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Warm-night temperature delays spike emergence and alters carbon pool metabolism in the stem and leaves of *Phalaenopsis aphroide*

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ABSTRACT

Different night temperature treatments have been adopted for spike emergence in *Phalaenopsis*. The aim of this research was to study the dawn-dusk carbohydrate and organic acid levels responding to warm- (28 °C day/28 °C night, spike inhibitory) and cool-night (28 °C day/20 °C night, spike inducing) temperatures for six weeks with respect to different ages of leaves, including the new and not fully expanded 1st leaf (L1), the new and fully expanded 2nd leaf (L2), the older 5th leaf (L5) and the stem, the locus of spiking, and the dawn-dusk oscillations of metabolites associated with spiking. The results showed that L2 exhibited the maximum amplitude of nocturnal malate accumulation and daytime starch deposition on both treatments. Compared with the control, the warm-night treatment dramatically reduced the soluble sugar concentration, particularly the hexose levels in L2 and L5. However, in the stem, the sucrose concentration after warm-night treatment was significantly higher than that of the control at dawn and dusk. We deduce that the potential role of sucrose in the stem is to sustain the viability of the dormant spike bud. The occurrence of a large amount of soluble sugars in the L5 of the control implies that the breaking of bud dormancy may rely on the supply of sugars from the older leaves. The warm-night treatment significantly increased the citrate concentration in all leaves at dawn, implying that citrate might be playing a crucial role in protecting the leaves from warm-night stress.

1. Introduction

Temperature is one of the most important environmental factors regulating flower development in *Phalaenopsis*. To induce year-round flowering, many orchid growers in Taiwan and Japan have adopted a warm-night temperature (~28 °C) treatment to inhibit spike induction during autumn and winter, and a cool treatment (~20 °C) to stimulate spiking during summer (Blanchard and Runkle, 2006; Chen et al., 2008; Guo and Lee, 2006; Pollet et al., 2011; Sakanishi et al., 1980). Nevertheless, the biochemical mechanism underlying spiking development in *Phalaenopsis* through alterations in the temperature regime remains poorly understood.

Phalaenopsis is an obligate crassulacean acid metabolism (CAM) plant and exhibits typical diurnal fluctuations of malate and starch content (Chen et al., 2008; Endo and Ikusima, 1989; Guo and Lee, 2006; Pollet et al., 2011), implying a close association with carbohydrate and organic acid metabolism. Popp et al. (2003) demonstrated that the extents of nocturnal accumulated malate and daytime deposited starch could be estimated by the levels at dawn minus that at dusk, and at dusk minus that at dawn, respectively, in CAM plants.

Phalaenopsis is a monopodial orchid with leaves located alternatively on the opposing side of the plant, in which causes the upper leaves to heavily shade the lower leaves. The stem had short internodes with many leaves embedded at the base and one or two dormant flower stalk bud(s), which developed from the stem at the base of the third to the fifth leaves from the apex. The dormant bud emerges under suitable conditions. Qin et al. (2012) suggested that orchid leaves primarily control the breaking of bud dormancy through temperature regulation. However, in Arabidopsis, the leaf is the site of photoperiodic signal perception, and vernalization primarily occurs in the shoot apical meristem, thus, some cues associated with the transition from the vegetative to the reproductive stage possibly coexist in the leaves and shoot apical meristem of Arabidopsis (Taiz and Zeiger, 2010). Since the spike bud connects with the stem, it is reasonable to speculate that the signal in the stem of *Phalaenopsis* is involved more directly to the spike development than that in the leaf. Many authors have asserted that sucrose

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plays a crucial role in flower stalk development of *Phalaenopsis*, but their deduction was only based on the results from studies using new and fully expanded leaves and one sampling time (Guo and Lee, 2006; Kataoka et al., 2004; Konow and Wang, 2001; Qin et al., 2012; Tsai et al., 2008). To our knowledge, little is known about the dawn-dusk changes in the carbohydrate levels in leaves of different ages and, particularly, in the stems of *Phalaenopsis* in relation to spike development. Therefore, the aim of this study was to focus on the dawn-dusk levels of carbohydrates and organic acids for elucidating the impact of warm-night treatment on the biochemical changes in leaves of three different ages and the stem to provide insight into the role of carbohydrates on *Phalaenopsis* spike emergence.

2. Materials and methods

2.1. Plant material and growth conditions

Mature *Phalaenopsis aphrodite* subsp. *formosana* were purchased from the Wusulin farm at the Taiwan Sugar Corporation and transplanted in 10.5 cm transparent plastic pots filled with sphagnum moss in an environment-controlled greenhouse at 28 °C for 4 months to inhibit spike induction. The plants were transferred for six weeks to a growth chamber under a photoperiod of 14 h (06:00 to 20:00) light and 10 h (20:00 to 06:00) dark with 150 μmole m^{-2} s^{-1} of photosynthetically active radiation (Philips cool-white fluorescence tubes) at the tops of the plants. The day temperature was 28 °C and the night temperature was either 28 °C or 20 °C for warm-night or control treatment, respectively. The plants were irrigated once a week, alternating between water and Peters fertilizer (1.0 g L^{-1}) (Hyponex Corp., Marysville, OH, USA) at approximately 1 mS cm^{-1}, as measured using a conductivity meter (Model 3250, JENCO Inc., CA, USA).

2.2. Definition of spiking and leaf age and sampling

Spiking was defined as the appearance of flower stalk greater than 0.5 cm in length. A total of three batch experiments, using 36 plants, were performed to determine the spiking ratio for each treatment. The spike number indicated the average number of flower stalk per spiking plant. The spike length was measured from the base of flower stalk at the junction between the base of the leaves and short internode to the apex of the flower stalk.

The leaves were numbered basipetally (Fig. 1A and B). The first leaf was new and not fully expanded leaf (L1); the second leaf was a new fully expanded leaf (L2) of approximately 1.5- to 2.0-fold the size of the first leaf; and the fifth leaf was located at the bottom of the old leaf (L5) with an age of at least 180 days older than the 2nd leaf (Guo and Lee, 2006). After completely removing all leaves, the stem was obtained by a knife cut from the connection between the base of flower stalk bud and the short internode (Fig. 1C).

Four plant replicates were obtained at dawn (06:00 to 07:30) and at dusk (18:30 to 20:00) for each treatment. Leaf discs (ca. 0.28 cm^2) were cut from the middle and approximately 0.5 cm away from the main vein of the leaf using a borer. The leaf discs and stems were weighed and frozen in liquid N_2.

2.3. Metabolite analyses

To determine the levels of organic acid, the leaf discs were boiled in distilled water (Callaway et al., 1997). The organic acid levels were analyzed through anion exchange chromatography on a Dionex AS-11 column (2 mm diameter, Dionex Corporation, Sunnyvale, CA, USA) using 5–100 mM NaOH for linear gradient elution, and the eluted acids were quantified using a Dionex Electrochemical Detector (ED50) (Chen et al., 2008).

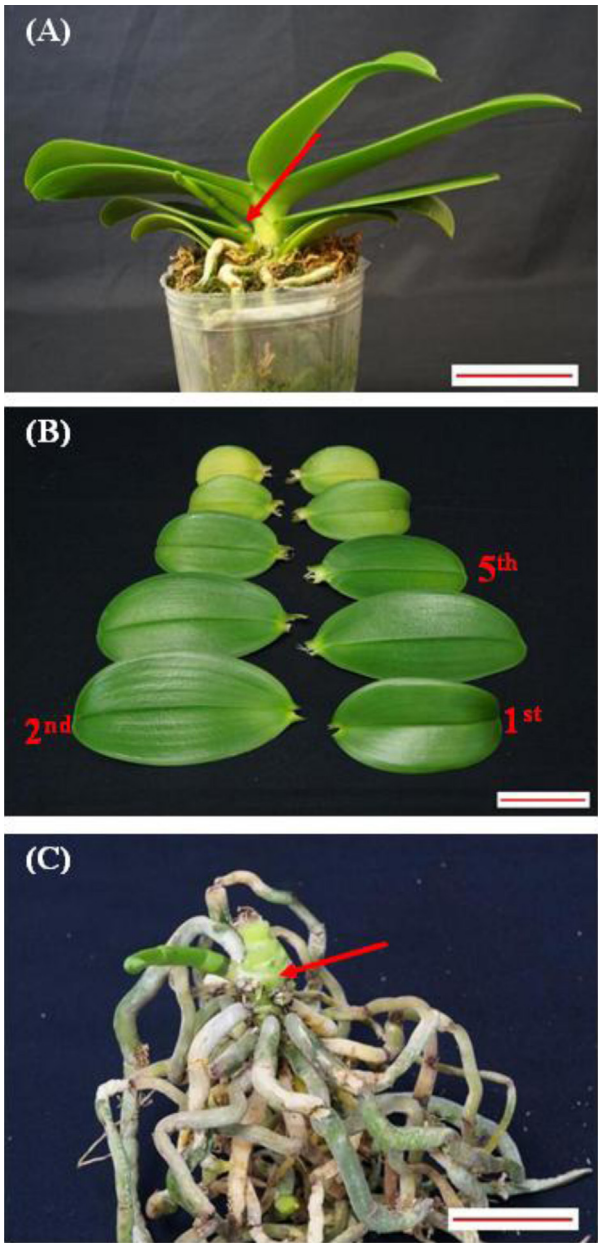

Fig. 1. (A) The appearance of mature *Phalaenopsis aphrodite* with spikes indicated with an arrow. (B) The leaves were numbered basipetally. (C) The stem was excised from the connection between the base of the flower stalk bud and the short internode indicated with an arrow. Bar = 5 cm.

To determine the soluble sugar concentration, the leaf discs and stems were homogenized and extracted with 80% (v/v) ethanol at 80 °C (Chen et al., 2008). The sugars were separated on a Dionex CarboPac PA10 column (2 mm diameter) using 18 mM NaOH as an eluent and quantified using a Dionex Pulsed Amperometric Detector (Chen et al., 2008). To determine the starch content, the sediment was digested with a mixture of pullulanase and amyloglucosidase, and the liberated glucose was estimated using a glucose oxidase and peroxidase method (Chen et al., 2008).

2.4. Statistical analysis

The data were evaluated for the effects of warm-night temperature, leaf age and the interaction between temperature and leaf age for statistical significance using two-way ANOVA. Fisher's protected least significant difference (LSD) test was adopted to