

Impact of girdling and leaf removal on *Alhagi sparsifolia* leaf senescence

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Abstract Leaf senescence can be described as the dismantling of cellular components during the terminal stage in the development of plant organs and tissues. In order to determine the leaf senescence process when stem girdling and leaf removal both exist. An experiment was carried out in *Alhagi sparsifolia*, which grew in the Cele oasis-desert transitional zone with the treatment of control (CK), phloem girdling (PG), leaf removal (LR), and combined girdling and removal (GR). Some parameters related to leaf senescence were measured at the 1st and 30th day post-girdling. The results showed that after PG and GR, leaf soluble sugar content, starch content, abscisic acid content, proline content, and malondialdehyde content increased substantially and leaf photosynthetic rate, stomatal conductance, transpiration rate, photosynthetic pigment content, and water potential decreased substantially compared with CK. It also changed much more in PG leaves than in GR leaves. The change in LR leaves was opposite to that of PG and GR leaves, but the change was rather slight. The result of the present work implied that senescence of leaves treated with PG greatly accelerates, and the accumulation

of carbohydrates and ABA in leaves is probably the main reason for this. Separate LR could play a role in delaying leaf senescence in plants; however, this delay effect was not obvious. Nevertheless, partial removal of leaves led to a significant compensation of girdling effects, i.e., senescence will be delayed significantly in girdled leaves when treated with partial LR.

Keywords *Alhagi sparsifolia* · Carbohydrate · Phloem girdling · Phloem transport · Leaf removal · Senescence

Introduction

Senescence is a highly regulated process that constitutes the final stage of a plant's organ and tissue development (Buchanan-Wollaston et al. 2003; Parrott et al. 2010). Subject to genetic control, it is a highly regulated plant development process that rescues nutrients prior to cell death (Parrott et al. 2005, 2007). In-depth research has demonstrated that the leaf senescence process is far more complicated than the degradation of chlorophyll and protein; declines in photosynthetic activity (Wingler et al. 2006) and change of resources distributed to plant vegetative organs are also obvious characteristics of leaf senescence (Miller et al. 2000).

The initiation of senescence, and its acceleration and delay, can be triggered by various internal and external factors, such as alterations of the source/sink interactions and hormonal balance, or the supply of light, ultraviolet light, ozone, hormones, drought, nutrients, and water (Gregersen et al. 2008; Parrott et al. 2010). In addition to the above factors, it has been demonstrated that high levels of leaf carbohydrates (the so-called carbon “feast”) are highly involved with the leaf senescence process (Pourtau

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et al. 2004, 2006; Parrott et al. 2005, 2007, 2010; Wingler et al. 2006).

The leaf senescence process is also closely related to the source/sink relationship (Masclaux et al. 2000). Because a rise in the proportion of source organs is always accompanied by high levels of carbohydrates, and sink organs often exist as organs that consume carbohydrates, it is also possible for senescence induced by changes in the source/sink ratio to take hexokinase as a signal factor for induction. In previous experiments, researchers investigated changes in the plant senescence process by the removal of sink organs as the relationship between source and sink can influence senescence. Some results revealed that plant senescence had been dramatically inhibited by the removal of sink organs, especially of reproductive organs, such as soybean (Wittenbach 1982). One mechanism may be that seeds, as a powerful sink organ, always extracted a lot of nutrients from plants (e.g. leaves), eventually leading to plant senescence, and removal of sink organs can significantly decline this process (Nooden et al. 1978). In addition, the appearance of seeds (sink organs) may lead to a deprivation of water relations, a depletion of available nutrients, or may produce several hormonal signals which regulate senescence (e.g. ethylene), and the removal of sink organs will slow down this process thus delay senescence (Mondal et al. 1978). While in other studies, researchers found that removal of sink organs did not influence the process of senescence, but rather altered the partitioning of plant constituents and the visual manifestations of senescence (Crafts-Brandner et al. 1984). Nevertheless, little empirical research has been conducted to observe the effects of removal of the sink organs (leaves) on senescence in the remaining leaves. Because leaf, as the organ for photosynthesis, is the main source of carbohydrates in plants, leaf removal (LR) will generally lessen whole plants' carbohydrate levels. Moreover, phloem girdling (PG) typically results in accumulation of carbohydrates in leaves, which ultimately accelerates the process of leaf senescence (Nooden et al. 1978; Krapp and Stitt 1995). These two change plant carbohydrates through inconsistent mechanisms, since LR gives rise to declining concentrations of carbohydrates in plants by reducing the synthesis of photosynthetic products; whereas, girdling leads to accumulation of carbohydrates in leaves by blocking the transport of materials through phloem sieve tubes. However, they both have an obvious antagonistic effect that exists in the changes of carbohydrates in leaves.

We use *A. sparsifolia* as our plant material which is a perennial leguminous shrub. *A. sparsifolia* was able to reach the groundwater table in the river valleys (6.5 m) within a time span of 5–6 months after germination, so at early stage of plant growth, *A. sparsifolia* allocated more

substance for the root growth to adapt the extreme drought (Vonlanthen et al. 2010). The formation of root biomass conformed to a logistic 'slow–quick–slow' growth curve, and total biomass decreased with intensity of soil drought (Zeng et al. 2012). Normal senescence of *A. sparsifolia* begins in late August, and during leaf senescence, the maximum photochemical quantum yield remained relatively high (Xue et al. 2012). In addition, leaf senescence of *A. sparsifolia* can be induced by the reduction of daily sunshine period and increase of temperature (Xue et al. 2012). In our experiment, we increased the carbohydrate concentrations in leaves and observed the process of leaf senescence by conducting stem girdling of *A. sparsifolia*. We also achieved the purpose of reducing the concentrations of carbohydrates in plants by removing *A. sparsifolia* leaves, and thus observed whether the senescence process could be delayed by the removal of source organs. The main purpose of our study is to detect which factor, i.e., phloem transport or production of carbohydrates in source organs, is dominant in plant senescence, as well as investigating whether or not the acceleration of leaf senescence as a result of blocked transport of sieve tubes could be effectively delayed by the removal of source organs. This research will deepen our understanding of leaf senescence.

Materials and methods

Study site

Experiments were performed in the Desert Experimental Area in Cele National Field Research Station for Desert Steppe Ecosystems, Chinese Academy of Sciences. The research area was 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. which was 3-year old was used for this study. This is a spiny, clonal, perennial leguminous herb growing in arid and semi-arid regions with few leaves, and is about 1 m tall (Zeng et al. 2008). It is widely distributed in the transition zone between oasis and desert in the southern fringe of the Taklimakan Desert in Western China, and is one of the important dominant species in this region (Xue et al. 2011). As a deep-rooted plant, the stem girdling of *A. sparsifolia* may be interesting because the sink organ (root) is more active and consumes more carbohydrates.

Experimental design

On 21 August 2013, 12 square plots, each 4×4 m and with 10–12 plants, were set up on flat land. Of these, each quadrat was randomly selected for four different treatments: CK: control (remain intact); PG: phloem girdling (girdle phloem at the bottom of the stem); LR: leaf removal (remove about half of the total leaves); and GR: combine girdling and removal (we first girdled phloem at the bottom of the stem and then removed about half of the total leaves in branches), as shown in Fig. 1. Every treatment (CK, PG, LR, and GR) had three quadrats (each 4×4 m and with 10–12 plants). Girdling consisted in removing a 10–12 mm wide band of bark at the bottom of the main stem of each branch. LR consisted of removing one leaf, every other leaf, from the bottom to the top of each branch, so that each kind of leaf (young, mature, and elderly) was both removed and remained. Soluble sugar content, starch content, net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), leaf abscisic acid (ABA) content, photosynthetic pigment content, leaf water status, proline (Pro) content, and malondialdehyde (MDA) content of *A. sparsifolia* were determined respectively on day 1 and 30 after the above treatments.

Measurement of parameters

Measurement of photosynthetic parameters

Pn, Gs, and Tr were measured in the field as Mittler et al. (2001) described using a Portable Photosynthesis System (Li-COR 6400 Inc., Lincoln, NE, USA). The parameters were measured every 2 h from 06:00 to 18:00 (GMT + 6) on August 22 and September 20, 2013. As the measured leaves did not reach the standard size of the leaf chamber

(2 cm \times 3 cm), a scanner was employed to scan the measured leaves. In addition, Image Pro Plus 6.0 software (Media Cybernetics, Silver Springs, MD, USA) read the surface area of the measured leaves, and was later used to calculate the actual values of Pn, Gs, and Tr. We selected the third leaf (count from top to base) on the eighth branch (count from base to top) as our measured leaf. Five replicate measurements were performed for each treatment.

Measurement of pigment, soluble sugar, and starch content

All leaves were of similar size and selected from the second to fifth leaf on each of the branches. We picked five leaves from each *A. sparsifolia*; thus, each quadrat had 50 leaves (5×10), for a total of 150 leaves (50×3). Every 30 leaves (total of five groups and 150 leaves) were homogenized using sand and $MgCO_3$, and then 0.2 g leaves were extracted with 25 mL 80 % acetone. After centrifugation at 2500 rpm for 2 min, the absorbance of the solution was measured by a UV–Vis spectrophotometer (Jenway, UK), at 470, 647, and 663 nm, with a correction for scattering, measured at 750 nm. The concentrations of Chl per unit leaf fresh weight were determined using the equation of Lichtenthaler (1987).

Leaves and roots were harvested at the 1st and 30th day, respectively, and then they were dried and ground to powder. We collected twenty leaves from each *A. sparsifolia*; thus, each quadrat had 200 leaves (20×10), for a total of 600 leaves (200×3). All leaves were selected from the third to twelfth leaf (count from top to base) on the sixth and seventh branches (count from base to top) on each plant. We collected one root on each plant in each quadrat, so we totally collected 30 roots ($1 \times 10 \times 3$). The roots we selected for measurement were all from the third secondary root (count from above to below). As the root

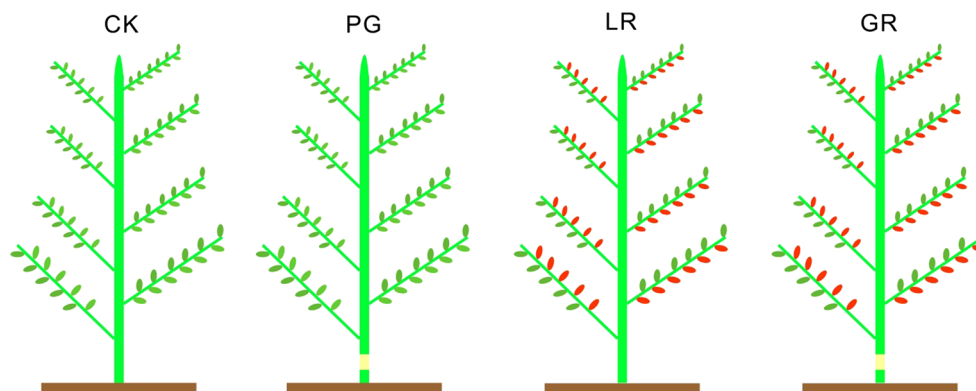


Fig. 1 Sketch of treatment of *Alhagi sparsifolia* girdling and leaf removal. CK: control (remain intact); PG: phloem girdling (girdle phloem at the bottom of the stem); LR: leaf removal (remove about half of the total leaves); GR: combine girdling and removal (we first girdled phloem at the bottom of the stem and then removed about half

of the total leaves in the branches). The stems with *yellow* mean that they were girdled, the leaves with *red* mean that they will be removed. From the bottom of the stem to the top, every other leaf was removed. The growth directions of leaves are random, but not shown in the sketch. (Color figure online)

system in *A. sparsifolia* was large, one secondary root will not influence the growth conditions of *A. sparsifolia*, so we can measure this parameter for twice on the 1st and 30th day respectively.

Six hundred leaves (each replicate was 120 leaves) and 30 roots (each replicate was six roots) were harvested at each treatment, and five replicate measurements were performed for each treatment. 0.5 g of ground leaves and roots were extracted with 4 mL of 80 % ethanol at 80 °C for 40 min (boiling ethanol), followed by two extractions with 2 mL of 80 % ethanol (20 % is water). The supernatants were combined and purified by 10 g activated carbon at 80 °C for 30 min. Ethanol (80 %) was added to the product to maintain a constant volume (25 mL) for measuring the amount of soluble sugar. Then, the remaining sample was dried at 45 °C to remove the ethanol, and boiled for 10 min with 3 mL double-distilled water in 7.5 mL centrifuge tubes. The samples were then cooled to 28 °C, and 4 mL HClO₄ was added to decompose the starch. Starches were hydrolyzed for 15 min, and the resulting soluble sugar glucose was determined at 630 nm, as described previously (Li et al. 2011). Starch content was calculated using the following formula (Tang et al. 2015):

$$\text{Starch content \%} = G(\text{glucose weight got from standard curve}) \times 0.90 (\text{coefficient of glucose converted to starch}) / \text{DW}(\text{dry weight}) \times 100 \%$$

Leaf water potential measurement

Leaf water potential readings were conducted according to previous studies (Williams and Araujo 2002; Williams et al. 2012). Predawn water potential measurements began at about 4:30 am and were finished before sunrise using a pressure chamber (PMS Instruments Co., Corvallis, Oregon, USA). Midday measurements occurred between 11:30 pm and 12:30 pm (GMT + 6). Leaf blades used for the determination of water potential were covered with a plastic bag and quickly sealed. Petioles were then cut within 1–2 s, and the time between leaf excision and chamber pressurization was generally <10–15 s. We selected the third leaf (count from top to base) on the eighth branch (count from base to top) as our measured leaf just like the leaf selected for measurement of photosynthesis. Leaves chosen for midday water potential determinations were exposed to direct solar radiation. Five replicate measurements were performed for each treatment.

Measurement of ABA content

All leaves were selected from the second to seventh leaf on the ninth branch (count from base to top) on each plant. We

collected five leaves from each *A. sparsifolia*; thus, each quadrat had 50 leaves (5 × 10), for a total of 150 leaves (50 × 3). ABA extraction was determined by the method as described by Veselov et al. (2008). All samples were taken in the morning at about 10:00 am (GMT + 6), weighed, and then frozen in liquid nitrogen. Frozen samples were ground to fine powder in liquid nitrogen. ABA was extracted in 80 % ethanol and incubated overnight at 4 °C. Distilled water was added to dilute the aqueous residue, then acidified with HCl (1 mol/L) to pH 2.5, then partitioned twice with peroxide-free diethyl ether (ratio of organic to aqueous phases was 1:3). The organic phase was transferred into 1 % sodium hydrocarbonate (pH 7–8, ratio of organic to aqueous phases was 3:1). The solution was re-extracted with diethyl ether, methylated with diazomethane, and immunoassayed using antibodies to ABA (Vysotskaya et al. 2004). For more details on the method and calculations, see Veselov et al. (2008).

Measurement of proline and MDA level

All leaves for Pro measurement were selected from the second to seventh leaf on the tenth branch (count from base to top) on each plant. We collected five leaves from each *A. sparsifolia*; thus, each quadrat had 50 leaves (5 × 10), for a total of 150 leaves (50 × 3). The measurement of proline level was conducted based on the method of Demiral and Türkan (2005). Each group of plant sample (0.5 g) was ground and mixed with 3 % (w/v) sulfosalicylic acid. Afterwards, homogenate was filtered through filter paper. After adding acid ninhydrin and glacial acetic acid, the mixture obtained was put in boiling water bath at 100 °C for 1 h. The reactant was then placed in an ice bath to terminate the reaction. The mixture was extracted with toluene, which was taken as a control. The extract was placed in a cuvette, and its absorbance was read at a wavelength of 520 nm. Proline concentration was calculated using a calibration curve and expressed as molproline g⁻¹ FW (fresh weight).

All leaves for Pro measurement were selected from the second to seventh leaf on the eleventh branch (count from base to top) on each plant. We collected five leaves from each *A. sparsifolia*; thus, each quadrat had 50 leaves (5 × 10), for a total of 150 leaves (50 × 3). The measurement of MDA level was conducted based on the method of Rahman et al. (2012). Thiobarbituric acid (TBA) reactive substances representing the lipid per oxidation products were extracted through the homogenization of a 0.2 g leaf sample in 5 mL of solution containing 20 % trichloroacetic acid and 0.5 % 2-TBA. The mixture was heated at 95 °C for 30 min, and the reaction was arrested by a sudden ice bath. The cooled mixture was centrifuged at 5000×g for 10 min at 25 °C, and the absorbance of the

supernatant at 532 and 600 nm was recorded. After subtracting the nonspecific turbidity at 600 nm, the MDA concentration was determined by its molar extinction coefficient, $155 \text{ M}^{-1} \text{ cm}^{-1}$ (Kosugi and Kikugawa 1985).

Data analysis

The differences of *A. sparsifolia* between treatments of CK, PG, LR and GR at day 1 and 30 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 significant differences were detected, LSD multiple range tests at $p < 0.05$ were employed to identify the statistically significant differences. Results shown in graphs are presented as the mean value \pm standard deviation.

Results

Carbohydrate characteristics after phloem were girdled and leaf were removed

In our study, we found that only after 30 days, significant changes were observed in its soluble sugar content. First, in terms of the leaves, 30 days after treatment, compared to CK, soluble sugar content in *A. sparsifolia* treated with PG and GR increased by 86.6 and 38.8 % ($p < 0.05$), respectively; whereas, that under the treatment of LR decreased by 8.7 % ($p > 0.05$) (Fig. 2a). With respect to the roots, 30 days after treatment, compared to CK, soluble sugar content in *A. sparsifolia* treated with PG and GR dropped by 30.7 and 35.8 % ($p < 0.05$), respectively (Fig. 2b).

Just as with soluble sugar, on the first day of girdling, no significant changes in the starch content of leaves and roots in *A. sparsifolia* were observed ($p > 0.05$). On the 30th day of girdling, the starch content of leaves in *A. sparsifolia* increased 40.3 and 26.8 %, respectively, under FG and SG compared to CK ($p < 0.05$). The starch content of leaves in *A. sparsifolia* treated with LR dropped by 7.3 % ($p > 0.05$); whereas, that in roots, *A. sparsifolia* treated with PG, LR and GR declined by 22.9 % ($p < 0.05$), 9.4 % ($p > 0.05$) and 24.2 % ($p < 0.05$) respectively, compared to CK.

Photosynthesis characteristics after phloem were girdled and leaf were removed

Pn, Gs, and Tr were measured and summed up over all measurement time points in the whole day, and then their differences were compared. In our experiment, no significant changes in the Pn of *A. sparsifolia* treated with LR and GR on the first day were observed; however, Pn of *A.*

sparsifolia treated with PG decreased 20.8 % ($p < 0.05$). Accordingly, Gs and Tr of *A. sparsifolia* treated with PG decreased by 13.3 % (Fig. 3a) and 28.3 % ($p < 0.05$) (Fig. 3e), respectively, on the first day.

On the 30th day of treatment, the Pn of *A. sparsifolia* under PG decreased by 84.2 % ($p < 0.05$) compared to CK; and a decrease of 58.3 % was observed in GR ($p < 0.05$) (Fig. 3d). Compared to CK, 30 days after girdling, Gs of *A. sparsifolia* treated with PG and GR decreased 71.5 and 41.2 % ($p < 0.05$), respectively; whereas, it rose 4.9 % when treated with LR ($p > 0.05$) (Fig. 3b). Changes in Tr were largely influenced by Gs. In our experiment, 30 days after treatment, Tr under PG and GR reduced by 46.1 and 21.4 % ($p < 0.05$), respectively; whereas, that under LR increased 4.9 % ($p > 0.05$) (Fig. 3f).

Photosynthetic pigments characteristics after phloem were girdled and leaf were removed

Our experiment found that 30 days after treatment, photosynthetic pigment content varied drastically among each kind of treatment. Compared with CK, Chl a, Chl b, total Chl, Car content, Chl a/b, and Chl/Car in *A. sparsifolia* leaves treated with PG fell decreased significantly ($p < 0.05$) (Fig. 4a–f). However, these parameters in the treatment of LR increased slightly. Similar to PG, Chl a, Chl b, total Chl, Car content, Chl a/b, and Chl/Car in *A. sparsifolia* leaves treated with PG had a great degree of decline; whereas compared with PG, the degree of decline was much less (Fig. 4a–f).

Water potential after phloem were girdled and leaf were removed

Our study found that on the 30th day of treatment, a significant change was observed in leaf water potential. Specifically, on the 30th day, predawn water potential in *A. sparsifolia* subjected to PG decreased by 70.9 % compared to CK ($p < 0.05$); whereas, midday water potential reduced by 38.5 % ($p < 0.05$) (Fig. 5a, b). Predawn water potential in *A. sparsifolia* subjected to GR treatment decreased by 30.0 % ($p < 0.05$); whereas, midday water potential reduced by 21.4 % ($p < 0.05$) (Fig. 5a, b).

Pro, MDA and ABA characteristics after phloem were girdled and leaf were removed

Our research found that after 30 days, Pro content had undergone significant changes, as demonstrated by an increase of 70.9 % ($p < 0.05$) under PG compared to CK, a rise of 23.2 % ($p < 0.05$) under GR treatment, and a fall of 4.9 % ($p > 0.05$) under LR compared to CK (Fig. 5c). In

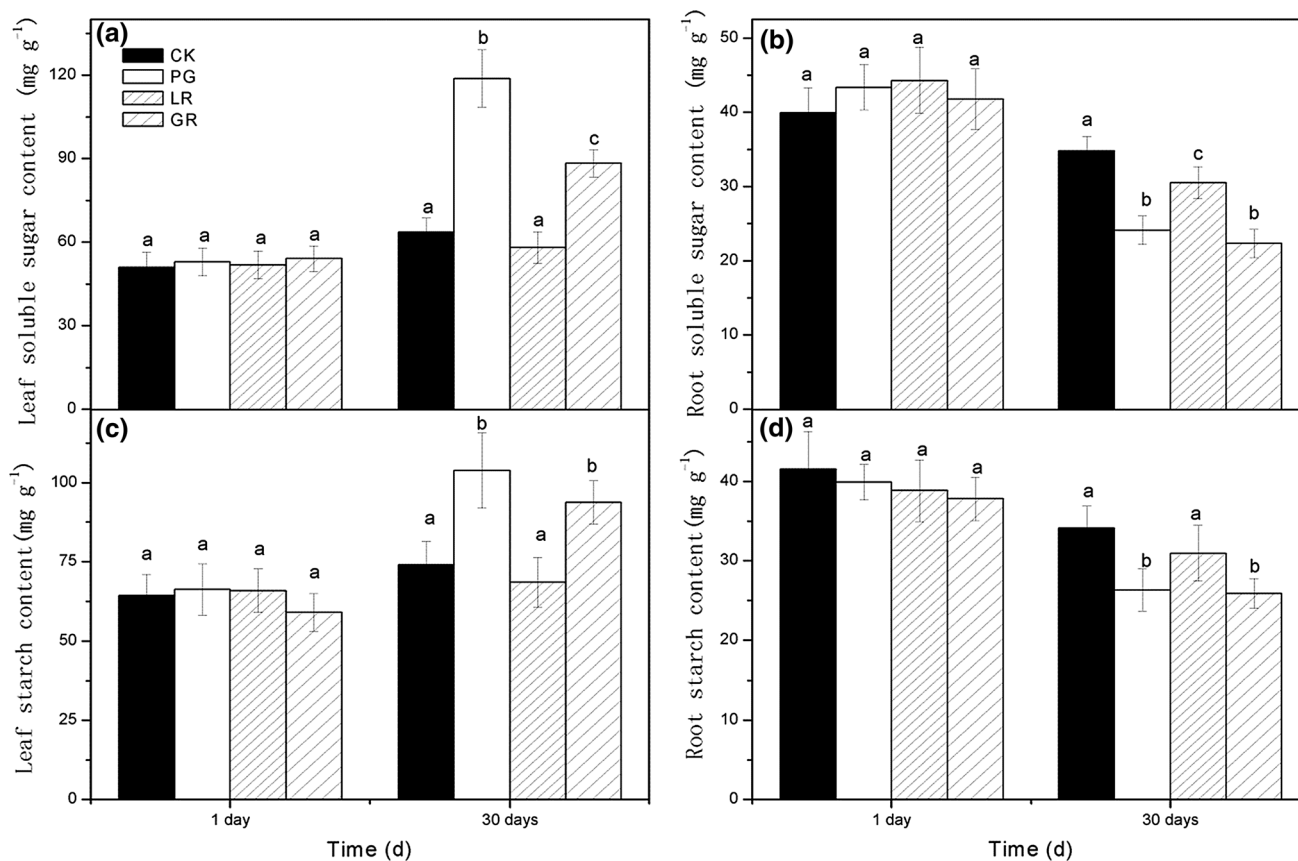


Fig. 2 Changes of soluble sugar and starch in control, phloem girdling, leaf removal, and combine girdling and removal after *A. sparsifolia* were treated at 1 and 30 days. **a** Leaf soluble sugar content. **b** Root soluble sugar content. **c** Leaf starch content. **d** Root

starch content. Different letters indicate a significant difference ($p < 0.05$). The data represent means of five biological replicates \pm SD. Meaning of CK, PG, LR, and GR. see Fig. 1

addition, 30 days of treatment made MDA content significantly change (Fig. 5d). Specifically, it increased 66.7 % ($p < 0.05$) under PG compared to CK, decreased 7.8 % ($p > 0.05$) under LR, and increased 15.1 % ($p > 0.05$) under GR (Fig. 5d). On the 30th day, compared to CK, ABA content in *A. sparsifolia* leaves subjected to PG and GR increased 248.3 and 80.0 % ($p < 0.05$), respectively; whereas, that in leaves subjected to LR fell 20.2 % ($p > 0.05$) (Fig. 5e).

Discussion

Senescence is a naturally occurring process for plant development, but high carbohydrate levels will accelerate the onset of the senescence process (Parrott et al. 2005, 2007). In the present study, changes in both soluble sugar and starch of *A. sparsifolia* leaves followed the same pattern in each treatment. Thirty days after treatment, soluble sugar and starch content in leaves treated with PG increased most obviously in leaves (Fig. 2), while the accumulation of

carbohydrates in the leaves of many girdled plant species was also reported (Li et al. 2003; Regier et al. 2010). Soluble sugar and starch content of *A. sparsifolia* leaves subjected to GR also significantly increased, but to a less obvious extent in relation to PG; whereas, soluble sugar and starch content of *A. sparsifolia* leaves subjected to LR displayed a slight decline. Here, we believe that sugar, whether directly used to supply or store energy, could significantly accumulate after PG; whereas, individual LR did not have a significant impact on carbohydrate content. Nevertheless, when combining LR with girdling, we could find that the accumulation of carbohydrates had been distinctly slowed.

Previous research shows that there is a correlation between leaf carbohydrate content and senescence rate (Mahouachi et al. 2009; Pourtau et al. 2004, 2006; Parrott et al. 2005, 2007, 2010; Wingler et al. 2006). In the present study, accompanied by changes in carbohydrate concentration, the rate of senescence of *A. sparsifolia* also exhibited noticeable changes, which could be seen from the velocity of plant yellowing. As observed from the

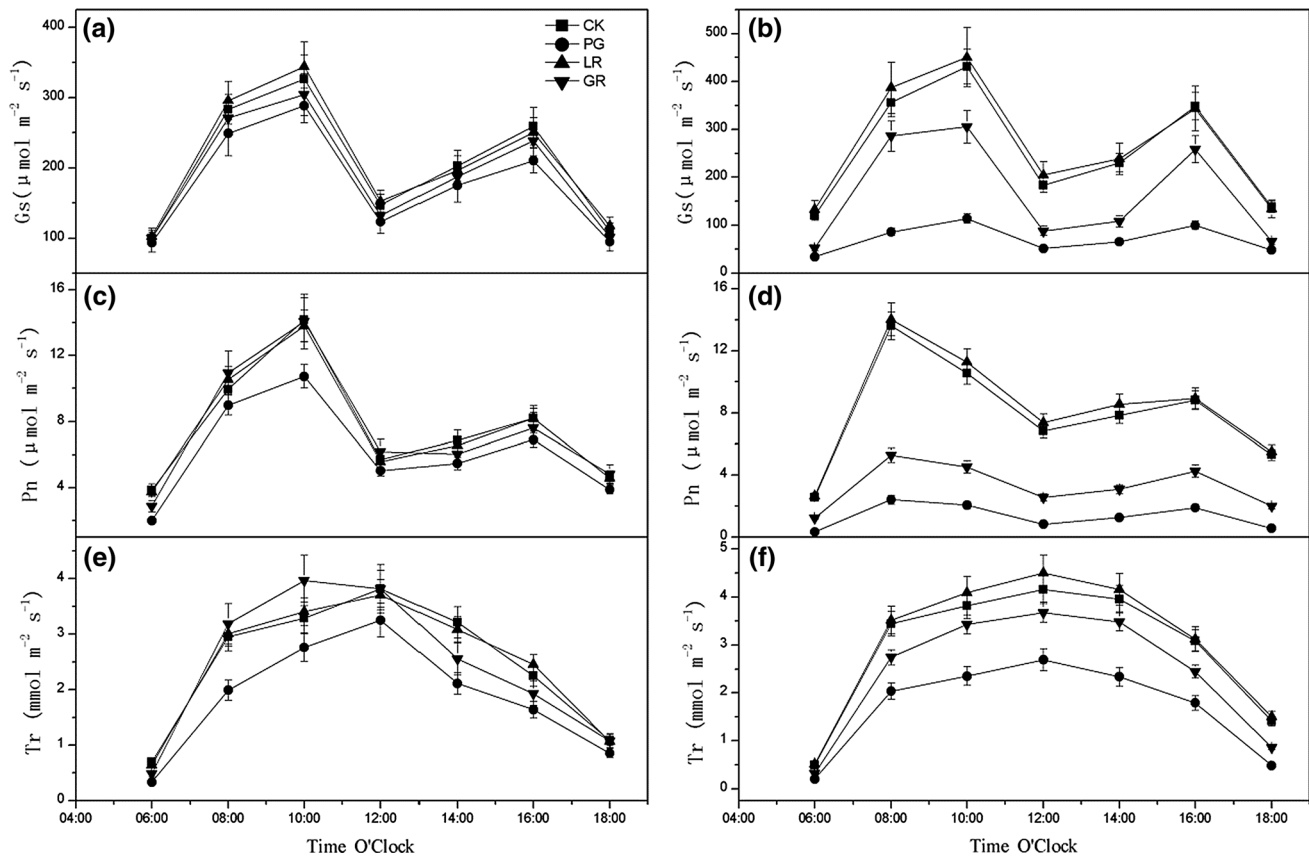


Fig. 3 Changes of stomatal conductance, photosynthesis, and transpiration rate of *A. sparsifolia* after girdling. **a** Stomatal conductance (1 day). **b** Stomatal conductance (30 days). **c** Photosynthesis rate (1 day). **d** Photosynthesis rate (30 days). **e** Transpiration rate (1 day).

f Transpiration rate (30 days). The data represent means of five biological replicates \pm SD. Meaning of CK, PG, LR, and GR. see Fig. 1

indicators of photosynthetic pigments (Fig. 4), *A. sparsifolia* leaves subjected to PG demonstrated greatly accelerated senescence; whereas, changes in photosynthetic pigments in leaves under GR also showed a consistent variation curve with photosynthetic pigments in the plant senescence process (Fig. 4). Regarding LR, senescence seems to be delayed to a certain degree (Fig. 4). In light of this result, we believe that PG and GR could clearly promote plant senescence, while LR could slightly reduce or delay plant senescence. Because of the strong relationship between this result and the contents of carbohydrates, our study demonstrates a similar conclusion to that made previously: in the process of senescence, whether it is natural or onset, the emergence or delay of senescence may be, to certain extent, induced by carbohydrate levels. Nevertheless, since we did not establish a linear relationship between the two factors, we will not exclude the role of other factors in the senescence induction process.

Weakened photosynthetic capacity of a plant is the principal symptom of senescence (Zhang et al. 2012). In our study, the reduction speed of photosynthesis under PG

is the most obvious, followed by GR treatment. This has also proven that PG and GR treatments could greatly accelerate the senescence process; whereas, LR has clearly and effectively delayed senescence, and caused a drop in Pn induced by girdling. Curiously, however, a reduction in Pn does not seem entirely consistent with variation in carbohydrates, because our experiment found that 1 day of PG also significantly reduced Pn, although the decline was still quite weak in relation to 30 days after treatment (Fig. 3). Our result is similar to that found in previous studies: a decline in Pn as a result of girdling treatment has been evidenced by many experiments (Di Vaio et al. 2001; Zhou and Quebedeaux 2003; Urban et al. 2004), and an increase in Pn as a result of LR treatment has also been evidenced by previous study (Petrie et al. 2000).

On the first day after treatment, since we did not find any accumulation of carbohydrates in plant leaves, the reduction in Pn on the first day should not be attributed to a feedback regulation of carbohydrates. So, what causes the appearance of a reduced rate of photosynthesis to always occur before plant senescence? We ascribe it to Gs, i.e.,

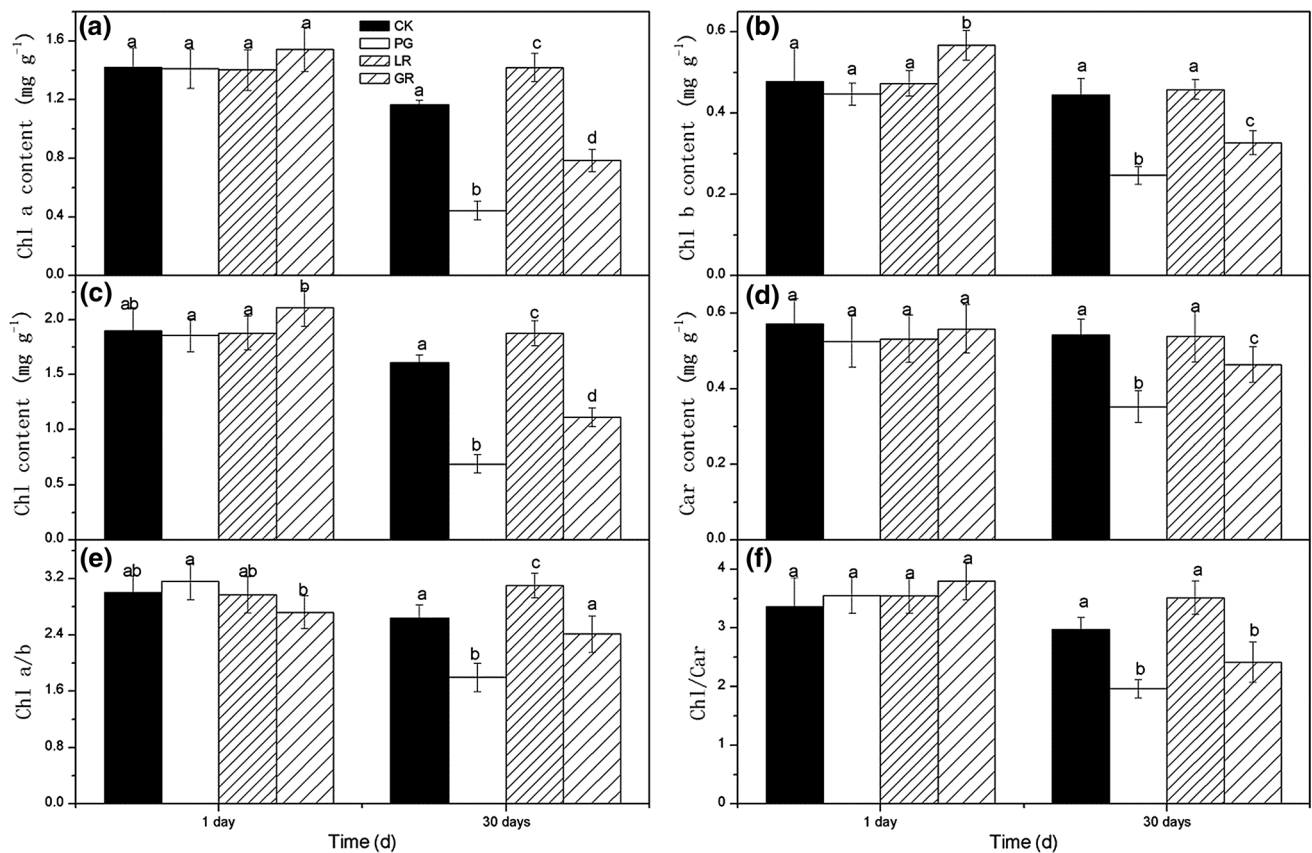


Fig. 4 Chl a content, Chl b content, Chl content, Car content, Chl a/b, and Chl/Car of *A. sparsifolia* under control, phloem girdling, leaf removal, and combine girdling and removal after 1 and 30 days. **a** Chl a content. **b** Chl b content. **c** Chl content. **d** Car content. **e** Chl a/b.

f Chl/Car. Chl: chlorophyll, Car: carotenoids. The data represent means of five biological replicates \pm SD. Different letters indicate a significant difference ($p < 0.05$). Meaning of CK, PG, LR, and GR. see Fig. 1

similar to light, stomata are one of the most sensitive indicators that control the photosynthetic rate of plants (Li et al. 2015). Our experiment indeed observed that Gs under PG showed a noticeable decline on the first day; whereas, the other two treatments (LR, GR) exhibited no noticeable alteration compared to CK, which was quite close to changes in Pn as demonstrated on the first day. From the diurnal variations of Gs and photosynthetic rate, we could also observe consistency between the two factors; thus, we think that the reduction in Pn on the first day was mainly caused by limitations of Gs.

It is impossible to explain a reduction in Pn induced by girdling and leaf removal entirely by using senescence as a result of carbohydrate concentrations which was showed by previous study (Pérez et al. 2011); however, it is possible by using changes in Gs. In this case, we need to identify some factors that lead to changes in Gs for verification. As ABA has been proven to be an important factor leading to reduced Gs in many experiments (Zhang et al. 2015), our experiment confirmed the relationship between girdling and ABA content, as well as Pn of *A. sparsifolia* leaves. In other words, on the first day, carbohydrate content did not change significantly, but

changes in ABA content began to appear under PG (Fig. 5e), which corresponded with declines in Gs, Tr, and Pn (Fig. 3). On the 30th day, ABA content in *A. sparsifolia* leaves subjected to PG and GR treatments rose dramatically, in which PG increased more significantly, while LR decreased slightly. This was probably because the obstruction in phloem sieve tubes caused by girdling blocked ABA from transporting downwards, thereby accumulating in leaves. Previous studies also support this view (Setter et al. 1980, 1981). ABA is a hormone that induces senescence. However, *A. sparsifolia* leaves subjected to PG which have more ABA content did not show symptoms of senescence on the first day, which might be entirely because ABA-induced senescence had a certain hysteretic nature. Therefore, we believe that in this experiment, induced senescence of leaves subjected to PG and GR should be resulted from a joint effect of increased carbohydrate concentrations and increased ABA content. Our result is similar to previous studies that have demonstrated that high ABA is always associated with induced senescence (Kumar et al. 2014).

A reduction of transpiration rate was found in the treatment of PG and GR, which may be caused by the

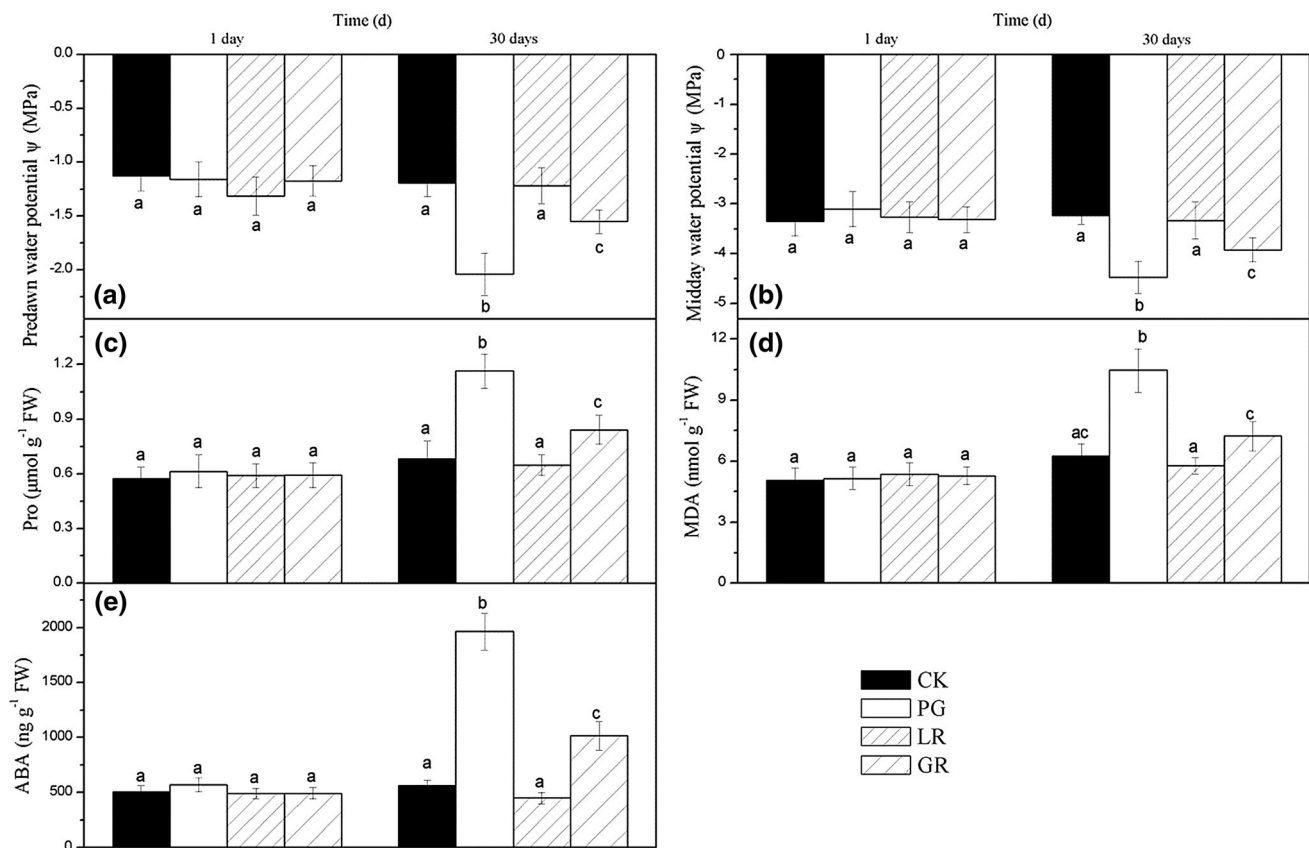


Fig. 5 Predawn water potential, midday water potential, Pro content, MDA content, and ABA content of *A. sparsifolia* under control, phloem girdling, leaf removal, and combine girdling and removal after 1 and 30 days. **a** Predawn water potential. **b** Midday water potential. **c** Pro content. **d** MDA content. **e** ABA content. Ψ : water

potential, Pro: proline, MDA: malonaldehyde, ABA: abscisic acid, FW: fresh weight. The data represent means of five biological replicates \pm SD. Different letters indicate a significant difference ($p < 0.05$). Meaning of CK, PG, LR, and GR. see Fig. 1

increase of ABA levels inducing stomatal closure, which was found by previous studies (Weaver and Amasino 2001; Agüera et al. 2012).

Although Setter et al. (1980, 1981) explained changes in ABA content as a result of ABA's failure to be transported from leaves to other organs, such as roots, we will not rule out the effect of other factors. Among the many environmental factors that have been proven to be able to cause changes in ABA content, drought is probably the most studied. Because water stress often leads to changes in plant ABA content, our experiment also studied the water status of *A. sparsifolia*, especially its predawn and midday leaf water potential. On the 30th day after treatment, the same variation pattern was displayed in both predawn and midday leaf water potential. Still, no noticeable changes were observed in the water potential of leaves subjected to LR; whereas, predawn and midday leaf water potential was significantly reduced under PG and GR, in which PG reduced more significantly (Fig. 5a, b). As a consequence, the reduction of water potential in *A. sparsifolia* might be caused by a change of carbohydrates, as we have evidenced

above. Girdling caused high concentrations of carbohydrates to accumulate in leaves, resulting in relatively high osmotic potential which was shown by our results above. Moreover, with low water status, leaf senescence was accelerated, which was proven in both our study and previous work (Wolfe et al. 1988). Redistribution of most of the minerals takes place through phloem (Palmer et al. 2014), which is closely related to senescence (Mae and Ohira 1981; Distelfeld et al. 2014). So in our study, obstruction of mineral nutrients from roots to leaves may another reason for leaf senescence in girdled *A. sparsifolia*. In addition, stem girdling interrupts phloem transport of acropetal flow of cytokinin (Kong et al. 2011), thus reduced levels of cytokinin which subsequently resulted in the acceleration of leaf senescence (Dai and Dong 2011). So induced senescence may also regulated by the change of phytohormones (e.g. cytokinin) in girdled *A. sparsifolia*.

When plants exposed to adversity stress (e.g. osmotic pressure and temperature stress), the content of Pro and MDA tend to increase dramatically (An et al. 2015; Brand 2011). Drought, high salinity, and high temperature often

lead to a substantial accumulation of Pro. In the present study, Pro increased in the treatment of PG and GR (Fig. 5c), which may be caused by low water potential, which was shown above. Under PG and GR, induced senescence was found, and a similar result has been obtained previously that demonstrated that Pro always accumulates during senescence (Rabiza-wider et al. 2004).

In senescence process in plants, a reduction in photosynthetic pigment content is an obvious fact, and has been considered as an important indicator of plant senescence (Hörtensteiner 2006; Wingler et al. 2006). In the present study, all of the pigments (Chl a, Chl b, and Car) decreased in the PG and GR leaves, in which senescence was obviously accelerated.

In some organs of species, such as radish cotyledons, the ratio of Chl a to Chl b increases slightly during senescence, which suggests that Chl b is degraded faster than Chl a during senescence (Suzuki and Shioi 2004). Some studies have also shown that Chl a is most strongly affected by leaf senescence as revealed by a significant decrease in the Chl a/Chl b ratio in leaves (Agüera et al. 2012). Our results support the assertion that the Chl a/Chl b ratio will decrease during senescence (Fig. 4e). Depending on the distinct divisions of Chl a and Chl b, Chl a has a greater absorption capacity of light energy than Chl b in the photosystem. Therefore, the ratio of Chl a/Chl b drops in the senescence process, which might partly explain why reduced Pn could not be entirely ascribed to reduced Gs, as we noted previously.

In addition to reduced Chl a/Chl b, we also observed reduced Chl/Car in the senescence process, which was evident in both GR and PG (Fig. 4f). It is not uncommon to get high degradation of Chl and high retention of Car in the senescence process (García-Plazaola et al. 2003). On the one hand, this shows that Chl is unstable and easier to be decomposed in relation to Car; whereas, on the other hand, it manifests the role of Car in the senescence process. While Car can also absorb some light energy and participate in the photochemical reaction in photosynthesis, one of its most important functions is to absorb residual light energy, quench reactive oxygen species and, thus, prevent membrane lipid peroxidation (Polívka and Frank 2010; Singh and Shah 2015; Ying et al. 2013). We have also proven this hypothesis by determining the content of MDA, because MDA is an indicator of plant cell membrane lipid peroxidation (Gawel et al. 2003). The rising levels of MDA (Fig. 5d) are likely to represent plant membrane lipid peroxidation; whereas, the high retention of Car probably exists to respond to this situation during senescence.

In conclusion, PG treatment is able to significantly accelerate plant senescence, as demonstrated by leaf yellowing, and a sharp decline in photosynthetic pigments

content and Pn. Induced senescence in PG leaves may be caused by high levels of carbohydrates and ABA content; low water condition may be also an important factor. Under PG treatment, carbohydrates and ABA formed by leaves could not be effectively transported to other organs and, thus, accumulated in leaves, thereby leading to accelerated senescence. Although LR would slow down the senescence process of plants, its delay on plant senescence was very limited. The treatment of GR is similar to PG, in that it significantly accelerates plant senescence, as demonstrated by the same parameter as PG. However, compared with PG, the senescence rate in GR leaves was much slower. This means that partial removal of leaves led to a compensation of girdling effects in the process of senescence.

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