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Research article

Exposure of *Catasetum fimbriatum* aerial roots to light coordinates carbon partitioning between source and sink organs in an auxin dependent manner

Paulo Marcelo Rayner Oliveira*, Maria Aurineide Rodrigues, Ana Zangirolame Gonçalves, Gilberto Barbante Kerbauy

Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, 05508-900, São Paulo, SP, Brazil

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ABSTRACT

Light energy is essential for carbon metabolism in plants, as well as controlling the transport of metabolites between the organs. While terrestrial plants have a distinct structural and functional separation between the light exposed aerial parts and the non-exposed roots, epiphytic plants, such as orchids, have shoots and roots simultaneously fully exposed to light. The roots of orchids differ mainly from non-orchidaceous plants in their ability to photosynthesize. Since the roots of *Catasetum fimbriatum* can synthesize auxin which is acropetally transported to the shoot region, we decided to investigate whether: (1) light treatment of *C. fimbriatum roots* raises the auxin levels in the plant; and (2) distinct auxin concentrations can change the source-sink relationships, altering the amounts of sugars and organic acids in leaves, pseudobulbs and roots. Among the organs studied, the roots accumulated the highest concentrations of indole-3-acetic-acid (IAA); and when roots were exposed to light, IAA accumulated in the leaves. However, when polar auxin transport (PAT) was blocked with *N*-(1-Naphthyl)phthalamic acid (NPA) treatment, a significant accumulation of sugars and organic acids occurred in the pseudobulbs and leaves, respectively, suggesting that auxin flux from roots to shoots was involved in carbon partitioning of the aerial organs. Considering that *C. fimbriatum* plants lose all their leaves seasonally, it is possible the roots are a substituting influence on the growth and development of this orchid during its leafless period.

1. Introduction

Due to their sessile nature, plants need to deal with environmental changes to maintain their developmental processes. Among these environmental changes, light is one of the most prominent physical factors, acting both as an energy source (Hohmann-Marriott and Blankenship, 2011) and controlling photomorphogenic responses (de Wit et al., 2016). For most terrestrial plants, the aerial vegetative organs are responsible for light sensing using a wide variety of specialized photoreceptors, such as UV RESISTANCE LOCUS 8 (UVR8) responsible for mediating mechanisms in response to UV wavelengths, phototropins and cryptochrome responsive to blue light, and phytochrome that perceives red and far-red light (de Wit et al., 2016). By contrast, the underground root system in the absence of direct light exposure is mainly involved with plant anchorage and acquisition of water and nutrients. However, light signaling can indirectly control root development. For example, Lee et al. (2016) showed that the light incident on leaves of *Arabidopsis* controlled the mobilization of phytochrome B from the cytosol to the nucleus in roots cells.

Signals reciprocally exchanged between shoot and root systems coordinate a series of responses connecting the atmospheric and soil conditions, and affecting plant growth and development (Shabala et al., 2016). For example, polar auxin transport (PAT) mediated by the *pin-formed* (PIN) proteins, a family of efflux auxin transporters, is modulated by light. This represents the key pathway behind phototropic responses (Friml et al., 2002), with light controlling PIN polarization on the plasma membrane (Laxmi et al., 2008; Xu et al., 2013). However, PAT also plays a prominent role in virtually all phases of root growth and development (Saini et al., 2013).

Light has also been shown to play a conspicuous role in controlling the transport of metabolites between different organs in a number of plant species (Häusler et al., 2014; Keiller and Smith, 1989). Sucrose, the main product of photosynthesis, is translocated from source to sink organs through the phloem, where it is either used for energy in growth and development or stored (Griffiths et al., 2016). Remarkably, Chen et al. (2016) showed that the transcription factor ELONGATED

Abbreviations: IAA, Indole-3-acetic-acid; NPA, *N*-(1-Naphthyl)phthalamic acid; PAT, Polar auxin transport

* Corresponding author.
E-mail address: paulorayner@usp.br (P.M.R. Oliveira).

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HYPOCOTYL 5 (HY5) expressed on leaves and regulated by light is transported to the roots and coordinates sucrose transport from the shoot to the root, controlling the source-sink relationship. Furthermore, the interaction between hormones and sugars, essential for plant development, can involve the expression of some genes up-regulated by glucose in *Arabidopsis* (Mishra et al., 2009), such as those for PIN2, YUCCA2 (an enzyme involved in the auxin biosynthetic pathway), ARFs (Auxin Response Factors) and ABP1 (a receptor for auxin). Beside sugars, organic acids, such as malate and citrate, are also thought to act as both carbon source and signaling molecules. Malate can participate in photosynthetic reactions as a CO_2 concentrator, as well as a substrate for ATP production through the tricarboxylic acid cycle in mitochondria (Peckmann et al., 2012). In the same way, citrate can be used as an energy source for carbon skeleton recycling (Borland and Griffiths, 1989; Luttge, 1988) and also reducing the cell osmotic potential, preventing plant dehydration (Lee et al., 2008).

Most terrestrial plants possess green photosynthetic shoots, and underground root systems for anchorage and water and nutrient uptake. By comparison, epiphytic plants display a wide range of adaptations due to the different environment in which they live, especially with regard to intermittent availability of light, nutrients, and water (Benzing, 1990; Benzing et al., 1983). One of the most numerous and diverse families of epiphytic plants is Orchidaceae, which has approximately 29,000 species (Hinsley et al., 2018). The epiphytic orchids have several morphological specializations that allow them to colonize their habitat, such as the velamen, a dead multilayer epidermis that covers the roots and is related to water retention and absorption (Benzing, 1990; Joca et al., 2017). In addition, most aerial roots of epiphytic orchids can also photosynthesize when exposed to light, a fact best exemplified by the extreme situation with the shootless orchid *Campylocentrum* sp. which depends entirely on its green aerial roots (Benzing et al., 1983). In another case, the Neotropical epiphytic orchid *Catasetum* sp. can form a nest-like structure consisting of tiny and short lateral roots that traps humidity and organic debris (Benzing, 1986). Since *Catasetum* sp. loses all its leaves seasonally, especially in the dry season (Benzing, 1990; Benzing et al., 1982), its roots might assume functions other than just nutrient and water uptake. Interestingly, while its tiny lateral roots are typically negatively gravitropic, the first thick roots are positively gravitropic and grow inside the nest-like structure (Fig. 1A and B).

The epiphytic orchid *Catasetum fimbriatum* is a deciduous species whose root system receives light in its natural environment. Since PAT is controlled by light, and the roots of *C. fimbriatum* are able to produce auxin (Peres et al., 2009), we aimed to investigate whether distinct light conditions on *C. fimbriatum* roots could induce and/or alter the acropetally PAT in the whole plant, changing its metabolism and carbon partitioning (Fig. 1C and D). Specifically, we conducted an experiment to evaluate: (1) whether light incident on the roots raises the auxin levels in the plant; and (2) whether distinct auxin concentrations in the plant resulting from light incident on the roots alters the source-sink relationships, altering the levels of sugars and organic acids among the different orchid organs, such as leaves, pseudobulbs, and roots.

2. Materials and methods

2.1. Plant growth conditions and experimental design

Micro-propagated plants of *Catasetum fimbriatum* Lindl (Orchidaceae) (SisGen number A89A94C) were grown *in vitro* for 6 months in the culture medium of Vacin and Went (1949), according to the procedure described by Suzuki et al. (2004). Plants were then transferred to pots containing dried moss fiber as a substrate, with approximately 15 plants per pot, and these were kept for 12 months in the greenhouse. Following this period, the plants were transferred to a climatic chamber with 200 μmol $m^{-2}s^{-1}$ of light intensity emitted by fluorescent lamps on a photoperiod of 12 h, temperature of 25 ± 2 °C, and 70% relative humidity. After 30 days under these conditions, plants were gently removed and transferred to transparent glass vials (10 cm height x 3.6 cm wide), filled with the inorganic solution of Vacin and Went (1949) without Phytagel®, and with their root systems fully exposed to light. Only 1 cm of the roots was inserted in the nutritional solution. During the following 30 days of incubation, the nutrient solution was renewed every 3 days. Subsequently, plants were incubated for 20 days in the same type of vials containing distilled water. They were then separated into 2 treatment groups of 30 plants each. One set of plants received distilled water at pH 5.8, and represented the control group. The other group of 30 plants received 10 μM N-(1-Naphthyl) phthalamic acid (NPA) in distilled water, pH 5.8, a well-known inhibitor of PAT. Each treatment group was then further divided into 2 sub-groups. For each treatment, 15 plants had their roots exposed to constant light; while the other 15 plants had their roots protected from light with aluminium foil covering all the root system and the vial. The plants were then placed in a climatic chamber for 15 days at 25 ± 2 °C with 150 μmol $m^{-2}s^{-1}$ of continuous light. At the end of 15 days, leaves, pseudobulbs and roots were harvested from the plants for analysis of levels of auxin, sugars and organic acids.

2.2. Analysis of auxin levels

Endogenous auxin (indole-3-acetic acid; IAA) amounts were quantified by the GC-MS procedure described in Melo et al. (2016) with some modifications. 200 mg of frozen roots were grounded in liquid nitrogen with 20 mg poly(vinylpolypyrrolidone) (PVPP) and then homogenized in 1.25 mL extraction solution composed of 10 mM ascorbic acid, 10 mM ethylenediamine tetraacetic acid and 10 mM dithiothreitol. One μg [$^{13}C_6$]-IAA (Cambridge Isotopes, Inc.) was added to each sample as an internal standard. After centrifugation (25,000 g, 20 min, 4 °C), the supernatant was purified via solid phase extraction columns (Supelclean LC-NH2, Supelco) as described in Chen et al. (1988). Purified samples were dried by evaporation, resuspended in 200 μL methanol, and dried again. Afterwards, samples were resuspended in 50 μL pyridine and then 50 μL N-*tert*-Butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA) followed by 60 min of derivatization at 92 °C. Analysis of samples was performed on a gas chromatograph coupled to a mass spectrometer (GCMS-QP2010 SE, Shimadzu) with a selective ion monitoring mode. The chromatograph was equipped with a fused-silica capillary column (30 m, ID 0.25 mm, 0.25 μm thick internal film; DB-5 MS, Agilent Technologies) stationary phase using helium as the carrier gas at a flow rate of 4.5 mL min^{-1} in the following program: 2 min at 100 °C, followed by ramps of 10 °C min^{-1} to 140 °C, 25 °C min^{-1} to 160 °C, 35 °C min^{-1} to 250 °C, 20 °C min^{-1} to 270 °C and 30 °C min^{-1} to 300 °C. The injector temperature was 250 °C and the following MS operating parameters were used: ionization voltage of 70 eV (electron impact ionization); 230 °C on ion source, 260 °C on interface. Ions with a mass ratio/charge (m/z) of 244, 202 and 130 (corresponding to the endogenous IAA), 250, 208 and 136 (corresponding to [$^{13}C_6$]-IAA) were monitored. Endogenous concentrations of IAA were calculated based on the extracted chromatograms at m/z 244 and 250.

2.3. Analysis of sugars and organic acids

Quantitation of soluble sugars and organic acids was performed as described by Roessner et al. (2001), with some modifications. 100 mg of fresh root samples were grounded in liquid nitrogen and extracted with a solution of methanol:chloroform:water in the proportion by volume of 12:5:1. 40 μg salicylic acid was added as an internal standard for organic acids and 200 μg phenyl-β-glucopyranoside as an internal standard for soluble sugars; then, samples were vigorously mixed and incubated at 60 °C for 30 min. Following incubation, 500 μL water was added, the samples were centrifuged at 16,000 g for 10 min, and the supernatant was collected. For the analysis of soluble sugars, an aliquot

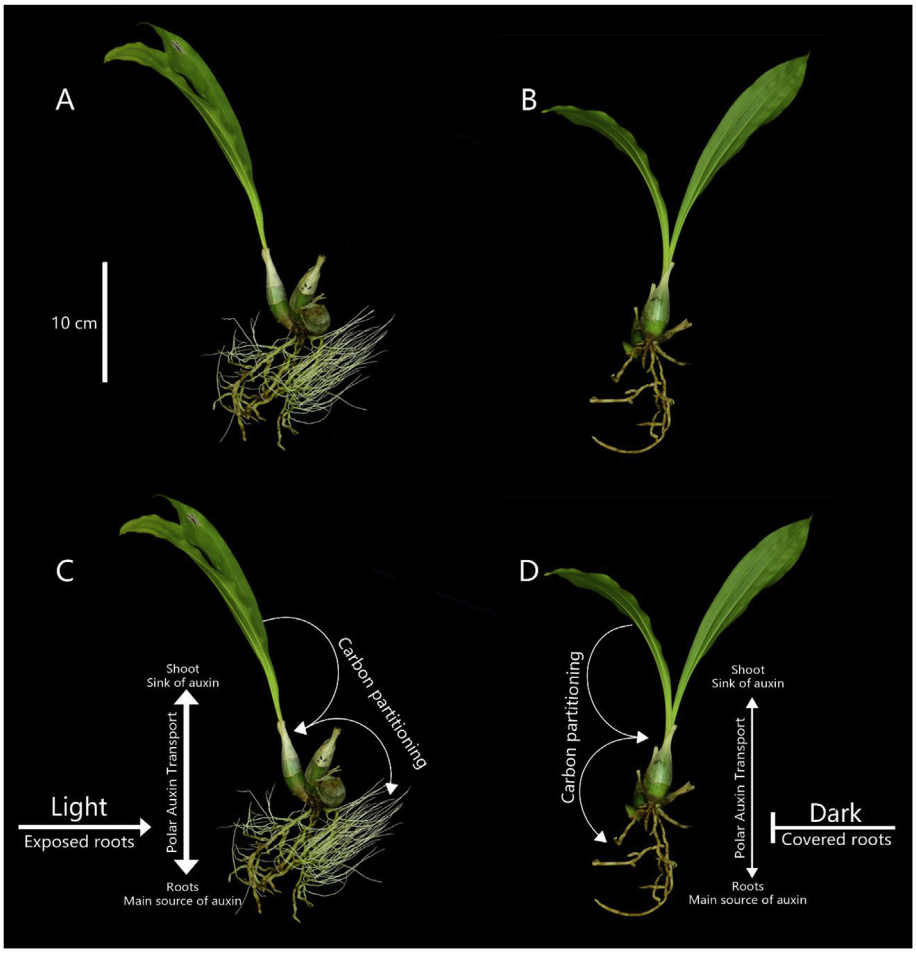

Fig. 1. Adult plants of *Catasetum fimbriatum* with their roots grown exposed to light [white roots] (A), or protected from light [covered roots] (B). Physiological model of polar auxin transport and the carbon partitioning between shoot and roots when roots are exposed to light (C), or protected from light (D).

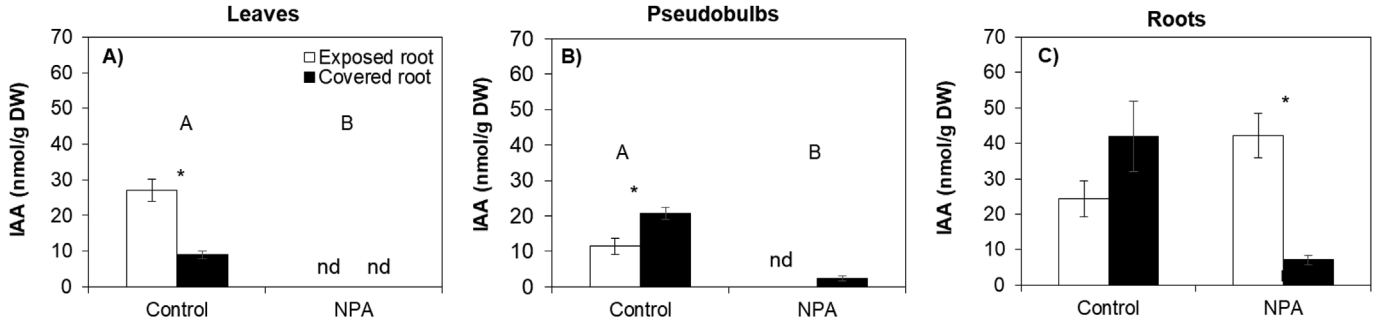

Fig. 2. Free indole-3-acetic-acid (IAA) content in leaves (A), pseudobulbs (B) and roots (C) of *Catasetum fimbriatum* in response to the root light conditions. White columns represent plants where roots were exposed to light (exposed root), while black columns represent plants where roots were protected from light (covered root). Control treatment was composed of plants where roots were kept in distilled water. NPA group were plants where roots were exposed to *N*-(1-Naphthyl) phthalamic acid (NPA), a polar auxin transport blocker. Bars indicate means and the standard error; nd, not detected. Asterisks (*) indicate statistical differences between plants with light exposed and covered roots; letters indicate statistical differences between treatments (ANOVA/Tukey HSD post-hoc test, $\alpha = 0.05$).

of 25 μL was dried under vacuum at 60 °C, 25 μL pyridine was then added, and derivatization was performed with 25 μL N,*O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane incubated for 60 min at 75 °C. For the analyses of organic acids, an aliquot of 50 μL was dried under vacuum at 60 °C, 25 μL pyridine was added, and derivatization was performed with 25 μL MTBSTFA incubated for 60 min at 92 °C. Analysis of sugars and organic acids were both performed on the GCMS-QP2010 SE, Shimadzu in scan mode. The chromatograph was equipped with a fused-silica capillary column (30 m, ID 0.25 mm, 0.25 μm thick internal film; DB-5 MS, Agilent Technologies) stationary phase using helium as the carrier gas at a total flow rate of 24 mL min^{-1} and column flow of 1 mL min^{-1} in the following program: initial temperature at 100 °C, followed by a ramp of 6 °C min^{-1} to 300 °C, 300 °C 10 min. The injector temperature was 290 °C and the following MS operating parameters were used: ionization voltage 70 eV (electron impact ionization), ion source temperature 200 °C, interface temperature 250 °C, scan mode, event time 0.3 s, scan speed 3333 amu/s. Due to concerns of possible interference, we demonstrated that endogenous levels of salicylic acid were not detectable in the scan mode.

2.4. Statistical analyses

The response variables, IAA, sucrose, glucose, fructose, malate and citrate from orchid roots, leaves and pseudobulbs were compared using analyses of variance (ANOVA), and the Tukey HSD *post-hoc* test was used for pair-wise comparisons. All statistical analyses were conducted with the statistical platform R (R Core Team, 2017).

3. Results

The free IAA content in the leaves, pseudobulbs, and roots of *Catasetum fimbriatum* changed depending on the light conditions and treatments of the root system (Table S1; Fig. 2). The leaves of orchids without the PAT blocker NPA and with roots exposed to light showed approximately 70% more IAA than leaves of plants with covered roots, (Table S1; Fig. 2A). NPA reduced the levels in leaves from both light exposed and covered roots to non-detectable levels. Interestingly, the concentration of IAA in pseudobulbs showed an opposite response, as pseudobulbs of orchids without NPA and with exposed roots showed lower IAA content compared with pseudobulbs of plants with covered roots (Table S1; Fig. 2B). Similar to the leaves, NPA reduced the concentration of IAA in pseudobulbs of plants with both exposed and covered roots (Table S1; Fig. 2B). Orchid roots presented the highest values of IAA compared to leaves and pseudobulbs; without NPA, the IAA concentration in roots exposed to light decreased by approximately 58% compared to covered roots, although this result was not statistically significant (Table S1; Fig. 2C). However, exposed roots that received NPA showed a higher concentration of IAA compared to covered roots (Table S1; Fig. 2C).

The content of glucose, fructose and sucrose differed among orchid organs and varied depending on the treatments and the light conditions of the roots (Table S1; Fig. 3). The concentrations of glucose, fructose and sucrose were similar in orchid leaves of plants with exposed or covered roots that did not receive NPA; however, orchid leaves showed higher concentrations of glucose and sucrose when its roots were covered and received NPA (Table S1; Fig. 3A–C). The pseudobulbs showed an elevated concentration of glucose, fructose, and sucrose compared to the leaves and roots, and showed an elevated content of all sugars when treated with NPA (Table S1; Fig. 3D–F). Additionally, the pseudobulbs of orchids with covered roots showed a higher sucrose content after receiving NPA (Fig. 3F). By contrast, the roots of *C. fimbriatum* showed the lowest concentration of sugars (Table S1). The concentration of sucrose was higher in the light exposed roots of plants without NPA, while the glucose content was higher in the light exposed roots of plants that received NPA (Table S1; Fig. 3G–I).

The concentration of malate and citrate varied depending on the treatments and the light conditions of the roots, and they were lower in the roots compared to leaves and pseudobulbs (Table S1; Fig. 4). Leaves of *C. fimbriatum* showed lower contents of malate and citrate in the control treatment compared to plants that received NPA (Table S1; Fig. 4AandB). By contrast, the pseudobulbs showed a higher content of malate in the control treatment compared to plants that received NPA (Table S1; Fig. 4C and D). In addition, the roots showed higher concentration of malate and citrate in the plants without NPA, and the citrate concentration was even higher in the covered roots (Table S1; Fig. 4E and F).

4. Discussion

Our results highlight the diverse relevance of the roots of *Catasetum fimbriatum*, as changing the light condition incident on the roots affected the IAA content and the carbon partitioning in the whole plant.

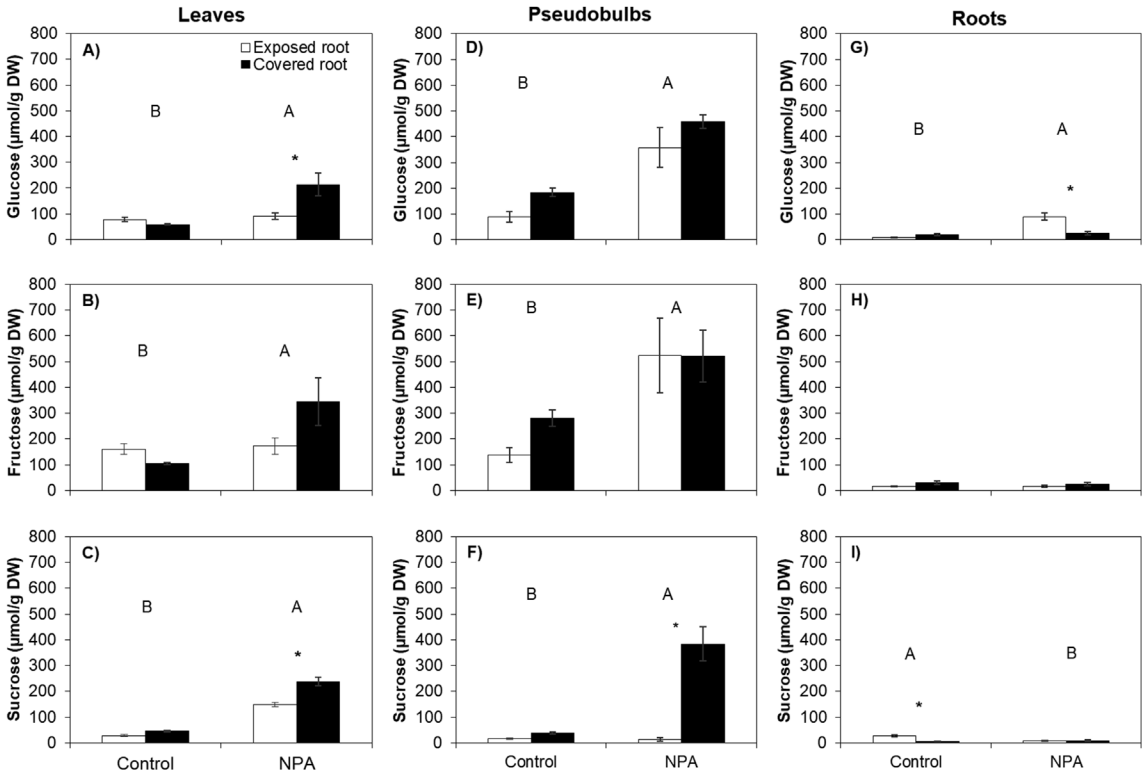

Fig. 3. Concentration of glucose, fructose and sucrose in leaves (A, B, C), pseudobulbs (D, E, F) and roots (G, H, I) of *Catasetum fimbriatum* in response to the root light conditions. White columns represent plants where roots grew exposed to light (exposed root), while black columns represent plants where roots grew protected from light (covered root). Control treatment was composed of plants where roots were kept in distilled water. NPA group were plants where roots were exposed to *N*-(1-Naphthyl)phthalamic acid (NPA), a polar auxin transport blocker. Bars indicate means and the standard error; nd, not detected. Asterisks (*) indicate statistical differences between plants with light exposed and covered roots; letters indicate statistical differences between treatments (ANOVA/Tukey HSD post-hoc test, $\alpha = 0.05$).

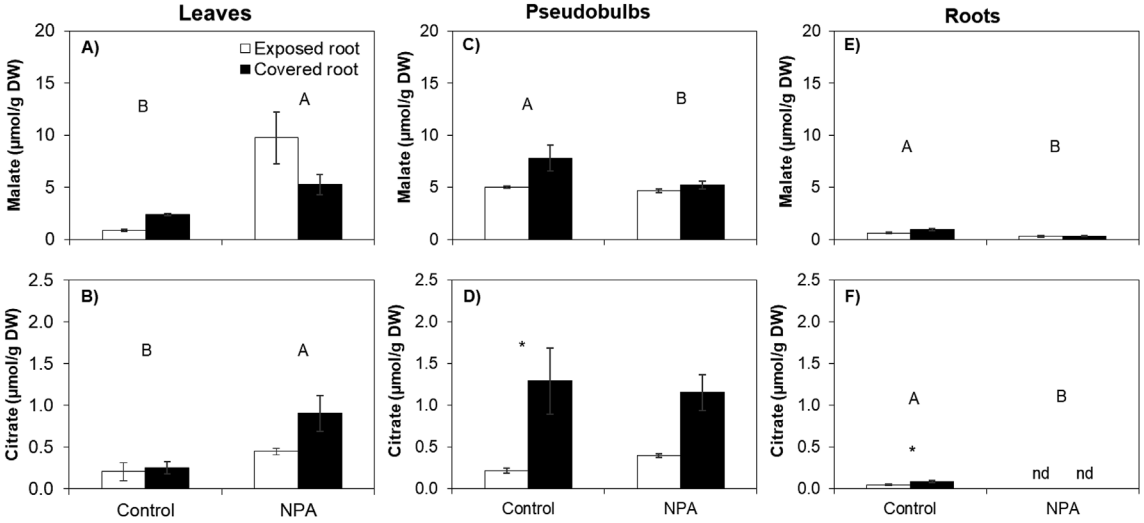

Fig. 4. Concentration of malate and citrate in leaves (A, B), pseudobulbs (C, D) and roots (E, F) of *Catasetum fimbriatum* in response to the root light conditions. White columns represent plants where roots grew exposed to light (exposed root), while black columns represent plants where roots grew protected from light (covered root). Control treatment was composed of plants where roots were kept in distilled water. NPA group were plants where roots were exposed to *N*-(1-Naphthyl) phthalamic acid (NPA), a polar auxin transport blocker. Bars indicate means and the standard error; nd, not detected. Asterisks (*) indicate statistical differences between plants with light exposed and covered roots; letters indicate statistical differences between treatments (ANOVA/Tukey HSD post-hoc test, $\alpha = 0.05$).

Notably, *Catasetum fimbriatum* roots showed the highest concentration of IAA and light incident on them favored the accumulation of this hormone in the leaves, suggesting that the roots may act as a source of auxin. Moreover, even covered roots contributed to the amount of IAA in the pseudobulbs. In addition, when PAT was blocked, sugars accumulated in the pseudobulbs and organic acids in the leaves, suggesting that acropetal auxin flux from roots to shoots controls carbon partitioning among the orchid organs. Since *C. fimbriatum* is a deciduous species that loses all its leaves seasonally, its roots could be responsible for maintaining plant growth and development during these periods by assuming such a function. In fact, the importance of this role by the roots, when compared to its other functions of substrate anchoring and nutrient uptake, assumes even more relevance as this species lives in an epiphytic environment.

This report provided evidence that the root system of *C. fimbriatum* acted as an auxin source and favored IAA accumulation in the leaves when the roots were exposed to light. It is known that the PAT in roots is mediated by efflux transporters, the PIN proteins, that are upregulated by light, which promotes transcription of the *PIN* genes and PIN protein trafficking from endosome to cell membrane (Laxmi et al., 2008; Xu et al., 2013). Therefore, the increase of auxin in leaves when roots were exposed to light is consistent with the remobilization of the PIN proteins to the cell membrane, increasing the acropetal PAT and consequently high free IAA content in leaves. In addition, the use of NPA blocked PAT and strongly reduced IAA levels in leaves and pseudobulbs, as it prevented free auxin exportation, resulting in IAA accumulation at the site of biosynthesis, the roots. By contrast, we observed a low amount of IAA in covered roots treated with NPA; however, this might be explained if there was conjugation of free IAA. Elevated levels of free IAA can induce the expression of genes involved in auxin conjugation, generating low molecular weight peptide, sugar, ester, and amide forms of IAA, thus controlling the cellular content of free IAA for storage or transportation (Ludwig-Müller, 2011). Future studies are necessary to detect whether these conjugates are produced in *C. fimbriatum* and how they are distributed with regard to storage or transportation.

The roots of *C. fimbriatum* naturally produce a nest-like structure that accumulates humidity and organic debris, where the tiny negatively gravitropic lateral roots wrap it and are exposed to light (Benzing, 1986). Inside this nest-like structure are positively gravitropic thick roots. Since the lateral root formation, as well as the root gravitropism, are controlled by auxin (De Smet, 2012; Su et al., 2017), and light signaling is involved in root gravitropic and phototropic responses (Lee et al., 2017), we believe that the size of the nest-like structure and consequently the amount of humidity and nutrients that roots will collect will be dependent on the light incident on the lateral roots and the resulting IAA production. We showed here that light was a prominent factor inducing IAA production in light-exposed roots of *C. fimbriatum* and this light-dependent IAA content might be responsible for: (i) the production of lateral roots, allowing the expansion of the nest-like structure and its accumulation of organic debris; (ii) the production of the thicker roots inside the nest-like structure; and (iii) increasing the ability of the roots to obtain nutrients for this orchid species.

The mechanisms involving the action of carbohydrates, such as glucose and sucrose, on auxin biosynthesis are well known, including their light-dependency (Ljung et al., 2015). On the other hand, the role of auxin in the molecular mechanisms of sugar metabolism is poorly understood. However, using mutants impaired in auxin signaling, Anegawa et al. (2015) showed that auxin was involved in the control of some primary metabolism steps, such as organic phosphate levels. Indeed, our data provide good evidence that auxin flux controls some photo-assimilate contents, as we showed that pseudobulbs presented the highest content of sugars, leaves presented intermediary values, while roots had the lowest levels. Moreover, disturbing the acropetal transport of auxin by NPA induced an increase in the sugar content of pseudobulbs. These differences among orchid organs are probably related to their functions, while orchid leaves are the primary source of photo-assimilates they may not also be the sink organ due to the deciduous nature of *C. fimbriatum*, an uncommon phenomenon in epiphytic orchid plants. Hence, sugar compounds produced in the leaves may be transported and stored in pseudobulbs, providing energy for the formation of new leaves, roots, and flowers during the next wet season (Benzing et al., 1982). Alternatively, the roots of *C. fimbriatum* exposed to light may be the organ requiring more energy sources, such as glucose or sucrose. Since sucrose is the main sugar transported from the source to the sink organs (Lemoine et al., 2013), the higher amount of sucrose observed in roots exposed to light in control treatment may have ramification for the roots. In addition, roots exposed to light that received NPA showed a higher amount of IAA and glucose. Since both cytosolic and vacuolar invertase activity is induced by auxin (Gasperl et al., 2016), the exposed roots of *C. fimbriatum* may have a higher

auxin-modulated invertase activity, converting sucrose in glucose and eliciting root development.

Besides carbohydrates, organic acids can also function as an energy source for plants. In addition to the inhibitory NPA effect on auxin-mediated acropetal transport of sugars from pseudobulbs, NPA also favored an increase in malate and citrate levels in leaves. Interestingly, the mutant *SOLITARY-ROOT* (*slr*) of *Arabidopsis*, which is less responsive to auxin, showed higher concentrations of organic acids compared to the wild-type when IAA-treated, with lower malate content in the shoots (Anegawa et al., 2015). Therefore, increased organic acid levels in leaves may be the result of decreased local production of energy products, such as glucose and sucrose, a process very likely triggered by a decrease of auxin in leaves. The energy liberation by organic acids can occur by the decarboxylation process, making CO_2 available for the production of hexoses and malate which can be used in mitochondria (Borland et al., 2011; Lüttge, 2006) and can be translocated throughout the plant (Hibberd and Quick, 2002). Altogether, the light incident on the roots controls the auxin content that modulates organic acids and sugars accumulation.

In conclusion, we suggest that the roots of *C. fimbriatum* are functioning as an auxin source and that increased IAA levels in illuminated roots may favor a positive relationship with regard to lateral root formation in this epiphytic orchid, leading to the establishment of its characteristic nest-like root system architecture. Unfortunately, so far nothing is known on the negative gravitropic growth of these lateral roots. Additionally, changing the auxin distribution in the shoots by blocking PAT modified their sugar and organic acid contents, and we observed an incremental increase of sugars in pseudobulbs and malate and citrate in leaves. We propose that light modulates the acropetal PAT which changes the carbon partitioning between the source and sink organs of *C. fimbriatum*. Therefore, *C. fimbriatum* roots may have acquired features that may have allowed its divergence into its current epiphytic environment. This study also highlighted the need for future studies focused on understanding the relationship of light to the production and destination of auxin conjugates, and whether invertase activity is induced by auxin or modulated by light incident on the roots of *C. fimbriatum*.

CRediT authorship contribution statement

Paulo Marcelo Rayner Oliveira: Formal analysis. **Maria Aurineide Rodrigues:** Formal analysis. **Ana Zangirolame Gonçalves:** Formal analysis. **Gilberto Barbante Kerbauy:** Formal analysis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2018.12.022.

References

Anegawa, A., Ohnishi, M., Takagi, D., Miyake, C., Shichijo, C., Ishizaki, K., Fukaki, H., Mimura, T., 2015. Altered levels of primary metabolites in response to exogenous indole-3-acetic acid in wild type and auxin signaling mutants of Arabidopsis thaliana: a capillary electrophoresis-mass spectrometry analysis. Plant Biotechnol. 32, 65–79.

Benzing, D.H., 1990. Vascular Epiphytes. General Biology and Related Biota, first ed. Cambridge University Press, Cambridge, UK.

Benzing, D.H., 1986. The vegetative basis of vascular epiphytism. Selbyana 9, 23–43.

Benzing, D.H., Bent, A., Moscow, D., Peterson, G., Renfrow, A., 1982. Functional correlates of deciduousness in *Catasetum integerrimum* (Orchidaceae). Selbyana 7, 1–9.

Benzing, D.H., Friedman, W.E., Peterson, G., Renfrow, A., 1983. Shootlessness, velamentous roots, and the pre-eminence of Orchidaceae in the epiphytic biotope. Am. J. Bot. 70, 121–133.

Borland, A.M., Barrera Zambrano, V.A., Ceusters, J., Shorrock, K., 2011. The photosynthetic plasticity of crassulacean acid metabolism: an evolutionary innovation for sustainable productivity in a changing world. New Phytol. 191, 619–633.

Borland, A.M., Griffiths, H., 1989. The regulation of citric acid accumulation and carbon recycling during CAM in *Ananas comosus*. J. Exp. Bot. 40, 53–60.

Chen, K., Miller, A.N., Patterson, G.W., Cohen, J.D.A., 1988. Rapid and simple procedure for purification of indole-3-acetic acid prior to GC-SIM-MS analysis. Plant Physiol 86, 822–825. https://doi.org/10.1104/pp.86.3.822.

Chen, X., Yao, Q., Gao, X., Jiang, C., Harberd, N.P., Fu, X., 2016. Shoot-to-root mobile transcription factor HY5 coordinates plant carbon and nitrogen acquisition. Curr. Biol. 26, 640–646.

De Smet, I., 2012. Lateral root initiation: one step at a time. New Phytol. 193, 867–873.

de Wit, M., Galvão, V.C., Fankhauser, C., 2016. Light-mediated hormonal regulation of plant growth and development. Annu. Rev. Plant Biol. 67, 513–537.

Friml, J., Wiśniewska, J., Benková, E., Mendgen, K., Palme, K., 2002. Lateral relocation of auxin efflux regulator PIN3 mediates tropism in *Arabidopsis*. Nature 415, 806–809.

Gasperl, A., Morvan-Bertrand, A., Prud'homme, M.-P., van der Graaff, E., Roitsch, T., 2016. Exogenous classic phytohormones have limited regulatory effects on fructan and primary carbohydrate metabolism in perennial ryegrass (*Lolium perenne* L.). Front. Plant Sci. 6, 1–19.

Griffiths, C.A., Paul, M.J., Foyer, C.H., 2016. Metabolite transport and associated sugar signalling systems underpinning source/sink interactions. Biochim. Biophys. Acta Bioenerg. 1857, 1715–1725.

Häusler, R.E., Heinrichs, L., Schmitz, J., Flügge, U.-I., 2014. How sugars might coordinate chloroplast and nuclear gene expression during acclimation to high light intensities. Mol. Plant 7, 1121–1137.

Hibberd, J.M., Quick, W.P., 2002. Characteristics of C_4 photosynthesis in stems and petioles of C_3 flowering plants. Nature 415, 451–454.

Hinsley, A., de Boer, H.J., Fay, M.F., Gale, S.W., Gardiner, L.M., Gunasekara, R.S., Kumar, P., Masters, S., Metusala, D., Roberts, D.L., Veldman, S., Wong, S., Phelps, J., 2018. A review of the trade in orchids and its implications for conservation. Bot. J. Linn. Soc. 186, 435–455.

Hohmann-Marriott, M.F., Blankenship, R.E., 2011. Evolution of photosynthesis. Annu. Rev. Plant Biol. 62, 515–548.

Joca, T.A.C., de Oliveira, D.C., Zotz, G., Winkler, U., Moreira, A.S.F.P., 2017. The velamen of epiphytic orchids: variation in structure and correlations with nutrient absorption. Flora 230, 66–74.

Keiller, D., Smith, H., 1989. Control of carbon partitioning by light quality mediated by phytochrome. Plant Sci. 63, 25–29.

Laxmi, A., Pan, J., Morsy, M., Chen, R., 2008. Light plays an essential role in intracellular distribution of auxin efflux Carrier PIN2 in *Arabidopsis thaliana*. PLoS One 3 (1), e1510. https://doi.org/10.1371/journal.pone.0001510.

Lee, H.-J., Ha, J.-H., Kim, S.-G., Choi, H.-K., Kim, Z.H., Han, Y.-J., Kim, J.-I., Oh, Y., Fragoso, V., Shin, K., Hyeon, T., Choi, H.-G., Oh, K.-H., Baldwin, I.T., Park, C.-M., 2016. Stem-piped light activates phytochrome B to trigger light responses in *Arabidopsis thaliana* roots. Sci. Signal. 9 (452), ra106.

Lee, H.-J., Park, Y.-J., Ha, J.-H., Baldwin, I.T., Park, C.-M., 2017. Multiple routes of light signaling during root photomorphogenesis. Trends Plant Sci. 22, 803–812.

Lee, M., Choi, Y., Burla, B., Kim, Y.-Y., Jeon, B., Maeshima, M., Yoo, J.-Y., Martinoia, E., Lee, Y., 2008. The ABC transporter AtABCB14 is a malate importer and modulates stomatal response to CO_2. Nat. Cell Biol. 10, 1217–1223.

Lemoine, R., La Camera, S., Atanassova, R., Dédaldéchamp, F., Allario, T., Pourtau, N., Bonnemain, J.-L., Laloi, M., Coutos-Thévenot, P., Maurousset, L., Faucher, M., Girousse, C., Lemonnier, P., Parrilla, J., Durand, M., 2013. Source-to-sink transport of sugar and regulation by environmental factors. Front. Plant Sci. 4, 272. https://doi.org/10.3389/fpls.2013.00272.

Ljung, K., Nemhauser, J.L., Perata, P., 2015. New mechanistic links between sugar and hormone signalling networks. Curr. Opin. Plant Biol. 25, 130–137.

Ludwig-Müller, J., 2011. Auxin conjugates: their role for plant development and in the evolution of land plants. J. Exp. Bot. 62, 1757–1773.

Lüttge, U., 1988. Day-night changes of citric-acid levels in crassulacean acid metabolism: phenomenon and ecophysiological significance. Plant Cell Environ. 11, 445–451.

Lüttge, U., 2006. Photosynthetic flexibility and ecophysiological plasticity: questions and lessons from *Clusia*, the only CAM tree, in the neotropics. New Phytol. 171, 7–25.

Melo, N.K.G., Bianchetti, R.E., Lira, B.S., Oliveira, P.M.R., Zuccarelli, R., Dias, D.L.O., Demarco, D., Peres, L.E.P., Rossi, M., Freschi, L., 2016. Nitric oxide, ethylene, and auxin crosstalk mediates greening and plastid development in deetiolating tomato seedlings. Plant Physiol. 170, 2278–2294.

Mishra, B.S., Singh, M., Aggrawal, P., Laxmi, A., 2009. Glucose and auxin signaling interaction in controlling *Arabidopsis thaliana* seedlings root growth and development. PLoS One 4 (2) e4502. https://doi.org/10.1371/journal.pone.0004502.

Peckmann, K., von Willert, D.J., Martin, C.E., Herppich, W.B., 2012. Mitochondrial respiration in ME-CAM, PEPCK-CAM, and C_3 succulents: comparative operation of the cytochrome, alternative, and rotenone-resistant pathways. J. Exp. Bot. 63, 2909–2919.

Peres, L.E.P., Zsögön, A., Kerbauy, G.B., 2009. Abscisic acid and auxin accumulation in *Catasetum fimbriatum* roots growing *in vitro* with high sucrose and mannitol content. Biol. Plant. 53, 560–564.

R Core Team, 2017. R: a Language and Environment for Statistical Computing. R foundation for statistical computing, Vienna, Austria. http://www.R-project.org/.

Roessner, U., Willmitzer, L., Fernie, A.R., 2001. High-resolution metabolic phenotyping of genetically and environmentally diverse potato tuber systems. identification of phenocopies. Plant Physiol. 127, 749–764.
Saini, S., Sharma, I., Kaur, N., Pati, P.K., 2013. Auxin: a master regulator in plant root development. Plant Cell Rep. 32, 741–757.
Shabala, S., White, R.G., Djordjevic, M.A., Ruan, Y.-L., Mathesius, U., 2016. Root-to-shoot signalling: integration of diverse molecules, pathways and functions. Funct. Plant Biol. 43, 87–104.
Su, S.-H., Gibbs, N.M., Jancewicz, A.L., Masson, P.H., 2017. Molecular mechanisms of root gravitropism. Curr. Biol. 27, R964–R972.
Suzuki, R.M., Kerbauy, G.B., Zaffari, G.R., 2004. Endogenous hormonal levels and growth of dark-incubated shoots of *Catasetum fimbriatum*. J. Plant Physiol. 161, 929–935.
Vacin, E.F., Went, F.W., 1949. Some pH changes in nutrient solutions. Bot. Gaz. 110, 605–613.
Xu, W., Ding, G., Yokawa, K., Baluška, F., Li, Q.-F., Liu, Y., Shi, W., Liang, J., Zhang, J., 2013. An improved agar-plate method for studying root growth and response of *Arabidopsis thaliana*. Sci. Rep. 3, 1273. https://doi.org/10.1038/srep01273.