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https://doi.org/10.5109/1833814

出版情報：九州大学大学院農学研究院紀要. 62 (2), pp.337-343, 2017-09-08. Faculty of Agriculture, Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **62** (2), 337–343 (2017)

Assessment of Cell Membrane Thermostability and Silicon Supplement on *Dendrobium* Lucky Girl

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(*Received April 25, 2017 and accepted May 10, 2017*)

Dedrobium Lucky Girl leaves position presented different cell membrane thermostability, respectively. It has enhancing the relative injury value when temperature exceeded 50°C. In terms of silicon dioxide treatments decreased leaves ion leakage, malondialdehyde content, and existed higher chlorophyll content which evaluated by water bath temperature at 50°C. Result not only presented higher root activity and chlorophyll SPAD value at 150 mg•L^{-1} silicon dioxide treatment, but also showed more leaves at 300–600 mg•L^{-1} silicon dioxide treatments during vegetative stage. Even though, silicon dioxide treatments had no significant difference among some horticultural characteristic, however it decreased flower bud abortion rate at 150 mg•L^{-1} treatment.

Key words: cell membrane thermostability, heat–tolerance, ion leakage, Nobile *Dendrobium*, silicon dioxide

INTRODUCTION

Temperature is an important factor for growth and development of plant, high temperature cause morpho–anatomical, physiological and biochemical changes of plant during the growing season (Wahid *et al*., 2007). The mesophyll cell of thermo–sensitive cultivar cabbage (*Brassica oleracea* var. *capitata* L.) showed significant ultrastructural change than thermo–resistant cultivar, such as chloroplasts membrane rupture, structural alteration of thylakoid membrane and disruption of nuclear envelope (Miao *et al*., 1994). High temperature lead to roll up of leaves in Chinese cabbage (*Brassica campestris* ssp. L. *pekinensis*) (Wu *et al*., 1995). The photosynthesis or photosynthetic rate was inhibited by high temperature in common bean and salvia taxa (Chaisompongpan *et al*., 1990; Lasseigne *et al*., 2007). Even Liu and Huang (2000a) reported that carbohydrate availability was associated with heat stress in creeping bentgrass. Moreover, high temperature stress also caused to affect chlorophyll content, chlorophyll fluorescence parameter and antioxidant systems in early cauliflower (Wang *et al*., 2004).

Silicon is not an essential element on plant growth and development. It has absorbed, translocated and accumulated of plant by silicic acid and silica gel form (Epstein, 1999; Yoshida, 1975). In fact, silicon has accelerated growth, development and enhanced stresses tolerance of plant, such as inhibited serious extra transpiration and increased water utility and photosynthetic activity of rice (Agarie *et al*., 1992, 1998), it also changed morphological and density of stomata in maize (Gao *et al*., 2006), increased chlorophyll and iron content, decreased proline content in cowpea (Mail and Aery, 2009), appeared thicker flower stalk, bigger diameter and blooming earlier affected leaf macro and micro element content in gerbera (Kamenidou *et al*., 2010), previous research indicated silicon also enhanced stresses tolerance such as increased cell wall polysaccharide content, decreased leakage at drought and heat stresses in rice (Agarie *et al*., 1998). Moreover, silicon applied increased superoxide dismutase, root H^+–ATPase activity, decreased lipid peroxidation and malondialdehyde content at salt stress in barley (Liang, 1999; Liang *et al*., 2003, 2006). Silicon could alleviate cell damage in norway spruce during aluminum stress (Prabagar *et al*., 2011).

Cell membrane structure, integrity and stability are most important of plant during heat stress, because it caused lipid liquefaction and electrolytes leakage of cell membrane (Ke, 2006; Yao *et al*., 2000). Cell membrane thermostability (CMT) has been becoming an efficiency method for evaluating heat tolerance in plants. The method measured as ion leakage from leaves over a range of temperature, it is rapid, inexpensive, requirement little space and highly correlation (Marcum, 1998; Chen *et al*., 2014; Yang and Hu, 2015; Yeh and Lin, 2003).

Nobile *Dendrobium* is belong *Dendrobium nobile* type and it has been found from native habitats of Himalayas, Thailand, Laos and South China (Kamemoto *et al*., 1999). Kuo (2011) indicated that high temperature has an influenced on development of nobile *Dendrobium*. In aspect of micro–propagation and breeding among *Doritaenopsis* and *Phalaenopsis* had difficultly problem to solve (Chuang *et al*., 2014; Liao *et al*., 2015), similarly to nobile *Dendrobium* orchid usually do not get used to higher temperature during vegetative growth around June to September (Wu, 2016).

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Therefore, it should be a limited factor during summer growing season in Taiwan. The purpose of this studies were determined cell membrane stability by CMT technique and silicon dioxide supply on development of nobile *Dendrobium*.

MATERIALS AND METHODS

Plant materials

Dedrobium Lucky Girl plants were purchased in Mar. 2014 from an orchid nursery in Douliu, Taiwan. Those plants were brought up serval pseudobulb with 10–17 nodes and grown in 9–cm diameter soft black plastic pot in greenhouse of National Chung Hsing University horticultural research station. The light intensity was 185–216 μmol·m^{-2}·s^{-1} and average temperature about 23.6–25.3ºC of experimental locations. The plants with two pseudobulb and vegetative buds were transplanted that switched old sphagnum moss medium, then pruned part of root and reporting with new sphagnum moss in 13–cm diameter pots. Those plants were watered as needed with tap water and no fertilizer applied for control. The plants were selected for experiment in Jan. 2015.

Experiment processes

On Mar. to Oct. 2015, the plants were supplied 2 g per pot of Hi–Control No.1 (14N–11P_2O_5–13K_2O, 180 type, JCAM Inc., Japan), while the pseudobulb terminal leaf had already formed and matured after that measured leaves cell membrane thermostability. In terms of silicon dioxide experiment, the plants were supplied nutrient solution (16.0 g·L^{-1} NH_4NO_3, 11.5 g·L^{-1} $NH_4H_2PO_4$, 48.6g·L^{-1} $MgSO_4$·7H_2O, 47.2g·L^{-1} $Ca(NO_3)_2$·4H_2O, 60.6g·L^{-1} KNO_3, 81.4 g·L^{-1} $CaCl_2$·2H_2O, 47.8 g·L^{-1} P_2O_5, 2.86 g·L^{-1} H_3BO_3, 0.22 g·L^{-1} $ZnSO_4$·7H_2O, 0.08 g·L^{-1} $CuSO_4$·5H_2O, 1.54 g·L^{-1} $MnSO_4$·H_2O, 0.017 g·L^{-1} H_2MoO_4·H_2O) with 0, 75, 150, 300 and 600 mg·L^{-1} silicon dioxide (Sigma–Aldrich, USA), 200 mL per pot twice a weeks. Furthermore, we mentioned the leaves chlorophyll SPAD value during vegetative stage, when pseudobulb terminal leaf has already formed and matured after that measured leaves ion leakage, chlorophyll content, root activity and malondialdehyde concentration. The horticultural characteristics were also investigated.

Leaves cell membrane thermostability

The leaves for analysis were harvested from the 3–5 nodes (the location downstream from terminal leaf), and were evaluated for CMT (Cell membrane thermostability) following procedures described by Yeh and Lin (2003) with some modification. Each samples for assay consisted of five leaf discs (6 mm diameter), has rinsed three times thoroughly tap water and distilled water before assay. Leaf discs were placed in 25 mL flask containing 10 mL distilled water then was measured at 25, 30, 35, 40, 45, 50, 55, 60, 65, and 70ºC water bath temperature for 30 min, after that the solution was measured with conductivity meter (Isfet, IQ–180, USA). The flask were capped with foil autoclaved (HY–300SV, 121ºC, 1.05 kg·cm^{-2}, Taiwan) for 20 min. The calibrated relative injury (RI) was calculated as follows:

RI(%)={1−[1−(T_I/T_F)]/[1−(C_I/C_F)]}×100, where T and C refer to conductance value for treatment and control, respectively, and subscript I and F refer to initial and final conductance value, respectively.

Leaves ion leakage

The leaves for analysis were harvested from the 3–5 nodes (the location downstream from terminal leaf), and were evaluated for ion leakage following procedures described by Martineau *et al*. (1979) with some modification. Each samples for assay consisted of five leaf discs at 6 mm diameter, has rinsed three times thoroughly tap water and distilled water before assay. Leaf discs were placed in 25 mL flask containing 10 mL distilled water then was measured at 50ºC water bath temperature for 30 min, after that the solution was measured with conductivity meter (Isfet, IQ–180, USA). The flask were capped with foil autoclaved (HY–300SV, 121ºC, 1.05 kg·cm^{-2}, Taiwan) for 20 min. The calibrated ion leakage was calculated as follows:

Leakage (%)=(R_1/R_2)×100, where R_1 and R_2 refer to conductance value for initial and final conductance value, respectively.

Malondialdehyde concentration (MDA)

MDA concentration was measured by using Heath and Packer (1968) with some modification. The sample of 0.3 g leaves tissue were harvested from the 3–5 nodes (the location downstream from terminal leaf), then homogenized with sand and 1.8 mL TCA (trichloroacetic acid 5%), after that was added to 10 mL tube and centrifuged (Kubata, KN–70, Japan) 3500 rpm for 15 min. A 1 mL supernatant solution was added to tube containing 4 mL of 20% (v/v) TCA and 0.5% (v/v) thiobarbituric acid. The sample was heated in a water bath at 95ºC for 30 min then quickly cooled in an ice bath followed by centrifugation at 3500 rpm for 15min. The absorbance of supernatant was determined with a spectrophotometer at 532–600 nm (Hitachi, U–2001, Japan). The MDA concentration was calculated as follow: MDA (nmol·g^{-1}) =(A532–A600 nm)÷155(extinction coefficient, mM^{-1}·cm^{-1})×5 (reaction volume, mL)×1.8 (dilution factor) ×1000÷sample fresh weight (g).

Chlorophyll content

The leaves sample were harvested from the 3–5 nodes (the location downstream from terminal leaf), and were measured by using the method of Arnon *et al*. (1954) and Marker (1972) with some modification. Sample of 0.1 g leaf tissue added 10 mL mixture solution (acetone: methanol=80: 20) soaked in darkness overnight, then determined with a spectrophotometer (Hitachi, U–2001, Japan) at 645, 652 and 663 nm.

Chlorophyll SPAD value

Each leaf sample has been determined on same position, and measured with Chlorophyll Meter (SPAD–502, Minolta Co. Ltd., Japan) during vegetative stage.

The results expressed as SPAD units.

Root activity

Root activity of plants was determined by using Steponkus and Lanphear, (1967) with modified. The sample of 0.01 g root tip added 0.6% TTC solution (triphenyl tetrazolium chloride, 0.05 mM Na_2HPO_4 and KH_2PO_4 buffer pH 7.4) then soaked in darkness over 17 hours. The sample has been rinsed distill water and added 20 mL alcohol (95%) into tube, when it being heated in a water bath at 78ºC for 20 min after that quickly cooled in room temperature and determined with a spectrophotometer (Hitachi, U–2001, Japan) at 480 nm.

Statistical analysis

The data were subjected to an analysis of variance using a completely randomized design. Separation of means among treatments was used least significant difference test at $P \leqq 0.05$. Statistical analyses were used Costat 6.1 (CoHort Software, Monterey, CA, USA). SigmaPlot software 12.5 (Systat Software Inc., Chicago, CA, USA) was used to assay regression analysis. The figures and tables were made out from the data using Microsoft Excel 2010 (Microsoft Office Software, Redmond, WA, USA).

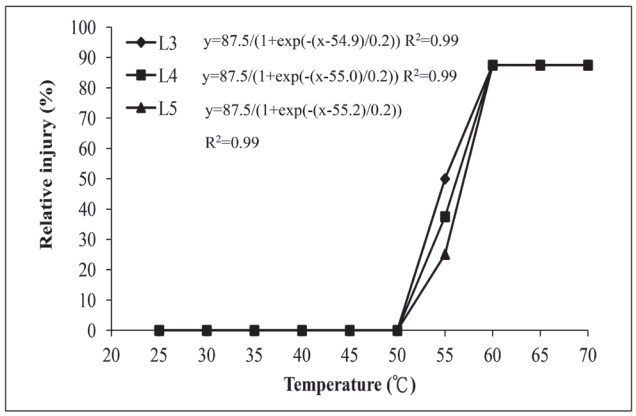

Fig. 1. Effect of water bath temperature on leaves relative injury of *Dendrobium* Lucky Girl. Formula represent sigmoidal regression, n=3.
L3, L4, L5: downstream from terminal leaf 3–5 location of node

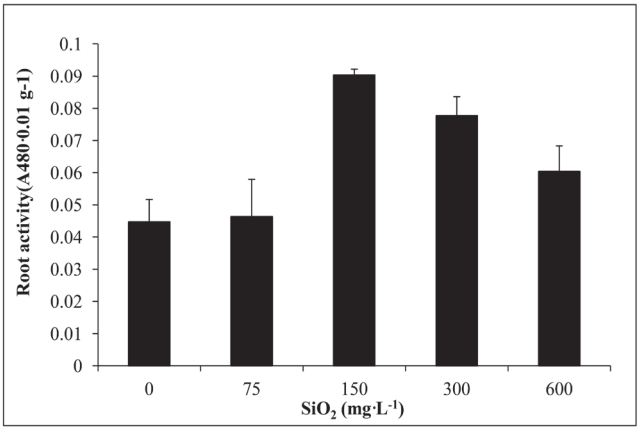

Fig. 2. Effect of silicon dioxide on root activity of *Dendrobium* Lucky Girl. Bar = ±SE; n=3.

RESULTS

The relationship between the relative injury value of leaves and water bath temperature were sigmoidal in *Dedrobium* Lucky Girl. The temperature has been increasing over 50.0ºC that leaves (L3–L5) enhancing the relative injury value, the leaf (L3) appeared higher value than others (L4–5). The relative injury value midpoint of leaves position occurred the sigmoid response curve between 54.9–55.2ºC (Fig. 1). In terms of silicon experiment, result showed higher root activity and chlorophyll SPAD value at 150 mg·L^{-1} treatment (Fig. 2 and Fig. 3). The leave ion leakage emerged fluctuation when heating at 50.0ºC for 30 min. The leave position (L3–L5) showed lower ion leakage at 150–600 mg·L^{-1} treatments compared with control (Fig. 4). Silicon dioxide treatments have gradually increasing leave chlorophyll content and decreasing MDA concentration (Table 1). In terms of horticultural characteristic, silicon dioxide treatments did not improve plant height and number of nodes, but it enhanced number of leaves during vegetative growth stage (Table 2), moreover, silicon dioxide treatments had no significant difference among numbers of flower bud, numbers of node with flower bud, flower per inflorescence and flower per pseudobulb, however 150 mg·L^{-1} treatments occurred lower flower bud abortion rate during reproductive growth stage (Table 3 and Fig. 5).

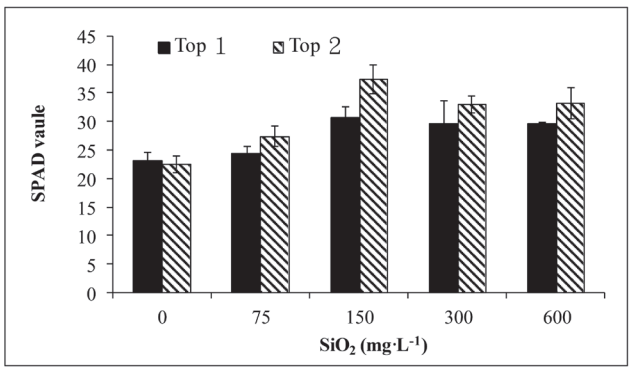

Fig. 3. Effect of silicon dioxide on SPAD value of *Dendrobium* Lucky Girl vegetative bud. Bar = ±SE; n=3.

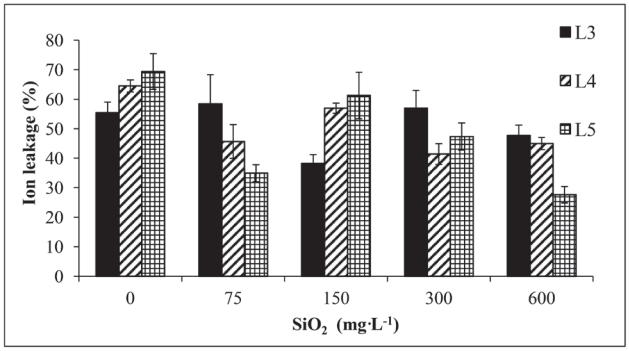

Fig. 4. Effect of silicon dioxide on leaves ion leakage of *Dendrobium* Lucky Girl. Bar = ±SE; n=3.
L3, L4, L5: downstream from terminal leaf 3–5 location of node

Table 1. Effect of silicon dioxide on leaves malondialdehyde and chlorophyll content of *Dendrobium* Lucky Girl

SiO_2 ($mg \cdot L^{-1}$)	L3[z]		L4		L5	
	MDA ($nmol \cdot g^{-1}$)	Total Chl. ($mg \cdot g^{-1}$)	MDA ($nmol \cdot g^{-1}$)	Total Chl. ($mg \cdot g^{-1}$)	MDA ($nmol \cdot g^{-1}$)	Total Chl. ($mg \cdot g^{-1}$)
0	11.9 a[y]	0.54 c	3.5 a	0.62 c	6.0 a	0.61 b
75	4.1 b	0.70 bc	3.4 ab	0.72 bc	3.9 ab	0.65 b
150	3.2 b	0.81ab	3.6 ab	0.89 ab	2.7 b	0.97 a
300	4.2 b	0.95 a	2.4 b	0.87 ab	2.7 ab	0.87 ab
600	1.8 b	0.98 a	2.1 b	0.98 a	2.8 b	1.01 a

[z] L3, L4, L5: downstream from terminal leaf 3–5 location of node
[y] Mean separation within each column by LSD test at $P \leqq 0.05$. n=3

Table 2. Effect of silicon dioxide on pseudobulb growth of *Dendrobium* Lucky Girl

SiO_2 ($mg \cdot L^{-1}$)	Plant height (cm)	Number of nodes	Number of leaves
0	28.7 a[z]	15.3 a	4.0 b
75	30.0 a	16.7 a	7.5 b
150	29.7 a	17.7 a	9.7 a
300	28.3 a	16.0 a	10.7 a
600	28.0 a	15.7 a	10.7 a

[z] Mean separation within each column by LSD test at $P \leqq 0.05$. n=3.

Table 3. Effect of silicon dioxide on blooming of *Dendrobium* Lucky Girl

SiO_2 ($mg \cdot L^{-1}$)	Numbers of flower bud	Numbers of node with flower bud	Flowers per inflorescence	Flowers per pseudobulb	Flower bud abortion (%)[z]
0	7.7 a[y]	5.7 a	1.8 a	10.3 a	25.0 a
75	7.3 a	6.3 a	2.0 a	12.3 a	12.5 ab
150	8.0 a	7.7 a	2.1 a	15.6 a	2.8 b
300	6.3 a	5.0 a	2.1 a	10.0 a	23.1 a
600	6.0 a	5.0 a	2.3 a	11.3 a	16.7 ab

[z] Percentage data were logarithmic transformed prior to analysis
[y] Mean separation within each column by LSD test at $P \leqq 0.05$. n=3.

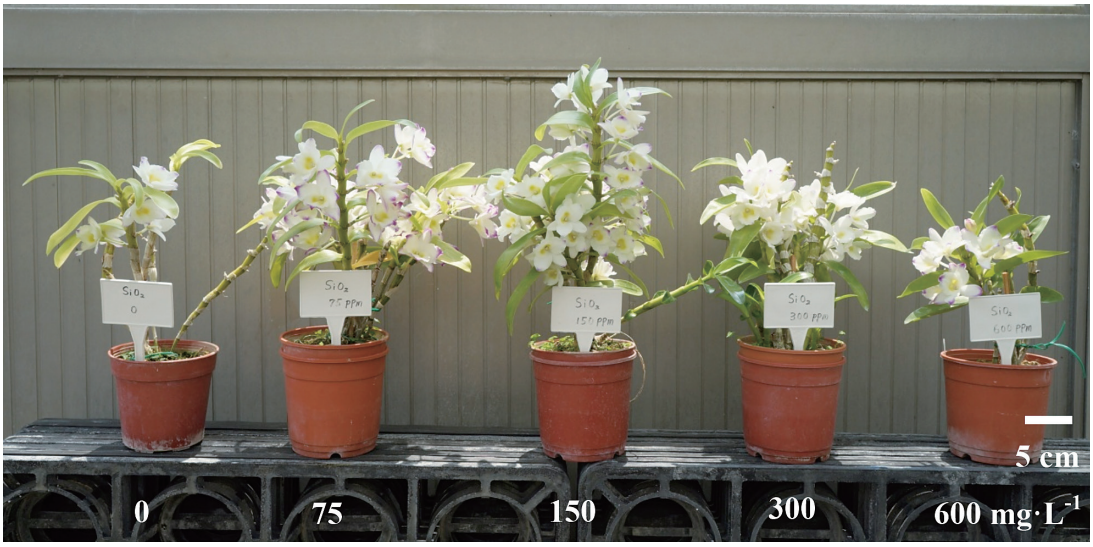

Fig. 5. Effect of silicon dioxide treatments on flowering of *Dendrobium* Lucky Girl.

DISCUSSION

The cell membrane thermostability technique has been screening crop for heat tolerance in soybeans (Martineau *et al*., 1979), wheat (Saadalla *et al*., 1990), chrysanthemum (Wang and Yeh, 2013; Wang *et al*., 2008; Yeh and Lin, 2003), leafy radish (Chen *et al*., 2014), marsh–rosemary (Chang, 2014) and *Phalaenopsis* (Yang and Hu, 2015). The exposure time and midpoint of sigmoidal curve that would able to evaluate heat tolerance of crop (Ingram and Buchanan, 1981; Lester, 1985). Result indicated the position of leaves have increasing the relative injury value after heating at 50ºC, the leaves ion leakage showed nearly midpoint among 54.9–55.2ºC, therefore cell membrane thermosstability should be able to determine at 55ºC in *Dendrobium* Lucky Girl (Fig. 1). The position of leaf (L3) showed more sensitively than others leaves.

Some research indicated, silicon improved development and production of crop (Kamenidou *et al.*, 2010; Mattson and Leatherwood, 2010; Sivanesan *et al*., 2010), In present experiment showed silicon dioxide treatments enhanced root activity and chlorophyll SPAD value (Fig. 2 and Fig. 3). Agarie *et al*. (1998) reported that silicon treatment reduced ion leakage in rice leaves, when it is heating at 42.5ºC. Ma (2004) also reported that silicon played a major role against stresses such as drought, freeze, metal ion toxin, salt, pest and disease. Result indicated that leaves (L3–5) ion leakage emerged fluctuation data, it should be discussed the assimilated difference of *Den*. Lucky Girl on ion leakage due to silicon dioxide concentration (Fig. 4), because silicon element absorbed, translocated and accumulated of plant by silicic acid and silica gel form, it should be consider to the ability of the roots to take up this element in monocotyledonous and dicotyledons (Epstein, 1999; Ma and Yamaji, 2006). In addition, silicon dioxide treatments increased the leaves chlorophyll content (Table 1), the result similar like Ahmad *et al*. (1992), Duan *et al*. (2013), Liang (1999) and Ma *et al*. (2002). Agarie *et al*. (1992, 1993) indicated that silicon treatments accelerated development, accumulation of dry weight and photosynthetic rate that attributed silicon maintained the chlorophyll stability. In present experiment also showed that silicon treatments decreased the malondialdehyde concentration of leaves (Table 1). Cell membrane has been received injury in adversity condition that lead to lipid peroxidation and produced the precursor is malondialdehyde (MDA), it would be able to integrate protein and enzyme in order to cause damage of cell membrane structure (Zhang and Yin, 2009; Bailly *et al*., 1996; Blokhina *et al*., 2003; Gutteridge and Halliwell, 1990; Liu and Huang, 2000b; Malencic *et al*., 2000). Lombardi and Wangersky (1991) reported that the marine diatom (*Chaetoceros gracilis*) less silicon supplement lead to break of cell membrane. Agarie *et al*. (1998) indicated silicon treatment prevented cell membrane function and structure damage in rice during stress environment. Wang and Galletta (1998) also reported that silicon treatment caused alternation glycolipids and phospholipids ratio of cell membrane. In other hand, silicon treatment also decreased lipid peroxidation and malondialdehyde content at salt stress on barley (Liang, 1999; Liang *et al*., 2003, 2006). Even silicon treatments did not enhance the plant height and number of node, however it has been increased number of leaves during vegetative growth stage, 150 mg·L^{-1} silicon dioxide treatments appeared lower flower abortion rate (Table 2 and Table 3).

In summary, silicon element has been protected the cell membrane to stress that discussed respect to membrane integrity, stability and function of crop. Furthermore, beneficial element silicon on physiological advantage and mechanism of horticultural crop should be more investigated.

AUTHOR CONTRIBUTIONS

Shing–Kuan WU, designed this study and wrote the initial draft of the manuscript. Ikuo MIYAJIMA, offered advices on tropical horticultural crops research for efficiency experiments on heat tolerance, and revised the manuscript and inspected final data. Kuang–Liang HUANG, joined orchid research team work, designed the cultural environment for orchid research under light intensity and temperature control for studies, and offered suggestion for research. Ya–Chin KUO, worked on experimental process of fertilizer supplement and data collection. Ruey–Song LIN, organized the research protocol through physiological and biochemical experiments on heat tolerance of crops and managed lab process. The final version of this manuscript was approved by all authors.

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