Kastamonu Uni., Orman Fakültesi Dergisi, 2024, 24(2): 209-219 Research Article
Kastamonu Univ., Journal of Forestry Faculty
Doi:10.17475/kastorman.1557438

Total Chlorophyll Content Variations in Some Oriental Beech (*Fagus orientalis* L.) Origins Exposed to Drought Stress

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Received Date: 14.04.2024 Accepted Date:11.06.2024

Abstract

Aim of study: Oriental beech (*Fagus orientalis* Lipsky.) seedlings of 4 different origins were divided into different irrigation regimes as "irrigation once a day (control)", "irrigation once every 2 days" and "no irrigation at all" in the 2nd growth period. The main aim of this study was to determine which origin was more tolerant to drought.

Area of study: Beech seedlings were produced from seeds originating from Bolu, Istanbul, Zonguldak and Giresun in the Greenhouse of Artvin Coruh University, Faculty of Forestry.

Material and methods: The beech seeds used in the study were collected by the Regional Directorates in 2021. The seeds were planted in polyethylene tubes Greenhouse. As of July 2023, Oriental beech seedlings in their second growth period were subjected to three different irrigation, total chlorophyll contents were measured by using the hand-held SPAD-502 Plus device and the data were analyzed statistically.

Main results: In the variance analysis made according to different irrigation regimes in terms of total chlorophyll contents, statistically significant ($p<0.05$) differences were detected between the origins, and in the Duncan Test used to determine which groups the differences originated from, it was found that all the origins were in 12 separate groups. It was also understood that the chlorophyll content of the origins was less in the regimes of irrigation once every 2 days and no irrigation at all, compared to the regime of irrigation once a day (control).

Research highlights: It was determined that total chlorophyll contents decreased in all origins in regimes exposed to drought stress compared to the control regime, but this change was less in the Istanbul (180 m) origin than in the other origins.

Keywords: Oriental Beech, SPAD-502 Plus, Drought Stress, Total Chlorophyll Content, Irrigation

Kuraklık Stresine Maruz Bırakılan Bazı Doğu Kayını (*Fagus orientalis* L.) Orijinlerinde Toplam Klorofil İçeriği Değişimleri

Öz

Çalışmanın amacı: 4 farklı orijinli Doğu kayını (*Fagus orientalis* Lipsky.) fidanları 2. büyüme döneminde "günde 1 sulama (kontrol)", "2 günde 1 sulama" ve "hiç sulamama" olarak farklı sulama rejimlerine ayrılmıştır. Hangi orijinin kuraklığa daha toleranslı olduğunun belirlenmesi bu çalışmanın ana amacıdır.

Çalışma alanı: Bolu, İstanbul, Zonguldak ve Giresun orijinli tohumlardan Artvin Çoruh Üniversitesi, Orman Fakültesi Serasında kayın fidanları yetiştirilmiştir.

Materyal ve yöntem: Araştırmada kullanılan kayın tohumları 2021 yılında ilgili Bölge Müdürlükleri tarafından toplanmıştır. Toplanan tohumlar Serada polietilen tüplere ekilmiştir. Temmuz 2023 itibariyle 2. büyüme dönemindeki Doğu kayını fidanları üç farklı sulama rejimine tabi tutulmuştur. Toplam klorofil içerikleri SPAD-502 Plus el tipi cihazı kullanılarak ölçülmüştür ve veriler istatistiksel olarak analiz edilmiştir.

Temel sonuçlar: Toplam klorofil içerikleri bakımından farklı sulama rejimlerine göre yapılan varyans analizlerinde orijinler arasında istatistiksel olarak anlamlı ($p<0.05$) farklılıklar olduğu, farklılıkların hangi gruplarda olduğunu belirlemek için yapılan Duncan testinde ise orijinlerin tamamının 12 ayrı gruplarda yer aldığı tespit edilmiştir. Orijinlerin klorofil içeriklerinin 2 günde 1 sulama ve hiç sulama yapılmayan rejimlerinde, günde 1 sulama (kontrol) yapılan rejimine kıyasla daha az olduğu anlaşılmıştır.

Araştırma vurguları: Toplam klorofil içerikleri kuraklık stresine maruz bırakılan rejimlerde, kontrol rejimine kıyasla, her orijinde azaldığı fakat İstanbul (180 m) orijininde diğer orijinlere göre bu değişimin daha az olduğu belirlenmiştir.

Anahtar Kelimeler: Doğu Kayını, SPAD-502 Plus, Kuraklık Stresi, Toplam Klorofil İçeriği, Sulama

Citation (Atıf): Isik Cakmakci, S., & Guner, S. (2024). Total Chlorophyll Content Variations in Some Oriental Beech (*Fagus orientalis* L.) Origins Exposed to Drought Stress. *Kastamonu University Journal of Forestry Faculty*, 24 (2), 209-219.

Introduction

With its effects and consequences, climate change is a fundamental problem closely concerning the entire world as one of the most important global and regional environmental problems threatening the future (Anonymous, 2022; Bakhoum et al., 2023). Water deficiency due to drought damages photosynthetic pigments, changes cell structure and function, disrupts metabolism, slows down nutrient uptake and transport rate, increases plant energy consumption, reduces plant growth and plant quality and might even cause plant death (Ali & Ashraf, 2011; Dawood & Sadak, 2014; Zhu, 2016; Chakraborty et al., 2016; Nan et al., 2018; Bakhoum et al., 2019). If drought continues, plants become stressed and may respond differently in biological and phenological terms.

If the environmental conditions of a plant change enough to affect growth and development negatively, the condition that occurs in the plant is defined as "stress" (Büyük et al., 2012; Bakry et al., 2012). Plants are affected by many stress factors, which are examined in two groups (biotic and abiotic) (Levitt, 1972). Abiotic stress factors consist of cold, heat, drought, salinity, excess water, radiation, various chemicals, oxidative stress, wind, and nutrient deficiency, and biotic factors consist of pathogens, insects, and herbivores, including viruses, bacteria, and fungi (Yılmaz et al., 2011; Sadak, 2016; Bakhoum et al., 2023).

Drought stress occurs when the amount of water needed by the plant in the soil decreases and water loss continues as a result of transpiration and evaporation under the effect of atmospheric conditions (Kacar et al., 2002; Khajeh-Hosseini et al., 2003; Elewa et al., 2017a). Metabolic irregularities occurring because of drought stress affect chlorophyll synthesis negatively. It has been reported that chlorophyll content decreases in plants under drought stress (Mishra & Singh, 2011; Arbona et al., 2013; Elewa et al., 2017b; Ezzo et al., 2018). They reported that one symptom of drought stress in leaves was the loss of chlorophyll, which indicates some kind of degradation in chloroplasts (Dawood & Sadak, 2014; Sadak, 2016). Drought stress decreases the chlorophyll content in leaves, causing a decrease in photosynthetic activity (Anjum et al., 2011b).

The plant closes its stomata when a water loss occurs in plants and tries to preserve the existing water not to lose more of it (Türkan et al., 2005; Osakabe et al., 2014). After the stomata closure, there is a decrease in CO_2 intake, which causes CO_2 limitation needed for photosynthesis fixation (Chaves et al., 2003). Water deficiency during photosynthesis leads to decreased photosynthetic efficiency because of increased accumulation of reactive oxygen species (Hasanuzzaman et al., 2013). The decreased photosynthesis rate and chlorophyll content under dry conditions is an indicator of oxidative stress, which causes chlorophyll deterioration (Anjum et al., 2011a; Marcińska et al., 2013; Sadak et al., 2019).

The forest area was 20.2 million ha in 1973 in our country, 20.8 million ha in 1999, 21.2 million ha in 2004, 21.7 million ha in 2012, 22.3 million ha in 2015, 22.9 million ha in 2020, 23.1 million ha in 2021, 23.2 million ha in 2022 (URL-1). In the distribution of Türkiye's forest areas according to tree species, Oriental beech (*Fagus orientalis* Lipsky.) ranks 4th and covers an area of 1.9 million hectares (URL-2). For this reason, it is very important in terms of forestry. Oriental beechhas many usage areas. Beech wood is hard, strong and highly resistant to impacts and can be steamed. It can be used as fuel, and in the coating, furniture, parquet, mining pole and paper industries (Kandemir & Kaya, 2009). Oriental beech has a wide distribution area in Thrace, Istranca Mountain, Tekirdağ and Belgrad forest, Aegean and Marmara basins, and Northern Anatolia in our country. It is also distributed in the Adana Pos Forests in the south of Anatolia, in the north of the Amanos Mountains, and the Maraş-Andırın section (Saatçioğlu, 1969; Kayacık, 1980). Oriental beech requires a climate where there is balanced rainfall, temperature extremes are not too high and relative humidity is high. Also, it continues to spread in climates where summer temperatures are less than 22°C and winters are cold. High sunshine periods and strong insolation damage the barks and shade leaves (Ata, 1995). The maritime climate meets the demands of the oriental beech species because of the amount of precipitation

and the absence of frost in areas with sea influence. Annual rainfall is around 1200 mm in the areas where oriental beech is distributed (Saatçioğlu, 1976; Suner, 1982; Anonymous, 1985). Severe summer droughts (such as those in 1976, 2003 and 2018/19) caused petals to fall and branches and trees to die in various beech forests (Rennenberg et al., 2004; Geßler et al., 2007).
Studies conducted to identify plant species that can resist drought stress and have high yields have been increasing recently. Because drought-resistant genotypes have the ability to increase yield in conditions where water is scarce, maintain important functions of cellular metabolism, and recover water balance and plant function after stress quickly (Waseem et al., 2011). It is important in breeding studies to investigate the biochemical, genetic, and physiological metabolism of plants in the production and selection of plant species that are resistant to drought stress (Yüksel & Aksoy, 2017). In this study, seedlings originating from Bolu, Istanbul, Zonguldak and Giresun were grown. The aim of the study was to determine which origin was more tolerant to drought according to their total chlorophyll contents by applying drought stress to oriental beech seedlings in the second growth period. It is expected that the results of the study will contribute to determining drought-tolerant origins and using them in suitable growing environments, determining future afforestation strategies, and creating the infrastructure for seed transfer, breeding studies and active gene protection.

Material and Method

The seeds used in the study were collected from the beech trees in Bolu (875 m), Istanbul (180 m), Zonguldak (500 m) and Giresun (1290 m) regions, which are among the regions where beech is naturally distributed in our country, in the regional directorates in 2021. The locations of Oriental beech origins on the map of Turkiye are given in Figure 1 and information about the origins is given in Table 1.


Figure 1. Locations of Oriental beech origins on the map of Türkiye

Table 1. The general datas on the origins

General datas	Origins			
	Bolu	İstanbul	Zonguldak	Giresun
Regional Directorate of Forestry	Bolu	İstanbul	Zonguldak	Giresun
Forest Management Directorate	Yığılca	Kanlıca	Alaplı	Ordu
Forest Management Directorate	Karadere	Ömerli	Bendere	Akkuş
Latitude	40^0 52 ׳ 58,17 ׳׳ N	41^0 05׳ 40,09 ׳׳ N	41^0 04 ׳ 48,14 ׳׳ N	40^0 47 ׳ 13,25 ׳׳ N
Longitude	31^0 24 ׳ 40,51 ׳׳ E	29^0 22 ׳ 47,33 ׳׳ E	31^0 34 ׳ 04,42 ׳׳ E	37^0 02 ׳ 33,35׳׳ E
Stand	Knd2	Knbc3	Knd3	Kndcd3
Section	79	93	63	168
Altitude (m)	875	180	500	1290
Slope (%)	35	14	34	25
Exposure	East	North	Southeast	East

The soil mixture in the greenhouse was prepared as forest soil, perlite, peat and animal manure (5:1:1:1). Irrigation and maintenance (e.g., weeding) operations were performed at regular intervals until the 2nd growth period of the seedlings. As of 2023, 120 tube seedlings were produced from each origin. Three different irrigation regimes were applied to each origin group for 15 days. The total chlorophyll contents of all seedlings of the origins allocated to irrigation regimes were measured at the end of 15 days and 3 leaves of each seedling were selected randomly. When chlorophyll contents were measured (absorbance), a portable handheld SPAD-502 Plus device was used, which can measure the chlorophyll content of the leaves without removing them from the branch. The measurements were made from three different points of each leaf (tip, middle and close to the petiole) with the device that quickly measures the chlorophyll content in the leaf as a numerical SPAD data and the average of the measurement values was taken. Chlorophyll Concentration Index (CCI) values were obtained with SPAD measurements. The effects of three different irrigation tests on the origins were determined with the Analysis of Variance (ANOVA), and the differences in which origins and irrigation regimes were determined were determined by the Duncan test.

Results

The seedlings that were produced from the seeds collected from Zonguldak (500 m), Istanbul (180 m), Bolu (875 m), and Giresun (1290 m) were subjected to irrigation treatments such as “once a day”, “once every 2 days” and “not irrigation at all”. According to the analysis of variance, these processes caused significant ($p<0.05$) changes in the total chlorophyll contents of the leaves (Table 2). In the Duncan Test, which was used to determine which groups the differences were in, it was found that each process significantly created 12 different groups (Table 3). Variations in the chlorophyll content of the origins are given in Figure 2.

Table 2. Analysis of variance results for total chlorophyll content

	Variance source	Sum of squares	Degrees of freedom	Mean squares	F value	Confidence Level (P)
SPAD(CCI)	Intergroup	18901.98	11.00	1718.36	5560.92	0.000
	Intragroup	139.05	450.00	0.31		
	Total	19041.03	461.00			

Table 3. Total chlorophyll contents measured at the origins according to irrigations SPAD (CCI)

Irrigation regimes	Origins	Mean ± Standard Deviation
Irrigation once a day (control)	Bolu	24.91±0.6[j*]
	Istanbul	40.09±0.54[a]
	Zonguldak	34.81±0.61[d]
	Giresun	30.2±0.49[g]
Irrigation once every 2 days	Bolu	21.51±0.31[k]
	Istanbul	37.49±0.33[b]
	Zonguldak	32.51±0.33[e]
	Giresun	27.61±0.96[h]
No irrigation at all	Bolu	18.87±0.59[l]
	Istanbul	36.47±0.31[c]
	Zonguldak	31.53±0.33[f]
	Giresun	25.28±0.75[i]

* Different letters indicate different groups in the columns.

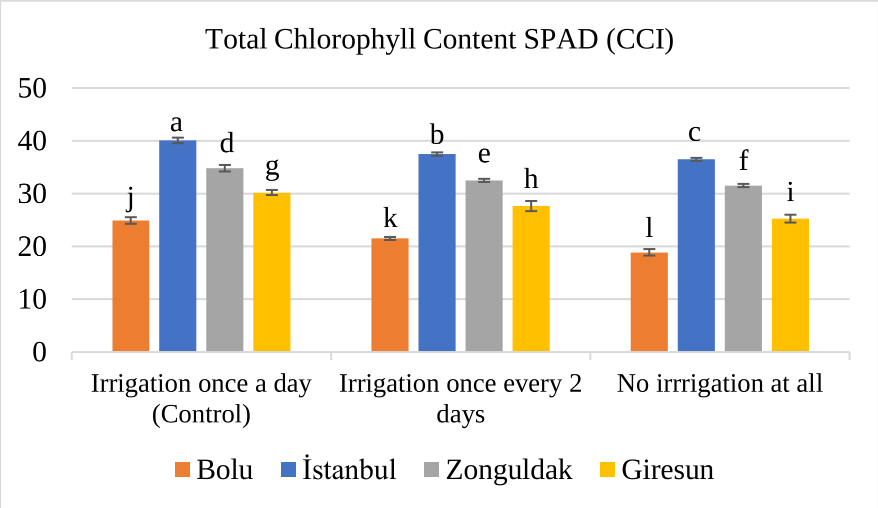

Figure 2. Variations in chlorophyll content according to origins

In all origins, it was found that the chlorophyll content decreased compared to the control, as expected when the seedlings were not irrigated and were irrigated once every 2 days. Among the origins tested, the origin with the highest chlorophyll content was the Istanbul (180 m) origin (40.09 CCI), which was irrigated once a day (control), and the lowest was the Bolu (875 m) origin (18.87 CCI), which was no irrigated at all. Similarly, the total chlorophyll content was the highest in the Istanbul origin (36.47 CCI) and the least in the Bolu origin (18.87 CCI) in the no-watering regime.

Discussion and Conclusions

Chlorophyll pigments show the response of plants to their habitat, weather conditions, and anthropogenic conditions, thus the vitality of plants and their resistance to stressful conditions because chlorophyll pigments are effective in photosynthesis (Bakry et al, 2019; Zielewicz et al, 2020). In general, there is decreased chlorophyll content under drought stress (Ziska et al., 1990, Sairam & Saxena, 2000; Khan & Beena, 2002; Ashraf, 2003; Hayatu & Mukhtar, 2010; Kocaçınar et al., 2010; Alizadeh et al., 2011; Zanjani et al., 2012; El Atta et al., 2012; Fini et al., 2012; Kulaç et al., 2012; Wu et al., 2013; Maatallah et al., 2016; Yavaş & Unay, 2016; Tariq et al., 2018; Guo et al., 2019; Sadak et al., 2020a; Abd Elhamid et al., 2021; Sadak & Bakhoum, 2022). Similarly, in our study, chlorophyll content was found to be lower in drought stress compared to the control in all origins. However, there was less difference in total chlorophyll content in the Istanbul origin compared to other origins. Based on this, Bolu (875 m), Zonguldak (500 m) and Giresun

(1290 m) origins tested in this study have low tolerance to drought stress and Istanbul (180 m) origin have high tolerance to drought stress. The chlorophyll content is one of the main factors for plant growth in plants (Farquhar & Richards, 1984). Annual and seasonal differences in pigments in plants are directly associated with adverse development conditions such as high light intensity in summer, very low temperatures in winter and seasonal water stress (Sauceda et al., 2008; Kancheva et al., 2014). It was reported that high temperature and light cause the chlorophyll content to decrease (Brett & Singer, 1973). Mafakheri et al. (2010) reported that drought stress applied during plant growth significantly reduced the total chlorophyll content. Arslan (2017) conducted a trial in the second vegetation period of Narrow Leaf Ash (*Fraxinus angustifolia* Vahl.) 1+0-year-old tube seedlings, S1: 280 ml every 2-3 days, S2: 140 ml every 3-4 days and S3: 70 ml every 3-4 days, and reported that the total chlorophyll content decreased from June to September. In their study, Akça & Yazıcı (1999) applied 4 different irrigation amounts to 2-year-old seedlings grown from red pine seeds and stated that the highest chlorophyll amount was seen in the 675 mm/year irrigation regime, while there was a decrease in the chlorophyll amount in the samples applied at 225 and 450 mm/year. Gallé & Feller (2007) conducted another study in which they applied 36-day drought stress by dividing approximately 1.3 m tall, 4-year-old beech seedlings (*Fagus sylvatica* L.) into 2 groups (control and unirrigated) and reported that the chlorophyll content per leaf area decreased on the 36th day in stressed plants compared to control plants. In the study conducted by Hájíčková et al. (2024) beech seedlings were exposed to three different irrigation regimens for 25 days (well-irrigated (W), moderately drought-exposed (M), and severe drought-exposed (S) and M and S seedlings were exposed to three different irrigation intensities. It was reported that there was a decrease in photosynthesis rate and chlorophyll fluorescence parameters. They also reported that the breakdown of photosynthetic pigments, which causes a decrease in photosynthesis and a delay in growth, is a known sign of drought stress (Sadak & Ramadan 2021; Abdalla et al., 2022).

It was reported in previous studies that drought causes chlorophyll synthesis to slow down and the chlorophyll content in the leaves to decrease (Ramanjulu et al., 1998; Singer et al., 2002; He et al., 2016; Chen et al., 2019; Sadak et al., 2020b; Bakhoum et al., 2022). In his study conducted on Scots pine (*Pinus sylvestris*), Kulaç (2010) reported that there was a decrease in leaves chlorophyll content in all irrigation methods (twice a week, once a week, and once every 15 days) during the growth period from May to July. In her study on the *Quercus petraea* subsp. iberica, Atar (2021) reported that the total chlorophyll amount in the drought group was lower than the control group. In a study conducted on juniper species, it was reported that there was a decrease in the amount of chlorophyll pigment because of drought and water loss (Brett & Singer, 1973). In their study investigating the effects of long-term drought on the chlorophyll capacity in the leaves of the Mango plant (*Mangifera indica* L.), Damour et al. (2009) reported that the amount of chlorophyll decreased in the months when drought stress intensified. Baquedano & Castıllo (2007) investigated the chlorophyll contents of four Mediterranean evergreen plants (*Quercus ilex*, *Quercus coccifera*, *Pinus halepensis*, and *Juniperus phoenicea*) to determine drought resistance. As a result of the study, it was reported that the chlorophyll content of the tested trees decreased in summer. Yang et al. (2007) investigated the effects of drought stress in the region where there were *Picea asperata* Mastters seedlings in two different irrigation regimes with field capacity (100% and 30%) and two different light systems (100% high light and 15% low light) and reported that severe drought stress reduced chlorophyll contents. In their 10-day drought stress trial, Pšidová et al. (2015) reported that the sample most resistant to water stress among the beech seedlings taken from 3 different regions (Divín-warm-530 m, Dobšiná-moderately warm-625 m, Pohorelá-cool-1.250 m) was the one taken from drier seedlings taken from low-altitude Divín. Özden (2009) conducted a study on Volcanic oak (*Quercus vulcanica* Boiss & Heldr, Ex, Kotschy), and Gray holm oak (*Quercus*

Aucheri Jaub & Spach) for a certain period (1, 2 and 4 weeks), and studied drought. He determined that chlorophyll contents decreased as a result of drought stress. It is seen that the chlorophyll content findings obtained in the present study are compatible with the findings of many other studies conducted on drought stress.

In conclusion, it was determined that the most tolerant origin to drought stress was the Istanbul (180 m) origin since there was less change in total chlorophyll content in the Istanbul origin compared to the other origins in the regimes exposed to drought stress, compared to the control regime. This change was as follows in %: Total chlorophyll contents (compared to the regime watered once a day (control)), were measured little in Bolu origin (13.65%), Giresun origin (8.58%), Zonguldak origin (6.61%), Istanbul origin (6.49%) in the regime watered once every 2 days, and were measured little in Bolu origin (24.25%), Giresun origin (16.29%), Zonguldak origin (9.42%), and Istanbul origin (9.03%) in the regime that was not watered at all compared to the regime irrigated once a day (control). When the climate and habitat conditions represented by the origins under drought stress and the findings obtained from this study were evaluated, it was found that the origins reflected the characteristics of the climate values they represented in terms of drought resistance, and the origins in regions where precipitation was low and temperature was higher were more tolerant to drought stress. Also, considering the difference in altitude values, it was found that the origins at lower altitudes, where drought was more common, responded more to drought stress. It is considered that the success of afforestation in semi-arid and arid regions will be increased by producing seedlings from the origins of the lower slopes, where the amount of precipitation is less and the temperature is higher, without leaving the seed transfer areas. In this context, considering the results of the study, it is recommended to use Istanbul origins, which are found to be more tolerant to drought stress, in places where summer drought is more, by taking into account the altitude and seed transfer areas in the afforestation works to be performed in the Western Black Sea and Marmara regions. In a possible future drought in our country, it is considered that Oriental beech seeds originating from Istanbul will survive if the severity of the drought is not very serious. To reach more detailed results, more drought stress studies must be conducted with trials on Oriental beech origins, which are distributed throughout the Black Sea and locally in the Hatay-Amanos mountains in the Mediterranean region.

Acknowledgment

The present study was produced from a part of the doctoral thesis "Investigation of basic morphological and physiological changes in some Oriental beech origins (*Fagus orientalis* Lipsky.*)* exposed to drought stress" conducted at Artvin Coruh University, Faculty of Forestry, Department of Forestry Engineering. We sincerely thanks to Artvin Coruh University Graduate Education Institute.

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conceptualization: S.G.; Investigation: S.I.Ç., S.G.; Material and Methodology: S.I.Ç.; S.G., Supervision: S.G.; Visualization: S.I.Ç.; Writing-Original Draft: S.I.Ç., S.G.; Writing-review & Editing: S.I.Ç., S.G.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The author has no conflicts of interest to declare.

Funding

The author declared that this study has received no financial support.

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