



SEASONAL CHANGE OF CHLOROPHYLL CONTENT (SPAD VALUE) IN SOME TREE AND SHRUB SPECIES

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ABSTRACT: Chlorophyll, which is of vital importance for living things, is the main active ingredient of photosynthesis, and the amount of chlorophyll in the plant can show very sensitive responses to various environmental factors, especially site conditions and plant species. In this study, it was aimed to determine the change of chlorophyll content (SPAD value) at the beginning (20 May) and the end (15 October) of growth period in some native and exotic tree and shrub species in Kanuni campus of Karadeniz Technical University and to reveal the differences between the species. Within the scope of the study, measurements were made on a total of 20 species including five native (*Quercus hartwissiana* Steven, *Fagus orientalis* Lipsky, *Ulmus minör* Mill., *Liquidambar orientalis* Mill., *Quercus pubescens* Willd.) and seven exotic (*Eucalyptus camadulensis* Dehnh., *Ginkgo biloba* L., *Quercus castaneifolia* C.A.Mey., *Cinnamomum camphora* (L.) Sieb., *Acer negundo* L., *Quercus rubra* L., *Aesculus hippocastanum* L.) tree species and seven native (*Cercis siliquastrum* L., *Laurus nobilis* L., *Osmanthus decorus* (Boiss.&Balansa) Kasapligil, *Laurocerasus officinalis* M.Roem., *Ostrya carpinifolia* Scop., *Corylus avellana* L., *Arbutus unedo* L.) and one exotic (*Weigela coraensis* Thunb.) shrub species. Chlorophyll content was determined with four replications by portable chlorophyll meter (Minolta SPAD-502, Osaka, Japan) which indirectly measures the amount of chlorophyll in the leaf. The data obtained were subjected to Wilcoxon test, variance analysis (one-way ANOVA) and Duncan's test by using SPSS 23.0 statistical program. As a result of the study, it was determined that there are statistically significant differences ($p<0.05$) between SPAD values of May and October of species except *Aesculus hippocastanum*, *Laurus nobilis*, *Cinnamomum camphora*, *Eucalyptus camaldulensis* and *Liquidambar orientalis*. In addition, it was found that there are significant differences at 99% confidence level among the species

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in both periods. As a result of Duncan's test, nine different groups occurred in May measurements and 15 different groups occurred in October measurements. While the average SPAD value was the highest (78.6 and 70.6, respectively) in *Laurocerasus officinalis* in both periods, the lowest was in *Acer negundo* (25.0) in May and *Quercus hartwissiana* (24.0) in October.

Keywords: Species, photosynthesis, chlorophyll, minolta

BAZI AĞAÇ VE ÇALI TÜRLERİNDE KLOROFİL İÇERİĞİNİN (SPAD DEĞERİ) MEVSİMSSEL DEĞİŞİMİ

ÖZET: Canlılar için hayati öneme sahip olan klorofil, fotosentez olayının başlıca etken maddesi olup, bitkideki klorofil miktarı yetiştirme yeri koşulları ve bitki türü başta olmak üzere çeşitli çevresel faktörlere karşı oldukça hassas tepkiler gösterebilmektedir. Bu çalışmada, Karadeniz Teknik Üniversitesi Kanuni yerleşkesi içerisinde bulunan bazı doğal egzotik ağaç ve çalı türlerinde klorofil içeriğinin (SPAD değeri) büyüme dönemi başlangıcında (20 Mayıs) ve sonundaki (15 Ekim) değişiminin belirlenmesi ve türler arasındaki farkların ortaya konması amaçlanmıştır. Çalışma kapsamında Kanuni yerleşkesinde yer alan beş adet doğal (*Quercus hartwissiana* Steven, *Fagus orientalis* Lipsky, *Ulmus minör* Mill., *Liquidambar orientalis* Mill., *Quercus pubescens* Willd.) ve yedi adet egzotik (*Eucalyptus camadulensis* Dehnh., *Ginkgo biloba* L., *Quercus castaneifolia* C.A.Mey., *Cinnamomum camphora* (L.) Sieb., *Acer negundo* L., *Quercus rubra* L., *Aesculus hippocastanum* L.) ağaç türü ile yedi adet doğal (*Cercis siliquastrum* L., *Laurus nobilis* L., *Osmanthus decorus* (Boiss.&Balansa) Kasapligil, *Laurocerasus officinalis* M.Roem., *Ostrya carpinifolia* Scop., *Corylus avellana* L., *Arbutus unedo* L.) ve bir adet egzotik (*Weigela coraensis* Thunb.) çalı türü olmak üzere toplam 20 adet türde ölçümler gerçekleştirilmiştir. Klorofil içeriği, yapraktaki klorofil miktarını dolaylı olarak ölçen, taşınabilir klorofil metre cihazı (Minolta SPAD-502, Osaka, Japan) ile 4 tekerrürlü olarak tespit edilmiştir. Elde edilen veriler SPSS 23.0 istatistik programı yardımıyla değerlendirilerek, Wilcoxon testi, varyans analizi (one-way Anova) ve Duncan's testi yapılmıştır. Çalışma sonucunda *Aesculus hippocastanum*, *Laurus nobilis*, *Cinnamomum camphora*, *Eucalyptus camaldulensis* ve *Liquidambar orientalis* dışındaki türlerin Mayıs ve Ekim ayına ait SPAD değerleri arasında istatistiksel olarak anlamlı ($p<0.05$) farklılıklar olduğu belirlenmiştir. Ayrıca, her iki dönemde de türler arasında %99 güven düzeyinde anlamlı farklılıklar bulunduğu tespit edilmiştir. Duncan's testi sonucunda Mayıs ayı ölçümlerinde dokuz farklı grup, ekim ayı ölçümlerinde 15 farklı grup meydana geldiği saptanmıştır. Ortalama SPAD değeri her iki dönemde de *Laurocerasus officinalis* türünde en yüksek (sırasıyla 78.6 ve 70.6) iken en düşük değer Mayıs ayında *Acer negundo* (25.0), Ekim ayında *Quercus hartwissiana* (24.0) türünde belirlenmiştir.

Anahtar kelimeler: Tür, fotosentez, klorofil, minolta

INTRODUCTION

In terms of energy, all living organisms are dependent on photosynthesis and the origin of nutrients and oxygen in the atmosphere is photosynthesis. The plants that photosynthesize creates the bottom of the food pyramid and the life of the world depends on the plants (Öncel et al., 2004; Yiğit, 2016). This importance of plants is due to their ability to perform

photosynthesis and the life cycle in the world depends on the phenomenon of photosynthesis (Monsi et al., 1973; Kacar et al., 2009; Şevik et al., 2016).

Chlorophyll content in the plant is one of the most important factors for plant growth (Farquhar & Richards, 1984). Leaf chlorophyll level is directly related to plant stress and aging (Hendry et al., 1987; Terzi et al., 2010; Zhang et al., 2011; Gholamin & Khayatnezhad, 2011). Chlorophyll are pigments that are essential in the conversion of light energy into chemical energy. The amount of radiation absorbed from the sun also depends on the photosynthetic amount in the leaf. Therefore, the content of chlorophyll is related to photosynthetic activity and primary production (Curran et al., 1990; Nageswara et al., 2001; Saeidi et al., 2009). Green plants have many important functions that affect the life of other living creatures. Plants reduce noise (Arıcak et al., 2016), air pollution (Kaya, 2009; Kaya et al., 2015; Çetin, 2017; Şevik et al., 2017) and wind speed in their environment, provide save energy (Çetin, 2015b) and have a positive psychological effect on people (Çetin, 2015a).

One of the important activity areas for people living in the city is urban green. Plants add aesthetic value to their environment and are therefore indispensable elements of landscape studies. Plants used in landscape studies fulfill many functions (social, aesthetic, ecological, economical) at the same time (Fallahchai et al., 2013). Since the color of the leaves of the plants is especially important in terms of aesthetics, it plays an important role in the selection of plants to be used in landscape studies. Plants with different shades of green colors, like leaves of different colors, are also highly preferred for aesthetic uses. The differences in the green color tone of the leaves of plants are directly related to the amount of chlorophyll in the content of the leaf (Kaya, 2009; Kaya et al., 2015; Çetin, 2017). In addition, the chlorophyll pigments in the plant can show very sensitive responses to various environmental factors (Lepeduš et al., 2003).

Although there are many studies for determining chlorophyll content of various plant species (Brett & Singer, 1973; Saucedo et al., 2008; Atar et al., 2013; Çetin, 2016; Zeren et al., 2017b; Zeren et al., 2018), there are not many studies that demonstrate the effect of seasonal change on chlorophyll content in Turkey. In this study, it was aimed to determine the change of chlorophyll content at the beginning (20 May) and the end (15 October) of the growth period in some natural and exotic tree and shrub species in Kanuni campus of Karadeniz Technical University and to reveal the differences between the species.

MATERIALS AND METHODS

The study was carried out on native and exotic tree and shrub species located in the Kanuni campus of Karadeniz Technical University in Trabzon (Figure 1). Accordingly, a total of 20 deciduous species including 5 native and 7 exotic tree species, and 7 native and 1 exotic shrub species were selected as examples. Information about the selected species are given in Table 1. Chlorophyll measurements were carried out in two different periods to reveal the change in chlorophyll content at the beginning of the growth period (20 May 2019) and at the end (15 October 2019). Additionally, the climate data 2019 year belonging to Trabzon province, where Karadeniz Technical University is located, is given in Table 2. According to the climate data of the area where the selected species is located, the average temperature in May and October is 18.6 °C and 19.1 °C, respectively.

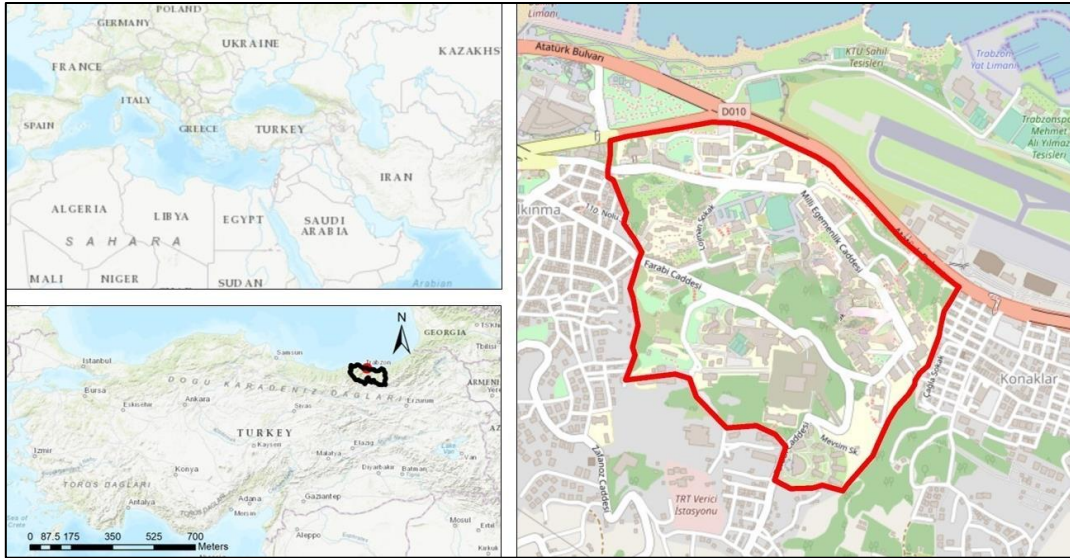


Figure 1. Geographical location of the study area

Table 1. Information on the species whose chlorophyll content (SPAD value) is determined

		Scientific name of taxon	
		Native species	Exotic species
Life Form	Tree	<i>Quercus hartwissiana</i> Steven	<i>Eucalyptus camaldulensis</i> Dehnh.
		<i>Fagus orientalis</i> Lipsky	<i>Ginkgo biloba</i> L.
		<i>Ulmus minor</i> Mill.	<i>Quercus castaneifolia</i> C.A.Mey.
		<i>Liquidambar orientalis</i> Mill.	<i>Cinnamomum camphora</i> (L.) Sieb.
		<i>Qercus pubescens</i> Willd.	<i>Acer negundo</i> L.
Shrub			<i>Quercus rubra</i> L.
			<i>Aesculus hippocastanum</i> L.
		<i>Cercis siliquastrum</i> L.	
		<i>Laurus nobilis</i> L.	
		<i>Osmanthus decorus</i> (Boiss.&Balansa) Kasapligil	
		<i>Laurocerasus officinalis</i> M.Roem.	<i>Weigela coraensis</i> Thunb.
	<i>Ostrya carpinifolia</i> Scop.		
	<i>Corylus avellana</i> L.		
	<i>Arbutus unedo</i> L.		

Chlorophyll content was determined with four replications from the leaves in the north, south, east and west directions on each individual with the portable chlorophyll meter device (Minolta SPAD-502, Osaka, Japan), which indirectly measures the amount of chlorophyll in the leaf. Chlorophyll measurement was determined by measuring three times on a leaf (from the tip and middle of the leaf, and the part near the petiole) and taking the average SPAD value. The chlorophyll meter was manufactured by designing with the principles of Inada (1963). It determines the relative chlorophyll density by measuring the red and infrared regions (650 nm and 940 nm wavelength, respectively) in leaf tissue.

Table 2. The average meteorological values of the study area

	Climate Year (2019)											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1	10.1	8.8	9.1	12.0	18.6	24.4	23.6	24.4	21.7	19.1	15.5	12.2
2	13.0	10.6	11.4	14.4	21.5	26.6	26.1	26.6	24.1	21.4	18.8	14.8
3	7.3	7.0	6.5	9.5	15.4	22.2	21.2	22.2	19.3	16.8	12.2	9.6
4	7.0	12.0	13.0	16.0	9.0	11.0	9.0	13.0	5.0	11.0	3.0	7.0
5	37.0	13.6	22.4	57.3	28.6	55.0	48.4	83.2	22.4	126.2	2.4	77.2

1. Avg. Temperature (°C); 2. Avg. Highest Temperature (°C); 3. Avg. Lowest Temperature (°C);
4. Avg. Number of Rainy Days; 5. Monthly Total Precipitation Avg. (mm)

Data were analyzed using the SPSS 23.0 statistical program. The analyses included Wilcoxon test, variance analysis (one-way ANOVA) and Duncan's test. The statistical significance of the differences between SPAD value at the beginning and end of the growth period of tree and shrub species was analyzed by the Wilcoxon test. In addition, the significance of the differences between species in terms of chlorophyll content was tested with one-way analysis of variance and groups were revealed with the Duncan's test.

RESULTS AND DISCUSSION

The study conducted in Kanuni campus of Karadeniz Technical University in May and October was determined the minimum, maximum, and mean and standard deviation values related to chlorophyll content of 20 species. Also, the results of the Wilcoxon test performed to determine the significance of the differences between the average SPAD values of the species for May and October are given in Table 3.

Table 3. SPAD values and Wilcoxon test results of tree and shrub species

Species	Minimum		Maximum		Mean±Std.dev.		Wilcoxon test Sig.
	May	Oct	May	Oct	May	Oct	
<i>Quercus hartwissiana</i>	28.1	21.2	32.7	28.8	30.6±2.2	24.0±3.4	0.011*
<i>Fagus orientalis</i>	27.6	41.2	28.6	42.8	28.1±5.3	41.9±0.7	0.011*
<i>Cercis siliquastrum</i>	27.7	36.4	29.5	42.5	28.9±0.4	38.7±2.6	0.011*
<i>Aesculus hippocastanum</i>	33.7	30.6	35.8	40.2	35.1±0.8	36.5±4.2	0.325
<i>Laurus nobilis</i>	48.5	45.4	53.8	53.0	50.9±0.9	49.1±3.3	0.122
<i>Quercus castaneifolia</i>	30.7	31.4	34.7	39.5	32.5±2.2	36.6±2.4	0.012*
<i>Quercus rubra</i>	23.1	29.6	28.0	38.6	26.3±1.8	33.2±3.8	0.011*
<i>Quercus pubescens</i>	30.8	44.0	34.4	48.2	32.1±2.2	46.6±1.8	0.011*
<i>Cinnamomum camphora</i>	41.2	39.1	49.7	47.2	44.4±2.8	42.1±2.9	0.160
<i>Osmanthus decorus</i>	63.2	59.6	78.6	66.6	71.8±3.2	62.8±2.9	0.011*
<i>Eucalyptus camaldulensis</i>	48.0	45.8	53.9	62.8	51.8±6.9	53.5±6.9	0.624
<i>Weigela coraensis</i>	30.7	32.4	35.0	45.8	33.2±2.7	40.0±5.7	0.011*
<i>Laurocerasus officinalis</i>	71.5	67.2	89.3	73.1	78.6±1.8	70.6±2.8	0.011*
<i>Corylus avellana</i>	30.6	33.8	32.8	48.9	31.7±7.9	40.5±6.3	0.011*
<i>Acer negundo</i>	21.7	26.6	6.9	34.2	25.0±1.0	31.3±3.3	0.011*
<i>Ulmus minor</i>	21.7	39.0	37.8	46.2	28.3±2.3	43.0±3.2	0.011*
<i>Ostrya carpinifolia</i>	32.8	34.8	34.6	38.4	33.7±2.0	36.7±1.6	0.011*
<i>Liquidambar orientalis</i>	22.6	25.1	29.0	37.0	25.6±6.8	29.0±5.6	0.260
<i>Arbutus unedo</i>	53.9	58.5	58.7	70.2	56.9±0.8	64.8±4.9	0.011*
<i>Ginkgo biloba</i>	16.7	20.9	52.2	49.8	35.5±2.7	32.9±8.7	0.026*

* $p < 0.05$: There is statistically significant difference at 95% confidence level.

In the measurements made in May, the minimum SPAD value was determined in *Ginkgo biloba* with 16.7, while the maximum SPAD value was determined in *Laurocerasus officinalis* with 89.3. The mean SPAD values were found to range between 25.0 and 78.6 and the lowest mean SPAD value was obtained in *Acer negundo* and the highest mean SPAD value was obtained in *Laurocerasus officinalis*. Similarly, the minimum SPAD value was determined in the *Ginkgo biloba* and the maximum SPAD value in the *Laurocerasus officinalis* in October measurements. The mean SPAD values are between 24.0 and 70.6 and the highest mean SPAD value was found in *Laurocerasus officinalis*. It was determined that there are statistically significant differences ($p < 0.05$) between SPAD values of May and October of species except *Aesculus hippocastanum*, *Laurus nobilis*, *Cinnamomum camphora*, *Eucalyptus camaldulensis* and *Liquidambar orientalis*. While the chlorophyll content for May was higher in the species of *Quercus hartwissiana*, *Laurus nobilis*, *Cinnamomum camphora*, *Osmanthus decorus*, *Laurocerasus officinalis* and *Ginkgo biloba*, the chlorophyll content for October was higher in other species (Table 3).

The statistical significance of the differences between chlorophyll values of the measured tree and shrub species was determined by variance analysis (Table 4). Accordingly, in both May and October measurements, there are statistically significant differences between chlorophyll values of the species at 99% confidence level.

Table 4. Results of variance analysis of SPAD values of tree and shrub species

		Sum of Squares	df	Mean Square	F	P
May	Between Groups	36173,385	19	1903,862	58,167	0,000*
	Within Groups	5367,899	164	32,731		
	Total	41541,284	183			
Oct	Between Groups	26657,420	19	1403,022	52,549	0,000*
	Within Groups	5019,450	188	26,699		
	Total	31676,870	207			

* $p < 0.01$: There is statistically significant difference at 99% confidence level.

After determining statistically significant difference between SPAD values of the species by variance analysis, the grouping of species was determined by the Duncan's test and the results are shown in Figure 2. As a result of the Duncan's test, it was determined that 9 different groups occurred in May measurements and 15 different groups occurred in October measurements. In the results for May, the first group was formed by the species of *Laurocerasus officinalis* and *Osmanthus decorus*, which have the highest SPAD value. The second group was created by *Arbutus unedo* alone, while the third group included *Laurus nobilis* and *Eucalyptus camaldulensis*. *Acer negundo*, which has the lowest chlorophyll content, constituted the last group alone. In the results of October, while *Laurocerasus officinalis* having the highest value was in the first group alone, and *Quercus hartwissiana* having the lowest value was in the last group.

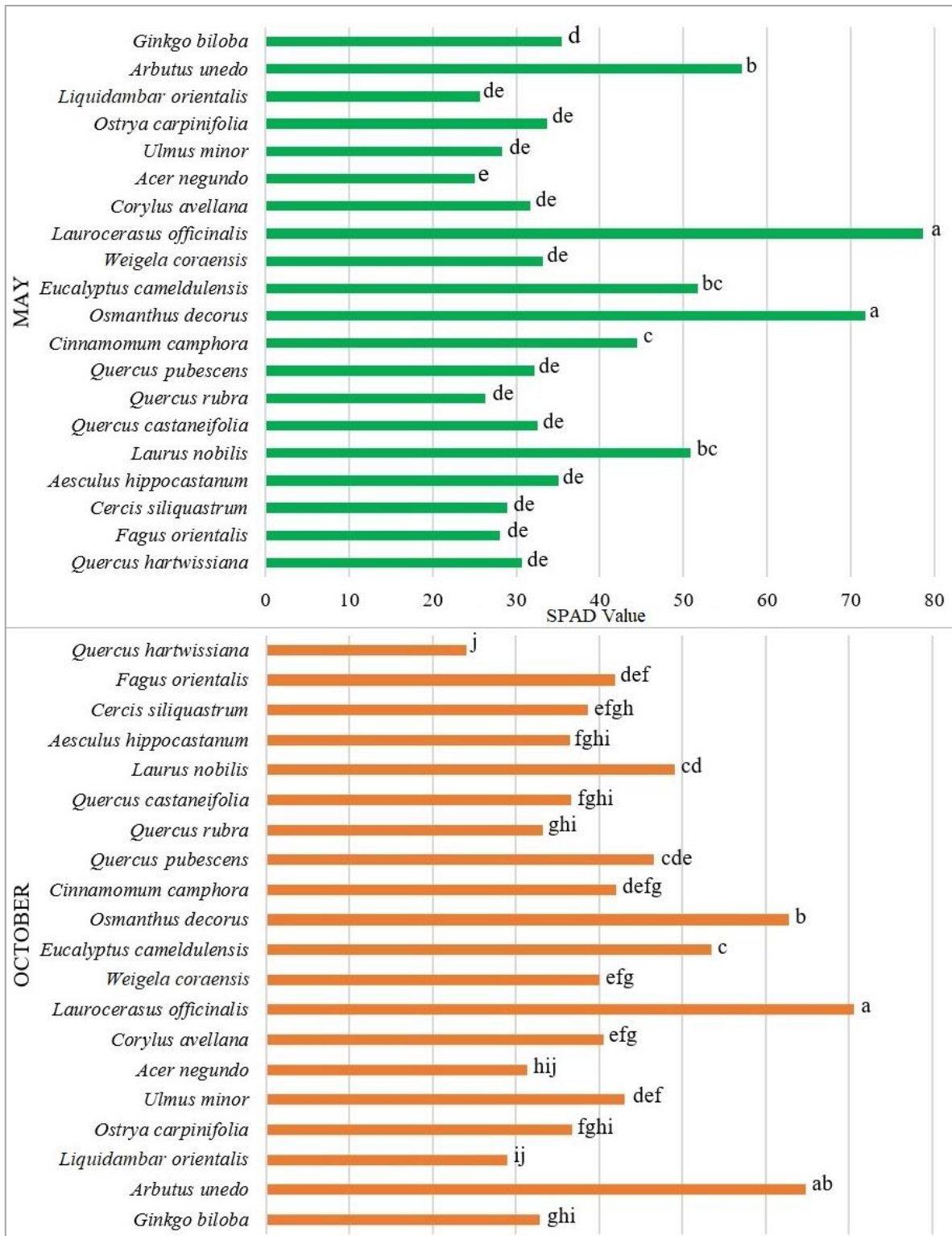


Figure 2. Duncan's test results of May and October according to SPAD value

It is known that the differences between the morphological, physiological, anatomical and phenological features of the plants depend not only on the genetic structure but also on the environmental factors (Güney et al., 2016; Atar & Turna, 2018; Atar et al., 2020). It has been reported in many studies that the amount of chlorophyll in leaves also varies by being affected by many environmental factors (Gond et al., 2012; Atar et al., 2013; Kaya et al., 2015).

Ecological conditions and especially light-related factors stand out among the factors affecting the change in chlorophyll content (Dai et al., 2009; Çetin, 2017).

The most important factor in differentiating chlorophyll levels of plants is the genetic structure like all other characters (Taner & Sade, 2005). It is also stated that leaf structure is one of the important factors that determine the chlorophyll amount. In a study, it was stated that the amount of chlorophyll in polyploid plants is higher than diploids and therefore the leaves of these plants are dark green in color (Tepe et al., 2002). As a result of the study carried out on the tree and shrub species in the Kanuni campus of Karadeniz Technical University, it was revealed that the chlorophyll values of the species showed statistically significant differences.

It was determined that there are statistically significant differences in chlorophyll values between the measurements made at the beginning (May) and end (October) of the growth period. Similar to the results of the study, it is stated in many studies that the amount of chlorophyll varies during the growth period (Zavoruev & Zavorueva, 2002; Çetin, 2017; Şevik et al., 2017). Hyyryläinen et al. (2015) reported that the reason for the low chlorophyll content at the beginning of the growth period may be due to the fact that the chlorophyll biosynthesis rate does not match the shoot development, the rapid growth of the shoots and the active growth. In another study, Faria et al. (1998) determined that the total chlorophyll concentration decreased from July to September. Annual and seasonal changes of pigments in plants are closely related to adverse development conditions such as high light intensity in summer, very low temperatures in winter and seasonal water deficit (Sauceda et al., 2008; Kancheva et al., 2014). High temperature and light can cause chlorophyll content to decrease (Brett & Singer, 1973). In the study results, while chlorophyll values belonging to six species were high in May, high values were obtained in October for 14 other species. Seasonal change of chlorophyll content among the species may result from the genetic characteristics of the species. Also, being relatively high values of many of the measured species in October may be caused by changes in environmental conditions such as temperature, light. As a matter of fact, when looking at the 2019 climate data, it is seen that the monthly average temperatures for May and October are determined as 18.6 °C and 19.1 °C, respectively, and are very close to each other. However, when the monthly total precipitation data are investigated, 28.6 mm of precipitation occurred in May, while in October there was approximately 5 times more precipitation with 126.2 mm.

As a result of measurements made in both periods, the highest average SPAD value was determined in *Laurocerasus officinalis*. In a study on indoor plants, the average amount of chlorophyll in *Begonia coccinea* was 11.86 CCI, and the average amount of chlorophyll in *Ficus elastica* was 145.12 CCI and it was reported that there were more than 10 times difference between these species (Çetin, 2016). Zeren et al. (2017a) reported that the amount of chlorophyll had more than seven times the difference between the *Prunus ceracifera* with the lowest chlorophyll value and the *Citrus reticulata* with the highest chlorophyll value. In another study, chlorophyll values of plant species used in landscape studies in Sivas city center were determined and the lowest amount of chlorophyll was in *Platanus orientalis* with 11.48 CCI and the highest amount of chlorophyll was in *Elaeagnus angustifolia* with 129.04 CCI (Zeren et al., 2017b). Zeren et al. (2018) in another study, the amounts of chlorophyll in 26 plant species used in landscape studies in Samsun city center were determined. As a result of the study, it was reported that chlorophyll amounts in *Robinia pseudoacacia* and *Yucca gloriosa* were 11.04 CCI and 144.82 CCI, respectively, and the average chlorophyll amount in other species varied between these two values.

In the process of rapid change on earth during centuries, nature is negatively affected in many ways and deteriorations in ecological balances are revealed. Plants have been heavily affected by all these negative changes and studies on plant health and sustainability have become important. Determination of chlorophyll content in plants can be used in many application areas such as determining the water stress of the plant (Demirel et al., 2010; Kulaç, 2010), determining the tolerance to cold (Rose & Haase, 2002; Perks et al., 2004; Çolak, 2012), and the determination of ozone damage (Knudson, 1977). For this reason, studies for revealing the different properties of plants in a more practical way with determining the chlorophyll content should be increased and their continuity by developing should be provided.

AUTHOR CONTRIBUTIONS

Fahrettin Atar: Original idea, study design and statistical analysis. Manuscript preparation. Interpret the data. **Deniz Güney:** Original idea, study design and statistical analysis. **Ali Bayraktar:** Field measurement. Manuscript preparation. **Nebahat Yıldırım:** Field measurement. **İbrahim Turna:** Interpret the data. All authors discussed the results and contributed to the final manuscript.

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