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Research Article

## Examining Seed Germination Rate and Seedlings Gas Exchange Performances of Some Turkish Red Pine Provenances Under Water Stress

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### ABSTRACT

With climate change, global warming has increased adverse effects on living things in our country. In these adverse effects, water scarcity is the most crucial problem due to the increase in temperature and decrease in precipitation. Forests are the most affected ecosystem among others by water scarcity in our country. This study tried to determine the 5-year-old seeds and 1-year-old seedlings (produced from the same seeds) of some Turkish red pine provenance' responses to different water stress levels. First, how the water stress levels (0, -0.2, -0.4, -0.6, -0.8 MPa) affect seed germination of these provenances was determined. Secondly, gas exchange parameters [net photosynthesis (*Anet*), stomatal conductance (*gs*), transpiration (*E*), and intrinsic water use efficiency (*iWUE*)] under different water stress were determined in the seedlings obtained from these species' seeds. As a result of the germination test, Denizli-Çameli (DC) provenance had the highest rate (48%), while Maraş-Suçatı had the lowest rate (29%) under control treatment. The highest germination rate was obtained in Burdur/Bucak provenance (5%) under -0.2 MPa osmotic potential. For gas exchange parameters, Antalya/Gündoğmuş provenance had the highest *Anet*, *gs* values while DC provenance had the lowest *Anet*, *gs*, and *E* values when provenance is considered as a single factor. Besides, increasing in irrigation increased *Anet*, *gs*, and *E* while decreased the *iWUE*. The lowest seedling *E* under water stress can be explained because this species responds to the water shortage by closing its stomata. Among the Turkish red pine origins, DC provenance showed higher drought tolerance than others.

**Keywords:** Gas exchange, Germination, PEG, Turkish red pine, Water stress

## Su Stresi Altındaki Bazı Kızılçam Kaynaklarının Tohum Çimlenme Oranı ve Fidelerin Gaz Değişim Performanslarının İncelenmesi

### ÖZET

İklim değişikliğiyle beraber küresel ısınmanın ülkemizdeki canlılar üzerindeki olumsuz etkileri artmıştır. Bu olumsuz etkilerden su kıtlığı, sıcaklık artışı ve yağışların azalmasıyla birlikte en önemli sorun olarak ortaya çıkmıştır. Ülkemizde su kıtlığından en çok etkilenen ormanlar ekosistemleridir. Bu çalışmada, bazı Kızılçam orijinlerinin 5 yaşındaki tohumları ve 1 yaşındaki fidelerinin (aynı tohumlardan üretilmiş) farklı su stresi seviyelerine tepkilerinin belirlenmesine çalışılmıştır. İlk olarak, su stresi seviyelerinin (0, -0.2, -0.4, -0.6, -0.8 MPa) bu tohum kaynaklarının çimlenmesini nasıl etkilediği belirlendi. Daha sonra, bu türlerin tohumlarından elde edilen fidelerin su stresi altında gaz değişim parametreleri [net fotosentez (*Anet*), stoma iletkenliği (*gs*), terleme (*E*), ve içsel su kullanma verimliliği (*iWUE*)] belirlenmiştir. Kontrol işleminde altındaki çimlenme testi sonucunda, en yüksek çimlenme oranı Denizli/Çameli (DC) orijininde (%48) belirlenirken, Maraş/Suçatı orijini en düşük orana (%29) sahiptir. -0.2 MPa ozmotik su gerilimi altında en yüksek çimlenme oranı Burdur/Bucak orijininde tespit edilmiştir. Orijin tek değişken olarak ele alınıp fidelerin gaz değişim parametreleri incelendiğinde ise en yüksek

*Anet* ve *gs* Antalya/Gündoğmuş orijininde belirlenirken en düşük *Anet*, *gs* ve *E* ise DC orijininde tespit edilmiştir. Ayrıca sulamanın artması *Anet*, *gs* ve *E*'yi artırırken *iWUE*'yi düşürmüştür. Türlerin en düşük *E* değerine sahip olması onların su stresine karşı somatalarını kapatarak tepki vermesiyle açıklanabilir. Kızılcam orijinleri arasında DC orijini diğerlerinden kuraklığa toleransı daha yüksektir.

**Anahtar Kelimeler:** *Gaz değişimi, Çimlenme, PEG, Kızılcam, Su stresi*

## **I. INTRODUCTION**

Turkish red pine (*Pinus brutia* Ten.) naturally occurs in a wide range of the Eastern Mediterranean region, from sea level to 1600 meters altitude and covering roughly 5.6 million ha of natural forest areas in Turkey [1,2]. Turkish red pine is a light-demanding, fast-growing, and drought-tolerant conifer species due to a deep tap-rooting system [3], and approximately 52.5 million seedlings of Turkish red pine were produced across the country in 2015 [4]. This species is the most critical forest tree species in Turkish forestry owing to its ability to grow in unfavorable environments such as calcareous, dry, and poor soil conditions [4,5]. Also, this species is widely used for various purposes such as afforestation, plantation, and recreation due to a high ecologically and economically crucial roles in arid and semi-arid areas in Turkey [1,6,7].

Water is the most vital compound in all terrestrial plants for their growth, development, and physiological process. The physiological processes, such as photosynthesis and carbon assimilation, are directly linked to the xylem water transportation efficiency for plants [8]. The amount of water used to produce a unit of biomass or fixed carbon unit during photosynthesis is called water use efficiency [9]. It is a key trait that indicates the physiological adaptation of plants to deal with limited water reserves. The proportion of stomatal conductance and photosynthesis reflects intrinsic water use efficiency in the leaf-level via gas exchange measurement [10]. This trait is a useful metric for investigating the link between plants' growth and development, especially in arid and semi-arid environments. Because the atmosphere is usually dry, resulting in loss of water from plant leaves via evaporation. Also, plants receive less precipitation due to global warming, and increasing in temperature progresses the evapotranspiration in the Mediterranean region recently [11].

The Mediterranean region is considered an arid and semi-arid area, is one of the risky areas in the temperate zone due to global warming. According to Intergovernmental Panel on Climate Change (IPCC) [12], climate projections for future scenarios, the region will become hotter and drier with more numerous and severe climatic events. With the future global climate change scenarios, the temperature may increase 2.5 °C and 5.4 °C by 2050 and 2100, respectively [12]. Thus, water stress has become one of the major problems limiting woody plants' seed germination, growth, and development in a warmer region. Understanding how critical physiological processes in conifer seed and seedlings associated with water – particularly seed germination, leaf photosynthesis, and respiration – respond to increasing temperature crucial for afforestation programs in warmer regions.

Environmental stresses have an increased effect on trees, such as water and salt stress in arid and semi-arid regions, reducing tree growth and development due to global warming [13]. Water stress has gained much attention in recent years that its effects on all stages of tress from germination to mature stage. One of the main and or maybe the most critical process is germination because this process provides some vital information on the species' strategy while fighting against the drought (avoidance, tolerance) [14,15]. Drought stress has substantial adverse effects on seed germination and early seedling growth [16]. Suitable conditions (water or moisture, oxygen, temperature) are the most important factors for seed germination and growth [17].

Seed germination is the most susceptible stage in plant growth and development and seedling under various environmental conditions [18]. The germination process can be separated into three stages in terms of sequential steps: inhibition, initiation of radicle growth, and radicle emergence. A decrease in

water potential and drought prevents water absorption, which is needed to start the germination process [19]. Moisture condition plays a crucial role during germination in terms of regulation [20] and the process of photosynthesis [21,22]. As mentioned earlier, water scarcity is one of the most critical factors that limit tree growth, development, and physiological activities in tree cells. Hence, achieving tree growth in arid and semi-arid regions depends on seeds' ability to germinate [23] and maintain photosynthesis balance under lack of soil moisture.

Polyethylene Glycol-6000 (PEG), one of the most common solutions, is applied to the seeds in different concentrations. Although PEG is incapable of entering the seed coat due to its high molecular mass, it is an osmotically active but physiologically inactive substance [23]. Molecules of PEG are too large enough not to be captivated by plants, but it is tiny enough to stimulate the osmotic potential [24]. This method has been used in many species to examine the effect of water stress on germination, and it has given successful outcomes [25,26].

To quantify needle gas exchange parameters, including net photosynthesis, stomatal conductance, and transpiration rate, the LI-6400XT portable photosynthesis system (LI-COR Biosciences, Lincoln, NE, USA) has been widely used last decades. This system is a closed system that controls CO<sub>2</sub>, IRGA, light intensity, and relative humidity, which provides rapid simultaneous and robust response and eliminates time delays. Gas exchange parameters are calculated as gas exchange ratios per unit area of the needle or leaves.

Previous studies have often focused on the germination percentage of different species or different species' origins under different osmotic potential. This study is focused on determining the responses of 5-year-old seeds of red pine species collected from different provenances to osmotic potential and the gas exchanges under various water stresses in 1-year-old seedlings produced from seeds of same origins. The study goal was to investigate the water stress effects on the relationships of Turkish red pine seed and young seedlings' provenances. Determining the physiological response to water stress will support the potential use of Turkish pine provenances for afforestation and reforestation programs in Mediterranean regions with warmer climates.

## **II. MATERIAL AND METHODS**

### **A. SEED GERMINATION**

The Turkish red pine seeds were collected in late 2010 on the trees from five different provenances by the Forest Nursery Directorate, Ministry of Forestry, Turkey, and the characteristics of seeds are shown in Table 1. All damaged and empty seeds were discarded and eliminated using the floating process in distilled water before the germination tests. Seed surface sterilized in 5% sodium hypochlorite solution for one minute, and then distilled water was used to rinse the seeds. Seeds were placed in polyethylene bags, stored in a cooler at 4 °C in Turkey and the United States before being used. The germination rate was over 90% for all species in 2011.

*Table 1. Locations (coordinates), mean summer and winter temperature, and elevation of Turkish red pine provenances.*

<b>Provenances</b>	<b>Label</b>	<b>Location</b>	<b>Mean summer temp. (°C)</b>	<b>Mean winter temp. (°C)</b>	<b>Elevation (m)</b>
Antalya- Gündoğmuş	AG	36°42'N–32°12'E	26.5	10.6	1150
Antalya-Kumluca	AK	36°25'N–30°18'E	26.1	11.6	900
Burdur-Bucak	BB	37°26'N–30°37'E	22.7	4.8	850
Denizli- Çameli	DC	37°06'N–29°07'E	27.2	6.9	800
Maraş-Suçatı	KMS	37°46'N–36°42'E	16.5	1.3	800

## **B. METHODS**

### **B. 1. Polyethylene Glycol Application**

The germination test was conducted at the Michigan State University laboratory, Department of Horticulture, Michigan, USA, at the beginning of January 2015. Before the germination test, damaged and empty seeds were removed by using the same process mentioned above. Glass petri dishes (11 cm diameter) on two layers of filter paper were saturated with distilled water for the germination test. Four 50-seed replicates for each provenance and each stress condition were used in this test. A total of 1000 seeds (50 seeds x 4 replicates x 5 water potentials) were used for each provenance, and 5000 seeds were used for this experiment.

Polyethylene Glycol-6000 (PEG) solution was applied to ensure drought stress with five different osmotic potential levels (0.0, -0.2, -0.4, -0.6, and -0.8 MPa) described by Michel and Kaufmann (1973) [27]. Distilled water was used as a control (0 MPa). First, two layers of filter paper then seeds were placed in 9 cm diameter glass petri dishes. The filter paper was saturated with prepared PEG solution for germination. Experiments were carried out at  $25 \pm 1$  °C under 12 h photoperiod using artificial lighting. Filter papers and PEG solutions were renewed every other day to keep relatively constant osmotic potential on the seeds during the tests. Seeds with abnormal radicals and deteriorated and moldy ones were eliminated from the germination counts. When the radicle protruded 2 mm from the seed coat, it was considered to seed germination occurred, and the cumulative germination sums were performed day-to-day for a month.

### **B. 2. Irrigation Treatment**

In this study, five years later from the seed collection, seeds were sown on January 05, 2015, into containers (7.2 x 6 x 23 cm) in the greenhouse at the Tree Research Center at Michigan State University in East Lansing, Michigan, USA. The greenhouse was automatically controlled, and the average temperature was 20.6 °C during the day, and there was no artificial lighting used for this experiment. The non-fertilized Fafard 52 mix was used as a soil media (Conrad Fafard Inc, Agawam, MA). The soil media contains Canadian sphagnum peat moss (30%), processed pine bark, perlite, vermiculite, dolomitic limestone, and wetting agents, with a pH range of 5.5 to 6.5 after wetting. After sowing, pots watered daily until the germination was finalized. When the germination was completed (late February), the seedlings were thinned to one (leave the vigorous seedling) per pots. The liquid form of fertilizer (Peters Professional Peat Lime nutrients; at N: 150, P: 60, K: 150 ppm) is included in irrigation water every weekday during March and May.

Before the irrigation treatment started (last week of March), ten containerized seedlings from each provenance were used to determine seedlings' water requirements as the following procedure. The selected seedlings well-watered and allowed the drain for about 2 hours, then weighted them. A week later, the selected containerized seedlings were weighed again. The difference between the two measured days was how much water was used by seedlings and evaporated on the container in a week. Irrigation rates were established at 25 (Low), 50 (Medium), and 100% (High) of the water requirement as determined above. Low, medium, and high irrigation treatment levels received 35, 70, 140 mL/week water. The irrigation volumes were applied manually once a week from June to July 11, 2015.

### **B. 3. Gas Exchange Measurements**

Gas exchange measurements were conducted on four randomly selected seedlings from each treatment using a LI-COR conifer chamber (LI-6400XT, Lincoln, NE, USA) with an attachment of the RGB (Red, Green, Blue) light source (640-18A). The calibration was done before each measurement. Then, the photosynthesis photon flux density (PPFD), the reference CO<sub>2</sub>, and the airflow rate were set and maintained  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $400 \mu\text{mol mol}^{-1} \text{s}^{-1}$ ,  $500 \mu\text{mol s}^{-1}$ , respectively. Three readings were taken on each seedling (5-10 cm in size) on July 11, 2015. The ImageJ software program was used to determine projected leaf area from scanned images of needles [28]. The leaf area values were then entered as a

section in the LI-COR system to adjust of gas exchange parameters previously measured in the field for the specimen.

Several physiological parameters, including the net photosynthetic rate ( $A_{net}$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\mu\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ ), transpiration rate ( $E$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ), and intrinsic water use efficiency ( $iWUE$ ) were simultaneously measured and calculated by the LI-6400XT software.

#### B. 4. Statistical Analysis

Germination percentage (GP%) was calculated every day, and the germination was completed after 30 days.

Complete randomized design was used with 4 replications for each treatment of water stress, and 5 Turkish red pine provenances and 20 seedlings for each replication, 3 water stress levels, and a total of 1440 seedlings were used. Data were analyzed using SAS 9.1 software (SAS Institute Inc., Cary, NC, USA). All gas exchange variables were tested for normality using PROC UNIVARIATE function. PROC MIXED was used to conduct an analysis of variance (ANOVA) for all these variables. Mean separation was performed using Tukey's adjustment. The relationships between gas exchange parameters were determined by linear and logarithmic regression.

### III. RESULTS

#### A. GERMINATION TREATMENT

The percentage of germinations for the five provenances of Turkish red pine seeds under various water stress is given in Table 2.

There is a significant difference between control and stress treatment that control treatment had a higher germination percentage than the water-stressed seeds for all provenances (Table 2). Increased water stress decreased the germination percentage for all provenances even at the low osmotic potential. Among provenances, DC provenance had the highest germination rate (48%), while KMS had the lowest germination rate (29%) under the control treatment. However, BB provenance had the highest germination rate, which is just 5% followed by AK provenance with 4% under the water stress level of -0.2. The seeds from provenances did not germinate under the water stress level of -0.4 to -0.8 MPa.

*Table 2. Effects of water potential on germination percentage.*

Provenance	Label	Osmotic potential (MPa)				
		Control	-0.2	-0.4	-0.6	-0.8
Antalya/ Gündoğmuş	AG	45	1	0	0	0
Antalya/Kumluca	AK	42	4	0	0	0
Burdur/Bucak	BB	30	5	0	0	0
Denizli/ Çameli	DC	48	0	0	0	0
Maraş/ Suçatı	KMS	29	1	0	0	0

#### B. GAS EXCHANGE PARAMETERS AT THE LEAF LEVEL

The mean values, Tukey's test result, F-values general mean of the main factors (provenance and irrigation) obtained from variance analysis of net photosynthesis rate in terms of the interaction of provenance and irrigation level are presented in Table 3.

**Table 3.** The mean of *Anet* rates ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) by provenances and irrigation levels.

Provenance	Irrigation			Mean	F-value
	High	Medium	Low		
AG	7.95 a	5.84 a	4.98 a	6.26 C	4.44**
AK	7.53 a	6.01 a	4.66 a	6.07 BC	
BB	8.17 a	5.58 a	4.99 a	6.24 C	
DC	6.88 a	5.48 a	4.53 a	5.63 A	
KMS	7.58 a	5.32 a	4.76 a	5.89 AB	
Mean	7.44 C	5.53 B	4.63 A		
F-Value	223.73***				1.57 ns

\*Uppercase letters vertically indicate the significance of provenance, whereas uppercase letters horizontally indicate the significance of irrigation. The lowercase letters indicate the significance within the interaction of provenance and irrigation combination. **High, medium** and **low** irrigation received 35, 70 and 140 mL/week, respectively. \* significant at 0.05 level. \*\* significant at 0.01 level. \*\*\* Significant at 0.001 level. ns: not significant.

The provenance and irrigation as a single factor were significant ( $P < 0.05$ ) on the *Anet*, while the interaction of provenance and irrigation was not significant ( $P > 0.05$ ) (Table 3). The highest *Anet* was observed on AG provenance seedlings, while the lowest value was observed on DC provenance in terms of comparing provenance means. Increasing irrigation was increased the *Anet* values in general while the interaction of provenance and irrigation was not significant ( $P > 0.05$ ), so the interactions were not statistically tested.

The mean values, Tukey's test result, F-values general mean of the main factors (provenance and irrigation) obtained from variance analysis of stomatal conductance rate in terms of the interaction of provenance and irrigation level are presented in Table 4.

**Table 4.** The mean of *gs* ( $\mu\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) by provenances and irrigation levels.

Provenance	Irrigation			Mean	F-value
	High	Medium	Low		
AG	0.17 e	0.06 b	0.05 a	0.09 C	4.88**
AK	0.13 d	0.07 b	0.04 a	0.08 BC	
BB	0.13 d	0.07 b	0.06 ba	0.09 BC	
DC	0.10 c	0.06 ba	0.05 a	0.07 A	
KMS	0.14 d	0.06 ba	0.04 a	0.08 AB	
Mean	0.12 C	0.06 B	0.04 A		
F-value	219.77***				4.49**

\*Uppercase letters vertically indicate the significance of provenance, whereas uppercase letters horizontally indicate the significance of irrigation. The lowercase letters indicate the significance within the interaction of provenance and irrigation combination. **High, medium** and **low** irrigation received 35, 70 and 140 mL/week, respectively. \* significant at 0.05 level. \*\* significant at 0.01 level. \*\*\* Significant at 0.001 level. ns: not significant.

The provenances, irrigation, and their interaction were significant ( $P < 0.05$ ) on the *gs* of Turkish red pine seedlings (Table 4). When comparing provenances as a single factor, the highest *gs* values were observed on AG provenance seedlings, while the seedlings from DC had the lowest *gs* values (Table 4). Increasing irrigation was increased the *gs* values when irrigation is a single factor. Highly irrigated AG provenance had the highest *gs*, while the lowly irrigated AK provenance seedlings had the lowest *gs* under the interaction of provenance and irrigation.

The mean values, Tukey's test result, F-values general mean of the main factors (provenance and irrigation) obtained from variance analysis of transpiration rate in terms of the interaction of provenance and irrigation level are presented in Table 5.

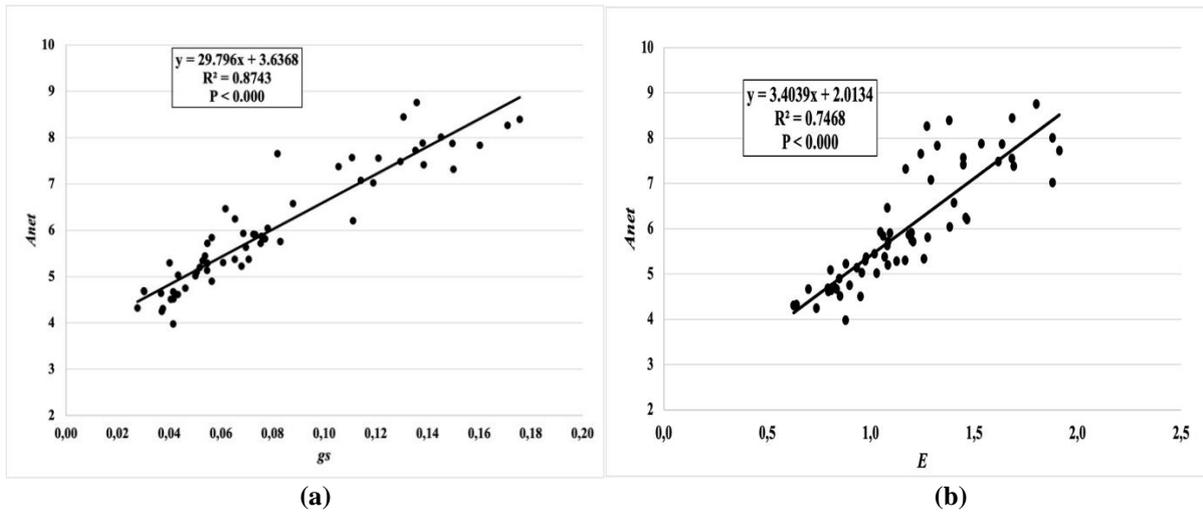
**Table 5.** The mean of  $E$  ( $\text{mmol m}^{-2} \text{s}^{-1}$ ) by provenances and irrigation levels.

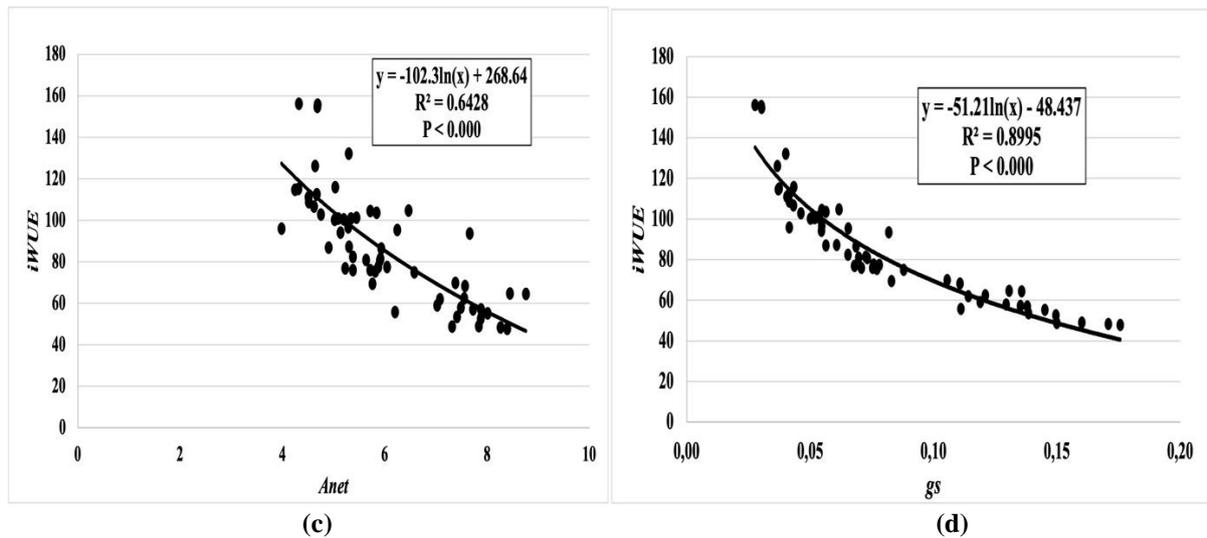
Provenance	Irrigation			Mean	F-value
	High	Medium	Low		
AG	1.29 d	1.33 d	0.89 b	1.17 B	8.95***
AK	1.84 f	1.10 c	0.92 b	1.29 C	
BB	1.62 e	1.14 c	0.90 b	1.22 BC	
DC	1.35 d	1.08 c	0.72 a	1.05 A	
KMS	1.60 e	1.08 c	0.78 b	1.15 B	
Mean	1.53 C	1.12 B	0.82 A		
F-value	237.71***				8.88***

\*Uppercase letters vertically indicate the significance of provenance, whereas uppercase letters horizontally indicate the significance of irrigation. The lowercase letters indicate the significance within the interaction of provenance and irrigation combination. **High, medium** and **low** irrigation received 35, 70 and 140 mL/week, respectively. \*\*\* Significant at 0.001 level.

The single factors (provenance and irrigation) and their interaction were significant ( $P < 0.05$ ) on  $E$  (Table 5). The highest  $E$  was observed in AK provenance seedlings, while the lowest values were observed in DC provenance seedlings when provenance as a single factor (Table 5). In general, increasing irrigation increased the  $E$ . There was not a clear trend within the provenance and irrigation interactions. Highly irrigated AK provenance had the highest  $E$ , while the lowly irrigated DC provenance seedlings had the lowest  $g_s$  under the interaction of provenance and irrigation.

Examining the changes  $iWUE$  (data not shown) by provenances and the interaction of irrigation and provenances, there was no statistically significant difference ( $P > 0.05$ ) between the provenance and the interactions of provenance and irrigation. The single factor of irrigation was significant ( $P < 0.05$ ) in  $iWUE$  that increasing irrigation levels declined the  $iWUE$ .





**Figure 1.** Relationships of gas exchange parameters for all provenances; (a) *Anet* and *gs*, (b) *Anet* and *E*, (c) *iWUE* and *Anet*, (d) *iWUE* and *gs*.

In this study, *Anet* was strongly correlated with *gs* and *E* (Fig. 1a, b) that increasing *Anet* increased *gs* and *E*. On the other hand, *iWUE* negatively correlated ( $P < 0.05$ ) with *Anet* and *gs* (Fig. 1c, d), increasing *Anet* and *gs* reduced *iWUE*.

## IV. DISCUSSION

### A. WATER STRESS EFFECTS ON SEED GERMINATION

In the present study, increasing water stress decreases the germination rates in Turkish red pine, similar to previous studies [25,26,29,30]. Control treatments had a 100% germination rate for all species [25,26]), while the highest germination rate was varied 45% in Turkish red pine provenances in the present study. These studies also stated that the 1-year-old-seeds germination rate decreased roughly 15-25%, 20-45%, 45-60%, 55-60% for Turkish red pine under each water stress level. In contrast, the 5-year-old Turkish red pine seed germination rate drastically decreased under the same osmotic potential levels in the present study because the seed's viability was caused by reducing the germination rate in control and water stress conditions. In this study, Turkish red pine from Burdur/Bucak provenance seed germination rate was less than 5% under -0.2 MPa water stress level for Turkish red pine, which was the highest germination rate. Turkish red pine seeds had no germination for the other water stress levels.

Besides the seed age effect on the seed viability, the germination percentage varied between provenances, and the increasing water stress results in the reduction of germination rate for Turkish red pine. Burdur/Bucak and Antalya/Kumluca provenance had a higher resistance to water stress under -0.2 MPa osmotic level in the current study. This may cause by the seed genetic and environmental factors. All phenotypic characteristics of plants emerge as a result of mutual interaction of genetic structure and environmental condition [31,32,33], and it is known that each genetic structure can react differently to the same environmental conditions [34,35,36]. Studies have shown a significant difference in terms of resistance to water stress both between origins of the same species [15] and between the clonal seed gardens establish with cuttings from the same origin [37]. Therefore, it is crucial that the species exhibits the best performance in terms of resistance to water stress, then determining the origin and even individuals, and using it in afforestation practices in arid and semi-arid areas. The species and origin are also a critical factor determining the tolerance for drought stress in plants [25,26,29,38,39].

## **B. RELATIONSHIPS BETWEEN GAS EXCHANGES PARAMETERS AND WATER STRESS**

Water stress has been more intense and frequent and has become the most adverse environmental factor for plant growth and development in the world, predominantly arid and semi-arid regions. Water plays a critical role directly or indirectly in each physiological process in plants; thus, water stress strongly inhibited seedling gas exchange, and increasing water stress increased the inhibitory effects of gas exchange parameters. It is found that  $g_s$  decreased more than  $A_{net}$  and  $E$  for all provenances in this study.  $A_{net}$ ,  $g_s$ ,  $E$ , and  $iWUE$  are significant parameters for estimating plant productivity in arid and semi-arid regions [40]. The decreases in  $A_{net}$  and  $E$  controlled by  $g_s$  caused increases in  $iWUE$  under water stress conditions.  $A_{net}$  decreases due to the significant reduction in  $CO_2$  assimilation caused by stomatal closure under water stress [41].

The present study in line with the previous study that  $g_s$  is often used as an indicator to measure the level of water stress that the increasing water stress reduces declined the  $g_s$  rates [41]. In periods when there is less water in the soil, water potential decreases in plants, which causes a decrease in  $A_{net}$  due to the decrease in stomatal conductivity [42]. Decreasing  $E$  helps maintain the cell turgor, which is called an acclimation response to water stress [43]. Denizli/Çameli provenances had the lowest  $g_s$  and  $E$  values indicating they have a robust stomatal control to bypass excessive water loss throughout water deficits which help better drought adaptation.

It is found that there is a strong positive linear relationship between  $A_{net}$  and  $g_s$ ,  $A_{net}$  and  $E$ ; however, there is a negative correlation between  $iWUE$  and  $A_{net}$ , and  $iWUE$  and  $g_s$  in the present study.  $A_{net}$  increases with the increasing  $g_s$  and  $E$ . Stomatal regulation is one of the critical factors that control the  $A_{net}$ ,  $E$ , and  $iWUE$  under water stress [44] in line with the present study. However, there is a balance between  $A_{net}$  and  $g_s$  that decreased  $A_{net}$  and  $g_s$  results in increased  $iWUE$  [44].

## **V. CONCLUSION**

Water stress has a suppression effect on the seed germination for conifer seeds. The increasing water stress effect decreased the germination rates. Some conifers may show some adaptation strategies to germinate in an arid and semi-arid environment for afforestation programs. It is also concluded that we must keep the desired seed moisture content from the collecting date to the sowing date from these species' optimum germination. The viability of seeds decreases year by year. It is concluded that when it is needed to use 5-years-old Turkish red pine for afforestation programs, it is better to use seeds from Denizli/Çameli provenance due to higher drought-resistant. However, it is always better to use new seed sources to increase the success of the germination.

## **VI. SUGGESTIONS**

Global warming caused by climate change has many devastating effects on tree species around the world. The most important of these is water scarcity due to both irregularity and a decrease in precipitation. Therefore, it is crucial to identify and use species that need less water or are resistant to drought in afforestation studies. Such studies need to be done both at the seed and seedling stages. In the seed stage, the fact that both new and old seeds are the subject of these studies will provide the necessary seed supply in the processes that may arise due to the interruption of abundant seed years due to future global warming. The fact that studies on seeds shorten the duration of such studies and are less costly increases the importance of these studies.

Although studies have revealed the tolerance of some mature forest tree species to water stress, such studies should also be carried out on seeds, and studies on seedlings should support these results. Studies to reveal the relationship between seeds and seedlings of tree species under drought will benefit afforestation studies in arid and semi-arid areas in the region. Turkey to expanding along with the

temperature in the IPCC's climate change scenarios was put forward to be further affected by the drought. Therefore, our country should preserve seeds for future afforestation programs and focus on scientific studies on their drought tolerance.

Some studies have shown that seeds accumulate some osmolites such as proline and soluble sugars to cope with water stress. Continuous studies are necessary to test the tree species in our country for such studies. These can provide more information about germination's physiological process under drought stress, increasing establishment success in afforestation areas in arid and semi-arid areas. All these studies will increase the success of future afforestation practices.

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