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Identification of Chlorophyll and Color Content in Grape Leaves During Two Growth Stages (Flowering And Set)

Turhan YILMAZ[1✍], Abdurrahim BOZKURT[2], Adem YAĞCI[3]
[1]Kahramanmaraş Sütçü İmam Üniversitesi, Ziraat Fakültesi, Bahçe Bitkileri Bölümü, Kahramanmaraş, Türkiye, [2]T.C. Tarım ve Orman Bakanlığı, Erzincan Bahçe Kültürleri Araştırma Enstitüsü Müdürlüğü, Erzincan, Türkiye, [3]Tokat Gaziosmanpaşa Üniversitesi Ziraat Fakültesi, Bahçe Bitkileri Bölümü, Tokat, Türkiye
[1]https://orcid.org/0000-0002-3756-4497, [2]https://orcid.org/0000-0001-7315-202X, [3]https://orcid.org/0000-0002-3650-4679
✉: turhanyilmaz@ksu.edu.tr

ABSTRACT
Chlorophyll concentration is a measure of the leaf's ability to photosynthesize. It is essential for advancing the physiological state of plants. A rapid estimate of in situ leaf chlorophyll concentration is frequently provided by SPAD meters. When utilizing SPAD meters to accurately quantify leaf chlorophyll content, it is important to consider the variations in growth conditions of vegetation species. This study aimed to calculate the chlorophyll (a*, b*) and L* (Lightness) levels from SPAD values in grape leaves for pickling during two vine different growth stages: blooming and set. In this study, 189 plants from Narince x Isabella combination, 39 plants from Narince x Kishmish Vatkana combination, and 218 plants from Narince x Regent combination were used. In addition, Narince, Künefi, Erciş, Dökülgen, Fenerit, Hatun Parmağı, Horoz Karası, Muhammedi, Karaerik, and Vakkas grape varieties from this local germplasm and Italia and Kyoho varieties known to be susceptible to powdery mildew were included. In this study, chlorophyll content has decreased statistically from flowering to setting for L*, a*, and b* concentration. Analyzing two factors (genotypes and periods) have statistical differences for both main and interactions. The study reported here indicates that a statistical separation ratio of chlorophyll a*/b* was discovered. According to the results of the study, concentrations of L*, a*, and b* decreased from flowering to berry set phases. This study points out that various developmental stages in plant species might affect the link between leaf chlorophyll content and SPAD readings.

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Üzüm Yapraklarındaki Klorofil ve Renk Içeriğinin Iki Asma Büyüme Evresinde (Çiçeklenme ve Tane Tutumu) Belirlenmesi

ÖZET
Klorofil konsantrasyonu yaprağın fotosentez yapma yeteneğinin bir ölçüsüdür. Bu bitkilerin fizyolojik durumunu ilerletmek için önemlidir. SPAD metreler tarafından sıklıkla yaprak klorofil konsantrasyonunun hızlı bir şekilde tahmin edilebilmektedir. SPAD metreler bitki türlerinin büyüme koşullarındaki değişiklikler dikkate alınarak yaprak klorofil içeriğini doğru bir şekilde ölçmek için kullanılabilirler. Bu çalışmanın amacı, salamura için üzüm yapraklarındaki SPAD değerlerinden klorofil (a*, b*) ve L* (Parlaklık) seviyelerini iki farklı asma büyüme aşaması olan çiçeklenme ve tane tutma sırasında hesaplamaktır. Bu çalışmada, Narince x Isabella kombinasyonundan 189, Narince x Kishmish Vatkana kombinasyonundan 39, Narince x Regent kombinasyonundan ise 218 bitki kullanılmıştır. Ayrıca, yerel gen kaynaklarımızdan Narince, Künefi, Erciş, Dökülgen, Fenerit, Hatun Parmağı, Horoz Karası, Muhammedi, Karaerik ve Vakkas üzüm çeşitleri ile küllemeye duyarlı olarak bilinen Italia ve Kyoho çeşitleri dahil edilmiştir. Bu çalışmada, klorofil içeriği (L*, a* ve b* konsantrasyonları için) çiçeklenmeden tane tutumuna istatistiksel olarak azalmıştır. Genotipler ve dönemler

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hem ana faktör hemde etkileşim faktörleri için farklılıklar göstermektedir. Burada bildirilen çalışma hibrit ve kültür asması çeşitleri arasında, klorofil a*/b* oranının farklılaştırıldığını göstermektedir. Çalışmanın sonuçlarına göre, L*, a* ve b* konsantrasyonları çiçeklenmeden tane tutumu evresine doğru azalmıştır. Bu çalışma, bitki türlerindeki çeşitli gelişim evrelerinin yaprak klorofil içeriği ile SPAD okumaları arasındaki bağlantıyı etkileyebileceğini göstermektedir.

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INTRODUCTION

Photosynthesis is a vital biochemical process in plants, and the key pigment involved in this process is chlorophyll (Samdur et al. 2000). Leaf chlorophyll content can be measured in the laboratory by applying several techniques (Monje and Bugbee 1992; Richardson et al. 2002). However, many researchers have reported that extraction under laboratory conditions is time-consuming, destructive, laborious, and expensive (Monje and Bugbee 1992; Uddling et al. 2007). Researchers have found that leaf chlorophyll content and leaf color can be determined easily and quickly by some optical methods without damaging the leaf (Markwell et al. 1995; Madeira et al. 2003). Different plant species which are reported that the relative chlorophyll content of leaves was estimated with a portable SPAD-502 meter based on the spectral transmittance properties of the leaves (Campbell et al. 1990; Madeira et al. 2003; Leon et al. 2007; Anand and Byju 2008; Ruiz-Espinoza et al. 2010; Ling et al. 2011; Jiang et al. 2017). There are many studies on determining leaf chlorophyll content, and leaf chlorophyll and leaf color content have a considerable positive correlation (Zotarelli et al. 2003; Netto et al. 2005; Anand and Byju 2008; Jiang et al. 2017). Grapevine leaves are a product whose quality decreases with storage. It has also been stated that grapevine leaves have a very high nutritional value in terms of fiber sources (Mürtezaoğlu, 2006). Underhill and Critchley (1995) reported an increase in the activity of peroxidases and polyphenol oxidase enzymes, which are frequently responsible for tissue darkening, in aging leaves. Considering that aging is directly related to leaf chlorophyll content, it is important for leaf consumers and producers to know the chlorophyll content before and after harvest. After establishing a general correlation relationship for a plant species, it is possible to use a chlorophyll meter in applications where exact values are not required. For example, a rapid assessment of relative chlorophyll in grape leaves to be used for pickling would be useful for leaf producers or researchers to detect senescence, nutrient deficiencies, and decreased chlorophyll content in leaves. To this knowledge, there is a piece of limited information on the accuracy and usefulness of the SPAD-502 chlorophyll meter for estimating chlorophyll content in pickling grape leaves. Some studies have reported differences between the regression equations for chlorophyll content and SPAD index (Campbell et al. 1990; Fanizza et al. 1991), and that the difference may be due to specific leaf weight (Yamamoto et al. 2002). The mathematical correlation calculation between SPAD value and chlorophyll content may be essential to optimize the advanced interpretation with the chlorophyll meter. The research was conducted to determine chlorophyll (a* and b*) and L* (lightness) levels from SPAD values in two growth stages (flowering and set) of grape leaves for pickling.

MATERIALS AND METHOD

Plant material

The research was carried out in the grapevine seedling production greenhouse located in the Application and Research Area of the Faculty of Agriculture at Gaziosmanpaşa University ın 2024. In the study, F1 plants obtained from Narince x Isabel (NVL, 189 plants), Narince x Kişhmişh Vatkana (NKV, 39 plants), and Narince x Regent (NRG, 218 plants) hybrids were used. In addition, Narince, Künefi, Erciş, Dökülgen, Fenerit, Hatun Parmağı, Horoz Karası, Muhammedi, Karaerik, and Vakkas grape varieties from thislocal germplasm and Italia and Kyoho varieties known to be susceptible to powdery mildew were included (Fig. 1A).

Experiment Design and determination of chlorophyll (a* and b*), and L* (Lightness) values

The study was conducted using a randomized complete block design with 3 replications, with 1 shoot and 3 leaves on each shoot in each replication. Leaf samples were harvested from the 4th, 5th, and 6th leaves on the shoots, which reached 2/3 of the mature leaf size from the tip (Kılıç, 2007). Nine leaves were taken from each genotype in

the F1 plants of the combinations during the flowering (end of May) and berry set (beginning of July) periods (Fig. 1A). Chlorophyll contents were determined by measuring the leaves of each F1 individual in 3 directions (right, middle, and left lobes) with Konica Minolta (SPAD-502 Plus, serial number: 20005480) branded SPAD reader for chlorophyll (a* and b*) values, and then L* (Lightness) was determined with Konica Minolta branded color measurement device with serial number 8203581 (Fig. 1B).

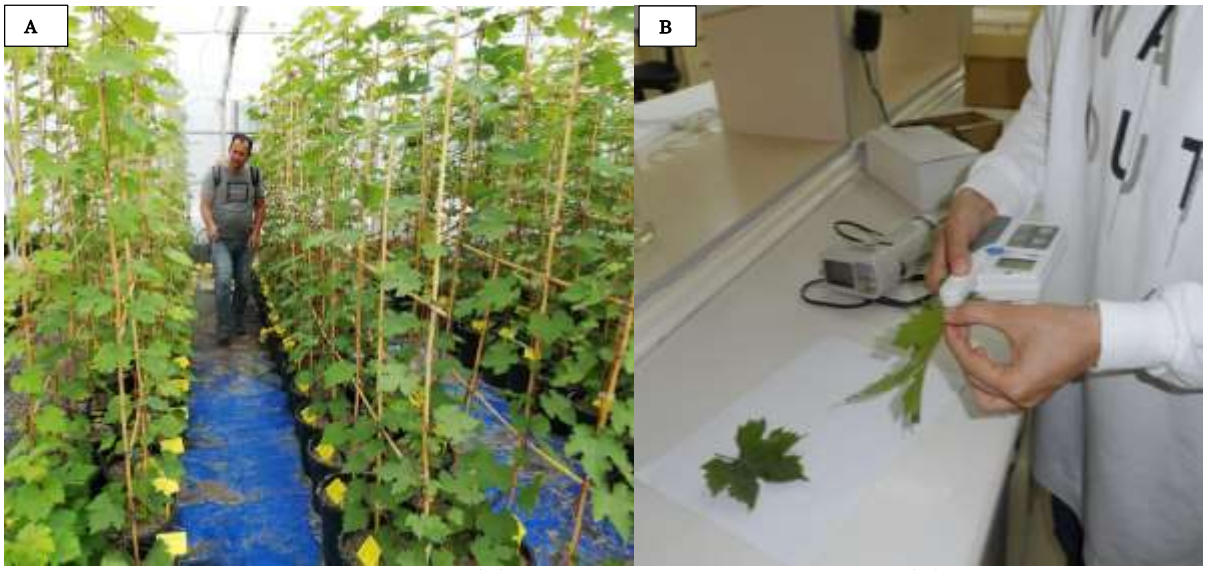

Figure 1. Images of the research area (A) and the use of the SPAD meter (B)
Şekil 1. Araştırma alanı (A) ve SPAD-502 metrenin kullanımına (B) ait görüntüler

Statistical Analysis

The Agricola package was used in R Studio to perform all descriptive analyses. Analysis of Variance (ANOVA) in R Studio was used to examine the importance of genotypes and periods, as well as how they interacted with various (L*, a*, b*, and a*/b*). The chi-square test was utilized to confirm that all the data were normal before the analysis was conducted. The primary impacts (genotypes and periods) on various (L*, a*, b*, and a*/b*) were assessed using linear models. Tukey HSD post-hoc analysis was performed using R Studio's agricolae package. The ggbiplot2 package in R Studio was used to perform Principal Component Analyses (PCAs) for the various (L*, a*, b*, and a*/b*) datasets R Core (2013).

RESULTS and DISCUSSION

Thisresults showed that the hybrids NRG had the lowest L* content (40.50 nm) while cultivars had the highest L* content (44.38 nm). The L* content was highest in the flowering (42.77 nm) period compared with the setting (40.68 nm) in leaves. Chlorophyll a* in the NRG hybrids had the highest content (-14.15 µg cm^{-2}), whereas cultivars had the lowest content of Chlorophyll a*, which is -18.72 µg cm^{-2}. The chlorophyll a* saw an increase from flowering (-14.65 µg cm^{-2}) to setting (-16.90 µg cm^{-2}). The cultivars (30.26 µg cm^{-2}) were shown to have the greatest chlorophyll b*, while NRG hybrids had little chlorophyll b* (19.22 µg cm^{-2}). The chlorophyll b* was decreased from flowering (23.84 µg cm^{-2}) to setting (20.44 µg cm^{-2}). The ratio of a*/b* was greatest in NKV hybrids with 0.8, while it was least in cultivars with 0.63 (Table 1).

Figure 2A displays four different compounds present in leaves: chlorophyll (a* and b*), L*, and a*/b*. PCA was used to analyze and display the complex relationships between genotypes and periods in leaves. To ensure a reliable and representative dataset, each data point represented the centroid of four measurements for each parameter. The Cos2 value, which illustrates the relative influence of each variable on the principal components, was used to show each variable's contribution to the primary components. The first PC in Figure 2A is responsible for 65.3% and 25.3% of the total variation in the data. Consequently, there was a negative correlation between the levels of chlorophyll a* and chlorophyll b*, L*, and a*/b*contents (Fig. 2A). Chlorophyll b* and L* were shown to have an especially strong association, as seen by the dark blue square. On the other hand, as the dark red square shows, there were strong negative associations between both chlorophyll a* and L* (as seen by the moderate red square), and a* and b* (as seen by the dark red square) (Fig. 2B). Every variable is displayed, and along PC1's vertical axis, various colored circles signifying the genotypes and periods are dispersed. The NVL hybrids are represented by the purple circle, which also contributes significantly to various chlorophyll (a* and b*) and L*

contents. In contrast, cultivars have the lowest total variance. NRG and NKV hybrids differed in their contributions and were found between NVL hybrids and cultivars (Fig. 2C). However, along the horizontal axis, flowering was more broadly distributed than the setting, suggesting that this time had a bigger impact on various chlorophyll (a* and b*) and L* contents (Fig. 2D).

Table 1. Chlorophyll (a* and b*), L*, and a*/b* contents of different Cultivars and F1 hybrids (NKV, NRG, and NVL) in flowering and set periods.

Çizelge 1. Çiçeklenme ve tane tutumu dönemlerinde farklı çeşit ve F1 hibritlerinin (NKV, NRG ve NVL) klorofil (a ve b*), L* ve a*/b* içerikleri.*

Genotypes (G)[x]	L*(nm)	a* (µg cm^{-2})	b* (µg cm^{-2})	a*/b*
Cultivars	44.38±0.32a	18.72±0.26d	30.26±0.45a	0.63±0.00d
NKV	42.17±0.21c	15.70±0.17b	21.85±0.29c	0.81±0.02a
NRG	40.50±0.08d	14.15±0.07a	19.22±0.12d	0.74±0.01b
NVL	42.82±0.09b	17.41±0.07c	24.86±0.13b	0.71±0.00c
Periods (P)[y]				
Flowering	42.77±0.08a	16.90±0.07a	23.84±0.12a	0.72±0.00b
Setting	40.68±0.01b	14.65±0.02b	20.44±0.09b	0.74±0.00a
G	< 2e-16 ***	< 2e-16 ***	<2e-16 ***	1.49e-10 ***
P	< 2e-16 ***	< 2e-16 ***	<2e-16 ***	0.00644 **
G x P	7.29e-15 ***	8.46e-08 ***	<2e-16 ***	9.78e-13 ***

x, Mean separation in Genotips; y, Mean separation in Periods; G, Genotips; P, Periods; G x P, interactions; For a given factor (different letters within a column represent significant differences (Tukey test, ***, Significant at p-value < 0.001). Data are stated as averages of the data and their standard deviations.

A study was conducted to state the total chlorophyll contents such as (a* and b*) in Solanum lycopersicum L. leaves that had interveinal chlorosis caused by continuous lighting by using the SPAD-502. According to their results, there is a positive correlation between the chlorophyll content index and the contents of chlorophyll a* and chlorophyll b* in the leaf. These findings imply that the portable chlorophyll meter SPAD-502 Plus can be a useful tool for nondestructive estimation of chlorophyll content in leaves (Shibaeva et al., 2020). In addition, many writers have described the SPAD-502 as a trustworthy instrument for determining the amount of chloride in the leaves of plants cultivated in comparable environmental conditions (Campbell et al., 1990; Yang et al., 2014). The concentration of both chlorophyll and carotene pigments decreased as a result of an unfavorable precipitation distribution throughout the May–July summer growing season (Zielewicz et al., 2020). It is seen that the chlorophyll content has decreased statistically from setting to flowering for L*, a*, and b* concentrations (Table 1, Figure 1A and B). A study was conducted to compare the methods that are frequently employed to SPAD readings and absolute leaf chlorophyll concentration. Three field datasets and one synthetic dataset were used to compare these methods. It is reported that SPAD readings obtained in the field for different vegetation types and the leaf chlorophyll concentration measured in the lab using a destructive approach are better suited for smaller data sets than the polynomial functions as the linear and exponential functions have fewer fitting parameters (Zhang et al., 2022). According to the findings from a butterhead lettuce study, butterhead lettuce leaves' chlorophyll concentration may be accurately estimated using SPAD measurements (León et al., 2007). The relationship between leaf chlorophyll concentration and SPAD readings in plant species may change as a result of varying growth vigor, leading to distinct functions for even the same species. This is comparable to the findings that showed the mathematical model utilized to calculate the concentration of chlorophyll in leaves using SPAD readings varies depending on the stage of growth of the leaves. Researchers have examined leaves at the vegetative and reproductive growth stages of the plant to determine the most appropriate function model, and they used correlation analysis to examine the link between tomato (Solanum lycopersicum) leaf chlorophyll concentration and the Minolta SPAD-502 plus chlorophyll meter. Another finding has demonstrated there is a substantial link between the SPAD value and the total chlorophyll contents (a*, b*, and total chlorophyll) in tomato leaves (Jiang et al., 2017). As a demonstration from thisstudy two factors (genotype and periods) have statically differences for both main and interactions (Table 1, Figure 2C and D). One of the most crucial chemical components of plants is the amount of chlorophyll since it directly regulates photosynthesis and biomass production (Zielewicz et al., 2020). Pheoporphyrin, a magnesium atom joined to the nitrogen atoms in the middle of the porphyrin system, is the building block of a chlorophyll molecule. All plants and green algae include chlorophyll, which comes in two types: chlorophyll a* and chlorophyll b*. In addition, plants with higher levels of chlorophyll have larger levels of total chlorophyll, and the ratio of chlorophyll a* to b* is roughly 3:1 (Rajalakshmi et al., 2015). Another study has impressed the importance of chlorophyll (a*/b*) connections and has reported testing plant leaves at various developmental stages, physiological conditions, and environmental conditions is necessary (Chen and Roca, 2018).

The chlorophyll a*/chlorophyll b* ratio is a sign of the functional balance between the effectiveness of light collection and electron transport in plants (Zhang et al., 2020). It is evident from the study presented here have a statistical differentiation ratio of chlorophyll a*/b* was found as from 0.63 to 0.81 between cultivars and hybrids (Table 1). Other studies have also reported that the feature of correlation analysis of leaf chlorophyll content might be affected by the leaf condition (Bullock and Anderson, 1998). The study presented currently has reported that the concentrations L*, a*, and b* have been negatively affected from the setting to the flowering periods (Table 1).

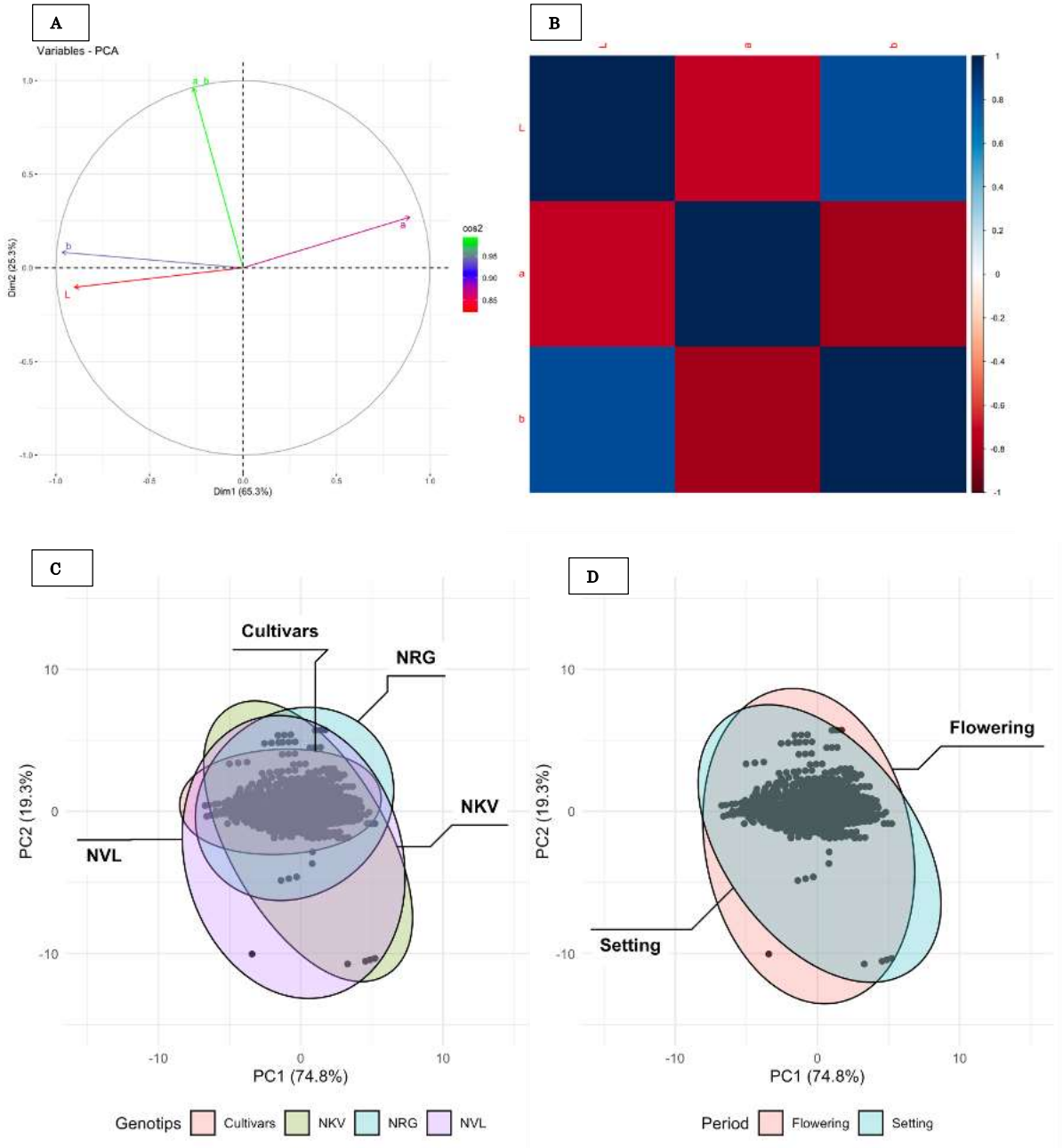

Figure 2. PCA biplot of colored by genotypes and periods. All variables (A), correlations (B), genotypes (C), and periods (D) are demonstrated.

Şekil 2. Genotiplere ve periyotlara göre renklendirilmiş PCA biplot'u. Tüm değişkenler (A), tüm değişkenlerin korelasyonu (B), genotipler (C) ve periyotlar gösterilmektedir.

CONCLUSION

The amount of chlorophyll in a leaf is a gauge of its capacity for photosynthetic activity, and it is crucial for improving plants' physiological conditions. Variations in vegetation species' growing conditions must be taken into account when using SPAD meters to measure the amount of chlorophyll in leaves precisely. As a result of this

study, chlorophyll content has statistically decreased from flowering to setting. In addition, there are statistical differences for both main and interaction factors when analyzing two factors (genotypes and times). A statistical separation ratio of chlorophyll a*/b* was also found. This study highlights that the relationship between leaf chlorophyll concentration and SPAD readings may vary depending on the developmental stage of the plant species.

Author's Contributions

The authors declare that they have contributed equally to the article.

Conflicts of Interest Statement

The author has stated that there is no conflict of interest.

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