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Regulation of flower development in *Dendrobium crumenatum* by changes in carbohydrate contents, water status and cell wall metabolism

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ABSTRACT

The involvement of carbohydrates, water potential, cell wall components and cell wall-based enzymes in regulating flower development in *Dendrobium crumenatum* was investigated. Plants were subjected to cold treatment to release floral buds from dormancy, and the various parameters were investigated from young floral bud stage till flower senescence. Development of floral buds was accompanied by progressive decrease in concentrations of fructans and starch. Upon full flower opening, concentration of soluble sugars was maximum, accompanied by a more negative water potential. High pectin methylesterase activity was observed during early bud development and decreased thereafter. Significant increase in activities of β-galactosidase, β-mannosidase and β-xylosidase was also observed during floral bud development. The cell walls of sepals and petals were modified extensively during floral bud and flower development, as observed by changes in the amounts of celluloses, hemicelluloses and total pectin. Pectin solubilisation was also observed to commence during early floral bud development. These results indicated that carbohydrate hydrolysis, osmotic changes and cell wall dissolution that began early in young floral buds, all regulated flower development in this sympodial orchid. Possible applications of the findings in the horticultural industry are discussed.

1. Introduction

Flowering is a critical event in the life-cycle of angiosperms, allowing for the reproduction of these plants. Flowers of various plants are also highly prized objects of beauty and are commercially valuable. Hence, understanding the various processes that regulate flower opening and senescence could help to enhance the visual quality and vase-life of flowers, and thus increasing their commercial value. Besides, with the world's increasing interest in 'green buildings' to aid energy efficiency and the accompanying issue of using flowers for aesthetic benefits (Spala et al., 2008), understanding flower physiology is very important. However, publications on the physiology of tropical flowers are limited and the few detailed studies focus mainly on flowers of temperate species such as carnation (*Dianthus caryophyllus* L.), daylily (*Hemerocallis* spp.), Asiatic lily (*Lilium* hybrid), rose (*Rosa*) and sandersonia (*Sandersonia aurantiaca* (Hook.)).

In the opening of flowers, changes in carbohydrate metabolism and cell osmolarity are considered important driving forces behind petal movements (van Doorn and van Meeteren, 2003). Rapid flower opening in many species, including roses (Ho and Nichols, 1977), daylily (Bieleski, 1993), Asiatic lilies (Bieleski et al., 2000) and creeping bellflowers (*Campanula rapunculoides*) (Vergauwen et al., 2000), was related to the hydrolysis of reserve carbohydrates. Rapid petal movements are also highly correlated with cell sap osmolarity changes, which regulate the direction of water movements, resulting in turgor changes and cell expansion (Ho and Nichols, 1977; Hew et al., 1989; Bieleski, 1993). Changes in carbohydrate metabolism and cell sap osmolarity are, therefore, intimately linked in the process of petal expansion and flower opening.

In recent years, besides the roles of carbohydrates and cell osmolarity, there has been some focus on the possible involvement of cell wall metabolism in the regulation of flowering (de Vetten and Huber, 1990; O'Donoghue et al., 2002). These studies also addressed the question on whether flowering could be a process induced by the senescence programme of the plant and/or specific plant organ. Some supporting evidences included the upregulation of a phosphoenolpyruvate mutase mRNA, involved in regulating hydrolytic enzymes that resulted in membrane degradation in senescing carnation petals (Wang et al., 1996), the upregulation in levels of a mRNA that codes for proteins controlling the oxidation of membrane lipids, prior to or during flower senescence in daylily (Panavas et al., 1999), and the increasing trend of DNA laddering

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throughout petal development in *Alstroemeria* (Wagstaff et al., 2003). While molecular evidence is increasing, structural and physiological evidences are quite limited. Studies on carnation and sandersonia flowers demonstrated that the transition of floral stages from opening to fully mature flower till senescence was accompanied by changes in the levels of various cell wall polymers such as cellulose and pectins, and activities of cell wall-based enzymes (de Vetten and Huber, 1990; O'Donoghue et al., 2002). These observations were similar to the loss of cell wall integrity in ripening fruits of carambola (*Averrhoa carambola*) and grapes (*Vitis vinifera*) (Chin et al., 1999; Deng et al., 2005). In daylily flowers, analyses of cell wall composition were not published, but reported changes in activities of cell wall-based enzymes during flower development suggested the involvement of cell wall metabolism in flowering (Panavas et al., 1998). While cellulase activity was detected in daylily flowers, it was reported to be absent in sandersonia flowers, indicating the possibility of a species-specific variation in cell wall metabolism that regulates flowering (O'Donoghue et al., 2002; Panavas et al., 1998). In the above-mentioned studies on carnation and sandersonia flowers, cell wall changes were compared only between stages of late bud (just prior to opening), opening flower, mature flower, wilting flower and senesced flower (de Vetten and Huber, 1990; O'Donoghue et al., 2002). To fully understand if flowering is a consequence of a senescence programme that has already started, investigating the physiological changes occurring throughout the development of a newly induced young floral bud till flower senescence would be advantageous. Pollination and fertilisation of flowers promote sepal/petal senescence, while keeping the fertilised ovary viable; in non-pollinated and unfertilised flowers, whole flowers senesce and die (van Doorn, 1997). The possible onset of senescence prior to flower opening would thus infer a modification of the senescence programme, due to a cascade of signals generated upon pollination and fertilisation that results in the senescence of sepals/petals, but not the ovaries.

Few studies on the physiology of flowering in tropical orchids have been conducted to date. As orchid cultivation continues to be a highly profitable commercial market (Hew and Yong, 2004), characterisation of tropical orchid flowering is of paramount importance. *Dendrobium crumenatum* (Swartz), also known as the pigeon orchid, is a common native epiphytic orchid species of South-east Asia. It exhibits an interesting and unique diversion of the normal flowering process: upon transition of the meristem from a vegetative to a reproductive phase, floral buds develop to a certain stage and then become 'dormant'. These floral buds resume growth and development after cold-induction, such as after a heavy rainfall, and culminating into the opening of the flowers exactly nine days after (Holttum, 1953; Corner, 1988). Full flower opening is achieved before dawn and the flowers last for only 24 h under natural conditions. Senescence of the flowers is indicated by the flaccid sepals and petals. Knowledge on the physiological processes controlling dormancy release, floral bud development, flower opening and senescence in *D. crumenatum* can be applied to commercially important flowers where it would be advantageous to be able to control the timing of floral bud development. For example, it would be beneficial to be able to force floral buds into dormancy during shipment, releasing them from dormancy and to resume normal floral bud development when required.

In this study, carbohydrates, water potential, cell wall components and activities of cell wall-based enzymes of *D. crumenatum* were analysed throughout the development of newly induced floral buds till flower senescence. These data would allow us a better understanding of the physiological processes, especially the possible involvement of cell wall metabolism, in the regulation of flowering.

2. Materials and methods

2.1. Plant material

Dendrobium crumenatum (Swartz) plants were maintained under partially shaded conditions (PAR ranged from 100 to 250 μmol m^{-2} s^{-1}; average air temperature ranged from 25 to 33 °C) in a planthouse of the Department of Biological Sciences, National University of Singapore. All plants were watered daily, and fertilised weekly with a foliar fertiliser (N18:P36:K18). Pots of *D. crumenatum* with inducible inflorescences carrying dormant floral buds were acclimatised at 30 °C for 24 h in temperature-controlled growth chambers. They were then subjected to a cold induction at 20 °C for 24 h. Growth chambers were maintained on a 12 h day/12 h night cycle and photosynthetic active radiation (PAR) ranged from 10 to 20 μmol m^{-2} s^{-1}. Plants of *D. crumenatum* exhibit crassulacean acid metabolism, demonstrating different carbon dioxide exchange patterns during different times of the day. Thus, all plants were moved into the growth chambers at 1600 h, to minimise the effects of any possible temporal variations in the plant physiology. Plants were also watered daily to minimise dehydration stress. Floral buds or flowers for analyses of carbohydrates, water potential, cell wall composition and cell wall enzyme activities were selected according to their age and features (Table 1, Fig. 3). Sepals and petals of the harvested floral buds and flowers were separated and stored at −80 °C until use.

2.2. Carbohydrate analyses

Sepals or petals (0.1 g) were boiled in 10 ml of distilled water for 90 min. The supernatant was collected as the total soluble sugar fraction. The residue was re-suspended in 10 ml of 10 mM sodium

Table 1
Stages of floral bud development in *D. crumenatum*

Features	Time (day)
Exposure of dormant floral buds to cold induction at 20 °C for 24 h	0
Green bud (*ca.* 1 cm long) with reddish brown tinges along ventral side, elongation of mentum, mentum reddish brown	4
Light green bud (*ca.* 2.5 cm long), reddish brown tinges only at beginning and tip of mentum, further elongation of mentum, length of mentum almost half of length of whole bud	7
White bud (*ca.* 3 cm long), no splitting of sepals, elongated mentum pointing downwards away from tip of bud	9
Full flower opening, sepals and petals fully expanded, lip fully protruded with visible yellow ridges running down from midlobe to foot of column	10
Sepals and petals shrivelled and brownish	12

Timing of events is reported in relation to the time during which floral buds were released from dormancy by cold induction (denoted as day 0).