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Contents of photosynthetic pigments and ratios of chlorophyll *a/b* and chlorophylls to carotenoids $(a+b)/(x+c)$ in C4 plants as compared to C3 plants

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Abstract

The content of chlorophylls (Chl) $(a+b)$, total carotenoids $(x+c)$, and the pigment ratios of Chl a/b and Chls to carotenoids $(a+b)/(x+c)$ of green leaves of five C4 plants were determined and compared to those of C3 plants. The C4 plants were: Pacific and Chinese silvergrass (*Miscanthus floridulus* and *Miscanthus sinensis*), sugar cane (*Saccharum officinarum*) as well as feed and sugar maize (*Zea mays*). The three C3 plants were beech, ginkgo, and oak. C4 plants possess higher values for the ratio Chl a/b (3.4–4.5) as compared to the C3 plants (2.6–3.3). Sugar maize had the highest values for Chl a/b (4.04–4.70) and exceptionally high contents of total carotenoids and consequently lower values for the ratio of $(a+b)/(x+c)$ (mean: 3.75 ± 0.6). During autumnal senescence also C4 plants showed a faster decline of Chl b as compared to Chl a yielding high values for Chl a/b of 6 to 8. Chlorophylls declined faster than carotenoids yielding low $(a+b)/(x+c)$ values below 1.0.

Introduction

C3 plants are known to adapt to the light environment they have been exposed to during their development. This adaptation response to high irradiance refers to growth and development of the entire plant, to leaves, cells, and particularly to the structure, thylakoid arrangement, and photosynthetic function of chloroplasts (Boardman 1977, Björkman 1981, Lichtenthaler *et al*. 1981, Meier and Lichtenthaler 1981, Anderson *et al*. 1995). Thus, a leaf

Highlights

- C4 plants possess higher Chl a/b ratios (mean of 3.4 to 4.5) as compared to C3 plants (2.6 to 3.3)
- Sugar maize (sweet corn) exhibits the highest Chl a/b value and exceptionally high carotenoid contents
- During senescence, Chl b is broken down faster than Chl a resulting in high values for the ratio Chl a/b of 6 to 8

developed under full sunlight (sun leaf) has a higher photosynthetic activity when receiving high irradiance as compared to a leaf that developed in the shade (shade leaf) (Lichtenthaler and Babani 2004, Lichtenthaler *et al.* 2000, 2007; Sarijeva *et al.* 2007). These physiological light adaptations are also seen in particular differences in the pigment composition of thylakoids (*see* a review of Lichtenthaler 2007, Lichtenthaler *et al.* 2013). Sun leaves with their sun chloroplasts (low and narrow grana stacks) possess higher values for the ratio Chl *a/b* and lower values for the mass ratio of total chlorophylls to total carotenoids, ratio $(a+b)/(x+c)$, as compared to the shade leaves with broad and high grana stacks (*see* reviews of Lichtenthaler 1983, 2007).

C$_4$ plants are known to be 'light plants' which require full sunshine for normal growth and development and also higher temperatures for full photosynthetic rates as compared to C$_3$ plants. C$_4$ plants, in general, grow in warmer, often tropical Earth regions. In C$_4$ plants, the wasteful photorespiration process is blocked, hence they usually possess higher photosynthetic rates as compared to C$_3$ plants. Their photosynthetic CO_2 fixation and reduction are partitioned between mesophyll cells (initial carbon fixation by phospho*enol*pyruvate carboxylase) and bundle sheath cells (final carbon fixation by ribulose-bisphosphate carboxylase) which differ in structure and function of their chloroplasts (*see* reviews of Hatch 1999, Kanai and Edwards 1999). Mesophyll chloroplasts possess the normal differentiation into stroma and grana thylakoids, whereas bundle sheath chloroplasts only exhibit stroma thylakoids (*e.g.*, Laetsch 1969, Kirchanski 1975, Evert *et al.* 1996). Some data indicate that – as compared to C$_3$ plants – C$_4$ plants can generally have higher values of the Chl *a/b* ratio in the range of 3.3 to 4.4 (*e.g.*, Lichtenthaler 1983, Tuba 1984). However, to our best information, the relative content of total carotenoids *x+c*, *e.g.*, on a Chl $(a+b)$ basis, has not yet been determined. In addition, also the question, whether leaves of C$_4$ plants may be similar in their pigment composition, *i.e.*, the ratios of Chl *a/b* and $(a+b)/(x+c)$, to sun-exposed tree leaves or leaves of high-light C$_3$ plants, has not yet been investigated.

To overcome this gap, we determined the chlorophyll and carotenoid contents of five C$_4$ plants and compared their ratio values for Chl *a/b* and chlorophylls to total carotenoids $(a+b)/(x+c)$ with that of sun leaves of three C$_3$ trees. In addition, it is not known for C$_4$ plants, whether during the autumnal leaf senescence – as it has been shown for C$_3$ plants (Lichtenthaler and Babani 2004) – Chl *a* is broken down faster than Chl *b* and whether the content of chlorophylls declines faster than that of total carotenoids. These points are also a matter of this investigation.

Materials and methods

Plant materials

C$_4$ **plants**: The leaves used in the experiments were taken from single standing fully light-exposed plants growing in the Botanical Garden on the campus of the Karlsruhe Institute of Technology (KIT). These were:

Pacific silvergrass (*Miscanthus floridulus* Warb.), Chinese silvergrass (*Miscanthus sinensis* Anderson), and sugar cane (*Saccharum officinarum* L.). Leaves of two maize varieties (*Zea mays* L.), *i.e.*, feed maize or field corn (var. 'Protador') and sugar maize or sweet corn (*Zea mays* var. *saccharata*, line 'yellow sh2'), were taken from two agricultural fields in the nearby Durlach area. Pigment determination was performed for green leaves at the beginning of August 2013 (green leaves) and at the end of September for senescing yellowish-green leaves. The leaf-disk samples were taken from the middle part of the elongated leaf blade of C$_4$ plants and also from leaf parts towards the tip of the leaf blade. The highest pigment contents were found in leaf disk samples from the middle part of the leaf blade.

C$_3$ **plants**: Sun and shade leaves of beech (*Fagus sylvatica* L.) and pedunculate oak (*Quercus robur* L.) leaves were taken from solitary standing trees in the park of the university campus. Ginkgo leaves (*Ginkgo biloba* L.) came from a solitary standing, 50-year-old tree in my private garden in Karlsruhe-Durlach. Sun leaves were taken from the outer fully sun-exposed tree crown facing south and shade leaves from the full shade of the inner tree crown. The pigments of sun and shade leaves of C$_3$ plants were determined in mid-July to the beginning of August 2012, when the differences between sun and shade leaves were fully pronounced. The observed differences in leaves in pigment contents and ratios between sun and shade (*see* below) show up every year as had been documented in earlier investigations (*e.g.*, Lichtenthaler and Babani 2004). For each pigment determination, small leaf disks were punched from the left and right half of the leaf blade.

Determination of photosynthetic leaf pigments

Chlorophylls *a* and *b*, as well as total carotenoids *x+c*, *i.e.*, xanthophylls and carotenes, were determined in a two-wavelength spectrophotometer using the specific extinction coefficients and equations by Lichtenthaler (1987). For more details and examples of several plants *see* Lichtenthaler and Buschmann (2001a,b). Moreover, practical instruction is found in the link: https://www.botanik.kit.edu/molbio/download/1A_Chlorophylls_Carotenoid_determin-HKLi_2010.pdf.

Green leaves: Extraction was performed with 100% acetone from usually two punched small green leaf pieces (leaf disks) which were obtained using a rim-sharpened laboratory cork driller (diameter of 6–8 mm). The leaf disks were ground, in presence of acetone, with quartz sand in a mortar with a pestle. Small amounts of magnesium oxide or carbonate (MgO or $MgCO_3$) were added to neutralize plants acids that would cause the formation of pheophytin *a* from chlorophyll *a*. The turbid leaf pigment extract was centrifuged at room temperature for 3–5 min at 300–500 × *g* to be fully clear. The latter is an absolute requirement, otherwise one obtains too high values for Chl *b* and total carotenoids *x+c*. Moreover, the faster decline of Chl *b* as compared to Chl *a* during

autumnal senescence as documented in this investigation, cannot be observed, if the absorbance of the pigment extract solution at the red Chl *a* absorption maximum is below 0.3. The optical density, the absorption A, was measured at 661.6, 644.8, and 470 nm using the usual path length of the spectrophotometer cuvette of 1 cm. The absorbance readings in the spectrophotometer in the red absorption maximum at 661.6 nm were performed in the absorbance range of 0.3–1.0 to obtain correct Chl *a* and Chl *b* values. At an absorbance reading at 661.6 nm below 0.3, the values of Chl *b* become too high, yielding too low values for the ratio Chl *a/b*.

The following equations were applied to determine from the absorbance readings (A): the concentration (C) of the pigments in µg per ml of extract solution: Chl *a*: $C_a = 11.24\ A_{661.6} - 2.04\ A_{644.8}$, Chl *b*: $C_b = 20.13\ A_{644.8} - 4.19\ A_{661.6}$, total carotenoids (*x+c*): $C_{(x+c)} = (1000\ A_{470} - 1.90\ C_a - 63.14\ C_b)/214$.

Yellowish-green leaves: In the case of senescing leaves of the C$_4$ plants, instead of one or two, up to 6 or 8 punched leaf disks were extracted to ensure an absorbance reading at 661.6 nm between 0.3–1.0. The whole procedure of pigment extraction, centrifugation steps, and absorbance readings were performed within 20 to 30 min to obtain reliable Chl *a* values. Longer standing times of the extract solutions were avoided, because the high narrow absorption peak at 661.6 nm for Chl *a* steadily decreases and broadens after 30 min due to the successive formation of allomeric Chl *a* forms. This would result in too low values for Chl *a* and too high values for Chl *b*.

Trend lines and correlation coefficients: In order to see trend lines of the relationship between two parameters, *e.g.*, the total carotenoid *x+c* content and the Chl (*a+b*) content of leaves as well as the ratio Chl *a/b* and the total Chl content of C$_4$ plants, we performed a regression analysis and calculated the correlation coefficients R-square (R^2). The latter, ranging from 0 to 1, is a primary measure of how well a regression model fits the data and is also known as the coefficient of determination. Values for R^2 of 0.4–0.5 indicate a moderate, of 0.6–0.7 a strong, and 0.7–1 a very strong correlation.

Statistical analysis: The differences in pigment contents and pigment ratios of sun and shade leaves were checked for significance using the *Student*'s *t*-test. The differences were assessed *via* the analysis of variance one-way (*ANOVA*). Differences with $p<0.05$ are significant and those with $p<0.01$ are highly significant.

Results

Green leaves of C$_4$ plants

Pigment contents: In four of five C$_4$ plants investigated here, the total Chl (*a+b*) content of fully green photosynthetically active leaves was found to range between *ca.* 320–580 mg m^{-2} leaf area. These were the two *Miscanthus* species, sugar cane, and feed maize as seen from the data

in Figs. 1 and 2, right part. The leaf blades of sugar maize had by far the highest Chl (*a+b*) contents per leaf area unit from 366 up to 785 mg m^{-2} as indicated in Fig. 2, right part. This relatively wide range of Chl (*a+b*) contents is because these C$_4$ plants possess far elongated leaves which have a considerably higher Chl (*a+b*) content in the middle region of the leaf blade as compared to the thinner leaf blade at the leaf tip. The mean Chl (*a+b*) contents of all five C$_4$ plants are shown in Table 1. The corresponding content of total carotenoids *x+c* per leaf area unit shows also a fairly wide range from 50 to 100 mg m^{-2}, except sugar maize with unusual high carotenoid contents of 126 up to 183 mg m^{-2} leaf area. The mean *x+c* contents of sugar maize leaf blades were significantly higher 149 ± 20 mg m^{-2} as compared to the four other C$_4$ plants which exhibited mean values of only 82 to 89 mg m^{-2} (Table 1).

Pigment ratio of (*a+b*)/(*x+c*): Concerning the relationship between the total Chl (*a+b*) and total carotenoid content, we found the general rule: the higher the Chl (*a+b*) content, the higher the total *x+c* content as is shown for green
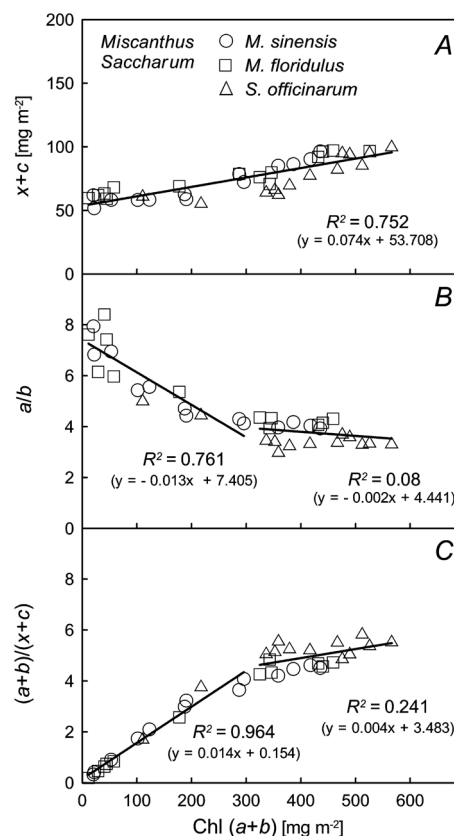

Fig. 1. Content of carotenoids (*A*), values of the ratio Chl a/b (*B*), and the ratio chlorophylls/carotenoids (*a+b*)/(*x+c*) (*C*), based on the total Chl (*a+b*) content per leaf area unit in three C$_4$ plants: two silvergrass species *Miscanthus sinensis* and M. *floridulus* and sugar cane (*Saccharum officinarum*). *Right part of the figure*: green leaves. *Left part of the figure*: yellowish-green leaves during autumnal senescence. Trend lines (correlation lines) are indicated for the changes in green leaves and during senescence.

leaves in the right part of Figs. 1A and 2A. This correlation is highly significant as indicated by high values for the correlation coefficients R-square (R^2) in all five C_4 plants. This correlation is also confirmed by the values for the mass ratio of total Chl ($a+b$) to total carotenoids, ($a+b$)/($x+c$), as shown in Figs. 1C and 2C. Sugar maize leaves with their particularly high $x+c$ contents consequently exhibited significantly lower mean values for the pigment ratio ($a+b$)/($x+c$) of 3.75 ± 0.58 as compared to the other four C_4 plants with distinctly higher mean $x+c$ values of 4.45 to 5.67 (Table 1).
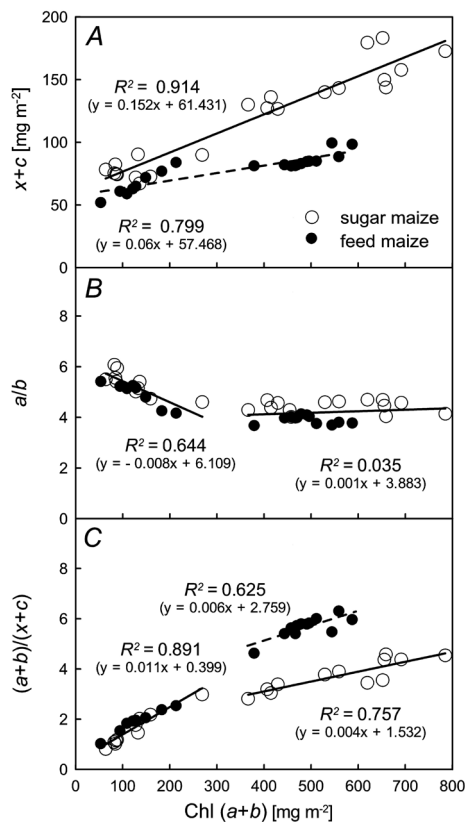
Ratio Chl *a/b*: We also checked the values of the ratio of Chl *a* to Chl *b* in the green leaf blades. These were more or less in the same range at low and high Chl contents and independent from the total Chl ($a+b$) content of green leaves. Also, in this case, the values for Chl *a/b* were significantly higher in sugar maize leaves (mean value of 4.50 ± 0.22) as compared to the other four C_4 plants (mean value range: 3.44–4.19) (Table 1 and right part of Figs. 1B and 2B).

Green leaves of C_3 plants

To detect potential differences in the content of photosynthetic pigments and the pigment ratios between C_4 plants and C_3 plants, we determined the Chl ($a+b$) and total carotenoid content of three typical trees growing in our South-German region. In contrast to the C_4 plants investigated here, where practically all leaves were exposed to full sunlight, we had to differentiate in the C_3 trees between the smaller and thicker sun leaves exposed to full sunshine and the usually larger and thinner shade leaves from the full shade of the inner tree crown (cf. Lichtenthaler 1983, Lichtenthaler and Babani 2004). The results of sun and shade leaves from beech, ginkgo, and pedunculate oak are shown in Table 2.

The contents of Chl ($a+b$) of sun leaves per leaf area unit were significantly higher as compared to those of shade leaves. This was paralleled by higher contents of total carotenoids $x+c$. In addition, sun leaves were found to possess significantly higher values for the ratio of Chl *a/b* (mean value range: 3.14–3.31) as compared to shade leaves (mean value range: 2.62–2.72) as presented in Table 2. This was paralleled by significantly lower values for the ratio of Chl ($a+b$) to total carotenoids ($a+b$)/($x+c$) (mean value range: 4.07–4.25 in sun leaves) as compared to those of shade leaves (mean value range: 5.18–5.54).

Fig. 2. Content of carotenoids (*A*), values of the ratio Chl *a/b* (*B*), and the ratio chlorophylls/carotenoids ($a+b$)/($x+c$) (*C*), based on the total Chl ($a+b$) content per leaf area unit in two maize varieties (C_4 plants): feed maize and sugar maize. *Right part of the figure*: green leaves. *Left part of the figure*: yellowish-green leaves during autumnal senescence. Trend lines (correlation lines) are indicated for the changes in green leaves and during senescence.

Table 1. Contents of Chl ($a+b$) and total carotenoids $x+c$ per leaf area unit [mg m^{-2}] and values of the pigment ratios Chl *a/b* and chlorophylls ($a+b$) to carotenoids, ($a+b$)/($x+c$), in green leaves of five C_4 plants: *Miscanthus floridus* and *Miscanthus sinensis*, sugar cane (*Saccharum officinarum*) as well as feed maize and sugar maize (*Zea mays*). Mean values with standard deviation. These are based on 6 and 4 determinations (*M. floridus* and *M. sinenesis*), on 11 (sugar cane) and on 12 determinations (for both maize varieties) from two separate plants in each case. The differences between feed maize and sugar maize in the level of $x+c$ and the pigment ratios Chl *a/b* and ($a+b$)/($x+c$) are highly significant ($p<0.01$).

C_4 plant	Chl ($a+b$) [mg m^{-2}]	$x+c$ [mg m^{-2}]	Chl *a/b*	($a+b$)/($x+c$)
M. floridus	391 ± 59	85 ± 11	4.19 ± 0.17	4.57 ± 0.23
M. sinensis	399 ± 34	89 ± 5	4.03 ± 0.11	4.45 ± 0.17
S. officinarum	435 ± 79	82 ± 14	3.44 ± 0.18	5.38 ± 0.25
Feed maize	491 ± 56	86 ± 6	3.92 ± 0.16	5.67 ± 0.42
Sugar maize	564 ± 134	149 ± 20	4.50 ± 0.22	3.75 ± 0.58

Table 2. Differences in the contents of Chl ($a+b$) and total carotenoids $x+c$ per leaf area unit [mg m^{-2}] and in the pigment ratios Chl a/b and chlorophylls ($a+b$) to carotenoids, ($a+b$)/($x+c$), between sun and shade leaves of three deciduous trees (C$_3$ plants). Mean values of 7 determinations per leaf type. The differences between sun and shade leaves were highly significant ($p<0.01$). For a better comparison of the differences in the pigments ratios between sun and shade leaves, those of sun leaves are shown here in bold numbers.

C$_3$ plant		Chl ($a+b$) [mg m^{-2}]	$x+c$ [mg m^{-2}]	Chl a/b	($a+b$)/($x+c$)
Fagus sylvatica	Sun leaf	521 ± 45	122 ± 12	**3.31** ± 0.13	**4.25** ± 0.27
	Shade leaf	383 ± 37	74 ± 7	2.65 ± 0.06	5.18 ± 0.16
Ginkgo biloba	Sun leaf	487 ± 35	117 ± 9	**3.14** ± 0.11	**4.16** ± 0.16
	Shade leaf	327 ± 21	59 ± 6	2.72 ± 0.07	5.54 ± 0.26
Quercus robur	Sun leaf	435 ± 36	107 ± 13	**3.24** ± 0.15	**4.07** ± 0.21
	Shade leaf	317 ± 24	61 ± 9	2.62 ± 0.07	5.20 ± 0.17

Yellowish-green leaves of C$_4$ plants

During autumnal senescence and Chl decline, the chlorophylls are broken down much faster than the carotenoids. This resulted in continuously declining values for the ratio of Chl ($a+b$) to total carotenoids, ($a+b$)/($x+c$), from about 4.5 (green leaves) to values below 0.4 in the two *Miscanthus* species (Fig. 1C, left part). Also for feed maize, the ratio values of ($a+b$)/($x+c$) decreased from 5.6 (green leaves) to values below 1, and in sugar maize, they decline from 3.75 to values below 1 as well (Fig. 2C, left part). This autumnal decrease of the pigment ratio, ($a+b$)/($x+c$), follows a linear correlation, as is documented by high values for the correlation coefficient R-square (R^2) of 0.964 (Fig.1C, left part) and of 0.891 (Fig. 2C, left part).

During this autumnal process of pigment breakdown, the content of Chl b was declining much faster than that of Chl a resulting in continuously increasing values of the ratio of Chl a/b with progressing Chl breakdown. In the two *Miscanthus* species, the values for Chl a/b increased from mean values of about 4 up to values of 8 and slightly higher (Fig. 1B, left part). In feed and sugar maize, the mean values of the ratio of Chl a/b increased from mean values of 4.5 and 3.9, respectively, to values of 6 (Fig. 2B, left part). This continuous increase of the Chl a/b values with decreasing Chl ($a+b$) content of leaves was highly significant as was indicated by the high values for the correlation coefficient R-square (R^2) of 0.761 (Fig. 1B) and 0.644 (Fig. 2B).

Discussion

The results of this investigation show again that green sun leaves of trees (C$_3$ plants) possess significantly higher values ($p<0.01$) for the ratio of Chl a/b as compared to shade leaves (Table 2). The Chl a/b values of green leaves continuously decrease from sun-exposed leaves (with the highest values) *via* leaves growing in the blue shade, *via* half shade with the lowest values in full shade (Lichtenthaler 1983, Lichtenthaler and Babani 2004, Lichtenthaler *et al.* 2013). This had been also described for herbaceous C$_3$ plants grown either under high or low irradiance conditions (Lichtenthaler *et al.* 1981, Lichtenthaler and Babani 2004).

These differences are because shade leaves and leaves from low-light plants contain a much higher proportion of light-harvesting chlorophyll-carotenoid-proteins, the LHCPs of photosystem II, as compared to sun leaves and leaves grown under high-light conditions. The LHCPs exhibit very low values for the ratio of Chl a/b of only 1.1 to 1.3 (Lichtenthaler *et al.* 1982a) and are also responsible for the stacking of thylakoids to grana stacks (Lichtenthaler *et al.* 1982b,c). As a consequence, the more LHCPs are present in the chloroplasts, the lower the values for the Chl a/b ratio and the higher the stacking degree, *i.e.*, the proportion of appressed thylakoid membranes. Sun-exposed leaves and green leaves from plants grown under high irradiance have much higher Chl a/b ratio values and a significantly lower stacking degree of their thylakoids (as reviewed in Lichtenthaler 1983, Lichtenthaler and Babani 2004).

Green leaves of the five C$_4$ plants analyzed here and taken as a sum exhibited significantly higher values ($p<0.05$) for the ratio of Chl a/b as compared to the sun-exposed green leaves of the three trees which are C$_3$ plants. This was less pronounced for the green sugar cane leaves investigated here (mean value for Chl a/b of 3.44), yet this relatively low mean value can at least partially be explained by the fact that the plant was shaded for *ca.* 3 h in the early afternoon. When compared with the Chl a/b values of the shade leaves of the three C$_3$ trees (mean values of 2.62 to 2.72), the differences in Chl a/b values of C$_4$ plants were highly significant ($p<0.01$). The higher values of the ratio of Chl a/b in C$_4$ plants can be explained because C$_4$ plants have dimorphic chloroplasts. Parenchyma cells contain chloroplasts with stroma and grana thylakoids, whereas bundle sheath cells exhibit agranal chloroplasts that only possess nonstacked stroma thylakoids (Laetsch 1969, Kirchanski 1975, Evert *et al.* 1996). The absence of grana stacks indicates that these agranal chloroplasts possess only very low amounts of the light-harvesting Chl a/b proteins, the LHCPs of PSII, which are known to have very low values for the ratio of Chl a/b (Lichtenthaler *et al.* 1982a). In addition, the C$_4$ plants investigated here are 'light plants' usually growing in full sunlight and show a much lower shade adaptation response of their photosynthetic pigment apparatus as

compared to C_3 plants. These facts then are the reasons for the significantly higher Chl a/b values of C_4 plants as compared to C_3 plants.

Among the C_4 plants, it is noteworthy to mention that the two maize varieties feed maize and sugar maize showed considerable and significant differences ($p<0.01$) in the ratio of Chl a/b, total carotenoid content $x+c$, and the ratio of $(a+b)/(x+c)$ (Table 1). The relatively high Chl a/b ratio value of 4.5 and the extremely high content of total carotenoids per leaf area unit and, as a consequence, an extremely low mean value of 3.75 for the ratio $(a+b)/(x+c)$, are exceptional. Sugar maize or sweet corn plants are a group of special maize varieties, where the photosynthetically produced sugar molecules transported into the individual corns of the maize cob are not directly or spontaneously transformed into starch as in normal feed maize. This rather occurs with a much-delayed rate and is the reason why the kernels contain a high soluble sugar content, taste sweet, and why the sugar maize cobs are used as a vegetable (Subaedah $et\ al.$ 2021). Based on our earlier observations on the accumulation of additional carotenoids at a high irradiance exposure of plants (Lichtenthaler 2007), the distinct pigment composition of sugar maize indicates that the photosynthetic pigment apparatus of sugar maize is more strongly adapted to high irradiance conditions as compared to feed maize and the other C_4 plants investigated here. Such high contents of total carotenoids (usually induced via a strong increase of ß-carotene and zeaxanthin) have also been observed in leaves of C_3 plants when these are exposed to continuous extreme high photosynthetic irradiances (Lichtenthaler 2007).

Concerning the values for the ratio of chlorophylls to total carotenoids, $(a+b)/(x+c)$, there are no clear differences between green leaves of the investigated C_3 and C_4 plants. The $(a+b)/(x+c)$ values of the investigated C_3 trees range in shade leaves from 5.18 to 5.54 and in sun leaves from 4.07 to 4.25. In the C_4 plants, the mean values for the ratio of $(a+b)/(x+c)$ were found to range from 3.75 to 5.67.

Moreover, also the total amounts of the Chl $(a+b)$ and total carotenoids $x+c$ contents per leaf area unit do not allow to differentiate between C_3 plants and C_4 plants as they are in a similar range. The total Chl $(a+b)$ amounts (mean values) of C_3 plants were found to range from 317 to 521 mg m^{-2}(leaf area), and those of the five investigated C_4 plants were in the range of 391 to 564 mg m^{-2}(leaf area). Similar behavior was also found for the $x+c$ contents. The mean values of C_3 plants were in the range of 59 to 122 mg m^{-2}(leaf area) and those of C_4 plants in the range of 85 to 149 mg m^{-2}(leaf area).

During autumnal leaf senescence and chlorophyll breakdown, the green leaves of C_3 plants and C_4 plants showed similar responses. Chlorophylls are broken down faster than carotenoids yielding very low values for the ratio $(a+b)/(x+c)$ of below 1.0 at a progressed Chl decline. In both plant types, Chl b is destroyed considerably faster than Chl a which yields continuously increasing values for the ratio of Chl a/b, up to values of 6 to 8 at a progressed Chl $(a+b)$ decline. This had been reported before for C_3

plants (Lichtenthaler and Babani 2004) and is confirmed here for C_4 plants. Such high values for Chl a/b indicate that the light-harvesting chlorophyll-proteins, the LHCPs of photosystem PSII with their low Chl a/b ratios of 1.1 to 1.3 (Thornber 1975, Lichtenthaler $et\ al.$ 1982a,b) are decomposed much faster than the reaction center pigment-proteins CPa and CPI.

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