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Vegetative Growth and Root Morphology in C*ymbidium* under Different Substrate Water Content Using a Sensor-based Irrigation System

센서 기반 관수 시스템 이용 시 상토 수분 함량에 따른 심비디움의 영양생장 및 뿌리 형태

BY

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MAJOR IN HORTICULTURAL SCIENCE AND BIOTECHNOLOGY

THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

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ABSTRACT

This study was conducted to investigate the optimum substrate volumetric water content (VWC, v/v) of *Cymbidium* (Exp. 1), and to understand *Cymbidium* response to water, and morphological characteristics of roots (Exp. 2). One-year-old *Cymbidium* 'Yang Guifei' plants were cultivated in an experimental greenhouse for 28 weeks. Four different set points, 0.1, 0.2, 0.3, and 0.4 $m^3 \cdot m^{-3}$, of coir dust-filled containers were used. VWC were measured by using soil moisture sensors every 20 min, and 32 mL of water was supplied whenever the level was less than each set of points. During the experiment, approximately 25.6, 58.0, 213.4, and 352.8 $mL \cdot d^{-1}$ of water was applied by sensor-based irrigation system at VWC of 0.1, 0.2, 0.3, and 0.4 $m^3 \cdot m^{-3}$, respectively. The 0.1 $m^3 \cdot m^{-3}$ was too dry to

Cymbidium to grow, because most of them wilted during the experiment. The diameter of mother bulb under 0.3 (37.0 mm) and 0.4 $m^3 \cdot m^{-3}$ (39.2 mm) treatment were biggest, while the bulb under 0.2 $m^3 \cdot m^{-3}$ (33.4 mm) was decreased due to withering symptoms. In the lead bulb plants, the pseudobulbs under 0.3 and 0.4 $m^3 \cdot m^{-3}$ showed the highest growth rate in diameter (27.6 and 26.6 mm), leaf length (57.9 and 56.4 cm), and the number of leaves (15.3 and 13.9), while 0.2 $m^3 \cdot m^{-3}$ showed the lowest growth rate, 24.0 mm, 51.5 cm, and 12.5, respectively. Contrary to the growth parameters, the 0.3 $m^3 \cdot m^{-3}$ treatment showed the highest rate in light response curve. There was no significant difference in maximum quantum efficiencies (0.79–0.81) of photosystem II (Fv/Fm) of leaves in mother and lead bulbs. In chemical properties, no significant difference was observed in pH, but EC value decreased with increasing VWC. In Exp. 2, ten-month-old plants as in Exp. 1 were cultivated for 12 weeks with the similar environment of Exp. 1. Deficit (479.6 $mL \cdot d^{-1}$) or moderate irrigations (5,122 $mL \cdot d^{-1}$) were employed. All growth parameters from the moderate irrigation treatment were significantly higher than to those from to the deficit irrigation treatment. In the root transverse section, cortex thickness and area increased significantly in the moderate irrigation treatment. Considering water usage and growth, 0.3 $m^3 \cdot m^{-3}$ can be recommended as optimum VWC for *Cymbidium* ‘Yang Guifei’ cultivation.

Additional keywords: orchids, photosynthesis, pseudobulbs, volumetric water content

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INTRODUCTION

Orchidaceae is one of the largest families in flowering plants, encompassing over 25,000 species. Among them, only a few genera are cultivated in large quantities as commercial ornamental crops such as *Cymbidium*, *Phalaenopsis*, *Oncidium*, and *Dendrobium*. *Cymbidium* is among the commercially valuable flower because of the size, shape, color, and longevity of the blooms (Lopez and Runkle, 2005).

Water is vital for plant growth and development. To do photosynthesis, plants continuously absorb water through the roots, transport it to the body, and evaporate it from leaf surfaces. Even slight imbalances of the process can cause water deficits, decrease photosynthesis, and severe malfunctioning of many cellular processes (Taiz and Zeiger, 2002). Photosynthesis is the first physiological response to water stress causing stomata closure, decreasing carboxylation efficiency, and suppressing leaf expansion. The overall activity of photosynthetic apparatus in leaves can be assessed by leaf gas exchange and chlorophyll a fluorescence analyses. Light saturation point was decreased in highbush blueberry (*Vaccinium corymbosum*) (Rho et al., 2012), and photosystem II photochemical efficiency (Fv/Fm) was decreased in oak tree (*Quercus petraea*) under water deficit environment (Epron et al., 1992). Water deficit also accompanies reducing shoot elongation, leaf area, and biomass production (Burnett and van Iersel, 2008; Garland et al., 2012; van Iersel et al., 2010).

Orchids developed their organs to sustain in water deficit environment. Among them, *Cymbidium* has pseudobulbs and velamen radicum that can contain more water compared to other plants (Zotz, 2016). The pseudobulb is an abundance of water-storing cells. It also accumulates massive amounts of carbohydrates during vegetative growth and subsequently remobilized to support new shoot and inflorescence development (Hew and Ng, 1996). Studies were conducted to investigate minimum pseudobulb diameter that could promote floral initiation, such as 4.4 cm for *Cymbidium* ‘Yokihi’ and 5.2 cm for ‘Red Fire’ (Kim et al., 2011b). Therefore, supplying water at an optimal rate is crucial to support vegetative growth and flowering of *Cymbidiums*.

Velamen radicum is also one of the adaptive features from the water deficit environment. It locates externally to the exodermis, the outer layer of the cortex, and constitutes multiple epidermises composed of dead cells. The layer confers mechanical protection for the root (Zotz, 2016), immobilization of solutions that arrive at the root via stem flow, and reduction of water loss at times of low availability of water (Zotz and Winkler, 2013). In case of features of the velamen, it is affected by water availability depending on environments. Aerial roots of *Acampae praemorsa* exhibited thicker velamen, cortical cell layers, and endodermis than terrestrial roots (Thangavelu and Ayyasamy, 2017). In *Vanilla*, metaxylem cells are always wider in terrestrial than in aerial roots (Stern and Judd, 1999). These features are clearly modified for rapid water transport.

Despite the features of the pseudobulb and velamen radicum, much water is wasted in *Cymbidium* cultivation, because of little or no quantitative data regarding specific water requirements of *Cymbidium*. In case of other nursery plant and flower production, there have been many researches going on controlling irrigation system to find proper water contents including *Rosa* sp. (Plaut et al., 1976), *Chrysanthemum* (Kiehl et al., 1992), *Dianthus caryophyllus* (Ucar et al., 2011), *Hydrangea* (Hagen et al., 2014) and *Petunia* (Kim et al., 2011a). In the optimum water content, *Lavandula angustifolia* was increased in total leaf number and shoot dry weight (Zhen and Burnett, 2015). However, *Cymbidium* is commonly cultivated with high air spaced bark substrate since it is epiphytic or terrestrial plants. This fact makes limitation to measure the water content of *Cymbidium*.

Recently, with a sensor-based irrigation system, potted plants can efficiently be irrigated with the desired level, suppling just the amount of water required for their growth. Thus, the objective of this study was to investigate optimum substrate volumetric water content (VWC, v/v) in a sensor-based irrigation system for *Cymbidium* by using growth measuring parameters and water usage depending on different VWC. To more effectively understand of *Cymbidium* response to water, anatomical characteristics of roots were also observed.

LITERACTURE REVIEW

Characteristics of *Cymbidium*

The genus *Cymbidium* consisting of approximately 50 species, is originated from the foothills of the Himalayas and distributed from tropical and subtropical Asia to northern Australia. The terrestrial, epiphytic, lithophytic, and semiepiphytic species require long days and day/night temperatures of 30/25 °C for rapid growth and pseudobulb maturity (Lopez and Runkle, 2005). There are three horticultural groups based on their temperature tolerance: cool, intermediate and warm. In general, *Cymbidium* requires a cool climate, porous medium, limited nutrition and judicious watering. A wide variety of substrates can successfully be used for growing the orchid and bark is preferential substrate to provide good aeration for roots (Naik et al., 2013; Shim and Kim, 2010).

Importance of water in growth

Water is vital for plant growth and development. As one of the major environment factors, water deficit influences plant growth at various levels from cell to community (Colom and Vazzana, 2001). Water deficit often influences reducing shoot elongation, leaf area, and biomass production (Burnett and van Iersel, 2008; Garland et al., 2012; van Iersel et al., 2010). Water deficit also causes wilting, leaf burn, and shoot dieback, thus substantially reducing visual quality of plants. Another improper irrigation practice in greenhouse operations is overwatering. Overwatering may increase plant susceptibility to diseases such as *Phytophthora* and *Pythium* (Nelson, 2012).

Anatomically, water stress reduces turgor pressure, and affects the quantity and quality of plant growth depending on cell division, enlargement and differentiation (Correia et al., 2001). Plants exposed to environments with low water availability have generally been reduced in cell size, increased in vascular tissue and cell wall thickness (Guerfel et al., 2009). Multiple characteristics of the vascular structure have been investigated, such as modifications to the wall architecture and alteration of xylem to phloem ratio, which are thought to be involved in the resistance of the plant to environmental stresses (Child et al., 2003).

Velamen radicum

Most of the orchid roots have stele, endodermis, cortex, and velamen radicum. The velamen is a multilayered envelope of dead and air-filled cells with a series of lignified branching strands. It forms a continuous longitudinal system over the surface of the cortex. There is a line of demarcation between the silvery velamen and the yellowish-green growing tip. This sudden transition corresponds with the disappearance of the nuclei, cytoplasm, and turgor of the velamen, but helical thickening of the cell wall provides a mechanical support from collapsing during desiccation (Noel, 1974).

The velamen absorbs water by capillary flow, until saturation is achieved. Then the water flows through parenchymatous cortex which is impermeable to water towards the conducting stele. According to Zotz and Winkler (2013), the velamen can take up water within seconds and prevent excess water loss from the cortex by retaining it more than 1 h. The features of the velamen are also affected by water availability depending on species environments. The orchids grown in dry environments tend to possess thick layers of velamen cells, while those grown in wet sites exhibit thin layers (Sanford and Adanlawo, 1973).

MATERIALS AND METHODS

Vegetative growth and water usage (Exp. 1)

***Plant materials and growth conditions*.** One-year-old *Cymbidium* ‘Yang Guifei’ (Mukoyama Orchids Co., Ltd., Yamanashi-ken, Japan) plants were purchased from a commercial grower (Hae Pyeung Orchids, Gongju, Korea) and transplanted into 2.3-L containers (52 cm diameter and 14 cm height) filled with 100% coir dust (Sivanthi Joe, Tuticorin, Tamil Nadu, India) on Dec. 16, 2016. They were acclimated and watered manually for 2 months in a greenhouse at the university farm, Seoul National University (Suwon, Korea), and 100 mL water soluble fertilizer (electrical conductivity (EC) 1.0 $mS{\cdot}cm^{-1}$; Technigro 20N-9N-20K, Sun-Gro Horticulture, Bellevue, WA, USA) was applied once a month during acclimation. The experiment was performed from Feb. 1 to Aug. 20, 2017. Average day/night temperatures inside the greenhouse were 26/24 °C monitored by using a data logger (CR1000; Campbell Scientific, Logan, UT, USA). Photoperiod was provided with natural day length with mean photosynthetic photon flux (PPF) 312 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$. High-pressure sodium high pressure sodium lamps (SKL-01; GEO, Hwasung, Korea) were used as additional supplemental lighting from 9:00 to 10:30 and from 15:30 to 17:00 during the winter season (from Feb. to Apr. 2017). During the experiment, plants were fertilized with 4 g of slow-release fertilizers (11N-4.4P-15.7K+1.2Mg+TE, Everris Co., Geldermalsen, The Netherlands) placed at the top of the substrate. Insecticides and fungicides were applied at their recommended rates as needed throughout the growing period. The pH and EC values of the fertilizer solution before application were about 6.54 and 0.7 $dS{\cdot}m^{-1}$, respectively.

Irrigation treatment. The experiment consisted of four levels of substrate VWC: 0.1, 0.2, 0.3, and 0.4 $m^3 \cdot m^{-3}$. The 12 capacitance soil moisture sensors (10HS; Decagon Devices, Pullman, WA, USA) were inserted diagonally from the top of the substrate and located between a plant and a pot. Every 20 min, if the VWC of the specific container was below the assigned VWC set points, the data logger opened the solenoid valve for 3 s using the relay controller and irrigated 32 mL per a container. To irrigate properly, two PC spray stakes (Netafim, Fresno, CA, USA) were inserted into each container. The number of each irrigation was recorded by the data logger and later multiplied by the average emitter output for each line to determine the average number of irrigations and water use per day.

Measurements. Pseudobulb diameter, number of leaves, leaf length and width, and relative chlorophyll content (SPAD) value from mother and lead bulb were measured monthly during the experimental period. The pseudobulb diameter was measured at the widest point using a digital vernier caliper (ABS Digimatic Caliper; Mitutoyo Co., Ltd., Tsukuba, Japan). The longest and widest parts of leaf measured from the base of pseudobulb were used to represent leaf length and width, respectively. SPAD of the third fully expanded leaf from the top was measured using a hand-held chlorophyll meter (SPAD 502; Konica Minolta Sensing Inc., Sakai, Osaka, Japan). At the end of the experiment, the leaves and bulbs were cut off from the upper part of roots, and weighed for the fresh weight. The dry weight of them was determined after drying in a dry oven at 80 °C for 7 days. The pH and EC values were measured randomly from three containers in each treatment.

Light response curves were determined by a portable photosynthesis system (Li 6400; Li-Cor Co., Inc., Lincoln, NE, USA) using three plants in each treatment.

Third fully expanded leaves from the top were exposed to ten different PPFs (1,000, 800, 600, 400, 200, 100, 80, 60, 40, 20, and 0 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$). Relative humidity, air temperature, and CO_2 concentration inside the head chamber were kept at 60%, 20 °C, and 400 $\mu mol{\cdot}mol^{-1}$, respectively during the daytime, the same value inside the greenhouse. The light response curves were fitted into the exponential model of $f(x) = y_0 + ax + bx^2$.

A chlorophyll fluorimeter (PAM-2000; Heinz Walz, Effeltrich, Germany) was used to measure photosynthetic activities. After 30 min dark adaptation period, a measuring light of 0.6 kHz and less than 0.1 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ PPF was irradiated to obtain the minimum fluorescence (Fo). Saturating light pulse (8,000 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$) was irradiated for 0.8 s to induce maximum fluorescence (Fm). The difference between Fo and Fm is the variable fluorescence (Fv), and the maximum quantum efficiency of photosystem II (PSII) photochemistry (Fv/Fm) could be obtained from (Fm-Fo)/Fm = Fv/Fm.

Experimental design and statistical analysis. The experiment used completely randomized design with 12 plants. Differences among the treatment means were assessed by Duncan's multiple range test at $P < 0.05$ with SAS 9.3 (SAS Inst. Inc., Cary, NC, USA).

Root morphology in different irrigation (Exp. 2)

Plant materials and growth condition. Ten-month-old *Cymbidium* 'Yang Guifei' (Mukoyama Orchids Co., Ltd.) plants were purchased from a commercial grower (Hae Pyeung Orchids) and transplanted into 2.2-L containers (52 cm diameter and 14 cm height) filled with 100% coir dust on Dec. 29, 2017. They were acclimated and watered manually for 2 months in a greenhouse at the university farm, Seoul National University (Suwon, Korea). Average day/night temperatures inside the greenhouse were 26/24 ℃ monitored by using a data logger (CR1000; Campbell Scientific, Inc. Logan). Photoperiod was provided with natural day light and supplemental lighting with 312 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF. During the experiment, plants were fertilized with 4 g of slow-release fertilizers (11N-4.4P-15.7K+1.2Mg+TE, Everris Co.) placed at the top of the substrate. Insecticides and fungicides were applied at their recommended rates as needed throughout the growing period.

Irrigation treatment. The experiment consisted of two levels of substrate VWC: deficit (479.6 $mL \cdot d^{-1}$) and moderate (5,122 $mL \cdot d^{-1}$). The number of each irrigation was recorded by the data logger and later multiplied by the average emitter output for each individual line to determine the average number of irrigations and water use per day.

Measurement. Pseudobulb diameter, number of leaves, leaf length and width, SPAD value, and dry weight were measured similarly in Exp. 1.

Examination of root morphology. For each treatment, three to five mature roots from the top (3 cm away from the bulb) and bottom (3 cm away from the root tip) parts were collected from plants. The roots were preserved in formalin-acetic acid-alcohol mixture (9 parts 70% ethanol, 0.5 parts 10% formalin and 0.5 parts glacial acetic acid) for 48 h and then, dehydrated by passage through the series of ethanol (70–100%), embedded with warm (60–61℃) paraffin. The resulting blocks were then cut into 15–30μm sections with rotary microtome (Leica RM2145: Leica, Nussloch, Germany), and stained with toluidine blue.

Transverse sections were examined and photographed with a digital camera (Axiocam 506 Color, Carl Zeiss, Jena, Germany) mounted on light microscopy

(DE/Axioplan 2; Carl Zeiss, Oberkochen, Germany). Photographs at 5, 10, and 40× magnification were used with the ZEN program, while the photographs at 40× magnification were used to observe root semidiameter, velamen thickness, cortex thickness, stele thickness, and metaxylem vessel cell area.

Experimental design and statistical analysis. The experiment used completely randomized design with ten plants. Differences among the treatment means were assessed by t-test at $p < 0.05$ with SAS 9.3 (SAS Inst. Inc.).

RESULTS AND DISCUSSION

Vegetative growth. In Exp. 1, sensor based irrigation system accelerated growth in all treatments of *Cymbidium* except 0.1 $m^3 \cdot m^{-3}$ (Table 1). It took 28 weeks to the first visible wilting in 0.1 $m^3 \cdot m^{-3}$. Similar to the result, *Hydrangea macrophylla* (Olson et al., 2002) also wilted below 0.1 $m^3 \cdot m^{-3}$. That means 0.1 $m^3 \cdot m^{-3}$ of VWC was too severe condition to grow these plants. There were no wilting symptoms in bulbs during Exp. 2, but deficit irrigation treatments caused root shrinkage (Fig. 1).

Pseudobulb is a major organ for storing water. *Oncidium* 'Goldiana' maintained relatively high water contents of 90–95% throughout development in its pseudobulbs (Hew and Ng, 1996; Zotz, 2016). As water storage organs, the size of the pseudobulb increased significantly ($P < 0.01$) with increasing VWC in all treatments in both experiments. The mother bulb diameter was 33.4, 37.0, and 39.2 mm when the plants were grown at 0.2, 0.3, and 0.4 $m^3 \cdot m^{-3}$, respectively (Table 1). In Exp. 2, the pseudobulb diameters were 23.2 and 18.9 mm in moderate and deficit irrigation treated, respectively (Table 2).

The pseudobulb diameter and leaf length of lead bulb grown at 0.2 $m^3 \cdot m^{-3}$ treatments (24.0 mm and 51.5 cm) were significantly 15% and 11% thinner ($p < 0.05$) and 12% and 10% ($p < 0.05$) shorter than those grown under 0.3 (27.6 mm and 57.9 cm) and 0.4 (26.6 mm and 56.4 cm) $m^3 \cdot m^{-3}$ treatments, respectively. The number of leaves was significantly ($P < 0.05$) influenced by different VWC and they were 12.5, 15.3, and 13.9 under 0.2, 0.3, and 0.4 $m^3 \cdot m^{-3}$ treatments, respectively (Table 1). Similar effects were shown in Exp. 2, which leaf length, leaf

Table 1. Effects of volumetric water content (VWC) of the substrate on the vegetative growth of *Cymbidium* 'Yang Guifei' after 28 weeks of treatment.

VWC ($m^3 \cdot m^{-3}$)	Pseudobulb diameter (mm)	Leaf length (cm)	Leaf width (cm)	No. of leaves	Chlorophyll content (SPAD)
			Mother bulb		
0.1	–[z]	–	–	–	–
0.2	33.4 b[y]	52.8	2.2 ab	6.5	69.2
0.3	37.0 a	55.0	2.1 b	6.7	64.9
0.4	39.2 a	55.2	2.3 a	6.9	65.6
Significance	**	NS	*	NS	NS
			Lead bulb		
0.1	–	–	–	–	–
0.2	24.0 b	51.5 b	2.1	12.5 b	55.2
0.3	27.6 a	57.9 a	2.1	15.3 a	53.6
0.4	26.6 a	56.4 a	2.2	13.9 ab	52.4
Significance	**	**	NS	**	NS

[z]Over half of the plants under 0.1 $m^3 \cdot m^{-3}$ treatments wilted.
[y]Means separation within columns by Duncan's multiple range test at $p < 0.05$.
[NS,*,**]Non-significant or significant at $p < 0.05$ and 0.01, respectively.

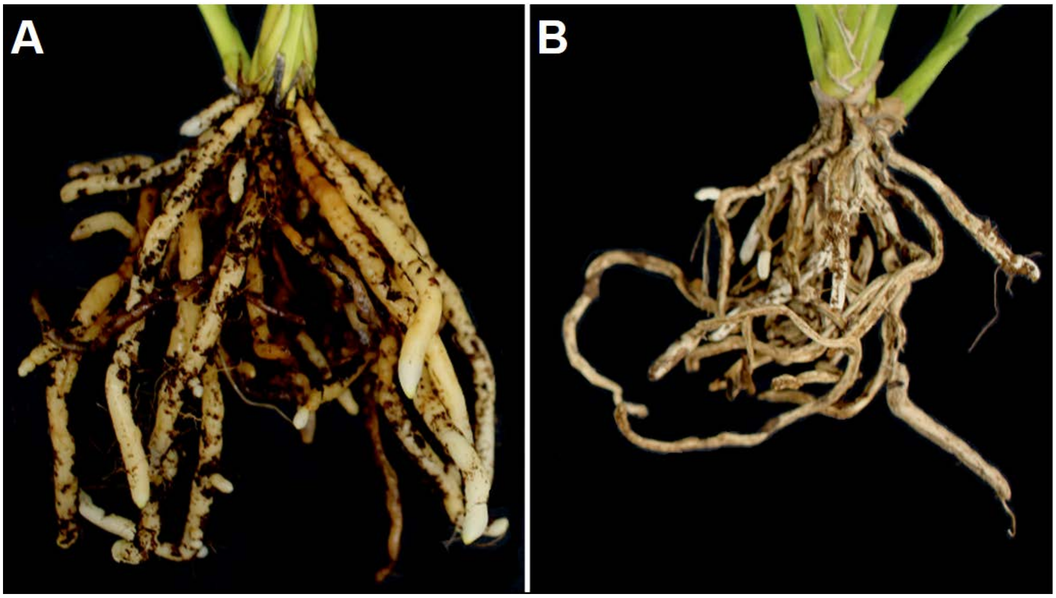

Fig. 1. *Cymbidium* 'Yang Guifei' roots of moderate (A) and deficit irrigation treated (B) after 12 weeks of treatment.

Table 2. Effects of volumetric water content (VWC) of the substrate on the vegetative growth of *Cymbidium* 'Yang Guifei' after 12 weeks of treatment.

Irrigation	Pseudobulb diameter (mm)	Leaf length (cm)	Leaf width (cm)	No. of leaves	Chlorophyll content (SPAD)
Deficit	18.9 b[z]	38.2 b	1.8 b	10.6 b	32.1
Moderate	23.2 a	40.4 a	2.0 a	12.6 a	34.9
Significance	***	*	***	***	NS

[z]Means separation within columns by t-test at $p < 0.05$.
[NS,*,***]Non-significant or significant at $p < 0.05$ and 0.001, respectively.

width, and the number of leaves were increased in more water supplied treatment (Table 2). The increased number of leaves ultimately increases the total leaf area of plants to photosynthesize. In general, the vegetative growth has been found to decrease with decreasing VWC (Bayer et al., 2013). However, the *Cymbidium* grown under 0.3 $m^3 \cdot m^{-3}$ treatment grew similar to those under 0.4 $m^3 \cdot m^{-3}$ treatments, and thus 0.3 $m^3 \cdot m^{-3}$ was optimum VWC for the plant.

Dry weights of pseudobulbs, leaves, and roots were measured after 28 weeks (Table 3) and 12 weeks of treatment (Table 4). All the measurements significantly ($P < 0.001$) increased with increasing water content. Despite different water usage (Table 5), 0.3 $m^3 \cdot m^{-3}$ treatment increased growth more than 0.4 $m^3 \cdot m^{-3}$ treatment during Exp. 1. Shoot dry weight of *Heuchera americana* 'Dale's Strain' did not have greater dry weights when maintained at VWC higher than 0.35 $m^3 \cdot m^{-3}$, indicating that *H. Americana* became saturated at 0.35 $m^3 \cdot m^{-3}$ (Garland et al., 2012). Perhaps the substrate of *Cymbidium* might also be saturated at 0.3 $m^3 \cdot m^{-3}$, which did not affect the growth in higher VWC. There was no significant difference in top/root ratio in Exp. 1, but root weight was heavier than shoot dry weights under 0.2 $m^3 \cdot m^{-3}$ treatment. In *Myrtus communis*, top/root ratio decreased under water deficit conditions, since the plants might need to maintain the root surface area under drought conditions in order to absorb water from the substrate (Navarro et al., 2009). The significant difference ($p < 0.05$) of top/root ratio could be confirmed in Exp. 2.

Vegetative Growth and Water Usage (Exp. 1)

Irrigation treatment. During the experiment period, the frequent irrigation was

Table 3. Effects substrate volumetric water content (VWC) on dry weights of pseudobulbs, leaves, and roots in *Cymbidium* 'Yang Guifei' after 28 weeks of treatment.

VWC ($m^3 \cdot m^{-3}$)	Shoot (g)		Root (g)	Top/root ratio
	Pseudobulb	Leaf		
0.1	–[z]	–	–	–
0.2	7.2[y]	10.5 b	18.9 b	0.9
0.3	10.7 a	18.9 a	26.7 a	1.1
0.4	10.7 a	16.6 a	25.4 a	1.1
Significance	***	***	***	NS

[z]Over half of the plants under 0.1 $m^3 \cdot m^{-3}$ treatments wilted.
[y]Means separation within columns by Duncan's multiple range test at $p < 0.05$.
[NS,***]Non-significant or significant at $p < 0.001$.

Table 4. Effects of substrate volumetric water content (VWC) on dry weights of pseudobulbs, leaves, and roots in *Cymbidium* 'Yang Guifei' after 12 weeks of treatment.

Irrigation	Shoot (g)		Root (g)	Top/root ratio
	Pseudobulb	Leaf		
Deficit	1.3 b[z]	2.5 b	3.7 b	1.1 a
Moderate	1.8 a	5.9 a	6.0 a	1.3 b
Significance	***	***	***	*

[z]Means separation within columns by t-test at $p < 0.05$.
*, ***Significant at $p < 0.05$ or 0.001.

Table 5. The average number of irrigations per day and water use for growing *Cymbidium* ‘Yang Guifei’ for 28 weeks.

VWC ($m^3 \cdot m^{-3}$)	Average No. of irrigations per day	Average daily water use (mL)
0.1	0.8	25.6
0.2	1.8	58.0
0.3	6.7	213.4
0.4	11.0	352.8

applied to maintain VWC above the thresholds (Fig. 2). It took 25 days to start irrigation for the 0.3 and 0.4 $m^3{\cdot}m^{-3}$ treatments, and 28 and 54 days for the 0.2 and 0.1 $m^3{\cdot}m^{-3}$ treatments, respectively. Short-term fluctuations in VWC were observed on May 6, because several electric wires were replaced from the data logger. Fungicides treatment every 4 weeks affected VWC threshold on June 16, July 7 and 28. Thereafter, VWC in each pot was maintained just above the allocated threshold consistently throughout the experiment.

Light response curve and chlorophyll fluorescence. Light saturation point for different VWC used in this study is shown in Fig. 3. The measurement of photosynthetic rates in the leaves showed the typical light response curve of C3 plants, initially increased, and then leveled off at saturating light intensity. Light saturation points were around 600 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ when photosynthetic rate were 2.1, 4.3, and 6.2 $CO_2{\cdot}m^{-2}{\cdot}s^{-1}$. Increasing light saturation point with increasing soil water content were observed in *Populus cathayana* (Xu et al., 2008) and *Vitis vinifera (*Escalona et al., 1999). However, *Cymbidium* under 0.4 $m^3{\cdot}m^{-3}$ treatment showed lower light saturation point than 0.3 $m^3{\cdot}m^{-3}$ treatments. The reason might be that 0.4 $m^3{\cdot}m^{-3}$ treatment caused water-logging stress. *Quercus pagoda* grown in flooding environment showed lower light saturation points than the control. Water logging causes root hypoxia that can disrupt Rubisco activity and thereby reduce photosynthetic capacity (Gardiner and Krauss, 2001).

Chlorophyll fluorescence is a rapid and sensitive measure of photosynthetic competence in higher plants and thus can be used to detect the impact of such stresses on them (Baker and Rosenqvist, 2004; Calatayud et al., 2006). Plants get photoinhibitory damages when water deficit stress occurs under the high light intensities (Hoch et al., 2001). These factors affect photosynthesis and chlorophyll

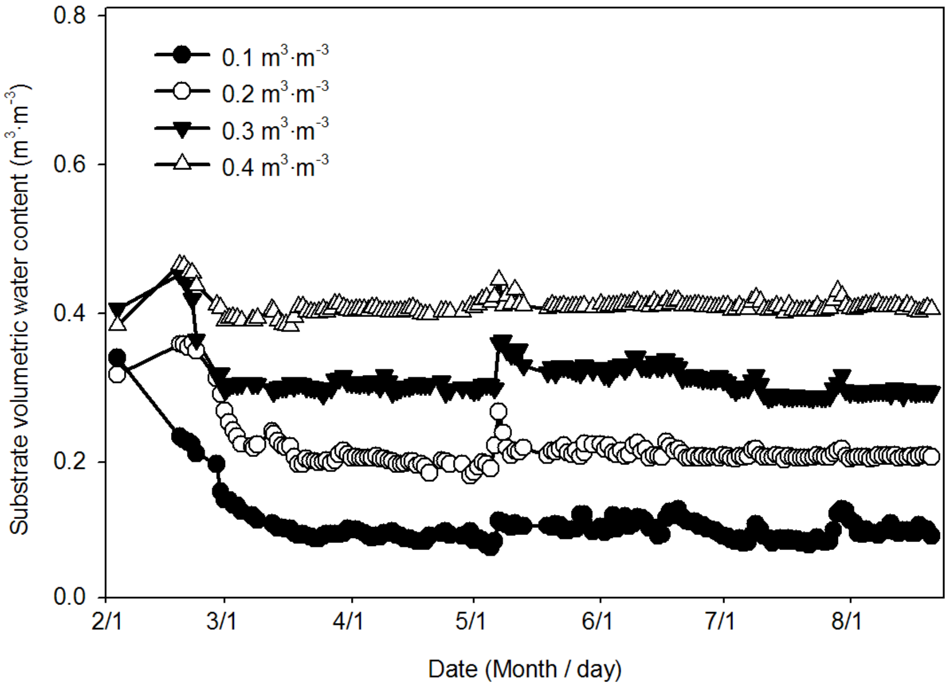

Fig. 2. Substrate volumetric water content (VWC) changes in *Cymbidium* ‘Yang Guifei’ during Exp. 1.

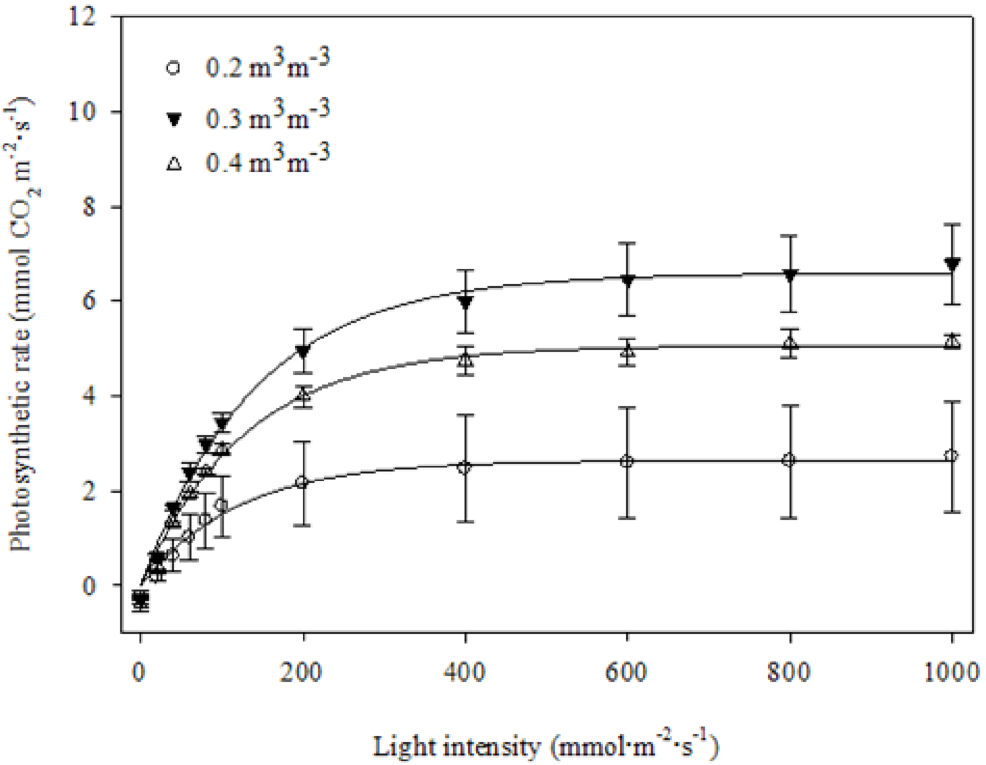

Fig. 3. Net photosynthetic assimilation rate (A_n) in response to incident light intensity of *Cymbidium* 'Yang Guifei' grown at 0.2, 0.3, and 0.4 $m^3{\cdot}m^{-3}$ after 28 weeks of treatment. Measurements were taken after 28 weeks of sensor based irrigation treatment. Vertical bars represent the standard errors of the means (n = 3).

fluorescence parameters directly or indirectly (Maxwell and Johnson, 2000), and negatively affect physiological processes (Thomas and Turner, 2001). However, there was no significant difference in chlorophyll fluorescence in *Cymbidium* during 28 weeks of the growth (Fig. 4). The treatments might not cause stress from light during the experiment, and the plants could manage water stress with their pseudobulbs.

EC and pH. There was no significant difference in pH among treatments (Table 6). However, EC showed significant ($p < 0.001$) increase with decreasing VWC after 28 weeks of experiment. EC were 3.9, 3.5, 2.0, and 1.7 $dS{\cdot}m^{-1}$ under 0.1, 0.2, 0.3, and 0.4 $m^3{\cdot}m^{-3}$ treatments, respectively. Despite using the same amount of slow release fertilizers to all treatments, differences in EC might be caused by the different amounts of irrigation. In case of the low VWC**,** average daily water use under 0.1 and 0.2 $m^3{\cdot}m^{-3}$ treatments (25.6 and 58.0 mL) might cause salt accumulation because of a low number of irrigations per day (0.8 and 1.8 irrigations per day, respectively), and increase EC (Tables 5, 6). According to Naik et al. (2013), *Cymbidium* 'Sleeping Nymph' enhanced flowering when NPK does of 12:30:10 at EC of 1 or 1.5 $mS{\cdot}cm^{-1}$. Plants treated with fertilizer solution with EC of 2.0 $mS{\cdot}cm^{-1}$ showed the similar vegetative growth, but did not produce flowers. During the vegetative growth of *Cymbidium* 'Yang Guifei' at 0.3 $m^3{\cdot}m^{-3}$ treatment EC value was 2.0 $dS{\cdot}m^{-2}$. However, more studies are required to test how different NPK ratio and EC will cause in flowering.

Water use. The average water use per day decreased with decreasing VWC level (Table 5). The water use applied at 0.1, 0.2 and 0.3 $m^3{\cdot}m^{-3}$ was 93, 85, and 40%, respectively, of those used by the plants grown at 0.4 $m^3{\cdot}m^{-3}$. The 0.1 and 0.2 $m^3{\cdot}m^{-3}$ saved a considerable amount of water, but they could not let plants grow

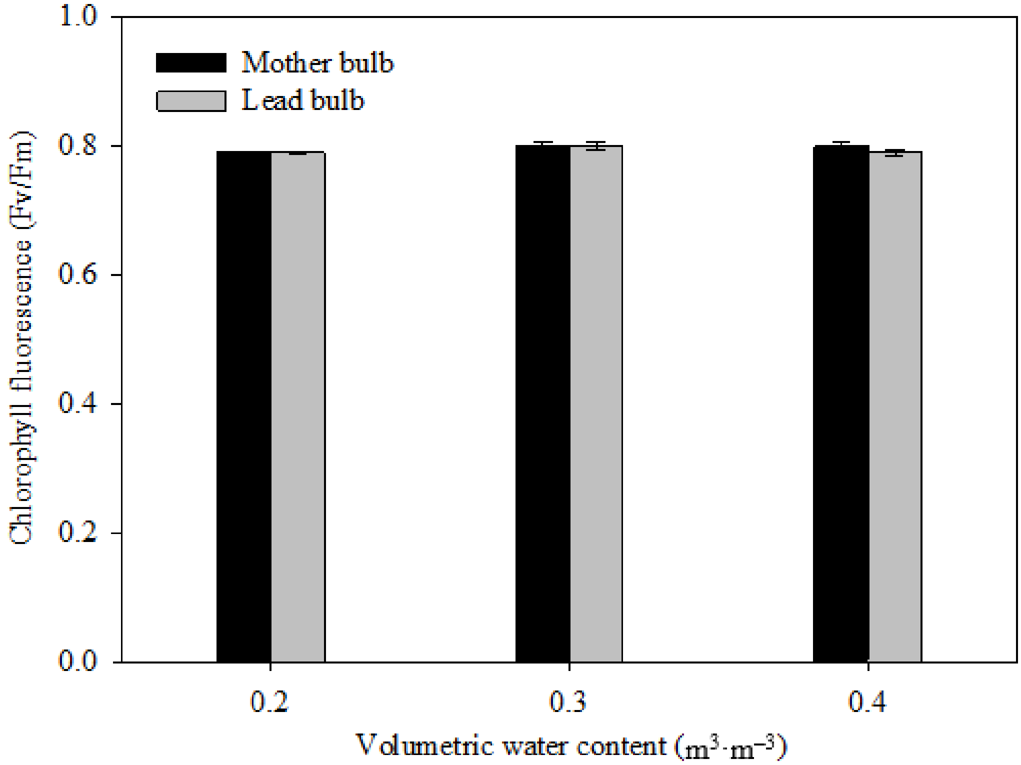

Fig. 4. Maximal quantum yield (Fv/Fm) of the third uppermost mature leaves of *Cymbidium* 'Yang Guifei' grown at 0.2, 0.3, and 0.4 $m^3 \cdot m^{-3}$ after 28 weeks of treatment. Vertical bars represent the standard errors of the means.

Table 6. The pH and EC of *Cymbidium* 'Yang Guifei' after 28 weeks as affected by volumetric water content (VWC).

VWC ($m^3 \cdot m^{-3}$)	pH	EC ($dS \cdot m^{-1}$)
0.1	4.9	3.9 a[z]
0.2	4.9	3.5 a
0.3	4.8	2.0 b
0.4	5.4	1.7 bc
Significance	NS	***

[z]Means separation within columns by Duncan's multiple range test at $p < 0.05$.
[NS,***]Non-significant or significant $p < 0.001$.

well, and 0.1 $m^3 \cdot m^{-3}$ treatments plants wilted. Previous research has shown that sensor based irrigation could reduce water use without adverse impacts on plant growth of blue bonnet (Niu et al., 2007), conifers (Pershey et al., 2015), geranium (Valdes et al., 2015), and woody ornamentals (Warsaw et al., 2009) to a certain extent. These results also indicate that maintaining the water contents at an optimum level could reduce the average water per day in *Cymbidium* ‘Yang Guifei’ cultivation.

Root morphology in different irrigation (Exp. 2)

Transverse section of root. Cross section of *Cymbidium* ‘Yang Guifei’ root consists of velamen radicum, cortex, and stele (Fig. 5A). Velamen radicum has ectovelamen and endovelamen. The ectovelamen has one cell layer, and the endovelamen has 4–6 cell layers with angular and radially elongated cells. Exodermis possess typical ꓵ-shaped thickening (Fig. 5B). Cortex has 20–23 cell layers of parenchymatous cells. The outermost and innermost one or two layers were smaller than those of the middle layer with lack of intercellular spaces. (Fig. 5A). Vascular cylinder shapes in polyarch contained phloem, xylem vessels, and pith (Fig. 5C). Phloem clustered elliptical to oval and circular alternate with xylem. Metaxylem cells were greatly broadened. Endodermis cells were adjacent to the sclerenchyma xylem and phloem (Fig. 5D).

Size of the root regions. Changes in root anatomical parameters under water deficit are shown in Fig. 6. All cortex layers were collapsed at 12 weeks after the start of deficit irrigation (Fig. 6A). Accordingly, a decrease in root diameter has been proposed as a trait for increasing plant acquisition of water and productivity

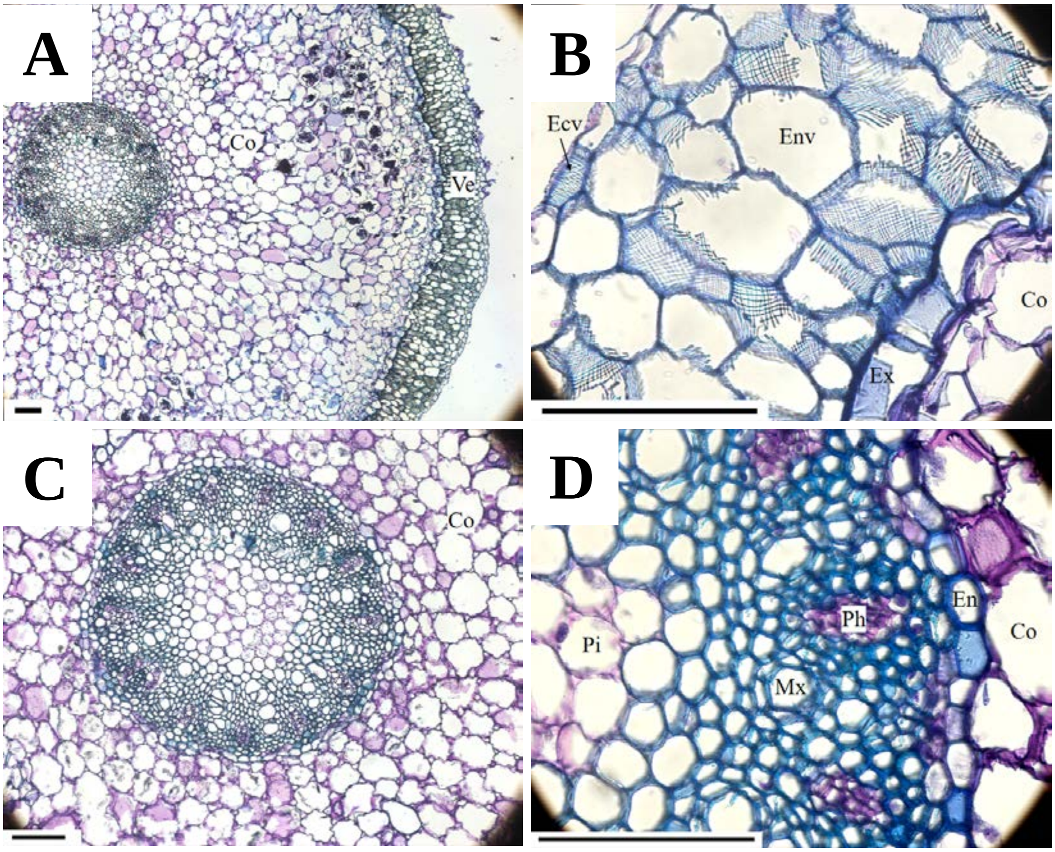

Fig. 5. Transverse section of *Cymbidium* ‘Yang Guifei’ roots. (A) Root cross-section with velamen (Ve) and cortex (Co); (B) Pericycle and vascular tissues; (C) Metaxylem elements (Mx), phloem elements (Ph), and sclerenchymatous pith (Pi); (D) Ectovelamen (Ecv), endovelamen (Env) with wall striations, Ω-shaped exodermis (Ex), and cortex (Co). Scale bars = 200 µm.

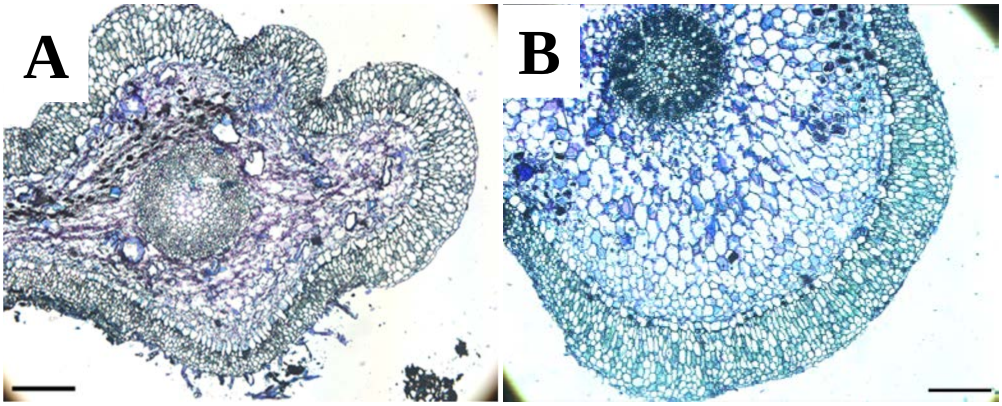

Fig. 6. Transverse sections of *Cymbidium* 'Yang Guifei' roots grown under the deficit (A) and moderate (B) irrigation after 12 weeks of treatment. Scale bars = 500 µm.

under drought (Wasson et al., 2012). However, there were only two significant differences in cortex thickness and cell area, which decreased with decreasing irrigation water (Table 7). No significance was found in root semi-cross section, velamen, and stele thickness. Epiphytic *Acampe praemorsa* has aerial roots that have higher ratios of velamen radicum thickness to root semi-diameter and xylem conduit diameter compared to its terrestrial root (Thangavelu and Ayyasamy, 2017). However, there were no differences in anatomical or morphological structure between aerial and terrestrial *V. planifolia* roots (Stern and Judd, 1999). Terrestrial *Cymbidium* has smaller ratios of velamen radicum thickness to root thickness than epiphytic *Cymbidium*, and these can be the reasons why the terrestrial root velamen was not affected by water deficit.

In summary, *Cymbidium* 'Yang Guifei' were grown under different VWC to determine the optimum VWC. Under the water deficit environment, cortex layer of *Cymbidium* roots and its vegetative growth were decreased. The findings in the present study suggest that increasing VWC promotes vegetative growth, but 0.4 $m^3 \cdot m^{-3}$ treatment requires 352.8 mL water per day, while 0.3 $m^3 \cdot m^{-3}$ treatment requires only 213.4 mL water. Considering long cultivation periods for *Cymbidium*, VWC of 0.3 $m^3 \cdot m^{-3}$ is recommended as optimum VWC for *Cymbidium* 'Yang Guifei' cultivation.

Table 7. Effects of the deficit (A) and moderate (B) irrigation treated on the transverse section of *Cymbidium* 'Yang Guifei' after 12 weeks of treatment.

Irrigation	Root half cross-section (μm)	Velamen layer thickness (μm)	Endovelamen cell area (μm^2)	Cortex thickness (μm)	Cortical cell area (μm^2)	Stele thickness (μm)	Protoxylem cell area (μm^2)
Deficit	2,244.6	391.9	2,778.0	1,278.5	9,293.4	729.8	1,466.9
Moderate	2,480.9	371.4	3,464.5	1,641.0	15,783.5	769.1	1,359.8
Significance	NS	NS	NS	*	*	NS	NS

[z]Means separation within columns by t-test at $p < 0.05$.
[NS, *]Non-significant or significant at $p < 0.05$.

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ABASTRACT IN KOREAN

본 연구는 *Cymbidium* ‘Yang Guifei’의 적정 수분 함량을 구명하기 위해 수행하였다. 첫 번째 실험에서는 상토 수분 함량에 따른 심비디움 영양 생장을 알아보기 위해 일년생 묘를 대상으로 온실에서 28주 동안 실험을 수행하였다. 수분 함량 처리로는 0.1, 0.2, 0.3, 그리고 0.4 $m^3 \cdot m^{-3}$를 코이어 화분에 심어 사용하였다. 상토 수분 함량은 토양수분 센서를 사용하여 20분마다 측정하였고, 설정값보다 적을 시 32 mL의 관수가 주어졌다. 실험하는 동안 0.1, 0.2, 0.3, 그리고 0.4 $m^3 \cdot m^{-3}$ 처리구 순으로 약 25.6, 58.0, 213.4 그리고 352.8 $mL \cdot d^{-1}$ 관수가 주어졌다. 0.1 $m^3 \cdot m^{-3}$ 처리구에서는 심비디움이 자라기에는 너무 건조하여 모든 식물이 시들었다. 마더벌브에서는 상토 수분 함량이 증가함에 따라 위구경과 엽폭이 증가 하였고, 리드벌브에서는 위구경, 엽장, 엽수에서 0.3 와 0.4 $m^3 \cdot m^{-3}$ 그리고 0.2 $m^3 \cdot m^{-3}$ 처리순으로 증가하였다. 건물중에서도 0.3 와 0.4 $m^3 \cdot m^{-3}$ 에서 동일하게 생육이 가장 왕성하였다. 하지만 광 반응 곡선에서는 0.3 $m^3 \cdot m^{-3}$ 처리구에서 가장 높은 광포화점을 보여주었다. 첫 번째 실험 결과, 생육지표와 하루 관수량을 비교하였을 때 0.3 $m^3 \cdot m^{-3}$ 가 *Cymbidium* ‘Yang Guifei’의 적정 상토 수분 함량이라는 것을 확인할 수 있었다. 두 번째 실험에서는 상토 수분 함량에 따른 난 뿌리형태를 비교하기 위해 10개월 된 같은 품종과 상토를 사용하여 적정 관수 (5,122 $mL \cdot d^{-1}$)와 건조 관수 (479.6

mL·d^{-1})처리 하에서 12 주 동안 실험을 진행하였다. 위구경, 엽장, 엽폭, 엽수, 건물중은 적정 관수 처리구에서 건조 관수 처리구에서보다 증가함을 확인 하였다. 실험 결과 피층과 피층 세포면적은 관수량이 증가함에 따라 유의성 있게 증가 되는 것을 알 수 있었다. 하지만, 벨라민층, 중심주, 물관부 크기에는 유의성이 나타나지 않았다. 두 번째 실험 결과, 관수량이 증가함에 따라 피층은 증가하였고, 지상부와 근권부 생육 모두가 증가하였다. 이런 결과를 통해 0.3 m^3·m^{-3} 수분함량이 *Cymbidium* ‘Yang Guifei’의 적정 수분 함량이라는 것을 알 수 있었고, 피층의 증가로 지상부와 근권부 생육도 증가한다는 것을 알 수 있었다.