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Research article

Influence of sucrose feeding on sugar metabolism and invertase activity in cut *Dendrobium* flowers

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Abstract

Importance of the work: The invertase enzyme is important for sugar metabolism; however, there is little information available on invertase activity in *Dendrobium* flowers.
Objectives: To investigate the changes in acid invertase activity and sugars in cut *Dendrobium* flowers held in a vase solution.
Materials & Methods: Inflorescences of *Dendrobium* cv. Khao Sanan were held either in distilled water or in solutions containing a combination of germicidal compounds and sucrose. The invertase activity and concentrations of sucrose, glucose and fructose in floral buds and open florets were determined.
Results: Cell wall invertase activity increased at bud opening; however, there was no increase in vacuolar invertase activity. Sucrose treatment increased cell wall invertase activity in the perianth but not in the column and did not increase vacuolar invertase activities in either the perianth or the column.
Main finding: Cell wall invertase activity was higher than vacuolar invertase activity for all flower developmental stages. Sucrose treatment stimulated the accumulation of hexoses in the floral buds and increased cell wall invertase activity in the perianth.

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Introduction

Inclusion of exogenous sugars in the holding or vase solution may have several impacts on cut orchid flowers, including an increase in the number of buds that open, a decrease in flower abscission and an extension of vase life (Ketsa and Boonrote, 1990; Ketsa and Teeracharoenpunya, 1990; Rattanawisalanon et al., 2003). Sucrose is the preferred sugar for inclusion in the holding solution because it is low cost, readily available and can be effectively transported in the phloem of most cut flowers (Halevy and Mayak, 1981). In intact plants, sucrose is the main sugar that is transported (through the phloem) from source organs, such as leaves, to sink organs, such as flowers and growing tissues. Sucrose is converted to hexoses by invertase, with this enzyme playing a key role in the regulation of sucrose metabolism, transport, and storage at various developmental stages of a particular plant tissue or organ and its activity can be impacted by different internal and external conditions (Tymowska and Kreis, 1998; Koch, 2004). Invertase catalyzes an irreversible cleavage reaction whereas, in contrast, sucrose synthase catalyzes a reversible reaction and can, therefore, also cleave sucrose (Kim et al., 2000). Invertase activity is found in both water soluble and insoluble fractions. Both vacuolar and neutral/alkaline invertases are soluble, with an acidic pI, while cell wall invertases are insoluble, with a mostly basic pI (Sturm and Tang, 1999). Invertase isozymes are localized to cell walls, the cytoplasm and the vacuole (Ma et al., 2000; Sherson et al., 2003). The cell wall (insoluble form) and vacuolar (soluble form) isozymes are called acid invertase, while the cytoplasmic isoform is termed neutral invertase. Insoluble acid invertase plays an important role in converting sucrose into hexoses when reaching the apoplast and hexose is taken up readily by petals (Roitsch and Gonzalez, 2004). Soluble acid invertase is presumed to hydrolyze sucrose to supply hexoses necessary for cell growth and development (Tymowska and Kreis, 1998; Tang et al., 1999). Flower petals, as sink tissues, often have relatively high invertase activity (Winkenbach and Matile, 1970; Hawker et al., 1976; Paulin and Jamain, 1982).

Thailand is currently the world's number one orchid flower exporter, with its top selling species being *Dendrobium*, *Aranda*, *Arachnis*, *Oncidium* and *Vanda*; the Ministry of Commerce of Thailand (MOC) has estimated a USD 400 million global orchid market, with most of the orchids available on the market coming from the tropics. There are currently more than 60 major orchid buyer markets worldwide, with the USA, Japan, Vietnam, China and Italy accounting for 68% of the world's orchid imports (Anon, 2022). The inclusion of sucrose in preservative solutions for various *Dendrobium* cultivars has been reported (Ketsa and Boonrote, 1990; Ketsa et al., 1995; Rattanawisalanon et al., 2003; Pattaravayo et al., 2013). The invertase enzyme has been shown to have an important role in the sugar metabolism of a range of different cut flower species (Sturm and Tang, 1999; Ito et al., 2007; Kumar et al., 2008; Shimizu-Yumoto et al., 2020). However, there is little information available on the activity of acid invertase in cut *Dendrobium* flowers, especially in flowers treated with sucrose. Therefore, the objective of this study was to investigate the changes in acid invertase activity and to determine the changes in different sugars within cut *Dendrobium* flowers during a holding period in a vase solution containing both antimicrobial compounds and sucrose.

Materials and Methods

Plant material and sugar treatment

Inflorescences of *Dendrobium* cv. Khao Sanan were purchased from a commercial grower in the Bangkok suburban area, Thailand. Export grade inflorescences, with 4–5 open florets and 6–7 floral buds were selected for freshness and uniformity. Upon arrival at the laboratory, the peduncles of individual inflorescences were recut 12 cm from the lowermost open floret. The entire inflorescence of cv. Khao Sanan comprised florets at different developmental stages (Fig. 1).

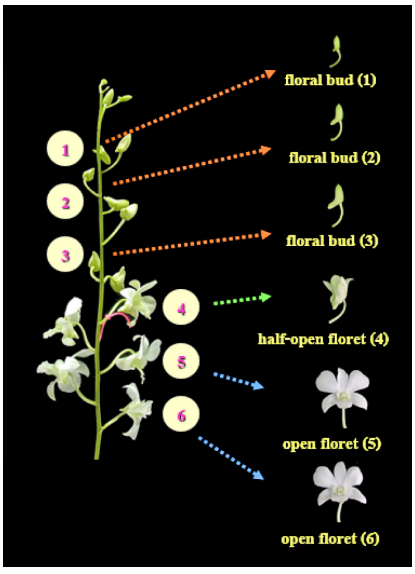

Fig. 1 Inflorescence samples of cut *Dendrobium* cv. Khao Sanan showing six different developmental stages (1–6)

Individual florets were assigned to six stages from the top of the inflorescence downward: stage 1, small floral bud; stage 2, medium floral bud; stage 3, large floral bud; stage 4, half-open floret; stage 5, open florets below the half-open floret; and stage 6, the lowermost open floret (Fig. 1).

Florets in all six developmental stages but without sucrose treatment were used to study cell wall and vacuolar invertase activity. Whole unopened floral buds at stages 1 and 2, and half-open florets and open florets at stages 5 and 6, were separated into petals and sepals and column plus labellum to study sugar metabolism and invertase activity in response to sucrose treatment.

The stem ends of individual inflorescences were placed in 15 cm centrifuge tubes containing either distilled water (control) or an aqueous solution of 4 g/L sucrose together with the antimicrobial compounds silver nitrate ($AgNO_3$) at 30 mg/L and 8-hydroxyquinoline sulfate (HQS) at 225 mg/L (Pattaravayo et al., 2013). Controls were included for these antimicrobial compounds combined without the inclusion of sucrose (data not reported here as the results were the same as those for the water treatment). Inflorescences were held indoors in a room with low light (about 15 µmol/m^2/s), an average temperature of 26.5 °C and a relative humidity of 70%, with a diurnal variation of about 2 °C and 5% relative humidity.

Determination of sucrose, glucose and fructose concentrations

Each 15 g sample of dried perianth (petals + sepals) or of the column + labellum was homogenized with 3 mL of 80% ethanol and kept at 80 °C for 20 min and then centrifuged at 12,000×g (Jouan KR22i Centrifuge; Curis-au-Mont-d'Or, France) for 20 min. The mixtures were filtered through four layers of cheese cloth and dried in a freeze-drier. The dried samples were dissolved with 3 mL double distilled water and filtered through a cellulose nitrate membrane filter (45 µm pore size). Soluble sugars were analyzed based on high performance liquid chromatography using a stainless-steel column (300 mm × 6.5 mm internal diameter) packed with a microparticulate cation exchange gel in calcium form (Sugar Pak 1; Waters Corporation; Milford, MA, USA). The filtered sample was pumped through the column at a flow rate of 0.5 mL/L and calcium ethylene diamine tetraacetate was used as the mobile phase. The temperature was adjusted to 90 °C. Sucrose, glucose and fructose were identified by their retention times and were quantified using standards.

Enzyme extraction and assay

Extraction of vacuolar (soluble) and cell wall (insoluble) invertase activity followed the method of Pramanik et al. (2004) with slight modification. Each 2 g sample of isolated petals plus sepals or column plus labellum was mixed with 1% polyvinylpolypyrrolidone and homogenized with 10 mL 0.2 M citrate-phosphate buffer (C-P buffer) at pH 5.0. Then, the resultant homogenate was filtered through four layers of cheese cloth and the filtrate was centrifuged at 11,000×g (Jouan KR22i Centrifuge; Curis-au-Mont-d'Or, France) for 10 min. The total supernatant was dialyzed for 12 h with 0.2 M C-P buffer at pH 5.0, diluted 40 times, and the inner solution was designated as vacuolar (soluble) invertase. The residual tissues were re-extracted in 10 mL 0.2 M NaCl in C-P buffer for 24 h with occasional stirring. The supernatant was dialyzed as described above and the dialyzed solution was designated as cell wall (insoluble) invertase. Assays of vacuolar and cell wall invertases followed the method of Pramanik et al. (2004). The protein content was determined using the standard Bradford method (Bradford, 1976).

Statistical analysis

A completely randomized design was used. Six replicates (florets) were used per treatment and two inflorescences were used in each replicate. All experiments were repeated at least once on separate occasions, with very similar results. Data were treated for multiple comparisons based on analysis of variance with the least significant difference (LSD) and a t test between averages determined at the 0.5% level using the SPSS Statistics 23.0 software (IBM Corp.; Armonk, NY, USA).

Results

At harvest, the concentrations of both sucrose and fructose were higher in the buds than in the open florets (Figs. 2A and 2D, respectively, compared to Figs. 2D and 2F, respectively), whereas the reverse was the case with glucose (Fig. 2B compared with Fig. 2E). The concentrations of both sucrose and fructose declined rapidly within the buds within 48 h of harvest. Subsequently, in the non-treated controls, the concentration of sucrose in the floral buds and in the open florets continued to decline but at a much slower rate and values were lower in the floral buds (Fig. 2A)

than in the open florets (Fig. 2D). A similar decline over time was noted for fructose but the concentrations were similar in each developmental stage (Figs. 2C, 2F). In contrast, large differences were found in the glucose concentrations, which were almost two times higher in the open florets (Fig. 2E) than in the floral buds (Fig. 2B). Furthermore, in the floral buds and in the open florets, the concentrations of glucose (Fig. 2B) were considerably higher than the concentrations of fructose and sucrose.

Treatment of the inflorescences with sucrose in the vase solution resulted in increased concentrations of all three sugars measured in the floral buds (Figs. 2A, 2B, 2C). However, such treatment had no effect on the concentrations of these sugars in the open florets during the first 6 days of vase life, but they increased slightly over the last 2 d (Figs. 2D, 2E, 2F). The patterns of decline in concentration were unchanged.

The cell wall invertase activity increased sharply at the time the florets were about to open, then progressively decreased in later stages of development. Such differences were not found in the vacuolar invertase activity that remained low in each developmental stage (Fig. 3). The activities of both invertases were considerably higher at harvest (day 0) than on day 2 of vase life and remained relatively low after day 4 (Fig. 4), especially with vacuolar invertase. Sucrose treatment significantly increased cell wall invertase activity in the floral buds for the first 6 d of vase life (Fig. 4A) but had no effect on the cell wall invertase in the open florets (Fig. 4B) or on the vacuolar invertase activity in either development stage (Figs. 4C, 4D).

In the half-open florets, the cell wall invertase activities in the perianth (Fig. 5A) were considerably less than in the column (Fig. 5C) at each assessment time for the 8 d for the control and treatment. In contrast, the cell wall invertase activities in the perianth and column of open florets were similar (Figs. 5B, 5D). The activity in the column reduced by 50% between the two development stages. Treatment with sucrose did not appreciably change the cell wall or vacuolar invertase activities in either the perianth or the column (Figs. 5 and 6).

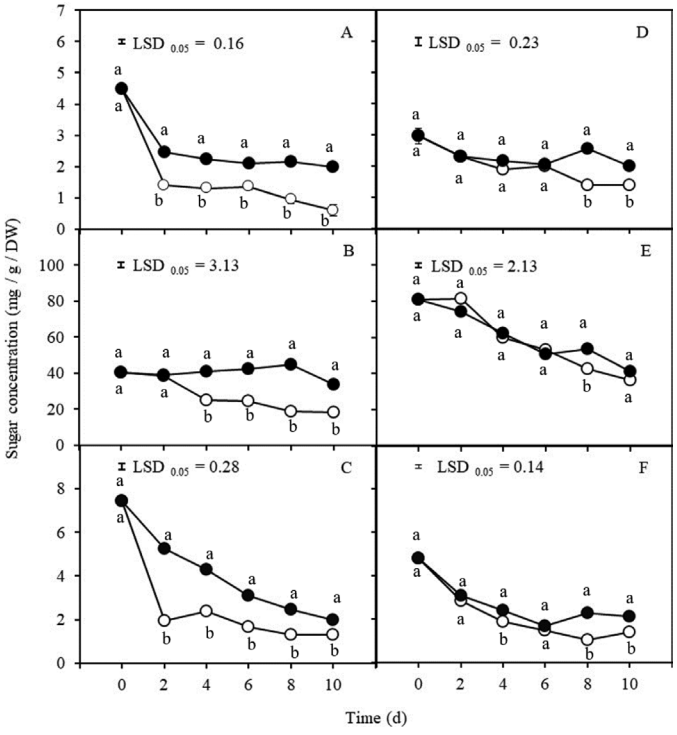

Fig. 2 Concentrations of sucrose (A, D), glucose (B, E) and fructose (C, F) in floral buds (stages 1 and 2; A, B, C) and open florets (stages 5 and 6; D, E, F) in cut *Dendrobium* cv. Khao Sanan inflorescences held in distilled water (○) or in solution of HQS + $AgNO_3$ + sucrose (●), where data are means of six replications ± SD, least significance difference (LSD) is indicated by a bar, significant ($p < 0.05$) differences between means at each time point are denoted by different lowercase letters.

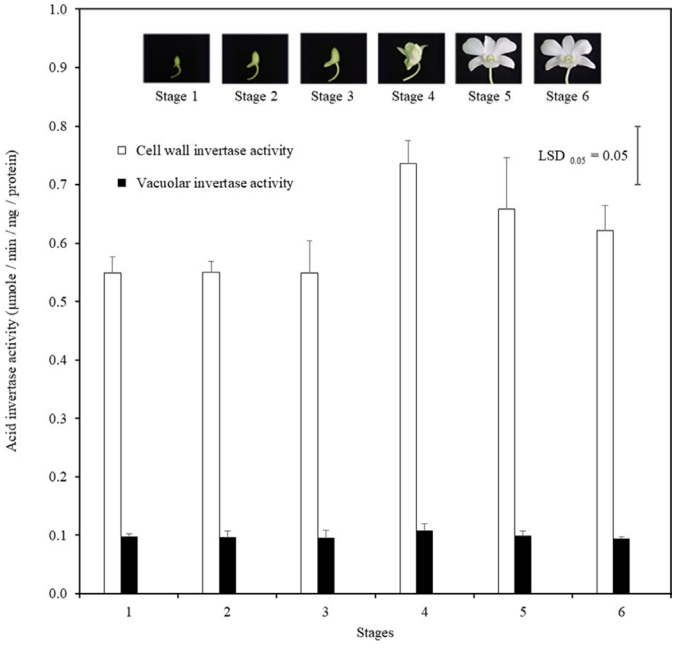

Fig. 3 Cell wall (□) and vacuolar (■) invertase activities at successive stages of floral bud development on untreated cut *Dendrobium* cv. Khao Sanan inflorescences, where data are means of six replications ± SD, least significance difference (LSD) is indicated by a bar. There was no significant different among mean cell wall invertase activity of different stages and neither was among the mean vacuolar invertase activity.

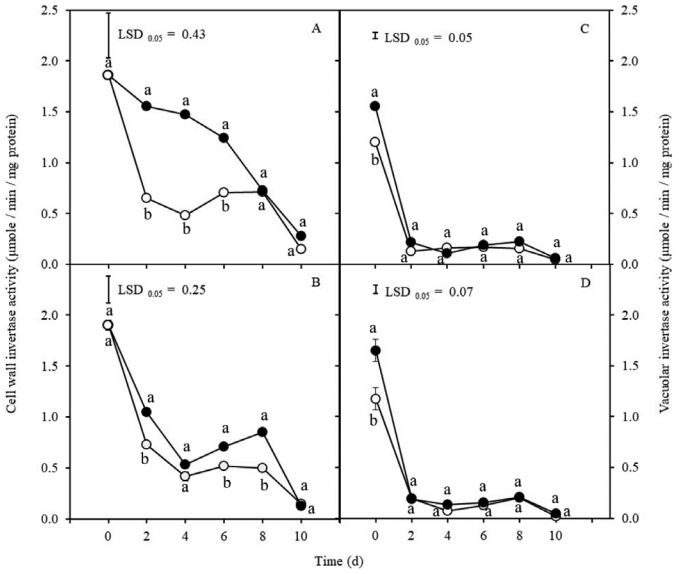

Fig. 4 Cell wall invertase (A, B) and vacuolar invertase (C, D) activities in floral buds (stages 1 and 2; A, C) and open florets (stages 5 and 6; B, D) in cut *Dendrobium* cv. Khao Sanan inflorescences held in distilled water (○) or in a solution of HQS + $AgNO_3$ + sucrose (●), where data are means of six replications ± SD, least significance difference (LSD) is indicated by a bar, significant ($p < 0.05$) differences between means at each time point are denoted by different lowercase letters.

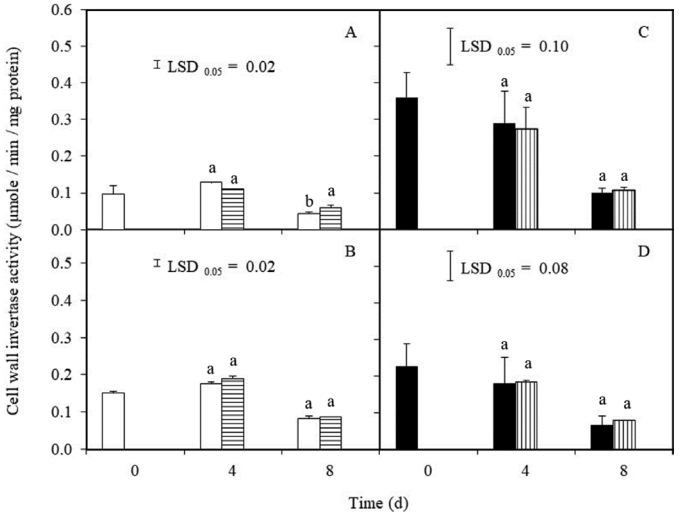

Fig. 5 Cell wall invertase activities in perianth (A and B; □, ▤) and column (C and D; ■, ▥) of half-open florets (stage 4; A, C) and open florets (stages 5 and 6; B, D) on cut *Dendrobium* cv Khao Sanan inflorescences held in distilled water (□, ■) or in a solution of HQS + $AgNO_3$ + sucrose (▤, ▥), where data are means of six replications ± SD, least significance difference (LSD) is indicated by a bar, significant ($p < 0.05$) differences between means at each time point are denoted by different lowercase letters.

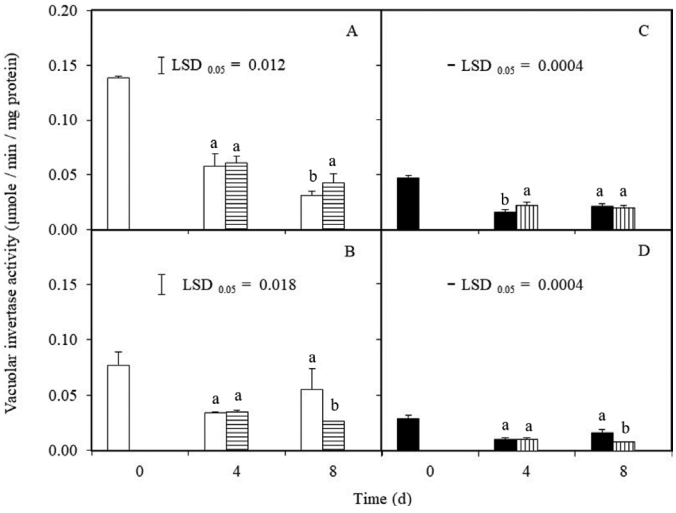

Fig. 6 Vacuolar invertase activities in perianth (A and B; □, ▤) and column (C and D; ■, ▥) of half-open florets (stage 4; A, C) and open florets (stage 5 and 6; B, D) of cut *Dendrobium* cv. Khao Sanan inflorescences held in distilled water (□, ■) or in a solution of HQS + $AgNO_3$ + sucrose (▤, ▥), where data are means of six replications ± SD, least significance difference (LSD) is indicated by a bar, significant ($p < 0.05$) differences between means at each time point are denoted by different lowercase letters.

Discussion

Glucose was the major sugar that accumulated in both the floral buds and the open florets of *Dendrobium* inflorescences (Figs. 2B, 2E), indicating that reducing sugars formed a large proportion of the carbohydrate pool in *Dendrobium*, in common with that in many other—but not all—cut flowers (Ho and Nichols, 1977; Trusty and Miller, 1991; Yamane et al., 1993; Lukaszewska, 1995; van Meeteren et al., 1995; Ichimura and Hisamutsu, 1999; van der Meulen-Muisers et al., 2001; Kumar et al., 2007; Yamada et al., 2007; Ketsa and Karunsatitchai, 2010; Shimizu-Yumoto et al., 2020). In contrast, fructose was the major reducing sugar that accumulated in chrysanthemum (Adachi, 1999), snapdragon (Ichimura and Hisamutsu, 1999) and rose (van Doorn et al., 1991; Ichimura et al., 2005; Yamada et al., 2007), while non-reducing sugars were the major carbohydrates that accumulated throughout the flower development of *Lilium pumilum* (Santos et al., 2016). Open florets (Fig. 2E) contained higher concentrations of reducing sugars than unopened floral buds (Fig. 2B) in *Dendrobium*. Studies have shown that floral buds are a strong sink during flower development (Kuiper et al., 1995;

Kumar et al., 2007; Ketsa and Karunsatitchai, 2010; Shimizu-Yumoto et al., 2020), supporting the assumption that floral buds are active metabolic centers and that they rapidly use reducing sugars for their growth and development, resulting in a lower level of reducing sugars in floral buds (Kumar et al., 2007; Yamada et al., 2007).

The positive effect of sucrose treatment on extending vase life was accompanied by an increase in the concentrations of sucrose, glucose and fructose in the floral buds (Figs. 2A, 2B, 2C); however, the treatment had little effect on the concentrations of these sugars in the whole open florets (Figs. 2D, 2, 2F). This response by floral buds likely accounted for the improvement in bud opening following sucrose treatment (Ketsa and Boonrote, 1990; Ketsa and Teeracharoenpunya, 1990; Rattanawisalanon et al., 2003). In contrast, the inclusion of sucrose in the vase solution increased the sucrose, glucose and fructose concentrations in the open florets of snapdragon (Ichimura and Hisamutsu, 1999; Asrar, 2012), rose (Kaltaler and Steponkus, 1974) and carnation (Hawker et al., 1976), suggesting that at least some of the sucrose treatment that had reached the floral buds had been converted into its constituent hexoses (Ho and Nichols, 1977; Kaltaler and Steponkus, 1974). Although fructose can be used for such treatments, glucose is preferred, while glucose, as the most directly usable form, is likely to be rapidly metabolized. Hence, a holding solution that contained glucose increased flower vase life longer than a solution containing sucrose and an anti-microbial compound (Ketsa and Boonrote, 1990; Cho et al., 2001; Ketsa, 2006; Watanabe et al., 2013; Hirose et al., 2020). In some cut flowers, sucrose treatment increased only the fructose content in open florets, although there was no increase in the sugars in the floral buds such as snapdragon (Ichimura and Hisamutsu, 1999), rose (Yamada et al., 2007) or dahlia (Shimizu-Yumoto et al., 2020).

Vacuolar invertase activity remained the same during the various stages of floral bud development. However, cell wall invertase activity markedly increased at the time that the floral buds were opening and then had declined by the time of full flower opening, though it remained higher than in the developing buds (Fig. 3). This suggested that vacuolar invertase activity has a relatively small role in and that cell wall invertase activity was largely involved in the processes leading to the accumulation of reducing sugars during this period of growth (Roitsch and Gonzalez, 2004). Cell wall invertases are central to phloem unloading in some—but not all—sucrose-importing structures. Their significance is most prominent in strong sinks (Koch, 2004). The changes in cell wall invertase activity at successive flower stages, which was shown to be related to an increase in the concentrations of reducing sugars, was similar to that found in rose (Kumar et al., 2008) and dahlia (Shimizu-Yumoto et al., 2020). Differences between cell wall and vacuolar invertase activity at successive flower stages in *Dendrobium* were also found in rose (Yamada et al., 2007) and dahlia (Shimizu-Yumoto et al., 2020). In contrast, vacuolar (soluble acidic) invertase activity was the major invertase involved in *Epidendrum ibaguense* vase life (Mapeli et al., 2015). Sucrose treatment appeared to increase only cell wall invertase activity in unopened floral buds (Fig. 3A) indicating that sucrose can induce such an increase, as suggested by Soni and Kaufman (1972) and Kaufman et al. (1973). Sucrose treatment did not increase cell wall or vacuolar invertase activity in either the perianth or in the column of half-open and open florets after 4 d and 8 d of vase life (Figs. 5, 6) as was similarly reported for rose (Yamada et al., 2007), supporting the observation that sucrose did not increase cell wall or vacuolar invertase activity in the open florets (Fig. 4). Overall, these results supported the major role of cell wall invertase activity in the floral bud development and floret expansion of *Dendrobium* flowers, as has been reported in rose (Yamada et al., 2007) and dahlia (Shimizu-Yumoto et al., 2020).

In conclusion, the sucrose treatment promoted an increase in the sucrose, glucose and fructose concentrations in the developing and expanding floral buds. However, the sucrose treatment had no effect on the concentrations of these sugars in whole open florets. Cell wall invertase activity was much higher than vacuolar invertase activity in all developmental stages of the flowers and cell wall invertase activity was higher in the column than in the perianth. Sucrose treatment enhanced cell wall invertase activity only in unopened floral buds.

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