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Research article

Water use and photosynthesis of *Dendrobium* Sonia 'Earsakul' under water deficit stress

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Abstract

Dendrobium Sonia 'Earsakul' orchids in three developmental stages (plantlet, mature, flowering) were exposed to water deficit stress for 7 d. The average daily evapotranspiration rate (ET) ± SD under the well-watered condition was lowest in the plantlet stage (10.51 ± 1.96 mL/d). The ET of the mature and flowering stages was 45.80 ± 6.90 mL/d and 50.97 ± 7.23 mL/d, respectively. However, the ET was not significantly different between the mature and flowering stages. Under the water-limited condition, the ET values of all stages for the orchid were continuously depleted from the well-watered control at the 2nd day in the plantlet stage and the 3rd day of the mature and flowering stages after withholding water. The CO_2 exchange rate (CER) of the water-stressed plant substantially decreased compared to the control due to strong decreases in the stomatal conductance (g_s) and the transpiration rate (TR) in all developmental stages. In addition, water deficit stress led to a substantial decline in the water use efficiency (WUE) in the plantlet stage while the WUE of the mature and flowering stages remained similar to the control. However, the water deficit stress induced increased C_i/C_a in the plantlet stage. Moreover, shrinking of the stressed pseudobulb was seen in the plantlet and flowering stages but leaf wilting occurred only in the stressed plant of the plantlet stage. These results demonstrated that water deficit stress led to decreased photosynthesis in *Den.* Sonia 'Earsakul' due to stomatal limitation.

Introduction

Water is the important factor for the growth and development of plants as it is a main component in plant structure and the metabolic process (Fang and Xiong, 2015). A sufficient water supply to a plant is usually considered to equal the evapotranspiration rate (water use of the plant) which includes evaporation from the planting medium material and transpiration by the plant that differ in each species and developmental stage (Zotarelli et al., 2010).

Drought stress can influence plant physiology mechanisms, especially photosynthesis (Osakabe et al., 2014). Under drought conditions, several studies have reported CO_2 assimilation reduction in both monopodial and sympodial orchids (Cui et al., 2004; He et al., 2013). The inhibition of photosynthesis under water limited stress possibly resulted from stomatal limitation or non-stomata limitation process (Ni and Pallardy, 1992). However, several orchids have a crassulacean acid metabolism (CAM), which is the adaptation of the photosynthesis pattern to water insufficient conditions, whereby the plant can prevent the water loss from transpiration through opening the stomata and taking up CO_2 during the night and closing the stomata during the daytime (Silvera et al., 2009). Moreover, the presence of a pseudobulb in some types of orchid is able to alleviate water content reduction in the leaves under the water deficit condition (Tay et al., 2015). In addition, the ability to maintain water use efficiency under stress is a characteristic of a tolerant plant (Kebbas et al., 2015).

Dendrobium Sonia 'Earsakul' is one of the important tropical hybrid orchids that is the most cultivated and exported in Thailand, especially as a cut flower (Lekawatana, 2010). This kind of orchid has the ability to flower during the whole year; however, currently, Thai orchid growers are encountering drought due to climate change. As a result, there are very large losses in orchid productivity. Nevertheless, orchid growers still use excess water for irrigation that exceeds the orchid's requirements because information on the water consumption of this commercial orchid remains unknown. In addition, there has not been a comprehensive study of the physiological response to drought in orchids cultivated commercially in Thailand.

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This research aimed to estimate the daily evapotranspiration rate of three different developmental stages (plantlet, mature, flowering) of *Den.* Sonia 'Earsakul' to assist in decreasing the amount of excess water used in irrigation on orchid farms and to investigate the impact of water deficit stress on physiological alterations.

Materials and Methods

Plant material and growth conditions

Dendrobium Sonia 'Earsakul' samples in three different developmental stages were obtained from an orchid farm and acclimatized in the greenhouse for 1 wk. The stages of plant development were: plantlet stage (3–4 mth after transplantation from tissue culture with two pseudobulbs) planted in a plastic pot (4 cm diameter), mature stage (6–8 mth) and flowering stage (24 mth) with three and four pseudobulbs, respectively, that were planted in plastic pots (8.5 cm diameter). Coconut husk was used as planting material in the experiment and the 21-21-21 foliar fertilizer was applied to each orchid once a week by spraying. The average temperature during daytime in the greenhouse was 36.03 ± 0.43°C, relative humidity was 57.71 ± 4.41% and the vapor pressure deficit of the air was 2.72 ± 0.31 kPa under 100–120 µmol/m^2/s of light intensity.

Water deficit condition

The parts of the plant growing in coconut husk were soaked in water for 15 min to saturate the medium. The water was applied to the plants daily to maintain the water content in the planting material at field capacity (control) and water deficit was induced by withholding water for 7 d (water-stressed plant).

Evapotranspiration rate

Whole plants were weighed daily for 7 d, including the plastic pot for both the control and the water-stressed plants. The daily evapotranspiration rate was calculated based on the weight loss of the plant day per day and 20 plants were measured in each treatment. The weight of water was converted to volume assuming 1 g = 1 mL.

Gas exchange measurement

The CO_2 exchange rate (CER), stomatal conductance (g_s), transpiration rate (TR) and internal-to-ambient CO_2 concentration ratio (C_i/C_a) were measured at the 3rd full expanded leaf from the apical shoot of the front pseudobulb at 0400 hours after 7 d of withholding water using a Portable Photosynthesis System (Licor-6400*XT*; Licor Inc.; Lincoln, NE, USA) with the CO_2 concentration from the ambient air (396.16 ± 0.23 parts per million). The water use efficiency (WUE) was calculated from the ratio of CO_2 exchange rate and the transpiration rate (TR) using WUE = CER/TR (Cui et al., 2009). Four plants were measured in each treatment.

Statistical analysis

The experiment was conducted as a completely randomize designed. Data were analyzed using one-way analysis of variance and a general linear model (GLM) at significance levels of at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

Results

Evapotranspiration rate

The average evapotranspiration rates (ET) of *Den.* Sonia 'Earsakul' throughout the 7 d of measurement of the plantlet, mature and flowering stages for the control were 10.51 ± 1.96 mL/d, 45.80 ± 6.90 mL/d and 50.97 ± 7.23 mL/d, respectively. However, the ET was not significantly different between the mature and flowering stages on average over the whole treatment period (Fig.1B and 1C). The ET of all stages of stressed plants decreased substantially as the time under stress was extended. In the plantlet stage, the ET of the water-stressed plants was lower than for the control at the 2nd day of water withholding and substantially declined after 2 d of stress and continuously decreased to nearly 0 mL/day from the 4th day until the 7th day of the experiment (Fig. 1A). In addition, the ET values of orchids in the mature and flowering stages under water deficit significantly declined in comparison with the control from the 3rd day and continuously decreased throughout the experiment.

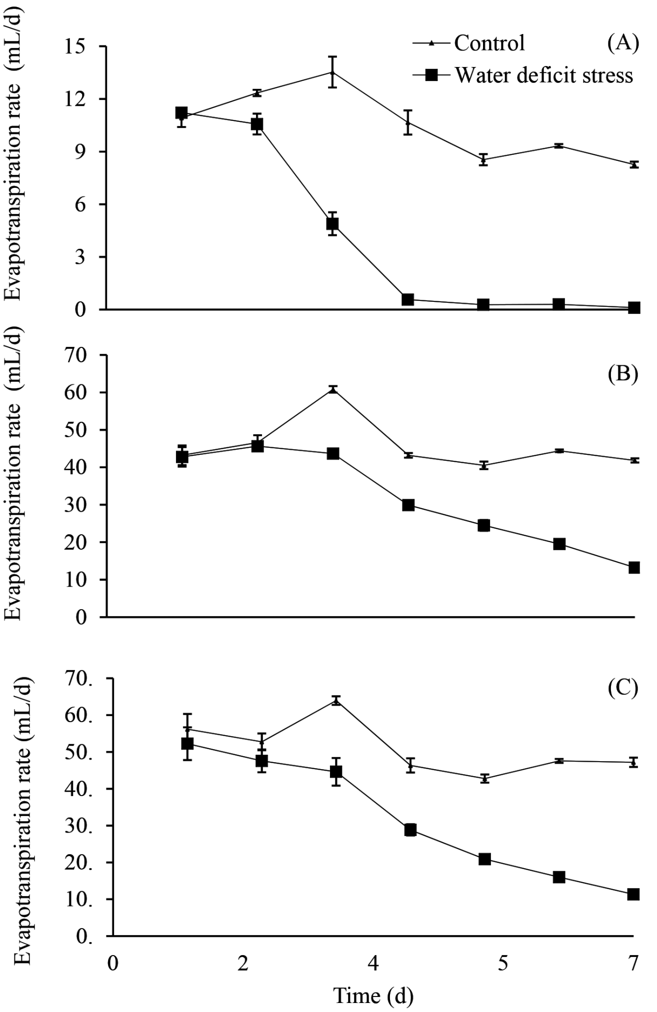

Fig. 1 Daily evapotranspiration rate of *Den.* Sonia 'Earsakul': (A) plantlet stage; (B) mature stage; (C) flowering stage exposed to well-watered condition (control) and water deficit stress for 7 d (error bar = ±SD).

Photosynthetic parameters

The CO_2 exchange rate (CER), stomatal conductance (g_s) and transpiration rate (TR) of *Den.* Sonia 'Earsakul' in the three developmental stages were measured at the 7th day after withholding water. The results demonstrated that the CER of orchids in the flowering stage was higher than in the plantlet and mature stages under the well-watered condition. However, the CER in all stages of orchids under water deficit stress substantially decreased and was 70% lower than the control (Fig. 2A). In addition, the values for g_s and TR of water-stressed plants in the three stages significantly declined compared with the well-watered plants (Fig. 2B–C). On the other hand, the values of WUE and C_i/C_a in the mature and flowering stages under stress remained similar to the control whereas there was a significant reduction in the WUE only in the plantlet stage under the water-limited condition (Fig. 2D–E). In contrast, the value of C_i/C_a significantly increased in the plantlet stage when exposed to water stress (Fig. 2E).

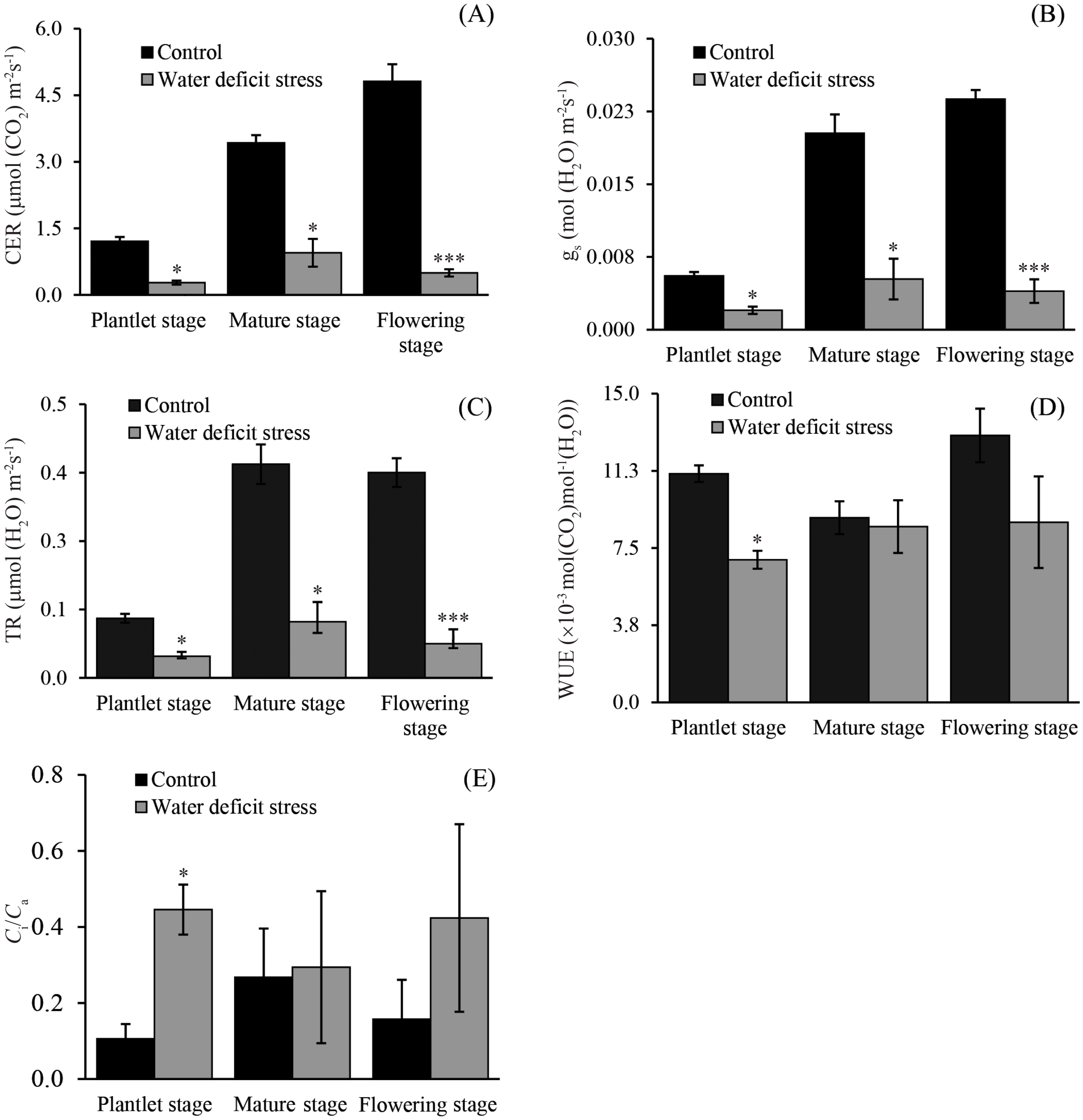

Fig. 2 Photosynthetic parameters of *Den.* Sonia 'Earsakul' in three developmental stages: (A) CO_2 exchange rate (CER); (B) stomatal conductance (g_s); (C) transpiration rate (TR); (D) water use efficiency (WUE); (E) internal-to-ambient CO_2 concentration ratio (C_i/C_a), where a significant difference from the control are shown as $^*p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$

Morphological features

The wilting of leaves occurred in the plantlet stage under water deficit stress and the color of the lower leaves turned yellow (Fig. 3E and 3F). There was shrinkage of the pseudobulb in the plantlet and flowering stages under stress, while the pseudobulb in the mature stage remained similar to that of the control (Fig. 3A and E, Fig. 3D and H and Fig. 3C and H, respectively).

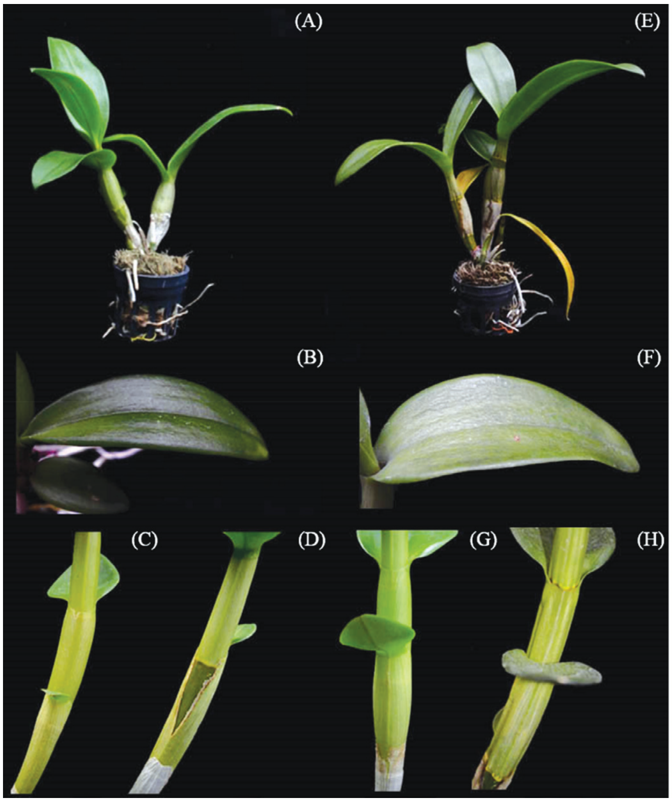

Fig. 3 Morphological features of *Den.* Sonia ‘Earsakul’ under well-watered control: (A) whole plant of plantlet stage, (B) leaf of plantlet stage, (C) pseudobulb of mature stage, (D) pseudobulb of flowering stage. Morphological features under water deficit stress: (E) whole plant of plantlet stage, (F) leaf of plantlet stage, (G) pseudobulb of mature stage (H) pseudobulb of flowering stage

Discussion

Dendrobium Sonia ‘Earsakul’ is a CAM plant that has thick leaves and a pseudobulb (Boonchai et al., 2017). The results of the current study showed that the different developmental stages of *Den.* Sonia ‘Earsakul’ required different quantities of water. The mature and flowering stages used more water than the plantlet stage because orchids in the mature and flowering stages had higher values of g_s and TR under sufficient water supply that was associated with an increased leaf size, more leaves and a greater total leaf area with age (Pallardy, 2008). Furthermore, the ET under the water deficit condition continuously decreased, unlike in the well-watered control at every stage. This finding suggested that the quantity of water in planting material was insufficient to cover the transpiration and evaporation demands when the plants were subjected to water deficit in comparison with the well-watered condition. With a sufficient water supply, the CER of the flowering stage was higher than for the other stages. Drought can lead to physiological alteration in various orchids especially in the photosynthesis mechanism (Fu and Hew, 1982; He et al., 2013). In *Den.* Sonia ‘Earsakul’, drought strongly impacted a decrease in CER, g_s and TR in all developmental stages. Although, CAM plants usually open their stomata at night and close them during the daytime to limit water loss during high temperature, the reduction in g_s due to water deficit stress was like stomatal closure to limit water loss by transpiration which is a water stress resistance mechanism (Fang and Xiong, 2015). However, the stomatal closure decreased transpiration but also inhibited CO_2 uptake which caused photosynthesis limitation (Kebbas et al., 2015). In the current study, *Den* Sonia ‘Earsakul’ in the mature and flowering stages under the water stress condition showed the ability to maintain WUE even though fewer stomata were open while the WUE significantly decreased in the plantlet stage, thus demonstrating that the plantlet stage was quite susceptible to water deficit stress. The water deficit led to a significant increase in C_i/C_a in the plantlet stage even though the CO_2 uptake and stomatal conductance decreased, so the increase in C_i/C_a may possibly have resulted from the rise in CO_2 in the intercellular space from the higher cellular respiration that was accelerated under stress to generate more ATP for maintenance of all metabolism in the cell (Pinheiro and Chaves, 2010). Nevertheless, the C_i/C_a values in the mature and flowering stages were not different under all two planting conditions studied and this could have been due to the ability to maintain WUE under water deficit stress.

Moreover, drought stress modifies plant morphological features (Zhang et al., 2004). The pseudobulb of *Den.* Sonia ‘Earsakul in the plantlet and flowering stages clearly shrank after 7 d of water deficit stress, possibly because of water transportation from the pseudobulb to maintain the water content in the leaves. The pseudobulb is a swollen stem which functions as a water storage organ and also stores both nutrients and carbohydrates (Pires et al., 2013). He et al. (2013) reported that water content reduction in the pseudobulb occurred before leaf water content depletion under droughting in the *Cattleya* orchid. Thus, the pseudobulb is an important water source to maintain the metabolism and prolong the survival of an orchid when the planting material lacks water (Tay et al., 2015). However, a shrinking pseudobulb did not occur in the mature stage and the pseudobulb remained similar under both the well-watered control and water deficit conditions. The shrinking pseudobulb occurred in the flowering stage under stress although the levels of water consumption in the mature and flowering stages were not significantly different. In the flowering stage, a plant requires water for blooming and so when there is insufficient water in the planting material, the water stored in the pseudobulb may possibly be transported to flowers, resulting in pseudobulb shrinkage.

This research provided basic information on water use (via the evapotranspiration rate) of an important commercial orchid, *Den.* Sonia ‘Earsakul’. This information could be applied in irrigation system management on orchid farms as a means of saving water. In addition, the ability of this orchid to tolerate water deficit stress was different in each developmental stage. The plantlet stage was susceptible to insufficient water more than the other stages; consequently, effective water management is important in each stage of orchid cultivation under drought conditions.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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