VARIATION IN PHOTOSYNTHETIC CHARACTERISTICS ALONG THE LEAF BLADE OF ONCIDIUM GOLDIANA, A C3 TROPICAL EPIPHYTIC ORCHID HYBRID

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Photosynthesis was studied in relation to leaf development in leaves of the tropical C3 epiphytic orchid hybrid Oncidium Goldiana. Variations in total chlorophyll, maximum Rubisco activity, and maximum photosynthetic capacity were detected along the leaf blade in both developing and fully expanded mature leaves. Increasing gradients of total chlorophyll, Rubisco, and photosynthetic activity were detected from the base to the apex in both developing and fully expanded mature leaves. Light response curves of basal and apical portions of leaves showed a higher photosynthetic capacity in the apex regardless of the stage of development. Correlation analyses revealed significant positive relationships between total chlorophyll, maximum Rubisco activity, and maximum photosynthetic capacity. Our results indicate a gradient of photosynthetic capacity along developing and fully expanded mature leaf blades of Oncidium Goldiana.

Introduction

Leaf photosynthesis serves as the major source of carbon for growth and development (Wardlaw 1990). The regulation of leaf growth and development is a critical factor in crop yield because these processes ultimately determine the photosynthetic area available for carbon assimilation (Tichá 1985; Turgeon 1989). Carbon metabolism in leaves can change profoundly during development. In general, young leaves are heterotrophic and depend on imported photoassimilates to sustain their growth. This dependence gradually diminishes as the leaf matures. Mature leaves are autotrophic and possess the capacity for photosynthesis and for export of photoassimilates to other parts of the plant (Turgeon 1989; Robinson-Beers et al. 1990).

Leaf development has been studied in a variety of plants, both dicotyledons (e.g., Cucumis sativus; Ho et al. 1984) and monocotyledons (e.g., Lolium multiflorum; Prioul et al. 1980). Grass leaves have been used substantially in the study of leaf development because (1) the young leaf blade is composed of a gradient of tissues of different ages, and (2) early differentiation takes place within the leaf sheath under low irradiance (Prioul et al. 1980). It was demonstrated in L. multiflorum that a decreasing gradient from apex to base is maintained for functional and structural characteristics like net photosynthesis, chlorophyll content, Rubisco activity, mesophyll cell numbers, and chloroplast numbers in fully expanded leaves (Prioul et al. 1980).

To date, no research on leaf maturation and the development of photosynthetic characteristics has been reported for orchids. Orchids are monocotyledons and form one of the largest families of angiosperms. They are distributed over a wide range of ecological habitats although a large proportion are epiphytic and are confined to tropical and subtropical regions (Dressler 1981; Arditti 1992).

We are interested in the development of photosynthetic characteristics in relation to leaf maturation in orchids, and how similar/different is the process of transformation from heterotrophic growth to autotrophic growth when compared to grass leaves. We examined various photosynthetic parameters (maximum photosynthetic capacity, chlorophyll content, and Rubisco activity) along the leaf blade. These characteristics were compared among developing and fully expanded leaves of Oncidium Goldiana.

Material and Methods

Plants

Experiments were performed with the sympodial, thin-leaved orchid hybrid Oncidium Goldiana (O. sphaecatum × O. flexuosum), an epiphytic C3 shade plant (Hew and Yong 1994). Plants were grown in sand contained in plastic pots (15 cm diam) and watered daily. The environmental conditions in the greenhouse were as follows: minimum temperature of 25°C, maximum temperature of 36°C; maximum light levels (PPFD) of 300–400 μmol m⁻² s⁻¹ (at noon); 12 h photoperiod; minimum RH of 63%, maximum RH of 99%. An optimal regime of fertilization (Foliar Fertilizer 67, Bluesky Agricultural Supplies, Singapore; 1.6 g in 1 L of water; nitrogen: phosphorus: potassium ratio of 13.5:27:27) was provided twice weekly throughout the experimental period (Hew and Ng 1996).

Growth Studies

Growth of leaf L2 (fig. 1) was monitored over a period of ca. 70 d. Blade length, area, fresh weight, and dry weight were measured at various times. Six independent samples were analyzed for each data point.

Chlorophyll Analysis

Total chlorophyll, chlorophyll a and chlorophyll b were analyzed using the Arnon (1949) method. In this experiment, the length of the developing leaf L2 was expressed as a percentage of the mean final blade length (FBL) of leaf L2 (30 cm). Leaf L2 at 33% FBL (10 cm), 57% FBL (17 cm), 80% FBL (24 cm), and 100% FBL (30 cm) was used for chlorophyll determination. Each leaf was cut transversely into 1-cm segments, weighed, and subjected to chlorophyll extraction using 80% acetone. For this and subsequent ex-

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Fig. 1 Diagrammatic representation of a single shoot of Oncidium Goldiana showing the relative positions of the various leaves. Experiments, four independent samples were analyzed for each data point.

Rubisco Analysis

Leaf L2 at four developmental stages (33% FBL, 57% FBL, 80% FBL, and 100% FBL) was used for Rubisco analysis. Fresh leaf tissue (harvested 6 h into the photoperiod) was cut transversely into segments of 2 cm or 3 cm, weighed, and rapidly ground in fine sand in a chilled mortar using a 1:10 tissue-to-buffer ratio. The buffer contained 100 mM Tricine-NaOH (pH 7.5), 5 mM MgCl₂, 10 mM DTT, and 12.5% glycerol (v/v). Magnesium chloride (5 mM) was included as a precaution against possible deactivation of the enzyme during extraction (Servaites et al. 1984). Before use, the extraction buffer was purged of CO₂ by flushing with purified nitrogen gas. The homogenate was centrifuged at 1200 g for 2 min. Preliminary experiments indicated that all assay conditions were optimal.

Maximum Rubisco activity was measured at 25°C by adding extract (100 μL) to an assay mixture (400 μL) containing (final concentrations) 100 mM Tricine-NaOH (pH 7.8), 25 mM MgCl₂, 10 mM DTT, 1 mM RuBP, and 20 mM NaH¹³CO₃ (2.05 GBq μmol⁻¹; Amersham International, England). The reaction was initiated by the addition of RuBP and terminated 10 min later by the addition of 200 μL of glacial acetic acid. After drying at 80°C, the acid-stable ¹³C was determined by liquid scintillation spectrophotometry using 5.5 mL of Ecoscint, a commercial scintillation cocktail (National Diagnostics, U.S.A.). Counting efficiency was high (93% and 96%), and counts in absolute disintegrations were corrected for background with a Beckman LS 6000LL scintillation counter (Beckman Instruments, U.S.A.).

Photosynthetic Measurements

Photosynthetic O₂ evolution was determined by the Hansatech leaf disc oxygen electrode according to Delieu and Walker (1981, 1983). Leaf L2 at four developmental stages (33% FBL, 57% FBL, 80% FBL, and 100% FBL) was selected. Leaf L2 was cut transversely into segments measuring 2 cm or 3 cm. Leaf discs were punched out of individual leaf segments, weighed, and used for measurements.

Fig. 2 Time course study of the growth of leaf L2 of Oncidium Goldiana. Data points represent means ± SE of six replicates.

Results

Growth Studies

The largest leaf (L2) of Oncidium Goldiana was selected for growth studies. Growth curves of leaf L2 demonstrated that leaf development is typically sigmoidal regardless of the parameters studied (fig. 2A–D). Maximum blade length of ca. 30 cm was attained after 60 d (fig. 2A). Maximum leaf blade area of ca. 60 cm² was also attained at about the same time (fig. 2B). Fresh weight (fig. 2C) and dry weight (fig. 2D) of leaf L2 continued to increase during development to maturation. Fresh weight and dry weight continued to increase after maximum blade length and area were attained.
Chlorophyll Content along the Leaf Blade

A gradient of increasing chlorophyll content was observed throughout the entire blade length in both developing (33% FBL, 57% FBL, 80% FBL) and fully expanded mature leaves (100% FBL). Chlorophyll $a$ content increased linearly from the base to the tip in both developing and fully expanded leaves (fig. 3). Similarly, an increasing gradient of chlorophyll $b$ content was observed in both developing and fully expanded leaves (fig. 3). Total chlorophyll content also increased linearly from the base to the tip in both developing and fully expanded leaves (fig. 3). Chlorophyll $a$, chlorophyll $b$, and total chlorophyll content when expressed in terms of dry weight and area also showed a similar pattern of distribution along the entire blade length in both developing and fully expanded leaves (data not shown). The greater variation of chlorophyll content at the leaf tip was the result of damage to the leaf apex in some of the replicates.

Chlorophyll $a/b$ ratio was lower in the developing tissues compared to mature tissues (fig. 3). Chlorophyll $a/b$ ratio was close to 1 in basal regions (0 cm to 5 cm) of developing leaf blades (33% FBL, 57% FBL, and 80% FBL). Chlorophyll $a/b$ ratio of regions of the leaf blade greater than 5 cm from the base to the leaf tip was maintained at a relatively constant value of 2.

Maximum Rubisco Activity

A gradient of increasing maximum Rubisco activity throughout the leaf blade was also observed in both developing and fully expanded mature leaves (fig. 4). A similar trend was also observed when maximum Rubisco activity was expressed both in terms of dry weight and area (data not shown).

Photosynthesis

Maximum photosynthetic rate (at PPFD: 555 μmol m$^{-2}$ s$^{-1}$) increased from the base to the tip in both
developing and fully expanded mature leaves (fig. 5). An increasing gradient of photosynthetic activity from the base to the tip of the leaf was also observed when maximum photosynthetic rate was expressed in terms of dry weight and area (data not shown).

Photosynthetic light response curves of the base and apex of the leaf L2 at various stages of development (33% FBL, 57% FBL, 80% FBL, 100% FBL) showed that the leaf tip possessed a higher photosynthetic capacity compared to the base regardless of the stage of development (fig. 5). Photosynthetic light saturation of both the basal portion and apex of developing and fully expanded leaf L2 occurred at ca. 300 μmol m⁻² s⁻¹. Maximum photosynthetic rate of the basal portion and apex of the leaf averaged 30 μmol O₂ gFW⁻¹ h⁻¹ and 120 μmol O₂ gFW⁻¹ h⁻¹, respectively.

Correlation Analysis

Total chlorophyll, maximum Rubisco activity, and maximum photosynthetic rates were positively correlated. The sample correlation coefficient (r) between total chlorophyll content and maximum photosynthetic rate was 0.679 (P < 0.01). Total chlorophyll content was also positively correlated with maximum Rubisco activity (r = 0.942; P < 0.01). In addition, a positive correlation (r = 0.882; P < 0.01) was also observed between maximum Rubisco activity and maximum photosynthetic rate.

Discussion

Our results showed that a period of ca. 70 d was required for leaves to be fully expanded in Oncidium Goldiana. Visual observations indicated a gradient of green coloration along the blade of leaf L2 of O. Goldiana. A similar observation was also made in the developing leaf of Lolium multiflorum (Prioul et al. 1980). The results of visual observations were supported by the observed gradient of chlorophyll content along the leaf blade of O. Goldiana.

The gradient of increasing chlorophyll content from the base to the apex was evident in both developing and fully expanded (mature) leaves of O. Goldiana. Such a gradient in chlorophyll content along the leaf blade has also been reported in developing and fully expanded leaves of sugarcane (Robinson-Beers et al. 1990) and L. multiflorum (Prioul et al. 1980). However, chlorophyll content in sugarcane peaked at 80 cm from the base, and this peak corresponded to the widest portion of the leaf blade (Robinson-Beers et al. 1990). There was a decline in chlorophyll content after 80 cm. This is consistent with the onset of senescence from the tip down to the base in sugarcane (Robinson-Beers et al. 1990). In O. Goldiana, however, chlorophyll content was always highest at the apex regardless of the developmental stage of the leaf.

Chlorophyll a/b ratio was lower in basal regions of the leaves compared to mature apical regions of leaf blades of O. Goldiana. Chlorophyll a/b ratio was maintained at ca. 2 throughout the blade of the fully expanded leaf. There was an increase in chlorophyll a/b ratio during leaf ontogeny in O. Goldiana. In other plants, chlorophyll a/b ratio usually declines during leaf ontogeny. This is evident in both dicotyledons and monocotyledons (for review, see Sesták 1985). The decrease in chlorophyll a/b ratio during leaf ontogeny is the result of the increase in light-harvesting chlorophyll protein complex (LHC) with age (Ho et al. 1984). The chlorophyll a/b ratio of close to 1 in basal regions of the developing leaves may be the result of the low irradiance encountered because of shading by other leaves.

Rubisco is the main enzyme for carbon fixation and its role in the regulation of photosynthesis is well documented (Woodrow and Berry 1988). However, little attention has been devoted to study how Rubisco regulates photosynthesis during leaf biogenesis. In developing leaves of Populus deltoides (Dickmann 1971) and Zea mays (Miranda et al. 1981), it was found that positive relationships exist among chlorophyll content, Rubisco activity, and photosynthesis. We observed an
increasing gradient of maximum Rubisco activity from the base to the tip in both developing and fully expanded leaves of O. Goldiana. This is highly correlated with the observed gradient of chlorophyll content in leaves of O. Goldiana.

Consistent with the distribution of chlorophyll and Rubisco activity along the leaf is the observation that there is also an increasing gradient of maximum photosynthetic capacity along the leaf blade in both developing and fully expanded leaves. Basal portions of leaves of O. Goldiana have a lower photosynthetic capacity compared to the apex. Correlation analyses revealed significantly positive relationships among chlorophyll content, maximum Rubisco activity, and maximum photosynthetic capacity.

We have shown that photosynthetic capacity varies along the leaf blade of O. Goldiana, and the variation in photosynthetic capacity is evident in both developing and fully expanded leaves. The situation in O. Goldiana is similar to L. multiflorum where a decreasing gradient of functional characteristics (net maximum photosynthesis, chlorophyll content, and Rubisco activity) was observed from the leaf tip to the base. The functional gradients that exist along the developing leaf are maintained and expressed in the fully expanded leaf.

Our objective in undertaking this study was to lay a foundation for subsequent studies to identify regions undergoing import/export transition in leaves of O. Goldiana during development. Future work would involve the use of radiocarbon to identify regions in the leaf undergoing import/export transition, studying changes associated with shifts in carbohydrate metabolism, and examining ultrastructural changes (e.g., sieve tube maturation) associated with the process of transformation from heterotrophic growth to autotrophic growth during leaf biogenesis.

Literature Cited


