

# Comparison of the Gas Exchange Parameters of Two Maple Species (*Acer negundo* and *Acer pseudoplatanus*) Seedlings under Drought Stress

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## Research Article



**Abstract** – In this study, two-year-old bare-root *Acer negundo* and *Acer pseudoplatanus* seedlings were grown indoors (control vs. drought-stressed) and outside the hoop house to examine leaf gas exchange parameters in the late growing period. Besides morphological features, several gas exchange parameters, such as net photosynthetic rate (*Anet*), transpiration rate (*E*), stomatal conductance (*gs*), intercellular CO<sub>2</sub> to ambient CO<sub>2</sub> (*Ci/Ca*), water use efficiency ( $WUE=Anet/E$ ), intrinsic water use efficiency ( $iWUE=Anet/g_s$ ) were measured. As a result, *A. pseudoplatanus* seedlings had a higher stem height growth. *A. negundo* had a higher *Anet*, and a lower *iWUE* and *Ci/Ca* than *A. pseudoplatanus*. Seedlings grown under control treatment had a higher *Anet*, *gs*, *E*, and a lower *iWUE*, *WUE*, and *Ci/Ca* values than other treatments. In conclusion, there was no significant difference between the two *Acer* species in terms of gas exchange parameters. However, it can be said that *A. negundo* and *A. pseudoplatanus* are drought resistant.

**Keyword** – *Acer negundo*, *Acer pseudoplatanus*, stomatal conductance, net photosynthetic rate, water use efficiency

## Kuraklık Stresi Altındaki İki Akçaağaç Türünün (*Acer negundo* ve *Acer pseudoplatanus*) Fidanlarının Gaz Değişim Parametrelerinin Karşılaştırılması

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## Araştırma Makalesi

**Öz** – Bu çalışmada, iki yaşında çıplak köklü *Acer negundo* ve *Acer pseudoplatanus* fidanlarının büyüme dönemi sonundaki yaprak gaz alışveriş parametrelerini incelenmek için sera içinde (kontrol vs. kuraklık stresi altında) ve dışında açık alan koşulları altında yetiştirilmiştir. Morfolojik özellikler yanında, net fotosentez (*Anet*), terleme oranı (*E*), stoma iletkenliği (*gs*), hücreler arası CO<sub>2</sub>'in ortamdaki CO<sub>2</sub>'ye oranı (*Ci/Ca*), anlık bitki su kullanım etkinliği ( $WUE=Anet/E$ ) ve içsel (gerçek) su kullanım etkinliği ( $iWUE=Anet/g_s$ ) ölçülmüştür. Sonuç olarak *A. pseudoplatanus* fidanları *A. negundo*'ya göre daha fazla boy büyümesi yapmıştır. *A. negundo*'nun *A. pseudoplatanus*'tan daha yüksek *Anet* ve daha düşük *iWUE* ve *Ci/Ca*'ya sahip olduğu tespit edilmiştir. Kontrol uygulaması altında yetişen fidanlar, diğer uygulamalara göre daha yüksek *Anet*, *gs*, *E* ve daha düşük *iWUE*, *WUE* ve *Ci/Ca* değerlerine sahip olduğu tespit edilmiştir. Sonuç olarak, gaz değişim parametreleri açısından iki akçaağaç türü arasında önemli bir fark bulunmamıştır. Ancak, *A. negundo* ve *A. pseudoplatanus*'un kuraklığa dayanıklı bir ağaç türü olduğu söylenebilir.

**Anahtar Kelimeler** – *Acer negundo*, *Acer pseudoplatanus*, stoma iletkenliği, net fotosentez, su kullanım verimliliği.

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## 1. Introduction

The world has faced many calamities, such as volcanic eruptions, wildfires, earthquakes, pollution, melting ice in the Arctic, and global warming resulting from climate change. Extreme weather events' frequency started to increase with the Industrial Revolution due to heavy usage of natural resources resulting in changing climate and increased pollution, especially in urban areas (Koç, 2021a). Under the social pressure caused by the increasing world population, forest ecosystems and climate change are negatively affected (Kaptan et al., 2021). Climate change has become one of the irreversible critical problems worldwide. Global climate change affects plant compositions as well as land cover change (Kaptan, 2021). As a result of climate change, the temperature has been increased by almost 1 °C in the last century and keeps increasing (IPCC, 2014a), and drought events have become more frequent and severe worldwide (Koç, 2019; Koç, 2021b, c; Koç et al., 2021; Koç and Nzokou, 2022). Elevation can play a critical role in the effect of drought events on plant species distribution in their natural areas (Ozel et al., 2021). Urban areas are about 10 °C warmer than adjacent rural environments because of heat islands (Zhou et al., 2017). Plants may face some positive or negative consequences from warmer air temperatures and relations between heating, drought stress, and air pollution.

The intensity and frequency of drought will likely be more usual recently due to global warming because of increased greenhouse gases concentration in the air (IPCC, 2014a; Varol et al., 2021) that more likely to be related to a decrease in soil moisture (Xu et al., 2020). Based on the future climate change projection scenarios, the temperature may increase 2.5 °C by 2050 and 5.4 °C by 2100 in the world (IPCC, 2014b) as well as in the cities of Turkey (Canturk and Kulaç, 2021; Koç, 2021d, e). Increasing air temperature accelerates the evaporation and may decrease or increase the precipitation (rain, snow) in some regions that trigger global warming, which tree species will face drought stress in their natural forests and plantation sites. Remarkably species in temperate forests commonly responded positively to increases in temperature in their natural habitat (Sendal et al., 2015; Lahr et al., 2018); however, the responses of these tree species found in urban areas are inconsistent.

As a result of global warming, plants have been faced drought in their natural and plantation areas (Koç, 2019; Koç, 2021b, c; Koç et al., 2021). Drought stress in plants is caused by increased temperature and evaporation in the air, and reduced precipitation. Drought stress is critical abiotic stress that limits tree growth, development, biochemical activities, physiology (Sevik and Erturk, 2015; Li et al., 2020; Guo et al., 2021; Koç, 2021b, c; Koç et al., 2021; Seleiman et al., 2021; Koç and Nzokou, 2022), even germination (Koc and Nzokou, 2018). However, each plant species has different response mechanisms to drought stress due to genetic differences and environmental factors.

The anatomical, morphological, and phenotypic features of tree species result from the combination of genetic and environmental conditions (Koç, 2021a; Ozel et al., 2021). Within environmental factors, water is one of the essential compounds for all processes in plants started with seed germination (Koc and Nzokou, 2018; Koç, 2021b), followed by growth, development, physiological and biochemical reactions such as photosynthesis and carbon assimilation (Yildiz et al., 2014; Koç, 2021b, c; Seleiman et al., 2021; Koç and Nzokou, 2022). All these processes are directly and indirectly associated with water use efficiency in woody plants (Soba et al., 2020; Wang et al., 2020) that may differ under various environmental conditions, such as heavy metal contamination, salt stress, and cold and drought stress. Water use efficiency in plants is described as the quantity of water used to produce a unit of fixed carbon or biomass unit throughout the process of photosynthesis (Maier et al., 2019). Water use efficiency is a key index that uncovers the plant physiological adaptation to arid and semi-arid regions.

Plants are hindered water and nutrient uptake in arid and semi-arid regions (Fageria et al., 2016), marginal lands (Shults et al., 2020), and urban areas, resulting in drought (Seleiman et al., 2021), which restricts many biochemical processes in plants. A decline in turgor pressure triggers stomatal closure with a signaling mechanism that reduces transpiration under drought (Dayer et al., 2020; Seleiman et al., 2021; Yang et al., 2021).

Among biochemical processes in plants, leaf gas exchange is used to determine whether the plants are affected by drought. Gas exchange measurement in plants occurs instantly from their fully grown leaves that reflect the status of plants.

The Li-Cor portable photosynthesis system (Li-Cor Biosciences, Lincoln, NE, USA) has recently gained fame in measuring leaf gas exchange parameters due to proving robust, reliable instant responses excepts time delays. The recent model of Li-Cor 6800 is a closed system that controls relative humidity, IRGA, CO<sub>2</sub>, and light intensity; that whole measured is based on a unit area on the broad leaves. This instrument provides information from the plant, such as net photosynthetic (assimilation) rate, stomatal conductance to water vapor, transpiration rate, intrinsic water use efficiency, and water use efficiency. Water use efficiency is indicated the ratio of net photosynthetic rate and transpiration, while intrinsic water use efficiency reflects the proportion of net photosynthetic rate and stomatal conductance (Lambers et al., 2010). Robust stomatal control induces to improve long-period (de Miguel et al., 2012) and short-period water use efficiency (Comstock, 2002). Besides stomatal conductance and net photosynthesis, these two gas exchange parameters have been usually used to determine the correlation between plant status and plant physiology under drought.

Among deciduous species, the maple (*Acer*) species is an important tree species consisting of over 150 species globally, primarily distributed in the northern hemisphere and distributed in Asia, Europe, North America, and North Africa (Yaltrık, 1971). About 10 maple species are naturally found in Turkey (Yaltrık, 1967, 1971; Davis et al., 1988). Besides the natural maple trees, exotic maple species have been used in Turkey's many cities' landscapes. Within maple trees, *Acer negundo* (L.) (Ash-leaved maple) and *Acer pseudoplatanus* (Sycamore maple) are one of two species primarily seen in many urban landscaping in Turkey due to desired color and resistance to environmental stress such as heavy metal (Turkyilmaz et al., 2018) and drought stress. *A. negundo* is considered an invasive species in seminatural regions in Eurasia (Gusev et al., 2017; Veselkin et al., 2021). It is also a transformer species that significantly changes its ecosystem's conditions (Richardson et al., 2000). Conversely, *A. pseudoplatanus* is also an invasive species, but it is also a critical silvicultural and ecological role in European forests (Beikircher et al., 2021). Besides these, *Acer* species as urban plants provide ecosystem services that range from carbon sequestration and heavy metal accumulation to shading urban surroundings and improving urban life quality.

Plant species can adapt under distinct arid and semi-arid regions (Allen et al., 2010; Seleiman et al., 2021). The easiest way to determine the physiological changes in plants is by monitoring the gas exchange parameters. The results will provide some information to improve our understanding of the gas exchange mechanism in these two plant species under drought conditions. It also provides farm managers and landscapers with better knowledge for preparation under future global warming conditions. So, this study aimed to determine the effect of drought stress on the *A. negundo* and *A. pseudoplatanus* seedlings gas exchange parameters.

## 2. Material and Method

This experiment was conducted at the Düzce University Forestry Department hoop house, Turkey. A layer of clear plastic was used to cover the hoop house. Two long sides of the hoop house were kept open between 0.5 - 1.5m above the surface level to allow unrestricted airflow circulation and prevent increased temperatures due to a greenhouse effect. During the experiment period (June-September), the minimum and maximum temperatures were approximately 20 and 45 °C in the hoop house during the experiment period (May-late September 2021).

### 2.1. Plant Material and Containerization Substrates

Two-year-old bare-root *A. negundo* (L.) and *A. pseudoplatanus* (L.) tree species seedlings were obtained from Sakarya-Hendek and Ordu Forest Enterprise nurseries, respectively, on the second week of December 2021. Then, their roots were pruned approximately in 15-20 cm length to fit into plastic tubes. The potting mix

consisted of soil, peat moss, and perlite (1:1:1 volume). The seedlings were planted in the plastic tubes as soon as possible (1<sup>st</sup> week of February) after receiving seedlings. A total of 30 seedlings from each species were potted into plastic tubes and well-watered until the beginning of May 2021.

The initial stem height ( $\pm$  S.D) were 89.00 ( $\pm$ 12.26), 80.70 ( $\pm$ 17.56) cm, and root collar diameters ( $\pm$  S.D) were 10.68 ( $\pm$ 1.90), 9.87 ( $\pm$ 1.76) mm for *A. negundo* and *A. pseudoplatanus*, respectively.

## 2.2. Irrigation Treatment

The three-irrigation treatment methods were assigned in each tree species as follows. Ten seedlings were subjected to well-watered (every other day) and drought stress treatment (well-watered once in two weeks) separately in the hoop house, while 10 containerized seedlings were move outside and grown there. The irrigation treatments were started at the beginning of May 2021. The seedlings outside did not receive any water artificially. They just received water when it rained. The precipitation in the study area in May, June, July, August, and September were about 62.9, 69.5, 44, 47.5, and 47.8 mm, respectively. The minimum and maximum temperatures within every month during the experiment period were 0.4-39.5, 6.6-38.9, 8.8-42.4, 7.6-40, and 4.5-38.7, respectively.

## 2.3. Relative Height Growth and Relative Root Collar Diameter

Seedling's height and diameter growth were measured using a tape measurer and caliper, respectively, at the beginning and end of the treatment period. Root collar diameter was measured at the soil surface. Relative growth for root collar diameter (RRCD) and height (RHG) was calculated as the change between the final and the initial growth divided by initial values.

## 2.4. Gas Exchange Measurement

In the study, ten seedlings were chosen on gas exchange measurements from each treatment using Li-Cor broad leaf chamber (6 cm<sup>2</sup>) (LI-6800, Lincoln, NE, USA) with an attached small light source (6800-02 – red, blue, light). The calibration was done as recommended by the manufacturer. Then, the airflow rate, the photosynthesis photon flux density (PPFD), and reference CO<sub>2</sub> were set and maintained automatically at 500  $\mu\text{mol s}^{-1}$ , 500  $\mu\text{mol s}^{-1}$ , 400  $\mu\text{mol mol}^{-1} \text{ s}^{-1}$ , respectively. Three readings were taken on each seedling on the measurement days on September 26, 2021.

Numerous gas exchange variables, particularly the net photosynthetic (assimilation) rate ( $A_{net}$ ,  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ), transpiration rate ( $E$ ,  $\text{mmol m}^{-2} \text{ s}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), intercellular CO<sub>2</sub> to ambient CO<sub>2</sub> ( $C_i/C_a$ , ( $\mu\text{mol mol}^{-1}$ )) water use efficiency ( $WUE=A_{net}/E$ , ( $\mu\text{mol mol}^{-1}$ )), intrinsic water use efficiency ( $iWUE=A_{net}/g_s$ , ( $\mu\text{mol mol}^{-1}$ )) were directly measured and calculated.

## 2.5. Statistical Analysis

A complete randomized design was used in this study consisting of two species and three treatment levels. Ten seedlings (considered as replication) for each treatment and a total of 60 containerized seedlings were used. SAS 9.1 statistical software (SAS Institute Inc., Cary, NC, USA) was used to analyze all gas exchange and growth data. The function of PROC UNIVARIATE was used for normality, while PROC MIXED function was used to perform an analysis of variance (ANOVA). For the mean separation, Tukey's adjustment was used.

## 3. Results

### 3.1. Relative Height Growth (RHG) and Relative Root Collar Diameter (RRCD)

Species, treatment, and their interaction were significant ( $p<0.05$ ) on RHG and RRCD except for species on

RRCd ( $p>0.05$ ). In the current year, *A. pseudoplatanus* statistically had a higher RHG than *A. negundo*. Although *A. pseudoplatanus* had a higher RRCd than *A. negundo* seedlings, it was not statistically significant ( $p>0.05$ ). When we examined the treatment factors, plants are grown outside (90.50 cm), and control treatment had a higher RHG than drought-stressed seedlings. There were no statistical differences between control seedlings and seedlings grown outside ( $p>0.05$ ). The seedlings grown outside had a statistically highest RRCd followed by control treatment when drought-stressed seedlings had the lowest RRCd. Lack of irrigation decreased RHG and RRCd that the control and plants grown outside treatments had a higher RHG and RRCd growth than drought-stressed seedlings for both *Acer* species.

Table 1

The mean of relative height and root collar diameter growth by the interaction of species and treatment

Treatment	Height (cm/cm)			Root Collar Diameter (mm/mm)		
	<i>Acer negundo</i>	<i>Acer pseudoplatanus</i>	Mean	<i>Acer negundo</i>	<i>Acer pseudoplatanus</i>	Mean
Control	86.60	72.20	79.40	5.41	2.16	3.78
Outside	64.10	116.90	90.50	4.44	9.35	6.90
Drought	44.20	47.07	45.64	1.20	1.04	1.12
Average	64.97	78.72		3.68	4.19	

### 3.2. Gas Exchange Parameters at The Leaf Level

The analysis of variance (ANOVA) of gas exchange parameters, such as *Anet*, *gs*, *E*, *iWUE*, *WUE* and *Ci/Ca* among species and treatments are given in Table 2.

Table 2

Degrees of freedom (df), F-values of ANOVA for *Anet*, *gs*, *E*, *iWUE*, *WUE* and *Ci/Ca* among two species and three treatments

Source of variation	df	<i>Anet</i>	<i>gs</i>	<i>E</i>	<i>iWUE</i>	<i>WUE</i>	<i>Ci/Ca</i>
Species (S)	1	51.83***	1.65ns	1.67ns	16.59**	12.60**	148.39***
Treatment (I)	2	89.70***	69.07***	93.16***	247.55***	1267.12***	79.91***
SxT	2	18.76***	6.05**	5.25**	57.45***	34.11***	53.26***

\*\*  $p < 0.001$ . \*\*\*  $p \leq 0.0001$ . ns: not significant.

Among the gas exchange parameters, all single (species and treatment) factors and their interaction were significant except species for *gs* and *E*.

The mean values and Tukey's test results of *Anet* rate in terms of the interaction of species and treatment are presented in Table 3.

As a result, species (S), treatment (T) as a single factor, and the interaction of SxT were significant ( $p < 0.05$ ) for *Anet* (Table 2). *A. negundo* seedlings had a higher *Anet* value than *A. pseudoplatanus* (Table 3). The seedlings under control treatment had a higher *Anet*, followed by seedlings grown outside, and the drought-stressed seedling had the lowest values. Under the interaction of species and treatment, *A. negundo* seedlings under control treatment had the highest values, while drought-stressed *A. pseudoplatanus* seedlings had the lowest values (Table 3).

Table 3

The average of *Anet* Rates ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) by the Interaction of species and treatment

Treatment	Species		Average
	<i>Acer negundo</i>	<i>Acer pseudoplatanus</i>	
Control	5.56 A	4.97 A	5.26 a
Outside	5.80 A	2.47 B	4.14 b
Drought	2.19 B	1.58 B	1.88 c
Average	4.51 a	3.00 b	

Note: The lowercase letter horizontally indicates the significance of species, whereas the lowercase letter vertically indicates the significance of treatment. The uppercase letters indicate the significance within the interaction of species and treatment.

The mean values and Tukey's test results of *gs* rate in terms of the interaction of species and treatment are presented in Table 4.

Table 4

The mean of *gs* ( $\mu\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) by the Interaction of species and treatment

Treatment	Species		Average
	<i>Acer negundo</i>	<i>Acer pseudoplatanus</i>	
Control	0.060 A	0.071 A	0.065 a
Outside	0.023 B	0.010 C	0.016 c
Drought	0.017 BC	0.033 B	0.025 b
Average	0.033 a	0.035 a	

Note: The lowercase letter horizontally indicates the significance of species, whereas the lowercase letter vertically indicates the significance of treatment. The uppercase letters indicate the significance within the interaction of species and treatment.

As a result, only T and SxT interactions were significant ( $p < 0.05$ ) on the *gs* (Table 2). Seedlings under the control treatment had a higher *gs*, followed by drought treatment, and seedlings grown outside had the lowest *gs* values (Table 4). *A. pseudoplatanus* seedlings had the highest *gs* while *A. pseudoplatanus* seedlings grown outside had the lowest values under the interaction of SxT (Table 4).

The mean values and Tukey's test results of *E* rate in terms of the interaction of species and treatment are presented in Table 5.

Table 5

The mean of *E* ( $\text{mol m}^{-2} \text{s}^{-1}$ ) by the interaction of species and treatment

Treatment	Species		Average
	<i>Acer negundo</i>	<i>Acer pseudoplatanus</i>	
Control	0.0021 A	0.0025 A	0.0023 a
Outside	0.0007 B	0.0003 C	0.0005 c
Drought	0.0008 B	0.0012 B	0.0010 b
Average	0.0012 a	0.0013 a	

Note: The lowercase letter horizontally indicates the significance of species, whereas the lowercase letter vertically indicates the significance of treatment. The uppercase letters indicate the significance within the interaction of species and treatment.

As a result, only T and SxT interactions were significant ( $p < 0.05$ ) on the *E* (Table 2). Seedlings under the control treatment had a higher *E*, followed by drought treatment, while the seedlings grown outside had the lowest *gs* values (Table 5). *A. pseudoplatanus* seedlings had the highest *E* while *A. pseudoplatanus* seedlings grown outside had the lowest values under the interaction of SxT (Table 5).

The mean values and Tukey's test results of *iWUE* (*Anet/g<sub>s</sub>*) rate in terms of the interaction of species and treatment are presented in Table 6.

As a result, S, T as a single factor and the interaction of SxT were significant ( $p < 0.05$ ) for *iWUE* (Table 2). *A. negundo* seedlings had a lower *iWUE* value than *A. pseudoplatanus* (Table 6). The seedlings grown outside had a higher *iWUE*, followed by seedlings under drought-stressed and control treatment had the lowest values. There was no statistical difference between control and drought-stressed seedlings. Under the interaction of species and treatment, *A. pseudoplatanus* seedlings grown outside had the highest values, while drought-stressed *A. pseudoplatanus* seedlings had the lowest values (Table 6).

Table 6

The mean of *iWUE* (*Anet/gS*) ( $\mu\text{mol mol}^{-1}$ ) by the interaction of species and treatment

Treatment	Species		Average
	<i>Acer negundo</i>	<i>Acer pseudoplatanus</i>	
Control	92.52 CD	75.55 CD	84.03 b
Outside	333.57 B	653.64 A	493.61 a
Drought	156.94 C	59.87 D	108.40 b
Average	195.68 b	248.35 a	

Note: The lowercase letter horizontally indicates the significance of species, whereas the lowercase letter vertically indicates the significance of treatment. The uppercase letters indicate the significance within the interaction of species and treatment.

Species, T, and SxT were significant on *WUE* (Table 2). *A. negundo* had a higher *WUE* than *A. pseudoplatanus*. Seedlings grown outside had the highest *WUE* compared to control and drought-stressed seedlings. Under the interaction of species and treatment, *A. pseudoplatanus* seedlings grown outside had the highest values, while drought-stressed *A. pseudoplatanus* seedlings had the lowest values (data not shown).

The mean values and Tukey's test results of *Ci/Ca* in terms of the interaction of species and treatment are presented in Table 7.

Table 7

The mean of *Ci/Ca* ( $\mu\text{mol mol}^{-1}$ ) by the interaction of species and treatment

Treatment	Species		Average
	<i>Acer negundo</i>	<i>Acer pseudoplatanus</i>	
Control	0.55 BC	0.66 B	0.61 b
Outside	0.59 BC	1.76 A	1.17 a
Drought	0.39 C	0.73 B	0.56 b
Average	0.51 b	1.05 a	

Note: The lowercase letter horizontally indicates the significance of species, whereas the lowercase letter vertically indicates the significance of treatment. The uppercase letters indicate the significance within the interaction of species and treatment.

As a result, S, T as a single factor and the interaction of SxT were significant ( $p < 0.05$ ) for *Ci/Ca* (Table 2). *A. negundo* had a lower *Ci/Ca* than *A. pseudoplatanus*. Seedlings grown outside had the highest *Ci/Ca* values than control and drought-stressed seedlings. There was no statistical difference between control and drought-stressed seedlings. Under the interaction of species and treatment, *A. pseudoplatanus* seedlings grown outside had the highest values, while drought-stressed *A. negundo* seedlings had the lowest values (Table 7).

## 4. Discussion

### 4.1. The Effect of Drought Stress on Seedlings RHG and RRCD

Under water deficit (drought) conditions, plant growth and development are restricted due to various drought response mechanisms (Yigit et al., 2019; Koç, 2021b, c; Seleiman et al., 2021), such as drought resistance, alleviation, and avoidance (Koç, 2019; Liu et al., 2020; Koç et al., 2021; Seleiman et al., 2021). Under these mechanisms, some plant species grow their roots while others increase their shoot growth under unfavorable climates (Pang et al., 2018). In this study, *A. pseudoplatanus* seedlings had a higher RHG and RRCD than *A.*

*negundo* under each treatment factor. Under severe drought-stressed, *A. pseudoplatanus* seedlings had higher RHG and a lower RRCD growth than *A. negundo* seedlings. Lack of water availability declines cell elongation, especially in the shoot and cambium, which results in declined RHG and RRCD in seedlings. The RHG of control seedlings increased almost 2-fold in *A. negundo* seedlings while 1.5-fold in *A. pseudoplatanus* compared to drought-stressed seedlings. The RRCD of control seedlings increased almost 4-fold in *A. negundo* seedlings and a 2-fold in *A. pseudoplatanus* compared to drought-stressed seedlings.

#### 4.2. Drought Stress Effect on Gas Exchange Parameters

In recent decades, drought stress has become the most irreversible environmental calamity for plants due to lack of precipitation and increased mean temperature worldwide (Koç et al., 2021; Seleiman et al., 2021). Water is essential for plants due to development and many physiological processes such as carbon assimilation and photosynthesis. Water deficit causes drought stress in plants; increasing temperature increases drought severity and prevents plant gas exchange (Koç, 2021b; Koç, 2021c). Moreover, declining water availability in the root zone reduces stem xylem pressure potential causing a reduction in *Anet*, *gs*, and *E* in plant leaves similar to the present study. The aperture of stomata is regulated by water potential and hormonal activities such as abscisic acid (Hsu et al., 2021) that reduces carbon dioxide assimilation resulting from hindered gas exchange under drought stress (Song et al., 2020). Under prolonged drought stress, seedlings had a declined *Anet*, *gs*, and *E* in the current study, which was observed in *A. saccharum* ssp. *saccharum* and ssp. *nigrum* (Hauer et al., 2021).

In contrast, if the water in the soil becomes a limiting factor, plant *iWUE* and *WUE* values usually increase either due to elevated *Anet* values and low stomatal conductivity or under the effect of both (Koç, 2019; Koç, 2021b, c). Reduced stomatal aperture declined *gs* in the leaf level that reduced the transpiration rates; consequently, this will increase the *WUE* in seedlings (Belmecheri et al., 2021) in line with the current study. When drought stress is exacerbated, seedlings increase their growth with more efficient water use mechanisms (Li et al., 2020). Seedlings with higher *iWUE* and *WUE* values enhance growth and development, especially under drought stress settings (Xu et al., 2020).

In this study, we expected to see that the seedlings in control and sit outside treatment had a higher *Anet*, *gs*, and *E* while lower *iWUE* and *WUE* values than drought-stressed seedlings. However, *A. pseudoplatanus* seedlings grown outside had the lowest *gs* and *E* values and highest *iWUE* and *WUE* values than seedlings under drought stress. This may be caused by a couple of reasons, such as genetic differentiation on plant species, container type, wind factor, and leaf structure. The genetic differences and environmental conditions play a crucial role in the response of plant physiology (Lahr et al., 2018; Koç et al., 2021). Some species develop different strategies to withstand drought stress. Some plants are called 'isohydric' or 'water savers' due to strong stomatal reduction when soil water potential decrease. However, some plants species maintain relatively high *gs* and are less sensitive to low soil water potential is called 'anisohydric' or 'water spenders' (Kunz et al., 2016). *A. negundo* is acted more likely water spenders than *A. negundo*. In addition, the use of plastic materials on containerized *Acer* seedlings in this study may have been caused by an increased soil temperature and evaporation from soil level that reduced available soil water for *A. pseudoplatanus* seedlings. Also, the wind can increase the evapotranspiration that may lead seedlings to close their stomata results in reduced *E* rates. *A. pseudoplatanus* leaves are thick and leathery, while *A. negundo* has a compound leaf, so this plays a critical role in terms of the process of gas exchange.

#### 5. Conclusion and Suggestion

Water is an essential compound for all living organisms. In water deficit conditions, plant species are exposed to drought stress. Drought stress inhibits a plant's growth, development, and all biochemical processes in the plant. In conclusion, the control seedlings in this study had a higher growth (height and root collar diameter) than drought-stressed seedlings of *A. negundo* and *A. pseudoplatanus*. Due to adaptive mechanisms, some



plant species have drought resistance, such as closing their stomata and reducing transpiration via their leaves. The effects of drought stress can detect using leaf gas exchange measurements. As a result of this study, it is concluded that there was no significant difference between the two *Acer* species in terms of gas exchange parameters. However, it can be said that *A. negundo* is a drought resistance while *A. pseudoplatanus* is particularly drought resistant in line with the literature. In addition, it can be said seedlings for both *Acer* species under control treatment generally had higher *Anet*, *gs*, and *E* and lower *iWUE* than the other two treatments. There was no clear separation in gas exchange parameters between the two treatments (seedlings grown under drought stress and outside). Also, seedlings under drought treatments had lower *Anet*, *E*, *iWUE*, and *Ci/Ca* and higher *gs* and *E* than seedlings grown outside. If one of these two species is to be preferred in afforestation practices in urban areas, it is more appropriate for this species to be *Acer negundo* in terms of gas exchange parameters and morphological data. Having irrigation systems in the areas where these seedlings are planted is a critical issue in seedling growth and less exposure to drought.

The adverse effect of global warming is visible in daily life, especially in urban areas. The severity of global warming will affect urban sites more than surrounding environments, so both species can be useful for planting in urban areas due to providing ecosystem services, reducing air pollution and heavy metals, resistance to drought stress, and particular leaf traits, such as size and color. Moreover, these species can improve the urban life quality with a well-organized landscape design. So, future studies are necessary to test all the varieties of *Acer* species and other species seen in the urban environments in terms of how they respond under drought stress. The results of future studies will improve our knowledge of which species are better grown in urban areas where the air temperature was higher than its surroundings under future climate change scenarios.

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