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Variability in chlorophyll fluorescence spectra of eggplant fruit grown under different light environments: a case study

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The main goal of the present work was to clarify physiological strategies in plants whose chloroplasts were developed under different light environments. The specific objective was to elucidate the influence of the spectral distribution of light on the chlorophyll fluorescence ratio and on photosynthetic parameters. To achieve this purpose, three species of eggplant fruit (black, purple and white striped and white) were used as a case study and their chlorophyll fluorescence was analyzed in detail. Spectra of the non-variable fluorescence in each part of the fruit were corrected for distortions by light reabsorption processes using a physical model. The main conclusion of this work was that the corrected fluorescence ratio was dependent on the contribution of each photosystem to the fluorescence and consequently on the environmental lighting conditions, becoming higher when illumination was rich in long wavelengths. Variable chlorophyll fluorescence, similar to that observed from plant leaves, was detected for the pulp of the black eggplant, for the pulp of the purple and white striped eggplant and for the intact fruit of the black eggplant. The maximum quantum efficiency of photosystem II in the light-adapted state (F'_v/F'_m), the quantum efficiency of photosystem II (Φ_{PSII}), and the photochemical and non-photochemical quenching coefficients (qP and qNP/NPQ respectively) were determined in each case. The results could be explained very interestingly, in relation with the proportion of exciting light reaching each photosystem (I and II). The photochemical parameters obtained from variable chlorophyll fluorescence, allowed us to monitor non-destructively the physiological state of the black fruit during storage under both chilled or room-temperature conditions.

Introduction

In plant leaves, the energy absorbed or received from energy transfer by chlorophyll-a molecules, can undergo different deactivation pathways: initiate the photosynthesis process by beginning the electron transfer process, dissipate excess energy as heat or emit excess energy as fluorescence light. As the three processes take place in competition, a change in the efficiency of one of them has an effect on the efficiency of the other two.[1] This is the reason why chlorophyll fluorescence is strongly connected with photosynthesis and why its analysis has become a relevant and practical tool to obtain information on the physiological state of plants in a non-destructive way.[2,3] Many fruits also contain chloroplasts and display photosynthetic activities similar to leaves.[4] Basically, two types of experiments are performed when analyzing chlorophyll fluorescence: steady-state fluorometry and pulse amplitude modulated (PAM) fluorometry.[1–9]

When a dark-adapted photosynthetic tissue is illuminated with low photon flux (usually lower than 20 μmol m^{-2} s^{-1}), there is no significant induction of photosynthesis and a constant spectral distribution may be obtained for the fluorescence emission. This emission is usually called ground fluorescence (F_0) and may be recorded by means of a standard steady-state fluorometer.[10] This spectrum is characterized by two bands: one in the red region (about 680 nm usually denoted as F_{red}) and one in the far-red region (about 735 to 740 nm denoted as $F_{far\text{-}red}$). These bands are referred to as chlorophyll fluorescence and are due more precisely to photosystems emission. At room temperature photosystem II (PSII) contributes to both F_{red} and $F_{far\text{-}red}$ bands while photosystem I (PSI) contributes only to the $F_{far\text{-}red}$.[11,12]

The fluorescence ratio ($F_{red}/F_{far\text{-}red}$) has been extensively studied as a function of stress in plants.[13–17] The ratio observed for the intact tissue differs appreciably from the value for isolated chloroplasts due to the important re-absorption of the fluorescence that takes place in media with a high pigment content. As chloroplast fluorescence and not the distorted observed emis-

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sion is related to the physiological state of the tissue, it is important to perform corrections for these artifacts. Some authors have developed physical models to take into account these processes allowing spectra correction.[13,18,19]

However, relatively few papers have been published in the literature where corrections for light re-absorption processes have been performed on chlorophyll fluorescence spectra.[10,18–24]

The experimental (non-corrected) fluorescence ratio ($F_{red}/F_{far\text{-}red}$) varies according to the chlorophyll content of the tissue. In fact, due to re-absorption of light, as chlorophyll concentration increases, the red band (which overlaps with the chlorophyll absorption spectrum) decreases while the far red band remains almost unchanged.[13] Some authors support that this ratio depends not only on the chlorophyll concentration but also on the particular contribution of each photosystem to the fluorescence.[11,25,26]

When correcting experimental spectra for re-absorption artifacts an important question arises: should the corrected fluorescence ratio $F_{red}/F_{far\text{-}red}$ be a constant value for all photosynthetic tissues?

A priori one would expect that it varies (i) according to the PSII/PSI ratio in the tissue and/or according to the sizes of their respective antenna complexes and (ii) whenever the electron transfer from excited P680 in PSII to the primary acceptors in the photosynthetic chain is blocked (disconnection between both photosystems).[23]

In shaded leaves and in the abaxial part of leaves, the corrected fluorescence ratio was found to be higher than for sun-leaves and adaxial parts of leaves.[21] Corrected fluorescence spectra for the peel of Granny Smith apples were similar to those for sun-leaves,[20] while the corrected emission spectra for the pulp of Kiwi resulted like spectra for shaded leaves.[22] Additionally, it is already known that shade plants, which grow under low light conditions with wavelengths enriched in the far red, synthesize very large amounts of light harvesting complex II, which is mostly associated with photosystem II.[27] The light quality also determines State I/State II transitions and thus the relative antenna sizes of PSI and PSII.[28]

So, previous evidence found in the literature strongly suggest that the fluorescence ratio, free of light re-absorption artifacts, might depend on the light conditions in which the chloroplasts grew. On this basis we build our hypothesis: as is already known that the spectral distribution of light during development of chloroplasts determines the proportion of photosystems and/or their antenna sizes the light quality will influence accordingly the corrected fluorescence ratio.

To test this hypothesis, in the present work, we analyzed in detail chlorophyll fluorescence of eggplant pulps that received different light distributions due to differential filtering through their peels.

In another aspect, when transferring photosynthetic materials from the dark to intense light, an increase in the yield of the chlorophyll fluorescence is observed due to saturation of the electron transfer chain (closed reactions centers) and variable chlorophyll fluorescence is recorded. Later, fluorescence starts to fall in a process called "fluorescence quenching" that lasts several minutes to finally reach a stationary state (F_s). The fluorescence quenching has a photochemical and a non-photochemical contribution. The photochemical quenching (qp) is due to activation of enzymes involved in the carbon metabolism induced by light and the opening of stomata. The non-photochemical quenching (qNp) is due to an increase in the yields of heat dissipation.[1] The described phenomenon is known as Kautsky kinetics and it is recorded by means of a pulse amplitude modulated (PAM) chlorophyll-fluorometer.[29] From this experiment different photosynthetic parameters such as: the ground fluorescence in the dark adapted state (F_0), the ground fluorescence (F'_0) in the light-adapted state, the maximum fluorescence in the light-adapted state (F'_m), the maximum quantum yield of PSII photochemistry in the light adapted state (F'_v/F'_m, calculated as $(F'_m - F'_0)/F'_m$), the ratio between variable and ground fluorescence in the light adapted state (F'_v/F'_0), the quantum efficiency of PSII ($\Phi_{PSII} = (F'_m - F'_0)/F'_m$), the photochemical quenching coefficient ($qP = (F'_m - F_s)/(F'_m - F_0)$), the non-photochemical ($qNp = (F_m - F'_m)/(F_m - F_0)$) and the alternative definition of non-photochemical quenching ($NPQ = (F_m - F'_m)/F'_m$), may be determined.[29]

Photosynthetic parameters may also depend on the lighting conditions in which the chloroplasts were developed and a study on this point was performed in this work by analyzing Kautsky kinetics[1] of the pulps receiving different lighting conditions.

As knowledge about chlorophyll fluorescence of fruits and vegetables may be used to non-destructively monitor their physiological state during stress or storage,[4,30–33] practical applications in this context were also explored in this research.

Materials and methods

Samples

Different species of eggplant (*Solanum melongena* L.: Solanaceae) were purchased at a local market. The three species used are shown in Fig. 1.

Measurements were performed for the whole fruit, the peel and the pulp. To separate the pulp and the peel, it was removed carefully and was scraped to eliminate the thin layer of pulp that remained attached to the peel. All the experiments were performed as soon as possible after the separation of the parts of the fruit to avoid sample degradation.

Reflectance and transmittance

For reflectance and transmittance measurements of the scattering samples, a spectrophotometer (UV3101PC, Shimadzu, Tokyo, Japan) equipped with an integrating sphere (ISR-3100, Shimadzu, Tokyo, Japan) was used for the reflectance and transmittance measurements. As white reference standard, barium sulphate was employed to adjust the 100% reflectance level. Diffuse reflectance (R_λ) was recorded as a function of wavelength from 400 to 800 nm for whole fruits, for the pulps, and for the group of stacked peels. Additionally, diffuse transmittance (T_λ) was measured in the same wavelength range for the individual peels. All data were obtained at room temperature.

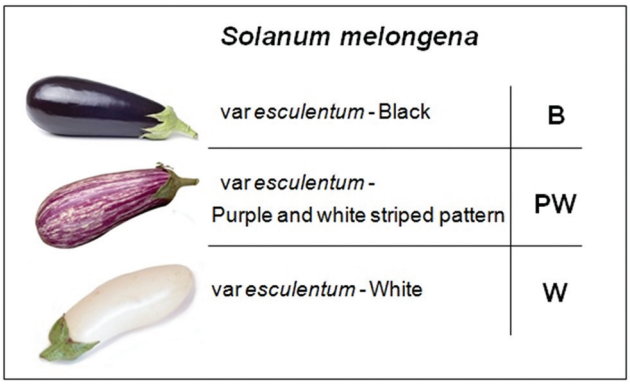

Fig. 1 The three varieties of *Solanum melongena* var. *esculentum* studied in this work. The letters B, PW and W are used as abbreviations for black, purple and white striped and for white species respectively.

Non-variable chlorophyll fluorescence

Fluorescence spectra were obtained for the whole fruit, the pulps and the peels of each eggplant species. The measurements were made at room temperature, with low photon irradiation to avoid induction of Kautsky kinetics: photon flux <20 μmol m^{-2} s^{-1}, excitation slit = 1 nm, emission slit: 8 nm. Under these conditions, all the electron acceptors in the photosynthetic centers were open and the recorded fluorescence corresponded to the ground emission F_0.

All the studied samples were measured under conditions of zero transmittance to allow application of the correction model to account for light reabsorption processes. Spectra were obtained from 600 to 800 nm with a steady-state spectrofluorometer (QuantaMaster, PTI-Photon Technology International-Brunswick, USA) in front face geometry. The working excitation wavelength was selected as 460 nm because it led to the most intense emission in the red/far-red region. Emission spectra were obtained on samples previously adapted to the dark for 15 minutes and they were corrected for the detector response to different wavelengths.

Corrections of fluorescence spectra to account for light reabsorption processes

The experimental fluorescence spectra for the whole fruits and for their different parts were corrected additionally for light reabsorption processes by dividing the experimental spectra by the gamma function (eqn (1)) which depends on the excitation (λ_0) and emission (λ) wavelengths:

$$\gamma(\lambda,\lambda_0) = \frac{1}{1+\sqrt{\frac{F(R_\lambda)}{F(R_\lambda)+2}}}\frac{1}{1+\sqrt{\frac{F(R_\lambda)(F(R_\lambda)+2)}{F(R_{\lambda_0})(F(R_{\lambda_0})+2)}}}, \quad (1)$$

where $F(R_\lambda)$ is the remission function at wavelength λ, defined by eqn (2):

$$F(R_\lambda) = \frac{(1-R_\lambda)^2}{2R_\lambda}. \quad (2)$$

The absence of light transmission through the sample is a necessary condition for the application of the correction model.

This method was originally developed for inert systems[34] and has successfully been applied to correct fluorescence spectra of leaves,[10] fruits[22] and pigments immobilized on solid matrixes.[35–38]

From the emission spectra corrected for re-absorption, the ratios $F_{red}/F_{far\text{-}red}$ were calculated as the quotients between the emission intensity at about 682 nm (F_{red}) and 732 nm ($F_{far\text{-}red}$).

Fluorescence modeling and spectra reconstruction

The observed spectrum was reconstructed by modeling the interaction between light and the fruit.[22] The fluorescence intensity I_f may be calculated by the product between the absorbed photon flux I_a and the fluorescence quantum yield of the tissue Φ_f (eqn (3)):

$$I_f = I_a \cdot \Phi_f. \quad (3)$$

For the pulp without peel, the fluorescence intensity is given by eqn (4):

$$I_{f(\text{pulp alone})} = I_0(\lambda_0) \cdot (1 - R_{\lambda_0}) \cdot \Phi_f, \quad (4)$$

where $I_0(\lambda_0)$ is the incident photon flux at the excitation wavelength λ_0, and $(1 - R_{\lambda_0})$ stands for the fraction of incident light absorbed by the pulp (assuming no-transmittance through the pulp).

The product $I_0(\lambda_0)\cdot T_{peel(\lambda_0)}$ represents the incident light flux that passes across the peel reaching the pulp. When this product is multiplied by the fraction of incident light absorbed by the pulp $(1 - R_{\lambda_0})$ and by the fluorescence quantum yield of the pulp Φ_f, the number of fluorescent photons originating in the pulp are obtained (eqn (5)):

$$\begin{aligned} I_{f(\text{pulp in the fruit})} &= I_0(\lambda_0) \cdot T_{peel(\lambda_0)}(1 - R_{\lambda_0}) \cdot \Phi_f \\ &= I_{f(\text{pulp alone})} \cdot T_{peel(\lambda_0)}, \end{aligned} \quad (5)$$

where $T_{peel(\lambda_0)}$ is the peel transmittance at the excitation wavelength λ_0.

Finally, the fluorescence intensity emerging from the whole fruit has contributions from the pulp and from the peel and may be calculated by eqn (6):

$$\begin{aligned} I_{f(\text{whole fruit})} = {} & I_{f(\text{pulp alone})} \cdot T_{peel(\lambda_0)} T_{peel(\lambda)} + I_{f(peel)} \\ & + I_{f(peel)} \cdot R_{pulp} \cdot T_{peel}. \end{aligned} \quad (6)$$

At the right hand side of eqn (6), the first term represents the contribution of the pulp filtered by the peel in its way out of the fruit, the second term is the fluorescence intensity of the peel and the third term stands for the peel fluorescence generated internally, reflected by the pulp and filtered by the peel.

The contribution of the peel fluorescence due to the absorption of the pulp fluorescence $(I_{f(pulp)}(1 - R_{peel(\lambda)} - T_{peel(\lambda)})\cdot\Phi_{fpeel})$ was considered negligible and was not included in eqn (6). To estimate the contribution of this term, which was 1% at most, we assumed a fluorescence quantum yield value similar to that of plant leaves (0.01–0.02).[1]

Eqn (6) allows estimation of the whole fruit fluorescence spectra from the experimental fluorescence spectrum of the separate peel, the experimental fluorescence spectrum for the pulp, the pulp reflectance and the peel transmittance. The results from the model were compared in this work with the experimental fluorescence spectra for the whole intact fruit.

Variable fluorescence

Variable fluorescence was investigated in the whole fruits and in their separate parts (pulp and peel) using a pulse-modulated chlorophyll fluorometer (Hansatech FMS1).

The excitation modulated beam (594 nm) induced a pulse fluorescence signal under conditions where ambient light was excluded. The modulating beam used had very short duration pulses (1.8 μs) with long off periods between pulses. In this way, the integrated amount of radiation incident upon the sample was smaller than 0.05 μmol m^{-2} s^{-1}, avoiding significant physiological changes in the sample during measurements. The saturating pulse (halogen light) had a duration of 0.4 s with an intensity of 14 400 μmol photons per m^2 per s. The actinic light was provided by a halogen light source of about 600 μmol m^{-2} s^{-1}. A 735 nm far-red LED source was used for preferential PSI excitation in order to determine accurately F'_0. The instrument was used with a low sampling-rate frequency (10 Hz) for F_0 measurements and a high frequency (20 kHz) during application of actinic or saturating light. A PIN photodiode with a long pass filter for wavelengths longer than 700 nm (Schott RG 695, 3 mm thickness) was used for signal detection. The experiments were started by recording the minimum fluorescence signal from dark-adapted samples (F_0) with the modulating beam. Then the saturating pulse was applied and the maximum fluorescence F_m was recorded. Next, the samples were exposed to the actinic light. When a steady state (F_s) was reached, a new saturating pulse was applied to record the maximum fluorescence for the light-adapted state (F'_m). Then, another saturating pulse was applied, the actinic light was automatically turned-off and the far-red pulse was applied for F'_0 determination. From these measurements, the parameters: F'_v/F'_m, F'_v/F'_0, Φ_{PSII}, qp, qNp and NPQ were calculated whenever a variable fluorescence signal was detected.

The photosynthetic parameters were also obtained as a function of storage time, for the intact fruit of black eggplant, during 12 days, at both 4 °C and room temperature.

Results

The spectral distributions of the ground fluorescence determined experimentally for the peel, the pulp and the whole fruit in each eggplant type are shown in Fig. 2.

Chlorophyll emission is observed for the different parts of the three types of eggplants with its characteristic bands located at the red region (682 nm) and at the far red region (732) nm. The band in the red region is almost imperceptible for the peel of the white species.

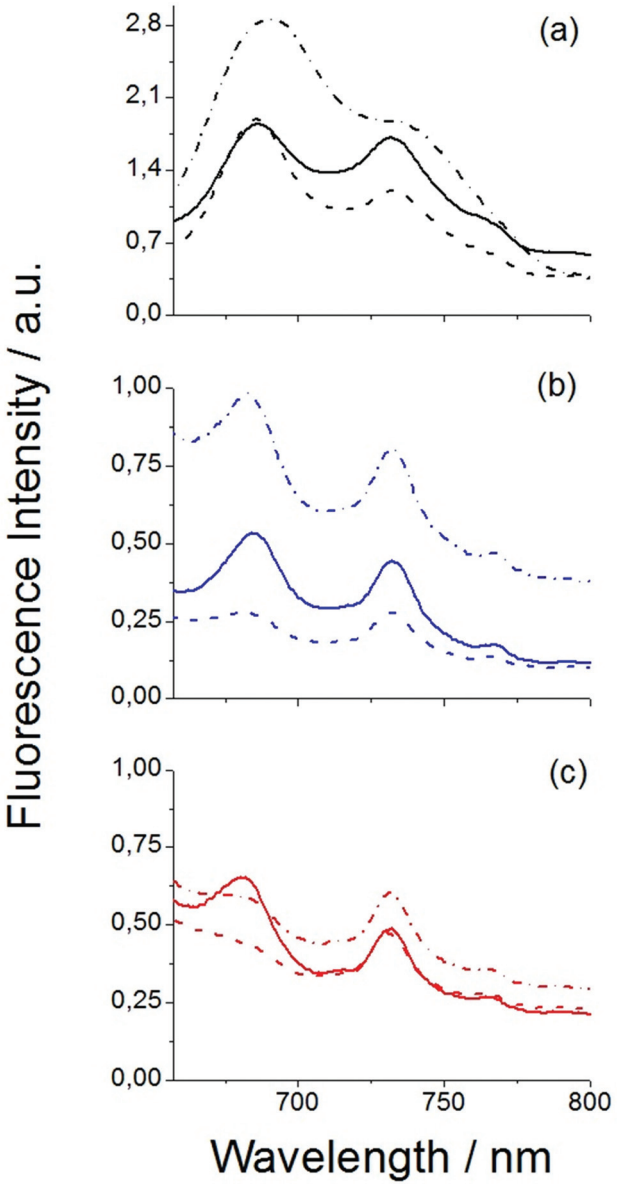

Fig. 2 Experimental fluorescence spectra (F_0) of the different parts of black eggplant (a), purple and white striped eggplant (b) and white eggplant (c). Whole fruit (–), peel (---) and pulp (-·-). All spectra are corrected for the detector response.

The fluorescence signal is higher for the pulp of black eggplant, following in decreasing order the pulps of the purple and white striped and the white one.

The fluorescence spectra corrected for light re-absorption processes, free from distortion artifacts and representing the true spectral distribution of chloroplasts emission are presented in Fig. 3.

Comparing Fig. 2 and 3 it may be observed that the experimental fluorescence ratios $F_{red}/F_{far\text{-}red}$ were lower than the corresponding value corrected taking into account light re-absorption artifacts. Additionally, the maximum in the red region was shifted to shorter wavelengths upon correction. As

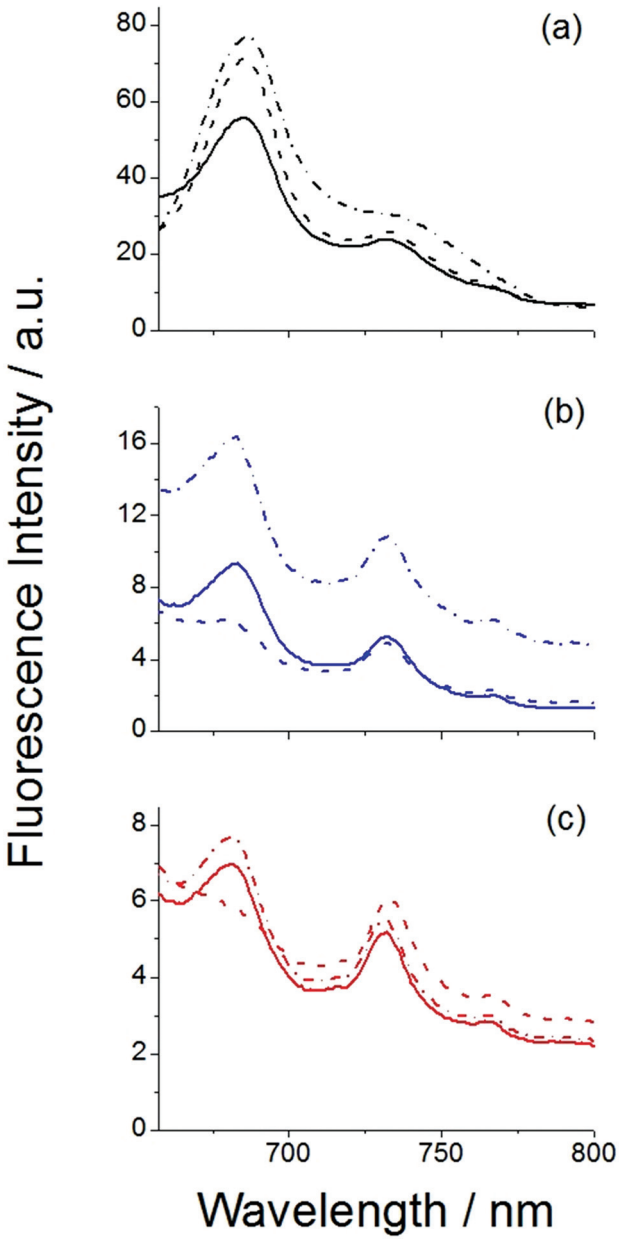

Fig. 3 Fluorescence spectra corrected for the detector response (F_0) and additionally corrected for light re-absorption processes for the different parts of black eggplant (a), purple and white striped eggplant (b) and white eggplant (c). Whole fruit (–), peel (---) and pulp (-·-).

examples, B pulp shifted from 691 to 686 nm, PW pulp from 683 to 682 nm, and W pulp from 682 to 681 nm.

The values for the fluorescence ratio corrected for light re-absorption processes for the different parts of the three eggplants species are shown in Table 1. This table shows a total of 81 measurements: three fruits of each type were taken and three spectra were recorded for each of their parts. Each reported value in this table is so an average of nine determinations.

Regarding pulps, which received different spectral distribution of light during growing, a decreasing order for this quotient: B > PW > W was observed. This order was also observed for the other parts of the fruit.

Table 1 Fluorescence ratio (F_{682}/F_{732}) corrected for light re-absorption processes for the different parts of the three eggplants species. The values reported in this table for each part of the fruits are averages from nine fluorescence spectra (three fruits were taken for each aubergine type and three spectra were recorded for each of their parts)

	$F_{red}/F_{far\text{-}red}$ corrected		
	Fruit	Peel	Pulp
Black	2.34 ± 0.04	2.7 ± 0.1	2.51 ± 0.08
Purple white	1.77 ± 0.01	1.26 ± 0.03	1.51 ± 0.01
White	1.35 ± 0.01	0.94 ± 0.01	1.39 ± 0.01

The spectral distribution of light reaching the pulps in intact fruits may be estimated following the spectral distribution of light transmitted by their respective peels. The differences in the transmittance of the peels may be observed in Fig. 4.

For the black peel, there was almost no transmittance below 600 nm while above 700 nm an abrupt increase was observed. This peel acts almost as a cut-off filter allowing the passage of far-red light through it. The white peel has a more constant response, without any sharp leap and with similar transmittance in the red and far-red regions. The striped purple-white peel, meanwhile, has an intermediate behavior transmitting more light in the red than the black one but less than the white peel. For the light transmitted through the peels, the ratio of the contributions red/far-red followed then the following order: W > PW > B.

The reconstruction of the fluorescence spectrum predicted for the whole fruit by eqn (6), together with the average experimental spectrum are shown in Fig. 5 for the black aubergine. The reconstruction was only performed for the black fruit because fluorescence levels for the other types were too low for modelling.

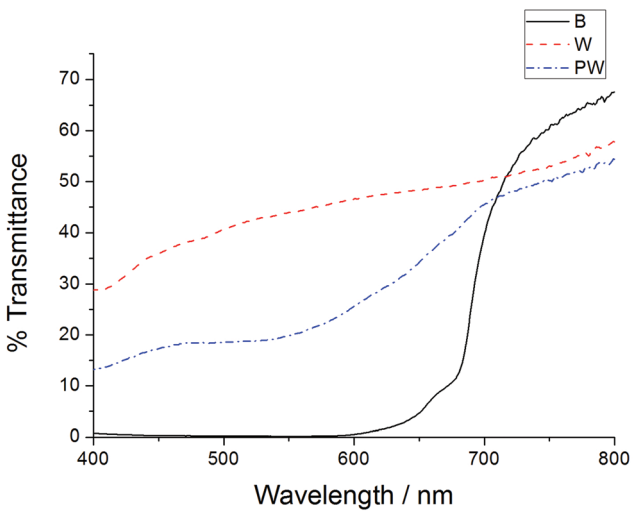

Fig. 4 Diffuse transmittance spectra for the peels of black (–), white (---) and striped purple and white (-·-) eggplant.

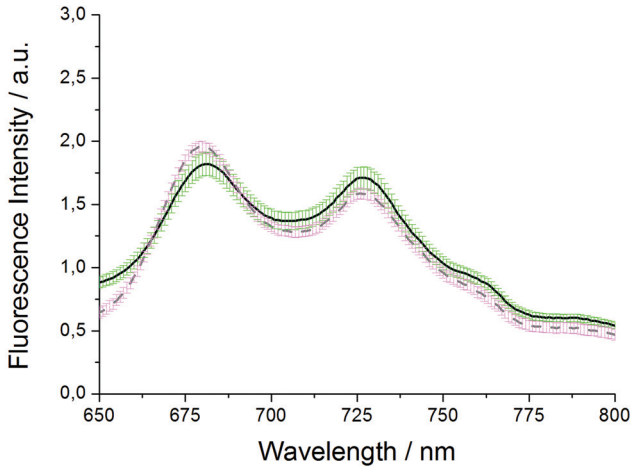

Fig. 5 Modelled fluorescence spectrum estimated for the whole fruit (– – –), and observed fluorescence spectrum for the whole fruit (–). Both spectra are shown with their respective error bars.

Variable fluorescence was explored in the different parts of the three eggplant species by means of the PAM fluorometer. Nil or negligible variable fluorescence was found for all the peels, for the pulp of the white species and for the intact fruit of the purple and white striped eggplant (see Table 2).

For the pulps of both B and PW fruits, the variable fluorescence signal was higher near the peel and decreased towards the center of the fruit, becoming almost null at the core. The photosynthetic parameters obtained from variable fluorescence for the pulps of both B and PW eggplants are compared in Fig. 6.

All the parameters, except those related with the non-photochemical quenching were lower for the pulp of the B eggplant.

The photosynthetic parameters were followed during storage time for intact eggplants of the black species as it was the only one that presented detectable variable chlorophyll fluorescence for the whole fruit. In Fig. 7 the results for room temperature and refrigerated conditions (4 °C) are shown. Only those parameters that resulted more sensitive to storage time are presented: F'_v/F'_m, Φ_{PSII} and F'_v/F'_0.

An extreme variability in values was observed at room temperature (specially for Φ_{PSII} and F'_v/F'_0). In contrast, dispersions in measurements among refrigerated samples were markedly lower (see error bars in Fig. 7). A strong decrease in the parameters and consequently in the photosynthetic activity was observed for the refrigerated eggplants. In contrast, at room temperature only a slight reduction after 12 days was observed.

Table 2 Variable chlorophyll fluorescence in the different parts of eggplants. The letters B, PW and W stands for black, purple-and-white striped and white eggplant, respectively

Variable chlorophyll fluorescence			
	Intact fruit	Pulp	Peel
B	Yes	Yes	No
PW	No	Yes	No
W	No	No	No

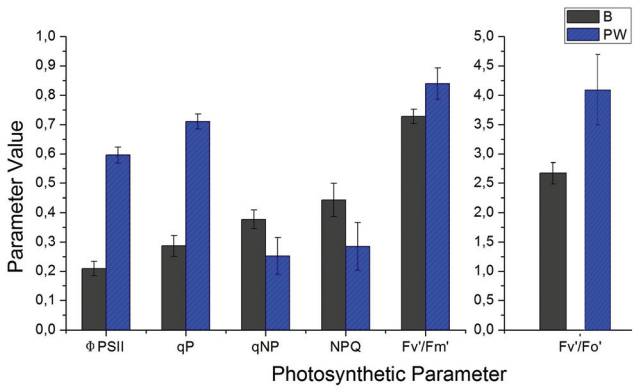

Fig. 6 Values of photosynthetic parameters obtained from variable chlorophyll measurements for the pulps of B and PW eggplants.

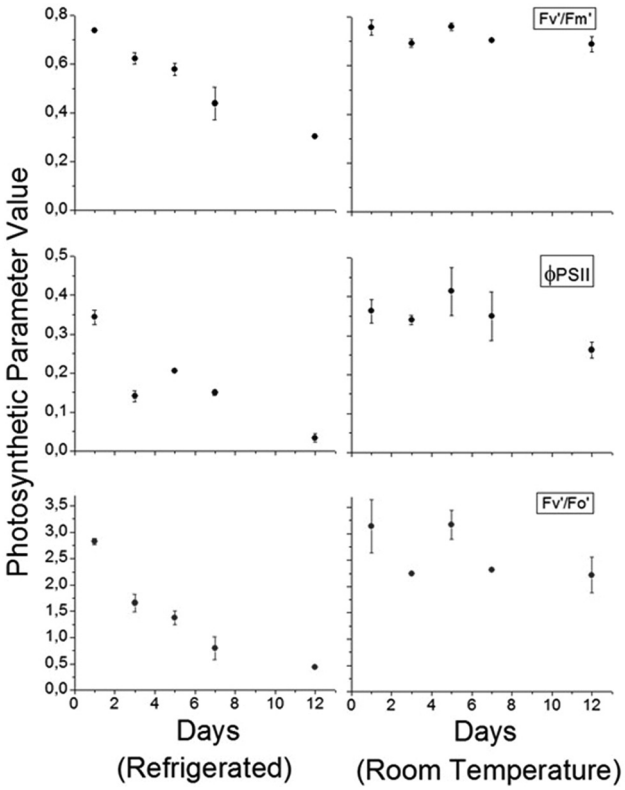

Fig. 7 Variations in the photosynthetic parameters: F'_v/F'_m, Φ_{PSII} and F'_v/F'_0, obtained from variable chlorophyll fluorescence of intact fruits of black eggplants as a function of storage time. Eggplants kept at 4 °C (left) and at room temperature –20 °C (right).

Discussion

The experimental ground fluorescence intensities of the eggplants followed the order B > PW > W for all their respective

parts. This fact is in agreement with a higher chlorophyll content for the B type, an intermediate concentration in PW and a lower content in the W variety.

The corrected fluorescence ratio for the pulps followed the order B > PW > W and this trend may be nicely explained taking into account the light transmitted through the peels. The B peel, compared to the W and PW peels, showed a lower transmittance in the visible region and higher transmittance in the far-red zone. The peel transmittance in the visible region is connected with the respective pigment content. Above 700 nm, in the far-red region, it is known that transmittance of plant tissues increases with the number of intercellular air spaces present.[39] In fact, a higher transmittance in the far-red region for the black peel may be due to structural features such as increased number of water–air interfaces or internal refractive index discontinuities. So, as shown in Fig. 4, the transmittance spectra of the peels clearly showed that the pulps received very different spectral distribution of light. For B, photons reaching the pulp were far-red enriched. Under these conditions, a preferential excitation of PSI occurred given that PSI absorbs mainly in the far-red region (maximum absorption at 700 nm) while PSII absorbs basically in the red region (maximum absorption at 680 nm).[3,40–42] In response to this distribution of light, there was an increase in the amount of PSII in the chloroplast and/or its antenna size. As a consequence, the corrected fluorescence ratio was high. On the other hand, the pulp of the W fruit received a more balanced proportion of red and far-red light and so no preferential excitation of one of the photosystems took place. In this case, the fluorescence ratio was accordingly lower. Finally, for the PW eggplant whose peel presented an intermediate situation between B and W, the corrected fluorescence ratio also displayed an intermediate value. These results supported our first hypothesis stating that the spectral distribution of light during development of chloroplasts determined the proportion of photosystems and/or their antenna sizes and influenced accordingly the corrected fluorescence ratio.

It has already been reported in the literature that low light intensities lead to a drastic increase in the light-harvesting antenna unit size of PSII and a slight augmentation of that of PSI.[43] Ambient light intensity also was proved to modulate the content of thylakoid components as well as the PSII/PSI ratio.[44]

The results obtained in this work also complement previous studies[20–22] reinforcing evidence that the higher the far red/red ratio in excitation light, greater will be the corrected fluorescence ratio. It was previously shown in the literature that shade light, rich in the far-red light, induced in plant leaves a tendency to augment PSII antenna[45] in agreement with our findings.

Spectra for the black fruit, reconstructed from the fluorescence of each part and reflectance and transmittance data, were in good agreement, within the experimental error, with the experimental spectra. A similar physical model was previously presented for kiwi.[22] However, in this case, as the peel fluorescence is not negligible compared to the pulp emission, a third term was included (see eqn (6)) to take into account the peel fluorescence generated internally and reflected by the pulp.

Variable chlorophyll fluorescence found in the different parts of eggplant fruits is a sign of the presence of photosynthetic activity, which has been reported previously in several fruits.[4,22]

For the peels and for the pulp of W eggplant, the changes in fluorescence upon application of a light saturating pulse was so small that it was difficult to separate signals from the instrumental noise and consequently it was impossible to infer any photosynthetic parameters from them.

The green color of the pulps followed the order B > PW > W indicating the order of their chlorophyll content. The lack of appreciable chlorophyll fluorescence for W eggplant was probably due to its low chlorophyll concentration.

The photosynthetic parameters F'_v/F'_m, F'_v/F'_0, qp and Φ_{PSII} were lower for the shaded pulp of the B eggplant than for the pulp of the PW fruit. These results agreed with previous knowledge regarding the fact that leaves adapted to full sunlight displayed higher photosynthetic parameters.[2,46] On the other hand, the non-photochemical parameters, qNp and NPQ, representing the heat dissipated by the system were higher for the B variety. A comparison of non-photochemical quenching parameters between sun and shade leaves has shown different behaviour depending on the light intensity used for their determination. However, for values of light intensities similar to those used in our experiments, these coefficients were higher for shade leaves[46] in agreement with our results. The lower value for Φ_{PSII} observed for the pulp of black eggplant is consistent with its higher values for the non-photochemical quenching parameters. In fact, a lower efficiency for Φ_{PSII} may be explained by an increase in heat dissipation indicated by qNP and NPQ.[46]

The whole B fruit displayed appreciable variable chlorophyll fluorescence and their photosynthetic parameters could be measured non-destructively as a function of storage time. The variability in photosynthetic parameters at room temperature were due to variations in F'_m values among the samples of black eggplants. While F'_0 values were similar for all the fruits, F'_m values displayed appreciable oscillations. These oscillations were not present for the refrigerated samples. The decrease in F'_v/F'_m, F'_v/F'_0, and Φ_{PSII} observed especially for the eggplants which were kept in the refrigerator at 4 °C showed the great sensitivity of the technique to indicate the physiological state of the fruit. The methodology clearly demonstrated the damage suffered by these fruits stored under cooling conditions and the differences with storage at room temperature. The results showed that the variable fluorescence of the B eggplant fruit may be a potential tool for detecting its quality.

Conclusion

The main conclusions arising from this work can be summarized as:

-Eggplants displayed variable and non-variable chlorophyll emission. The variable fluorescence, however, was practically negligible for the white variety due to its very low chlorophyll concentration.

-The presence of variable chlorophyll fluorescence is indicative of the photosynthetic activity in the fruit.

-Chloroplasts of eggplant fruits grown under far-red enriched light showed a higher corrected fluorescence ratio and displayed greater values for the photosynthetic parameters.

-The state of the eggplant fruit during the post-harvesting period may be non-destructively monitored from the variable fluorescence analyzed with a PAM fluorometer.

-A contribution is presented supporting the statement that the corrected fluorescence ratio depends on the relative proportion of photosystems.

-A second contribution is furnished in favor of the assertation that photosynthetic parameters are also functions of the lighting conditions in which the chloroplasts were developed.

Abbreviations

PSI	Photosystem I
PSII	Photosystem II
F_0	Ground chlorophyll fluorescence triggered by the measuring light in the dark-adapted state
F'_0	Ground chlorophyll fluorescence measured after application of 735 nm far-red light
F_m	Maximum chlorophyll fluorescence caused by application of a saturating light pulse on dark-adapted leaves
F'_m	Maximum chlorophyll fluorescence caused by application of a saturating light pulse in the light-adapted state of the leaf
F'_v	Variable fluorescence in the light-adapted state ($F'_m - F'_0$)
F'_v/F'_0	Maximum quantum yield of PS2 photochemistry (alternative expression more sensitive than F'_v/F'_m)
Φ_{PSII}	Quantum efficiency of photosystem II
qP	Photochemical quenching coefficient
NPQ and qNP	Non-photochemical quenching coefficients
F'_v/F'_m	Maximum quantum yield of photosynthesis of leaves adapted to light or antennae efficiency of PSII

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References

1 K. Maxwell and G. N. Johnson, Chlorophyll fluorescence–a practical guide, *J. Exp. Bot.*, 2000, **51**, 659–668.
2 N. R. Baker and K. Oxborough, in *Chlorophyll a Fluorescence: A Signature of Photosynthesis*, ed. G. C. Papageorgiou and Govindjee, Springer, Dordrecht, 1st edn, 2004, vol. 1, ch. 3, pp. 68–74.
3 M. G. Lagorio, Chlorophyll fluorescence emission spectra in photosynthetic organisms, in *Chlorophyll: Structure, Production and Medicinal Uses*, ed. H. Le and E. Salcedo, Nova Publisher, Hauppauge, NY, 2011, ch. 4, p. 115.
4 J. R. DeEll and P. M. A. Toivonen, Use of chlorophyll fluorescence in postharvest quality assessments of fruits and vegetables, in *Practical applications of Chlorophyll fluorescence in plant biology*, ed. J. R. DeEll and P. M. A. Toivonen, Kluwer Academic Publishers, London, 2003, ch. 7, p. 203.
5 M. H. Kalaji, V. Goltsev, K. Bosa, S. I. Allakverdiev, R. J. Strasser and Govindjee, Experimental in vivo measurements of light emission in plants: a perspective dedicated to David Walker, *Photosynth. Res.*, 2012, **114**, 69–96.
6 H. M. Kalaji, G. Schansker and M. Brestic, Frequently asked questions about chlorophyll fluorescence, the sequel, *Photosynth. Res.*, 2016, DOI: 10.1007/s11120-016-0318.
7 K. Kowalczyk, J. Gajc-Wolska, M. Marcinkowska, M. D. Cetner and H. M. Kalaji, Response of growth, quality parameters and photosynthetic apparatus of endive plant to different culture media, *Folia Hort.*, 2016, **28**, 25–30.
8 V. Goltsev, M. H. Kalaji, M. Paunova, V. Babak, T. Horachekd, J. Moyskid, H. Kotsel and S. I. Allahverdieve, Using a variable chlorophyll fluorescence for evaluation of physiological state photosynthetic apparatus plants, *Russ. J. Plant Physiol.*, 2016, **63**, 1–28.
9 M. H. Kalaji, G. Schansker, R. J. Ladle, *et al.*, Frequently Asked Questions about in vivo chlorophyll fluorescence: practical issues, *Photosynth. Res.*, 2014, **122**, 121–158.
10 M. E. Ramos and M. G. Lagorio, True Fluorescence Spectra of leaves, *Photochem. Photobiol. Sci.*, 2004, **3**, 1063–1066.
11 F. Franck, P. Juneau and R. Popovic, Resolution of the Photosystem I and Photosystem II contributions to chlorophyll fluorescence of intact leaves at room temperature, *Biochim. Biophys. Acta*, 2002, **1556**, 239–256.
12 P. Mazzinghi, G. Agati and F. Fusi, Interpretation and physiological significance of blue-green and red vegetation fluorescence, in *Geoscience and Remote Sensing Symposium, 1994 (IGARSS) '94, Surface and Atmospheric Remote Sensing: Technologies, Data Analysis and Interpretation*, 1994, vol. 1, pp. 640–642.
13 C. Buschmann, Variability and application of the chlorophyll fluorescence emission ratio red/far-red of leaves, *Photosynth. Res.*, 2007, **92**, 261–271.
14 R. Hák, H. K. Lichtenthaler and U. Rinderle, Decrease of the chlorophyll fluorescence ratio F690/F730 during greening and development of leaves, *Radiat. Environ. Biophys.*, 1990, **29**, 329–336.

15 N. D'Ambrosio, K. Szabo and H. K. Lichtenthaler, Increase of the chlorophyll fluorescence ratio F690/F735 during the autumnal chlorophyll breakdown, *Radiat. Environ. Biophys*, 1992, **31**, 51–62.

16 R. Pedrós, I. Moya, Y. Goulas and S. Jacquemoud, Chlorophyll fluorescence emission spectrum inside a leaf, *Photochem. Photobiol. Sci.*, 2008, **7**, 498–502.

17 G. Agati, Response of the in vivo chlorophyll fluorescence spectrum to environmental factors and laser excitation wavelength, *Pure Appl. Opt.*, 1998, **7**, 797–807.

18 G. Agati, F. Fusi, P. Mazzinghi and M. Lipucci di Paola, A simple approach to the evaluation of the re-absorption of chlorophyll fluorescence spectra in intact leaves, *J. Photochem. Photobiol., B*, 1993, **17**, 163–171.

19 G. B. Cordon and M. G. Lagorio, Re-absorption of chlorophyll fluorescence in leaves revisited. A comparison of correction models, *Photochem. Photobiol. Sci.*, 2006, **5**, 735–740.

20 M. E. Ramos and M. G. Lagorio, A model considering light reabsorption processes to correct in vivo chlorophyll fluorescence spectra in apples, *Photochem. Photobiol. Sci.*, 2006, **5**, 508–512.

21 G. B. Cordon and M. G. Lagorio, Optical properties of the adaxial and abaxial faces of leaves. Chlorophyll fluorescence, absorption and scattering coefficients, *Photochem. Photobiol. Sci.*, 2007, **6**, 873–882.

22 J. Mendes Novo, A. Iriel and M. G. Lagorio, Modelling chlorophyll fluorescence of kiwi fruit (Actinidia deliciosa), *Photochem. Photobiol. Sci.*, 2012, **11**, 724–730.

23 A. Iriel, J. Mendes Novo, G. Cordon and M. G. Lagorio, Atrazine and Methyl viologen effects on Chlorophyll-a fluorescence revisited. Implications in Photosystems Emission and Ecotoxicity Assessment, *Photochem. Photobiol.*, 2014, **90**, 107–112.

24 A. Iriel, G. Dundas, A. Fernández Cirelli and M. G. Lagorio, Effect of arsenic on reflectance spectra and chlorophyll fluorescence of aquatic plants, *Chemosphere*, 2015, **119**, 697–703.

25 E. Pfündel, Estimating the contribution of Photosystem I to total leaf chlorophyll fluorescence, *Photosynth. Res.*, 1998, **56**, 185–195.

26 G. Agati, Response of the in vivo Chlorophyll fluorescence spectrum to environmental factors and laser excitation wavelength, *Pure Appl. Opt.*, 1998, **7**, 797.

27 J. M. Anderson, P. Horton, E. Kim and W. S. Chow, Towards elucidation of dynamic structural changes of plant thylakoid architecture, *Philos. Trans. R. Soc. London, Ser. B*, 2012, **367**, 3515–3524.

28 J. Minagawa, State transitions. The molecular remodeling of photosynthetic supercomplexes that controls energy flow inchloroplasts, *Biochim. Biophys. Acta*, 2011, **1807**, 897–905.

29 H. K. Lichtenthaler, C. Buschmann and M. Knapp, How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio RFd of leaves with the PAM fluorometer, *Photosynthetica*, 2005, **43**, 379–393.

30 M. Lechaudel, L. Urban and J. Joas, Chlorophyll fluorescence, a nondestructive method to assess maturity of mango fruits (Cv. 'Cogshall') without growth conditions bias, *J. Agric. Food Chem.*, 2010, **58**, 7532–7538.

31 Z. G. Cerovic, N. Moise, G. Agati, G. Latouche, N. Ben Ghozlen and S. Meyer, New portable optical sensors for the assessment of winegrape phenolic maturity based on berry fluorescence, *J. Food Compos. Anal.*, 2008, **21**, 650–654.

32 P. Dąbrowski, A. H. Baczewska, B. Pawluśkiewicz, M. Paunovc, V. Alexantrov, V. Goltsev and M. H. Kalaji, Prompt chlorophyll a fluorescence as a rapid tool for diagnostic changes in PSII structure inhibited by salt stress in Perennial ryegrass, *J. Photochem. Photobiol., B*, 2016, **157**, 22–31.

33 H. M. Kalaji, A. Jajoo, A. Oukarroum, M. Brestic, M. Zivcak, A. Samborska, M. D. Cetner, I. Łukasik, V. Goltsev and R. J. Ladle, Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions, *Acta Physiol. Plant*, 2016, **38**, 102.

34 M. G. Lagorio, L. E. Dicelio, M. I. Litter and E. San Román, Modeling of Fluorescence Quantum Yields of Supported dyes. Aluminum carboxy-phthalocyanine on cellulose, *J. Chem. Soc., Faraday Trans.*, 1998, **94**(3), 419–425.

35 H. B. Rodriguez, M. G. Lagorio and E. San Román, Rose Bengal adsorbed on microgranular cellulose. Evidence of fluorescent dimers, *Photochem. Photobiol. Sci.*, 2004, **3**, 674–680.

36 A. Zeug, J. Zimmermann, M. G. Lagorio and E. San Román, Microcrystalline cellulose as a carrier for hydrophobic photosensitizers in water, *Photochem. Photobiol. Sci.*, 2002, **1**, 198–203.

37 A. Iriel, M. G. Lagorio, L. E. Dicelio and E. San Román, Photophysics of supported dyes: phthalocyanine on silanized silica, *Phys. Chem. Chem. Phys.*, 2002, **4**, 224–231.

38 M. G. Lagorio, E. San Román, A. Zeug, J. Zimmermann and B. Roeder, Photophysics on surfaces: Absorption and luminescence properties of pheophorbide-a on cellulose, *Phys. Chem. Chem. Phys.*, 2001, **3**, 1524–1529.

39 H. W. Gausman and W. A. Allen, Optical Parameters of Leaves of 30 Plant species, *Plant Physiol.*, 1973, **52**, 57–62.

40 J. Barder, *The Photosystems: Structure, Function and Molecular Biology*, Elsevier, New York, 1992.

41 O. Nanba and K. Satoh, Isolation of a photosystem II reaction center consisting of D-1 and D-2 polypeptide and cytochrome, *Proc. Natl. Acad. Sci. U. S. A.*, 1987, **84**, 109–112.

42 J. Deisenhofer and J. R. Norris, *The Photosynthetic reaction center*, Academic Press, London, 1993, vol. II.

43 T. Y. Leong and J. M. Anderson, Adaptation of the thylakoid membranes of pea chloroplasts to light intensities. II. Regulation of electron transport capacities, electron carriers, coupling factor (CF_1) activity and rates of photosynthesis, *Photosynth. Res.*, 1984, **5**, 117–128.

44 T. Y. Leong and J. M. Anderson, Light-quality and irradiance adaptation of the composition and function of

peathylakoid membranes, *Biochim. Biophys. Acta*, 1986, **850**, 57–63.
45 M. S. Mc Donald, in *Photobiology of Higher Plants*, John Wiley and Sons Ltd, Chichester, England, 2003, ch. 4, pp. 121–122.
46 E. Brugnoli, A. Scartazza, M. C. De Tullio, M. C. Monteverdi, M. Lauteri and A. Augusti, Zeaxanthin and non-photohemical quenching in sun and shade leaves of C3 and C4 plants, *Physiol. Plant.*, 1998, **104**, 727–734.