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Effects of Kaolin Application on Leaf Chlorophyll Content and SPAD Values in Some Walnut Cultivars

**Abstract**

This study evaluated the effects of kaolin particle film application on leaf chlorophyll content and SPAD values in various walnut (*Juglans regia* L.) cultivars grown under Mediterranean climatic conditions. The plant material included Bilecik, Kaplan86, Malatya1, Şebin, Şen1, Tokat1, Yalova1, Yalova3, Yalova4, Yavuz, and 77H1. Compared with control trees, kaolin-treated trees had higher chlorophyll a, chlorophyll b, total chlorophyll, and SPAD values in most genotypes. On average, chlorophyll a increased from 0.85 to 1.18 mg/g FW, chlorophyll b from 0.38 to 0.53 mg/g FW, total chlorophyll from 1.23 to 1.72 mg/g FW, and SPAD values from 38.24 to 40.91 under kaolin treatment, indicating that kaolin particle film can effectively mitigate heat and light stress by preserving photosynthetic pigments and chlorophyll status. These results revealed that kaolin is a practical cultural strategy for walnut-growing regions where thermal and radiation stress during the summer months can reduce physiological performance.

Keywords: *Juglans regia*, genotype response, correlation, PCA analysis

**1. Introduction**

Walnut (*Juglans regia* L.) is one of the most important temperate nut crops, valued for its high-quality kernels, nutritional content, and timber. Taxonomically, it belongs to the family Juglandaceae (Ding et al., 2023). Among all *Juglans* species, *J. regia* is the most widely cultivated and commercially dominant species in global nut production (Bernard et al., 2018; Marrano et al., 2020). This species is generally considered native to a broad area extending from Central and West Asia to the Himalayan region, and it has spread widely through long-term human movement and cultivation (Aradhya et al., 2007; Bernard et al., 2018).

Türkiye is one of the major walnut-growing countries due to its ecological diversity, long horticultural tradition, and rich local germplasm resources (Bayazit et al., 2007; Şen, 2011). National production and tree inventories have increased substantially over the past decade, while improving competitiveness in high-value export segments remains a strategic objective (Güvenç and Kazankaya, 2019). The cultivation of walnuts in Türkiye's diverse climatic zones, ranging from the humid Black Sea region to the continental Eastern Anatolian plateaus, semi-arid inland basins, and Mediterranean coastal regions,

reveals a rich genetic resource for walnuts and highlights significant genotype-environment interactions in fruit quality and biochemical characteristics (Akça, 2009; Bayazıt et al., 2024; Bayazıt et al., 2025). This ecological diversity has enabled the development of different walnut varieties in terms of plant growth, yield, and nut and kernel size, and biochemical characteristics (Şen, 2011; Kafkas et al., 2017; Bayazıt and Çalışkan, 2020).

Climate change is a major ecological pressure on walnut production, as it intensifies summer heat stress, reduces precipitation, and weakens winter chill accumulation. In Mediterranean environments such as Türkiye, higher summer temperatures and more frequent heat extremes are expected to increase evaporative demand, exacerbate water deficits, impair photosynthetic performance, and elevate risks of sunburn and reduced productivity (Gauthier and Jacobs, 2011; IPCC, 2022). National projections for Türkiye indicate sustained warming throughout the 21st century, especially under higher-emission scenarios, implying progressively stronger thermal and hydrological constraints in current walnut-growing zones (Demircan et al., 2017). Regional walnut studies in Türkiye have also reported that low winter chilling and high summer temperatures are key limiting factors under Mediterranean conditions, with clear effects on yield and quality stability (Bükücü and Sütyemez, 2016; Bayazıt & Çalışkan, 2020; Bayazıt et al., 2024). In parallel, declining chill accumulation threatens proper dormancy release and synchronized budbreak in temperate fruit species, increasing the risk to flowering regularity, yield stability, and cultivar adaptation in walnuts (Aslamaraz et al., 2009; Çalışkan and Bayazıt, 2024).

In Mediterranean-type environments, walnut production is increasingly limited by compound climate stressors, particularly hotter and drier summers with stronger solar load. Elevated air temperatures, high radiation, and declining precipitation together increase evaporative demand and canopy heat load, which can accelerate chlorophyll degradation, reduce photochemical efficiency, and increase sunburn injury in leaves and nuts (Palliotti et al., 2009; Gauthier and Jacobs, 2011). For Türkiye, climate projections indicating sustained warming and increasing hydroclimatic pressure suggest that summer stress exposure in walnut-growing zones will intensify over time (Demircan et al., 2017).

Kaolin particle film is among the most widely used protective practices against heat and excess radiation in tree crops. By forming a reflective white layer on plant surfaces, kaolin can lower tissue temperature, redistribute light within the canopy, and help protect the photosynthetic machinery under stress conditions (Glenn and Puterka, 2005; Glenn et al., 2010). In walnut, previous studies have reported reductions in sunburn damage and improvements in physiological and fruit quality traits following kaolin application, although treatment efficacy depends on genotype and environment (Rosati et al., 2007; Gharaghani et al., 2018; Bayazıt et al., 2024). Therefore, this study evaluated whether kaolin treatment improves chlorophyll status and supports photosynthetic capacity under summer stress by assessing chlorophyll *a*, chlorophyll *b*, total chlorophyll, and SPAD values in selected walnut cultivars under Mediterranean conditions, with emphasis on cultivar-specific physiological responses.

2. Material and Methods

2.1. Plant material

The study was conducted in 2015 in a walnut adaptation orchard in Yayladağı (Hatay, Türkiye), at approximately 421 m above sea level (35° 55' N, 36° 05' E), under Mediterranean climatic conditions characterized by hot, high-radiation summers. The walnut cultivars Bilecik, Kaplan86, Malatya1, Şebin, Şen1, Tokat1, Yalova1, Yalova3, Yalova4, Yavuz, and 77H1, which were selected and improved from different regions of the country, were used as research material. In the experiment, seedlings of the cultivars were planted in 2001 at 7 × 7 m intervals, with six trees of each cultivar per plot. Trees with uniform growth were selected for the study. In addition, in the study area, maximum air temperatures were measured as 30.5°C, 33.1°C, and 36.9°C in June, July, and August, respectively.

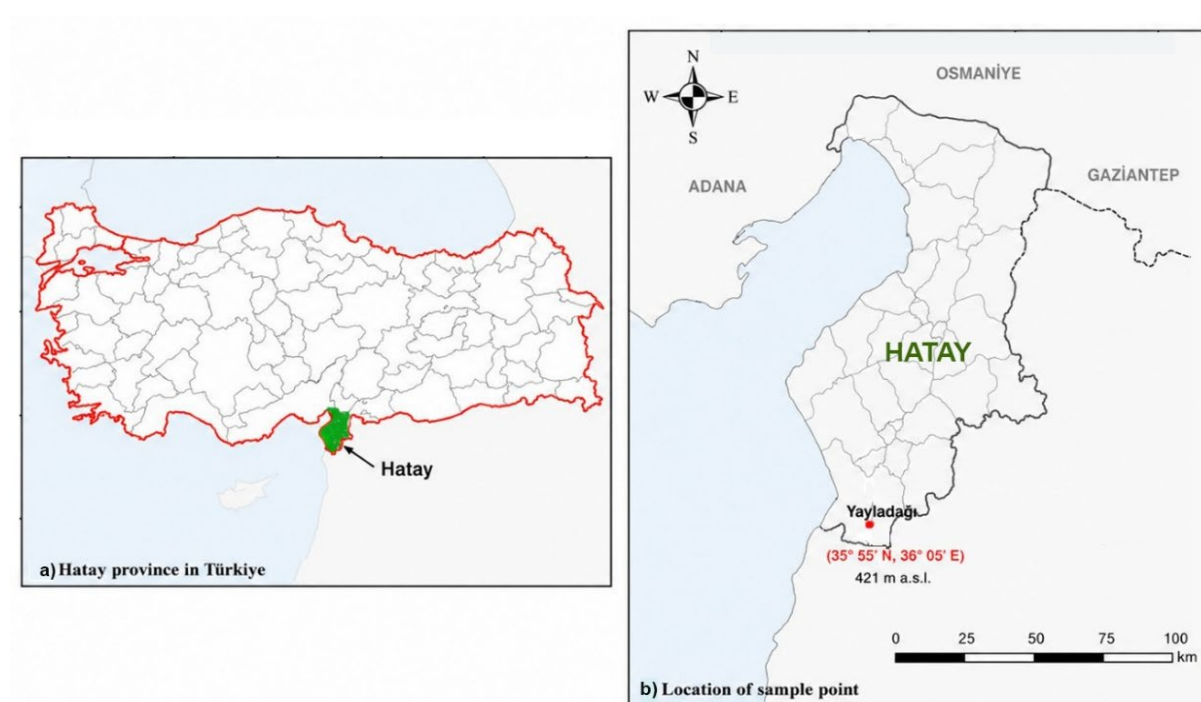


Figure 1. Geographic location of the sampling site of the studied walnut genotypes

2.2. Kaolin Treatments

The experiment used a randomized design with three replicates and one tree per replicate for each genotype. Kaolin particle film was applied using a commercial formulation (Surround WP; 95% kaolin and 5% adjuvant). Two foliar applications were performed. The first application (full dose) used a 6% suspension (6 kg per 100 L water) in early June during the fruit set period. The second application (half dose) used a 3% suspension (3 kg per 100 L water) in the second half of June. Applications were made with a sprayer to ensure full canopy coverage. Control trees received only a water spray.

2.3. Chlorophyll and SPAD Measurements

Leaf samples were collected on July 10 from fully expanded leaves after the second kaolin application. Chlorophyll extraction and calculation procedures followed the methods described by Arnon (1949) and Lichtenthaler and Wellburn (1983). After determining the fresh weights (FW) of the collected samples, the samples were homogenized on a porcelain plate with 1–2 ml of 80% acetone. The homogenates were filtered through coarse filter paper into 10 ml glass tubes and brought to a final volume of 10 ml with 80% acetone. Absorbance values were measured at 663, 645, and 652 nm using a spectrophotometer. Chlorophyll concentrations were calculated as chlorophyll a, chlorophyll b, and total chlorophyll using the following standard equations:

$$\text{Chlorophyll } a = \frac{(11.75 \times A_{663} - 2.35 \times A_{645}) \times 10}{\text{Sample weight} \times 1000}$$

$$\text{Chlorophyll } b = \frac{(18.61 \times A_{645} - 3.96 \times A_{663}) \times 10}{\text{Sample weight} \times 1000}$$

$$\text{Total Chlorophyll} = \frac{(\text{A}_{652} \times 27.8) \times 10}{\text{Sample weight} \times 1000}$$

The relative chlorophyll index was measured in the field using a portable SPAD meter (SPAD 502, Konica) on physiologically comparable leaves from each experimental unit.

2.4. Statistical Analysis

Data were analyzed using SAS/STAT procedures (SAS Institute, 2005). Treatment means were compared using the t-test at $P < 0.05$. In addition, correlation and PCA analyses were performed on the examined characteristics using JMP Pro 16.

3. Results and Discussion

Kaolin particle film application significantly enhanced the photosynthetic pigment profile across the evaluated walnut genotypes compared to the untreated controls. Chlorophyll *a* content across all cultivars increased by approximately 38.8%, rising from 0.85 mg/g FW in the control to 1.18 mg/g FW in the kaolin-treated group (Figure 2).

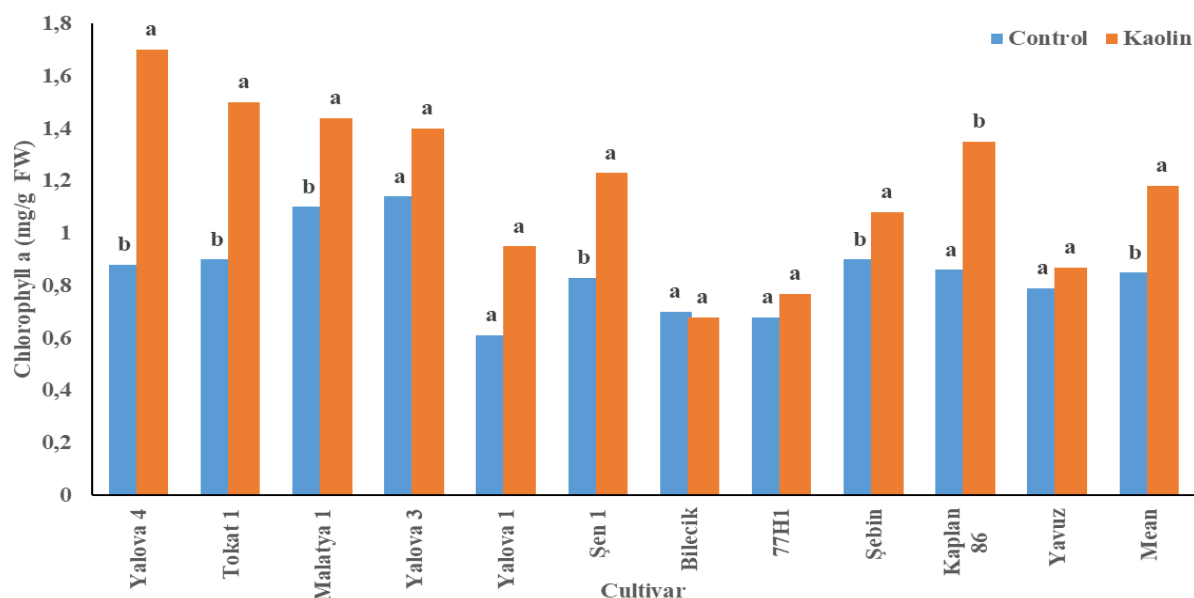


Figure 2. Effects of kaolin application on chlorophyll *a* content (mg/g FW) in different walnut genotypes. Different letters indicate significant differences at $p < 0.05$.

Among the studied genotypes, Yalova 4, Malatya 1, Şen 1, Şebin, and Kaplan 86 showed statistically higher chlorophyll *a* levels after kaolin treatment. This protection is critical under Mediterranean conditions, where August temperatures peaked at 36.9°C, suggesting that kaolin effectively shields photosynthetic reaction centers from photo-oxidative damage.

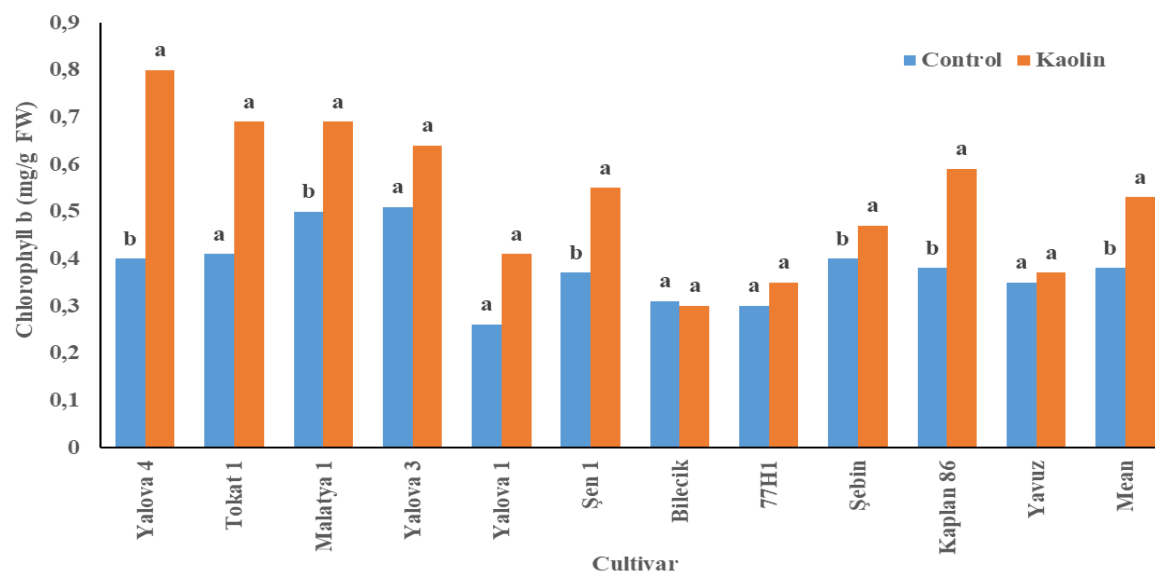


Figure 3. Effects of kaolin application on chlorophyll *b* content (mg/g FW) in different walnut genotypes. Different letters indicate significant differences at $p < 0.05$.

Similarly, chlorophyll *b* levels showed a significant upward trend, increasing from 0.38 mg/g FW to 0.53 mg/g FW (a 39.4% increase) with kaolin application (Figure 3). Significant increases in chlorophyll *b* were recorded in Yalova 4, Malatya 1, Şen 1, and Kaplan 86. Given the role of chlorophyll *b* in light harvesting, this increase is important for maintaining photosynthetic efficiency. The total chlorophyll content reached a mean of 1.72 mg/g FW in kaolin-treated trees, compared to 1.23 mg/g FW in the control group (Figure 4). The most notable enhancements were observed in the Yalova 4 and Tokat 1 genotypes. This significant increase (approximately %40) indicates that the reflective barrier formed by the kaolin significantly mitigates leaf thermal load, preventing the degradation of the pigment system that typically occurs under heat stress in walnuts. SPAD measurements were consistent with chlorophyll analyses. The mean SPAD value was 40.91 in kaolin-treated trees and 38.24 in the control. Kaolin application produced statistically significant SPAD increases in Yalova 4, Tokat 1, and Malatya 1 (Figure 5).

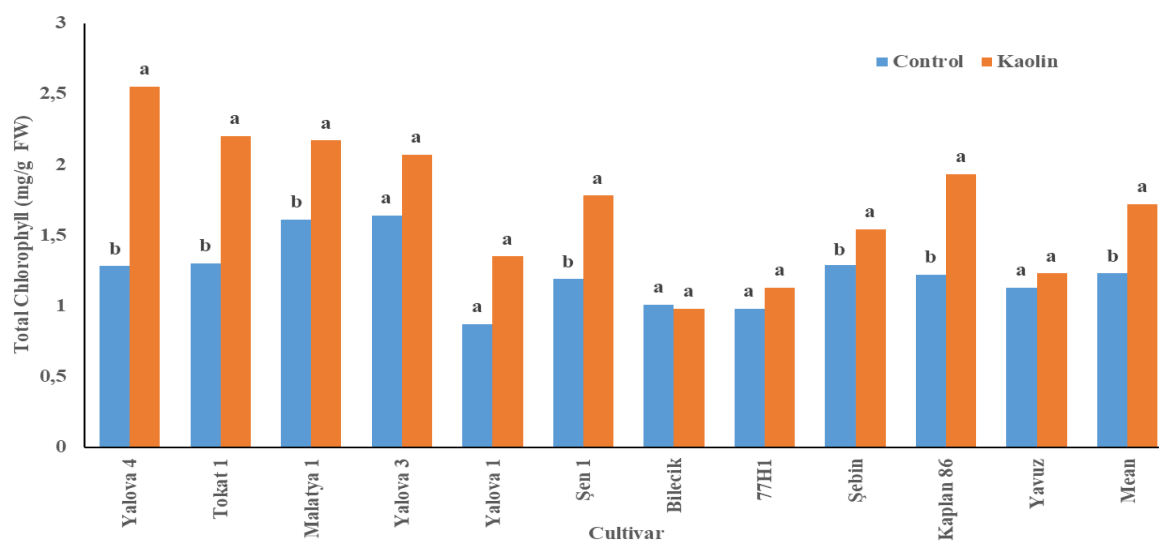


Figure 4. Effects of kaolin application on total chlorophyll content (mg/g FW) in different walnut genotypes. Different letters indicate significant differences at $p < 0.05$.

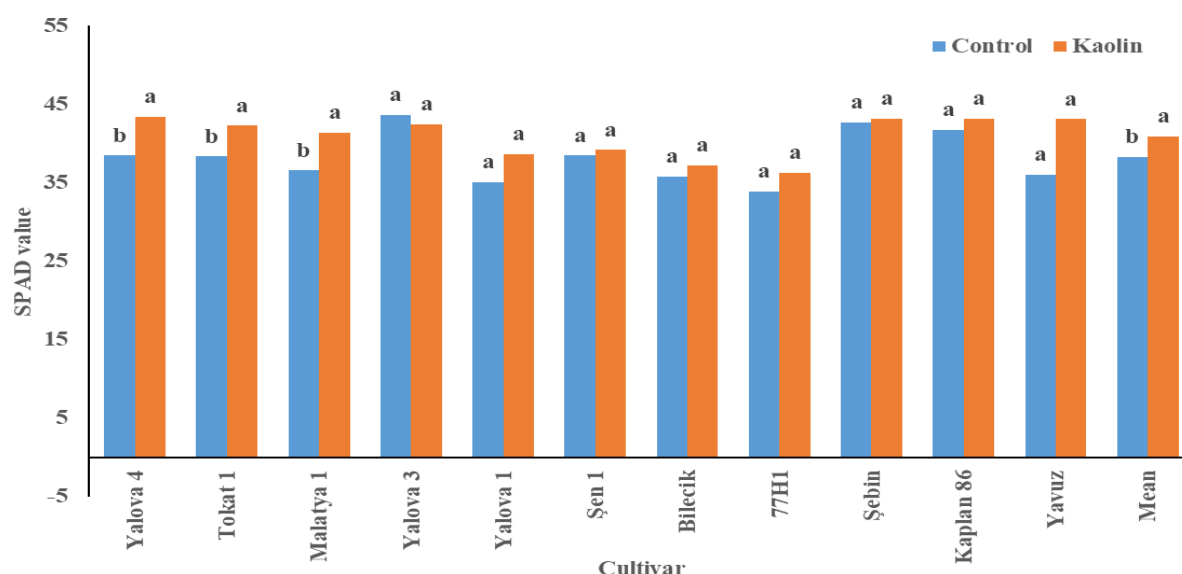


Figure 5. Effects of kaolin application on SPAD values different walnut genotypes. Different letters indicate significant differences at $p < 0.05$.

Kaolin treatment in this study consistently improved photosynthetic pigment status across genotypes, with mean chlorophyll *a* increasing from 0.85 to 1.18 mg/g FW, chlorophyll *b* from 0.38 to 0.53 mg/g FW, and total chlorophyll from 1.23 to 1.72 mg/g FW. SPAD values also increased from 38.24 to 40.91. These gains represent approximately 39-40% increases for chlorophyll fractions and about 7% for SPAD, indicating that the treatment effect was physiologically significant rather than marginal. The strongest responses in Yalova 4, Tokat 1, Malatya 1, Şen 1, and Kaplan 86 further suggest genotype-dependent sensitivity to particle film protection. Mechanistically, these results were consistent with the established role of kaolin in reducing leaf energy load and canopy thermal stress through increased reflectance (Glenn and Puterka, 2005). Previous studies showed that kaolin can lower tissue temperature and reduce sunburn risk under high-radiation conditions. For example, in pear, fruit surface temperature decreased by several degrees Celsius and sunburn incidence was markedly reduced (Colavita et al., 2011). In addition, our results were in agreement with those of Kılıç et al. (2017), who reported in walnut that kaolin reduced plant overheating and sunburn injury while not causing adverse effects on total chlorophyll, supporting the protective role of kaolin under high-radiation conditions. Similar physiological benefits reported in other fruit crops, including improved gas exchange and water-use efficiency in grape fruit under hot, high-light conditions (Jifon and Syvertsen, 2003), and enhanced photo assimilate-related metabolism in grapevine leaves (Conde et al., 2018). Gharaghani et al. (2018) also supported this interpretation with studies on walnuts, reporting that kaolin alleviated light and heat stress in Iranian walnuts and improved leaf physiology, gas exchange, and walnut quality. Earlier canopy-scale studies in walnut and almond indicate that responses may vary with canopy structure and light redistribution dynamics (Rosati et al., 2007), consistent with the cultivar differences observed here. These results indicate that kaolin is an effective tool for mitigating heat stress in walnut orchards, especially under Mediterranean summer conditions, where preserving chlorophyll stability is critical for sustaining photosynthetic capacity.

The correlation results between the examined characteristics were presented in Table 1. Very strong positive correlations were found among *Ca* (chlorophyll *a*), *Cb* (chlorophyll *b*), and TC (total chlorophyll) ($r = 0.99$, $p < 0.01$). This confirmed that chlorophyll characteristics change together and that the pigment system behaves as a whole. The correlations between SPAD and *Ca*, *Cb*, and TC were also positive and

statistically significant ($r = 0.66\text{--}0.69$, $p < 0.05$), but lower than the correlations among the chlorophyll characteristics. These results confirmed that SPAD values reflect chlorophyll status well; however, they can differ from direct chlorophyll measurements. These results were consistent with literature reporting a generally strong correlation between SPAD measurements and leaf chlorophyll content, although this relationship can vary depending on species, leaf structure, and environmental conditions (Uddling et al., 2007; Palliotti et al., 2009; Wang et al., 2024).

Table 1. Pearson correlation coefficients among the examined characteristics in walnuts

Trait	Ca	Cb	TC	SPAD
Ca	1	0.99**	0.99**	0.69*
Cb		1	0.99**	0.66*
TC			1	0.67*
SPAD				1

* and ** indicate significance at the 5% and 1% levels, respectively.

Principal Component Analysis was performed to determine the multivariate relationships between the measured physiological parameters and the treatments across different walnut genotypes (Figure 6). The PCA analysis revealed that the first two principal components explained 99.6% of the total variation, with PC1 and PC2 accounting for 88.3% and 11.3% of the variance, respectively.

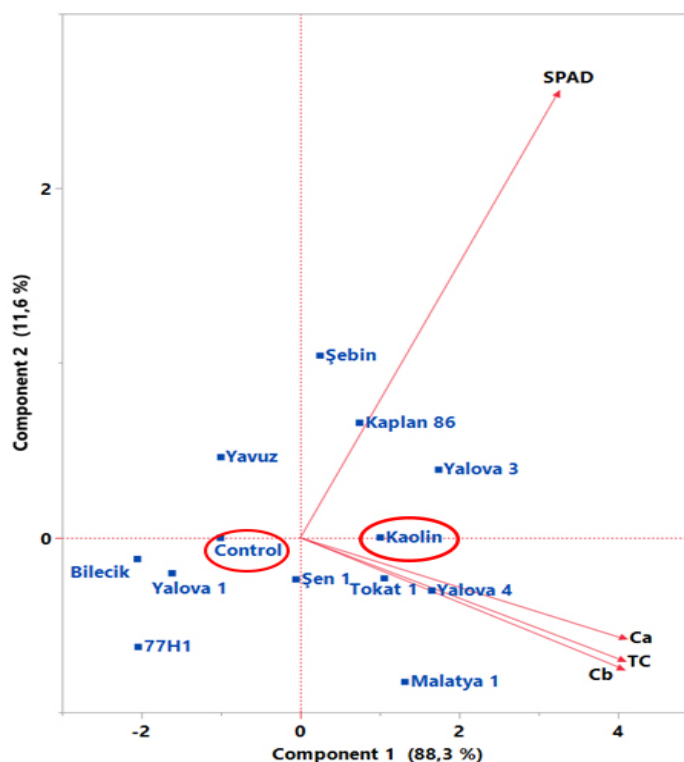


Figure 6. PCA results of kaolin application response on chlorophyll parameters and SPAD value in walnut cultivars.

PC1 primarily represented chlorophyll-related properties, with *Ca*, *Cb*, and TC closely aligned, indicating a strong positive correlation among these variables. In contrast, the SPAD was the primary driver for PC2. While SPAD was positively associated with the biochemical chlorophyll variables, its orientation on the second axis demonstrates that it captures a distinct dimension of the physiological variation within the leaf tissue.

The distribution of genotypes and treatments on the biplot further revealed a clear separation between kaolin-applied groups and the control. All walnut cultivars subjected to kaolin application were positioned on the positive side of the PC1 axis, indicating a systematic enhancement in the leaf pigment profile regardless of the genotype. Conversely, the untreated control genotypes were clustered on the negative side of the PC1 axis. Different positions of walnut cultivars were distributed around these vectors, indicating genotype-dependent responses in chlorophyll and SPAD behavior. These results were physiologically consistent with the known mode of action of kaolin particle films (Glenn and Puterka, 2005; Glenn et al., 2010). By increasing reflectance and reducing leaf thermal load, kaolin can decrease photo inhibitory pressure and help maintain pigment systems under high radiation and high temperature conditions (Dinis et al., 2016; Palliotti et al., 2009). Some researchers reported that kaolin mitigates heat and light damage in walnuts, improves leaf physiological performance, reduces sunburn damage, and enhances walnut quality (Gharaghani et al., 2018; Rosati et al., 2007). Similar protective effects have been observed in other fruit crops, such as grapevine and citrus, where kaolin improves photoprotection and stabilizes photosynthetic function under stress (Palliotti et al., 2009; Terán et al., 2024). These results confirmed that the effect of kaolin application on chlorophyll can vary depending on the genotype, stress intensity, and application timing (Gharaghani et al., 2018; Glenn et al., 2010; Terán et al., 2024).

4. Conclusion

This study demonstrated that foliar kaolin particle film application was an effective strategy for protecting walnut trees against the detrimental effects of thermal and radiation stress. The results showed that kaolin treatment significantly prevented the degradation of photosynthetic pigments during periods of intense solar radiation. The reflective barrier provided by the particle film successfully mitigated the heat load on the leaf surface, which proved vital during the peak summer temperatures recorded in the Mediterranean region. The research also highlighted distinct genotype-dependent responses to the treatment among the evaluated walnut cultivars. Specifically, genotypes such as Yalova 4, Tokat 1, and Malatya 1 exhibited superior physiological resilience, maintaining higher pigment stability and chlorophyll index values compared to the other genotypes. The multivariate analysis further validated these results by clearly separating the physiological performance of kaolin-applied genotypes from the heat-stressed control groups. In conclusion, kaolin application was found to be a sustainable and practical management tool to safeguard the photosynthetic apparatus and maintain the physiological vigor of walnut trees under extreme heat conditions.

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