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Photosynthesis and photoinhibition in two differently coloured varieties of *Oxalis triangularis* – the effect of anthocyanin content

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Abstract

The purpose of this study was to clarify effects of anthocyanins on photosynthesis and photoinhibition in green and red leaves of *Oxalis triangularis*. Gas analysis indicated that green plants had the highest apparent quantum yield for CO_2 assimilation [0.051 *vs.* 0.031 μmol(CO_2) μmol^{-1}(photon)] and the highest maximum photosynthesis [10.07 *vs.* 7.24 μmol(CO_2) m^{-2} s^{-1}], while fluorescence measurements indicated that red plants had the highest PSII quantum yield [0.200 *vs.* 0.143 μmol(e^-) μmol^{-1}(photon)] and ETR_{max} [66.27 *vs.* 44.34 μmol(e^-) m^{-2} s^{-1}]. Red plants had high contents of anthocyanins [20.11 mg g^{-1}(DM)], while green plants had low and undetectable levels of anthocyanin. Red plants also had statistically significantly ($0.05>p>0.01$) lower contents of xanthophyll cycle components [0.63 *vs.* 0.76 mg g^{-1}(DM)] and higher activities of the reactive oxygen scavenging enzyme ascorbate peroxidase [41.2 *vs.* 10.0 nkat g^{-1}(DM)]. Anthocyanins act as a sunscreen, protecting the chloroplasts from high light intensities. This shading effect causes a lower photosynthetic CO_2 assimilation in red plants compared to green plants, but a higher quantum efficiency of photosystem II (PSII). Anthocyanins contribute to photoprotection, compensating for lower xanthophyll content in red plants, and red plants are less photoinhibited than green plants, as illustrated by the F_v/F_m ratio.

Additional key words: anthocyanin; ascorbate peroxidase; chlorophyll fluorescence; photoinhibition; photosynthesis; superoxide dismutase; xanthophyll.

Introduction

Light is required for photosynthesis, but may also be damaging to the plant. The decrease in photosynthetic efficiency caused by light is known as photoinhibition, and can be divided into two types: reversible photoinhibition, in which excess absorbed light energy is quenched, and chronic photoinhibition, due to photodamage of the D1 protein of PSII (Kyle *et al.* 1984). Photodamage to PSII is a result of reactive oxygen species (ROS) produced in the thylakoids when light is in excess. Under high light intensities or other stress conditions an imbalance between the absorption of light energy and the assimilation of the absorbed energy through carbon fixation and other metabolic processes may occur. Consequently, plants have developed several different mechanisms to prevent or cope with photodamage and several pathways exist for the dissipation of excess energy, known as reversible photoinhibition, as well as for scavenging ROS. One of the most important pathways is the xanthophyll cycle, in which excess excitation energy is converted to heat. The light-dependent conversion of violaxanthin through antheraxanthin to zeaxanthin takes place under conditions in which excessive light is absorbed by chlorophyll (Chl) (Demmig *et al.* 1987). When light is no longer in excess, zeaxanthin is epoxidized back to violaxanthin (Siefermann and Yamamoto 1975). The changes in the levels of antheraxanthin and zeaxanthin in illuminated leaves are strongly correlated with nonphotochemical quenching (NPQ), which is a measure of the rate of heat dissipation. Large amounts of excess absorbed light energy have been showed

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Abbreviations: APX – ascorbate peroxidase; Chl – chlorophyll; ETR – electron transport rate; F_v/F_m – maximum quantum yield of PSII; HPLC – high performance liquid chromatography; Ic – light compensation point of photosynthesis; Isat – light saturation point of photosynthesis; LED – light-emitting diodes; NPQ – nonphotochemical quenching; Φ_{CO2} – apparent quantum yield of CO_2 assimilation; Φ_{PSII} – quantum yield of PSII; PAM – pulse-amplitude modulated; PAR – photosynthetically active radiation; PSII – photosystem II; ROS – reactive oxygen species; SOD – superoxide dismutase; UV – ultraviolet.
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to be dissipated through this pathway (Demmig-Adams and Adams 1992, Demmig-Adams and Adams 1996).

While the role of the xanthophyll cycle is to dissipate excess energy before it can give rise to the formation of ROS, plants possess several defense mechanisms for the quenching of ROS, once formed. Two of the most important of these are superoxide dismutase (SOD), reducing superoxide to hydrogen peroxide and free oxygen, and ascorbate peroxidase (APX), reducing hydrogen peroxide to water (Asada 2006).

Recently, the role of anthocyanins in photoprotection has attracted some attention. Anthocyanins may be present only in limited periods of plant development, *e.g*. in juvenile or senescing tissue, and their presence may be controlled by photoperiod and temperature. Since anthocyanins are photoinducible, it has been hypothesized that they have a photoprotective role in plant leaves. Anthocyanins are often found in or just below the upper epidermis of leaves (Burger and Edwards 1996). They absorb large quantities of green and UV light as well as smaller quantities of blue light, while absorbing negligible quantities of red light (Harborne 1958). Consequently, less blue-green light is available to Chl when anthocyanins are present (Pietrini and Massacci 1998, Smillie and Hetherington 1999). Overall, anthocyanins are capable of absorbing up to 17% of the incident PAR (Neill and Gould 1999). Thus, since anthocyanins modify both quantity and quality of the incident light on chloroplasts, they can be said to act as a sunscreen. Another role of anthocyanins that could potentially decrease the adverse effect of ROS is their effect as antioxidants, scavenging ROS (Wang *et al.* 1997). The antioxidant effect of anthocyanins has been illustrated *in vitro* (Wang *et al.* 1997) as well as *in vivo* (Gould *et al.* 2002a). Anthocyanins thus possess at least two mechanisms acting to relieve the problem with excess electrons, including the ability to protect chloroplasts from excess light, and the ability to scavenge ROS.

In this study, we characterized the effect of anthocyanins on the photosynthesis and photoinhibition of two differently coloured varieties of *Oxalis triangularis* A. St.-Hil. and compare the content of xanthophyll cycle components and the activity of ROS scavenging enzymes SOD and APX in the two varieties.

We are testing the hypothesis that high anthocyanin content will reduce photosynthesis due to the absorption of light in anthocyanin, but that photoinhibition will be similarly reduced due to the photoprotective effect of anthocyanin. Furthermore, we hypothesize that as a consequence of the photoprotective effect of anthocyanin, plants with high anthocyanin content will downregulate their content of xanthophyll cycle components.

Materials and methods

Plant material and growth conditions: We obtained two cultivars of *Oxalis triangularis* from a commercial source, *O. triangularis* with green leaves and white flowers and *O. triangularis* ssp. *atropurpurea* with dark purple leaves and pink flowers. Prior to experiments the plants were kept in a growth chamber at 20°C and an irradiance of 350 μmol m^{-2} s^{-1} (PAR) with a photoperiod of 16:8 h (light:darkness) for at least two weeks for acclimatization. The plants were kept in soil-filled pots in self-watering trays.

CO_2 assimilation: Photosynthetic CO_2 assimilation was measured with an infrared gas analyzer (*IRGA* type *225 mk3*; *Analytical Development Co*., Hoddesdon, England), measuring in differential mode. The IRGA was calibrated using pure nitrogen for zero values together with atmospheric air containing 350 ppm CO_2, both gases supplied from Strandmøllen, Denmark. A mature, undamaged leaf from the topmost light exposed part of the plant was cut off with a small part of the stem. The leaf was placed in a leaf chamber (*LSC-2-ASSY, Analytical Development Co*., Hoddesdon, England) kept at 20°C with the stem placed in water in the built-in leaf chamber compartment to prevent the leaf from drying out during measurements. The air supplied to both the *IRGA* and the chamber was passed through a washing bottle filled with water to ensure saturation with water vapour, thus preventing desiccation of the leaf with subsequent stomata closure. The light for photosynthesis measurements was supplied by a red LED-lamp (*Hansatech LC1*). The CO_2 uptake by the leaf was measured at 12 different light intensities, increasing from darkness to the highest light intensity. Dark adaptation lasted 30 min, while the measurement at each light intensity was completed within 10 min, when steady-state photosynthesis had been achieved. Measured dark respiration was added to the measured net photosynthetic rates to give gross photosynthesis.

Chl fluorescence (PAM): The electron transport rate (ETR) and maximum quantum yield of PSII (F_v/F_m) was measured using an *Imaging-PAM* Chl fluorometer (*Walz*, Effentrich, Germany). Plants were dark-adapted for one hour prior to measurements. A mature undamaged leaf from the top-most part of the dark-adapted plant was selected and cut off at the base. The leaf was placed in a leaf-holder at a fixed distance from the camera of the *Imaging-PAM* during measurements. Minimum fluorescence of the dark-adapted state (F_0) was recorded at a measuring light intensity of 0.5 μmol m^{-2} s^{-1} (PAR) and followed by an excitation pulse [2,400 μmol m^{-2} s^{-1}(PAR)] of 0.8 s from the 470 nm light-emitting diode (LED) ring array to acquire the maximum fluorescence of the dark-adapted state (F_m). Subsequently, the leaves were exposed to 16 illuminations of increasing light intensities provided by the LED ring array. Each illumination lasted

20 s, and each series of measurements was completed within 7 min. Three circular areas on the resulting fluorescence image of the leaf surface were randomly chosen and numerical values of fluorescence parameters were extracted from these points using the *ImagingWin* software (*Walz*, Effentrich, Germany).

Chl and xanthophyll content: The content of Chl *a* and *b* as well as xanthophyll cycle components was measured by High Performance Liquid Chromatography (HPLC) according to Nielsen and Nielsen (2006). Plant extracts were prepared from lyophilized and pulverized leaf samples that were extracted in methanol in an ice bath for 30 min. Extracts were filtered before analysis (*Mini-UniPrep PTFE*, 0.45 µm, *Whatman*, Maidstone, England). Analyses were performed on a *Dionex Summit HPLC* system (*Dionex*, Hvidovre, Denmark) applying a gradient method, using methanol:1M ammonium acetate (80:20, v/v) as eluent A and methanol:acetone (90:10, v/v) as eluent B. A gradient elution was run for 30 min going from 50% eluent B to 100 % eluent B in 5 min, then immediately back to 60% eluent B, continuing to 100% eluent B again in 3.5 min. This was then maintained for 15.5 min and was followed by a 6-min re-equilibration period, so that the total runtime for each sample was 30 min. The column was a *Waters Spherisorb* 3 µm *ODS2* 4.6 × 150 mm *C18* column. The flow rate was set to 0.8 ml min^{-1}. The detector was a *Dionex UltiMate 3000 DAD* (*Dionex*, Hvidovre, Denmark). Standards were provided by *DHI Water and Environment* (Hørsholm, Denmark).

Anthocyanin content: The anthocyanin content was determined by HPLC according to Fossen *et al.* (2005). Plant extracts were prepared from lyophilized and pulverized leaf samples, extracted in methanol in an ice bath for two hours. Extracts were filtered as previously described. Analyses were performed on a *Dionex Summit HPLC* system (*Dionex*, Hvidovre, Denmark), using a gradient method. The column was a *Phenomenex Luna C18 100A* column (4.6 × 250 mm, 5 µm), and the three eluents were 2.5% formic acid in 2 mM KCl (eluent A), 2.5% formic acid in acetonitrile (eluent B) and pure methanol (eluent C). A gradient elution was run for 66 min, going from 90:4:6 % A:B:C to 100% eluent C in 61 min, which was maintained for 3.5 min, then changing to 92:8 % A:C for the last 1.5 min. The flow rate was set to 1 ml min^{-1}. The content of individual anthocyanins was calculated as pelargonidin equivalents, based on a 3-O-β-glucopyranoside standard (Fossen *et al.* 2005), obtained from *Polyphenols Laboratories*, Norway, and integrated to yield total anthocyanin content.

Scavenging enzymes: SOD was analyzed using a SOD assay kit from *Sigma-Aldrich* (*Sigma-Aldrich product no. 19160*). The analysis is an indirect assay method based on xanthine oxidase and a water-soluble tetrazolium salt as colour reagent. The colour reagent is oxidized with superoxide in a reaction inhibited by SOD, the activity of which consequently can be determined colorimetrically. The guidelines enclosed with the assay kit were followed without modifications. APX was analyzed colorimetrically in an assay in which the oxidation of ascorbate was followed as a decrease in absorbance at 290 nm according to Nakano and Asada (1981) as modified by Chen and Asada (1989). For both SOD and APX assays, 0.2–0.3 g FM was homogenized in liquid nitrogen and extracted in the relevant buffers. The activity of both enzymes was expressed per dry mass, using a FM/DM relationship determined for both varieties of the plant.

Statistical analysis: Since data on pigment content and enzyme activity had a non-normal distribution and displayed heteroscedasticity, the nonparametric *Mann-Whitney U*-test (Siegel 1956) was used to test for differences in these parameters between the green and red form of *O. triangularis*. All tests were carried out at a level of significance of $p<0.05$.

Photosynthetic parameters were derived using curve-fits (Baly 1935, Steele 1962). Data on F_v/F_m were found to be normally distributed (*Kolmogorov-Smirnov*, $p>0.05$) with equal variances (*Levene*'s test, $p>0.05$), and were therefore tested for differences between green and red varieties of *O. triangularis* using one-way *ANOVA* (Sokal and Rohlf 1995), with a level of significance of $p<0.05$.

Results

CO_2 assimilation: Both green and red *O. triangularis* showed typical light responses, where the photosynthetic rate first increase linearly with irradiance, before leveling off and approaching saturation (Fig. 1). Initial slopes (α) of the light response curves were derived by curve fit together with values for maximum photosynthesis (P_{max}; Table 1). The green variety showed a higher apparent light affinity for CO_2 assimilation (α) and a higher P_{max} than the red variety. The light compensation point (I_c), found as the x-axis intercept of the net photosynthesis curve (not shown), was 53% lower for the green variety than for the red, corresponding to the higher initial slope of the green variety (Table 1), and the saturating irradiance (I_{sat}), found as the intercept between a line with a slope equal to α and a line through P_{max}, was also 40% lower for the green variety than for the red (Table 1).

Chl fluorometry: Light-response curves were generated from ETR data. The curves showed similarity to the light response curves of CO_2 assimilation with increasing ETR with increasing irradiance until saturation is reached (Fig. 2; Table 2). Unlike what is found for CO_2

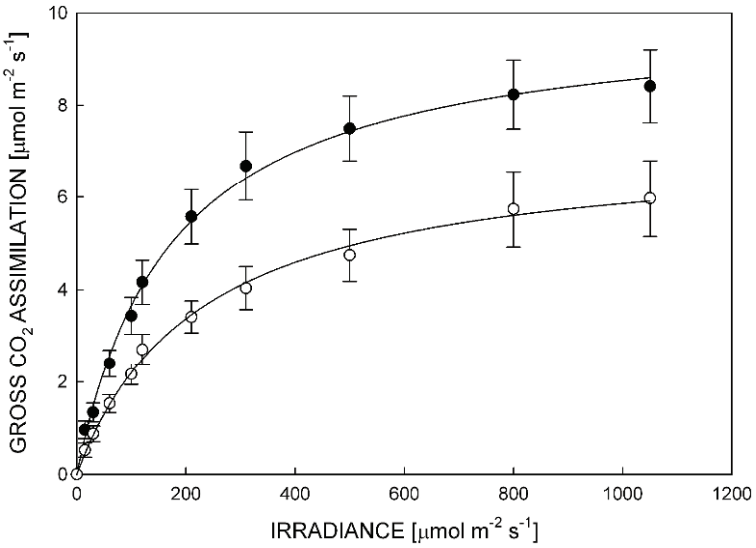

Fig. 1. Light-response curves for *Oxalis triangularis* CO_2 assimilation. ● – green variety; ○ – red variety (ssp. *atropurpurea*). Values are shown as means ± 1 SD; curves are curve fits according to Baly (1935). Parameters derived from the curve fits are given in Table 1 and discussed in the text.

Table 1. Photosynthetic parameters of *Oxalis triangularis* relating to gross CO_2 assimilation as measured by infrared gas analysis. The parameters are derived from curve fits according to Baly (1935), unless otherwise stated. The following parameters are given: α – initial slope of the light response curve; P_{max} – maximum photosynthetic rate; I_c – light compensation point, derived by linear regression on net photosynthetic rates at low light intensities; I_{sat} – saturating irradiance, derived from α and P_{max} values. Values for α and P_{max} are given as mean ± 95 % CL to allow direct comparisons. In addition the R^2 values from the curve fits are given. n = 10 in all cases.

Parameter	*O. triangularis* (green)	*O. triangularis* ssp. *atropurpurea* (red)
α [μmol(CO_2) μmol^{-1}(photon)]	0.056 ± 0.005	0.031 ± 0.003
P_{max} [μmol(CO_2) m^{-2} s^{-1}]	10.070 ± 0.803	7.240 ± 0.584
I_c [μmol m^{-2} s^{-1}]	23.153	49.170
I_{sat} [μmol m^{-2} s^{-1}]	179.8	233.5
R^2	0.955	0.964

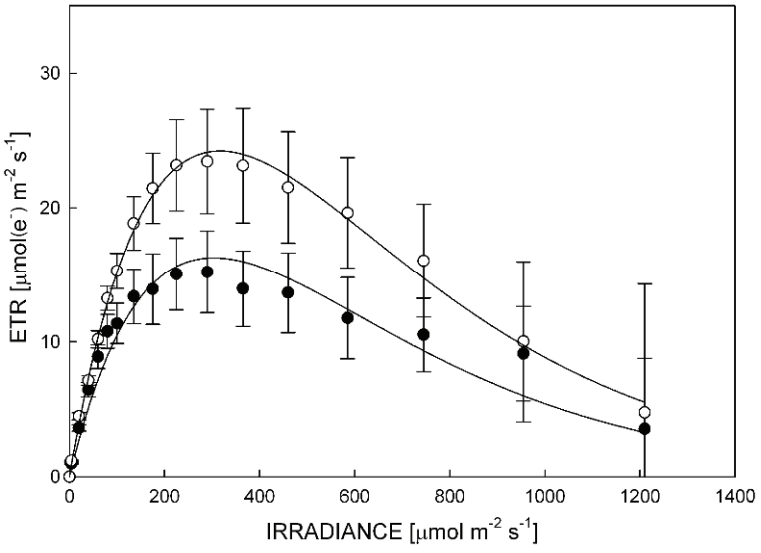

Fig. 2. Light-response curves for *Oxalis triangularis* electron transport rates (ETR). ● – green variety; ○ – red variety (ssp. *atropurpurea*). Values are shown as means ± 1 SD; curves curve fits according to Steele (1962). Parameters derived from the curve fits are given in Table 2 and discussed in the text.

assimilation, the red variety showed a significantly higher apparent light affinity for electron transport (initial PSII quantum yield) and a higher maximum rate of electron transport than the green variety. As above, the I_{sat} was found as the intercept between a line with a slope equal to α, and a line through ETR_{max}. The value for the green variety was found to be 310.1, 6 % lower than for the red variety (331.4; Table 2). The F_v/F_m was significantly

Table 2. Photosynthetic parameters relating to electron transport rates as measured by PAM fluorometry of *Oxalis triangularis*. The parameters are derived from curvefits according to Steele (1962), unless otherwise stated. The following parameters are given: α – initial slope of the light response curve; ETR_{max} – maximum electron transport rate; I_{sat} – saturating irradiance, derived from α and ETR_{max} values; F_v/F_m – calculated as $(F_m–F_0)/F_m$ values from the PAM measurements (Genty *et al.* 1989). Values for α and ETR_{max} are given as mean ± 95 % CL to allow direct comparisons. Values for F_v/F_m are given as mean ± 1SD. n = 10 in all cases. In addition the R^2 values from the curve fits are given.

Parameter	*O. triangularis* (green)	*O. triangularis* ssp. *atropurpurea* (red)
α [μmol(e^-) μmol^{-1}(photon)]	0.143 ± 0.011	0.200 ± 0.015
ETR_{max} [μmol(e^-) m^{-2} s^{-1}]	44.340 ± 1.923	66.270 ± 2.646
I_{sat} [μmol m^{-2} s^{-1}]	310.1	331.4
F_v/F_m	0.726 ± 0.055	0.769 ± 0.030
R^2	0.831	0.878

Table 3. Content of plant pigments and activity of antioxidant enzymes in the two different varieties of *Oxalis triangularis* used in this study. Values are given as mean ± 1 SD. n = 10 in all cases. The level of significance for the difference among the two varieties is given. DM – dry mass; Chl – chlorophyll; N.S. – $p>0.05$, * – $0.05>p\geq0.01$, ** – $0.01>p\geq0.001$, *** – $p<0.001$. [1] – below detection limits.

	O. triangularis (green)	*O. triangularis* ssp. *atropurpurea* (red)	Significance level
Chl *a* [mg g^{-1}(DM)]	1.95 ± 0.44	2.45 ± 0.69	N.S.
Chl *b* [mg g^{-1}(DM)]	0.47 ± 0.91	1.21 ± 0.86	N.S.
Chl *a*/*b*	4.15	2.02	
Xanthophyll cycle components (V+A+Z) [mg g^{-1}(DM)]	0.76 ± 0.09	0.63 ± 0.11	*
Total anthocyanins [mg g^{-1}(DM)]	≈ 0[1]	20.11 ± 6.73	***
Superoxide dismutase [nkat g^{-1}(DM)]	166.0 ± 63.0	226.7 ± 72.3	N.S.
Ascorbate peroxidase [nkat g^{-1}(DM)]	10.0 ± 4.8	41.2 ± 13.8	***

higher for the red variety of *O. triangularis*, than for the green variety (Table 2, one-way *ANOVA*, $p<0.0001$). The ETR curves show an apparent decline in ETR at high light intensities which may seem surprising since a similar trend is not observed in the CO_2 assimilation curves. However, this is probably a technical flaw related to the *Imaging PAM* used, since the saturating pulse of 2,400 μmol m^{-2} s^{-1} is probably not strong enough to achieve saturation at high intesities of actinic light (Nielsen and Nielsen 2008). ETR values at high light intensities are therefore not considered further, but values measured at lower light intensities are considered reliable.

Pigment content: Not surprisingly, the anthocyanin content was highly different between the two varieties of *O. triangularis* (*Mann-Whitney U*, $p<0.0001$; Table 3). The green plants contained very little if any anthocyanin, the mean value was not significantly different from 0, so anthocyanin content in green *O. triangularis* must be said to below detection limits (Table 3). Both Chl *a* and *b* content showed nonsignificant trends towards higher values in leaves of the red variety (Table 3; *Mann-Whitney U*, $p>0.05$ in both cases), but as this trend, although nonsignificant, was stronger for Chl *b* than for Chl *a*, the Chl *a*/*b* ratio in the leaves of the green variety was more than twice as high as in the leaves of the red variety. The green variety contained significantly higher concentrations of xanthophyll cycle components than the red variety (Table 3; *Mann-Whitney U*, $0.05>p>0.01$).

Scavenging enzymes: The SOD activity was not significantly different between the differently coloured varieties (Table 3, *Mann-Whitney U*, $p>0.05$), but the red variety had more than four times higher APX activity than the green variety (Table 3; *Mann-Whitney U*, $p<0.001$).

Discussion

Plants of the red variety had, not surprisingly, given their colour, significantly higher anthocyanin contents than plants of the green variety. Red plants also had significantly lower contents of xanthophyll cycle components than green plants. This is in agreement with what has been found in other plants that have both green and red forms such as *Quercus coccifera* (Manetas *et al.* 2003), in plants that change colour during the year (Zeliou *et al.* 2009), as well as in plants that have young red leaves, gradually turning green as they mature

(Gamon and Surfus 1999). In the latter case, gradual changes in anthocyanin and xanthophyll cycle components have been observed, so that anthocyanin content decreased and xanthophyll cycle components increased during leaf development (Gamon and Surfus 1999).

Previous observations indicate that young red leaves are less susceptible to photoinhibition than older green leaves, although the young red leaves have lower contents of xanthophyll cycle components (Manetas *et al.* 2002). This suggests that the presence of anthocyanins decreases the need for energy dissipation by the xanthophyll cycle, or conversely, provide photoprotection in situations where xanthophyll production may be suppressed. Red leaves may thus compensate for an inferior photoprotective capability due to xanthophyll-deficiency by achieving an equivalent photoprotection due to anthocyanins, although red leaves do not have the photosynthetic capabilities of green leaves (Manetas *et al.* 2003, Zeliou *et al.* 2009).

Our results show a nonsignificant tendency to higher Chl content in red plants. This is in contrast to previous findings that indicated that Chl content is inversely related to anthocyanin content in young developing leaves as well as in mature leaves (Manetas *et al.* 2002, Zeliou *et al.* 2009). The tendency to higher Chl content in the leaves of the red variety of *O. triangularis*, could, together with the lower Chl *a*/*b*-ratio in red leaves, suggest that anthocyanins shade the chloroplasts from irradiance, since shade leaves usually display a higher Chl concentration than sun leaves (Björkman 1981), as well as a lower Chl *a*/*b* ratio (Anderson 1986).

In agreement with previous findings (Neill *et al.* 2002) we found a significantly higher activity of ascorbate peroxidase in red leaves compared to green leaves in this study, as well as a nonsignificant tendency for a higher activity of superoxide dismutase in red leaves. It is possible that the high APX activity found in the leaves of the red variety, compared to the green variety, is a result of APX in green leaves being degraded as a result of the high formation of ROS in these leaves, compared to the red leaves with their higher content of photoprotective anthocyanins (Hegedüs *et al.* 2001).

Photosynthetic rates of *O. triangularis* were measured both as CO_2 assimilation and as Chl fluorescence. Curvefit analysis of CO_2 assimilation data showed that green plants had a higher initial slope of the photosynthesis curve and a higher P_{max} than red plants, while red plants had the highest I_{sat} and I_c. However, direct comparisons of CO_2 assimilation curves of green and red leaves are difficult since they are based on incident rather than absorbed light and anthocyanins in the red leaves absorb part of the incident light. The lower CO_2 assimilation in red leaves under limiting light is probably due to the shading effect of anthocyanins, while the lower apparent P_{max} of red leaves can be due to a shade acclimation consistent with the lower Chl *a*/*b* ratio and tendency towards higher total Chl content in red leaves, or it may be due to a lower leaf nitrogen content as it is sometimes found in anthocyaninc leaves (Steyn *et al.* 2002), but this parameter has not been measured in this study. In contrast, red plants had the highest initial slope, the highest ETR_{max} and the highest I_{sat} when measuring photosynthesis as Chl fluorescence. As above, the interpretation of these results are complicated by the ETR – curves being based on incident, rather than absorbed light. However, the higher ETR of red leaves under limiting light is again consistent with a shading effect of anthocyanins, resulting in a higher apparent PSII effective quantum yield. The higher ETR_{max} in red leaves that have a lower P_{max}, measured as CO_2 assimilation, is more difficult to explain, but could indicate a higher engagement of alternative electron sinks in red leaves (Miyake 2010), however, in the context of this study this remains speculative. Previous comparisons of photosynthesis in green *vs.* red leaves of the same plant species are partly conflicting. Lower photosynthetic capacity in red relative to green forms has been found in some species (Gould *et al.* 2002b), while other studies show a higher photosynthetic capacity in red forms, when measured as CO_2 assimilation (Gould *et al.* 1995, Liakopoulos *et al.* 2006). Overall, our findings suggest that anthocyanin acts as a kind of sunscreen, shading chloroplasts from incident light and protecting them from the adverse effects of high light irradiances (Timmins *et al.* 2002). Due to high anthocyanin contents, less light reaches the photosystems in the red plants and the CO_2 assimilation rates are accordingly reduced. Since less light reaches the photosystems in red leaves, the photosynthesis is more efficient as more of the incoming light is used in photosynthesis, expressed as a higher apparent quantum yield of PSII. Red leaves showed a strong, statistically significant, tendency to lower photoinhibition than green plants, expressed as F_v/F_m. The lower degree of photoinhibition may be attributed to anthocyanins, similarly to what has been observed in other studies (Li *et al.* 2008). The shading effect of anthocyanins is also consistent with the lower Chl *a*/*b* ratio in red leaves. The lower photoinhibition in the red variety is observed despite the lower content of xanthophyll cycle components in these plants, indicating that anthocyanins to some extent fill the photoprotective role played by the xanthophyll cycle in nonanthocyanic leaves.

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