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Carbohydrates Are Associated with the Flowering Ability of *Oncidesa* Gower Ramsey 'Honey Angel'

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***Abstract. Oncidesa* Gower Ramsey 'Honey Angel' is a cut flower crop of high economic value worldwide. The regulation of flowering is important for cut flower production scheduling. However, its flowering transition mechanism is still unclear. *Oncidesa* usually flowers at the end of the growth cycle for each pseudobulb; this timing is probably related to carbohydrate accumulation. During this study, we investigated the carbohydrates in the pseudobulbs from juvenile plants to adult plants and compared the carbohydrates in flowering and nonflowering adult plants. The current pseudobulb and back pseudobulbs of the plants at 0, 0.5, 1.0, 1.5, and 2.0 years after having been moved out of the tissue culture flask were collected. The first pseudobulb formed at 0.5 years, and plants had fulfilled four growth cycles and flowered at 2.0 years. Each successive current shoot grew larger and the back shoot number progressively increased after each new growth cycle. The concentration of total soluble sugars in the current shoot increased from 5.5% of dry weight at 0.5 years to 20.2% of dry weight at 1.5 years. Conversely, the starch concentration decreased in the current pseudobulb as the plants matured. The starch concentration in the back pseudobulbs did not change when the plant grew a new shoot. The starch concentrations in the back pseudobulbs ranged from 33.2% to 57.5% of dry weight, but the combined content of starch in all of the back pseudobulbs increased significantly from 168 mg at 0.5 years to 4608 mg at 2.0 years because of the increasing number of back shoots. The starch in the first back pseudobulb of the nonflowering adult plants accounted for 18.0% of dry weight, which was lower than that of the flowering plants (48.3%). There was no significant difference in total soluble sugars in the current pseudobulb of the nonflowering and flowering plants. Overall, we revealed that the increase in the back shoot number increased the total amount of reserve carbohydrates as the plant reached reproductive maturity. A low starch level was observed in nonflowering adult plants. In both cases, flowering plants had higher starch storage in the back pseudobulbs, suggesting that carbohydrates might regulate the flowering of *Oncidesa* Gower Ramsey 'Honey Angel'.**

Oncidesa Gower Ramsey 'Honey Angel' is an important cut flower orchid cultivar with a high global commercial value. The global cut flower market is valued at approximately USD $8490 million, including USD $214 million for cut orchids. The type of orchids exported depends on the location; however, the *Oncidesa* is mainly exported from Taiwan and Thailand (Yuan et al. 2021). It is an epiphytic orchid with a pseudobulb at the base of each shoot that stores water and nutrients. A growth cycle of *Oncidesa* starts from the emergence of a vegetative shoot from a base node of the immediate last mature back pseudobulb. As the shoot grows, an internode starts to swell and forms a pseudobulb, followed by the inflorescence initiation on the leaf axil immediately below the pseudobulb (Li and Chang 2023). The growth cycle ends after inflorescence development and flowering. The developing shoot is called the current shoot, and it becomes a back shoot after the new vegetative shoot for the next growth cycle emerges. The back shoot connected to the new current shoot and the number of back shoots increased after each growth cycle. For *Oncidesa* Goldiana, a growth cycle comprises half a year, 133 to 182 d; however, sometimes the inflorescence does not develop, and the length of the growth cycle is shortened to 77 to 140 d (Hew and Yong 1994).

During our observation, the flowering of *Oncidesa* depends on its nutritional status. The adult plants bloom at the end of each growth cycle, which is unaffected by the seasonal climate changes or through photoperiod or vernalization pathways. Hsiao et al. (2011) also indicated that the flowering of *Oncidesa* might be more regulated by an autonomous pathway linked to the nutritional status of the pseudobulbs. Carbohydrates interact with several flowering transition pathways (Bolouri Moghaddam and Van den Ende 2013; Wahl et al. 2013). The carbohydrate availability is an important signal during flower induction that ensures that nutrients are sufficient during the flowering process (Cho et al. 2018; Wahl et al. 2013). Sucrose in *Arabidopsis thaliana* advances the flowering time by suppressing the juvenile-related mRNA, mi156, and it is essential for the expression of the *FLOWERING LOCUS T* (*FT*) gene in the phloem companion cells that ensure that the carbohydrates are available to support the energy demand for flowering (Wahl et al. 2013). In laelia orchid (*Laelia anceps* subsp. *ancep*), a sympodial orchid with a growth habit similar to that of *Oncidesa*, the balance of sugars between the back and current pseudobulbs and endogenous hormones are likely involved in flowering regulation (Tejeda-Sartorius et al. 2022). The sugars increase in the leaves of *Phalaenopsis* just after the plants are forced under cool temperatures, and the sugar content is strongly correlated with the inflorescence development rate (Kataoka et al. 2004; Lee et al. 2020). Studies have shown that the carbohydrate supply is important for the production of the floral meristem.

Oncidesa Gower Ramsey 'Honey Angel' is propagated by tissue culture in commercial production, with a juvenile stage of 1.5 to 2 years after deflasking. Additionally, the inflorescence of adult plants is sometimes not induced. Carbohydrates in plants are involved in the signaling network of flowering transition pathways. Therefore, we investigated the carbohydrate profiles of the juvenile and adult *Oncidesa* Gower Ramsey 'Honey Angel' to determine the relationship between carbohydrates and the flowering ability during this study.

Pseudobulbs are carbohydrate storage organs in *Oncidesa*. When *Oncidesa* Goldiana plants have more shoots, they produce higher-quality cut flowers with more florets, increased branches, and longer inflorescence (Yong and Hew 1995a). Soluble sugars accumulate in the expanding pseudobulb on the current shoot of *Oncidesa* Gower Ramsey 'Honey Angel'. Glucose and fructose are the main carbohydrates in the current pseudobulb. The total soluble sugars account for 25% to 30% of the current pseudobulb dry weight when they reach the highest concentration (Lin and Chang 2023; Wang et al. 2003). Inflorescence growth consumes these soluble sugars in the current pseudobulb. After the plants enter the next growth cycle, the "current" pseudobulb becomes a back pseudobulb and the pseudobulb begins storing starch (Lin and Chang 2023). In nonflowering plants, the soluble sugars accumulated in the current pseudobulb remain and are used for vegetative growth during the next growth cycle.

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The *Oncidesa* flowers on the current shoot at the end of a growth cycle, and the carbohydrates in the current shoot support the development of the inflorescence (Lin and Chang 2023). However, the carbohydrates in the current pseudobulb might not be associated with the flowering transition of *Oncidesa.* The inflorescence of *Oncidesa* forms from the axillary bud below the pseudobulb. At first, the node number of the axillary bud increases, and the primordia of branches and florets are formed later in the bud (Li and Chang 2023; Tanaka et al. 1986). Based on the anatomical features, the transition from the vegetative phase to the reproductive phase was confirmed after the formation of branch primordia on the axillary bud. In *Oncidesa* Gower Ramsey 'Honey Angel', the formation of branch primordia and expansion of the current pseudobulb occur simultaneously (Li and Chang 2023). The ortholog of the *FT* gene is expressed in the axillary bud of *Oncidesa* Gower Ramsey before the expansion of the pseudobulb (Hou and Yang 2009). In brief, the flowering transition happens before sugar is accumulated in the current pseudobulb. If the flowering transition of *Oncidesa* is linked to the carbohydrate status, then it might be associated with the carbohydrates in the back pseudobulbs.

During this study, we investigated the relationship between flowering ability and carbohydrates in *Oncidesa* Gower Ramsey 'Honey Angel' using juvenile and nonflowering adult plants and compared them with plants that were mature and able to flower.

Materials and Methods

Expt. 1: Changes in carbohydrates in pseudobulbs of *Oncidesa* Gower Ramsey 'Honey Angel' from deflasking to flowering.

Plant materials. The tissue culture-propagated 'Honey Angel' plants were collected at Jin-Chang Orchid Nursery in Taichung, Taiwan (lat. 24.195907°N, long. 120.762995°E, elevation 310 m), on 20 Dec 2019. The plants were grown in the open air with a shade net in the nursery, and the average annual temperature was 23.7 °C. Plants at five stages, 0, 0.5, 1, 1.5, and 2 years after deflasking, were used during this experiment. We collected 10 plants for each stage, and a single plant served as one replication. Plants just moved out of the flask (0 years) were the bud-like plantlets consisting of unfolding leaves; the pseudobulb had not formed (Fig. 1A). After 0.5 years from deflasking, the bud-like shoot had fully developed, and another new shoot emerged from the leaf axil of the base node (Fig. 1B). The shoot number continued to increase from 1 to 2 years after deflasking (Fig. 1C–E). We collected the plants when a new shoot had just emerged from the leaf axils at each stage to ensure the current shoot had fully developed and accumulated carbohydrates. Plants were at the juvenile stages from 0 to 1.5 years. At 2 years after deflasking, the plants were mature and flowered before being collected.

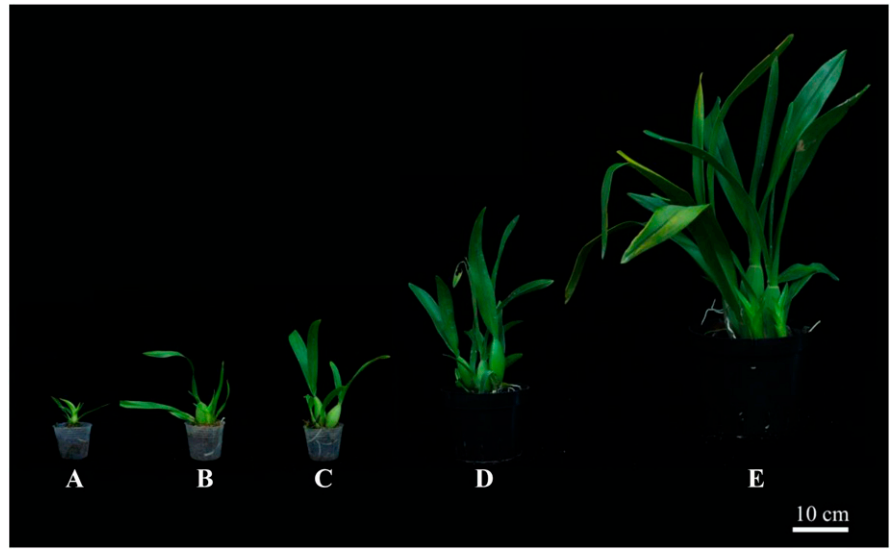

Fig. 1. The appearance of *Oncidesa* Gower Ramsey 'Honey Angel' after deflasking for 0 (**A**), 0.5 (**B**), 1 (**C**), 1.5 (**D**), and 2 (**E**) years.

Data and sample collection. Plants were separated into a bud-like new shoot, current shoot, and multiple individual back shoots. The new shoots were further divided into leaves and roots, and the current shoot and back shoots were divided into leaves, pseudobulbs, and roots. We weighed the fresh weight of each separated part immediately after plant collection and dry weights after freeze-drying the pseudobulbs for the carbohydrate analysis.

The pseudobulb of the current shoot accumulated glucose and fructose as it developed, which were different from the starch stored in the pseudobulbs of back shoots (Lin and Chang 2023). During this experiment, we collected the plants when the new shoots just emerged, indicating that the current shoots had fully developed and became the first back shoots. However, for ease of understanding, we still called it the current shoot during this experiment and separated it from other back shoots during analyses. The carbohydrates, including glucose, fructose, sucrose, water-soluble polysaccharides, and starch concentration (dry weight basis), in the current pseudobulb and other back pseudobulbs were analyzed using the following method.

Expt. 2: Carbohydrate analysis of the flowering and nonflowering 'Honey Angel'

Plant materials. The 'Honey Angel' plants used in this experiment were collected from Jin-Chang Orchid Nursery in Taichung, Taiwan, on 5 Mar 2019. Ten flowering and 10 nonflowering plants of the same age were picked. The flowering and nonflowering plants were 2.5 years from deflasking and grown in the same environment in the nursery with a shade net. Visible inflorescence buds that just emerged on the leaf axil determined the flowering plants. However, the nonflowering plants had vegetative buds just emerging on the leaf axil; no developing inflorescence or cut stalk residue was observed.

Data and sample collection. Plants were collected and transported to the laboratory on the same day. The connected shoots of the plants were divided. We used two shoots from each plant, the latest developed shoot and the back shoot connected to the first one, for analyses. Although the nonflowering plants had just entered the next growth cycle and the first shoots of nonflowering plants had just become the first back shoots, we still referred to the first shoots of both flowering and nonflowering plants as the current shoots and the second shoots as the first back shoots during this experiment to allow a consistent comparison. Each shoot was divided into leaves, pseudobulbs, and roots. We weighed and recorded the fresh weight when we collected the samples. We weighed and recorded the dry weight of the samples after freeze-drying. We also analyzed the carbohydrates in these pseudobulbs.

Carbohydrate analysis. During both experiments, content and concentration (dry weight basis) of glucose, fructose, sucrose, water-soluble polysaccharides, and starch were analyzed. We fixed the samples in liquid nitrogen during the morning, collected them, and ground them into powder after freeze-drying. We placed 50 mg of sample powder in a tube for the soluble sugar analysis and added 100 μL of 1% raffinose as an internal standard. The extractions were conducted with 3 mL 80% ethanol at 70 °C for 30 min; then, we performed centrifuging at 5000 rpm for 10 min. The samples were vortexed twice during each extraction. We performed the extraction processes three times for each sample and gathered 9 mL of supernatants. Then, we purified the supernatants using an ion-exchange resin column consisting of 1 mL anion exchange resin (Amberlite IRA-67, acetate form; Sigma-Aldrich, St. Louis, MO, USA) and 1 mL cation exchange resin (Dowex-50W, hydrogen form; Sigma-Aldrich). We washed the column with 4 mL of 80% ethanol at the end to collect 13 mL of purified supernatants. We concentrated the purified supernatants to less than 1 mL using

a vacuum evaporator (Labconco Co., Kansas, MO, USA); then, we increased it to 10 mL with Milli-Q water.

The insoluble residue pellets from the soluble sugars extraction were used to extract the water-soluble polysaccharides (WSPs). We followed the method of Ranwala and Miller (2008). We adjusted the pH of Milli-Q water with $CaCO_3$ to 8 and used 5 mL adjusted Milli-Q water to extract WSPs at 70 °C for 30 min. Two extractions were conducted. After each extraction, the samples were centrifuged at 5000 rpm for 10 min. The supernatants of two extractions were combined, and 2 mL of supernatants were used for hydrolyzation with 0.5 mL 5 M HCl for 3 h.

We used potato starch (Merck, Darmstadt, Germany) as an external standard for the starch analysis. The leftovers of the WSP extraction were gelatinized in 2 mL Milli-Q water at 100 °C for 6 h. We added 2 mL of starch-degrading enzymes, α-amylase (100 units/sample; Sigma-Aldrich), pullulanase (9 units/sample; Sigma-Aldrich), and amyloglucosidase (10 units/sample; Sigma-Aldrich) in pH 4.7 sodium citrate buffer solution after cooling to room temperature, followed by a 37 °C water bath for 6 h and 55 °C water bath for 18 h to break down the starch. Then, we centrifuged the samples, collected the supernatant, and brought the volume to 10 mL with Milli-Q water.

High-performance anion exchange chromatography with pulsed amperometric detection (Dionex Co, Sunnyvale, CA, USA) was used to analyze the carbohydrate extractants. The GS50 gradient pump, an ED50 detector, and a CarboPac PA-1 analytical (4 × 250 mm) column comprised the chromatography system. The samples of soluble sugars and glucose hydrolyzed from starch were eluted with 200 mM NaOH for 30 min at 1 mL·min^{-1} at 800 psi, and the samples of the soluble sugars hydrolyzed from WSPs were eluted with 10 mM NaOH for 30 min at 1 mL·min^{-1} at 800 psi. The potential and duration of waveform were as follows: E1 = 0.1 V, 400 ms; E2 = −2.0 V, 20 ms; E3 = 0.6 V, 10 ms; and E4 = −0.1 V, 60 ms.

Statistical analysis. A completely randomized design was used during both experiments. Multiple comparisons were performed using an analysis of variance (ANOVA) during Expt. 1, and means separation was conducted using the least significant difference (LSD) test when the ANOVA $P \leq 0.05$. Expt. 2 used the Student t test with $P \leq 0.05$ for mean separation. We used Costat (version 6.1; CoHort Software, Monterey, CA, USA) for the statistical analysis and SigmaPlot software (version 10.0; Systat Software, San Jose, CA, USA) to plot figures.

Results

Expt. 1: Changes in carbohydrates in pseudobulbs of 'Honey Angel' from deflasking to flowering. The pseudobulb of *Oncidesa* Gower Ramsey 'Honey Angel' plantlets formed at the base of the shoot, and a new shoot emerged from the leaf axil by 0.5 year safter deflasking (Fig. 1B). By 1.0 year after deflasking, the plants had the first back shoot, and more back shoots connected to the current shoot as the plants grew. The fresh and dry weights of all back shoots of plants increased from 6.3 to 111.7 g and 0.8 to 20.8 g, respectively, between 1.0 and 2.0 years (Figs. 2A and 3A). The current shoot became larger during each successive new growth cycle. The pseudobulb of the current shoot was 1.8 g in fresh weight at 0.5 years that increased to 31.5 g at 2.0 years after deflasking (Fig. 2C). The fresh weights of leaves and roots of the current shoot also increased from 0.9 to 22.6 g and 1.1 to 14.7 g, respectively (Fig. 2B and D).

The concentration of total soluble sugars increased in the current pseudobulb from 55.5 to 202.2 mg·g^{-1} (dry weight basis) from 0.5 to 1.5 years, and the concentration decreased to 95.9 mg·g^{-1} when plants flowered at 2.0 years (Fig. 4A). However, the starch concentration of the current pseudobulb decreased from 221.5 to 51.2 mg·g^{-1} from 0.5 to 1.5 years, and it increased to 352.8 mg·g^{-1} at 2.0 years (Fig. 4A). The concentrations of WSPs of the current shoot at 0.5 to 2.0 years were between 19.2 and 37.2 mg·g^{-1} (Fig. 4A).

Plants did not have a back shoot at 0.5 years after deflasking, and the current shoot at 0.5 years became the back shoot at 1.0 year. The carbohydrate concentrations of the back shoots were stable, and there were no significant changes as the plants aged. The concentrations of soluble sugars were between 54.3 and 94.9 mg·g^{-1}. The WSP concentrations were between 12.7 and 17.7 mg·g^{-1}, and the starch concentrations were between 332.7 and 575.5 mg·g^{-1} (Fig. 4C).

When plants were at the juvenile stage, 0.5 to 1.5 years, the concentration of soluble sugars increased and that of starch decreased in the current pseudobulb as plants grew (Fig. 4A). Plants had flowered before being collected at 2.0 years after deflasking. The carbohydrate concentrations in the current pseudobulb of the plants at 2.0 years were similar to those in the back pseudobulbs (high in starch and low in soluble sugars) (Fig. 4A and C).

Because the current shoot grew increasingly larger and the number of back shoots progressively increased after each growth cycle, the carbohydrate contents changes in the pseudobulbs differed from carbohydrate concentrations. The total content of soluble sugars in the current pseudobulb increased from 7.8 to 413.2 mg, and the starch content increased from 31.6 to 1680.9 mg as plants increased in age from 0.5 to 2.0 years (Fig. 4B). The starch stored in all of the back pseudobulbs combined also increased significantly from 168.6 to 4608.2 mg (Fig. 4D) as plants grew.

The main soluble sugars in the current pseudobulb are glucose and fructose. The concentrations of glucose and fructose at 1.5 years were 89.2 mg·g^{-1} and 93.7 mg·g^{-1}, respectively (Fig. 5A). The sucrose concentrations in the current pseudobulb were between 9.8 and 26.6 mg·g^{-1} (Fig. 5A). In the back pseudobulbs, the soluble sugars remained at low concentrations (Fig. 5B).

Expt. 2: Carbohydrates in the flowering and nonflowering Oncidesa Gower Ramsey 'Honey Angel'. The current and first back shoots on flowering and nonflowering 'Honey Angel' were collected. In both types of plants, the fresh weights of the

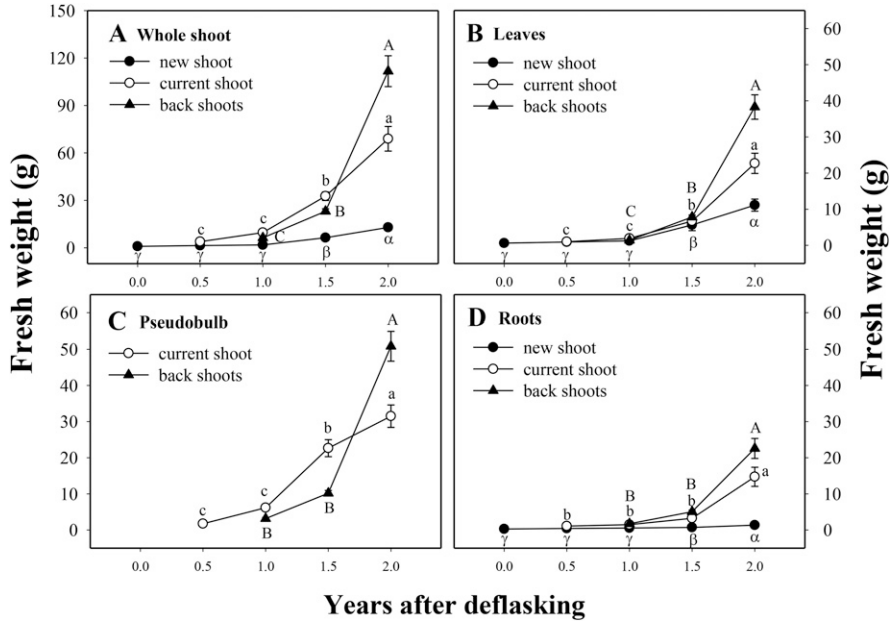

Fig. 2. Changes in the fresh weight of the whole shoot (**A**), leaves (**B**), pseudobulb (**C**), and roots (**D**) of various shoots in *Oncidesa* Gower Ramsey 'Honey Angel' plants after deflasking. Bars indicate the *SEM* (n = 10). Different Greek, lowercase, and capital letters represent significant differences in new shoots, current shoots, and back shoots, respectively, at $P \leq 0.05$ according to the least significant difference test.

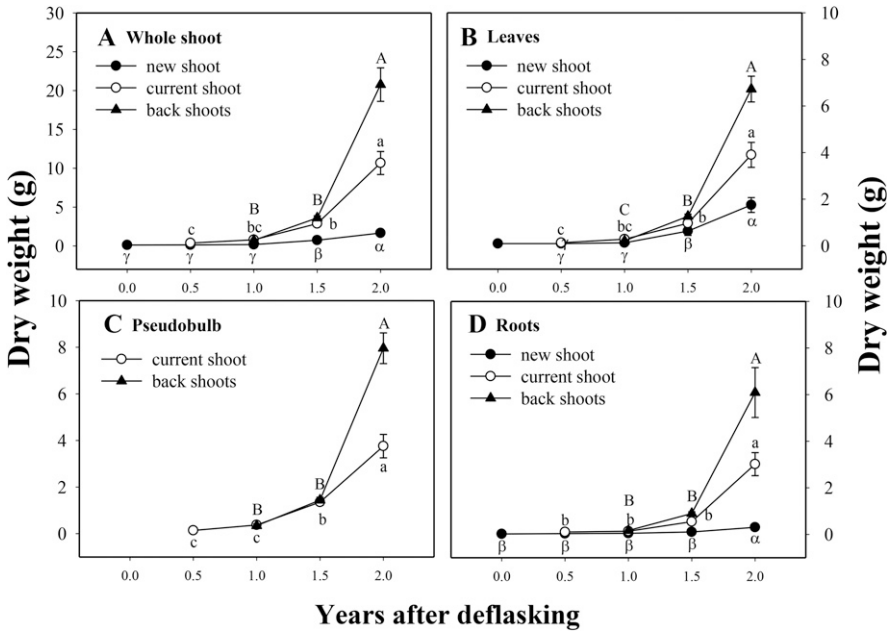

Fig. 3. Changes in dry weights of the whole shoot (**A**), leaves (**B**), pseudobulb (**C**), and roots (**D**) of various shoots in *Oncidesa* Gower Ramsey 'Honey Angel' plants after deflasking. Bars indicate the *SEM* (n = 10). Different Greek, lowercase, and capital letters represent significant differences in new shoots, current shoots, and back shoots, respectively, at $P \leq 0.05$ according to the least significant difference test.

current shoot were similar. Only the roots on the current shoot of flowering plants were heavier than those of the nonflowering plants, resulting in heavier total dry weights of the entire current shoot (Table 1). In the first back shoot, the fresh weight and dry weight of the pseudobulb of flowering plants were heavier than those on the nonflowering plants, resulting in a heavier dry weight of the whole first back shoot (Table 1).

The current pseudobulb of the nonflowering plants had a lower glucose concentration, but nonflowering plants had a higher fructose concentration in the first back pseudobulb (Fig. 6). However, the concentrations of total soluble sugars in both the current and the first back pseudobulb of flowering and nonflowering plants were not significantly different (Fig. 7). The WSP concentration in the current pseudobulb of the flowering plants was higher than that of the nonflowering plants; however, the concentrations in both types of plants were low, 9.3 and 6.3 mg·g^{-1}, respectively (Fig. 7). The most significant difference between flowering plants and nonflowering plants was the concentration of starch in the first back pseudobulb. The starch concentrations were 483 mg·g^{-1} in flowering plants and 181 mg·g^{-1} in nonflowering plants (Fig. 7).

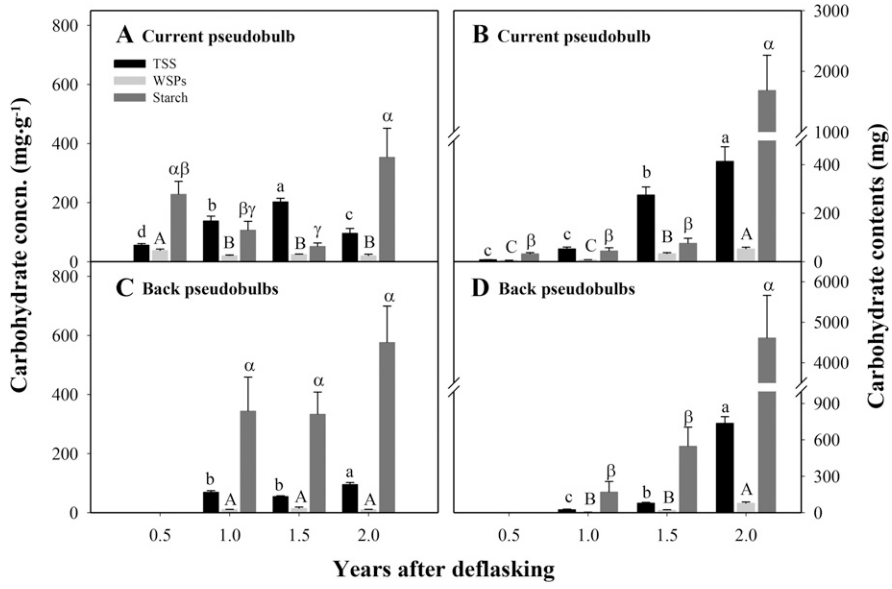

Fig. 4. Changes in total soluble sugars (TSS), water-soluble polysaccharides (WSPs), and starch concentrations and contents in the current pseudobulb (**A**, **B**) and all other back pseudobulbs (**C**, **D**) of *Oncidesa* Gower Ramsey 'Honey Angel'. Bars indicate the *SEM* (n = 10). Different lowercase, capital, and Greek letters represent significant differences in TSS, WSPs, and starch, respectively, according to the least significant difference test at $P \leq 0.05$.

Discussion

The back pseudobulbs of *Oncidesa* store a large amount of starch and act as carbohydrate source organs for new growth (Lin and Chang 2023; Yong and Hew 1995a). Between 61% and 72% of the photoassimilates produced by the leaves of back shoots was transported to the current shoot in *Oncidesa* Goldiana, and the increase in the back shoot number enhanced the quality of the inflorescence (Yong and Hew 1995a). The pseudobulb numbers of *Catasetum viridiflavum* and *Dimerandra emarginata* were also important for plant growth (Zimmerman 1990; Zotz 1999). In *Oncidesa* Gower Ramsey 'Honey Angel', the starch concentration was approximately 50% of the dry weight in the back pseudobulbs, which contributed to the growth of the current shoot and affected the accumulation of soluble sugars in the current pseudobulb (Lin and Chang 2023).

The concentration of soluble sugars, 202.2 mg·g^{-1} (dry weight basis), in the current pseudobulb of 1.5-year plants in Expt. 1 (Fig. 4A) was close to that of the flowered adult plants, at 235.9 mg·g^{-1}, in Expt. 2 (Fig. 7). During previous studies, the concentrations of soluble sugars in the current pseudobulb of adult *Oncidesa* plants were also in the range of 200 to 300 mg·g^{-1} (Lin and Chang 2023; Wang et al. 2003), which could be an indicator of the maturity of *Oncidesa* plants.

Although the soluble sugar concentrations in the current pseudobulb of adult plants (Figs. 4A and 7) were similar to those reported by prior studies (Lin and Chang 2023; Wang et al. 2003), some factors affected the size of the current pseudobulb and the content of total soluble sugars. When two current shoots emerged on a back shoot and grew simultaneously, the current shoots competed for carbohydrates in the back pseudobulbs, resulting in smaller current pseudobulbs and a lower level of soluble sugars (Lin and Chang 2023). The nonflowering plants also had less starch to contribute to the current shoot during this study (Fig. 7), which might be the reason for the lower current shoot dry weight in nonflowering plants (Table 1). The soluble sugars in the current pseudobulb contributed to inflorescence development, and the starch concentration increased to approximately 50% of the dry weight when the next vegetative shoot emerged in 'Honey Angel' (Lin and Chang 2023). We collected the plants with new vegetative shoots at various times after deflasking during this study. Because the plants had matured and flowered on the current shoot at 2.0 years, soluble sugars

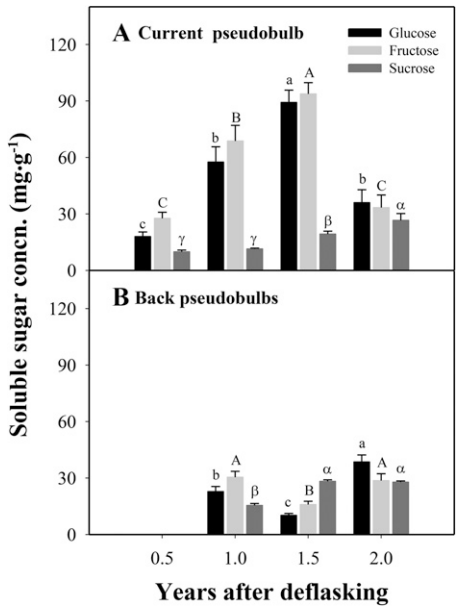

Fig. 5. Changes in glucose, fructose, and sucrose concentrations in the current pseudobulb (**A**) and back pseudobulbs (**B**) of *Oncidesa* Gower Ramsey 'Honey Angel'. Bars indicate the *SEM* (n = 10). Different lowercase, capital, and Greek letters represent significant difference in glucose, fructose, and sucrose, respectively, according to the least significant difference test at $P \leq 0.05$.

were consumed by flowering and the starch accumulated in the current pseudobulb when we collected the plants (Fig. 4A).

Although pseudobulbs of *Oncidesa* Goldiana contained chlorophyll and performed photosynthesis, the thick cuticle on the surface restricted gas exchange. The pseudobulbs only fixed carbon dioxide produced by itself internally (Hew and Yong 1994), and they were strong carbon sink organs during vegetative growth (Yong and Hew 1995b). As the pseudobulb of *Oncidesa* Gower Ramsey 'Honey Angel' developed, the hexoses, including glucose and fructose, first accumulated in the pseudobulb. Then, the pseudobulb began storing starch after the hexoses were consumed by reproductive growth (Lin and Chang 2023). The terrestrial orchids *Pleione aurita* and *Cymbidium sinense* also accumulated soluble sugars first, and then stored starch as the level of soluble sugars declined after the pseudobulb matured (Li and Zhang 2019; Zhang et al. 2023) The different carbohydrate forms in the young and old pseudobulbs were also found during this study. There was a higher soluble sugar level in the current pseudobulb and a higher starch level in the back pseudobulb (Figs. 4 and 7).

Resource storage is a major function of a plant, and the resources that accumulate in plants can be remobilized to support growth or other plant functions in the future (Chapin et al. 1990). Storage at different timeframes, such as daily, weekly, or lifetime, also has different patterns and compounds (Chapin et al. 1990). Glucose and fructose in the current pseudobulb of *Oncidesa* could be used as substrates for respiration, build-up the reserve organ and pseudobulb, and support inflorescence development. These two sugars benefited the growth of the pseudobulb and inflorescence. The concentrations of glucose and fructose in the pseudobulb were high, 20% to 30% of dry weight in total, for only one-third of a growth cycle to support current pseudobulb enlargement and inflorescence development (Lin and Chang 2023). Compared with starch that was stored for several growth cycles, glucose and fructose were the carbohydrate forms during a shorter timeframe and immediately supported the growth.

However, the young *Oncidesa* Gower Ramsey 'Honey Angel' plants had a different storage pattern in the pseudobulb. Higher starch was detected in the first formed pseudobulb (0.5 years) (Fig. 4A); the current pseudobulb had already started storing starch when we collected the plant. During the following growth cycles (1.0–1.5 years), the total soluble sugar increased and starch decreased in the current pseudobulb (Fig. 4A), likely because the current pseudobulb tended to grow more vigorously from 1.0 to 1.5 years (Figs. 2C and 3C), and this growth needed more soluble sugars for support. The *Oncidesa* had flowered and produced new vegetative shoots at 2.0 years. Compared with the current pseudobulb that formed between 1.0 to 1.5 years, the soluble sugars in the current pseudobulb had been used for inflorescence growth, and the pseudobulb had changed from a vigorous growth organ to a storage organ. Therefore, a high starch level and low total soluble sugar level were found in the current pseudobulb at 2.0 years (Fig. 4A).

Sugars control the flowering of plants. Elevating the endogenous sugar level, exogenously applying sugar, or regulating the sucrose transporter can effectively accelerate flowering in many species (Cho et al. 2018). Sucrose application at 50 mM promoted flowering even under the night break condition of the short day-induced *Chrysanthemum* 'Flora Yuuka' (Sun et al. 2017). Increasing sucrose in the shoot apex by silencing the sucrose transporter in tomato (*Solanum lycopersicum*) enhanced flowering (Liang et al. 2023). Silencing the *TREHALOSE-6-PHOSPHATE SYNTHASE 1* (*TPS1*), the synthase for sugar signal, delayed the flowering of *Arabidopsis thaliana* under an inductive photoperiod (Wahl et al. 2013). Sucrose application in *Oncidesa* Gower Ramsey 'Honey Angel' accelerated the expression of *TPS1* in the current pseudobulb and advanced flowering (Tsai 2018). During our previous studies, the flowering transition of *Oncidesa* 'Honey Angel' occurred when the current pseudobulb just started to expand and accumulate sugars (Li and Chang 2023). The soluble sugars in the current pseudobulb of *Oncidesa* 'Honey Angel' were significantly affected by the amount of starch that can be supplied in the back pseudobulbs (Lin and Chang 2023). During this study, we found that the flowering

Table 1. Fresh weight and dry weight of flowering and nonflowering *Oncidesa* Gower Ramsey 'Honey Angel' plants.

	Fresh wt (g)			Dry wt (g)		
Plant part	Flowering	Nonflowering	*t* test	Flowering	Nonflowering	*t* test
Whole current shoot	75.2	65.8	ns	7.65	5.95	*
Leaves	15.3	14.9	ns	2.48	2.10	ns
Pseudobulb	54.1	47.6	ns	4.22	3.33	ns
Roots	5.8	3.3	**	0.95	0.51	***
Whole first back shoot	54.6	43.9	ns	8.73	5.05	**
Leaves	19.5	17.2	ns	3.44	2.39	ns
Pseudobulb	31.9	23.3	*	4.55	1.99	***
Roots	3.2	3.4	ns	0.73	0.67	ns

ns, *, **, and *** represent nonsignificant differences and significant differences at $P \leq 0.05$, 0.01, and 0.001, respectively, between flowering and nonflowering plants in a row according to the Student *t* test.

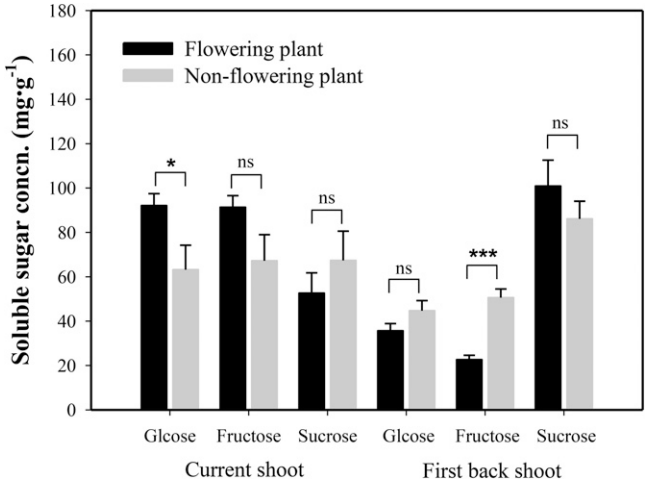

Fig. 6. Concentrations of soluble sugars in the current and first back pseudobulb of flowering and nonflowering *Oncidesa* Gower Ramsey 'Honey Angel' plants. Bars indicate the *SEM* (n = 10). ns, *, and *** represent nonsignificant and significant differences at $P \leq 0.05$ and 0.001, respectively, between flowering and nonflowering plants according to the Student *t*-test.

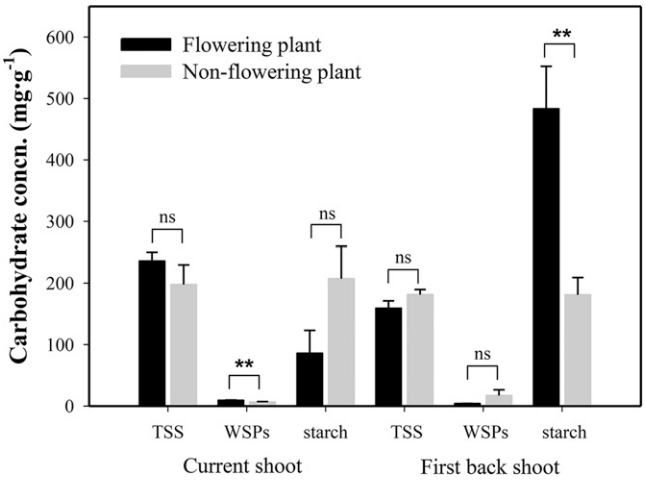

Fig. 7. Concentrations of total soluble sugars (TSS), water-soluble polysaccharides (WSPs), and starch in the current and first back pseudobulb of flowering and nonflowering *Oncidesa* Gower Ramsey 'Honey Angel' plants. Bars indicate the *SEM* (n = 10). ns and ** represent nonsignificant ($P > 0.05$) and significant differences at $P \leq 0.01$, respectively, between flowering and nonflowering plants according to the Student *t*-test.

plants had a significant higher starch level stored in the first back pseudobulb (Fig. 7). Although the soluble sugar might control the flowering transition, the starch in the back pseudobulbs played an more important role in mediating the flowering of *Oncidesa*.

Conclusions

By comparing the carbohydrates among the plants of various maturity levels and between flowering and nonflowering adult plants, we found that there were significant differences in the reserve carbohydrates in the back pseudobulbs. The carbohydrate storage of the plants increased as they matured; this was attributed to the larger size of the pseudobulb and increased number of the back pseudobulbs. In the adult plants, carbohydrates were also found to be related to the flowering ability. In the nonflowering plants, the back pseudobulb had a lower starch concentration, but there was no difference in carbohydrates in the current pseudobulb between nonflowering and flowering plants. In conclusion, the starch stored in the back pseudobulbs was strongly associated with the flowering of *Oncidesa* Gower Ramsey 'Honey Angel'.

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