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Source-to-sink relationship between green leaves and green pseudobulbs of C_3 orchid in regulation of photosynthesis

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Abstract

In this paper, photosynthetic characteristics of green leaves (GL) and green pseudobulbs (GPSB) of C_3 orchid *Oncidium* Golden Wish were first studied. Light saturation for photosynthesis and maximum photosynthetic rates (P_{max}) were significantly higher in GL than in GPSB. The results of the optimal PSII quantum yield (F_v/F_m ratio), electron transport rate (ETR), the effective photochemical quantum yield ($\Delta F/F_m'$) and nonphotochemical quenching (NPQ) of Chl fluorescence revealed that GPSB had lower light utilization than that of GL. Significantly higher photosynthetic pigments were found in GL than in GPSB. Alteration of source/sink ratio had no impact on all photosynthetic parameters for both GL and GPSB after a short term of 3 days or even a long term of 2 weeks of treatments although there were significant decreases in GL carbohydrate concentration of GL-darkened plants by the end of the day. However, decreases of all photosynthetic parameters of GL were observed in GL-darkened plants after 4 weeks of treatment compared to those of fully illuminated (FI) and GPSB-darkened plants. These results indicate that the level of carbohydrates in GL plays an important role in regulating their photosynthesis. Due to their lower photosynthetic capacities, GPSB function mainly as sinks. Darkening GPSB up to 2 weeks did not affect their own P_{max} and the P_{max} of GL and thus, did not result in significant decreases of total carbohydrate concentration of GPSB. As GPSB store a large amount of carbohydrates, it could also act as a source when the level of carbohydrates decreased. Thus, GL could depend on GPSB carbohydrates to regulate their photosynthesis when their source capacity was removed. However, 4 weeks after treatments, photosynthetic capacities of GL were significantly lower in GL- and GPSB-darkened plants than in FI plants, which could be due to the lower total soluble and insoluble sugar concentrations of both GL and GPSB in these plants.

Additional key words: carbohydrate; Chl fluorescence; green pseudobulbs; orchid; photosynthetic O_2 evolution.

Introduction

Orchids are either epiphytic or terrestrial in nature. Epiphytic orchids are typically found in tropical and subtropical environments. *Oncidium* species are native to Southeast Asia and the Pacific regions. They are an important ornamental crop in potted plants as well as cut flowers. These orchids have enlarged green pseudobulbs (GPSB). Evolution of epiphytism has been postulated to result in the formation of GPSB (Dressler 1990). The GPSB is characterized by the presence of very thick cuticle, absence of stomata and the abundance of water-storing cells (Arditti 1992). The GPSB of epiphytic orchids have been shown to be an integral organ in the survival and growth of orchids (Hew and Yong 1997). These 'false bulbs' are known to function as water-storage organs and as stores for carbohydrates and minerals. In addition, photosynthesis of GPSB can contribute positively to carbon balance by recycling respiratory carbon that would otherwise be lost. Therefore the GPSB are of physiological and ecological importance and survival value.

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Abbreviations: Chl – chlorophyll; DM – dry matter; ETR – electron transport rate; FI – fully illuminated; F_o – minimal fluorescence yield of a dark-adapted sample; F_m and F_v – maximal and variable fluorescence yields obtained from a dark-adapted sample upon application of a saturation pulse of radiation, respctively; F_m'– the maximum fluorescence at the steady state; F_t – the current fluorescence yield; GL – green leaf; GPSB – green pseudobulb; $\Delta F/F_m$' – the effective photochemical quantum yield; P_{max} – maximal photosynthetic O_2 evolution rate; NPQ – nonphotochemical quenching; PPFD – photosynthetic photon flux density.

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The partitioning of assimilates between sources and sinks results in phloem translocation. A source may be defined as an exporter of sugars to the phloem and a sink is an importer of sugar from the phloem. Sink organs utilize the imported assimilates for growth, maintenance, and storage (Zamski and Schaffer 1996). GL are the main sources of assimilates for growth especially in orchids (He and Woon 2008). In our previous study, we investigated source-to-sink relationship between GL and green flower petals (GFP) of CAM orchid *Dendrobium* cv. Burana Jade. It has been found that GFP function as sinks and depend on carbohydrates exported from GL for their development and growth (He and Woon 2008). Similarly, nonfoliar green organs such as GPSB may provide additional carbon through photosynthesis for growth. It was reported that GPSB could act as a storage organ for photoassimilates which support inflorescence development (Yong and Hew 1995a). In their natural habitat, would the GL and GPSB function efficiently as source if the photosynthetic capacity of either part was removed? Do GPSB function as both source and sink? How does the changing of source/sink ratio regulate the photosynthesis of GL or GPSB? Indeed, very little information is available to answer these questions. It was hypothesized as early as 1868 by Bouddinhault that the accumulation of photoassimilate in leaves has a role in regulation of photosynthetic rate (Paul and Foyer 2001). In plants, changes in the photosynthetic source/sink balance are important in regulating leaf photosynthetic rate through effects on the leaf carbohydrate status (Kasai 2008). Hence, the main objective of this study was to study the source-to-sink relationship between GL and GPSB of a C_3 orchid, *Oncidium* Golden Wish in the regulation of photosynthesis. We attempted to investigate the photosynthetic characteristics of GL and GPSB, and the source-to-sink relationship between GL and GPSB following a short- and long-term darkening of GL or GPSB. Source/sink ratios were manipulated by totally darkening either all GL or all GPSB while the remaining parts of the plants were kept fully illuminated. Photosynthetic pigments, Chl fluorescence, $P_{\max}$, and total soluble and insoluble carbohydrates of GL and GPSB were measured after altering the source-to-sink relationship between GL and GPSB for a short (a few days) or long term (a few weeks).

Materials and methods

Plant materials and experimental design: Mature plants of *Oncidium* Golden Wish at the pseudobulb stage without inflorescence (about 4–5 months from the bud stage) were obtained from a commercial nursery. These orchids are intermediate shade plants (He *et al.* 2004) and were acclimatized for two weeks in the greenhouse under a similar light condition with maximal photosynthetic photon flux density (PPFD) of 700–800 µmol m^{-2} s^{-1}. The daily ambient temperature ranged from 24 to 33°C. They were watered once a day, and fertilized and sprayed with fungicides twice a week. In order to study the source-to-sink relationship between GL and GPSB, all old or yellow leaves and axillary buds on the base of GPSB were removed. For some plants, all GL of one plant were wrapped with aluminium foils while GPSB were left uncovered. Another group of plants had the GPSB covered with aluminium foils, leaving the GL exposed to light. Hence, for these plants, either GL or GPSB were the only photosynthetic sources. For the third group, neither the GL nor the GPSB was wrapped and hence for these plants both GL and GPSB performed photosynthesis.

Measurement of photosynthetic O_2 evolution: The photosynthetic O_2 evolution of GL and GPSB were determined using a leaf disc O_2 electrode (*CB1D, Hansatech*, King's Lynn, Norfolk, UK). Each GL and GPSB section was placed in saturating CO_2 condition (1% CO_2 from 1 M carbonate/bicarbonate buffer, pH 9) as described by Ball *et al.* (1987). GL or GPSB disc was illuminated, starting from the lowest photosynthetic photon flux density, PPFD (96 µmol m^{-2} s^{-1}) to the highest one (1,200 µmol m^{-2} s^{-1}). The photosynthetic light-response curve was obtained by plotting the O_2 evolution rates against respective light intensity. For the $P_{\max}$ of GL and GPSB after 1, 2, and 3 days, and after 2 and 4 weeks of changing source/sink ratio, the plant materials were kept in a tray of distilled water in the laboratory under a PPFD of 1,200 µmol m^{-2} s^{-1} for 30 min to equilibrate. $P_{\max}$ was determined at a PPFD of 1,200 µmol m^{-2} s^{-1} at 25°C at saturating CO_2 conditions as described above.

Measurement of different Chl fluorescence parameters: GL and GPSB were harvested at 07:00 h for Chl fluorescence analysis. The optimal PSII quantum yield (F_v/F_m ratio), the effective photochemical quantum yield ($\Delta F/F_m{}'$), electron transport rate (ETR) and nonphotochemical quenching (NPQ) of Chl fluorescence were determined using the *Imaging-PAM* Chl Fluorometer (*Walz*, Effeltrich, Germany) at 25°C in the laboratory. GL and GPSB were predarkened under a piece of black cloth for 15 min prior to measurements. By using the *PAM* Chl fluorometer, images of fluorescence emission were digitized within the camera and *via* a *Firewire* interface (400 megabits/s) (*Firewire-1394*, Austin, TX, USA) to a personal computer for storage and analysis. Measuring light pulses were applied at low frequency (about 1 Hz) for measurement of initial Chl fluorescence, F_o images in quasi-dark state. During actinic illumination and saturation pulses, the frequency of measuring light pulses and image capture was automatically increased to about

10 Hz. The *Imaging-PAM* continuously measured the current fluorescence yield (F_t). In the absence of actinic illumination and upon application of a 0.8-s of maximal saturation pulse (2,400 μmol m^{-2} s^{-1}), the dark-level fluorescence yield (F_o) and the maximum fluorescence yield (F_m) were determined respectively, from which Chl fluorescence F_v/F_m ratio was calculated. After measurements of F_v/F_m ratio, rapid light curve measurements in the presence of actinic illuminations (Schreiber *et al.* 1997) were obtained through the application of a series of 10-s light exposures with increasing irradiance from 1 to 1,200 μmol(photon) m^{-2} s^{-1}. A 0.8-s of maximal saturation pulse (2,400 μmol m^{-2} s^{-1}) was applied after each actinic illumination to obtain maximal fluorescence yield (F_m'). Hence, in the presence of actinic illumination, the current fluorescence yield (F_t = F), and the maximum fluorescence (F_m') at the steady state, were determined, from which the effective PSII quantum yield, $\Delta F/F_m$' [(F_m'– F)/F_m')] and ETR (PPFD × $\Delta F/F_m$' × 0.5 × 0.84) (Rascher *et al.* 2000) could be calculated. The number of 0.5 represents a supposition that the excitations are equally distributed between PSII and PSI. Correction factor 0.84 takes into account that only a fraction of incident light is really absorbed by photosynthesis (Rascher *et al.* 2000). NPQ was defined as: NPQ = (F_m – F_m')/F_m'.

Measurement of photosynthetic pigments: 0.05 g of each GL and GPSB sample was extracted in dimethylformamide and quantified spectrophotometrically using the procedure of Wellburn (1994).

Soluble sugar extraction and determination: Both GL and GPSB were harvested at 07:00 h and 18:00 h respectively, and dried in the oven at 80°C for one week before carbohydrate analysis. Soluble sugars were extracted using the method described by Buysse and Merckx (1994). 0.05 g of dried GL and GPSB samples were extracted three times in 10 cm^3 of hot 80% (v/v) ethanol (65°C). For each time, the homogenate was centrifuged at 1,664 × *g* for 10 min and the supernatant collected. The supernatants were pooled and made to a convenient volume. To 1 cm^3 of extraction solution, 1 cm^3 of 28% (v/v) phenol was added, followed by 5 cm^3 of concentrated H_2SO_4. The reaction mixture was mixed thoroughly and then allowed to stand for 15 min. A small aliquot was poured into a 1 cm^3 glass curvette, and the absorbance was recorded at 490 nm using a spectrophotometer. Total sugar concentration of the sample was calculated based on a calibration curve from a glucose working standard.

Insoluble sugar extraction and determination: Insoluble sugars were extracted from the residual plant material from the soluble sugar extraction described above. This was done by incubating the dry pellet with 5 cm^3 of 3% (v/v) HCl in a boiling water bath for 3 h. The soluble products were assayed by the same phenol-sulphuric method as that of soluble sugars.

Table 1. Chlorophyll (Chl) fluorescence F_v/F_m ratio and photosynthetic pigments of GL and GPSB of *Oncidium* Golden Wish. The means and standard errors of 4 readings are given for each mean. *Letters* represent comparison between GL and GPSB (compared with a row). Means with *different letters* are statistically different (p<0.05).

Parameter	GL	GPSB
F_v/F_m ratio	0.81 ± 0.03 ^{a}	0.71 ± 0.02^b
Total Chl content [mg g^{-1}(DM)]	11.24 ± 0.64^a	1.55 ± 0.34^b
Chl *a*/*b* [mg mg^{-1}]	1.99 ± 0.04^a	1.25 ± 0.03^b
Total carotenoids content [mg g^{-1}(DM)]	1.89 ± 0.13^a	0.17 ± 0.05^b
Carotenoids/Chl [mg mg^{-1}]	0.17 ± 0.02^a	0.11 ± 0.01^b

Statistical analysis: For light-response curves of photosynthetic O_2 evolution (Fig. 1) and Chl fluorescence parameters (Fig. 2) and data shown in Table 1, *t*-test was used to test for differences of two different organs under the same PPFD. For Figs. 3 to 8, a separate *ANOVA* was used to discriminate means across all three treatments using *Tukey*'s multiple comparison test. All statistical analyses were carried out using *MINITAB* software (*MINITAB, Inc., Release 15*, 2007, USA).

Results

Photosynthetic characteristics of GL and GPSB: GL had gradual saturation for photosynthesis from PPFD of 600 μmol(photon) m^{-2} s^{-1}. However, the light saturation for GPSB photosynthesis was much lower, at about 400 μmol(photon) m^{-2} s^{-1}, than that of GL (Fig. 1). At each given PPFD, photosynthetic O_2 evolution rate was significantly higher in GL than in GPSB (Fig. 1, p<0.05). $\Delta F/F_m$', ETR and NPQ were used to explore the utilization of light energy by GL and GPSB. GL had $\Delta F/F_m$' values of 0.76 to 0.60 from PPFD of 50 to 400 μmol m^{-2} s^{-1}. A further decrease of $\Delta F/F_m$' in GL was observed when PPFD was higher than 400 μmol m^{-2} s^{-1} with its value almost zero at PPFD of 1,200 μmol m^{-2} s^{-1}. Although GPSB exhibited similar changes of

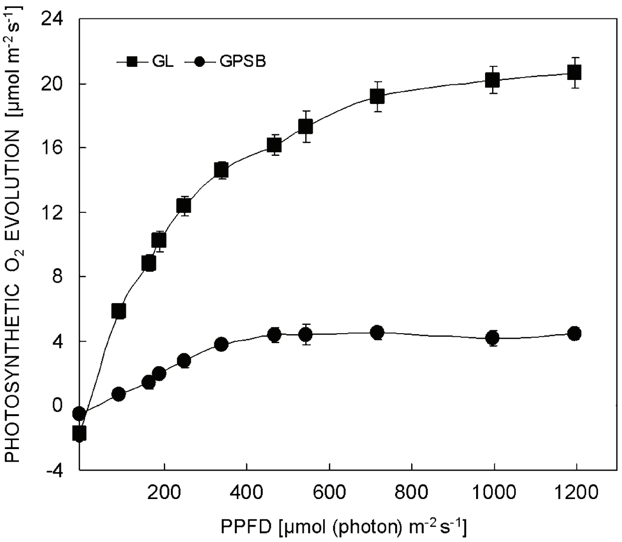

Fig. 1. Photosynthetic light-response curve of GL and GPSB of *Oncidium* Golden Wish under different PPFDs. Means of 4 measurements from 4 different GL and 4 different GPSB. *Vertical bars* represent standard errors. When the standard error bars cannot be seen, they are smaller than the symbols.

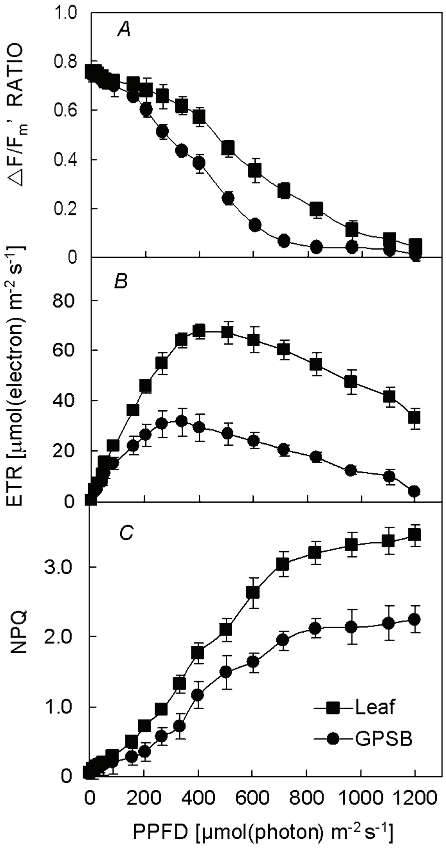

Fig. 2. Changes in $\Delta F/F_m$' ratio (*A*), ETR (*B*) and NPQ (*C*) of GL and GPSB of *Oncidium* Golden Wish under different PPFDs. Means of 4 measurements from 4 different GL and 4 different GPSB. *Vertical bars* represent standard errors. When the standard error bars cannot be seen, they are smaller than the symbols.

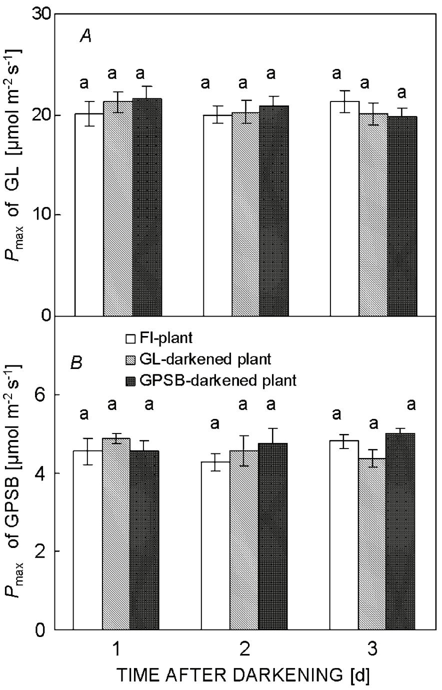

Fig. 3. P_{max} of GL (*A*) and GPSB (*B*) after altering source/sink ratio for 1, 2, and 3 days, respectively. Each value is the mean of 4 measurements of 4 different leaves. *Vertical bars* represent the standard errors. Means with *different letters* above the bars are statistically different ($p<0.001$) as determined by *Tukey*'s multiple comparison test.

increasing PPFD, a significant decrease of $\Delta F/F_m$' with increasing PPFD occurred at much lower PPFDs than that of GL at about 200 μmol m^{-2} s^{-1} (Fig. 2*A*). GL had significantly higher values of $\Delta F/F_m$' than that of GPSB at each PPFD from 200 to 1,200 μmol m^{-2} s^{-1} (Fig. 2*A*, $p<0.05$). Initially, there was a steep increase of ETR values in GL with increasing PPFD until 400 μmol m^{-2} s^{-1}, after which it decreased sharply (Fig. 2*B*). The ETR of GPSB was much lower at any given PPFD compared to that of GL from 200 to 1,200 μmol m^{-2} s^{-1}. There was a gradual increase in NPQ of GL from 50 to 800 μmol (photon) m^{-2} s^{-1} after which it reached a plateau around 3.2. The increase of NPQ was faster in GL than in GPSB (Fig. 2*C*) from 200 μmol m^{-2} s^{-1} onwards. The optimal PSII quantum yield F_v/F_m ratio measured from the dark-adapted tissues was significantly higher in GL than in GPSB ($p<0.05$) (Table 1). Photosynthetic pigments were also measured from the same plant tissues after recording the different Chl fluorescence parameters. GL had significantly higher total Chl concentration, Chl *a*/*b* ratio, total carotenoid concentration and carotenoids/Chl ratio compared to those of GPSB (Table 1) ($p<0.05$).

Effects of short-term alternation of the source-sink ratio on photosynthetic characteristics and carbohydrates: There were no significant differences in different Chl fluorescence parameters, photosynthetic

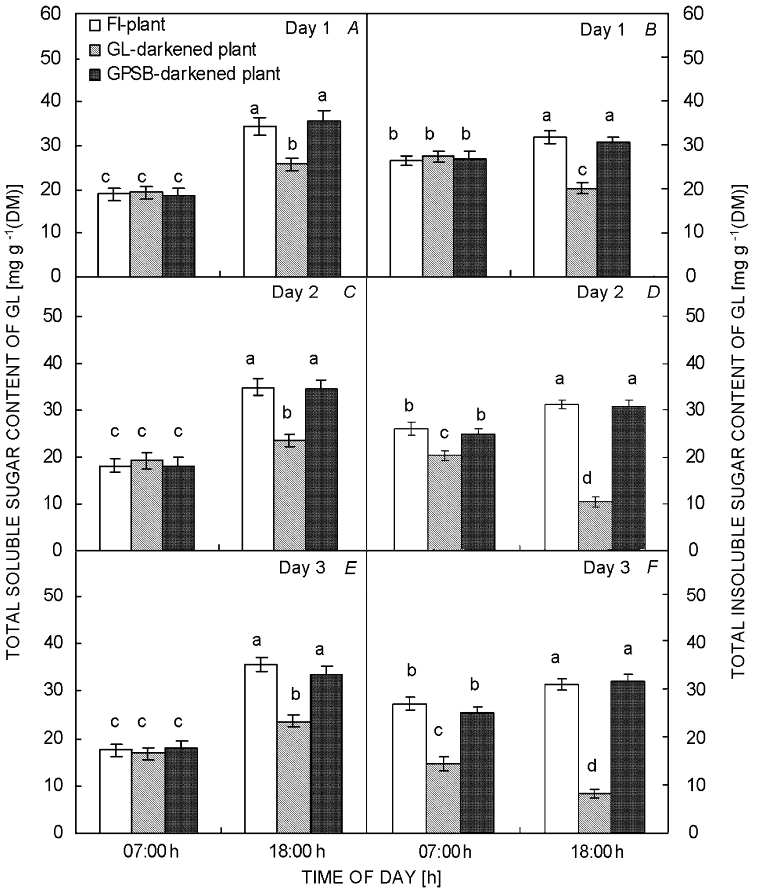

Fig. 4. Total soluble (*A,C,E*) and insoluble sugar concentrations (*B,D,F*) of GL measured at 07:00 h and 18:00 h after altering source/sink ratio for 1, 2, and 3 days, respectively. Each value is the mean of 4 measurements of 4 different leaves. *Vertical bars* represent the standard errors. Means with *different letters* above the bars are statistically different ($p<0.001$) as determined by *Tukey*'s multiple comparison test.

pigments (data not shown) and $P_{\max}$ of GL and GPSB during the 3-day period of altering source/sink ratio treatment (Fig. 3). Fig. 4 shows the total soluble and insoluble sugar concentration of GL after altering source/sink ratio for 1, 2, and 3 days, respectively. Total soluble sugar concentration was higher at 18:00 h than at 07:00 h for all GL regardless of treatments. There were no significant differences in total soluble sugar concentration of GL at 07:00 h among the different treatments while this parameter was lower in GL-darkened plants than in the other two plants at 18:00 h (Fig. 4*A,C,E*). For total insoluble sugar concentration, on day 1, there were no significant differences in total insoluble sugar concentration of GL among the different treatments at 07:00 h. Compared to those of FI and GPSB plants, GL-darkened plants had significantly lower total insoluble sugar concentration than their GL at 18:00 h, which was even lower than at 07:00 h. However, FI and GPSB-darkened plants had similar higher total insoluble sugar concentration at 18:00 h than at 07:00 h (Fig. 4*B*). On days 2 and 3, total insoluble sugar concentration of GL was significantly lower in GL-darkened plants than in GL of FI and GPSB-darkened plants at both 07:00 h and 18:00 h (Fig. 4*D,F*). Total soluble and insoluble sugar concentrations of GPSB are illustrated in Fig. 5. Similar to those of GL during the 3-day of different treatments, there were no significant differences in total soluble sugar concentration of GPSB at 07:00 h among the different treatments (Fig. 5*A*,*C*,*E*). On the day 1, total insoluble sugar concentrations of GPSB were similarly lower at 07:00 h than at 18:00 h for FI and GPSB-darkened plants but this value was lower at 18:00 h than at 07:00 h in GL-darkened plants (Fig. 5*B*). On days 2 and 3, total insoluble sugar concentrations of GPSB were lower in GL-darkened plants than in FI and GPSB-darkened plants at both 07:00 h and 18:00 h (Fig. 5*D*,*F*).

Effects of long-term alternation of the source-sink ratio on photosynthetic characteristics and carbohydrates: Two weeks after altering source/sink ratio, there were no differences in F_v/F_m ratio, $\Delta F/F_m$', ETR, and photosynthetic pigments of GL (Figs. 6, 7) and GPSB (data not shown). Regardless of treatments, all plants had similar values of $P_{\max}$ for GL (Fig. 8*A*) and GPSB (Fig. 8*B*), respectively. Four weeks after treatments, F_v/F_m ratio (Fig. 6*A*), $\Delta F/F_m$' (Fig. 6*B*), ETR (Fig. 6*C*), photosynthetic pigments (Fig. 7) and $P_{\max}$ of GL (Fig. 8*A*) were much lower in GL-darkened plants than in both FI and GPSB-darkened plants. However, there were no significant differences in F_v/F_m ratio, $\Delta F/F_m{}'$, ETR, photosynthetic pigments (data not shown), and $P_{\max}$ of GPSB (Fig. 8*B*) after 4 weeks of altering source/sink ratio.

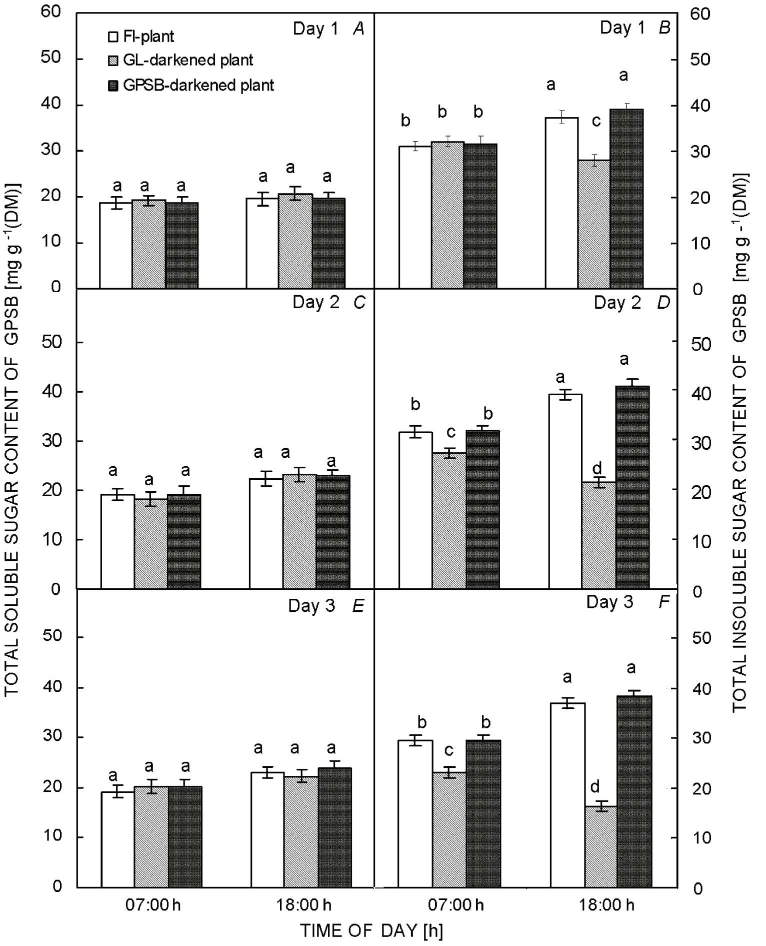

Fig. 5. Total soluble (*A,C,E*) and insoluble sugar concentrations (*B,D,F*) of GPSB measured at 07:00 h and 18:00 h after altering source/sink ratio for 1, 2, and 3 days, respectively. Each value is the mean of 4 measurements of 4 different leaves. *Vertical bars* represent the standard errors. Means with *different letters* above the bars are statistically different ($p<0.001$) as determined by *Tukey*'s multiple comparison test.

Fig. 9 shows the changes in total soluble and insoluble sugar concentrations in GL and GPSB after 2 and 4 weeks of darkening of GL or GPSB. Only the data obtained at 18:00 h are shown. The total soluble sugar concentrations of GL and GPSB were lower in GL-darkened plants than in GPSB-darkened plants and FI plants after 2 weeks of treatments. After the same periods of 2 weeks treatments, there were no significant differences in total soluble sugar concentration of GL between GPSB-darkened plants and FI plants (Fig. 9*A*,*C*). However, the total soluble sugar concentrations of GL and GPSB were lower in GL- and GPSB-darkened plants than in FI plants after 4 weeks of treatments and the decreases were much greater in GL-darkened plants than in GPSB-darkened plants (Fig. 9*A*,*C*). Both GL and GPSB had significantly lower total insoluble sugar concentration in both GL- and GPSB-darkened plants as compared to those of FI plants after 2 and 4 weeks of treatments (Fig. 9*B*,*D*). The decreases were greater in GL-darkened plants than in GPSB-darkened ones and also, they were greater after 4 weeks than after 2 weeks of treatments (Fig. 9*B*,*D*).

Discussion

The GPSB is an important water-storage organ. It maintains relatively high water content of 91.5% as compared to GL of 74.7%. There were no significant changes in water content for both GL and GPSB after short- and long-term altering of source/sink ratio (data not shown). In view of the ephiphytic habitat which is characterized by frequent periods of water shortage, it is conceivable that the process of evolution has resulted in the development of massive water-storage organs (Ng and Hew 2000). For GPSB of *Oncidium* used in the present study, they have photosynthetic capacities and function essentially for the refixation of respiratory carbon produced by the underlying massive parenchyma (Yong and Hew 1995a), though their Chl concentration and carbon fixation rates were lower than that of GL (Table 1, Fig. 1). Similar studies showed that nongreen petals of CAM *Dendrobium* Sonia were capable of photosynthesizing but at a lower amount of radiant energy (Khoo *et al.* 1997, He *et al.* 1998). He and Teo (2007) also reported that the photosynthetic rate of non-folia photosynthetic organ such as green petals of *Dendrobium* Burana Jade was only 30–40% that of GL. Results of the present study (Figs. 1, 2) show that the photosynthetic capacities of nonfolia photosynthetic organ, GPSB were much lower than that of GL. Quantum yield (QY) was also much lower in GPSB than in GL (Fig. 1). The lower QY of GPSB could be due to their lower photosynthetic pigments (Table 1). On the other hand, GPSB lack of functional stomata (unpublished data). This could also be partially responsible for the lower photosynthetic gas exchange (Fig. 1). Haupt-Herting and Fock (2002) reported that electron transport to O_2 increased relative to gross photosynthetic rate. Mehler reaction could act as a sink for electron transport (Heber 2002). However, the normal capacity of Mehler reaction to consume electron is probably small for GL (Biehler and Fock 1996, Haupt-Herting and Fock 2002). It is unclear if there is an enhanced Mehler reaction for GPSB in the present study which consumes O_2 so that the net O_2 evolution was small. NPQ normally increases with Mehler reaction (Blokhina *et al.* 2003). However, in this study, NPQ was significantly lower in GPSB than in GL (Fig. 2*C*). Organs or tissues that produce more assimilates than they require for their own metabolism and growth are the sources. Thus they are net exporters or producers of photoassimilate (Zamski and Schaffer 2006). GPSB of orchid plants functioning as additional photosynthetic organs could be due to the fact that most of the orchids are shade plants and are source-limited (Hew and Yong 1994, Yong and Hew 1995a). However, due to their lower photosynthetic capacities, GPSB could function primarily as sinks (Zamski and Schaffer 2006). Hence, in the present study, the authors attempted to address this question – how does alteration of source/sink ratio regulate the photosynthesis of GL or GPSB?

In the present study, it was observed that on day 1 (a sunny day) of altering source/sink ratio, the immediate photosynthetic product of GL, the soluble sugar concentration, decreased significantly by the end of photoperiod

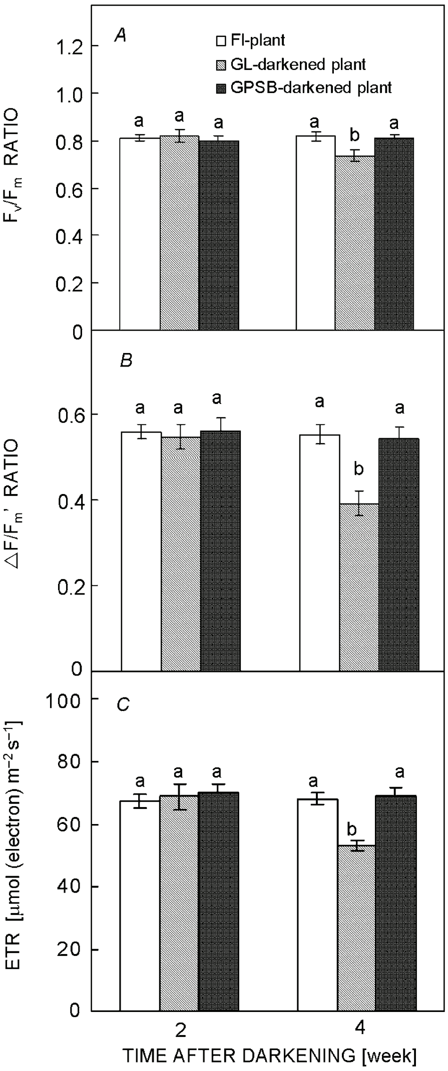

Fig. 6. F_v/F_m ratio (*A*), $\Delta F/F_m$' ratio (*B*) and ETR (*C*) of GL after altering source/sink ratio for 2 and 4 weeks, respectively. Both $\Delta F/F_m$' ratio and ETR were measured at 400 µmol m^{-2} s^{-1}. Each value is the mean of 4 measurements of 4 different leaves. *Vertical bars* represent the standard errors. Means with *different letters* above the bars are statistically different ($p<0.001$) as determined by *Tukey*'s multiple comparison test.

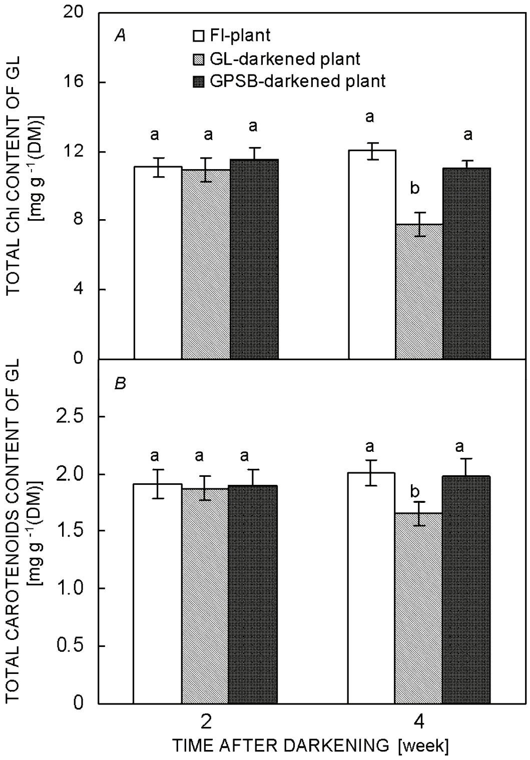

Fig. 7. Total Chl (*A*) and carotenoid concentrations (*B*) of GL after altering source/sink ratio for 2 and 4 weeks, respectively. Each value is the mean of 4 measurements of 4 different leaves. *Vertical bars* represent the standard errors. Means with *different letters* above the bars are statistically different ($p<0.001$) as determined by *Tukey*'s multiple comparison test.

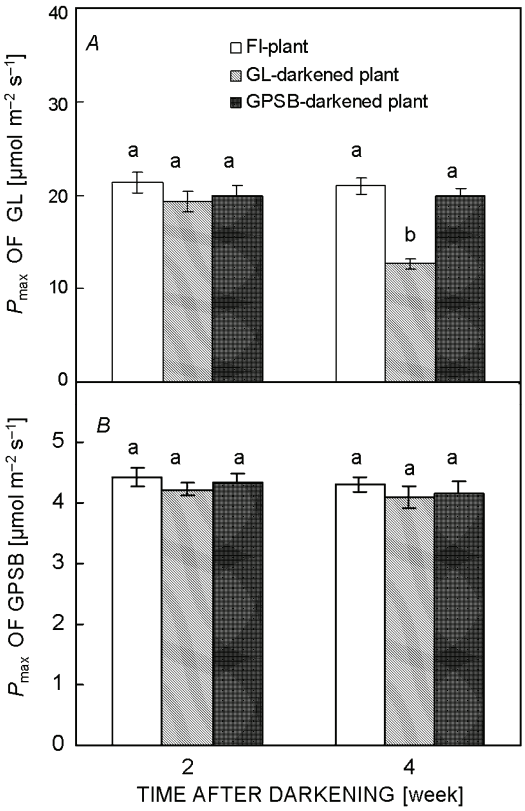

Fig. 8. P_{max} of GL (*A*) and GPSB (*B*) after altering source/sink ratio for 2 and 4 weeks, respectively. Each value is the mean of 4 measurements of 4 different leaves. *Vertical bars* represent the standard errors. Means with *different letters* above the bars are statistically different ($p<0.001$) as determined by *Tukey*'s multiple comparison test.

at 18:00 h when the photosynthetic source of GL was removed (Fig. 4*A*) and this was observed for the consecutive two sunny days (Fig. 4*C*,*E*). GPSB of GL-darkened plants also had lower total insoluble sugar concentration on the 3 consecutive days at 18:00 h (Fig. 5*B*,*D*,*F*), supporting the fact that GPSB functions as sink which stores the soluble sugar transported from GL and could also transport their stored soluble sugars to GL. These findings also imply that metabolism such as photosynthesis of GL could depend on the carbohydrates accumulated during the day and that carbohydrate stored in GPSB represents an important supplementary source of carbohydrates. These arguments could be supported by the fact that there were no significant differences in P_{max} of GL among all plants for any of the three days after altering source/sink ratio (Fig. 3*A*). Removal of GL capacity to produce carbohydrate for 3 days did not affect their $P_{max.}$ This could be due to the fact that darkened GL could regulate their P_{max} through the hydrolysis of insoluble sugar to soluble sugar of both GL and GPSB. Thus, maintaining the optimal level of soluble sugar required for cellular function, particularly in sustaining chloroplast structure and function, could help to maintain photosynthetic capacity. Hydrolysis of insoluble sugar to soluble sugar in GL and GPSB of GL-darkened plants was supported by the lower concentration of total insoluble sugar of GL and GPSB of GL-darkened plants. But constant concentrations of total soluble sugar were observed among different treatments at 07:00 h on day 2 regardless of low total soluble and insoluble sugars at 18:00 h from day 1 due to the removal of source capacity for GL compared to that of the FI plants (Fig. 4*A*,*B*,*C*,*D*). Our results also showed that darkening GPSB had no

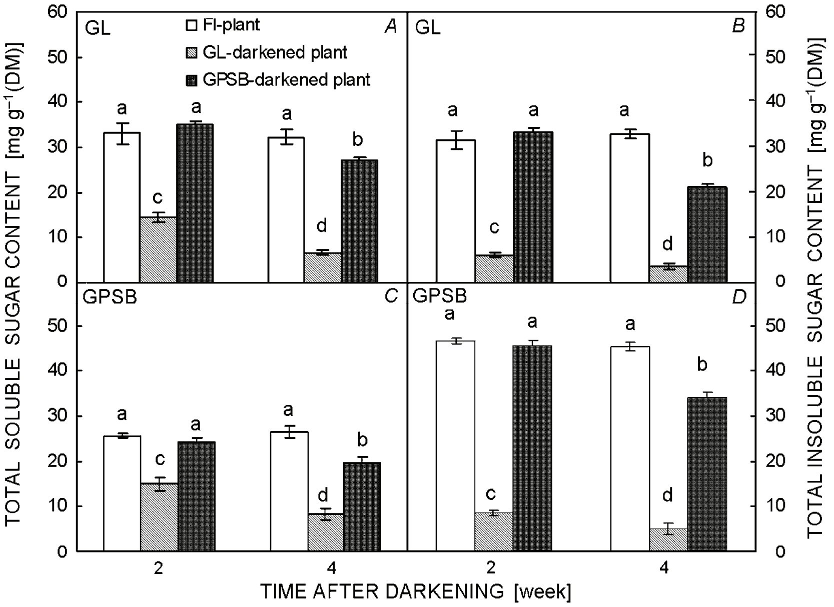

Fig. 9. Total soluble (*A, C*) and insoluble sugar concentrations (*B, D*) of GL and GPSB measured at 18:00 h after altering source/sink ratio for 2 and 4 weeks, respectively. Each value is the mean of 4 measurements of 4 different leaves. *Vertical bars* represent the standard errors. Means with *different letters* above the bars are statistically different ($p<0.001$) as determined by *Tukey*'s multiple comparison test.

effects on P_{max} of GL (Fig. 3*A*), total soluble and contributions to the GL in terms of carbohydrate status insoluble sugars of GL (Fig. 4) on any given day during the three days of treatment compared to FI plants. In another word, removing the capacity of GPSB to produce carbohydrates did not affect the P_{max} and carbohydrate production of GL. These results further confirmed that GPSB mainly acted as a sink and their source and P_{max} were negligible when plants were subjected to a short-term alteration of source/sink ratio. For GPSB, their P_{max} was not affected over the 3-day short period of source/sink alteration (Fig. 3*B*). Furthermore, over the same treatment period, there were no significant differences in total soluble sugar concentration of their GPSB among the different treatments at both 07:00 h and 18:00 h (Fig. 5*A*,*C*,*E*). Removing GL and GPSB capacity to produce carbohydrate for 3 days did not affect the levels of total soluble sugar concentration of GPSB; the steady total soluble sugar concentration could result from the hydrolysis of their total insoluble sugar that was reflected by their lower insoluble sugar concentration (Fig. 5*B*,*D*,*F*). Some researchers failed to relate rate of photosynthesis to source-sink relationships, suggesting that photosynthesis per se does not allow to arrive at specific conclusions on source or sink limitation (Plaut *et al.* 1987, Wada *et al.* 1993). However, in the present study, large GPSB of *Oncidium* Golden Wish constitutes almost two-third of the total biomass (data not shown). Maintaining such a large biomass of GPSB requires substantial amount of energy in the form of carbohydrates, which clearly plays an important role in regulation of GL photosynthesis and thus, the carbon balance of the plant.

Altering source/sink ratio by darkening GL for 2 weeks did not have significant impact on photosynthetic characteristics of both GL (Figs. 6, 7, 8) and GPSB (Fig. 8). For the same period, GL and GPSB of GL-darkened plants had significantly lower total soluble and insoluble sugar concentration compared to FI and GPSB-darkened plants (Fig. 9), indicating that the levels of carbohydrates were still sufficient to maintain the substantial level of photosynthesis. Maintaining moderate level of carbohydrates in GL without photosynthesis is obviously dependent on the supply from GPSB and this is possible as the DM of GPSB was 2.5 fold higher than that of GL for one single shoot (data not shown). Furthermore, GPSB had significantly higher concentration of insoluble sugar than that of GL. GPSB could switch from acting as a strong carbohydrate sink when GL were actively photosynthesizing to become a strong source when GL were subjected to unfavourable conditions such as long period of cloudy days. Although the amount of imported assimilates was lower and reasons for mature leaves acting as a sink were unclear, mature orchid leaves that imported assimilates from other nonfoliar photosynthetic organs were reported previously (Clifford *et al.* 1992, Wadasinghe and Hew 1995, Yong and Hew 1995a, b). Insoluble sugar reserves of GPSB act as important source of exported assimilates which can be remobilized to support the growth of GL and other plant tissues. Functioning primarily as sinks but with additional source capacity, GPSB points to the dynamism and plasticity in plant growth and development. For horticultural practice, it would be useful to further understand how environmental conditions would ultimately modulate the pattern of growth and development through their effects on source/sink relations between GL and GPSB. However, 4 weeks after removing the source capacity of GL, total soluble (Fig. 9*A*) and insoluble sugar concentrations of GL (Fig. 9*B*) were too low to sustain their optimal level of photosynthesis as reflected by their lower F_v/F_m ratio, $\Delta F/F_m'$, ETR (Fig. 6), photosynthetic pigment (Fig. 7) and P_{max} (Fig. 8). These findings suggest that removing GL source capacity for longer period affected the photosynthetic capacity of GL. There is evidence suggesting that high carbohydrate accumulation in leaves could negatively regulate leaf photosynthetic rate (Foyer and Galtier 1996, Paul and Foyer 2001, Kasai 2008). In the present study, low levels of carbohydrate in GL after reducing photosynthetic source/sink balance could also adversely affect the photosynthetic rate of GL for C_3 orchid *Oncidium* Golden Wish (Figs. 6, 7, 8*A*). Thus, the level of photoassimilate in GL has an important role in regulating photosynthetic rate (Paul and Foyer 2001). Four weeks after treatments, GPSB-darkened plants had significantly lower total soluble and insoluble sugar concentration not only in their GL but also in their GPSB (Fig. 9). These results imply that long-term subjecting of green nonfoliar tissue such as GPSB to darkness may influence their capability of importing carbohydrates from their source leaves. Ultimately, it may also affect their capability of exporting carbohydrates to GL when their source capacity was removed. The role of light in regulating import and export of carbohydrates from GPSB merits our further study.

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