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# Leaf chlorophyll characteristics and red/far-red fluorescence responses of two pear cultivars under contrasting canopy light conditions

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## Abstract

This study evaluated total chlorophyll content (Chl (a + b)) and chlorophyll fluorescence in sun-exposed and shaded leaves of two pear cultivars, 'Santa Maria' and 'Abbas', in September. The field experiment was conducted in the Zhurje area of Albania using four trees per cultivar. One fully developed leaf was sampled from each of the sun-exposed and shaded canopy positions on each tree, providing four biological replicates (n = 4) for each cultivar × light-condition combination and 16 leaves in total. Total chlorophyll content was determined by acetone extraction, and chlorophyll fluorescence was measured at an excitation wavelength of 470 nm. Data were analysed using two-way analysis of variance (ANOVA), with cultivar and light condition as fixed factors. Chl (a + b) content was higher in sun-exposed than in shaded leaves in both cultivars. In 'Santa Maria', the values were 6.420 ± 0.610 and 3.236 ± 0.557 mg $dm^{-2}$ in sun-exposed and shaded leaves, respectively; the corresponding values in 'Abbas' were 6.566 ± 1.820 and 4.715 ± 0.446 mg $dm^{-2}$. The F690/F735 ratio ranged from 1.407 ± 0.444 to 1.553 ± 0.264 in 'Santa Maria' and from 0.940 ± 0.076 to 1.386 ± 0.120 in 'Abbas' across the two light conditions. Cultivar and light condition had significant effects on the F690/F735 ratio ($P = 0.035$ and $P = 0.047$, respectively), whereas their interaction was not significant ($P = 0.284$). For Chl (a + b), light condition had a significant effect ($P < 0.001$), whereas cultivar ($P = 0.139$) and the cultivar × light-condition interaction ($P = 0.217$) did not. The combined assessment of Chl (a + b) content and the F690/F735 ratio provides complementary information for comparing the responses of pear leaves to contrasting canopy light conditions.

**Key words**: Chlorophyll content, chlorophyll fluorescence, fluorescence spectra, *Pyrus communis*, F690/F735.

## Introduction

Light conditions are associated with differences in leaf development, pigment accumulation and photosynthetic activity. Leaves located in exposed parts of the tree receive higher irradiance, whereas leaves within the inner canopy develop under lower and more variable light conditions. These contrasting environments can produce differences in leaf thickness, chloroplast organization, pigment composition and photosynthetic capacity (Lichtenthaler *et al*., 2000; Lichtenthaler *et al*., 2007a; Long *et al*., 2022; Swoczyna *et al*., 2022). Such adjustments are part of leaf acclimation to the prevailing light environment. Differences between sun and shade leaves have been reported in woody plants, including changes in chlorophyll content, photosynthetic activity and chlorophyll fluorescence characteristics (Lichtenthaler *et al*., 2000; Lichtenthaler *et al*., 2007a, b). Canopy architecture is an important factor influencing the amount and distribution of radiation within fruit trees. Leaf position, canopy density and branch arrangement influence the light reaching individual leaves and are associated with differences in their physiological performance. In pear trees, differences in canopy position and training systems have been associated with changes in leaf physiology and photosynthetic characteristics (Liu *et al*., 2020). Light conditions can influence photosynthetic pigment characteristics and leaf physiological responses in horticultural crops (Reyna-Ávila *et al*., 2025). The spatial distribution of radiation within fruit-tree canopies is also influenced by canopy structure and leaf arrangement (Tang *et al*., 2024). Therefore, differences between exposed and shaded leaves can reflect both the intensity of incident radiation and the structural characteristics of the canopy. Chlorophyll is the main photosynthetic pigment involved in light absorption during photosynthesis. Its concentration is related to the photosynthetic characteristics of leaves and can change in response to environmental conditions and leaf development (Baker, 2008; Maxwell and Johnson, 2000).

Leaves located in sun-exposed parts of the canopy commonly develop characteristics associated with greater photosynthetic capacity, including changes in pigment accumulation and leaf structure (Lichtenthaler *et al*., 2007a; Long *et al*., 2022). Shade leaves, in contrast, develop under reduced photon availability and adjust their photosynthetic organization to improve the use of available radiation (Lichtenthaler *et al*., 2007a, b; Long *et al*., 2022). Thus, comparison of total chlorophyll content between sun and shade leaves provides useful information on differences between leaves developing under contrasting light conditions. Cultivar characteristics can also contribute to differences in chlorophyll accumulation. Substantial variation in chlorophyll content among tomato genotypes exposed to dark treatment further demonstrates the influence of genetic background on pigment responses to environmental stress Genetic background, rootstock and growing conditions may influence leaf pigment concentration and photosynthetic performance. Faizi *et al*. (2023) reported differences in leaf chlorophyll content among European pear cultivars grafted on different rootstocks, indicating that

chlorophyll accumulation can vary according to plant genotype and rootstock combination.

Physiological differences among pear leaves can also occur according to their position within the canopy and the associated light environment (Liu *et al*., 2020). These findings support the comparison of chlorophyll content among pear cultivars growing under contrasting light conditions. Leaf structure is closely related to light absorption and distribution within photosynthetic tissues. Sun leaves commonly have greater thickness and a more developed palisade mesophyll, whereas shade leaves tend to have a thinner structure adapted to lower irradiance (Lichtenthaler *et al*., 2007a,b). Differences in leaf morphological characteristics have also been reported in *Pyrus communis* L. (Kārkliņa *et al*., 2021). Such structural differences can influence the amount of chlorophyll-bearing tissue per unit leaf area and can modify light propagation and fluorescence within the leaf. Therefore, differences in chlorophyll content between sun and shade leaves may reflect both pigment accumulation and structural organization of photosynthetic tissues. Photosynthetic acclimation also involves changes in resource allocation to the photosynthetic apparatus. Nitrogen is an important component of chlorophyll and photosynthetic proteins, and nitrogen distribution within leaves is closely associated with photosynthetic capacity (Evans, 1989). Under higher irradiance, greater investment in photosynthetic components can support increased carbon assimilation, whereas leaves developing under shade may allocate resources differently according to available radiation (Evans, 1989; Long *et al*., 2022). Although nitrogen concentration was not measured in the present study, this relationship may be relevant to the differences in chlorophyll content between sun and shade leaves.

Chlorophyll fluorescence provides a non-destructive approach for characterizing the optical and physiological properties of leaves. It provides information related to the photosynthetic apparatus and its response to environmental conditions (Baker, 2008; Kalaji *et al*., 2016; Kalaji *et al*., 2017). Chlorophyll fluorescence measurements have been demonstrated to provide useful complementary information on photosynthetic performance and physiological responses to environmental stress (Vishnu *et al*., 2025). Fluorescence emission from green leaves occurs mainly in the red and far-red regions, with characteristic bands near 690 and 735–740 nm. The relative intensity of these bands is influenced by chlorophyll concentration, fluorescence reabsorption and the optical properties of the leaf (Buschmann, 2007). The F690/F735 ratio has been used to characterize differences in chlorophyll-related fluorescence emission. Red fluorescence around 690 nm is more strongly reabsorbed by chlorophyll within the leaf than far-red fluorescence around 735 nm. Consequently, changes in chlorophyll concentration and leaf optical structure can modify the F690/F735 ratio (Buschmann, 2007). Differences in red and far-red fluorescence between sun and shade leaves have also been related to changes in chlorophyll content and photosynthetic activity (Lichtenthaler *et al*., 2000; Lichtenthaler *et al*., 2007a, b). The ratio can therefore provide complementary information when used together with direct measurements of chlorophyll concentration. The interpretation of chlorophyll fluorescence also depends on measurement conditions. Excitation wavelength influences the absorbed radiation and the resulting fluorescence signal, particularly when leaves differ in pigment concentration and optical properties. Lysenko *et al*. (2020) demonstrated that fluorescence characteristics can vary according to excitation conditions and the spectral properties of photosynthetic tissues. Using the same excitation wavelength for all samples is therefore important when comparing fluorescence characteristics among cultivars and leaf positions. Environmental stress, particularly salinity, can alter photosynthetic pigment accumulation and physiological characteristics in horticultural crops (Rahman *et al*., 2025). Fluorescence parameters can provide information on energy conversion, photochemical activity and energy dissipation in photosystem II (Maxwell and Johnson, 2000; Kalaji *et al*., 2016; Kalaji *et al*., 2017). Recent studies have also emphasized fluorescence-based measurements as a useful approach for detecting physiological changes associated with environmental stress (Park *et al*., 2024; Swoczyna *et al*., 2022). However, the F690/F735 ratio should not be considered equivalent to direct PSII efficiency measurements such as Fv/Fm or ΦPSII because it is strongly influenced by chlorophyll concentration and leaf optical properties (Buschmann, 2007; Kalaji *et al*., 2017). Pear trees provide a useful system for comparing these differences because individual leaves within the same tree can experience markedly different light environments. Differences in canopy position are associated with contrasting irradiance conditions that may be reflected in chlorophyll content, leaf structure and fluorescence characteristics (Liu *et al*., 2020; Tang *et al*., 2024). In addition, cultivar-dependent differences in chlorophyll content have been reported in European pear (Faizi *et al*., 2023). However, information combining total chlorophyll content and red/far-red chlorophyll fluorescence in sun and shade leaves of pear cultivars under field conditions remains limited.

The aim of this study was to compare total chlorophyll content (Chl (a + b)) and chlorophyll fluorescence characteristics in sun and shade leaves of two pear cultivars, '*Santa Maria*' and '*Abbas*', during September. Fluorescence measurements were performed using an excitation wavelength of 470 nm, with particular attention to the F690/F735 ratio. The study provides a direct comparison of chlorophyll accumulation and fluorescence characteristics between two pear cultivars growing under contrasting leaf light conditions.

## Materials and methods

Plants and determination of total chlorophyll content: The experiment was conducted on pear (*Pyrus communis* L.) trees of two cultivars, 'Santa Maria' and 'Abbas', grown in the Zhurje area, Albania. The trees were 10 years old and grafted onto quince rootstock. Four trees of each cultivar were included in the experiment. The orchard was managed under regular irrigation, fertilization, pruning, and disease-control practices. Disease-control treatments were applied in February. Leaf sampling was performed in September from two canopy positions: sun-exposed leaves and shaded leaves. One fully developed and healthy leaf was randomly selected from each canopy position on each tree. Thus, four biological replicates (n = 4) were obtained for each cultivar × canopy position combination. In total, 16 leaves were collected from the eight experimental trees. Leaves with visible damage or disease symptoms were excluded. For chlorophyll extraction, five leaf discs, each 9 mm in diameter, were collected from each sampled leaf using a cork borer, corresponding to a total leaf area of approximately 3.18 $cm^2$ per extraction. The discs were placed in a ground-glass mortar with 100–200 mg $MgCO_3$ or MgO and a small amount of quartz sand. The material was ground with 100% acetone until the leaf tissue was almost colourless. The extract was transferred to centrifuge tubes, and the extraction procedure was repeated until the remaining leaf material was nearly colourless. The extracts were homogenized for 10–15 s and centrifuged at 3000 rpm for 5 min. The final extract volume

varied among samples, depending on the amount of extract obtained from each leaf. The exact final volume of each sample was recorded and used in the chlorophyll calculation. Absorbance was measured using a single-beam SP-2000 spectrophotometer at 470, 644.8, 661.6, and 750 nm. Chlorophyll concentrations were calculated according to Lichtenthaler and Buschmann (2001b). Absorbance values at 661.6 and 644.8 nm were corrected for turbidity using the absorbance measured at 750 nm. Thus, total chlorophyll was calculated as:

Total chlorophyll concentration

$$\text{Chl (a + b)} (\mu g\ mL^{-1}) = 7.05 \times A_{661.6} - 18.09 \times A_{644.8}$$

Total chlorophyll content per unit leaf area

$$\text{Chl (a + b)} (mg\ dm^{-2}) = [(7.05 \times A_{661.6} - 18.09 \times A_{644.8}) \times V] / (1000 \times S)$$

where: $A_{661.6}$ = Absorbance at 661.6 nm. $A_{644.8}$ = Absorbance at 644.8 nm. V = Final extract volume (mL). S = Total leaf-disc area ($dm^2$).

Absorbance at 470 nm was measured as part of the spectrophotometric pigment procedure described by Lichtenthaler and Buschmann (2001b), but carotenoid content was not calculated or reported because it was outside the scope of the present study. The results were expressed as mg $dm^{-2}$ of leaf area. The extraction procedure followed Lichtenthaler and Buschmann (2001a).

**Chlorophyll fluorescence measurements**: Chlorophyll fluorescence was measured using a Jobin Yvon J.Y.3 CS spectrofluorometer. After zeroing the instrument, intact leaves were placed in the cuvette compartment. The leaves were oriented so that the measured surface faced the incident excitation beam. Four leaves were measured for each light condition. The excitation monochromator was set at 470 nm, and the emission spectrum was recorded from 660 to 800 nm at 2 nm intervals. The wavelength and fluorescence-intensity values obtained for each leaf were transferred to Excel, where the fluorescence emission spectra were plotted. The signal gain was set to ×300, with a slit width of 2 nm. The fluorescence bands at approximately 690 nm (F690) and 735 nm (F735) were used to calculate the F690/F735 ratio according to:

Chlorophyll fluorescence ratio (F690/F735) = Fluorescence intensity at 690 nm / Fluorescence intensity at 735 nm

where F690 = Fluorescence intensity at 690 nm; F735 = Fluorescence intensity at 735 nm.

The fluorescence spectra and F690/F735 ratio were compared between sun-exposed and shaded leaves of '*Santa Maria*' and '*Abbas*'. The measurements were based on steady-state fluorescence emission spectra. Therefore, parameters that require pulse-amplitude-modulated fluorometry or fast fluorescence induction measurements, including Fv/Fm, ΦPSII, qP, NPQ, and OJIP transients, were not determined.

**Statistical analysis**: Data were analysed using two-way ANOVA with cultivar and light condition as fixed factors. Means were separated using Tukey's HSD at $P < 0.05$. Student's t-test was used to compare sun and shade leaves within each cultivar. Four biological replicates were used for each cultivar × light condition combination (n = 4). Results are presented as mean ± SD; standard errors (SEs) are also reported. The mean, standard deviation (SD), and standard error (SE) were calculated using the following equations:

## Results

**Total chlorophyll content in sun-exposed and shaded leaves:** Chlorophyll content differed between sun-exposed and shaded leaves (Table 1, Fig. 1). In '*Santa Maria*', the mean chlorophyll content was 6.420 ± 0.610 mg $dm^{-2}$ in sun leaves and 3.236 ± 0.557 mg $dm^{-2}$ in shade leaves. The difference between the two canopy positions was statistically significant (t = 7.711, $P < 0.001$). In '*Abbas*', chlorophyll content was 6.566 ± 1.820 mg $dm^{-2}$ in sun leaves and 4.715 ± 0.446 mg $dm^{-2}$ in shade leaves, with no significant difference between canopy positions ($P = 0.096$).

Table 1. Total chlorophyll content (Chl (a + b)) in sun and shade leaves of *'Santa Maria'* and *'Abbas'* pear cultivars

| Cultiva r | Canopy position | Chl (a + b) (mg $dm^{-2}$) | SE |
|---|---|---|---|
| Santa Maria | Sun-exposed | $6.420 \pm 0.610^{a}$ | 0.305 |
| Santa Maria | Shaded | $3.236 \pm 0.557^{b}$ | 0.278 |
| Abbas | Sun-exposed | $6.566 \pm 1.820^{a}$ | 0.910 |
| Abbas | Shaded | $4.715 \pm 0.446^{ab}$ | 0.223 |

Values are presented as mean ± SD (n = 4). Different superscript letters indicate significant differences among treatment means according to Tukey's HSD test at $P < 0.05$.

Two-way ANOVA showed a significant difference between canopy positions in chlorophyll content (F = 24.181, $P < 0.001$), whereas the effects of cultivar (F = 2.517, $P = 0.139$) and the cultivar × canopy position interaction (F = 1.696, $P = 0.217$) were not significant. Tukey's HSD test confirmed differences among group means at $P < 0.05$ (Table 1).

Chlorophyll fluorescence spectra and F690/F735 ratio: The chlorophyll fluorescence spectra showed differences between sun and shade leaves of the two pear cultivars. The measurements were performed at an excitation wavelength of 470 nm, and the fluorescence bands at 690 and 735 nm were used to calculate the F690/F735 ratio (Fig. 2). The F690/F735 ratio differed between sun-exposed and shaded leaves of the two cultivars (Fig. 3).

In 'Santa Maria', the ratio was 1.407 ± 0.444 in sun leaves and 1.553 ± 0.264 in shade leaves, with no significant difference between the two canopy positions ($P = 0.593$). In 'Abbas', the ratio was higher in shade leaves (1.386 ± 0.120) than in sun leaves, and this difference was statistically significant (t = −6.283, $P < 0.001$). Two-way ANOVA indicated significant differences in the F690/F735 ratio between cultivars (F = 5.620, $P = 0.035$) and canopy positions (F = 4.885, $P = 0.047$). The cultivar × light

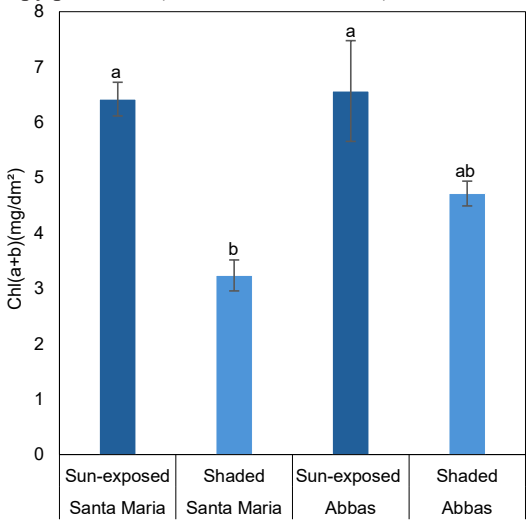



Fig. 1. Chlorophyll content (Chl (a + b)) (mg $dm^{-2}$) in sun and shade leaves of '*Santa Maria*' and '*Abbas*' pear cultivars. Bars represent mean values ± SE (n = 4). Different letters indicate significant differences according to Tukey's HSD test at $P < 0.05$.

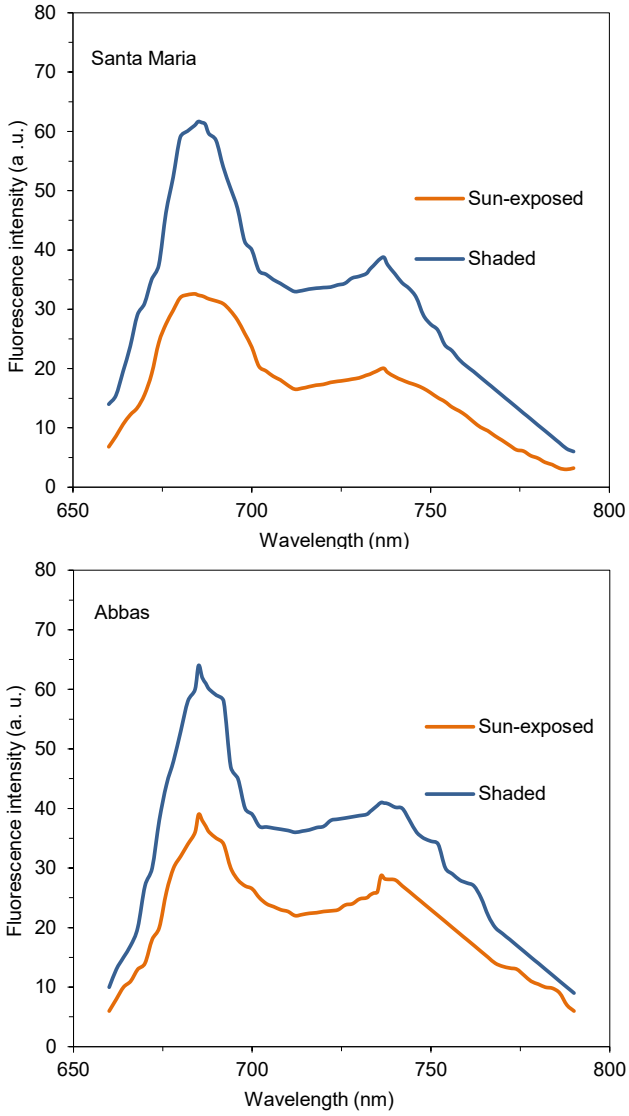



Fig. 2. Chlorophyll fluorescence emission spectra of '*Santa Maria*' and '*Abbas*' pear leaves from sun and shade positions

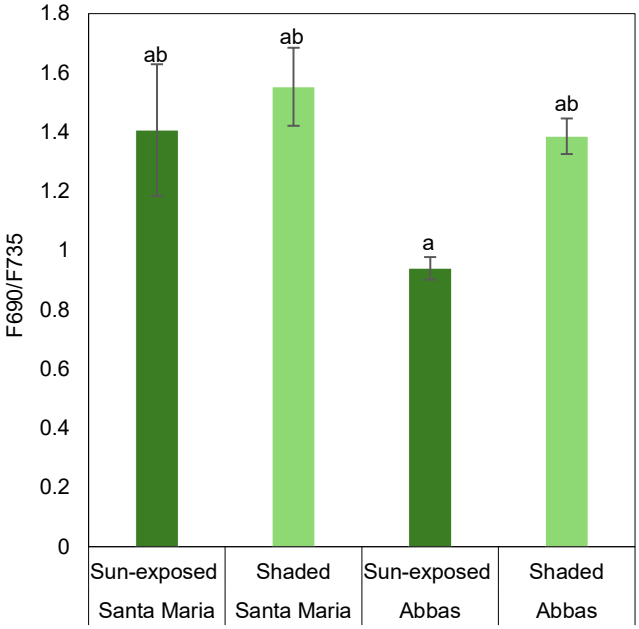



Fig. 3. F690/F735 fluorescence ratio in sun and shade leaves of '*Santa Maria*' and '*Abbas*' pear cultivars. Bars represent mean values ± SE (n = 4). Different letters indicate significant differences according to Tukey's HSD test at $P < 0.05$.

condition interaction was not significant ($F = 1.259$, $P = 0.284$). Tukey's HSD test was used to compare group means at $P < 0.05$ (Table 2).

## Discussion

**Chlorophyll content in sun and shade leaves**: Light availability within the canopy is an important factor associated with differences in leaf structure, pigment accumulation and photosynthetic acclimation in pear. Sun and shade leaves develop under contrasting irradiance conditions and can therefore differ in leaf thickness, chloroplast organization, pigment composition and photosynthetic capacity (Lichtenthaler *et al*., 2000; Lichtenthaler *et al*., 2007a, b; Long *et al*., 2022). In the present study, both 'Santa Maria' and 'Abbas' had higher Chl (a + b) content in sun leaves than in shade leaves. This pattern is consistent with the response commonly associated with acclimation to contrasting light environments. The higher chlorophyll content of sun leaves may be related to differences in leaf anatomy. Leaves developing under high irradiance commonly have greater thickness and a more developed palisade layer than leaves developing under shade conditions (Lichtenthaler *et al*., 2007a, b). Differences in leaf morphological characteristics have also been reported in *Pyrus communis* L. (Kārkliņa *et al*., 2021). Increased leaf thickness provides a greater volume of photosynthetically active tissue per unit leaf area and can support a larger photosynthetic apparatus. Shade leaves, in contrast, tend to develop a thinner structure adapted to lower irradiance. Thus, the differences in Chl (a + b) between sun and shade leaves may reflect not only pigment accumulation but also differences in structural organization of the leaf. Canopy structure can further influence the light environment experienced by individual pear leaves. The distribution of radiation within a fruit-tree canopy depends on leaf position, canopy architecture and shading by surrounding leaves. In pear, differences in canopy position and training systems have been associated with physiological differences among leaves (Liu *et al*., 2020). Light interception within fruit-tree canopies is also influenced by canopy structure and leaf arrangement (Tang *et al*., 2024). Therefore, the higher Chl (a + b) content in sun leaves in the present study is consistent with their exposure to greater irradiance compared with leaves located within the shaded part of the canopy. Chloroplast distribution and density may also contribute to differences in chlorophyll content. Sun leaves generally maintain greater investment in photosynthetic tissues under high irradiance, whereas shade leaves are associated with differences in photosynthetic apparatus under lower photon availability (Lichtenthaler *et al*., 2007a, b; Long *et al*., 2022). Changes in chloroplast organization and pigment-protein complexes form part of this acclimation process. However, chloroplast density and ultrastructure were not measured in the present study, so their contribution to the measured chlorophyll differences cannot be separated from other structural effects. Nitrogen allocation provides another possible explanation for the higher Chl (a + b) content in sun leaves. Chlorophyll and many proteins involved in photosynthetic electron transport and carbon assimilation require nitrogen. Leaf nitrogen allocation is closely associated with photosynthetic capacity because a substantial proportion of leaf nitrogen is invested in photosynthetic components (Evans, 1989). Under high irradiance, greater investment in photosynthetic tissues can support the increased demand for light utilization and carbon fixation. Therefore, the higher chlorophyll concentration measured in sun leaves could partly reflect greater investment in photosynthetic components.

Nitrogen concentration was not measured in this study, however, and this explanation should be considered a possible physiological mechanism rather than a directly tested relationship. The lower Chl (a + b) content in shade leaves is consistent with differences associated with reduced irradiance. Under shade conditions, leaves adjust their photosynthetic structure and pigment characteristics to capture available radiation efficiently rather than maintaining the same investment in photosynthetic tissue as sun leaves (Lichtenthaler *et al*., 2007a, b; Long *et al*., 2022). Similar differences in chlorophyll content between sun and shade leaves have been documented using fluorescence imaging and pigment analyses (Lichtenthaler *et al*., 2000; Lichtenthaler *et al*., 2007a). In pear, chlorophyll content also varies among cultivars and rootstock combinations, supporting the presence of genotype-dependent differences in pigment accumulation (Faizi *et al*., 2023). The difference between the two cultivars was also evident under shade, with 'Santa Maria' maintaining a higher Chl (a + b) content than 'Abbas'. This difference may reflect cultivar-specific regulation of pigment accumulation or differences in acclimation capacity under low irradiance. Differences in leaf chlorophyll content among European pear cultivars have been reported in relation to cultivar and rootstock (Faizi *et al*., 2023). Physiological differences among pear leaves can also be associated with canopy position and growing conditions (Liu *et al*., 2020). Nevertheless, because the present experiment did not measure photosynthetic rate, nitrogen content or chloroplast characteristics, the physiological basis of the difference between 'Santa Maria' and 'Abbas' remains unresolved. Leaf thickness may further be associated with differences in the measured chlorophyll concentration and the optical properties of the tissue. Thicker sun leaves can contain a greater amount of chlorophyll-bearing tissue along the optical path, whereas thinner shade leaves may have a different distribution of chloroplasts and pigments (Lichtenthaler *et al*., 2007a, b). Such structural differences can affect light absorption and the propagation of fluorescence within the leaf. Consequently, the higher Chl (a + b) content measured in sun leaves should be considered together with possible anatomical differences associated with irradiance acclimation. In general, the higher chlorophyll content in sun leaves of both cultivars is consistent with acclimation of the photosynthetic apparatus to the prevailing light environment. The response is consistent with sun–shade acclimation involving changes in pigment accumulation and potentially in leaf thickness, chloroplast organization and nitrogen investment (Lichtenthaler *et al*., 2007a, b; Long *et al*., 2022; Evans, 1989). Since leaf anatomy, chloroplast characteristics, nitrogen concentration and photosynthetic gas exchange were not directly measured, these mechanisms require further experimental confirmation.

**Chlorophyll fluorescence and F690/F735 ratio:** Chlorophyll fluorescence provides complementary information on the interaction of excitation light with chlorophyll-containing tissues and can be used to characterize changes in the physiological and optical properties of leaves (Baker, 2008; Kalaji *et al*., 2016; Kalaji *et al*., 2017). Chlorophyll fluorescence emission is characterized by a red band near 690 nm and a far-red band around 730–740 nm. The relative intensity of these bands depends partly on chlorophyll concentration and on the reabsorption and scattering of fluorescence within the leaf (Buschmann, 2007). In the present study, the F690/F735 ratio was higher in shade leaves than in sun leaves in both '*Santa Maria*' and '*Abbas*'. The increase was particularly pronounced in 'Abbas'. At the same time, shade leaves had lower Chl (a + b) content than sun leaves. The inverse relationship between chlorophyll content and the F690/F735 ratio is consistent with stronger reabsorption of red fluorescence in chlorophyll-rich tissues. Buschmann (2007) described the red/far-red fluorescence emission ratio as strongly affected by chlorophyll concentration and the optical properties of leaves. Leaf anatomy may also contribute to the fluorescence response. Differences in leaf thickness, palisade development and chloroplast distribution can modify the path of excitation light and emitted fluorescence through the tissue (Lichtenthaler *et al*., 2007a, b). In thicker sun leaves, greater chlorophyll content and increased reabsorption of red fluorescence may contribute to a lower F690/F735 ratio. In shade leaves, lower chlorophyll content and altered tissue structure may permit a relatively greater contribution of red fluorescence to the detected signal. Therefore, the changes in F690/F735 observed in this study may result from the combined effects of chlorophyll concentration and leaf optical structure. The cultivar response differed in magnitude. '*Abbas*' showed a larger increase in F690/F735 between sun and shade leaves than '*Santa Maria*'. This difference may reflect stronger differences in pigment accumulation or optical characteristics between sun-exposed and shaded '*Abbas*' leaves across the light gradient. Cultivar-dependent differences in chlorophyll content have been reported in European pear cultivars (Faizi *et al*., 2023), while physiological differences within pear canopies can also be associated with leaf position and light availability (Liu *et al*., 2020). Differences in chloroplast distribution, leaf thickness and pigment composition may therefore contribute to the cultivar-specific fluorescence response. These characteristics were not measured separately in the present study and cannot be identified as the direct cause of the observed difference. The excitation wavelength is also relevant when interpreting fluorescence measurements. Fluorescence emission depends on excitation conditions and on the absorption characteristics of photosynthetic tissues. Lysenko *et al*. (2020) demonstrated that fluorescence characteristics can vary with excitation conditions and the optical properties of chlorophyll-containing tissues. In the present study, all fluorescence measurements were performed using 470 nm excitation. The use of the same excitation wavelength for all samples provides a consistent experimental condition for comparing the two cultivars and the two leaf positions. The combined Chl (a + b) and F690/F735 results indicate that leaf position within the canopy is associated with differences in pigment accumulation and fluorescence characteristics. Sun leaves had higher chlorophyll content and lower F690/F735 values, whereas shade leaves had lower chlorophyll content and higher F690/F735 values. Similar changes in chlorophyll fluorescence between sun and shade leaves have been reported in woody plants (Lichtenthaler *et al*., 2000; Lichtenthaler *et al*., 2007a,b). The present results extend this type of observation to the two pear cultivars studied under the same experimental conditions. The stronger change in F690/F735 observed in 'Abbas' compared with 'Santa Maria' is relevant because the chlorophyll differences between the two cultivars were not equally pronounced under all light conditions. This suggests that fluorescence ratios may provide information about changes in leaf optical characteristics that are not fully described by total chlorophyll concentration alone. However, the fluorescence ratio is influenced by several factors, including chlorophyll concentration, tissue structure, reabsorption and scattering (Buschmann, 2007). It should therefore not be interpreted as a direct measurement of photosynthetic efficiency. Chlorophyll fluorescence parameters such as Fv/Fm, ΦPSII, qP and NPQ provide more direct information on different aspects of PSII

photochemistry and energy dissipation (Maxwell and Johnson, 2000; Kalaji *et al*., 2016; Kalaji *et al*., 2017). The F690/F735 ratio used in the present study should instead be considered primarily as a fluorescence parameter influenced by chlorophyll concentration and leaf optical properties. This distinction is important when interpreting the observed differences between sun and shade leaves. The results also agree with the broader concept of physiological acclimation to contrasting light environments. Sun and shade leaves differ not only in pigment content but also in the organization and operation of the photosynthetic apparatus (Lichtenthaler *et al*., 2007a, b; Long *et al*., 2022). Photosynthesis under variable irradiance requires coordinated changes in light capture, electron transport and carbon assimilation, particularly when leaves experience transitions between shaded and illuminated conditions (Long *et al*., 2022). Therefore, the differences in chlorophyll content and fluorescence ratio observed in the present study are consistent with the broader pattern of sun–shade acclimation. The study has some limitations. Leaf thickness, chloroplast density, nitrogen concentration, gas-exchange characteristics and direct PSII fluorescence parameters were not measured. Consequently, the relationships between chlorophyll concentration, leaf structure and fluorescence cannot be assigned to a single mechanism. Future studies combining pigment analysis with leaf anatomical measurements, nitrogen determination, gas exchange and fluorescence parameters such as Fv/Fm, ΦPSII, NPQ and OJIP transients would provide a more complete assessment of the mechanisms underlying sun–shade acclimation in '*Santa Maria*' and '*Abbas*'. Taken together, the results show that leaf position within the pear canopy was associated with clear differences in chlorophyll accumulation and F690/F735 fluorescence. The higher Chl (a + b) content and lower F690/F735 ratio in sun leaves, together with the cultivar-dependent response, support the use of pigment and fluorescence measurements as complementary approaches for characterizing differences between sun and shade leaves in pear. This study has some limitations that should be considered when interpreting the results. The study was conducted in one orchard and during one sampling period in September, with four trees per cultivar. Therefore, tree-level replication was limited. The findings should not be generalized broadly across different seasons, orchards or pear cultivars. In addition, non-significant differences should not be interpreted as evidence that the compared groups are biologically identical; rather, they indicate that a statistically significant difference was not detected under the conditions and sample size of this study. Further studies with a larger number of trees, multiple orchards and sampling across different seasons would help determine whether the observed patterns are consistent under a wider range of conditions.

Leaf position was associated with chlorophyll content and the F690/F735 fluorescence ratio in both pear cultivars. Chlorophyll content and the F690/F735 fluorescence ratio differed between sun-exposed and shaded canopy positions in both pear cultivars. Chl (a + b) content was higher in sun-exposed leaves, whereas the F690/F735 ratio increased in shade leaves. 'Abbas' maintained higher Chl (a + b) content in shade leaves than 'Santa Maria' and also showed a larger increase in the F690/F735 ratio from sun-exposed to shade conditions. Fluorescence measurements at 470 nm excitation provided additional information on differences between leaf positions and cultivars. The two measurements provided complementary information about differences between sun-exposed and shaded canopy leaves. Leaf position should be considered when evaluating chlorophyll content and fluorescence characteristics in pear cultivars. The results also show that chlorophyll concentration and fluorescence measurements can provide complementary information on the response of pear leaves to different light environments.

**List of abbreviations:** Chl (a + b): Total chlorophyll content; F690: Fluorescence emission at 690 nm; F735: Fluorescence emission at 735 nm; F690/F735: Ratio of fluorescence emission at 690 nm and 735 nm.

## Acknowledgments

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