



Investigation of Leaf Gas Exchange Parameters of Several Chestnut Population Seedlings at the End of the Growing Season

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ABSTRACT

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Changes in temperature and precipitation due to global climate change negatively affect plant species' growth, development, and adaptation to new places. However, genetic structure is the most critical criterion for determining a species's potential to adapt to changing environmental conditions. Monitoring gas exchange parameters in plants is the simplest way to monitor physiological changes in plants under changing environmental factors. Among species, the Anatolian chestnut (*Castanea sativa*) is native and economically important tree species (fruit and wood production). It is naturally distributed from the north side of Turkey, Marmara, and Western Anatolia. However, the Anatolian chestnut is one of the most affected tree species by global climate change. In this study, numerous Anatolian chestnut populations (3 years old) were used to determine leaf gas exchange parameters at the end of the growing season in Düzce. Stomatal conductance (g_s), transpiration rate (E), net photosynthetic rate (A_{net}), and other parameters were measured. As a result, the leaf gas exchange parameters of chestnut populations changed significantly based on the populations. Marigoule population seedlings had 2-fold A_{net} values compared to the Ibradı population. Regarding g_s , the differences between populations (Erfelek and Ibradı) changed approximately 2.5 folds and the differences (Erfelek and Ibradı) increased more than 3 folds in terms of E values. It can be said that Marigoule and Erfelek populations can adapt more to Düzce climate conditions than other populations. In contrast, Ibradı population seedlings have a low adaptation mechanism in terms of gas exchange traits.

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Introduction

Temperature and precipitation are the two main drivers for plant distribution around the world. With the Industrial Revolution, people have been heavily using natural resources, such as coal, gases and some other elements, resulting in increased air pollution (Çobanoğlu et al., 2022; Isinkaralar et al., 2022; Key et al., 2022; Koç et al., 2022), water contamination (Demir et al., 2021; Kutlu and Mutlu, 2021; Tokatli et al., 2021; Mutlu and Uncumusaoğlu, 2022) and temperature on a regional and global scale (IPCC, 2014), and therefore a change occurs in the climate (Koç, 2022a). The temperature has risen around 1 °C in the previous century (IPCC, 2014), and the mean annual air temperature may increase by 2.5 and 5.4 °C by 2050 and 2100, respectively, in the world (IPCC, 2014), and Türkiye (Cantürk and Kulaç, 2021). The temperature increases cause some adverse effects on plant species, such as drought (Seleiman et al., 2021; McDowell et al., 2022; Koç, 2022a) and cold stress (Yildiz et al., 2014; Raza et al., 2019; Chaudhry and Sidhu, 2021), which negatively affect

tree growth, development, and physiology (Koç and Nzokou, 2022; Koç and Nzokou, 2023). All these changes are related to the genetic variation of plants.

Genetic diversity is the fundamental component that enables species to survive in changing ecosystems. In addition, genetic diversity is the most important criterion that determines the potential of a species to adapt to different environmental conditions (Sevik et al. 2010). This situation has become much more important for plants, especially with global climate change and global warming, the effects of which are increasing daily. Therefore, it is crucial to determine how different species populations behave under these changing environmental stress factors (Koç, 2022a). Identifying the most resistant populations or adaptable to these changing stress factors is vital and encourages their use. Genetic variation may play an essential role during the photosynthetic process, especially in diverse environments.

As a physiological parameter of plants, leaf photosynthetic gas exchange directly affects plant growth, and therefore scientists have been aware of this subject recently. However, similar plant species differ even under the same environmental conditions (Bhattacharjee and Saha, 2014; Koç and Nzokou, 2022). Species have various abilities to respond to environmental fluctuations (Allen et al. 2010; Koç et al., 2022). All the response mechanism to environmental alterations is linked to species' genetic structure (Koç and Nzokou, 2022). Plant species' morphological, anatomical, and photosynthetic traits are formed by the interplay of genetic codes and environmental situations (Ozel et al. 2021).

Photosynthetic gas exchange from leaves, using the LI-COR photosynthesis method (LI-COR Biosciences, Lincoln, NE, USA), is one of the common, reliable, and robust methods to determine plant status instantly. The LI-COR 6800 is worked based on a unit area on the plant leaf and a closed system that maintains IRGA, relative humidity, light intensity, and CO₂. The portable LI-COR 6800 is the latest version that provides a series of measurements, such as transpiration rate, net assimilation (photosynthetic) rate, stomatal conductance, intrinsic CO₂ concentration, and many more. Using some of these measurements, water use and intrinsic water use efficiency are also calculated. Water use efficiency has reflected the percentage of net photosynthetic ratio and transpiration, while intrinsic water use efficiency indicates the percentage of net photosynthetic ratio and stomatal conductance (Lambers et al. 2008; Koç, 2022a).

Among broad-leaf species, the Anatolian chestnut (*Castanea sativa*) is native tree species in Turkey and is distributed from the north side of Turkey from Bulgaria to the Caucasus, besides Marmara and Western Anatolian provinces (Orman Genel Müdürlüğü (OGM), 2013). Anatolian chestnut is the main tree species and is economically significant in Turkish forestry due to its fruit and wood production. It has a smooth and plump trunk that has been used in many areas, such as decorative, furniture, and other construction purposes (Conedera et al. 2004; Kakava et al. 2018; Mirela, 2020). Türkiye has around 39-40 thousand ha chestnut production areas, which ranks third in the world and earns more than \$40 million from its export (about 60 thousand tons) worldwide in 2018 (Food and Agriculture Organization of the United State (FAO), 2019).

Türkiye is among the “countries at risk” against climate change (UNDP, 2019), and the most adverse impact of

global climate change will manifest itself in the Mediterranean region in the form of increased temperatures and decreased precipitation (Giorgi and Lionello, 2008). It is estimated that these changes will significantly affect chestnut forests as in many species (Talu et al. 2011). Species that do not have an adaptation mechanism to these rapid climate changes will suffer the most (Lindner et al. 2010), and therefore some local populations will be in danger of extinction (Keenan, 2012).

For these reasons, various studies have been conducted on the possible effects of climate change on the adaptation of different species populations and plant species in different ecosystems. Most such studies harm plant species; however, plant species' have some mechanisms against climatic changes in the regions that affect plants' morphological and physiological parameters. The most harmless method to determine the changes in these physiological structures is the examination of the leaf gas exchange parameters of the plants. Therefore, seeds were collected from chestnut populations adapted to different regions of Turkey, seedlings were grown, and the differences between gas exchange parameters of these populations in the same environment (Düzce province) were tried to be determined.

Material and Method

Seed Collection, Sowing, and Containerization Substrates

The chestnut seeds were collected from at least 10 different trees in each population from September to October 2017. The information about populations is given in Table 1. The seeds were subjected to wet, cold stratification for about 28-45 days for germination, and then germinated seeds were sown into 18x25 cm polyethylene plastic bag where the potting mix consisted of peat moss, forest soil, and perlite (1:1:1 volume). The seedlings were placed outside on the concrete floor and watered once every week when it was no rain for the following years. In the warmer summer (July-September), the top was covered with a shade of material to prevent sunburn on leaves and water on hot days. This experiment was conducted at the Düzce University Forestry Department nursery in 2021. The stem height and diameter growth (\pm S.D.) for each chestnut population during the measurement period are given in Table 1.

Table 1. The origins of chestnut populations and their seedling growth parameters

Codes	Origin	Height (cm)	Diameter (mm)
IBR	Ibradı (Akseki-Antalya)	90.50 \pm 19.21	16.20 \pm 3.73
OVA	Ovacık (İzmir)	87.20 \pm 19.92	12.95 \pm 2.95
DSA	Deli Sarnıç (Antalya)	96.70 \pm 22.43	11.18 \pm 2.17
SEL	Selge (Antalya)	84.50 \pm 22.23	10.76 \pm 2.20
BUR	Bursa	106.50 \pm 19.57	14.22 \pm 2.41
DKAB	Kabalak (Düzce)	98.70 \pm 24.29	11.76 \pm 1.54
ERF	Erfelek (Sinop)	79.10 \pm 25.54	11.30 \pm 2.28
AOM	Oluk Mahallesi (Antalya)	85.00 \pm 24.32	11.81 \pm 2.22
DKAP	Kaplanağı (Düzce)	95.10 \pm 20.99	11.66 \pm 1.59
MAR	Marigoule	60.50 \pm 15.76	8.07 \pm 0.82

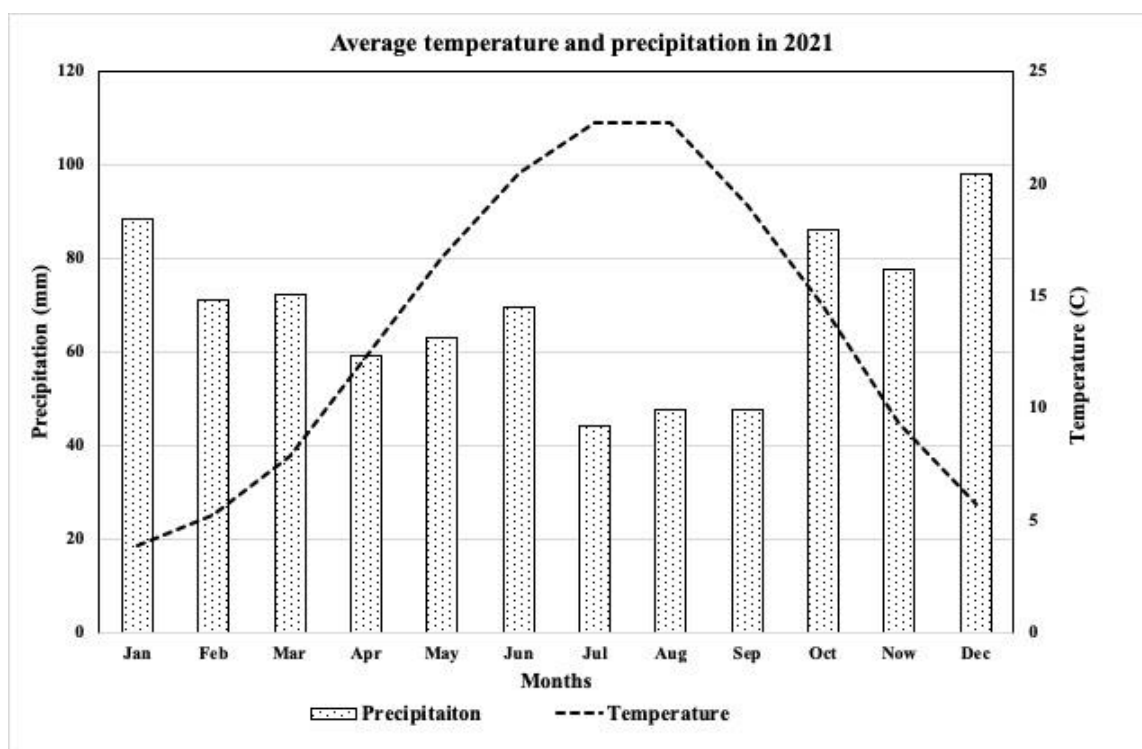


Figure 1. The mean monthly temperature and precipitation

The seeds were subjected to wet, cold stratification for about 28-45 days for germination, and then germinated seeds were sown into 18x25 cm polyethylene plastic bag where the potting mix consisted of peat moss, forest soil, and perlite (1:1:1 volume). The seedlings were placed outside on the concrete floor and watered once every week when it was no rain for the following years. In the warmer summer (July-September), the top was covered with a shade of material to prevent sunburn on leaves and water on hot days. This experiment was conducted at the Düzce University Forestry Department nursery in 2021. The stem height and diameter growth (\pm S.D.) for each chestnut population during the measurement period are given in Table 1.

Temperature and Precipitation

The meteorological data (temperature and precipitation) was obtained from Düzce Meteorological Station for 2021. The city's average monthly temperature and precipitation are given in Figure 1.

Growth Parameters and Gas Exchange Measurements

The height growth was measured with a wooden tape measure, while diameter growth was measured using a caliper in 10 seedlings for each population.

The gas exchange parameters measurement was performed on eight different seedlings (a single reading was taken from each seedling) from each population using a broad leaf chamber (9 cm²) of LI-COR (LI-6800, Lincoln, NE, USA) with an attached light source (6800-02 – red/blue/light). The calibration was done as recommended by the producer. Then, the airflow rate, PPFD (photosynthesis photon flux density), and reference CO₂ were set and held automatically at 500 $\mu\text{mol s}^{-1}$, 1500 $\mu\text{mol s}^{-1}$, and 400 $\mu\text{mol mol}^{-1} \text{s}^{-1}$, respectively. The measurement was taken on September 29, 2021.

The stomatal conductance (g_s , $\mu\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$), transpiration rate (E , $\text{mmol m}^{-2} \text{s}^{-1}$), net photosynthetic rate (A_{net} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), and intercellular CO₂ (C_i) within gas exchange parameters were directly measured when the intercellular CO₂ to ambient CO₂ (C_i/C_a), intrinsic water use efficiency ($iWUE=A_{net}/g_s$), water use efficiency ($WUE=A_{net}/E$), parameters were calculated.

Data Analysis

This experimental design was a complete randomized consisting of 10 populations, and each population had eight seedlings (replication) subjected to gas exchange measurement. Data analysis of all gas exchange parameters was done using SAS 9.1 statistical software (SAS Institute Inc., Cary, NC, USA). PROC MIXED function was used to perform analysis of variance (ANOVA), and mean separation of populations was done using Tukey's adjustment.

Result

Height Growth (HG) and Diameter Growth (DG)

The growth parameters (HG and DG) of populations are given in Table 1. The populations of Bursa seedlings had the highest HG (106.50 ± 19.57 cm), followed by the Kabalak population (98.70 ± 24.29 cm), while Mariguole population seedlings had the lowest HG (60.50 ± 15.76 cm). The other populations averaged HG values are close to each other. The highest DG was observed in the seedlings of the Ibradı population (16.20 ± 3.73 mm), followed by the Bursa population (14.22 ± 2.41 mm) and Ovacık population (12.95 ± 2.95 mm), while the seedlings of the Mariguole population s had the lowest DG (8.07 ± 0.82 mm). The rest of the population seedlings means DG is almost the same.

Table 2. F-values of ANOVA for leaf gas exchange traits of chestnut populations

Source of variation	df	Anet	gs	E	iWUE	WUE	Ci	Ci/Ca
Population	9	6.25***	4.75***	12.61***	8.92***	26.28***	6.27***	6.40***

*** $P \leq 0.0001$. Df = Degrees of Freedom.

Table 3. The Average Values of Gas Exchange Parameters among Chestnut Populations

Population	Anet	gs	E	iWUE	WUE	Ci	Ci/Ca
AOM	6.08 bc	0.051 abc	0.0017 bc	113.62 d	3350.37c	188.17 a	0.485 a
BUR	8.50 ab	0.068 ab	0.0020 ab	126.81 cd	4131.22 c	164.04 ab	0.430 ab
DKAB	6.21 bc	0.056 abc	0.0019 abc	114.8 d	3469.88 c	186.64 a	0.484 a
DKAP	6.47 bc	0.049 abc	0.0017 bcd	146.97 bcd	4135.37 c	135.16 abcd	0.350 abc
DSA	7.37 abc	0.048 abc	0.0012 cde	155.54 abc	5937.99 b	123.89 abcd	0.320 abc
ERF	8.79 ab	0.075 a	0.0024 a	126.6 cd	3893.91 c	163.00 abc	0.425 ab
IBR	4.71 c	0.029 c	0.0007 e	175.78 ab	6715.03 ab	98.02 cd	0.251 c
MAR	10.07 a	0.070 ab	0.0016 bcd	143.58 bcd	6238.45 ab	138.30 abcd	0.363 abc
OVA	7.64 abc	0.043 bc	0.0011 de	187.46 a	7538.61 a	84.94 d	0.220 c
SEL	8.80 ab	0.054 abc	0.0016 bcd	157.4 abc	5568.49 b	116.98 bcd	0.305 bc

Note: Different letters, such as a, b, c means that the parameters of gas exchange significantly ($p < 0.05$) differed among chestnut populations.

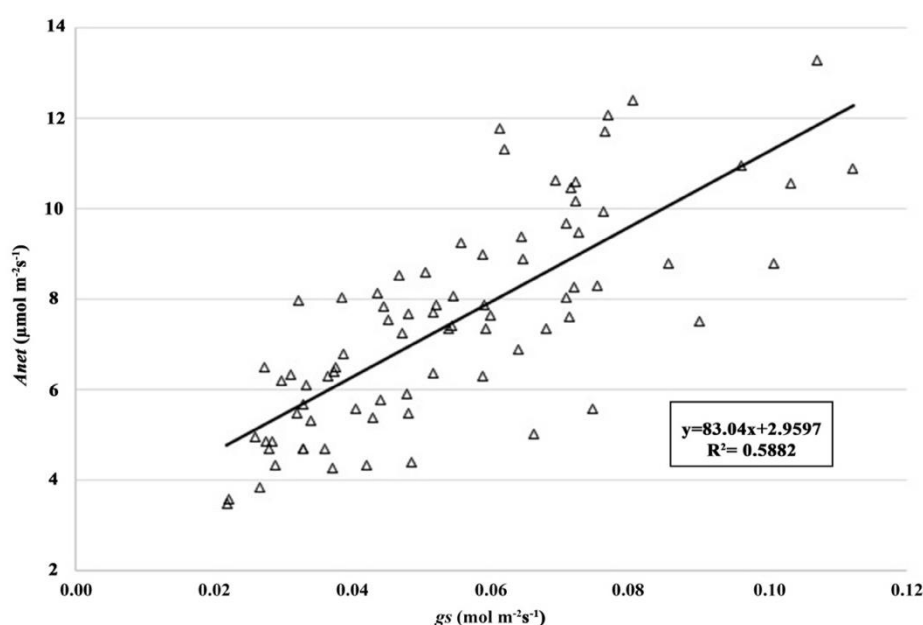


Figure 2. Relationship between gs and Anet within chestnut population

Leaf Gas Exchange Measurement

The ANOVA of leaf gas exchange measurements, such as *Anet*, *E*, *gs*, *WUE*, *iWUE*, *Ci*, and *Ci/Ca* among populations, are presented in Table 2. Chestnut population statistically differed ($P \leq 0.0001$) for each gas exchange parameter (Table 2). The averages and Tukey's test results of gas exchange parameters of chestnut populations are presented in Table 3.

When the chestnut populations are evaluated in terms of *Anet* amounts, the seedlings of the MAR population have the highest *Anet* amount. At the same time, the lowest average *Anet* value is found in the IBR population, followed by ERF and BUR populations (Table 3).

When the chestnut populations are evaluated in terms of *gs*, the seedlings of the ERF population have the highest average *gs* value, followed by MAR and BUR populations. The lowest *gs* values are determined in chestnut seedlings belonging to IBR populations (Table 3).

If we evaluate the chestnut populations in terms of *E*, the seedlings of the ERF population have the highest *E* values, followed by the BUR population. In contrast, the lowest *E* value is seen in the seedlings of the IBR population, followed by the seedlings of the OVA population (Table 3).

When the chestnut populations are evaluated in terms of *iWUE* amounts, the highest values are found in the OVA population seedlings, followed by the IBR population. The lowest *iWUE* values were determined in the AOM population, followed by the DKAB and BUR populations (Table 3).

When the chestnut populations are evaluated in terms of *WUE* amounts, the seedlings of the OVA population showed the highest value, followed by the group formed by the IBR, MAR, and SEL populations. The lowest *WUE* values were observed in a group consisting of AOM, DKAB, ERF, BUR, and DKAP populations (Table 3).

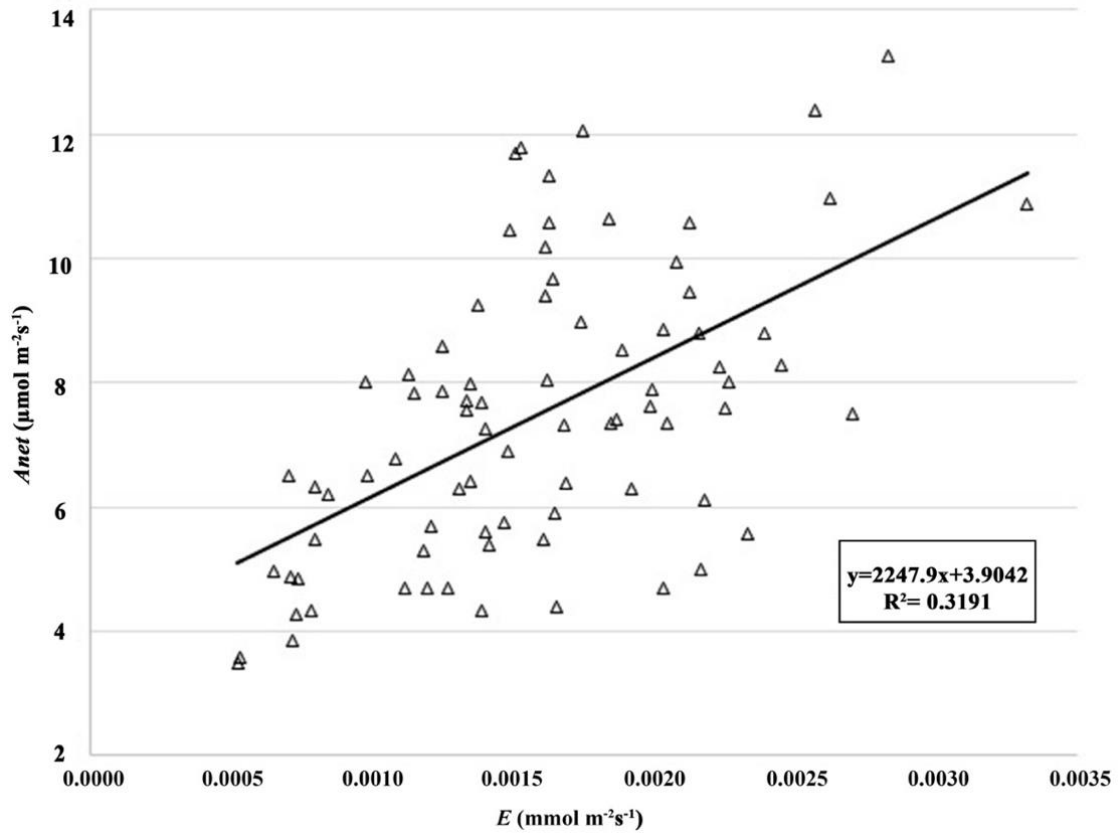


Figure 3. Relationship between E and Anet within chestnut population

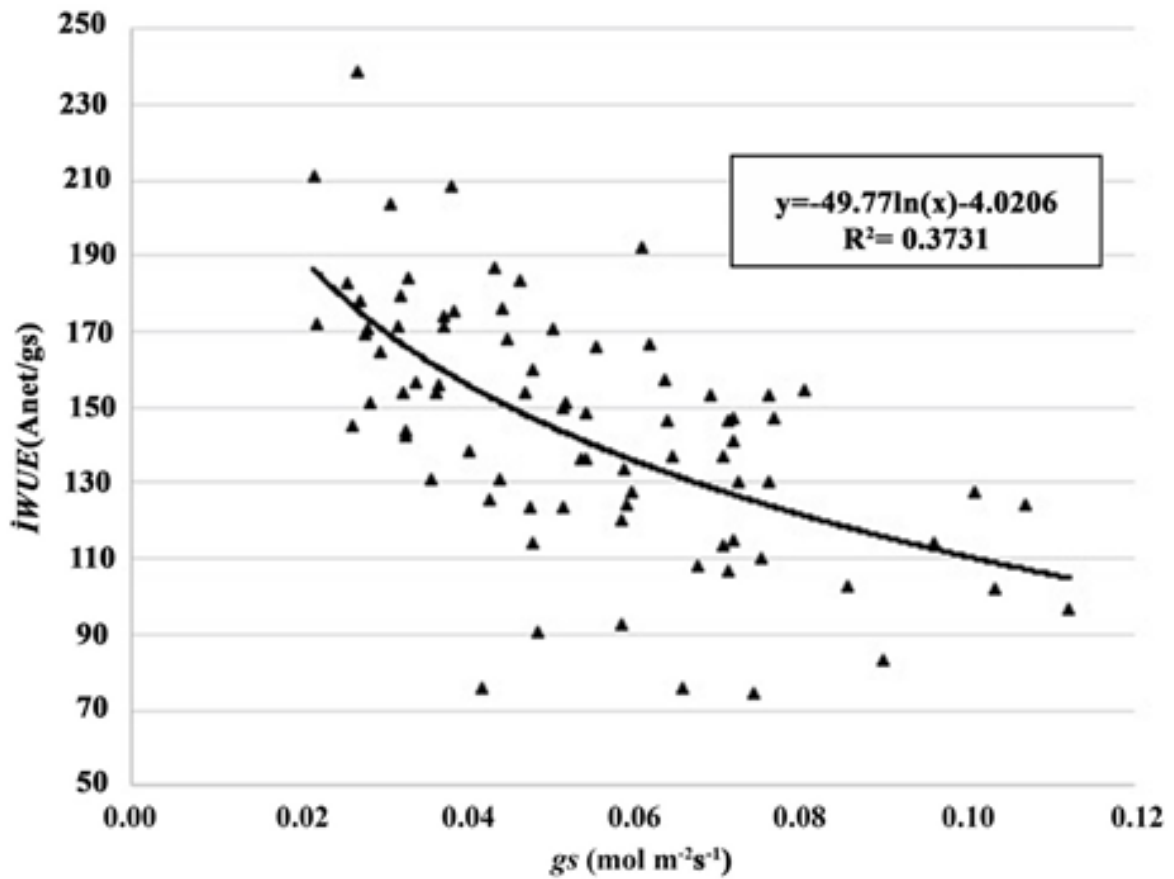


Figure 4. Relationship between g_s and $iWUE$ within chestnut population

When C_i values of chestnut populations were examined, AOM and DKAB populations had the highest values, whereas OVA and IBR showed the minimum C_i values (Table 3). When the C_i/C_a values of the chestnut populations are examined, the seedlings of the AOM and DKAB populations have the highest C_i/C_a values. In contrast, the OVA and IBR populations have the lowest values (Table 3).

In the current study, A_{net} correlated with g_s that increasing stomatal conductance increased A_{net} in chestnut populations (Figure 2). However, there was not a strong correlation between A_{net} and E in the seedlings of chestnut populations (Figure 3). There was a negative correlation between $iWUE$ and g_s , which was not robust (Figure 4).

Discussion and Conclusion

As a result of the study, it was determined that the leaf gas exchange parameters changed significantly based on the population, and even this change was determined to be approximately 2 times higher in terms of the A_{net} values amount among some populations (MAR and IBR). Regarding g_s , the differences between populations (ERF and IBR) changed approximately 2.5 times, and the differences (ERF and IBR) increased more than 3 times in terms of E values. These differences in $iWUE$ and C_i/C_a values were determined as 1.5 (OVA and DKAB) and 2 (AOM and OVA) folds, respectively. Reduced water content in the soil causes a decline in plant organs' water potential, causing a reduction in g_s , A_{net} , and E . Similar results were observed in *Acer negundo* and *Acer pseudoplatanus* (Koç, 2022a) and some conifer species seedlings (Koç 2021; Koç and Nzokou, 2022). A decline in g_s caused a reduction in A_{net} and E , which led to plants having different WUE and $iWUE$ traits. The different traits may play an essential role in plant growth and development (Xu et al. 2020).

Today, global climate change is one of the most critical glitches on a global scale, and this problem is considered irreversible (Varol et al. 2021; Tekin et al. 2022). It is estimated that global climate change will directly or indirectly affect all living things and ecosystems (Varol et al. 2022; Varol et al. 2022). Because the phenotypic features of all living things are shaped under the influence of genetic structure and environmental factors, the most apparent effects of global climate change will affect the climate parameters (Koç, 2022a; Yayla, 2022). It is estimated that the main effect of global climate change on climate parameters will manifest itself in the form of precipitation regime and temperature increase (Koç, 2022b; Koç, 2022c).

In this process, it is stated that factors such as fertilization deficiency, especially in marginal regions (Shults et al., 2018), increased temperature, more prolonged summer drought, and increased UV-B will significantly affect plant growth (Cantürk and Kulaç, 2021; Ozel et al. 2021). Because these factors, especially drought, are among the most critical stress facets affecting the development and growth of plants (Seleiman et al. 2021; Chaudhry and Sidhu, 2021; Koç, 2022a; Koç et al., 2022).

Summer temperatures and drought that will be prolonged due to global climate change will affect plant growth and may cause significant species and population losses (Varol et al. 2021). However, it is predicted that these impacts will have different levels of effects on a

species basis, and while some species have significantly narrowed their distribution areas, the appropriate distribution areas of some species will increase (Dyderski et al. 2018; Ning et al. 2021).

It is estimated that species resistant to drought conditions will be less affected in this process. Studies reveal that the drought tolerance of different species differs significantly (Sevik and Cetin, 2015; Yigit et al. 2016). In addition, it has been determined that different populations of the same species have different levels of drought tolerance (Sevik and Erturk, 2015; Koç and Nzokou, 2022). Therefore, less resistant species and population losses are inevitable due to the effects of global climate change. In order to minimize these losses, necessary measures should be taken without delay.

In order to diminish possible species and population losses in the global climate change process, it is necessary to determine the climatic changes that will occur in advance. Studies on the subject it has been tried to determine both the change of climate parameters (Koç, 2022b; Koç, 2022c) and how this change will change the appropriate distribution areas of various species (Cantürk and Kulaç, 2021; Tekin et al. 2022). It is essential to continue and diversify these studies to determine the climatic changes that may occur, which species will be affected by these changes, and to what extent.

Suggestions

In order to diminish the outcomes of climate change globally, especially species and population losses, these effects must be determined in advance. For this reason, projected model studies on the subject, determining the variables affecting this process, and determining possible changes in advance are priority issues.

In the next stage, to prevent deforestation, it is recommended to determine the most drought-resistant species and plant these species in appropriate areas, at least to include them in the mixture of the stands. Thus, although species and population losses are experienced in forested areas, at least the effects of deforestation and related erosion, loss of water resources, and large-scale loss of fauna can be reduced. Drought-resistant species in landscaping in urban areas are critical in preventing plant loss and saving water use.

One of the most critical measures that can be taken on the subject is the use of drought-resistant plant origins. Determining and using drought-resistant origins in the forest, agriculture, and landscape studies is very important in adapting to global climate change. In the studies to be carried out in this area, it is crucial to determine the drought-resistant origin and even individuals. For this purpose, the method used in the study can be recommended as a method that can be used to evaluate a large number of individuals in a short time, giving fast, cheap and reliable results.

Competing Interest / Conflict of Interest

The authors declare no conflict/competing interests.

Author Contribution

We declare that all authors equally contribute.

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