DOI: 10.1007/s11099-015-0148-8 PHOTOSYNTHETICA 53 (4): 585-596, 2015

Girdling-induced *Alhagi sparsifolia* senescence and chlorophyll fluorescence changes

G.-L. TANG[*,**,***,****], X.-Y. LI[*,**,***,+], L.-S. LIN[*,**,***], F.-J. ZENG[*,**,***], and Z.-Y. GU[#]

State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China[*]
Cele National Station of Observation and Research for Desert-Grassland Ecosystems, Xinjiang 848300, China[**]
Key Laboratory of Biogeography and Bioresource in Arid Zone, Chinese Academy of Sciences, Urumqi 830011, China[***]
University of the Chinese Academy of Sciences, Beijing 100049, China[****]
Xinjiang Key Laboratory of Soil and Plant Ecological Processes, College of Grassland and Environmental Sciences, Xinjiang Agricultural University, Urumqi 830052, China[#]

Abstract

Senescence constitutes the final stage of a plant organ and tissue development and is a subject to gene control and strict regulation. By the late growing season, when *Alhagi sparsifolia* entered the natural senescence period, a girdling treatment was carried out on the phloem to increase the sugar content in leaves and to investigate carbohydrate-induced leaf senescence. After the semi-girdling and full-girdling treatment, organic matter could not leave leaves due to the destruction of sieve tubes. This led to constantly increasing sugar contents in leaves. Girdling was shown to greatly accelerate the senescence of plants. In girdled leaves, chlorophyll (Chl) *a*, Chl *b*, carotenoids (Car), and both ratios of Chl *a*/*b* and Chl/Car were significantly reduced. On the donor side of PSII, the oxygen-evolving complex was inhibited under high concentrations of carbohydrates, which was manifested as the emergence of the K phase in fluorescence kinetic curves. On the acceptor side of PSII, the high carbohydrate content also led to the disruption of electron transport and reduced light-use efficiency, which was manifested as a reduction in numerous fluorescence parameters. We believe that the emergence and development of plant senescence was not necessarily induced by the high content of carbohydrates, because even a decrease in the carbohydrate concentration could not stop the senescence process. Although the high content of carbohydrates in plants could induce plant senescence, this kind of senescence was likely a pathological process, including degradations of physiological functions.

Additional key words: carbon; nutrient cycling; photosynthetic apparatus; photosynthetic pigment; reactive oxygen; stress.

Introduction

Senescence constitutes a plant development process controlled and highly regulated by genes that rescue nutritive elements before cell death (Masclaux *et al*. 2000, Parrott *et al*. 2005, 2007). Since early senescence impedes plants from absorbing carbon dioxide, late senescence interferes with the reactivation of nutritive elements. Since the photosynthetic activity of tender leaves and reproductive success is affected (Wingler *et al*. 2006), the senescence process may be subject to rigorous regulation of genes. So far, at least six categories of genes have been identified as being associated with senescence (Thomas and Smart 1993, Smart 1994, Rajcan *et al*. 1999).

Received 20 August 2014, *accepted* 2 March 2015.
[+]Corresponding author; tel: +86 991 7885320, e-mail: lixy@ms.xjb.ac.cn
Abbreviations: Car – carotenoids; Chl – chlorophyll; CK – control; DM – dry mass; FG – full-girdling; FM – fresh mass; F_v/F_m – maximal quantum yield of PSII photochemistry; MDA – malondialdehyde; M_0 – approximated initial slope of the fluorescence transient; OEC – oxygen-evolving complex; PI_{abs} – performance index on absorption basis; P_N – net photosynthetic rate; Pro – proline; PQ – plastoquinone; Q_A – primary quinone acceptor of PSII; Q_B – secondary quinone acceptor of PSII; SE – standard error; SG – semi-girdling; S_m – normalized total complementary area above the O-J-I-P transient; ψ_0 – probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (at t = 0); φ_{E0} – quantum yield for electron transport (at t = 0).
Acknowledgments: We thank the anonymous reviewers for their invaluable comments. We also express gratitude to Zhuyu Gu for assistance with experiment, and Jake Carpenter for polishing the English in this manuscript. This work was financially supported by the National Natural Sciences Foundation of China (41571057), Key Program of Joint Funds of the National Natural Sciences Foundation and the Government of Xinjiang Uygur Autonomous Region of China (U1203201), National Natural Sciences Foundation of China (41371516).

In addition to decomposition of chlorophyll (Chl) and proteins, a decline in photosynthetic activity (Humbeck *et al*. 1996, Wingler *et al*. 1998, 2006), and ectopia of resources allocated to plant vegetative organs (Bleecker and Patterson 1997, Buchanan-Wollaston 1997, Gan and Amasino 1997, Miller *et al*. 2000), the most obvious feature of leaf senescence is the transition of leaf color from green to yellow or red (Rajcan *et al*. 1999). Color change occurs because Chl degrades easily prior to carotenoids. In addition, it is also relevant to the synthesis of new compounds in the senescence process, such as the synthesis of anthocyanins and phenolic compounds (Hendry 1988, Matile 1992, Smart 1994, Rajcan *et al*. 1999). Therefore, the disappearance of Chl has been widely considered to be a symptom of leaf senescence (Hörtensteiner 2006). However, leaf senescence is not merely a degenerative process; it also plays a vital role in nutrient cycling, especially for the reactivation of nutrient elements (Himelblau and Amasino 2001, Wingler *et al*. 2006). Nitrogen reallocation in senescent nutritive organs is important, particularly, in the transfer of nitrogen from leaves and stems to mature seeds (Parrott *et al*. 2005).

Senescence can be initiated by various internal and external factors, or controlled by a serie of biological and non-biological factors, such as changes of source-sink interactions and hormonal balance, or by the supply of light, ultraviolet light, ozone, hormones, drought, nutrients and water, or induction by various pathogens or pests (Feller and Fischer 1994, Buchanan-Wollaston *et al*. 2003, Lim *et al*. 2007, Gregersen *et al*. 2008, Parrott *et al*. 2010). In previous studies, it has been demonstrated that high carbohydrate content (carbon "feast") is associated with the onset of the natural senescence process (Feller and Fischer 1994, Koch 1996, Wingler *et al*. 1998, 2006; Masclaux *et al*. 2000, Ono *et al*. 2001, Pourtau *et al*. 2004, 2006; Parrott *et al*. 2005, 2007, 2010). However, leaf senescence is not necessarily induced by high concentrations of carbohydrates, since low concentrations of carbohydrates can produce the same effect primarily by applying dark treatment, thereby resulting in carbohydrate famine, which is revealed by experiments where plants showed symptoms of senescence (Matile 1992, Pourtau *et al*. 2006). Plants are demonstrated to be able to induce or accelerate the senescence of blade under a high activity of hexokinase; whereas, plant senescence is delayed in the absence of hexokinase (Dai *et al*. 1999, Mickelson *et al*. 2003, Parrott *et al*. 2010). This also makes hexokinase generally accepted as the main signal factor by which carbohydrate activity induces senescence (Dai *et al*. 1999, Moore *et al*. 2003, Yoshida 2003, Parrott *et al*. 2005).

Because Jongebloed *et al*. (2004) showed that naturally occurring sieve tube occlusion and carbohydrate accumulation is relevant to Chl degradation, it appears that phloem girdling enhances the possibility of plant sieve tube occlusion, thereby enhancing the possibility of carbohydrate increase through experimentation. In fact, numerous experiments have shown that girdling could cause leaf carbohydrate accumulation and accelerate the senescence process (Krapp and Stitt 1995, Parrott *et al*. 2007).

Although experiments, where leaf senescence induced by girdling leads to a decreased photosynthetic activity, have been reported frequently (Parrott *et al*. 2007), there are only a few studies concerning the activity of photosynthetic apparatus and electron transfer in the senescence process (Lichtenthaler and Babani 2004). Although it is well known that plant senescence is always accompanied by yellowing and Chl decomposition, observation of leaf color and determination of Chl content is insufficient to determine the process of leaf senescence, particularly in relation to changes in photosynthetic apparatus performance and structure (Smart 1994).

The measurement of Chl fluorescence is able to elucidate more comprehensively and profoundly the changes in photosynthetic apparatus structure and performance in the process of leaf senescence in plants. Moreover, the literature concerning fast kinetic parameters of Chl fluorescence to study leaf senescence is very limited; thus, we examined the change of PSII structure and electron transfer during senescence utilizing fast kinetic parameters. Given the current lack of information in this area, we carried out phloem girdling in *A. sparsifolia* plants that just entered the senescence period at the Cele Oasis frontier on the southern rim of the Taklimakan Desert. In order to deepen our understanding, changes in leaf carbohydrates accumulation were studied during senescence using Chl fluorescence.

Materials and methods

Study site: The study was performed in the Desert Experimental Area in the Cele National Field Research Station for Desert Steppe Ecosystems, the Chinese Academy of Sciences. The research area is located in the Taklimakan Desert at an oasis-desert transitional zone on the southern rim of the Taklimakan Desert (35°17'55"–39°30'00" N, 80°03'24"–82°10'34" E).

Plant material: *Alhagi sparsifolia* Shap. is a kind of prickly, cloning, and perennial leguminous caudex in arid and semi-arid regions, with a height of about 1 m (Zeng *et al*. 2008). It is widely distributed in the oasis-desert transitional zone at the southern rim of the Taklimakan Desert in northwestern China, and is one of the most important plant species in the area (Xue *et al*. 2011).

Experimental design: On 21 August 2013, nine quadrats, each 4 × 4 m^2, containing 10–12 plants, were set up on flat land. In these quadrats, *A. sparsifolia* plants were two years old and about 80 cm tall. Of these, each quadrat was randomly selected for three different degrees of girdling treatments. Care was taken to a position of the quadrats in

such a way that all of the plants inside the quadrats were as far away as possible from the edges, to avoid possible edge effects from ingrowing roots of nontreated plants.

The three different girdling treatments were: control (plants left intact, CK), semi-girdling (girdle semi-circle of the phloem, SG), and full-girdling (girdle full-circle of the phloem, FG); every treatment was comprised of squares. Girdling consisted in removing a 10–12 mm wide band of bark at the bottom of the main stem of each branch. Studying the leaf senescence process and interrelated physiological parameters under SG may assist us in determining whether the system with half sieve function can work as well as phloem that is not damaged at all. After girdling, a soluble sugar content, starch content, net photosynthetic rate (P_N), photosynthetic pigment content, Chl fluorescence, proline (Pro) content, and malondialdehyde (MDA) content of *A. sparsifolia* were determined, respectively, on the 1st, 11th, 21st, and 31st day (days after treatment, DAT). The examined leaves were all fully developed and expanded, and were mainly selected from the second leaf on the inferior branches (not stems). After three DAT, we checked the survival rate of plants in the sample plot to determine whether plant death occurred due to improper treatment. In most squares, all of the plants survived. In one or two squares, however, individual plants expired; then we removed these plants and ensured that each square contained at least ten plants.

Measurement of P_N was conducted in the field as described by Mittler *et al*. (2001) using a portable photosynthesis system (*LI-COR 6400*, *LI-COR Inc*., Lincoln, NE, USA). Specifically, starting from August 22, 2013, we measured the P_N of *A. sparsifolia* once every 10 d within the square, totally four times. The measurement was conducted on cloudless days at 10:00 h (GMT+6), when the P_N of plants peaks during the day.

As the measured leaves were too small to meet the size of 6 cm^2 of the leaf chamber, a scanner (*HP Scanjet 3770*, *Hewlett-Packard*, Palo Alto, USA) was used to determine a particular leaf area. *Image Pro Plus 6.0* software (*Media Cybernetics*, Silver Springs, MD, USA) was used to calculate the actual area and values of P_N. Five replicates were performed at each treatment using fully developed and expanded leaves.

Photosynthetic pigments content: All leaves were fully developed and selected from the second to fifth leaves on each branch. The fresh leaves were homogenized using sand, $MgCO_3$, and 100% acetone, and then extracted with 80% acetone. After centrifugation at 2,500 rpm for 2 min, the absorbance of the solution was measured by a UV/Vis spectrophotometer (*Jenway 6400*, *Krackeler Scientific*, London, UK) at 470, 647, and 663 nm, with a correction for scattering measured at 750 nm. The concentrations of the Chl per unit of leaf fresh mass were determined using the equation of Lichtenthaler (1987).

The measurement of Chl fluorescence was carried out according to the procedure of Strasser and Govindjee (1992). Specifically, adequately dark-adapted (20 min) leaves were used at room temperature (25°C) using a *Plant Efficiency Analyzer* (*Handy PEA*, *Hansatech*, King's Lynn, Norfolk, UK). Irradiance was 3,600 μmol(photon) m^{-2} s^{-1} to generate fast fluorescence curves expanding from O (taken as F_0, minimal recorded fluorescence intensity) to P (F_m, maximal recorded fluorescence intensity). By using this technique, following parameters were calculated: (*1*) Maximum quantum yield for primary photochemistry (F_v/F_m); (*2*) approximated initial slope of the fluorescence transient (M_0); the total normalized complementary area above the O-J-I-P transient [reflecting single-turnover primary bound plastoquinone (Q_A) reduction events] (S_m); (*3*) quantum yield for electron transport (at t = 0) (φ_{E0}); (*4*) probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (at t = 0) (ψ_0); and (*5*) performance index on absorption basis (PI_{abs}).

Soluble sugars and starch content: Leaves for the measurement of soluble sugars and starch were selected selected from the second to fifth leaf on each of the branches of *A. sparsifolia*. The leaves were dried at 75°C for 24 h until a stable mass was maintained. Dried *A. sparsifolia* leaves were ground to powder for later study. The leaves (0.5 g of dry mass) were extracted with 4 mL of 80% ethanol at 80°C for 40 min, followed by two extractions with 2 mL of 80% ethanol. The supernatants were combined and purified by 10 g of activated carbon at 80°C for 30 min. We then added ethanol (80%) to the product to maintain a constant volume for measuring the amount of soluble sugar. Then, the remaining sample was dried at 45°C to remove ethanol, and boiled for 10 min with 3 mL of double-distilled water in 7.5 mL-centrifuge tubes. The samples were then cooled to room temperature (about 28°C), and we added 4 mL of $HClO_4$ to decompose starch. Starch in the paste was hydrolyzed for 15 min, and soluble-sugar glucose was determined at 630 nm as described previously by Li *et al*. (2011). The starch content was calculated using the formula: Starch content [%] = G (glucose mass obtained from standard curve) × 0.9 = DM (dry mass) × 100.

Pro content: The measurement of the Pro content was based on the method described by Demiral and Türkan (2005). We ground plant samples (0.5 g) and mixed them with 3% (w/v) sulfosalicylic acid. Afterwards, we filtered homogenate through filter paper. After adding acid ninhydrin and glacial acetic acid, the obtained mixture was boiled in a water bath at 100°C for 1 h. The sample reaction mixture was then placed in an ice bath to terminate the reaction. The mixture was extracted with toluene and pure toluene was taken as a control. The extract was placed in a cuvette and its absorbance was read at 520 nm (*Jenway 6400*, *Krackeler Scientific*, London, UK). The Pro

concentration was calculated using a calibration curve and expressed as mol(Pro) g^{-1}(FM, fresh mass).

MDA content was measured according to Rahman *et al*. (2012). Thiobarbituric acid (TBA) reactive substances representing the lipid peroxidation products were extracted by the homogenization of a 0.2 g leaf sample in 5 mL of solution containing 20% trichloroacetic acid and 0.5% TBA. The mixture was heated at 95°C for 30 min, and the reaction was stopped using an ice bath. The cooled mixture was centrifuged at 5,000 × *g* for 10 min at 25°C, and the absorbance of the supernatant at 532 and 600 nm was recorded (*Jenway 6400*, *Krackeler Scientific*, London, UK). After subtracting the nonspecific absorbance at 600 nm, the MDA concentration was determined by its molar extinction coefficient, *i.e*., 155 M^{-1} cm^{-1} (Kosugi and Kikugawa 1985, Rahman *et al*. 2012).

Data analysis: The significance of data differences between CK, SG, and FG after 1, 11, 21, and 31 DAT were analyzed by one-way analysis of variance (*ANOVA*) using *PASW Statistics 18.0* software (*Macintosh*, *SPSS Inc*., Chicago, IL, USA) for each parameter. Once a significant difference was detected, *post-hoc* LSD multiple range tests at $p<0.05$ were used to identify statistically significant differences.

Results

Carbohydrate characteristics: In our experiment, both soluble sugar and starch exhibited the same trend after girdling. On 1 DAT, there was little difference between treatments (CK, SG, and FG) in leaf soluble sugar. After 11 DAT, however, compared with CK, soluble sugar of *A. sparsifolia* subjected to SG and FG increased by 8.7 and 20.6%, respectively. After 31 DAT, soluble sugar of the SG and FG plants increased by 48.3 and 81.6%, respectively, compared with CK. For CK, soluble sugar increased by 24.2% from 1 to 21 DAT, while from 21 to 31 DAT, the soluble sugar content decreased by 12.7%. For SG, soluble sugar increased by 55.0% from 1 to 31 DAT; for FG, soluble sugar increased by 81.6% from 1 to 31 DAT (Fig. 1).

On 1 DAT, there was only a slight difference between treatments in leaf starch. After 11 DAT, however, compared with CK, starch of *A. sparsifolia* subjected to SG and FG increased by 5.8 and 18.1%, respectively. After 21 DAT, compared with CK, starch in the SG and FG plants increased by 14.7 and 37.8%, respectively, while after 31 DAT, it increased by 45.0 and 64.2%, respectively. For CK, starch increased by 14.8% from 1 to 21 DAT, while from 21 to 31 DAT, the starch content decreased by 9.5%. Starch increased by 48.0% from 1 to 31 DAT in SG, while it was enhanced by 68.5% in FG during the same period (Fig. 1).

Photosynthesis characteristics: In the present study, P_N declined with time independently on the treatment (Fig. 2). While after 1 DAT, P_N in the SG and FG leaves decreased by 1.9 and 6.4%, respectively, after 31 DAT, P_N declined by 43.4 and 81.9% in the SG and FG leaves, respectively, compared with CK (Fig. 2).

Photosynthetic pigment characteristics: All pigments, such as Chl *a*, Chl *b*, Chl (*a*+*b*), Car, and both their ratios of Chl *a*/*b* and Chl/Car decreased with time in each treatment. At the beginning, no difference was apparent between CK and both girdling treatments, while the decline in pigment contents became more apparent after 11 DAT (Fig. 3), particularly in the SG (10.9, 9.4, 10.5, 9.2, 1.7, and 1.3%, respectively, for Chl *a*, Chl *b*, Chl (*a*+*b*), Car, Chl *a*/*b*, and Chl/Car) and even more in FG plants (32.7, 26.4, 31.0, 14.4, 8.6, and 18.8%, respectively). The reduction in pigment contents continued with treatment duration and after 31 DAT; it decreased by 32.7, 26.4, 31.0, 14.4, 8.6, and 18.8%, respectively, in the SG for Chl *a*, Chl *b*, Chl (*a*+*b*), Car, Chl *a*/*b*, and Chl/Car compared with CK, while in the FG, pigments decreased by 62.0, 44.5, 57.1, 35.1, 31.9, and 33.9%, respectively (Fig. 3).

Chl fluorescence characteristics: The study revealed that, on the 1st day, there was no significant difference in: 1) F_v/F_m; 2) M_0; S_m; 3) φ_{E0}; 4) ψ_0; and 5) PI_{abs}. Chl fluorescence parameters, such as F_v/F_m, S_m, φ_{E0}, ψ_0, and PI_{abs} declined with time, and M_0 increased with time independently on the treatment (Fig. 4). The significant difference appeared after 11 DAT and became more obvious further on. On the 31st day, compared with CK, F_v/F_m, S_m, φ_{E0}, ψ_0, and PI_{abs} in SG leaves decreased by 24.3, 26.0, 31.2, 26.6, and 70.9%, respectively, while M_0 increased by 45.6%. After 31 DAT, compared with CK, F_v/F_m, S_m, φ_{E0}, ψ_0, and PI_{abs} in FG leaves decreased by 62.8, 47.0, 63.9, 40.6, and 90.7%, respectively, while M_0 increased by 99.3% (Fig. 4).

The Chl fluorescence kinetic curve of *A. sparsifolia* exhibited a typical O-J-I-P phase 1 DAT, whereas, regardless of the treatment, there was no K-phase. However, after 11 DAT, the Chl fluorescence kinetic curve of the FG plants showed the K-phase. Contrary to it, no K-phase was found in other treatments. After 21 and 31 DAT, both the SG and FG plants exhibited the apparent K-phase, whereas there was still no K-phase in the CK plants (Fig. 5).

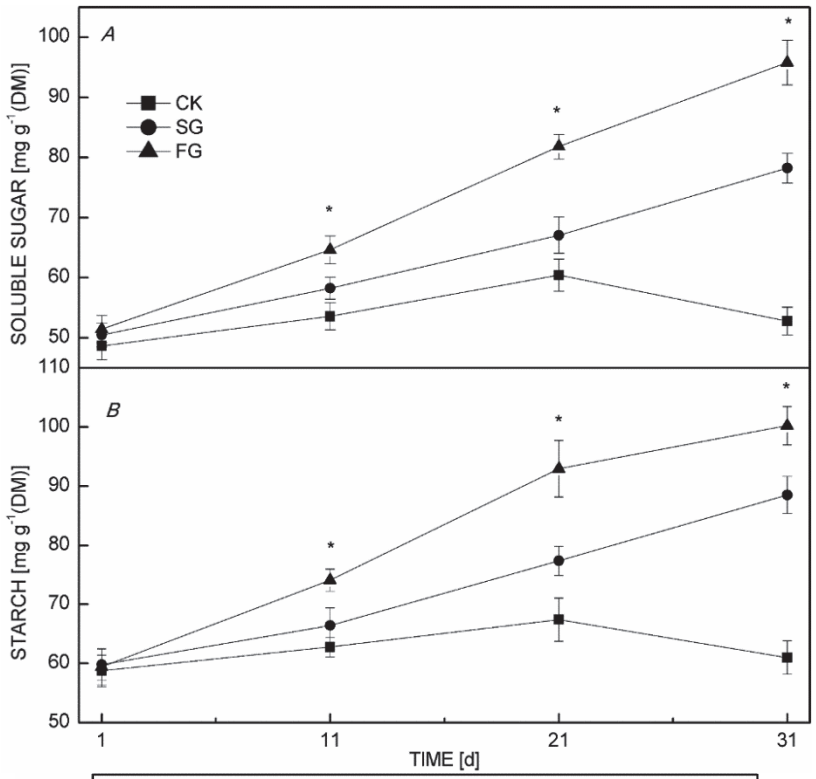

Fig. 1. Soluble sugar (*A*) and starch (*B*) contents of *Alhagi sparsifolia* under control (CK), semi-girdling (SG), and full-girdling (FG) after 1, 11, 21, and 31 d. * – significant difference at the 0.05 probability level according to LSD test (n = 5) estimated by two-tailed *ANOVA* between CK and other treatments (SG, FG). Data points represent the means of five biological replicates ± SE. DM – dry mass.

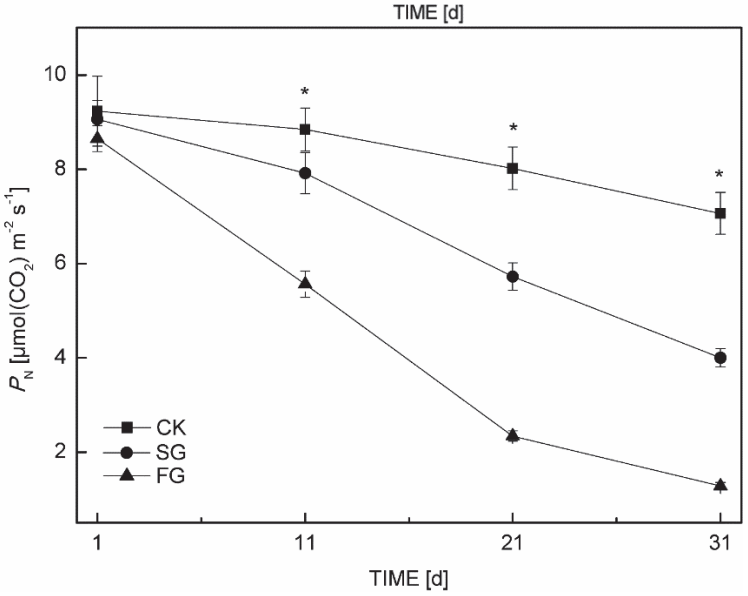

Fig. 2. Net photosynthetic rate (P_N) of *Alhagi sparsifolia* under control (CK), semi-girdling (SG), and full-girdling (FG) after 1, 11, 21, and 31 d. Data points represent the means of five biological replicates ± SE. * – significant difference at the 0.05 probability level according to LSD test (n = 5) estimated by two-tailed *ANOVA* between CK and other treatments (SG, FG).

Pro and MDA characteristics: Both Pro and MDA contents increased with time during our experiment (Fig. 6). At the beginning, the Pro and MDA contents in *A. sparsifolia* leaves changed only slightly. Starting 11 DAT, both Pro and MDA contents increased by 9.1 and 8.6%, respectively, in the SG plants, while they increased by 35.0 and 23.8%, respectively, in the FG compared with CK plants. After 31 DAT, the Pro and MDA contents increased by 26.0 and 22.1%, respectively, in SG, while they enhanced by 50.9 and 48.4%, respectively, in FG compared with CK (Fig. 6).

Discussion

The primary function of girdling is to remove a ring of phloem, thereby disrupting the basipetal movement of photosynthetic products through the phloem. This action may result in the accumulation of carbohydrates on the side of the girdle closer to the source(s) of metabolites, and in a reduction in tissue on the side opposite to the girdle

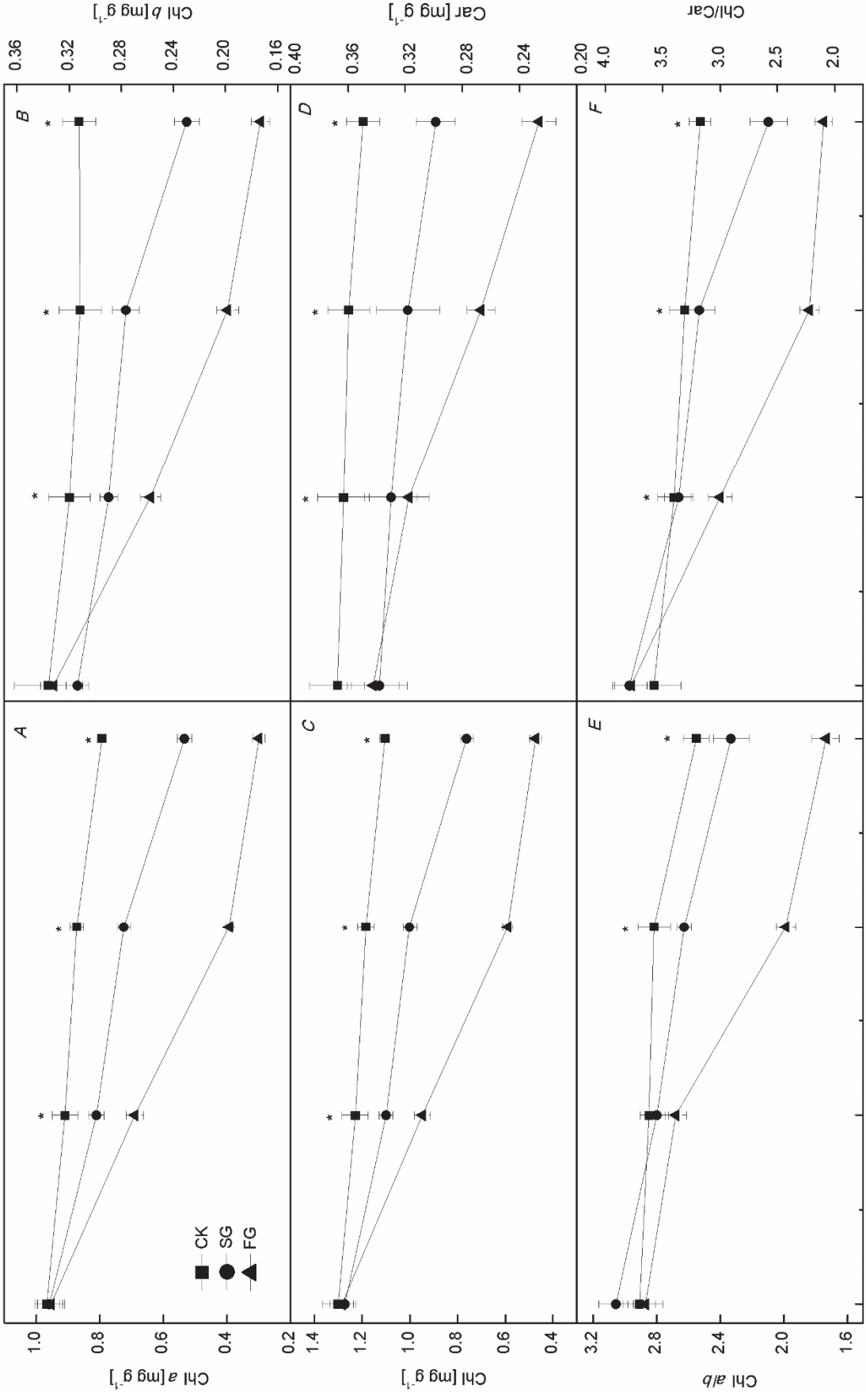

Fig. 3. Chlorophyll (Chl) *a* (*A*), Chl *b* (*B*), total Chl (*C*), carotenoids (Car) (*D*) content, Chl *a*/*b* (*E*), and Chl/Car (*F*) of *Alhagi sparsifolia* under control (CK), semigirdling (SG), and full-girdling (FG) after 1, 11, 21, and 31 d. * – significant difference at the 0.05 probability level according to LSD test ($n = 5$) estimated by two-tailed *ANOVA* between CK and other treatments (SG, FG). Data points represent the means of five biological replicates ± SE.

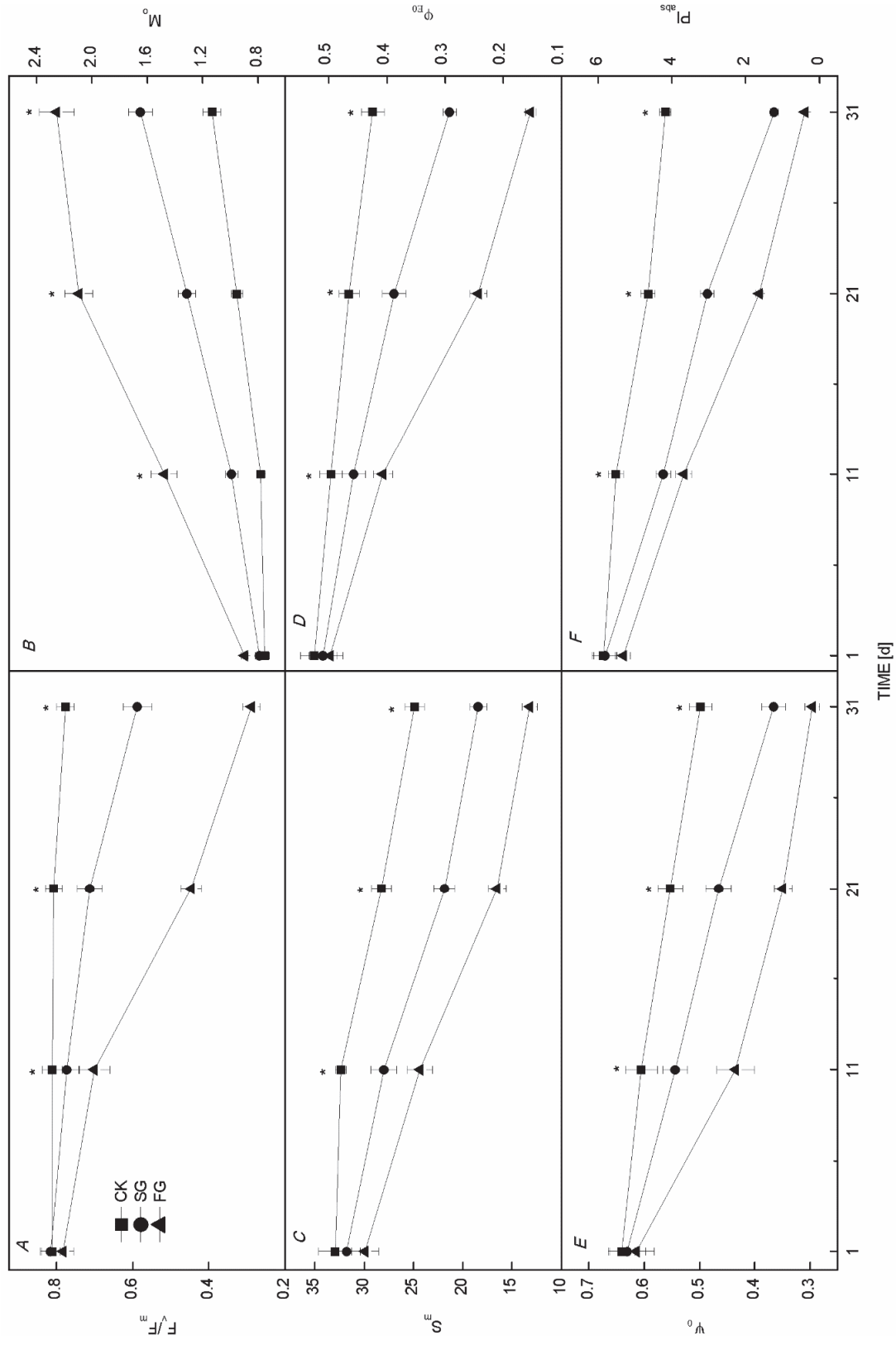

Fig. 4. Maximum photochemical efficiency of PSII (F_v/F_m) (*A*), approximated initial slope of the fluorescence transient (M_0) (*B*), normalized total complementary area above the O-J-I-P transient (reflecting single-turnover Q_A reduction events) (S_m) (*C*), quantum yield for electron transport (at $t = 0$) (φ_{E0}) (*D*), probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (at $t = 0$) (ψ_0) (*E*), and the performance index on absorption basis (PI_{abs}) (*F*) of *Alhagi sparsifolia* under control (CK), semi-girdling (SG), and full-girdling (FG) after 1, 11, 21, and 31 d. * – significant difference at the 0.05 probability level according to LSD test ($n = 5$) estimated by two-tailed *ANOVA* between CK and other treatments (SG, FG). Data points represent the means of five biological replicates ± SE.

(Rivas *et al*. 2006, Urban and Alphonsout 2007, Yang *et al*. 2013). Our study showed that both soluble sugar and starch content increased in the girdled (SG, FG) leaves. As leaf senescence can be induced by a carbohydrate "feast", the acceleration of leaf senescence in the girdled (SG, FG) leaves in our study might result from the increase of carbohydrates, which was consistent with previous studies (Parrott *et al*. 2005, 2010). In fact, our study was conducted during the late growing season; regardless of the type of treatment, *A. sparsifolia* basically exhibited the signs of senescence throughout the entire 31 d-lasting experiment. In our study, leaf soluble sugar and starch showed the trend of first increasing and then decreasing during the natural senescence (CK) process. After the girdling treatment (SG, FG), however, soluble sugar and starch increased continuously during the 31 d period. This may be because the function of photosynthetic apparatus did not rapidly decline during the early stage of senescence and carbohydrates could be still constantly synthesized and slowly accumulated in leaves through photosynthesis. Nevertheless, with proceeding senescence, the function of photosynthetic apparatus began to decline, as well as compounds synthesized *via* photosynthesis, while there was a continual stream of sugar outputting to storage tissues, such as roots. Consequently, at a particular moment of senescence, leaf carbohydrate concentration started to decline, which was evidenced previously by similar experiments (Masclaux *et al*. 2000). On the other hand, when the sieve tube is damaged as a result of phloem girdling, the products of photosynthesis accumulate in the leaf and cannot be exported. Even later in senescence the plant is unable to export excessive carbohydrates through phloem transport. Thus, we could not observe the decrease of the sugar content in the girdled leaves in *A. sparsifolia* in the late stage of the experiment.

P_N decreased the most rapidly in the FG leaves and slowly in natural (CK) leaves during leaf senescence. For this reason, we believe that girdling accelerated leaf senescence. Moreover, P_N after the girdling treatment (SG and FG) began to decline greatly, and this reduction was also accompanied by the accumulation of soluble sugar and starch and it increased with the degree of girdling. We considered that a rise in carbohydrate concentration cannot be the only factor limiting P_N, since the P_N was also continually reduced in the CK treatment in the course of the experiment (Fig. 2). On the other hand, the carbohydrate concentration showed no decline. This might be due to a reduction in P_N induced by changes in the structure and function of the photosynthetic apparatus which might be revealed by changes in the Chl content and Chl fluorescence. Whereas, it is clear that high concentrations of carbohydrates is one of the reasons limiting P_N of plants and inducing leaf senescence. Similar conclusions can be found for *Malus sylvestris* (L.) Mill. var. domestica (Borkh.) (Moore *et al*. 2003), *Anacardium occidentale* L. (Schaper and Chacko 1993), *Vitis vinifera* L. (Harrell and Williams 1987, Roper and Williams 1989), and *Mangifera indica* L. (Lu and Chacko 1998).

Decline of the Chl content and P_N has been used as an indication of leaf senescence (Dong *et al*. 2008, Dai and Dong 2011). In our study, Chl *a*, Chl *b*, and Car contents declined during the 31-d experiment, which indicated that each type of treatment (CK, SG, and FG) exhibited leaf senescence. In addition, the results also demonstrated that, regardless of the type of treatment, Chl *a* decreased more rapidly than Chl *b* during leaf senescence. According to the different function of Chl *a* and Chl *b* in photosynthesis (Argyroudi-Akoyunoglou and Akoyunoglou 1970), the higher ratio of Chl *a*/*b* indicated that Chl *a* contained more molecules that were excited directly by light and thus more molecules were directly involved in photochemical reactions, which is conducive to improving photosynthetic efficiency. In the senescence process, Chl *a* is more easily decomposed compared to Chl *b*, and Chl *a*/*b* tends to decrease, which may also explain why plants show low P_N in the senescence process. Our experiment also led to the same conclusion.

In addition, total Chl was reduced more rapidly than Car, which was revealed by changes in Chl/Car. For most of the higher plants, the Chl content decreased faster than carotenoids during leaf senescence, causing senescent leaves to turn yellow (Matile 1992, 1994; Biswal 1995), which was consistent with our results. Car can absorb excessive light energy in the cell and quench reactive oxygen species, thus preventing membrane lipid peroxidation (Willekens *et al*. 1994) and protecting photosynthetic function (Burton and Ingold 1984). So, we believe that during the senescence process, the decline in Chl/Car of leaves in *A. sparsifolia* occurred most likely because the plants needed to absorb residual light during photosynthesis and quench reactive oxygen species to maintain the plant cells in a more stable state. This was proven by the increase in MDA during the experiment, as well as greater MDA concentrations under SG and FG treatments compared with CK. High retention of Car in the senescence process prevents membrane lipid peroxidation, which has also been proven by previous studies (Rivas *et al*. 2011).

Plant senescence induced by girdling or by the high content of carbohydrates, and reduction in the Chl content and photosynthesis resulting from senescence, have been studied frequently, but there are few studies on plant Chl fluorescence parameters during senescence (Lu *et al*. 2002). The F_v/F_m value is the maximum photochemical efficiency after dark adaptation. It also represents the primary light energy conversion efficiency of PSII and is an important parameter to study physiological state. Under nonstress conditions, the F_v/F_m usually reaches the value of 0.80–0.85; in adverse conditions, however, this value was significantly lower (Björkman and Demmig 1987). The present study showed that each type of senescence led to the decline of F_v/F_m. In natural senescence (CK), F_v/F_m declined slightly, which indicated that the plant was still in a good status. However, the plants subjected to SG and FG exhibited the significant decline of F_v/F_m during

senescence, and the value was much lower than 0.8 after 31 DAT, which indicated that the plants were under stress; the factor causing stress might be the high content of carbohydrates. The girdling-induced stress could be indicated by the increase of Pro in the SG and FG leaves, because the increase of Pro is always associated with oxidative stress. Thus, Pro is a reliable indicator of stress (Jaleel *et al*. 2007). Performance index (PI_{abs}) involves three independent parameters (ABS/RC, φ_{P0}, and ψ_0); therefore, the PI_{abs} can more accurately reflect the state of photosynthetic apparatus. In addition, it is more sensitive to some stresses compared to F_v/F_m and can better reflect the impacts of stresses on the photosynthetic apparatus (Appenroth *et al*. 2001, Van Heerden *et al*. 2003, 2004). In our study, the trend of PI_{abs} was the same as that of F_v/F_m, but the degree was much greater. This result indicated that PI_{abs} was much more sensitive to leaf senescence compared to F_v/F_m.

Regarding the fast Chl fluorescence induction kinetic parameters, M_0, S_m, φ_{E0}, and ψ_0 primarily reflect changes on the acceptor side of PSII, including primary quinone acceptor (Q_A), second electron quinone acceptor (Q_B), and plastoquinone (PQ) pool. M_0 reflects the maximum rate of Q_A reduction, *i.e*., the reduction rate of Q_A in the O-J process (Strasser and Strasser 1995, Strasser *et al*. 2000, 2004). It is relevant to pigments in the reaction center, light-harvesting pigment, and the state of Q_A. S_m reflects the energy needed to reduce Q_A completely. The more electrons that enter the electron transfer chain from Q_A^-, the longer it takes to reach F_m and the greater is the value of S_m. When leaves are damaged by light, the degradation of D1 protein intensifies, causing electron transfer complex, especially Q_B, to easily detach from the protein complex, resulting in reduced storage capacity of the acceptor, shown as S_m decrease. In the present study, girdling accelerated leaf senescence, and in the senescence process, the depredation of D1 protein was intensified. This then cause Q_B shed from protein complexes, which resulted in reduced storage capacity of the acceptor pool, and was shown as S_m decrease.

Electron acceptor capacity (S_m) of the acceptor side of PSII becomes smaller, leading to the probability that a

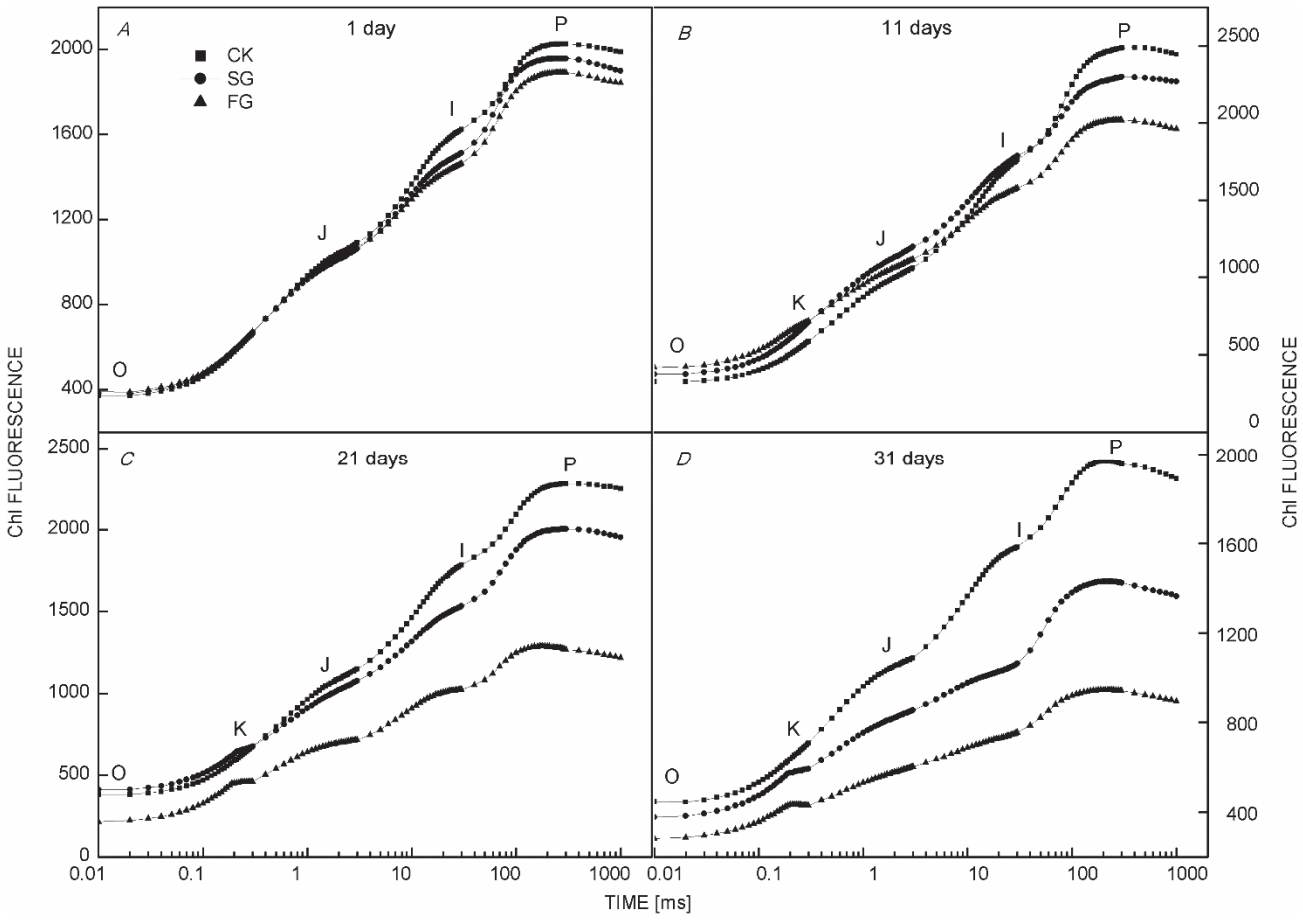

Fig. 5. Chlorophyll (Chl) fluorescence of *Alhagi sparsifolia* under control (CK), semi-girdling (SG), and full-girdling (FG) after 1 (*A*), 11 (*B*), 21 (*C*), and 31 d (*D*). * – significant difference at the 0.05 probability level according to LSD test (n = 5) estimated by two-tailed *ANOVA* between CK and other treatments (SG, FG). Data points represent the means of five biological replicates ± SE.

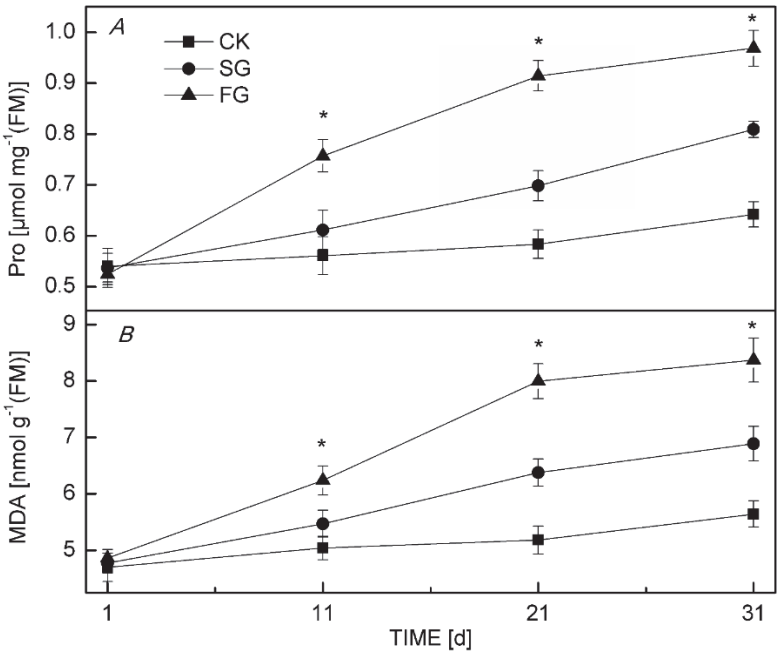

Fig. 6. Proline (Pro) (*A*) and malondialdehyde (MDA) (*B*) contents of *Alhagi sparsifolia* under control (CK), semi-girdling (SG), and full-girdling (FG) after 1, 11, 21, and 31 d. * – significant difference at the 0.05 probability level according to LSD test (n = 5) estimated by two-tailed *ANOVA* between CK and other treatments (SG, FG). Data points represent the means of five biological replicates ± SE. FM – fresh mass.

trapped exciton moves an electron into the electron transport chain beyond Q_A^- (ψ_0), and the quantum yield for electron transport (φ_{E0}) decreases. More energy is used to reduce Q_A, accelerating Q_A reduction (M_0), which is apparent as an increase of M_0.

Previous studies have shown that the emergence of the K-phase is a result of the suppression of the water-splitting system and the acceptor part before Q_A (Guissé *et al*. 1995, Strasser *et al*. 2000, 2004). In this suppression process, the oxygen evolution complex (OEC) is damaged, thus the K-phase can constitute a special indicator of OEC injury (Van Rensburg *et al*. 1996, Strasser *et al*. 2000, 2004). In our study, the differences between two kinds of senescence was found in Chl fluorescence kinetic curves on the donor side of PSII. During 30 d after girdling, the CK plants showed no K-phase and no OEC damage; under the SG treatment, the K-phase appeared after 21 d and in FG, the K-phase appeared after 11 d. In other words, after FG, damage of OEC became to be apparent within 11 d, while it postponed to 21 d after SG, contrary to CK, where OEC sustained very stable during senescence.

From the changes in the structure and function of PSII, we believe that the naturally occurring senescence process would not normally cause serious damage to the structure of PSII, at least in the prometaphase of senescence. However, plant senescence induced by girdling and high concentrations of carbohydrates could cause changes in the mechanism and function of PSII, and at least OEC was damaged. This seems to indicate that natural plant senescence may be a subject to strict regulation, and in the natural senescence process, PSII structure does not degenerate a lot, it still maintain a good performance. However, in the case of plant senescence caused by external factors, such as the high content of carbohydrates, senescence may surpass the regulatory capacity of the plant itself, which in turn leads to speeding up plant senescence. In this case, some organs or tissues may be alienated in terms of structure and function, and their physical conditions would be seriously affected. Photosynthetic rate declined more dramatically after the girdling than that in the natural senescence system, which exactly illustrated this problem. Nevertheless, the decline of photosynthetic rate was due to damage of PSII structure, reduction in energy use efficiency and reduction in content of photosynthetic pigments.

From the above discussion, we can draw the following conclusions. First, the beginning and process of senescence cannot be completely dominated by the concentration of carbohydrates. When the carbohydrate concentrations in leaves started to reduce in the later period of natural senescence, the plants still exhibited a significant senescence characteristic, so senescence in the later period was not because of the high concentrations of carbohydrates. Second, the high concentrations of carbohydrates indeed accelerated the process of senescence, which could be seen from the subsequent performance of leaves of *A. sparsifolia* under the SG and FG treatment. Third, such senescence probably cannot be entirely regulated by senescence genes. It was manifested as degeneration of some organs and tissues in their structure and function, as well as suppression of related physiological metabolism. Finally, plant senescence induced by the high concentrations of carbohydrates was likely to damage OEC on the donor side of PSII and electron transfer chain by the acceptor side of PSII, thereby accelerating the plant senescence.

References

Appenroth K.J., Stöckel J., Srivastava A., Strasser .R.J.: Multiple effects of chromate on the photosynthetic apparatus of *Spirodela polyrhiza* as probed by OJIP chlorophyll *a* fluorescence measurements. – Environ. Pollut. **115**: 49-64, 2001.

Argyroudi-Akoyunoglou J., Akoyunoglou G.: Photoinduced changes in the chlorophyll *a* to chlorophyll *b* ratio in young bean plants. – Plant Physiol. **46**: 247-249, 1970.

Biswal B.: Carotenoid catabolism during leaf senescence and its control by light. – J. Photochem. Photobiol. B **30**: 3-13, 1995.

Björkman O., Demmig B.: Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. – Planta **170**: 489-504, 1987.

Bleecker A.B., Patterson S.E.: Last exit: senescence, abscission, and meristem arrest in *Arabidopsis*. – Plant Cell **9**: 1169-1179, 1997.

Buchanan-Wollaston V., Earl S., Harrison E. *et al*.: The molecular analysis of leaf senescence – a genomics approach. – Plant Biotechnol. J. **1**: 3-22, 2003.

Buchanan-Wollaston V.: The molecular biology of leaf senescence. – J. Exp. Bot. **48**: 181-199, 1997.

Burton G.W., Ingold K.U.: Beta-carotene: an unusual type of lipid antioxidant. – Science **224**: 569-573, 1984.

Dai J., Dong H.: Stem girdling influences concentrations of endogenous cytokinins and abscisic acid in relation to leaf senescence in cotton. – Acta Physiol. Plant. **33**: 1697-1705, 2011.

Dai N., Schaffer A., Petreikov M. *et al*.: Overexpression of *Arabidopsis* hexokinase in tomato plants inhibits growth, reduces photosynthesis, and induces rapid senescence. – Plant Cell **11**: 1253-1266, 1999.

Demiral T., Türkan I.: Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. – Environ. Exp. Bot. **53**: 247-257, 2005.

Dong H., Niu Y., Li W. *et al*.: Effects of cotton rootstock on endogenous cytokinins and abscisic acid in xylem sap and leaves in relation to leaf senescence. – J. Exp. Bot. **59**: 1295-1304, 2008.

Feller U., Fischer A.: Nitrogen metabolism in senescing leaves. – Crit. Rev. Plant Sci. **13**: 241-273, 1994.

Gan S., Amasino R.M.: Making sense of senescence (molecular genetic regulation and manipulation of leaf senescence). – Plant Physiol. **113**: 313-319, 1997.

Gregersen P., Holm P., Krupinska K.: Leaf senescence and nutrient remobilisation in barley and wheat. – Plant Biol. **10**: 37-49, 2008.

Guissé B., Srivastava A., Strasser R.: The polyphasic rise of the chlorophyll a fluorescence (OKJIP) in heat stressed leaves. – Arch. Sci. **48**: 147-160, 1995.

Harrell D.C., Williams L.E.: Net CO_2 assimilation rate of grapevine leaves in response to trunk girdling and gibberellic acid application. – Plant Physiol. **83**: 457-459, 1987.

Hendry G.: Where does all the green go? – New Scientist **1637**: 38-42, 1988.

Himelblau E., Amasino R.M.: Nutrients mobilized from leaves of *Arabidopsis thaliana* during leaf senescence. – J. Plant Physiol. **158**: 1317-1323, 2001.

Hörtensteiner S.: Chlorophyll degradation during senescence. – Annu. Rev. Plant Biol. **57**: 55-77, 2006.

Humbeck K., Quast S., Krupinska K.: Functional and molecular changes in the photosynthetic apparatus during senescence of flag leaves from field-grown barley plants. – Plant Cell Environ. **19**: 337-344, 1996.

Jaleel C.A., Manivannan P., Sankar B. *et al*.: Calcium chloride effects on salinity-induced oxidative stress, proline metabolism and indole alkaloid accumulation in *Catharanthus roseus*. – C. R. Biol. **330**: 674-683, 2007.

Jongebloed U., Szederkényi J., Hartig K. *et al*.: Sequence of morphological and physiological events during natural ageing and senescence of a castor bean leaf: sieve tube occlusion and carbohydrate back-up precede chlorophyll degradation. – Physiol. Plantarum **120**: 338-346, 2004.

Koch K.: Carbohydrate-modulated gene expression in plants. – Annu. Rev. Plant Biol. **47**: 509-540, 1996.

Kosugi H., Kikugawa K.: Thiobarbituric acid reaction of aldehydes and oxidized lipids in glacial acetic acid. – Lipids **20**: 915-921, 1985.

Krapp A., Stitt M.: An evaluation of direct and indirect mechanisms for the "sink-regulation" of photosynthesis in spinach: changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady-state transcript levels after cold-girdling source leaves. – Planta **195**: 313-323, 1995.

Li N., Zhang S., Zhao Y. *et al*.: Over-expression of AGPase genes enhances seed weight and starch content in transgenic maize. – Planta **233**: 241-250, 2011.

Lichtenthaler H.K., Babani F.: Light adaptation and senescence of the photosynthetic apparatus. Changes in pigment composition, chlorophyll fluorescence parameters and photosynthetic activity. – In: Papageorgiou, G.C., Govindjee (ed.): Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Advances in Photosynthesis and Respiration. Pp. 713-736. Springer, Dordrecht 2004.

Lichtenthaler H.K.: Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. – Methods Enzymol. **148**: 350-382, 1987.

Lim P.O., Kim H.J., Gil-Nam H.: Leaf senescence. – Annu. Rev. Plant Biol. **58**: 115-136, 2007.

Lu P., Chacko E.: Evaluation of Granier's sap flux sensor in young mango trees. – Agronomie **18**: 461-471, 1998.

Lu Q., Lu C., Zhang J. *et al*.: Photosynthesis and chlorophyll *a* fluorescence during flag leaf senescence of field-grown wheat plants. – J. Plant Physiol. **159**: 1173-1178, 2002.

Masclaux C., Valadier M.-H., Brugière N. *et al*.: Characterization of the sink/source transition in tobacco (*Nicotiana tabacum* L.) shoots in relation to nitrogen management and leaf senescence. – Planta **211**: 510-518, 2000.

Matile P.: Fluorescent idioblasts in autumn leaves of *Ginkgo biloba*. – Bot. Helv. **104**: 87-92, 1994.

Matile P.: Chloroplast senescence. – Topics Photosynth. **12**: 413-440, 1992.

Mickelson S., See D., Meyer F.D. *et al*.: Mapping of QTL associated with nitrogen storage and remobilization in barley (*Hordeum vulgare* L.) leaves. – J. Exp. Bot. **54**: 801-812, 2003.

Miller A., Schlagnhaufer C., Spalding M., Rodermel, S.: Carbohydrate regulation of leaf development: prolongation of leaf senescence in Rubisco antisense mutants of tobacco. – Photosynth. Res. **63**: 1-8, 2000.

Mittler R., Merquiol E., Hallak-Herr E. *et al*.: Living under a 'dormant' canopy: a molecular acclimation mechanism of the desert plant *Retama raetam*. – Plant J. **25**: 407-416, 2001.

Moore B., Zhou L., Rolland F. *et al*.: Role of the *Arabidopsis* glucose sensor HXK1 in nutrient, light, and hormonal

signaling. – Science **300**: 332-336, 2003.
Ono K., Nishi Y., Watanabe A., Terashima, I.: Possible mechanisms of adaptive leaf senescence. – Plant Biol. **3**: 234-243, 2001.
Parrott D., Yang L., Shama L., Fischer, A.M.: Senescence is accelerated, and several proteases are induced by carbon "feast" conditions in barley (*Hordeum vulgare* L.) leaves. – Planta **222**: 989-1000, 2005.
Parrott D.L., Martin J.M., Fischer A.M.: Analysis of barley (*Hordeum vulgare*) leaf senescence and protease gene expression: a family C1A cysteine protease is specifically induced under conditions characterized by high carbohydrate, but low to moderate nitrogen levels. – New Phytol. **187**: 313-331, 2010.
Parrott D.L., McInnerney K., Feller U., Fischer, A.M.: Steam-girdling of barley (*Hordeum vulgare*) leaves leads to carbohydrate accumulation and accelerated leaf senescence, facilitating transcriptomic analysis of senescence-associated genes. – New Phytol. **176**: 56-69, 2007.
Pourtau N., Jennings R., Pelzer E. *et al*.: Effect of sugar-induced senescence on gene expression and implications for the regulation of senescence in *Arabidopsis*. – Planta **224**: 556-568, 2006.
Pourtau N., Marès M., Purdy S. *et al*.: Interactions of abscisic acid and sugar signalling in the regulation of leaf senescence. – Planta **219**: 765-772, 2004.
Rahman M.M., Chongling Y., Rahman M.M., Islam, K.S.: Effects of copper on growth, accumulation, antioxidant activity and malondialdehyde content in young seedlings of the mangrove species *Kandelia candel* (L.). – Plant Biosyst. **146**: 47-57, 2012.
Rajcan I., Dwyer L.M., Tollenaar M.: Note on relationship between leaf soluble carbohydrate and chlorophyll concentrations in maize during leaf senescence. – Field Crops Res. **63**: 13-17, 1999.
Rivas F., Erner Y., Alós E. *et al*.: Girdling increases carbohydrate availability and fruit-set in citrus cultivars irrespective of parthenocarpic ability. – J. Hortic. Sci. Biotechnol. **81**: 289-295, 2006.
Rivas F., Fornes F., Rodrigo J.M. *et al*.: Changes in carotenoids and ABA content in *Citrus* leaves in response to girdling. – Sci. Hortic. **127**: 482-487, 2011.
Roper T.R., Williams L.E.: Net CO_2 assimilation and carbohydrate partitioning of grapevine leaves in response to trunk girdling and gibberellic acid application. – Plant Physiol. **89**: 1136-1140, 1989.
Schaper H., Chacko E.: Effect of irradiance, leaf age, chlorophyll content and branch-girdling on gas exchange of cashew (*Anacardium accidentale* L.) leaves. – J. Hortic. Sci. **68**: 541-550, 1993.
Smart C.M.: Gene expression during leaf senescence. – New Phytol. **126**: 419-448, 1994.
Strasser B.J., Strasser R.J.: Measuring fast fluorescence transients to address environmental questions: the JIP test. – In: Mathis P. (ed.): Photosynthesis: From Light to Biosphere. Volume 5. Pp. 977-980. Kluwer Academic Publisher, Dordrecht 1995.
Strasser R.J., Govindjee: On the O-J-I-P fluorescence transient in leaves and D1 mutants of *Chlamydomonas reinhardtii*. – In: Murata, N. (ed.): Research in Photosynthesis. 2nd Ed., Vol. 2. Pp. 529-532. Kluwer Academic Publishers, Dordrecht 1992.
Strasser R.J., Srivastava A., Tsimilli-Michael M.: The fluorescence transient as a tool to characterize and screen photosynthetic samples. – In: Yunus M., Pathre U., Mohanty P. (eds.): Probing Photosynthesis: Mechanism, Regulation and Adaptation. Pp. 443-480. Taylor and Francis, London 2000.
Strasser R.J., Tsimilli-Michael M., Srivastava A.: Analysis of the chlorophyll *a* fluorescence transient. – In: Papageorgiou, G.C., Govindjee (ed.): Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Advances in Photosynthesis and Respiration. Pp. 321-362. Springer, Dordrecht 2004.
Thomas H., Smart C.M.: Crops that stay green. – Ann. Appl. Biol. **123**: 193-219, 1993.
Urban L., Alphonsout L.: Girdling decreases photosynthetic electron fluxes and induces sustained photoprotection in mango leaves. – Tree Physiol. **27**: 345-352, 2007.
Van Heerden P.D., Strasser R.J., Krüger G.H.: Reduction of dark chilling stress in N_2-fixing soybean by nitrate as indicated by chlorophyll *a* fluorescence kinetics. – Physiol. Plantarum **121**: 239-249, 2004.
Van Heerden P.D., Tsimilli-Michael M., Krüger G.H., Strasser, R.J.: Dark chilling effects on soybean genotypes during vegetative development: parallel studies of CO_2 assimilation, chlorophyll *a* fluorescence kinetics O-J-I-P and nitrogen fixation. – Physiol. Plantarum **117**: 476-491, 2003.
Van Rensburg L., Kruger G., Eggenberg P., Strasser, R.J.: Can screening criteria for drought resistance in *Nicotiana tabacum* L. be derived from the polyphasic rise of the chlorophyll *a* fluorescence transient (OJIP)? – S. Afr. J. Bot. **62**: 337-341, 1996.
Willekens H., Van Camp W., Van Montagu M. *et al*.: Ozone, sulfur dioxide, and ultraviolet B have similar effects on mRNA accumulation of antioxidant genes in *Nicotiana plumbaginifolia* L. – Plant Physiol. **106**: 1007-1014, 1994.
Wingler A., Purdy S., MacLean J.A., Pourtau, N.: The role of sugars in integrating environmental signals during the regulation of leaf senescence. – J. Exp. Bot. **57**: 391-399, 2006.
Wingler A., Von Schaewen A., Leegood R.C. *et al*.: Regulation of leaf senescence by cytokinin, sugars, and light effects on NADH-dependent hydroxypyruvate reductase. – Plant Physiol. **116**: 329-335, 1998.
Xue W., Li X., Lin L. *et al*.: Effects of elevated temperature on photosynthesis in desert plant *Alhagi sparsifolia* S. – Photosynthetica **49**: 435-447, 2011.
Yang X.-Y., Wang F.-F., Teixeira da Silva J.A. *et al*.: Branch girdling at fruit green mature stage affects fruit ascorbic acid contents and expression of genes involved in L-galactose pathway in citrus. – New Zealand J. Crop Hortic. Sci. **41**: 23-31, 2013.
Yoshida S.: Molecular regulation of leaf senescence. – Curr. Opin. Plant Biol. **6**: 79-84, 2003.
Zeng J., Zeng F., Arndt S. *et al*.: Growth, physiological characteristics and ion distribution of NaCl stressed *Alhagi sparsifolia* seedlings. – Chin. Sci. Bull. **53**: 169-176, 2008.