Acta Physiol Plant (2008) 30:1–9
DOI 10.1007/s11738-007-0070-1

ORIGINAL PAPER

Changes in activities of antioxidative system and monoterpene and photochemical efficiency during seasonal leaf senescence in *Hevea brasiliensis* trees

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Received: 30 December 2006 / Revised: 26 March 2007 / Accepted: 26 March 2007 / Published online: 12 June 2007

Abstract A variety of ecophysiological parameters were monitored in leaves of *Hevea brasiliensis* (rubber tree) during seasonal leaf senescence. Higher levels of hydrogen peroxide and malondialdehyde, and lower content of total protein and efficiency of photochemistry of photosystem II (PSII) were observed in the senescent leaves (SL) compared to the mature leaves (ML). A significant decrease in the contents of chlorophyll (Chl) and carotenoids (Car) in SL was also observed, but with increase in ratio of Car/Chl. Moreover, activities of superoxide dismutases, catalase, and glutathione reductase in SL were strongly suppressed. In contrast, the activities of guaiacol peroxidase (POD) and ascorbate peroxidase (APX), and the contents of reduced ascorbate, total ascorbate, reduced glutathione and total glutathione were considerably increased in SL compared to ML. In addition, α-pinene, β-pinene, sabinene and total monoterpene pool in SL were drastically decreased. Taken together, these results indicate that the enhanced activities of POD and APX, and further activation of ascorbate-glutathione cycle conferred an important photoprotection against oxidative stress in senescent leaves of rubber trees. The increased Car/Chl could give the protection against photoxidation as well.

Keywords Antioxidative enzymes · Antioxidative metabolites · Chlorophyll fluorescence · Isoprenoids · Photoprotection · Reactive oxygen species (ROS) · Leaf senescence

Abbreviations

H_2O_2	Hydrogen peroxide
MDA	Malondialdehyde
Chl	Chlorophyll
Car	Carotenoids
PSII	Photosystem II
SOD	Superoxide dismutase (EC 1.15.1.1)
CAT	Catalase (EC 1.11.1.6)
GR	Glutathione reductase (EC 1.6.4.2)
POD	Guaiacol peroxidase (EC 1.11.1.7)
APX	Ascorbate peroxidase (EC 1.11.1.11)
AsA	Reduced ascorbate
DHA	Oxidized ascorbate
GSH	Reduced glutathione
GSSG	Oxidized glutathione
F_v/F_m	Maximum photochemical efficiency of PSII
$\Delta F/F_m'$	Actual photochemical efficiency of PSII
ROS	Reactive oxygen species
TCA	Trichloroacetic acid
TBA	Thiobarbituric acid

Communicated by M. Filek.

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Introduction

The senescing process of leaves is characterized with dramatic exacerbation in lipid peroxidation, degradation of chlorophyll (Chl), proteins and other macromolecules, conversion of peroxisomes into gloxysomes, and a marked increase in production of reactive oxygen species (ROS;

Gan and Amasino 1997; Pastori and del Río 1997; Corpas et al. 2001; Procházková et al. 2001; Buchanan-Wollaston 1997). Leaf senescence can be, therefore, regarded as a process of oxidative stress due to the overproduction of ROS, such as hydrogen peroxide (H_2O_2), superoxide radicals ($O_2^{\cdot-}$), hydroxyl radicals (·OH) and singlet oxygen ($^1O_2$; Piquery et al. 2000; Scebba et al. 2004). To counteract the injurious effects of ROS, plants are equipped with antioxidative systems composed of enzymes such as superoxide dismutase (SOD), guaiacol peroxidase (POD) and catalase (CAT) as well as metabolites such as ascorbate, glutathione, tocopherol, and carotenoids (Alscher et al. 1997; Noctor and Foyer 1998). Some previous studies have reported both the decrease (Dhindsa et al. 1981; Hurng and Kao 1994) and increase (Bueno and del Rio 1992; Procházková et al. 2001) in the activities of various antioxidative enzymes during leaf senescence. On the other hand, a substantial decrease in all the components of the mitochondrial ascorbate-glutathione cycle has also been observed in senescing leaves of pea (Pastori and del Río 1997).

Although the physiological functions of volatile isoprenoids have not yet clearly been established, it has been shown that leaves producing isoprene and specific monoterpene (e.g. α- and β-pinene) withstand higher oxidative stress than those in which isoprene or monoterpene production is inhibited (Sharkey and Singsaas 1995; Peñuelas and Munné-Bosch 2005; Sharkey and Yeh 2001; Loreto et al. 2004). Therefore, volatile isoprenoids have been suggested to be involved in scavenging ROS and potentially protecting plants against photo-oxidative stress (Zeidler et al. 1997; Loreto et al. 2001; Loreto and Velikova 2001). Correspondingly, non-volatile isoprenoids, mostly carotenoids, are believed to confer photoprotection during dismounting of photosynthetic machinery in senescing leaves (Merzlyak and Solovchenko 2002). The volatile isoprenoids productions are exclusively dependent on photosynthesis (Sharkey and Yeh 2001) and share a common biochemical production pathway and localization with nonvolatile isoprenoids (carotenoids; Logan et al. 2002; Affeck and Yakir 2002). However, little information is available on the actual roles of volatile isoprenoids in scavenging ROS and protecting photosynthesis in relation to progression of leaf senescence.

It has been recently found that the leaves of *Hevea brasiliensis* (rubber tree) produce and emit monoterpene (Klinger et al. 2002). The rubber tree is widely cultivated in tropical regions for economical purpose, including the marginal tropical areas of China. However, it is an evergreen tree in its native habitats, but deciduous in the marginal tropical areas of China, such as in Xishuangbanna of southern Yunnan where the daily temperature in winter is about 6–8°C lower than in the summer. The change in leaf-habit of the rubber tree in these marginal tropical areas might be caused by chilly temperature. During this chilly period, although no visible injury to leaves of rubber trees has been observed, the low temperature could have adversely affected their physiology and hence ultimately result in senescence and defoliation.

The aim of the present study was to clarify the relationship between oxidative stress and leaf senescence, and antioxidative activities during leaf senescence in rubber trees in a marginal tropical area of China. For this purpose, the components of antioxidative metabolites of ascorbate, glutathione, nonvolatile isoprenoids (carotenoids) and volatile isoprenoids (monoterpenes), and the activities of antioxidative enzymes: SOD, POD, and CAT, as well as stress makers such as the contents of pigments, protein and malondialdehyde (MDA), and the photochemical efficiency of PSII were measured in leaves of rubber trees in relation to the progress of leaf senescence in response to chilly season.

Materials and methods

Study site and leaf sampling

This study was conducted in a rubber tree plantation stand at the Xishuangbanna Tropical Botanical Garden (21°41′N, 101°25′E, and 570 masl), Chinese Academy of Sciences, SW China. Mean annual air temperature is about 21.7°C and annual precipitation is about 1,560 mm. There is a rainy season from May to October, and a well-defined dry season from November to April.

Rubber tree is an evergreen tree in its native habitats but deciduous in the present region. Its old leaves defoliate in early March and the new leaves emerge about 2 weeks later. Uppermost canopy leaves of rubber trees by an iron tower within the rubber-tree plantation were sampled on clear midday on 10–11 November and 15–16 December 2005, 11–12 January, 7–8 February and 19–20 February 2006, respectively. The leaf samples were immediately submerged in liquid nitrogen and then taken to laboratory for assays.

Chl fluorescence

The midday maximum (F_v/F_m) and actual photochemical efficiency [$\Delta F/F_m' = (F_m'-F_t)/F_m'$] of photosystem II (PSII) were detected from the canopy leaves of the rubber trees under natural light with a portable fluorescence system (FMS-2.02, Hansatech, King's Lynn, U.K.). F_v/F_m was measured from the leaves after 20 min dark adaptation. There are not significant differences in maximum photosynthetic photo flux density values during study period

(data not shown), thus, it is possible to compare changes in $\Delta F/F_m'$ during leaf senescence.

Determination of contents of photosynthetic pigments, protein and H_2O_2

Contents of chlorophyll (Chl) and carotenoids (Car) of the leaves were measured according to Lichtenthaler and Wellburn (1983). Total content of foliar protein was measured according to Lowry et al. (1951) using bovine serum albumin as a standard. The level of H_2O_2 was determined according to Velikova et al. (2000).

Determination of MDA content

The content of MDA was analyzed by the method described by Hodges et al. (1999) with slight modification, for taking into account the possible influence of interfering compounds in the assay for thiobarbituric acid (TBA)-reactive substances. Leaf tissues were repeatedly extracted with 4 ml 5% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000×*g* for 15 min and an aliquot of appropriately diluted sample was added to a test tube with an equal volume of either: (1) –TBA solution containing 20% (w/v) TCA and 0.01% butylated hydroxytoluene (BHT); or (2) +TBA solution containing the above solution plus 0.65% (w/v) TBA. Samples were heated at 95°C for 25 min, then after cooling, the absorbance was read at 440, 532 and 600 nm. MDA equivalents were calculated as $10^6 \times ((A - B)/157{,}000)$, where $A = [(\text{Abs } 532_{+TBA}) - (\text{Abs } 600_{+TBA}) - (\text{Abs } 532_{-TBA} - \text{Abs } 600_{-TBA})]$, and $B = [(\text{Abs } 440_{+TBA} - \text{Abs } 600_{+TBA}) \times 0.0571]$.

Analysis of enzyme activity

Leaf fresh material was homogenized with a mortar and pestle in 4 ml ice-cold 0.2 M phosphate buffer (pH 7.8) containing 0.1 mM EDTA, 0.3% (w/v) Triton X-100, 1% (w/v) PVP, and 10 mM dithiothreitol. The homogenate was centrifuged at 15,000×*g* for 15 min and the supernatant was used for assays of enzyme activity and protein content. All the assay steps were carried out at 0 ± 4°C. CAT (EC 1.11.1.6) activity was measured in the presence of 10 mM H_2O_2 by monitoring the decrease in absorbance at 240 nm in phosphate buffer (pH 7.0), and expressed as $\Delta A_{240}\ min^{-1}\ mg^{-1}$ protein. APX (EC 1.11.1.11) activity was measured in the presence of 0.5 mM ascorbic acid and 1.0 mM H_2O_2 by monitoring the decrease in absorbance at 290 nm in phosphate buffer (pH 7.0), and expressed as $\Delta A_{290}\ min^{-1}\ mg^{-1}$ protein. GR (EC 1.6.4.2) activity was measured in the presence of 0.5 mM GSSG and 0.15 mM NADPH by monitoring the decrease in absorbance at 340 nm in phosphate buffer (pH 7.0), and expressed as $\Delta A_{340}\ min^{-1}\ mg^{-1}$ protein. POD (EC 1.11.1.7) activity was measured in the presence of 16 mM guaiacol and 10 mM H_2O_2 by monitoring the increase in absorbance at 470 nm in phosphate buffer (pH 7.0), and expressed as $\Delta A_{470}\ min^{-1}\ mg^{-1}$ protein. SOD (EC 1.15.1.1) activity was measured by the photochemical method as described by Giannopolitis and Ries (1977), and one unit of SOD activity was defined as the amount of enzyme which produced a 50% inhibition of nitroblue tetrazolium reduction at 560 nm.

Determination of antioxidative metabolites

Fresh leaf material was homogenized in an ice bath with 4 ml 5% (w/v) TCA. The homogenate was centrifuged at 15,000×*g* for 15 min and the supernatant was used for assays of contents of ascorbate and glutathione. The content of reduced ascorbate (AsA) was analyzed according to the methods described by Arakawa et al. (1981), which were based on the reduction of ferric ion to ferrous ion with AsA in acid solution, followed by formation of the red chelate between ferrous ion and bathophenanthroline, which absorb at 534 nm. After the reduction of oxidized ascorbate (DHA) to AsA by dithiothreitol, the total AsA content was measured as described above, DHA content was determined by subtraction of AsA from the total AsA content. The contents of reduced and oxidized forms of glutathione were measured by the method described by Doulis et al. (1997) via the increase in absorbance at 412 nm following addition of GR for determination of reduced glutathione (GSH) or GR and NADPH for determination of oxidized glutathione (GSSG) to a solution containing extract and 5,5′-Dithiobis (2-nitrobenzoic acid).

Analysis of monoterpene content

Leaf fresh material was submerged in liquid nitrogen and then the sample was homogenized in ice-cold pentane under liquid nitrogen. A non-terpenoid volatile internal standard, dodecane was used to avoid interference of terpenes. It was added to the pentane extraction procedure before grinding in order to quantify the recovery. Detailed assays of monoterpene concentration were conducted as described by Llusià and Peñuelas (2000).

Statistical analysis

Statistical analysis was performed with software SPSS (Chicago, IL, USA) using one-way ANOVAs (LSD method) to evaluate differences in assayed parameters among different months.

Results

During the study period from August 2005 to February 2006, the mean maximum monthly temperature (MAT) remained relatively stable from August to October, and then reached lowest value of 23.9°C in December (Fig. 1). Mean monthly temperature (MMT) was continuously decreased from August to December, and reached lowest value of 16.7°C in December. Thereafter, a moderate increase in MAT and a slight increase in MMT were observed. However, mean minimum monthly temperature attained lowest value of 13.1°C in January, then with a slight increase in February. The monthly rainfall remained relatively stable from September to December, and then reached lowest value of 4 mm. However, the monthly rainfall was increased to 16.6 mm in February.

The contents of photosynthetic pigments varied slightly in the leaves of the rubber trees from November 2005 to January 2006, thereafter, about 80% decrease in Chl ($P < 0.001$), 35–50% decrease in Car ($P < 0.05$), and 18% decrease in Chl*a*/Chl*b* ($P < 0.05$) as well as threefold increase in Car/Chl ($P < 0.001$) were observed in the leaves sampled on 19–20 February 2006 relative to in leaves from November 2005 to January 2006, respectively (Fig. 2). Similarly, the content of total protein increased from November 2005 to January 2006, afterwards sharply decreased, reaching the values approximately 60–70% lower in February 2006 than those from November 2005 to January 2006 ($P < 0.001$; Fig. 3a). Based on loss of photosynthetic pigments and total proteins, the following stages of leaf development of the present rubber trees were defined: mature leaves (ML; November 2005 until January 2006), and senescent leaves (SL; 19–20 February 2006).

The contents of H_2O_2 and MDA in the leaves of rubber trees were increased as leaf senescence progressed. H_2O_2 content of SL was about 20% higher than that of ML ($P < 0.05$; Fig. 3b), and approximately 2–5-folds increase in MDA content was observed in SL ($P < 0.001$; Fig. 3c). On the other hand, maximum photochemical efficiency of PSII (F_v/F_m) and actual photochemical efficiency of PSII ($\Delta F/F_m'$) at midday were decreased by about 20 and 30% in SL versus ML ($P < 0.001$), respectively (Fig. 4). In addition, the content of MDA was correlated negatively with the content of total protein ($r^2 = 0.48$, $P < 0.001$, Fig. 5a) and F_v/F_m ($r^2 = 0.47$, $P < 0.001$, Fig. 5b). Conversely, F_v/F_m was related positively with total protein ($r^2 = 0.62$, $P < 0.001$; Fig. 5c).

Activities of SOD, CAT, and GR were considerably lower in SL than in ML, decreasing by about 71, 54, 58% ($P < 0.001$), respectively (Fig. 6a–c). In contrast, activities of POD and APX were increased by 60–96% and 1.41–1.96-fold in SL ($P < 0.001$), respectively (Fig. 6d, e). The content of reduced ascorbate was increased by 43–69% ($P < 0.01$), and the content of total ascorbate was increased by 32–71% ($P < 0.05$) in SL when compared to ML (Fig. 7a). DHA content and ratio of AsA to total ascorbate (DHA + AsA) were increased slightly during the study periods (data not shown). On the other hand, the contents of reduced glutathione and total glutathione were increased by 7.2–35.3% and 10–22% in SL, respectively (Fig. 7b). However, there were no considerable differences in content of GSSG and ratio of GSH to total glutathione (GSH + GSSG) between SL and ML (data not shown). In addition, there were decreases of about 35% in ratio of H_2O_2 to AsA in SL versus ML ($P < 0.05$; Fig. 7c).

The contents of volatile isoprenoids were drastically decreased during leaf senescence, approximately 98% decrease of α-pinene ($P < 0.001$), 94% decrease of β-pinene ($P < 0.001$), and 80% decrease of sabinene ($P < 0.001$) as well as 94% decrease of total monoterpene pool ($P < 0.001$) were observed in SL versus ML (Fig. 8). These results suggested that leaf senescence strongly affected the biosynthesis and accumulation of volatile isoprenoids.

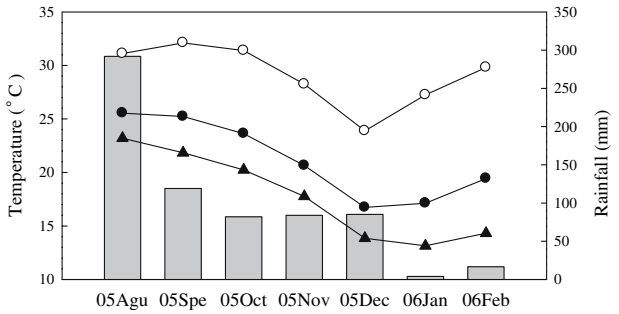

Fig. 1 The monthly rainfall (*bars*), mean maximum monthly temperature (*open circle*), mean monthly temperature (*filled circle*) and mean minimum monthly temperature (*filled triangle*) during the experimental period from August 2005 to February 2006, recorded by the Menglun Meteorological Station of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences

Discussion

In the present experimental period, although the monthly rainfall was decreased to 4 mm and minimum monthly temperature reached the lowest values of 13.1°C recorded in January (Fig. 1), rubber tree would not suffer from drought because its deep taproot could absorb deep underground water. However, tropical tree species such as rubber trees, coffee, and mango are most vulnerable to low temperature. Therefore, it could be speculated that low temperature could be the main factor triggering rubber tree leaves senescence. Indeed, an easily observed event during leaf senescence is the loss of chlorophyll. In the present

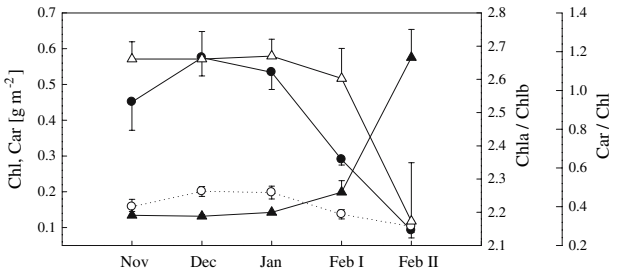

Fig. 2 The contents of chlorophyll (Chl, *filled circle*) and carotenoids (Car, *open circle*) levels as well as Chla/Chlb (*open triangle*) and Car/Chl (*filled triangle*) in leaves of *Hevea brasiliensis* trees from November 2005 to February 2006. Data are the mean ± SE (n = 6)

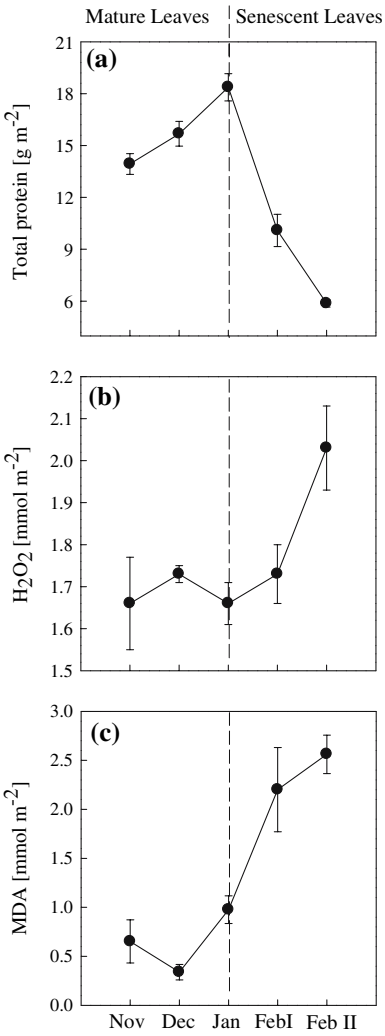

Fig. 3 The contents of total protein (**a**), hydrogen peroxide (H_2O_2, **b**), and malondialdehyde (MDA, **c**) in leaves of *Hevea brasiliensis* trees from November 2005 to February 2006. Data are the mean ± SE (n = 6)

study, the contents of Chl, Car, and total protein were significantly lower in SL than in ML (Figs. 2, 3a). This further supports the idea that leaf senescence involves the

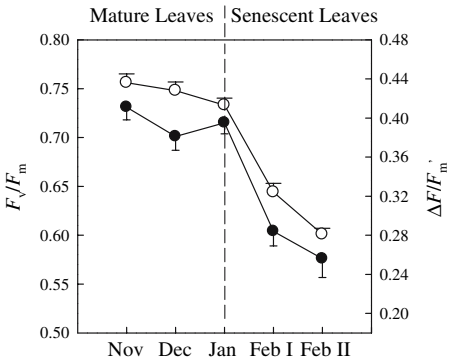

Fig. 4 The variations in midday maximum (F_v/F_m, *filled circle*) and actual ($\Delta F/F_m' = (F_m' - F_t)/F_m'$, *open circle*) photochemistry efficiency of PSII in leaves of *Hevea brasiliensis* trees from November 2005 to February 2006. Data are the mean ± SE (n = 6)

degradation of protein (Lutts et al. 1996), nucleic acids (Buchanan-Wollaston 1997), and membranes (Trippi and Thimann 1983), as well as the loss of chlorophyll (Smart 1994). In contrast, the contents of H_2O_2 and MDA (indicative of oxidative lipid metabolism) in SL were significantly higher than those in ML (Fig. 3b, c). This was consistent with results of the increase in contents of H_2O_2 and MDA during leaf senescence reported by other researchers (Dhindsa et al. 1981; Hurng and Kao 1994; Ye et al. 2000; Marie 1995). The simultaneous increase in contents of MDA and H_2O_2 reveals that elevated oxidative stress during leaf senescence results in increased lipid degradation or lipid peroxidation.

Increased oxidative stress during leaf senescence was accompanied with reduction of photochemical efficiency of PSII (Fig. 4). Midday F_v/F_m and $\Delta F/F_m'$ strongly decreased in SL versus ML (Fig. 4), indicating the down-regulation or even irreversible damage of PSII reaction centers (Someralo and Krause 1989). Furthermore, the content of MDA was negatively correlated with F_v/F_m (Fig. 5b), it was consistent with the report of Mishra and Singhal (1992) on the MDA accumulation with the decline in F_v/F_m and $\Delta F/F_m'$. The lipids are utilized in maintenance of protein conformations, which are required for optimal electron transport. However, the lipid peroxidation might result in dysfunction of the proteins, and thus could slow down PSII electron transport and decline in the photochemical efficiency. The positive correlation of total protein with F_v/F_m (Fig. 5c) and the negative correlation of MDA with total protein (Fig. 5a) support this idea as stated above.

Low levels of ROS especially H_2O_2 are known to act as signal molecules initiating several protective mechanisms against oxidative stress (Desikin et al. 2001; Knight and Knight 2001). However, excessive ROS load as the case in the present senescent leaves (Fig. 3b) can cause unrecoverable membrane damage (Rao et al. 1997). Therefore,

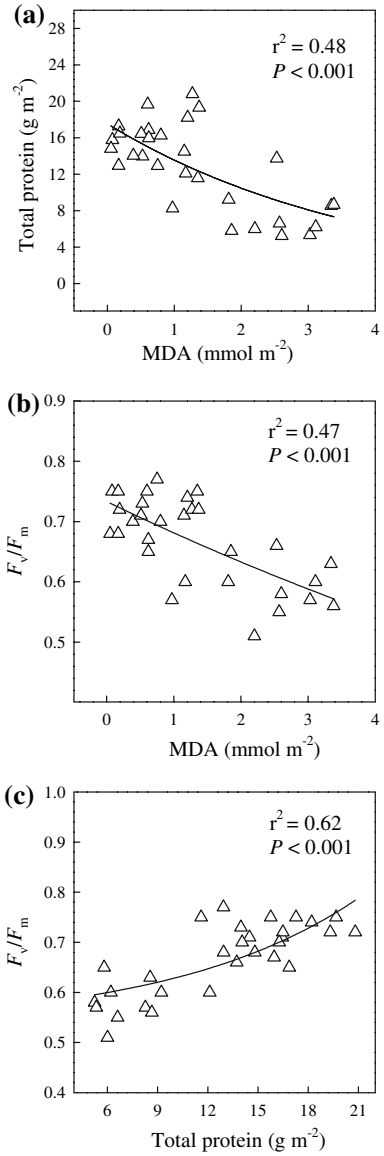

Fig. 5 The correlation of malondialdehyde (MDA) with total protein (**a**), maximum photochemical efficiency of PSII (F_v/F_m, **b**) and the correlation of total protein with F_v/F_m (**c**) (n = 30)

under this circumstance, plants must activate mechanism of antioxidative protection to withstand oxidative stress. In the present study, a significant decrease in SOD activity in SL was observed, with increased levels of H_2O_2, this was possibly caused by a significant decrease in CAT activity (Figs. 3b, 6a, b). It further supports the notion that ROS accumulations during leaf senescence were generally attributed to a decrease in antioxidative activities (Droillard et al. 1989; Pastori and Trippi 1993). Besides CAT, POD and APX also play important roles in scavenging of H_2O_2. In the present study, their activities were higher in SL than in ML (Fig. 6d, e). This supports the hypothesis that activation of POD and APX occurs under higher H_2O_2

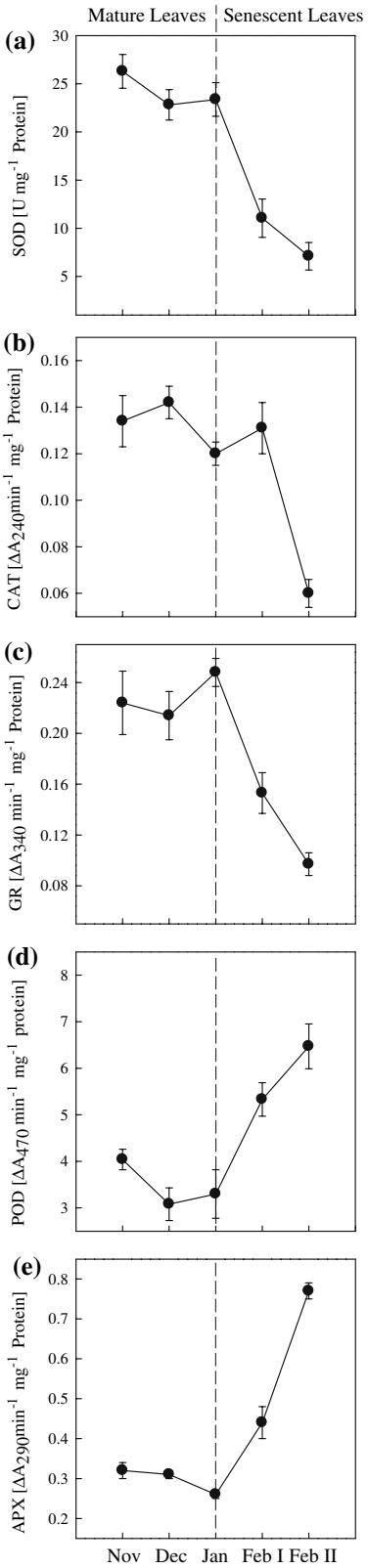

Fig. 6 The changes in activities of antioxidative enzymes of superoxide dismutases (SOD, **a**), catalase (CAT, **b**), glutathione reductase (GR, **c**), guaiacol peroxidase (POD, **d**), and ascorbate peroxidase (APX, **e**) in leaves of *Hevea brasiliensis* trees from November 2005 to February 2006. Data are the mean ± SE (n = 6)

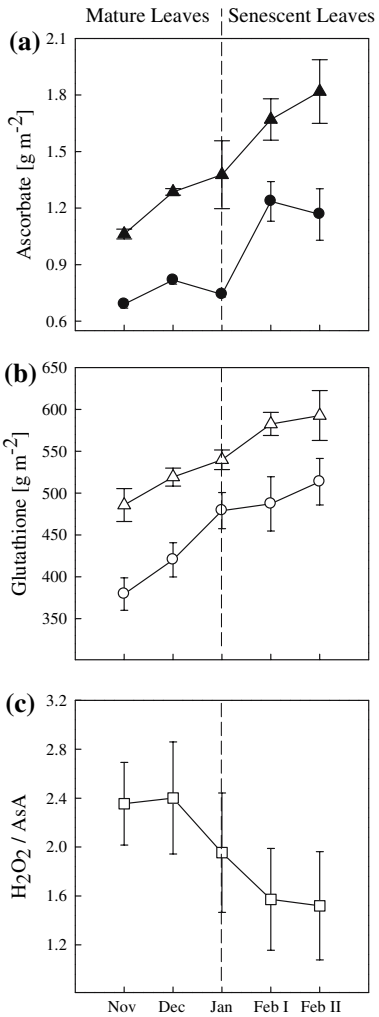

Fig. 7 The contents of reduced ascorbate (AsA, *filled circle*), total ascorbate (*filled triangle*) (**a**), reduced glutathione (GSH, *open circle*) and total glutathione (*open triangle*) (**b**) as well as hydrogen peroxide/reduced ascorbate (H_2O_2/AsA, **c**) in leaves of *Hevea brasiliensis* trees from November 2005 to February 2006. Data are the mean ± SE (n = 6)

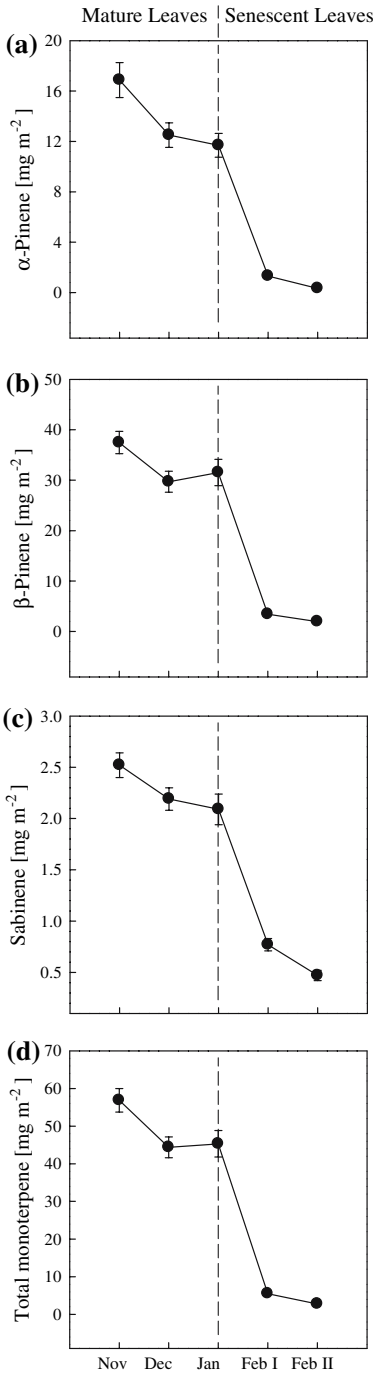

Fig. 8 The contents of α-pinene (**a**), β-pinene (**b**), sabinene (**c**), and total monoterpene (**d**) in leaves of *Hevea brasiliensis* trees from November 2005 to February 2006. Data are the mean ± SE (n = 6)

levels to prevent H_2O_2 reaching too high level (Kang et al. 2003a, b).

Under oxidative stress condition, it is believed that H_2O_2 generated in plant cells is mainly scavenged by the ascorbate–glutathione cycle (Noctor and Foyer 1998). In this cycle, APX reduces H_2O_2 to water using AsA as the electron donor; the resulting DHA recycled to AsA using GSH as the electron donor, and the GSSG is converted back to GSH by NAD(P)H-dependent GR (Foyer and Halliwell 1976). The contents of reduced ascorbate and glutathione were significant increased, together with a significant increase in APX activity in SL (Figs. 6e, 7a, b). This suggests that the capacity of antioxidative metabolites to scavenge H_2O_2 by the ascorbate–glutathione cycle is robust in SL. If so, however, it appears unreasonable that higher level of H_2O_2 accumulation was observed in SL relative to ML (Fig. 3b). It has been proposed that the ratio of H_2O_2 to AsA rather than the absolute H_2O_2 content is a better indicator of the redox balance in plant cells (Kingston-Smith et al. 1997). The lower H_2O_2/AsA ratio was observed in SL versus ML (Fig. 7c), indicating that, although SL undergo elevated oxidative stress as indicated

by H_2O_2 levels, they may potentially be able to accommodate higher level of H_2O_2 because some components of antioxidative systems such as POD, APX, and ascorbate–glutathione cycle as stated above are further activated at this stage.

Isoprenoids can also scavenge ROS and dissipate excess excitation energy, thus alleviating lipid peroxidation. In the present study, non-volatile isoprenoids, mostly carotenoids, was decreased with leaf senescence, but Car/Chl was significantly higher along with decreased level of Chl in SL (Fig. 2). This will lead the senescent leaves to reduce the amount of light energy harvested by the antenna complex, and thereby relatively enhance the capacities of Car to dissipate excess energy as heat, to scavenge ROS and to inhibit lipid peroxidation. On the other hand, it has been reported that volatile isoprenoids may serve as an antioxidative metabolites in leaves (Loreto et al. 1998, 2004; Peňuelas and Llusià 1999). In the present study, monoterpene concentrations (Fig. 8) as well as monoterpene/Chl (data not shown) was much lower in SL than in ML. The volatile isoprenoids biosynthesis is exclusively dependent on photosynthesis (Sharkey and Yeh 2001) and share a common biochemical synthesis pathway and localization with nonvolatile isoprenoids (carotenoids) (Logan et al. 2002; Affeck and Yakir 2002). Therefore, when photosynthetic pigments in SL were significantly degraded, volatile isoprenoids (α-pinene, β-pinene, and sabinene) biosynthesis and accumulation also significantly decreased.

In conclusion, the results obtained here have demonstrated that (1) the senescent leaves of rubber trees confronted to elevated oxidative stress, were able to further activate some components of antioxidative system such as POD, APX and ascorbate–glutathione cycle to withstand oxidative stress and thus possibly postpone the senescing process; (2) the biosynthesis of volatile isoprenoids (monoterpene) was suppressed in senescent leaves, but non-volatile isoprenoids (carotenoids) may in turn confer further photoprotection at this stage.

Acknowledgments This study was funded by the National Science Foundation of China (project no. 90302013). We are grateful to our colleagues Jiao-lin Zhang and Jun-jie Zhu for helpful comments on the manuscript.

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