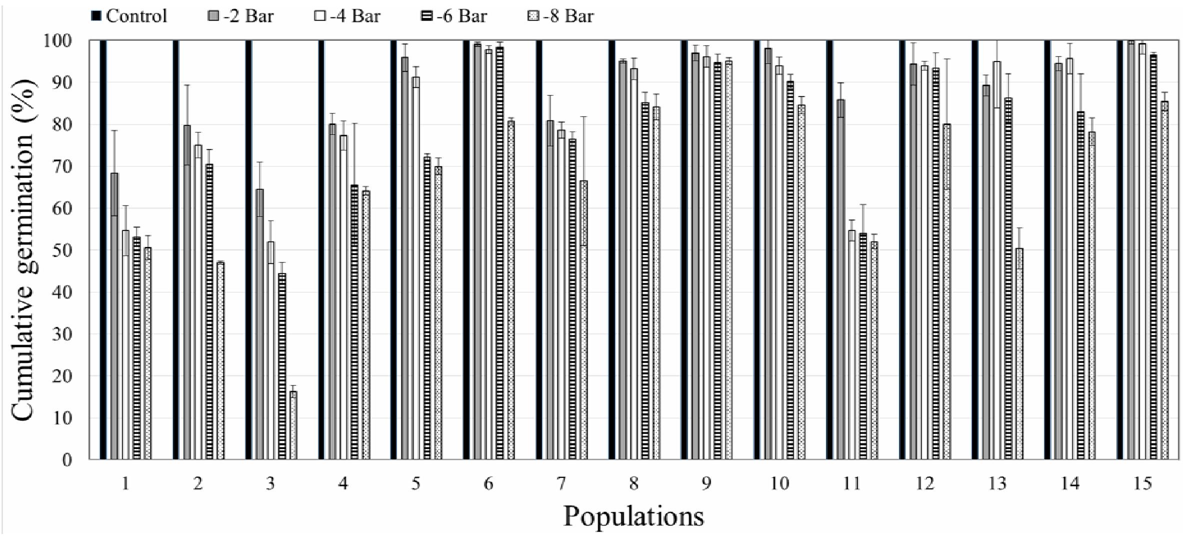

Fig. 1. Effects of water stress on cumulative germination percentage (CGP) of *Pinus nigra* seeds.

Discussion

Trees in their habitat live under extreme conditions on account of many stress factors, such as nutrient deficiencies, drought, dry climate and air pollution (Makineci & Sevgi, 2006). 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 & Var, 2010).

Some parameters were used for determination to influence of water stress on plants such as photosynthesis, chlorophyll fluorescence, PEG, Fv/Fm transpiration, CO_2, photosynthetic pigments, total carbohydrates and proline. These parameters were used on *Picea asperata* (Yang *et al.*, 2007), *Pinus sylvestris* (Sudachkova *et al.*, 2009; Kulac, 2010), *P. canariensis* (López *et al.*, 2010), *Quercus ilex*, *Q. coccifera*, *Pinus halepensis* and *Juniperus phoenicea* (Baquedano & Castillo, 2007).

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), *Solanum lycopersicum* (George *et al.*, 2015), *Triticum aestivum* (Shafeeq *et al.*, 2006; Mujtaba *et al.*, 2007; Raziuddin *et al.*, 2010), sugarcane (Errabii *et al.*, 2006; Errabii *et al.*, 2007), rice (Lefèvre *et al.*, 2001; Ahmad *et al.*, 2007; Liu *et al.*, 2007;), 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.*, 2006), *Suaeda salsa* and *Kalanchoe claigremontiana* (Kefu *et al.*, 2003).

In this study, the seeds of *P. nigra* from 15 regions were germinated under water stress. Decreasing the water stress in the substrate decreased germination, indicating that water stress inhibits germination. GP was adversely affected when moisture stress increased, while it was reduced at -8.0 bars by about 30%. This result agrees well with the findings of Boydak *et al.* (2003), Falusi & Calamassi (1982), Dirik (2000), Calikoglu (2002), and Tilki (2005). Similar trends have also been observed in some other conifer species; lowering the water stress to -8.0 bars reduced the germination of *Pinus nigra* (Buyurukcu, 2011), *P. pinaster* (Falleri, 1994), *P. contorta* and *Picea engelmanii* by approximately 50% (Kaufmann & Eckard 1977). Falusi *et al.* (1983) observed in *P. halepensis* that a reduction of the water potential of the germination substrate even to -2.0 bars lowers GP considerably, while at -8.0 bars germination was lowered to approximately 25%. Thanos & Skordilis (1987) reported that *Pinus brutia* seeds exhibited water inhibition of germination at values lower than -10.0 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 (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 *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 for *Pinus nigra* (Calikoglu, 2002; Buyurukcu, 2011), *P. elderica* and *P. halepensis* (Falusi *et al.*, 1983; Thanos & Skordillis, 1987), *P. sylvestris* (Tilki, 2005), *P. brutia* (Sevik & Erturk, 2015), *P. taeda* (Dunalp & Barnett, 1984).

Black pine one of the most widely distributed species of the Mediterranean basin, particularly in southern Europe and Anatolia. It occurs naturally as small clusters in Algeria and Morocco (Kaya & Temerit, 1993). It occurs throughout Turkey except the northeast Black Sea region and covers the second largest area of all native commercial forest tree species of Turkey (Yaltirik & Efe, 2000). Meanwhile, the stands of this species occupy roughly 4.2 million ha, of which about 1.8 million ha are considered to be nonproductive forest. Thus, Anatolian black pine is a high priority species in the National Forest Tree Breeding Programme in the country. Additionally Black pine is the most important species which can be spread to the steppe regions in Anatolia. Away from its native areas *P. nigra* is planted for its ornamental value and timber production purposes. It is extensively used in landscape projects. But water stress is a big threat in these areas. One of the solutions of this problem is to use plants surviving on minute amounts of water. According to results of this study, especially Uluhan (9), Tota (15), Sorgun (10), Bogazova (8), Golcuk (6) and D.Carsamba (12) provenance can be used in arid areas.

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