Bartın Orman Fakültesi Dergisi | Journal of Bartin Faculty of Forestry
2026, 28(2): 298 – 312 | DOI: 10.24011/barofd.1887589

Relationship Between Leaf Color–Associated Chlorophyll Content and Selected Vegetation Indices in *Photinia × fraseri* Seedlings

Ali Bayraktar[1,*], Fahrettin Atar[2], Deniz Güney[3], İbrahim Turna[4]

[1,2,3,4] Department of Forest Engineering, Faculty of Forestry, Karadeniz Technical University, Trabzon, Türkiye

Article History
Received: 12.02.2026
Accepted: 08.07.2026
Published: 15.08.2026

Research Article

Abstract − Leaf color is not only an aesthetic characteristic for plants, but also an important indicator reflecting the physiological status of the plant. This study aimed to determine chlorophyll differences arising depending on leaf color (green and red) in *Photinia × fraseri* Dress. seedlings and to reveal the relationship between these differences and vegetation indices derived from RGB-based image analyses. The study material consisted of green and red leaves collected from four-year-old seedlings grown under the same environmental conditions. Leaf chlorophyll content was determined using a portable chlorophyll meter (SPAD-502); red (R), green (G), and blue (B) color values of the same leaves were obtained from scanned images using ImageJ software. Based on RGB values, excess green (E×G) index, normalized green–red difference index (NGRDI), visible atmospherically resistant index (VARI), green leaf index (GLI), and greenness index (GI) values were calculated. The obtained data were evaluated using independent samples t-test, Spearman's rank correlation analysis, and regression analyses. The results revealed statistically significant differences between green and red leaves in all RGB color values except the blue value, in SPAD values, and in vegetation indices ($p < 0.01$). SPAD values in green leaves (79.83) were found to be markedly higher than those in red leaves (30.15). In particular, the E×G index showed higher performance than the other indices in reflecting chlorophyll differences associated with leaf color and in explaining SPAD values. Regression analyses revealed that the relationships between SPAD values and vegetation indices exhibited a non-linear structure and that quadratic models provided higher explanatory power. The findings indicate that RGB-based vegetation indices can be used for the non-destructive and practical monitoring of leaf color–associated physiological differences in ornamental plants such as *Photinia × fraseri*.

***Keywords** – Excess green index, image analysis, RGB color values, SPAD-502*

Photinia × fraseri Fidanlarında Yaprak Rengine Bağlı Klorofil İçeriği ile Bazı Vejetasyon İndeksleri Arasındaki İlişki

[1,2,3,4] Karadeniz Teknik Üniversitesi, Orman Fakültesi, Orman Mühendisliği Bölümü, Trabzon, Türkiye

Makale Tarihçesi
Gönderim: 12.02.2026
Kabul: 08.07.2026
Yayın: 15.08.2026

Araştırma Makalesi

Öz − Yaprak rengi, bitkiler için estetik bir özellik olmanın yanı sıra, bitkinin fizyolojik durumunu da yansıtan önemli bir göstergedir. Bu çalışmada, *Photinia × fraseri* Dress. fidanlarında yaprak rengine (yeşil ve kırmızı) bağlı olarak ortaya çıkan klorofil farklılıklarının belirlenmesi ve bu farklılıkların RGB tabanlı görüntü analizlerinden türetilen vejetasyon indeksleri ile ilişkisinin ortaya konulması amaçlanmıştır. Çalışma materyalini, aynı çevresel koşullarda yetiştirilen dört yaşındaki fidanlardan alınan yeşil ve kırmızı yapraklar oluşturmuştur. Yaprak klorofil içeriği taşınabilir klorofilmetre (SPAD-502) kullanılarak belirlenmiş; aynı yapraklara ait kırmızı (R), yeşil (G) ve mavi (B) renk değerleri taranmış görüntüler üzerinden ImageJ yazılımı ile elde edilmiştir. RGB verileri kullanılarak aşırı yeşil indeksi (excess green, E×G), normalize edilmiş yeşil–kırmızı fark indeksi (normalized green–red difference index, NGRDI), görünür atmosfere dayanıklı indeks (visible atmospherically resistant index, VARI), yeşil yaprak indeksi (green leaf index, GLI) ve yeşillik indeksi (greenness index, GI) değerleri hesaplanmıştır. Elde edilen veriler bağımsız örneklem t-testi, Spearman sıra korelasyon analizi ve regresyon analizleri ile değerlendirilmiştir. Sonuçlar, mavi renk değeri dışındaki tüm RGB renk değerlerinde, SPAD değerlerinde ve vejetasyon indekslerinde yeşil ve kırmızı yapraklar arasında istatistiksel olarak anlamlı farklılıklar bulunduğunu göstermiştir ($p < 0{,}01$). Yeşil yapraklardaki (79,83) SPAD değerleri kırmızı yapraklara (30,15) kıyasla belirgin biçimde daha yüksek bulunmuştur. Özellikle E×G indeksi, yaprak rengine bağlı klorofil farklılıklarını yansıtmada ve SPAD değerlerini açıklamada diğer indekslerden daha yüksek bir performans göstermiştir. Regresyon analizleri, SPAD değerleri ile vejetasyon indeksleri arasındaki ilişkilerin doğrusal olmayan bir yapı sergilediğini ve kuadratik modellerin daha yüksek açıklayıcılık sağladığını ortaya koymuştur. Elde edilen bulgular, RGB tabanlı vejetasyon indekslerinin *Photinia × fraseri* gibi süs bitkilerinde yaprak rengine bağlı fizyolojik farklılıkların tahribatsız ve pratik bir şekilde izlenmesinde kullanılabileceğini göstermektedir.

***Anahtar Kelimeler** – Aşırı yeşil indeksi, görüntü analizi, RGB renk değerleri, SPAD-502*

[1] alibayraktar@ktu.edu.tr
[2] fatar@ktu.edu.tr
[3] d_guney@ktu.edu.tr
[4] turna@ktu.edu.tr
*Sorumlu Yazar / Corresponding Author

1. Introduction

Plants not only perform fundamental functions for the continuity of ecosystems (Rawat and Agarwal, 2015), but also provide multifaceted benefits to human life in physiological, psychological, and socio-cultural dimensions (Alvarsson et al., 2010; Bowler et al., 2010; Tyrväinen et al., 2014; Elsadek et al., 2019). Among these benefits, the effects of aesthetic and visual qualities on mental well-being stand out; the literature emphasizes that plant characteristics such as flowers or leaves with different colors, forms, and textures create perceptual diversity, positively influence mood, and strengthen individuals' psychological interaction with space (Janeczko et al., 2019; Wang et al., 2019; Holzman et al., 2025). *Photinia × fraseri* Dress. (photinia), which is distinguished by its vivid leaf colors and high aesthetic value, is a member of the Rosaceae family and is an ornamental plant obtained through the hybridization of *P. glabra* and *P. serrulata*. The young leaves of this evergreen species display shades ranging from red to purple during spring and autumn, gradually turning green during summer and winter. This dynamic change in leaf color observed throughout the year adds significant ornamental value to the species in landscape applications (Larraburu et al., 2010; Li et al., 2025). Leaf color is one of the main characteristics determining the aesthetic value of ornamental plants, and its formation is associated with the quantity and relative proportions of plant pigments such as chlorophyll, anthocyanins, and carotenoids (Tang et al., 2020). High chlorophyll content causes green coloration to become dominant in leaves, whereas carotenoids are generally associated with the formation of yellow, orange, and red tones (Yang et al., 2015). On the other hand, anthocyanins—whose presence in photinia leaves has been demonstrated and which constitute a subgroup of flavonoids—are water-soluble pigments that play an important role in the emergence of a wide range of colors extending from red to purple and blue (Kytridis and Manetas, 2006).

The two main pigment groups that play a crucial role in photosynthesis—a fundamental biological process in which green plants capture light energy to convert carbon dioxide and water into organic compounds while simultaneously releasing oxygen—are chlorophylls and carotenoids (Cutolo et al., 2023; Li et al., 2024). Chlorophyll, the most important pigment due to its primary responsibility for light absorption and participation in photochemical reactions, characteristically absorbs red and blue-violet wavelengths while reflecting green light, thereby giving plant leaves their green color (Nishio, 2000; Li et al., 2024). In the measurement of leaf chlorophyll content, destructive methods based on chemical extraction and spectrophotometry under laboratory conditions can be used (Lichtenthaler and Wellburn, 1983). However, non-destructive methods performed using portable handheld devices (SPAD-502, Konica–Minolta, Inc., Osaka, Japan), which offer a simpler, faster, and more cost-efficient alternative, are also widely preferred (Richardson et al., 2002; Cerovic et al., 2012). In both approaches, leaf chlorophyll content is determined based on the measurement of chlorophyll absorption or transmittance in the red band (Castelli et al., 1996; Dong et al., 2019). Furthermore, it is emphasized that SPAD measurements reflect not only chlorophyll content but also leaf nitrogen status and photosynthetic activity; therefore, they can be used as an indirect yet powerful indicator in physiological evaluations (Chang and Robison, 2003; Reis et al., 2009; Cerovic et al., 2012; Zhang et al., 2022).

In recent years, with the development of digital camera and image processing techniques, vegetation indices based on visible spectrum bands have emerged as a practical alternative for the indirect estimation of leaf chlorophyll (Hunt et al., 2011; Saberioon et al., 2014; Barman and Choudhury, 2022; Wang et al., 2024). The RGB system, which defines colors through the simple combination of three primary colors (Red, Green, and Blue), generally represents the colors perceived by the human eye, which has three types of cone cells sensitive to the reddish, greenish, and bluish regions of the visible spectrum (Trussell et al., 2005; Hunt and Pointer, 2011). To date, many studies have listed and used various vegetation indices developed by different authors using RGB values—such as excess green (E×G) index, normalized green–red difference index (NGRDI), visible atmospherically resistant index (VARI), green leaf index (GLI), and greenness index (GI)—for the estimation of SPAD values (Hunt et al., 2011; Cen et al., 2019; Mao et al., 2020; Liu et al., 2021; Yuan et al., 2022; Zhang et al., 2022; Signorelli et al., 2025).

The aim of this study was to determine chlorophyll levels (SPAD values) associated with different leaf colors (green and red) in *Photinia × fraseri* seedlings using a portable chlorophyll meter and to examine the relationships between the obtained values and vegetation indices (E×G, NGRDI, VARI, GLI, and GI) derived from RGB-based image analyses. Additionally, the objective was to identify the indices with the highest explanatory power for estimating SPAD values based on leaf color differences.

2. Materials and Methods

The study was conducted on November 6, 2025, using *Photinia × fraseri* Dress. seedlings grown at the Of Forest Nursery Unit under the Trabzon Forest Nursery Directorate, Türkiye. A total of 30 four-year-old seedlings, grown under identical conditions and exhibiting similar growth patterns, constituted the study material. For each seedling, SPAD values were measured on five green and five red leaves using a portable chlorophyll meter (SPAD-502, Konica–Minolta, Inc., Osaka, Japan). In total, 300 leaves (150 green and 150 red) were used in the study. Measurements were taken from three points on each leaf—apex, middle, and base—and recorded as arithmetic means. The leaves whose SPAD values were determined were numbered, transported to the laboratory, arranged in sequence, and scanned individually according to seedling and leaf color using an HP Scanjet G4010 flatbed scanner at a resolution of 600 dpi. The use of scanned images provided standardized image acquisition conditions for all samples and reduced potential variability arising from differences in illumination, shadowing, and viewing geometry. Similar approaches based on scanned leaf images have been employed in studies investigating relationships between leaf color characteristics and chlorophyll content (Yadav et al., 2010; Treder et al., 2021).

In the scanned leaves, R (red), G (green), and B (blue) color values were obtained from three regions, corresponding to the locations used for SPAD measurements, using the ImageJ software, and the arithmetic means were recorded for further analyses. Images related to the *P. × fraseri* seedlings used in the study, the portable chlorophyll meter readings, and the acquisition of color values in the ImageJ software are presented in Figure 1.

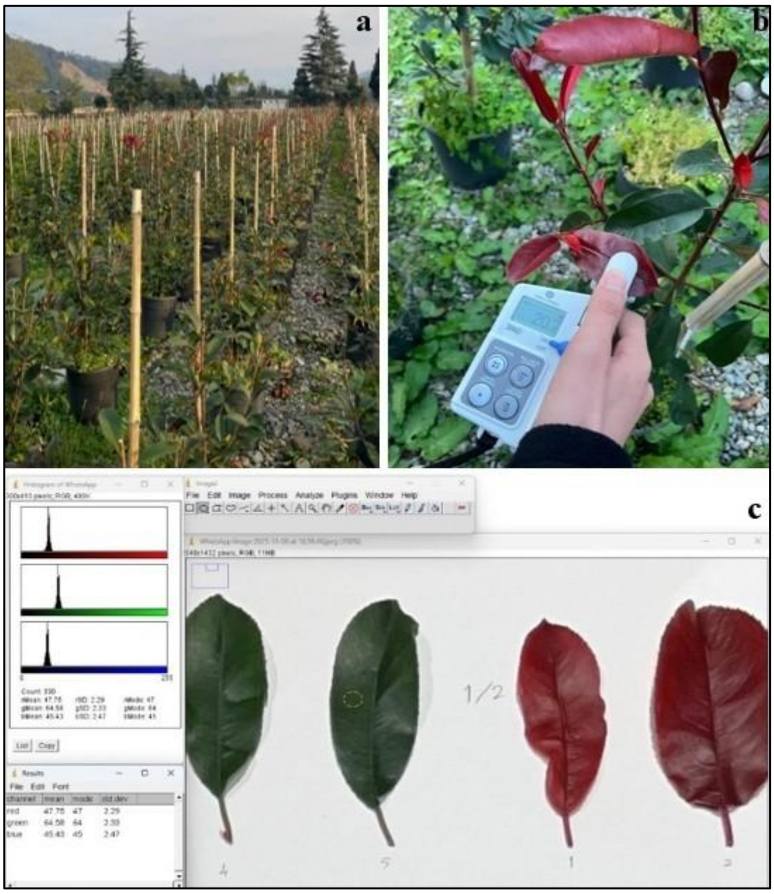

Figure 1. *P. × fraseri* seedlings (a), SPAD measurements (b), and acquisition of RGB color values (c)
Şekil 1. P. × fraseri fidanları (a), SPAD ölçümleri (b), ve RGB renk değerlerinin elde edilme süreci (c)

The mean RGB color values recorded for each leaf were substituted into the formulas presented in Table 1 to calculate the vegetation indices. These indices quantitatively represent the level of greenness in leaf tissues by emphasizing green reflectance within the visible spectrum and are widely used as indirect indicators of chlorophyll content (Mao et al., 2020; Yuan et al., 2022).

Table 1. Formulas of vegetation indices used in the study
Tablo 1. Çalışmada kullanılan vejetasyon indekslerinin formülleri

Index	Index Formula	References
Excess Green (E×G)	E×G=2×G-R-B	Woebbecke et al., 1995
Normalized Green-Red Difference Index (NGRDI)	NGRDI= (G-R)/(G+R)	Hunt et al., 2005
Visible Atmospherically Resistant Index (VARI)	VARI= (G-R)/(G+R-B)	Gitelson et al., 2002
Green Leaf Index (GLI)	GLI= (2×G-R-B)/(2×G+R+B)	Gobron et al., 2000
Greenness Index (GI)	GI= G/(R+G+B)	Kawashima and Nakatani, 1998

Although VARI was originally developed to reduce atmospheric effects in field imagery, it was included in this study as a visible-band-based greenness index to allow comparative evaluation under controlled scanning conditions. SPAD values, RGB color values (R, G, B), and vegetation indices were determined separately for green and red leaves. For statistical analyses, the mean values obtained from five leaves of the same color class were used to represent each seedling. Consequently, all statistical analyses were performed using 30 independent observations for each leaf color group. Prior to statistical analyses, data normality was evaluated using skewness and kurtosis statistics. All variables exhibited skewness and kurtosis values within acceptable limits (±2), indicating approximate normality of the data distribution. Homogeneity of variances was assessed using Levene's test. Differences between green and red leaves were evaluated using independent samples t-tests in IBM SPSS Statistics 27 software. When the assumption of homogeneity of variances was violated, the results based on unequal variances were considered. Furthermore, the direction and strength of the relationships among the parameters were determined using Spearman's rank correlation analysis in RStudio. In addition, non-linear relationships between SPAD values and vegetation indices were analyzed using the regression (curve estimation) module in IBM SPSS Statistics 27 to identify the mathematical models that best described these relationships. Model performance was evaluated using the coefficient of determination (R^2), root mean square error (RMSE), and mean absolute error (MAE), with higher R^2 values and lower RMSE and MAE values indicating better model performance (Özdamar, 1999; Quinn and Keough, 2002; Özkan, 2003; Crawley, 2013).

3. Results

Levene's test for homogeneity of variances indicated unequal variances between green and red leaves for SPAD values, red and blue color values, and the vegetation indices E×G, NGRDI, VARI, and GLI ($p < 0.05$). In contrast, variance homogeneity was confirmed for green color values (G) and GI ($p > 0.05$). Accordingly, independent samples t-test results were reported according to the appropriate variance assumption for each variable (Table 2).

Table 2. Independent samples t-test results for the measured parameters
Tablo 2. Ölçülen parametreler için bağımsız örneklem t-testi sonuçları

Variables	t	df	Mean Difference	*p*
SPAD	15.08	39.15	49.69	<0.001**
R	-8.40	34.74	-27.31	<0.001**
G	9.70	58.00	18.35	<0.001**
B	1.13	50.52	2.16	0.262
E×G	20.63	43.60	61.85	<0.001**
NGRDI	29.45	32.98	0.49	<0.001**
VARI	36.66	36.53	0.74	<0.001**
GLI	24.67	43.62	0.38	<0.001**
GI	26.64	58.00	0.17	<0.001**

**$p < 0.01$: Statistically significant at the 99% confidence level.

As shown in Table 2, statistically significant differences ($p < 0.001$) were detected between green and red leaves in all RGB color values except blue, in SPAD values, and in vegetation indices. The clear separation of SPAD values between groups was considered a strong indicator of physiological differences associated with leaf color. When RGB color values were evaluated, significant differences were observed between groups in the red and green values. The negative mean difference in the red value indicated that the first group (green leaves) had lower red color intensity compared to the second group (red leaves). Conversely, the positive mean difference in the green value showed that the first group had higher green color intensity. In contrast, the small mean difference and statistically non-significant result observed for the blue value ($p = 0.262$) suggest that this color component has limited sensitivity in distinguishing between groups. All vegetation indices consistently reflected the green–red leaf differentiation based on SPAD values in the same direction and with statistical significance. The mean and standard deviation values of all variables examined in the study are presented in Figure 2. To avoid visual distortion due to differences in variable scales, the graphs were arranged in two separate panels.

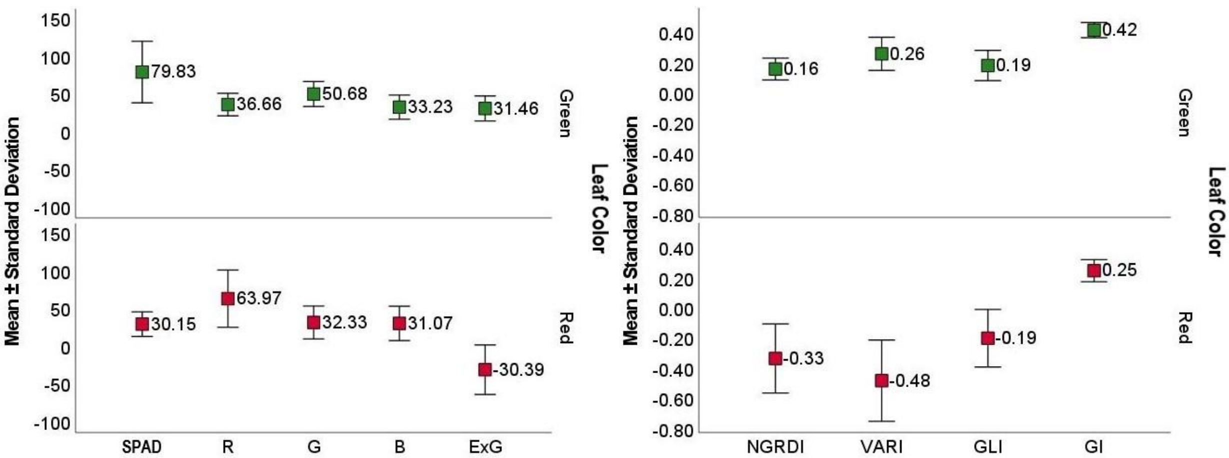

Figure 2. Mean and standard deviation values of all measured variables in green and red leaves
Şekil 2. Yeşil ve kırmızı yapraklarda ölçülen tüm parametrelerin ortalama ve standart sapma değerleri

According to Figure 2, the mean SPAD value obtained in green leaves (79.83) was approximately 165% higher than the mean value measured in red leaves (30.15). Consistent with expectations, the mean green (G) color value in green leaves (50.68) was markedly higher than that of red leaves (32.33), whereas the mean red (R) color value in red leaves (63.97) was higher than that measured in green leaves (36.66). The only variable that did not show a significant difference in the independent samples t-test was the blue (B) color value, with similar means observed in green (33.23) and red (31.07) leaves. These findings indicate that leaf color differentiation was clearly reflected through the dominant RGB color components. Regarding vegetation indices, all mean values obtained in green leaves were positive. In contrast, except for GI (0.25), for which negative values are mathematically not possible, E×G (-30.39), NGRDI (-0.33), VARI (-0.48), and GLI (-0.19) values were negative in red leaves. This pattern indicates that the dominance of green color intensity in green leaves, associated with higher chlorophyll content, resulted in positive values of indices, including E×G (31.46), NGRDI (0.16), VARI (0.26), GLI (0.19), and GI (0.42). Conversely, the increased red color intensity in red leaves, likely associated with anthocyanin accumulation and reduced chlorophyll content, led to negative values of these indices. Among the vegetation indices, E×G demonstrated clear separation between green and red leaf groups, whereas the GI index, although producing positive values in both leaf types, exhibited limited discriminative power when mean values and variation ranges were considered. These findings demonstrate that RGB-based vegetation indices successfully capture leaf color–associated differences and can serve as effective indicators for distinguishing between green and red leaves.

The results of the Spearman correlation analysis among SPAD values, RGB color values (R, G, and B), and the vegetation indices derived from these color components (E×G, NGRDI, VARI, GLI, and GI) are presented using correlation coefficients and a color scale (Figure 3). In the correlation matrix, the lower triangle represents the results for green leaves, whereas the upper triangle corresponds to red leaves. Only statistically significant correlations ($p < 0.05$) are shown in the figure, and blank cells indicate statistically non-significant relationships.

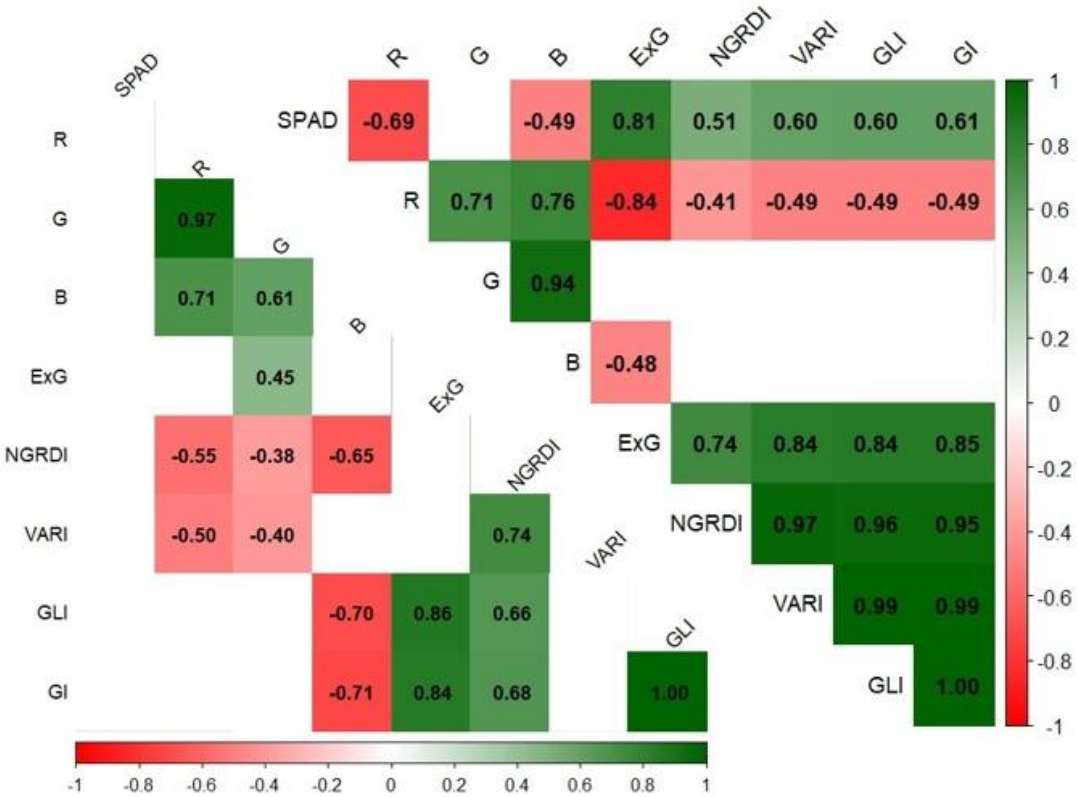

Figure 3. Spearman correlation matrix for green (lower triangle) and red (upper triangle) leaves

Şekil 3. Yeşil (alt üçgen) ve kırmızı (üst üçgen) yapraklara ait Spearman korelasyon matrisi

When the Spearman correlation results for green leaves were examined, no statistically significant relationship was detected between SPAD values and RGB color values or vegetation indices. However, significant relationships were observed among RGB color components and vegetation indices. Among RGB color values, strong positive correlations were found between R and G (r = 0.97), between R and B (r = 0.71), and a moderate positive correlation was observed between G and B (r = 0.61). Regarding the relationships between RGB color values and vegetation indices, R was negatively correlated with NGRDI (r = -0.55) and VARI (r = -0.50), while G was positively correlated with E×G (r = 0.45) and negatively correlated with NGRDI (r = -0.38) and VARI (r = -0.40). The B color value showed negative correlations with NGRDI (r = -0.65), GLI (r = -0.70), and GI (r = -0.71). Among the vegetation indices, E×G was strongly and positively correlated with GLI (r = 0.86) and GI (r = 0.84). NGRDI showed positive correlations with VARI (r = 0.74), GLI (r = 0.66), and GI (r = 0.68), while GLI and GI exhibited an almost perfect positive correlation (r = 1.00).

For red leaves, SPAD values showed significant negative correlations with the red color value (r = -0.69) and blue color value (r = -0.49). In contrast, SPAD values were positively correlated with all vegetation indices, with the strongest relationship observed for E×G (r = 0.81), followed by GI (r = 0.61), VARI (r = 0.60), GLI (r = 0.60), and NGRDI (r = 0.51). Among RGB color values, positive correlations were detected between R and G (r = 0.71), R and B (r = 0.76), and G and B (r = 0.94). Regarding the relationships between RGB color values and vegetation indices, R was negatively correlated with E×G (r = -0.84), NGRDI (r = -0.41), VARI (r = -0.49), GLI (r = -0.49), and GI (r = -0.49), while B was negatively correlated with E×G (r = -0.48). Among the vegetation indices, E×G showed strong positive correlations with NGRDI (r = 0.74), VARI (r = 0.84), GLI (r = 0.84), and GI (r = 0.85). Very strong positive correlations were also observed between NGRDI and VARI (r = 0.97), NGRDI and GLI (r = 0.96), NGRDI and GI (r = 0.95), VARI and GLI (r = 0.99), VARI and GI (r = 0.99), and GLI and GI (r = 1.00). Overall, SPAD values were more closely associated with vegetation indices in red leaves than in green leaves.

Different regression models were tested to evaluate the relationships between SPAD values and vegetation indices. Since the highest R^2 values were obtained from quadratic models, the regression results for both green and red leaves are presented based on these models. The general regression model applied in the study is expressed as follows:

$$SPAD = a + bx + cx^2 \tag{3.1}$$

In the model, *x* represents the respective vegetation index, while *a*, *b*, and *c* denote the regression coefficients. The quadratic model results indicated that the change in SPAD values as a function of vegetation index values did not follow a constant linear trend and that the second-order term contributed significantly to the model. Because NGRDI and VARI explained only a negligible proportion of the variation in SPAD values ($R^2 \approx 0.01$) in green leaves, these indices were excluded from further regression analyses. The relationships between SPAD values and the remaining vegetation indices were evaluated using quadratic regression models are presented in Figure 4.

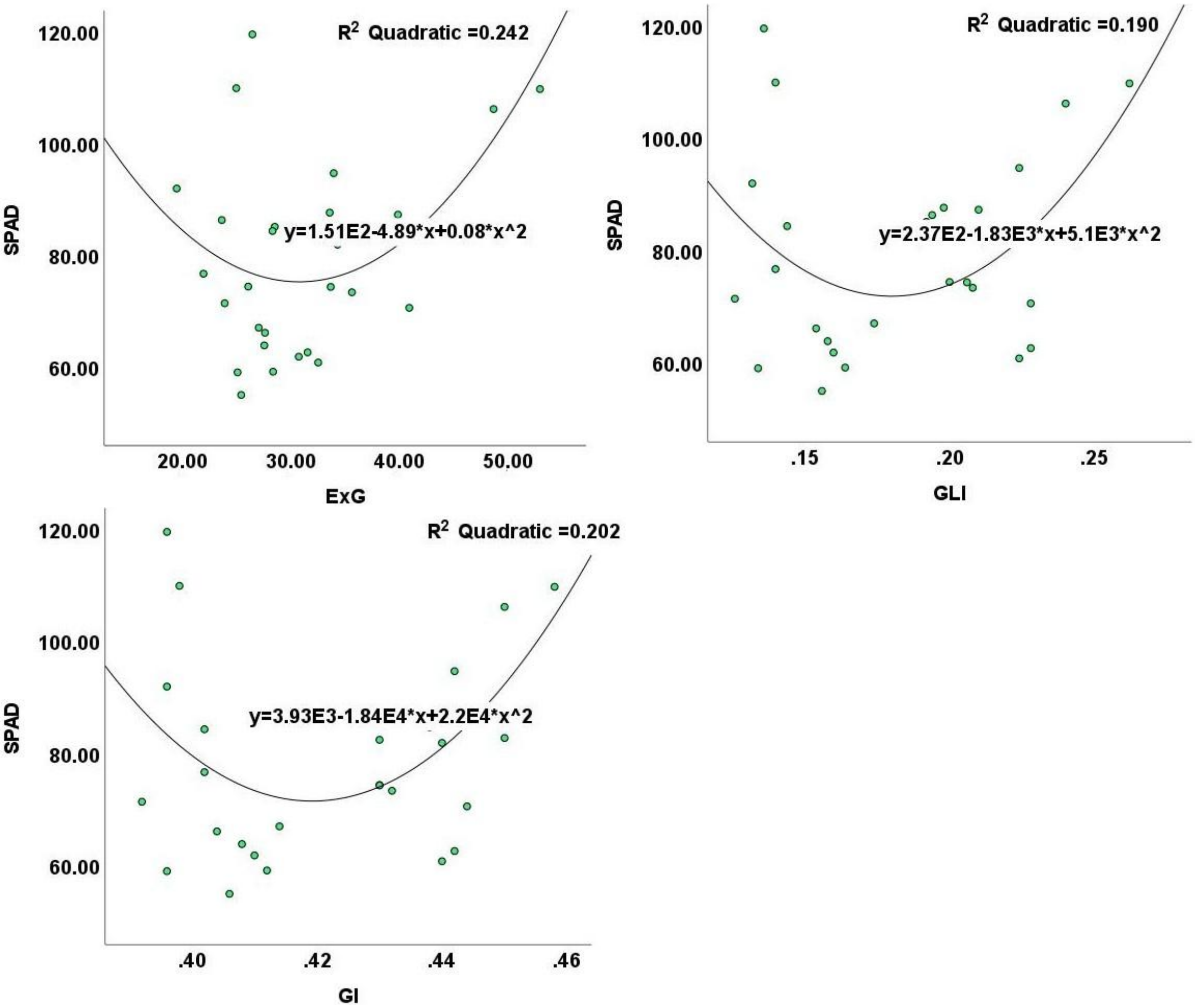

Figure 4. Quadratic regression models showing the relationships between SPAD values and vegetation indices in green leaves

Şekil 4. Yeşil yapraklarda SPAD değerleri ile vejetasyon indeksleri arasındaki ilişkileri gösteren kuadratik regresyon modelleri

The quadratic regression equations describing the relationships between SPAD values and vegetation indices in green leaves are presented below.

$$SPAD = 151 - 4.89E{\times}G + 0.08E{\times}G^2 \quad (3.2)$$

$$SPAD = 237 - 1830GLI + 5100GLI^2 \quad (3.3)$$

$$SPAD = 3930 - 18400GI + 22000GI^2 \quad (3.4)$$

Although the explanatory power of the quadratic models for green leaves was relatively limited, the relationships between SPAD values and the vegetation indices could still be described mathematically. Among the evaluated indices, E×G exhibited the highest explanatory power (R^2 = 0.242, RMSE = 14.252, MAE = 11.218). The corresponding values for GI and GLI were R^2 = 0.202, RMSE = 18.431, MAE = 14.860 and R^2 = 0.190, RMSE = 14.732, MAE = 11.990, respectively. These results indicate that E×G provided the strongest fit to the observed SPAD values among the vegetation indices evaluated for green leaves. The corresponding quadratic regression models for red leaves are presented in Figure 5.

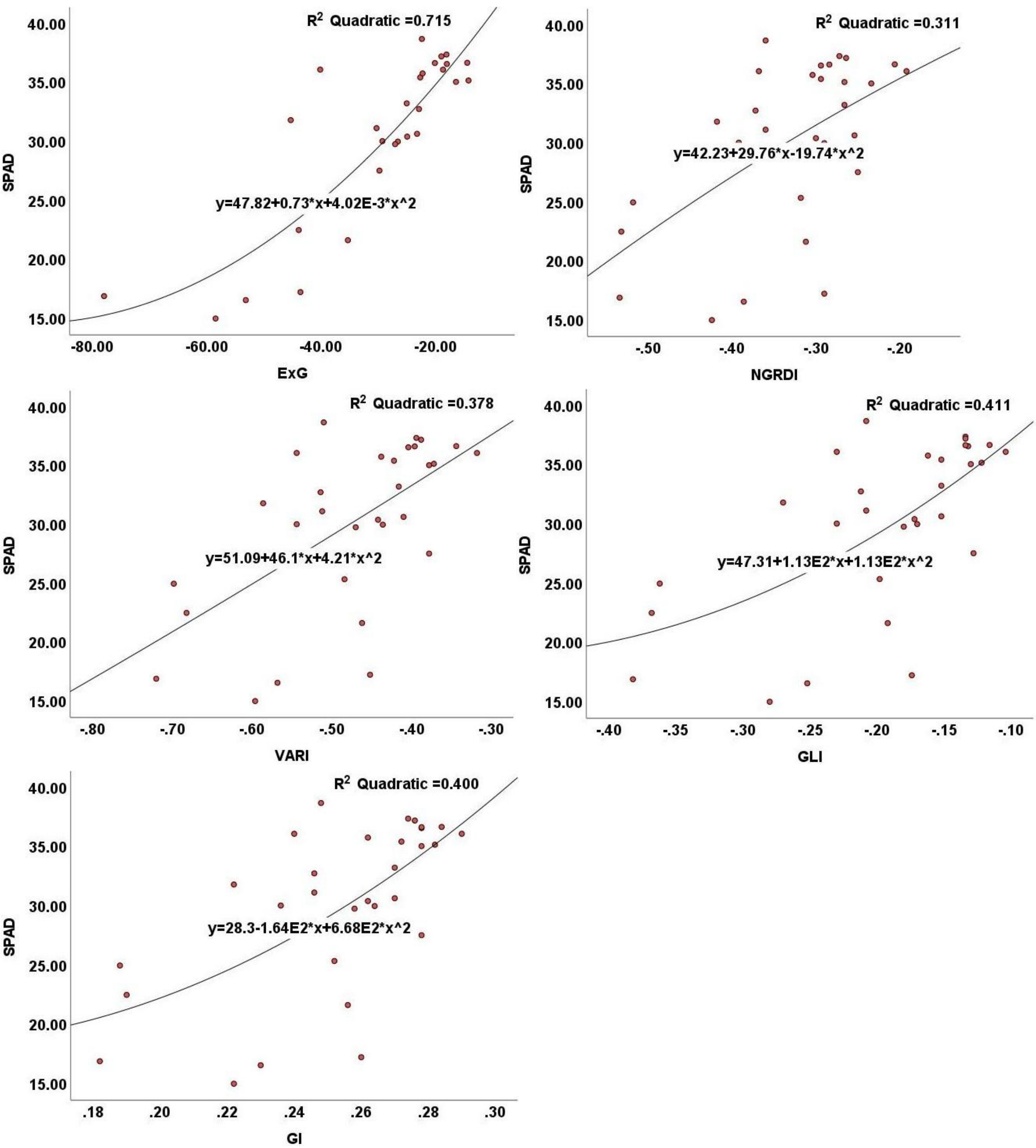

Figure 5. Quadratic regression models showing the relationships between SPAD values and vegetation indices in red leaves
Şekil 5. Kırmızı yapraklarda SPAD değerleri ile vejetasyon indeksleri arasındaki ilişkileri gösteren kuadratik regresyon modelleri

The corresponding quadratic regression equations for red leaves are presented below.

$$SPAD = 47.82 + 0.73E\times G + 0.00402E\times G^2 \quad (3.5)$$

$$SPAD = 42.23 + 29.76NGRDI - 19.74NGRDI^2 \quad (3.6)$$

$$SPAD = 51.09 + 46.1VARI + 4.21VARI^2 \quad (3.7)$$

$$SPAD = 47.31 + 113GLI + 113GLI^2 \quad (3.8)$$

$$SPAD = 28.3 - 164GI + 668GI^2 \quad (3.9)$$

For red leaves, the quadratic regression models generally exhibited higher explanatory power than those observed for green leaves. The strongest relationship was obtained with E×G (R^2 = 0.715, RMSE = 3.705, MAE = 2.727), indicating a substantial proportion of the variation in SPAD values could be explained by this index. The remaining vegetation indices also showed moderate explanatory performance, with R^2 values of 0.411 for GLI (RMSE = 5.331, MAE = 4.046), 0.400 for GI (RMSE = 5,376, MAE = 4.126), 0.378 for VARI (RMSE = 5.475, MAE = 4.236), and 0.311 for NGRDI (RMSE = 5.763, MAE = 4.562). These findings suggest that color-based vegetation indices, particularly E×G, were closely associated with SPAD values in red leaves. The obtained quadratic regression equations enabled a mathematical characterization of the relationships between SPAD values and vegetation indices.

4. Discussion

In this study, chlorophyll differences associated with leaf color (green and red) in *Photinia × fraseri* Dress. seedlings were determined using portable chlorophyll meter (SPAD-502) measurements. Furthermore, the relationships between vegetation indices derived from RGB-based image analyses and SPAD values obtained from green and red leaves were evaluated. The findings revealed clear and statistically significant differences between green and red leaves in terms of SPAD values, RGB color values (except blue), and vegetation indices. According to the results, the mean SPAD value in green leaves (79.83) was more than 2.5 times higher than that measured in red leaves (30.15). This finding indicates that green leaves possess higher chlorophyll content and, consequently, potentially greater photosynthetic capacity compared to red leaves. Since SPAD measurements were conducted simultaneously on green and red leaves from the same seedlings at the same developmental stage, inter-seedling physiological variation and environmental effects were minimized. Therefore, the observed differences can be attributed primarily to leaf color and associated pigment composition. Previous studies conducted on different plant species have demonstrated that chlorophyll content varies depending on leaf color (Jaleel et al., 2008; Zhang et al., 2018; Taques et al., 2021; Toța and Sala, 2023), which is consistent with the marked chlorophyll differences observed between green and red leaves in *P. × fraseri* seedlings. In this context, a study on *P. × fraseri* that examined variations according to leaf type and shoot position using a portable chlorophyll meter (SPAD 502Plus) reported that SPAD values ranged from 68.23 to 74.03 in green leaves and from 23.55 to 28.58 in red leaves (Toța and Sala, 2023). In another study, leaf chlorophyll content (SPAD) in *P. × fraseri* cuttings has also been reported to be sensitive to growing media and hormone applications. SPAD values averaged 76.38 in a peatmoss + sand + soil medium, while they decreased to 62.66 in a peatmoss + sand + clay medium. Regarding hormone treatments, SPAD values increased with IBA dose, reaching 66.90 in the control, 68.85 at 3500 mg L^{-1} IBA, and 72.82 at 7000 mg L^{-1} IBA (Edo, 2025). It has been reported that SPAD values obtained using portable chlorophyll meters exhibit considerable variation among plant species, largely associated with species-specific leaf characteristics (Atar et al., 2020; Atar and Bayraktar, 2022). For example, Atar et al. (2020) found that mean SPAD values ranged from 25.00 (*Acer negundo*) to 78.60 (*Laurocerasus officinalis*). Similarly, Atar and Bayraktar (2022) reported the highest mean SPAD value (73.68) in *Arbutus unedo* and the lowest (35.64) in *Hydrangea macrophylla*. Although these studies did not directly classify leaves according to color, the marked interspecific differences in SPAD values may be associated with differences in perceived leaf greenness related to chlorophyll content. Additionally, a study employing a non-destructive method to determine leaf chlorophyll content reported that SPAD values obtained from branches representing the four cardinal directions (north, south, east, and west) of the same species could vary significantly within the species (Atar et al., 2023). In this framework, the pronounced SPAD differences identified between green and red leaves in *P. × fraseri* seedlings may reasonably be attributed to differences in pigment composition reflected in leaf coloration, as also supported by previous findings (Toța and Sala, 2023).

Among the vegetation indices examined in this study, E×G emerged as the most sensitive variable reflecting leaf color–associated differentiation based on the overall findings of the correlation and regression analyses, despite the absence of significant correlations between SPAD values and vegetation indices in green leaves.

In particular, it exhibited higher explanatory power for predicting SPAD values in red leaves. In contrast, the relatively narrow range of chlorophyll content in green leaves and the associated saturation effect may have weakened the relationships with RGB-based vegetation indices. The higher performance of E×G in red leaves may be associated with the combined influence of chlorophyll and anthocyanin accumulation on leaf optical properties in *P.* × *fraseri*. The increase in red color values and the suppression of green color values due to anthocyanin accumulation may have enabled the E×G index, by its mathematical structure, to produce results that are more sensitive to differences between green and red color values. Conversely, indices such as NGRDI, VARI, GLI, and GI, which represent RGB color components in a more balanced manner, may have shown lower discriminative performance specifically in green leaves, where high chlorophyll levels and limited variability may have constrained index sensitivity. These findings indicate that the performance of RGB-based vegetation indices may vary depending on leaf pigment composition. Since all measurements were performed on the same species under identical sampling conditions, the contrasting relationships observed between green and red leaves are likely attributable to leaf-level optical and biochemical differences rather than external environmental factors. Vesali et al. (2017) reported that the red color value showed a much stronger relationship with chlorophyll-related measurements than the green color value. Similarly, Du et al. (2018) demonstrated that E×G exhibited the highest and significant relationship with SPAD-derived chlorophyll measurements. However, other vegetation indices have also been proposed for estimating chlorophyll content. For example, Signorelli et al. (2025) reported that GI, which showed strong correlations with leaf chlorophyll content, provides a simple and cost-effective alternative for monitoring chlorophyll dynamics. Likewise, Yuan et al. (2022) indicated that appropriate mathematical models can successfully quantify the relationship between vegetation indices and SPAD values using stable model structures and parameters.

The RGB-based vegetation indices used in this study were calculated based on mean RGB color values obtained from scanned leaf images using ImageJ software and were intended to compare relative color-based differences associated with leaf color. Therefore, the findings are not directly comparable with sensor-based field measurements but are primarily applicable to leaf-level physiological assessments conducted under controlled conditions. Indeed, the literature indicates that the color components used in RGB-based vegetation indices can be obtained through different data acquisition approaches depending on the measurement objective and experimental design. For example, in the study by Vesali et al. (2017), although the vegetation index ((G−R)/(G+R)) retained the same mathematical structure, it was calculated based on RGB color values obtained through contact imaging of light transmitted through the leaf using a smartphone camera. In that approach, the RGB color values represented relative spectral information derived from light absorption characteristics. In contrast, in the present study, RGB color components were derived from scanned leaf images and represent mean surface-based color values. In both approaches, the calculated indices aim to reveal relative color differences associated with leaf pigmentation. Similarly, numerous studies in the literature have employed RGB-based vegetation indices derived from scanned leaf images or digital camera–based color data (Kawashima and Nakatani, 1998; Karcher and Richardson, 2003; Yadav et al., 2010). In these studies, instead of sensor-based spectral measurements, mean RGB color values obtained from leaf surfaces were used to establish relationships between leaf color and chlorophyll content or nutritional status. Therefore, the use of different imaging techniques for deriving RGB-based indices should not be considered a limitation affecting methodological validity; rather, it should be interpreted as a methodological choice aligned with the specific application objective. At the same time, it should also be recognized that RGB values and vegetation indices may vary according to the image acquisition platform and measurement conditions. Previous studies have demonstrated that image-based approaches employing scanners, smartphones, digital cameras, and other imaging systems can successfully capture chlorophyll-related variation and provide reliable estimates of plant physiological status (Kawashima and Nakatani, 1998; Yadav et al., 2010; Vesali et al., 2017; Cen et al., 2019; Treder et al., 2021).

5. Conclusion

Based on the findings of this study, the marked differences observed in SPAD values between green and red leaves demonstrated a close association between leaf coloration and chlorophyll-related characteristics in *Photinia × fraseri*. Furthermore, RGB-based vegetation indices, particularly E×G, showed potential for quantifying leaf color–associated differences and estimating SPAD values in *P. × fraseri*. The practical applicability and repeatable nature of the method suggest that RGB-based vegetation indices can serve as useful tools for monitoring leaf color–associated physiological changes in ornamental plants such as *P. × fraseri*. In this context, incorporating RGB-based vegetation indices into future studies that simultaneously evaluate physiological and biochemical parameters would contribute to a clearer understanding of the relationships between leaf color and plant physiological status. In particular, statistically linking color-based indices with measurable physiological and biochemical indicators could strengthen their potential use as indirect markers for assessing plant health and stress conditions. Such an approach also offers potential for the development of rapid, non-destructive, and cost-effective monitoring methods. Furthermore, since no significant relationships were detected between SPAD values and the evaluated vegetation indices in green leaves, future studies should investigate additional vegetation indices and image-based approaches that may better capture chlorophyll variation within green foliage.

Author Contributions

AB: Conceived the study, developed the methodology, conducted the investigation, performed the formal analysis, prepared the visualizations, and wrote the original draft of the manuscript.

FA: Contributed to the methodology, participated in the formal analysis, validated the results, and reviewed and edited the manuscript.

DG: Contributed to the methodology, participated in the formal analysis, validated the results, and critically reviewed and edited the manuscript.

İT: Supervised the study, provided resources, and reviewed and edited the manuscript.

All authors read and approved the final version of the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Alvarsson, J. J., Wiens, S. and Nilsson, M. E. (2010). Stress recovery during exposure to nature sound and environmental noise. *International Journal of Environmental Research and Public Health, 7*(3), 1036–1046. https://doi.org/10.3390/ijerph7031036

Atar, F. and Bayraktar, A. (2022). Klorofil içeriğinin tespitinde ekstraksiyon yöntemi ve klorofilmetre (SPAD) ölçümleri arasındaki ilişki. In M. Çolak (Ed.), *Ziraat, orman ve su ürünleri alanında uluslararası araştırmalar IV* (pp. 5–23). Konya: Eğitim Yayınevi.

Atar, F., Bayraktar, A. and Güney, D. (2023). Determination of chlorophyll content variation dependent on direction and species using a portable chlorophyll meter (SPAD). In D. Güloğlu (Ed.), *Academic analysis and discussions in agriculture, forestry and fisheries* (pp. 145–161). Afyonkarahisar: Yaz Yayınları.

Atar, F., Güney, D., Bayraktar, A., Yıldırım, N. and Turna, İ. (2020). Seasonal change of chlorophyll content (SPAD value) in some tree and shrub species. *Turkish Journal of Forest Science, 4*(2), 245–256.

Barman, U. and Choudhury, R. D. (2022). Smartphone image based digital chlorophyll meter to estimate the value of citrus leaves chlorophyll using linear regression, LMBP-ANN and SCGBP-ANN. *Journal of King Saud University – Computer and Information Sciences, 34*(6), 2938–2950. https://doi.org/10.1016/j.jksuci.2020.01.005

Bowler, D. E., Buyung-Ali, L. M., Knight, T. M. and Pullin, A. S. (2010). A systematic review of evidence for the added benefits to health of exposure to natural environments. *BMC Public Health, 10*(1), 456. https://doi.org/10.1186/1471-2458-10-456

Castelli, F., Contillo, R. and Miceli, F. (1996). Non-destructive determination of leaf chlorophyll content in four crop species. *Journal of Agronomy and Crop Science, 177*, 275–283.

Cen, H., Wan, L., Zhu, J., Li, Y., Li, X., Zhu, Y., … and He, Y. (2019). Dynamic monitoring of biomass of rice under different nitrogen treatments using a lightweight UAV with dual image-frame snapshot cameras. *Plant Methods, 15*(1), 32. https://doi.org/10.1186/s13007-019-0418-8

Cerovic, Z. G., Masdoumier, G., Ghozlen, N. B. and Latouche, G. (2012). A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids. *Physiologia Plantarum, 146*, 251–260. https://doi.org/10.1111/j.1399-3054.2012.01639.x

Chang, S. X. and Robison, D. J. (2003). Nondestructive and rapid estimation of hardwood foliar nitrogen status using the SPAD-502 chlorophyll meter. *Forest Ecology and Management, 181*(3), 331–338. https://doi.org/10.1016/S0378-1127(03)00004-5

Crawley, M. J. (2013). *The R book* (2nd ed.). Chichester, UK: Wiley.

Cutolo, E. A., Guardini, Z., Dall'Osto, L. and Bassi, R. (2023). A paler shade of green: Engineering cellular chlorophyll content to enhance photosynthesis in crowded environments. *New Phytologist, 239*(5), 1567–1583. https://doi.org/10.1111/nph.19064

Dong, T., Shang, J., Chen, J. M., Liu, J., Qian, B., Ma, B., … and Zhou, G. (2019). Assessment of portable chlorophyll meters for measuring crop leaf chlorophyll concentration. *Remote Sensing, 11*(22), 2706. https://doi.org/10.3390/rs11222706

Du, S., Liu, X., Zhang, D., Zhang, X., Huang, L., Zhao, X., … and Xu, Y. (2018). Growth monitoring of weak gluten wheat using visible and multispectral UAV imagery. In *Proceedings of the 7th International Conference on Agro-geoinformatics* (pp. 1–6). IEEE.

Edo, N. Y. (2025). Enhancing cutting propagation in *Photinia × fraseri* plant through the synergistic effect of IBA, glycerol, and growing media mixture. *Zanin Journal of Science and Engineering, 1*(3), 80–92. https://doi.org/10.64362/zjse.86

Elsadek, M., Sun, M., Sugiyama, R. and Fujii, E. (2019). Cross-cultural comparison of physiological and psychological responses to different garden styles. *Urban Forestry & Urban Greening, 38*, 74–83. https://doi.org/10.1016/j.ufug.2018.11.007

Gitelson, A. A., Kaufman, Y. J., Stark, R. and Rundquist, D. (2002). Novel algorithms for remote estimation of vegetation fraction. *Remote Sensing and Environment, 80*(1), 76–87. https://doi.org/10.1016/S0034-4257(01)00289-9

Gobron, N., Pinty, B., Verstraete, M. M. and Widlowski, J. L. (2000). Advanced vegetation indices optimized for up-coming sensors: Design, performance, and applications. I*EEE Transactions on Geoscience and Remote Sensing, 38*(6), 2489–2505.

Holzman, D., Meletaki, V., Bobrow, I., Weinberger, A., Jivraj, R. F., Green, A. and Chatterjee, A. (2025). Natural beauty and human potential: Examining aesthetic, cognitive, and emotional states in natural, biophilic, and control environments. *Journal of Environmental Psychology, 104*, 102591. https://doi.org/10.1016/j.jenvp.2025.102591

Hunt, E. R., Cavigelli, M., Daughtry, C. S. T., McMurtrey, J. E. and Walthall, C. L. (2005). Evaluation of digital photography from model aircraft for remote sensing of crop biomass and nitrogen status. *Precision Agriculture, 6*(4), 359–378.

Hunt, E. R., Daughtry, C. S. T., Eitel, J. U. H. and Long, D. S. (2011). Remote sensing leaf chlorophyll content using a visible band index. *Agronomy Journal, 103*(4), 1090–1099. https://doi.org/10.2134/agronj2010.0395

Hunt, R. W. G. and Pointer, M. R. (2011). *Measuring colour* (4th ed.). John Wiley & Sons.

Jaleel, C. A., Gopi, R. and Panneerselvam, R. (2008). Growth and photosynthetic pigments responses of two varieties of *Catharanthus roseus* to triadimefon treatment. *Comptes Rendus Biologies, 331*(4), 272–277. https://doi.org/10.1016/j.crvi.2008.01.004

Janeczko, E., Czyżyk, K., Korcz, N., Woźnicka, M. and Bielinis, E. (2023). The psychological effects and benefits of using green spaces in the city: A field experiment with young Polish adults. *Forests, 14*(3), 497. https://doi.org/10.3390/f14030497

Karcher, D. E. and Richardson, M. D. (2003). Quantifying turfgrass color using digital image analysis. *Crop*

Science, 43(3), 943–951.

Kawashima, S. and Nakatani, M. (1998). An algorithm for estimating chlorophyll content in leaves using a video camera. *Annals of Botany, 81*(1), 49–54.

Kytridis, V. P. and Manetas, Y. (2006). Mesophyll versus epidermal anthocyanins as potential in vivo antioxidants: Evidence linking the putative antioxidant role to the proximity of oxy-radical source. *Journal of Experimental Botany, 57*(10), 2203–2210. https://doi.org/10.1093/jxb/erj185

Larraburu, E. E., Apóstolo, N. M. and Llorente, B. E. (2010). Anatomy and morphology of photinia (*Photinia × fraseri* Dress) in vitro plants inoculated with rhizobacteria. *Trees, 24*(4), 635–642. https://doi.org/10.1007/s00468-010-0433-x

Li, X., Li, J., Liu, A., Zhang, Y. and Zhao, K. (2025). Effects of different agricultural wastes on the growth of *Photinia × fraseri* under natural low-temperature conditions. *Horticulturae, 11*(9), 1055. https://doi.org/10.3390/horticulturae11091055

Li, X., Zhang, W., Niu, D. and Liu, X. (2024). Effects of abiotic stress on chlorophyll metabolism. *Plant Science, 342*, 112030. https://doi.org/10.1016/j.plantsci.2024.112030

Lichtenthaler, H. K. and Wellburn, A. R. (1983). Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions, 11*(5), 591–592. https://doi.org/10.1042/bst0110591

Liu, Y., Hatou, K., Aihara, T., Kurose, S., Akiyama, T., Kohno, Y., … and Omasa, K. (2021). A robust vegetation index based on different UAV RGB images to estimate SPAD values of naked barley leaves. *Remote Sensing, 13*(4), 686. https://doi.org/10.3390/rs13040686

Mao, Z. H., Deng, L., Duan, F. Z., Li, X. J. and Qiao, D. Y. (2020). Angle effects of vegetation indices and the influence on prediction of SPAD values in soybean and maize. *International Journal of Applied Earth Observation and Geoinformation, 93*, 102198. https://doi.org/10.1016/j.jag.2020.102198

Nishio, J. N. (2000). Why are higher plants green? Evolution of the higher plant photosynthetic pigment complement. *Plant, Cell & Environment, 23*(6), 539–548.

Özdamar, K. (1999). *Paket programlar ile istatistiksel veri analizi: SPSS MINITAB* (4. baskı). Eskişehir, Türkiye: Kaan Kitabevi.

Özkan, Y. (2003). *Uygulamalı istatistik 2*. İstanbul, Türkiye: Sakarya Kitabevi.

Quinn, G. P. and Keough, M. J. (2002). *Experimental design and data analysis for biologists*. Cambridge, UK: Cambridge University Press.

Rawat, U. S. and Agarwal, N. K. (2015). Biodiversity: Concept, threats and conservation. *Environment Conservation Journal, 16*(3), 19–28.

Reis, A. R., Favarin, J. L., Malavolta, E., Júnior, J. L. and Moraes, M. F. (2009). Photosynthesis, chlorophylls, and SPAD readings in coffee leaves in relation to nitrogen supply. *Communications in Soil Science and Plant Analysis, 40*(9–10), 1512–1528. https://doi.org/10.1080/00103620902820373

Richardson, A. D., Duigan, S. P. and Berlyn, G. P. (2002). An evaluation of noninvasive methods to estimate foliar chlorophyll content. *New Phytologist, 153*(1), 185–194.

Saberioon, M. M., Amin, M. S. M., Anuar, A. R., Gholizadeh, A., Wayayok, A. and Khairunniza-Bejo, S. (2014). Assessment of rice leaf chlorophyll content using visible bands at different growth stages at both the leaf and canopy scale. *International Journal of Applied Earth Observation and Geoinformation, 32,* 35–45. https://doi.org/10.1016/j.jag.2014.03.018

Signorelli, S., Casaretto, E., Etchemendy-Gamundi, M., Bentancor, M. and Harvey Millar, A. (2025). The green index: A widely accessible method to quantify the degree of greenness of photosynthetic organisms. *Plant, Cell & Environment, 48*(11), 8027–8043. https://doi.org/10.1111/pce.70102

Tang, Y., Fang, Z., Liu, M., Zhao, D. and Tao, J. (2020). Color characteristics, pigment accumulation and biosynthetic analyses of leaf color variation in herbaceous peony (*Paeonia lactiflora* Pall.). *3 Biotech, 10*(2), 76. https://doi.org/10.1007/s13205-020-2063-3

Taques, R. C. V., Giloni-Lima, P. C. and Lima, V. A. (2021). Vegetation inside: A comparative analysis of leaf pigments and chlorophyll fluorescence in variegated plants. *Scientia Plena, 17*(9), 1–12. https://doi.org/10.14808/sci.plena.2021.094901

Toța, C. and Sala, F. (2023). Photosynthetic pigments distributions in two leaves categories in *Photinia fraseri* 'Red Robin'. *Journal of Horticulture, Forestry and Biotechnology, 27*(2), 98–104.

Treder, W., Klamkowski, K., Sowik, I. and Maciorowski, R. (2021). Possibilities of using RGB-based image analysis to estimate the chlorophyll content of micropropagated strawberry plants. *Acta Scientiarum Polonorum. Hortorum Cultus, 20*(6), 105-115. https://doi.org/10.24326/asphc.2021.6.11

Trussell, H. J., Saber, E. and Vrhel, M. (2005). Color image processing (Basics and special issue overview). *IEEE Signal Processing Magazine, 22*(1), 14–22.

Tyrväinen, L., Ojala, A., Korpela, K., Lanki, T., Tsunetsugu, Y. and Kagawa, T. (2014). The influence of urban green environments on stress relief measures: A field experiment. *Journal of Environmental Psychology, 38*, 1–9. https://doi.org/10.1016/j.jenvp.2013.12.005

Vesali, F., Omid, M., Mobli, H. and Kaleita, A. (2017). Feasibility of using smart phones to estimate chlorophyll content in corn plants. *Photosynthetica, 55*(4), 603–610. https://doi.org/10.1007/s11099-016-0677-9

Wang, R., Helbich, M., Yao, Y., Zhang, J., Liu, P., Yuan, Y. and Liu, Y. (2019). Urban greenery and mental wellbeing in adults: Cross-sectional mediation analyses on multiple pathways across different greenery measures. *Environmental Research, 176*, 108535. https://doi.org/10.1016/j.envres.2019.108535

Wang, Z., Tan, X., Ma, Y., Liu, T., He, L., Yang, F., … and Ma, J. (2024). Combining canopy spectral reflectance and RGB images to estimate leaf chlorophyll content and grain yield in rice. *Computers and Electronics in Agriculture, 221*, 108975. https://doi.org/10.1016/j.compag.2024.108975

Woebbecke, D. M., Meyer, G. E., Von Bargen, K. and Mortensen, D. A. (1995). Color indices for weed identification under various soil, residue, and lighting conditions. *Transactions of the ASAE, 38*, 259–269. https://doi.org/10.13031/2013.27838

Yadav, S. P., Ibaraki, Y. and Dutta Gupta, S. (2010). Estimation of the chlorophyll content of micropropagated potato plants using RGB based image analysis. *Plant Cell, Tissue and Organ Culture, 100*(2), 183–188. https://doi.org/10.1007/s11240-009-9635-6

Yang, Y., Chen, X., Xu, B., Li, Y., Ma, Y. and Wang, G. (2015). Phenotype and transcriptome analysis reveals chloroplast development and pigment biosynthesis together influenced the leaf color formation in mutants of *Anthurium andraeanum* 'Sonate'. *Frontiers in Plant Science, 6*, 139. https://doi.org/10.3389/fpls.2015.00139

Yuan, Y., Wang, X., Shi, M. and Wang, P. (2022). Performance comparison of RGB and multispectral vegetation indices based on machine learning for estimating *Hopea hainanensis* SPAD values under different shade conditions. *Frontiers in Plant Science, 13*, 928953. https://doi.org/10.3389/fpls.2022.928953

Zhang, Q., Zhang, M., Ding, Y., Zhou, P. and Fang, Y. (2018). Composition of photosynthetic pigments and photosynthetic characteristics in green and yellow sectors of the variegated *Aucuba japonica* 'Variegata' leaves. *Flora, 240*, 25–33. https://doi.org/10.1016/j.flora.2017.12.010

Zhang, R., Yang, P., Liu, S., Wang, C. and Liu, J. (2022). Evaluation of the methods for estimating leaf chlorophyll content with SPAD chlorophyll meters. *Remote Sensing, 14*(20), 5144. https://doi.org/10.3390/rs14205144