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Comparative ecophysiological analysis of photosynthesis, biomass allocation, polysaccharide and alkaloid content in three *Dendrobium candidum* cultivars

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Abstract

The photosynthesis, plant biomass, alkaloid and polysaccharide contents of three *Dendrobium candidum* cultivars were compared in order to investigate the differences between their ecophysiological characteristics and provide recommendations for cultivation of *Dendrobium candidum.* The results demonstrate that leaf perimeter and leaf length in Kuanye was significantly higher ($P < 0.05$) than the other two cultivars. Compared to Zhaiye and Qingeng, Kuanye had the highest P_N value (0.346 μmol m^{-2} s^{-1}) at PAR of 70 to 90 μmol m^{-2} s^{-1}. Further, Kuanye had the highest ($P < 0.05$) stem polysaccharide content in autumn. On the other hand, stem alkaloid content of one-year old Qingeng was higher than that of Kuanye and Zhaiye in autumn, while two-year old stem of Zhaiye had higher alkaloid content than Kuanye and Qingeng. Interestingly, three-year old Kuanye had higher ($P < 0.05$) stem alkaloid content than the other two cultivars. These results suggest that the Kuanye cultivar has has superior ecophysiology than the other two cultivars and it can be recommended for cultivation in southeastern China.

Keywords: alkaloid; biomass; *Dendrobium candidum*; photosynthesis; polysaccharides; variety breeding.
Abbreviations: P_N_net photosynthetic rate; PAR_photosynthetically active radiation; LST_local summer time.

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Introduction

Dendrobium candidum is one of the most famous orchids and epiphytic herb in some countries of South and Southeast Asia (Bian et al., 2002; Zhao et al., 2007). In China, *D. candidum* has anciently been cultivated as herbal medicine and nutraceutical plant (Xing et al., 2013). According to the Pharmacopoeia of the People's Republic of China (2010), the *D candidum* stem has medicinal properties and is used to treat throat, lung, and ophthalmic disorders. The main compounds of *D. candidum* stem are polysaccharides and alkaloids, that have been demonstrated to benefit the stomach, promote the production of necessary body fluids, relieve throat inflammation and fatigue and enhance immune system (Chen and Wang, 2005; Zheng et al., 2012a). In addition, it has been reported that *D. candidum* present anti-hyperglycemic effects in adrenaline-induced glycemic mice and STZ-DM rats (Wu et al., 2004). It can stimulate the secretion of insulin from *beta* cells and inhibit the secretion of glucagons from cells. Moreover, it regulates the degradation and/or synthesis of liver glucogen (Wu et al., 2004). Although this herb has significant beneficial properties, the wild resources were in danger of extinction as a result of digging and deterioration of their natural habitats (Li et al., 2008). In China, it is epiphytic on cliffs or trunks commonly covered with moss and humus at altitudes of about 800 to 2000 m (Wang et al., 1998; Zhang et al., 2001; Xiao and Zhang, 2012). To overcome the danger of losing this important species, *in vitro* propagation and cryopreservation techniques have been recently developed (Wang et al., 1998; Bian et al., 2002). This has resulted in rapid increase in cultivation of this species. In China alone, more than 700 hectares is now grown of which about 85% is localized in Zhejiang Province. *D. candidum* sales in China amounted to 500 million dollars in 2012 (Zheng et al., 2012b). The main concern with *D. candidum* cultivation currently is availability of a cultivar that can produce high levels of bioactives. Different cultivars of the same medicinal species are significantly different in quality and bioactive components when cultivated at different locations. This can be attributed to the influence of climate, soil nutrients, and water (Leung et al., 2006; Yang et al., 2013). This variation in quality has not only ruined the efficacy of *D. candidum* but also resulted in reduced value to growers. Therefore there is demand for a variety capable of producing high levels of bioactives in native conditions. Until recently, only limited research has been done in *D. candidum* variety selection. Strategies for the cultivation of *D. candidum* should be developed with the aim to increase the production of bioactive compounds (Zheng et al., 2012b). Previous research on *D candidum* has been

Table 1 Fresh weight, dry weight and drying rate of the three *Dendrobium candidum* cutivars.[1]

Varieties [2]	Fresh weight (g)	Dry weight (g)	Dry rate (%)
K 1-year old stem	7.14 ± 0.28 b	1.15 ± 0.22 ab	16.04 ± 2.38 ab
K 2-year old stem	6.32 ± 0.09 bc	0.99 ± 0.08 b	15.66 ± 1.08 ab
K 3-year old stem	7.46 ± 0.28 b	1.13 ± 0.13 b	15.15 ± 1.15 ab
Z 1-year old stem	7.05 ± 0.34 b	1.41 ± 0.27 a	20.00 ± 2.97 a
Z 2-year old stem	5.51 ± 0.43 c	0.92 ± 0.16 bc	16.70 ± 2.98 a
Z 3-year old stem	5.56 ± 0.25 c	0.87 ± 0.02 bc	15.65 ± 1.18 ab
Q 1-year old stem	5.69 ± 0.29 c	0.88 ± 0.14 bc	15.48 ± 1.68 ab
Q 2-year old stem	7.74 ± 0.43 ab	0.96 ± 0.10 bc	12.40 ± 0.91 b
Q 3-year old stem	7.63 ± 0.25 ab	0.91 ± 0.09 bc	11.93 ± 1.02 b
K 1-year old leaf	7.41 ± 0.31 b	0.81 ± 0.14 c	10.89 ± 0.78 b
Z 1-year old leaf	8.45 ± 0.35 a	1.10 ± 0.10 b	13.03 ± 0.70 b
Q 1-year old leaf	7.04 ± 0.30 b	0.77 ± 0.09 c	10.95 ± 0.58 b

[1] Values represent mean ± SD determined from five replicates. Means followed by the same letter within a column are not significantly different at $P = 0.05$ according to Duncan's multiple-range test.
[2] Symbols K, Z Q along with the 1,2,3 years represents Kuanye (K), Zhaiye (Z), and Qinggeng (Q).

around developing microporpagation techniques and selection of optimal growing environment (Wei et al., 2007). However, there is little information about the photosynthetic characters, biomass and medical constituent of different *D. candidum* cultivars studied. Thus, the object of this study was to evaluate the medicinal properties and associated physiological parameters of three *D. candidum* cultivars for planting under shade conditions.

Results and discussion

Leaf analysis and biomass comparison

The leaf parameters of three *Dendrobium candidum* cultivars are showed in (Fig. 1). Leaf perimeter (11.29 cm ± 2.71) and leaf length (4.79 cm ± 1.42) of Kuanye were significantly ($P < 0.05$) higher than Qinggeng and Zhaiye, whereas both leaf area and leaf width of Kuanye was not significantly ($P > 0.05$) higher than Qinggeng. In addition, all leaf parameters of Zhaiye expect length-width ratio were significantly lower than that of the other two cultivars. Further, the dry weight of 3-year old stem of Kuanye (1.13 g ± 0.13) was significantly higher ($P < 0.05$) than Zhaiye and Qingeng (Table 1). For 1-year old stem, the dry weight (1.41 g ± 0.27) and drying rate (20.00% ± 2.97) of Zhaiye were significantly higher ($P < 0.05$) than other two cultivars. In addition, the leaf drying rate for three cultivars was no significantly different ($P > 0.05$) over the three years, although the fresh and dry weights of one-year old Zhaiye leaves were higher than that observed for Kuanye and Qingeng. Unlike fruits and vegetables, the herbal medicines are used for treating patients. It is therefore critical to ensure the quality of medicinal materials when the herbs are harvested. Combine to produce real, the best harvesting period of *D. candidum* is at the third year. Stem of Zhaiye with one-year old reached higher growth than two- and three-year old stem (Table 1), which presents downtrend basically as time passed. In opposite our results showed that was not occurred significant change in dry weight in stem of Kuanye along three years, which showed that stability and variety advantage.

Photosynthesis-irradiance response curves and diurnal variation of photosynthetic

The shade-induced photosynthetic responses in the three *D. candidum* cultivars were evaluated by constructing steady-state light response curves (Fig. 2A). It is important to note that the three cultivars were saturated at different irradiances. For example, the Kuanye cultivar reached its maximal P_N at about 100 μmol m^{-2} s^{-1} while the Qingeng and Zhaiye cultivars reached their maximal P_N at 70 μmol m^{-2} s^{-1} and 140 μmol m^{-2} s^{-1}, respectively. After the three cultivars reached their respective light saturation point, the P_N values reduced sharply. In fact, the net photosynthesis of Zhaiye cultivar decreased faster than the other two cultivars. Interestingly, the Kuanye cultivar had the highest P_N when three cultivars reached their light saturation point, however it had the lowest light compensation point (Fig. 2A). Diurnal changes in net photosynthetic rate (P_N) of three cultivars were evaluated for typical bimodal curves (Fig. 2B). The diurnal courses of P_N of three cultivars showed a midday depression that is frequently observed in Mediterranean species growing in the open air. The first peak value of Zhaiye was reached at about 9:00 h while for the other two cultivars this peak occurred at about 7:30 h LST (local summer time). The second peak occurred at almost 13:00 h LST and reached a value much lower than the first peak, except for Zhaiye. It is interesting to note that for all the three cultivars, minimum P_N values were found between 11:00 h and 12:00 h LST.

Photosynthesis plays an important role in plant productivity (Faville et al., 1999; Koç et al., 2003; Dai et al., 2009). Our data indicates that the light compensation point of Kuanye was lower while light saturation point was higher than Zhaiye and Qingeng cultivars. This indicates that Kuanye is better than Zhaiye and Qingeng in obtaining and utilizing photon energy. Nonetheless, the phenomenon of photosynthetic midday depression was found in all three cultivars. Shuting et al. (1997) demonstrated that in maize, cultivars with higher grain yield maintained higher rates of photosynthesis during plant development. This supports the idea that Kuanye which maintained higher photosynthetic rate has physiological advantage for obtaining high commercial yield (Zhang et al., 2004).

Polysaccharide content

Polysaccharides isolated from genus *Dendrobium* possess immunological activity (Hsieh et al., 2008). The polysaccharide content in stems of the three *D. candidum* cultivars was analyzed in four seasons (Fig. 3). One-year old Kuanye stem had significantly higher polysaccharide content ($P < 0.05$) than the other two cultivars in spring and summer (Fig. 3A). The average polysaccharide content of one-year old stems of the three cultivars increased rapidly from spring to autumn, while it decreased slowly from autumn to winter. The highest polysaccharide content for one-year old stems was found in Kuanye in autumn season. In the second year,

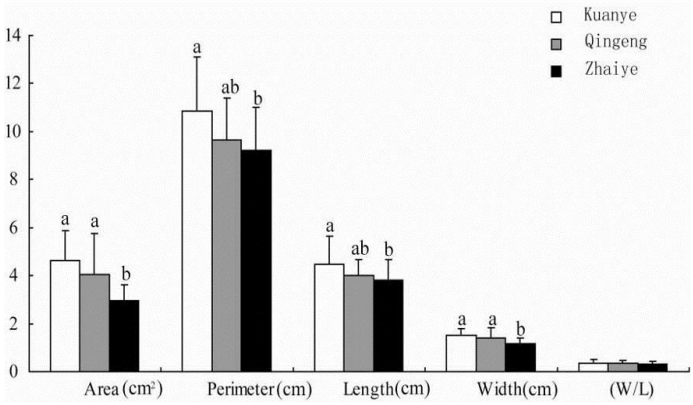

Fig 1. Leaf area, length, width and perimeter in different *Dendrobium candidum* cultivars.

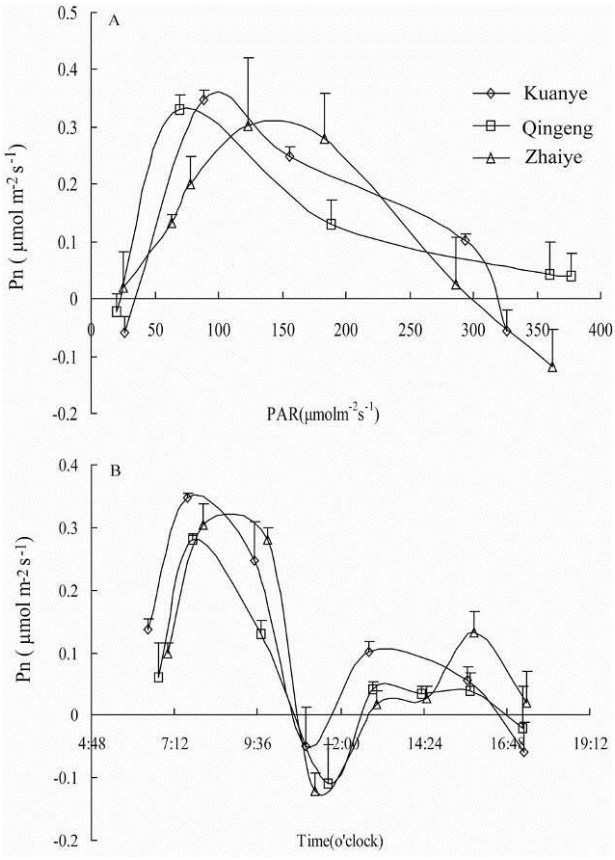

Fig 2. A: The photosynthesis irradiance (PAR) response curves in leaves of the three *Dendrobium candidum* cultivars. B: Diurnal variation of net photosynthetic rate, Pn, in the leaves of the three *Dendrobium candidum* cultivars.

polysaccharide content of the three cultivars increased from spring to summer and then decreased from autumn to winter (Fig. 3B). In the second year too, polysaccharide content was found to be highest ($P < 0.05$) in Kuanye (46.84% ± 0.77) in autumn. In the third year again, Kuanye had significantly higher polysaccharide content ($P < 0.05$) in autumn than the other two cultivars (Fig. 3C). Therefore, for all three years, polysaccharide content of Kuanye stem increased from spring to autumn and then decreased rapidly in winter. The highest percentage of polysaccharide is before blooming and it tends to decline at flowering stage. Hence, the optimal harvest time of *D. candidum* in Zhejiang province is autumn (November). Importantly, stem polysaccharide content of two-year old Kuanye was higher than that of one and three-year old plants, which indicates that the best time to harvest Kuanye is two years after planting. Further, our results show significant changes in leaf polysaccharide content of one- and two-year plants of all three cultivars (Fig. 4). The leaf polysaccharide content of one-year old plants increased from spring to summer and thereafter decreased (Fig. 4A), while in the two-year old plants, the maximum polysaccharide content was observed in summer (Fig. 4B). Comparing the three cultivars, one-year leaf of Kuanye had the highest polysaccharide content (18.68% ± 0.86, $P < 0.05$) in summer. Our results indicate that the polysaccharide content of stem is much higher than leaves, which is probably why stems of *D. candidum* are preferred for medicinal purposes (Lau et al., 2001). In addition, the leaves fell three years and therefore, only data for two years could be compared unlike stems where data for three years was analyzed. Normally, the leaves of *D. candidum* are removed before selling the stems for medicinal purposes. Our results demonstrate that although lower than stems, leaves also contain medicinally important polysaccharides and therefore should be used rather than wasted. Like some other herbal medicines, *D. candidum* leaves may be used as a fodder immunologic adjuvant in animal husbandry (Jiang et al., 2012). The leaves can also be processed into green tea for human consumption. Our results could therefore benefit nutraceutical, medicinal and veterinary industries.

Alkaloid content

Alkaloids from genus *Dendrobium* such as dendrobine have been known to possess antioxidant capacity and anticancer activity through improvement of human immunity (Bulpitt et al., 2007). The stem alkaloid content of the three *D. candidum* varieties in different harvest seasons was recorded (Fig. 5). The alkaloid content of three-year old stems was significantly ($P < 0.05$) higher than one and two-year old stem in all the three cultivars. Also, the highest alkaloid content was found in autumn (Fig. 5). Further, in autumn, the stem alkaloid content of one-year old Qingeng cultivar was higher ($P > 0.05$) than that of Kuanye and Zhaiye (Fig. 5A), while two-year old Qinggeng stem had significantly lower ($P < 0.05$) alkaloid content than Kuanye and Zhaiye (Fig. 5B). For three-year old plants in autumn, Kuanye had significantly higher ($P < 0.05$) alkaloid content than the other two cultivars (Fig. 5C). Moreover, for all three years of harvest, the highest ($P < 0.05$) alkaloid content (0.027% ± 0.001) was found in three-year old Kuanye stem in autumn (Fig. 5). This finding further supports our statement in previous section that the optimal harvest time for *D. candidum* is autumn (November). It also validates that Kuanye is better cultivar than Zhaiye and Qingeng as it is rich in alkaloids and polysaccharides of medicinal importance.

We also investigated the alkaloid content in leaves of the three cultivars in different harvest seasons (Fig. 6). Alkaloid content in leaf of one-year plants increased rapidly from spring to autumn and then decreased (Fig. 6A). The highest leaf alkaloid content ($P > 0.05$) was found in one-year old Qingeng cultivar in autumn. On the other hand, there was no significant difference ($P > 0.05$) in leaf alkaloid content of two-year old Zhaiye and Qingeng from summer to winter (Fig. 6B). However, alkaloid content in leaves of two-year old plants of all cultivars reached maximum values in autumn and then stopped increasing in winter. The highest leaf

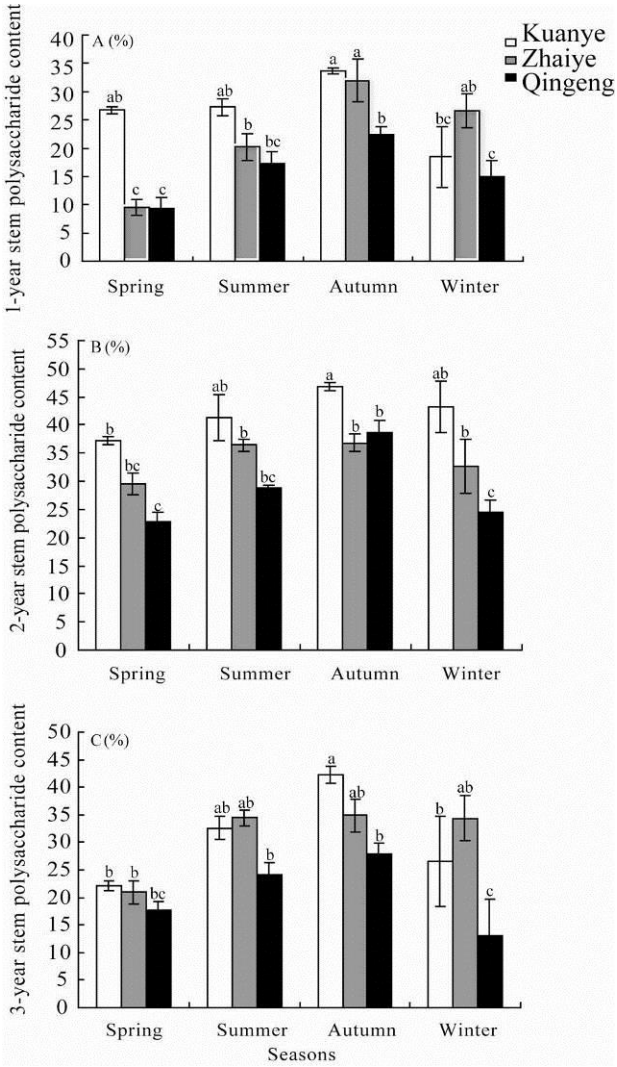

Fig 3. Polysaccharide content in stems of the three *Dendrobium candidum* cultivars. A: one-year old stem. B: two-year old stem. C: three-year old stem.

alkaloid content (0.0515% ± 0.001, $P < 0.05$) in two-year old plants was found in Kuanye cultivar in autumn. Comparing the leaf alkaloid content of one and two-year old plants, the leaves of two-year old Kuanye cultivar had highest alkaloid content ($P < 0.05$) in autumn.

Further, the dry matter accumulation of plants varied according to cultivar and the growing season (Wang et al., 2006). We found that the polysaccharide and alkaloid content of stem increased rapidly from spring to autumn (Fig. 3; Fig. 4). This phenomenon was also observed in leaves from spring to summer (Fig. 5; Fig. 6). We therefore propose that spring and summer are the best seasons for growing *D. candidum* and accumulation of polysaccharides and alkaloids. In three-year old stems, because of flowering, polysaccharide and alkaloid content decreased from the winter of the second year to the spring of third year.

Different cultivars accumulated different amounts of polysaccharides and alkaloids in stems and leaves. The highest amounts of polysaccharides and alkaloids were found in the Kuanye cultivar. In autumn, polysaccharide and alkaloid content in leaves and stems of Kuanye were higher ($P < 0.05$) than in other growing seasons. Kuanye also exhibited better photosynthetic performance (Fig. 2), which was consistent with the results of higher polysaccharide

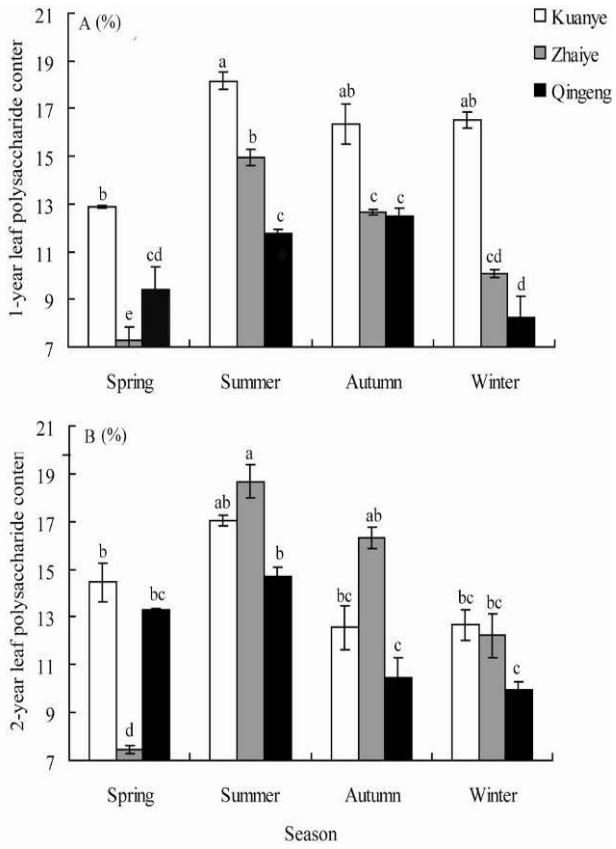

Fig 4. Polysaccharide content in leaves of the three *Dendrobium candidum* cultivars. A: one-year old leaves. B: two-year old leaves.

content. These facts suggest that for Chinese conditions, Kuanye may be a better cultivar than Zhaiye and Qingeng.

Materials and methods

Plants and growth conditions

The experiment was implemented in a nursery garden at Wenzhou Tiefengtang Co. Ltd, Zhejiang province, China. Three varieties of *D. candidum* namely, Kyanye, Zhaiye and Qingeng were cultivated. Plants were grown under 85% of natural incident irradiance shade treatment. Shading was accomplished by using sun shading net, the light intensities of five positions were measured at 8:00 h, 13:00 h and 16:00 h every day. All measurements were carried out in spring (March), summer (July), autumn (November), and winter (January) over three years, respectively. Irrigation and fertilization were applied based on local production practices.

Leaves analyze and plant drying rate determination

Biomass allocation was measured in the leaves and stems of plants by using WinFOLIA (Regent Instruments Inc., Quebec, Canada) according to Shen et al. (2007). Leaves were scanned with a Xatbeb graphics scanner and the images analyzed with WinFOLIA. The leaf drying rate and stem drying rate were measured as follows. Ratio of dry weight = Dry weight / Fresh weight × 100%. The fresh weight was measured soon after harvest, while the dry weight was determined after the samples pre-dried to a constant weight in

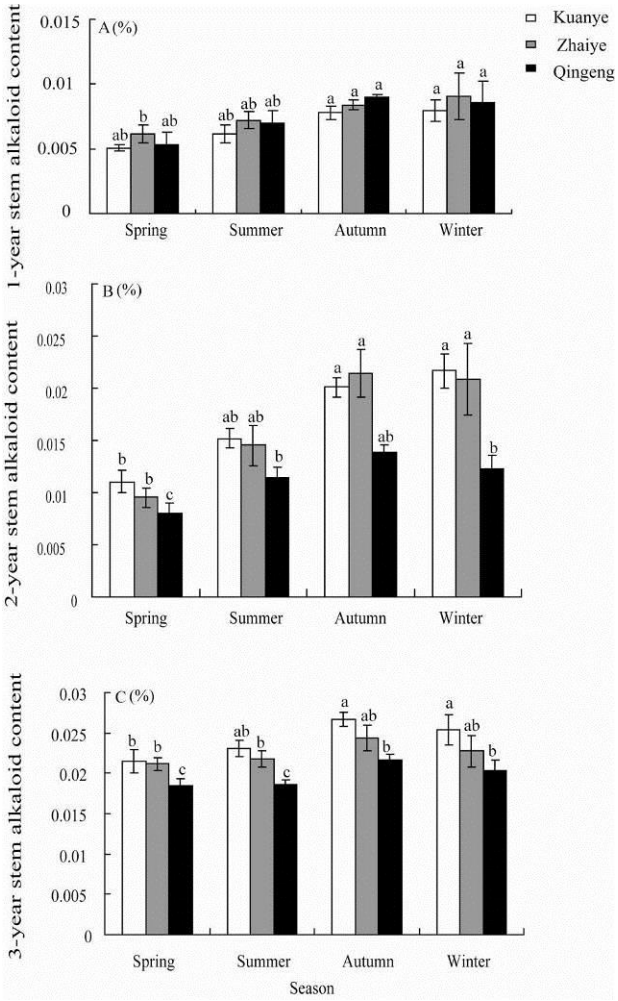

Fig 5. Alkaloid content in stems of the three *Dendrobium candidum* cultivars. A: one-year old stem B: two-year old stem. C: three-year old stem.

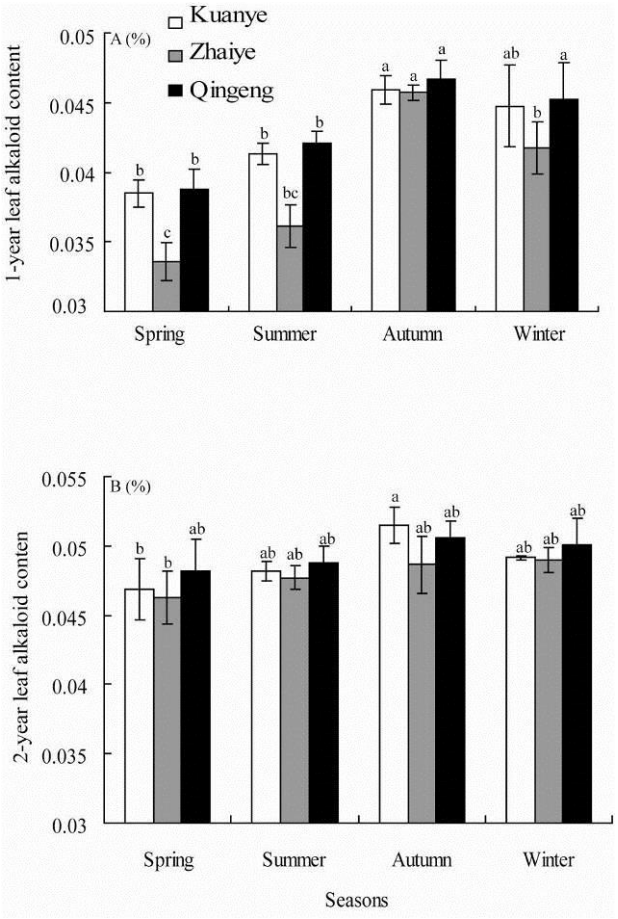

Fig 6. Alkaloid content in the leaves of the three *Dendrobium candidum* cultivars. A: one-year old leaves. B: two-year old leaves.

oven (103 ± 2 ^{o}C, 24 h). In each season five individuals of *Dendrobium* plants were sampled with shovel keeping the root as intact as possible.

Photosynthesis

The measurements of net photosynthetic rate (P_N), PAR response curves and diurnal course of photosynthesis were carried out according to Mao et al. (2007) using the Portable Gas Exchange Fluorescence System LCA-4 (*ADC Bio Scientific Ltd.* Hoddesdon, UK). The parameters of diurnal course of photosynthesis were measured on uppermost, fully expanded leaves from 7:00 to 18:00 in bright sunlight on a clear, cloudless day. Measurements were done on five replicates of five plants each. Results were given as the mean of these determinations.

Polysaccharide content analysis

Polysaccharide content was determined in one, two and three year old leaves and stems of the three cultivars. Both leaves and stems were washed with distilled water before processing. Leaf samples were taken from green, disease free leaves horizontally arranged on the plants. Polysaccharides were extracted from dried, ground material. Initially, samples (0.1 g ground to powder with 300 µm in diameter) were dried in oven at 105 ^{o}C for 30 min, followed by heating at 60 ^{o}C until constant mass was achieved (12h). For polysaccharide isolation, the residue extracted from powder with 80% ethanol was dried, and then ultrasonic extraction was performed twice with 3 ml of distilled water for 30 min. The extracts were filtrated, and extracted with chloroform for three times. After the supernatant was concentrated, ethanol was added to a final concentration of 80% (v/v). The polysaccharides were allowed to precipitate by overnight incubation at 4^{o}C, and subsequently washed with ethanol, acetone, ether and dried in a vacuum to produce crude polysaccharide. The polysaccharide content was determined by the phenol-sulphuric acid method (Dubios, 1956).

Alkaloids content analysis

The alkaloid content of leaves and stems of one, two and three year old plants of the three varieties was determined. The leaves and stems samples were washed with distilled water before processing. Samples were initially dried in the oven at 105 ^{o}C for 30 min, and followed by 60 ^{o}C until a constant weight was achieved. The triturated powders (0.4 g) were used for the determination of alkaloids. The powder moistened with ammonia solution was extracted with chloroform (10 ml) in a 100 ml conical flask. The flask was weighed and placed in a water bath at 65 ^{o}C for 2 h. The flask

was subsequently cooled bought to original weight with chloroform. 2 ml of filtrated samples were mixed with 8 ml chloroform. The 4 ml of diluted solution was poured in a separatory funnel and mixed with 6 ml chloroform, 5 ml buffer (pH 4.6), and 1 ml of 0.04% bromocresol green solution. The samples were filtered and 6 ml of sample was mixed with 1 ml of 0.01mol/L NaOH and used to alkaloid analysis. Absorbance was measured at the 630 nm wavelength (Bush et al., 1997).

Data analysis

Each experiment was carried out as a randomized complete block design and was repeated five times. Data was expressed as mean ± SD (standard deviation). Statistical significance between mean values was assessed by Duncan's multiple range tests ($P \leq 0.05$ was considered significant). Data were analyzed by SPSS version 20.0 for windows (SPSS Inc., Chicago, IL).

Conclusion

Our study revealed significant differences in the photosynthesis, dry matter accumulation and polysaccharide and alkaloid content in the three *D. candidum* cultivars. Kuanye was the best performing cultivar based on the physiological parameters analyzed for all the cultivars. We propose that Kuanye can be chosen as ideal cultivar for *D. candidum* commercial plantation in southeastern China.

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References

Bian HW, Wang JH, Lin WQ, Han N, Zhu MY (2002) Accumulation of soluble sugars, heat-stable proteins and dehydrins in cryopreservation protocorm-like bodies of *Dendrobium candidum* by the air-drying method. J Plant Physiol. 159:1139-1145.

Bush LP, Wilkinson HH, Schardl CL (1997) Bioprotective alkaloids of grass-fungal endophyte symbioses. Plant Physiol 114:1-7.

Chen YG, Wang HY (2005). Advance on the chemical and pharmacological studies on plants of Flickingeria genus. Lishizhen Med Mater Med Res. 16:725-727.

Commission of Chinese Pharmacopoeia (2010). Pharmacopoeia of the People's Republic of China. Chemical Industry Press, Beijing

Dai YJ, Shen ZG, Liu Y, Wang LL, Hannaway D, Lu HF (2009) Effects of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of *Tetrastigma hemsleyanum* Diels et Gilg. Environ Exp Bot. 65:177-182.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem. 28:350-356.

Faville MJ, Silvester WB, Green TGA, Jermyn WA (1999) Photosynthetic characteristics of three asparagus cultivars differing in yield. Crop Sci. 39:1070-1077.

Jiang W, Liu Y, Zheng H, Zheng Y, Xu H, Lu H (2012) Immune regulation of avian influenza vaccine in hens using *Hypericum perforatum* L. methanol extraction. Plant Omics. 5:40-45.

Koç M, Barutçular C, Genç I (2003) Photosynthesis and productivity of old and modern durum wheats in a Mediterranean environment. Crop Sci. 43:2089-2098.

Lau DTW, Shaw PC, Wang J, But PPH (2001) Authentication of medicinal *Dendrobium* species by the internal transcribed spacer of ribosomal DNA. Planta Med. 67:456- 460.

Leung PC (2006) Good agricultural practice-GAP: Does it ensure a perfect supply of medicinal herbs for research and drug development? In: Leung PC, Fong HHS, Xue CC (eds.) Current review of Chinese medicine: quality control of herbs and herbal materials, World Scientific Publishing Co. Pte. Ltd, Singapore, p 27-55.

Li B, Wei J, Wei XL, Wei X, Tang K, Liang Y, Shu K, Wang B (2008) Effect of sound wave stress on antioxidant enzyme activities and lipid peroxidation of *Dendrobium candidum*. Colloid Surface B. 63:269-275.

Mao LZ, Lu HF, Wang Q, Cai MM (2007) Comparative photosynthesis characteristics of *Calycanthus chinensis* and *Chimonanthus praecox*. Photosynthetica. 45:601-605.

Shen J, Li X, Wang D, Lu H (2007) *In vitro* culture of Croftonweed (*Ageratina adenophora*): Considerable potential for fast and convenient plantlet production. Weed Sci. 21:445-452.

Wang GG, Bauerle WL, Mudder BT (2006) Effects of light acclimation on the photosynthesis, growth, and biomass allocation in American chestnut (*Castanea dentata*) seedlings. Forest Ecol Manag. 226:173-180.

Wang JH, Ge JG, Feng L, Bian HW, Huang CN (1998) Cryopreservation of seeds and protocorms of *Dendrobium candidum*. Cryo Lett. 19:123-128.

Wei M, Jiang ST, Luo JP (2007) Enhancement of growth and polysaccharide production in suspension cultures of protocorm-like bodies from *Dendrobium huoshanense* by the addition of putrescine. Biotechnol Lett. 29:495-499.

Wu HS, Xu JH, Chen LZ, Sun JJ (2004) Studies on anti-hyperglycemic effect and its mechanism of *Dendrobium candidum*. Chin J Chin Mater Med. 29:160-163.

Xiao F, Zhang JZ (2012) First report of *Fusarium oxysporum* causing wilt of *Dendrobium candidum* in Zhejiang province, China. Plant Dis. 96:1377-1377.

Xing X, Cui SW, Nie S, Phillips GO, Goff HD, Wang Q (2013) A review of isolation process, structural characteristics, and bioactivities of water-soluble polysaccharides from *Dendrobium* plants. Bioac Carbohyds Diet Fibre. 1:131-147.

Yang B, Zheng J, Laaksonen O, Tahvonen R, Kallio H (2013) Effects of latitude and weather conditions on phenolic compounds in currant (*Ribes* spp) cultivars. J Agric Food Chem. 61:3517-3532.

Zhang JZ, Shi L, Shi AP, Zhang QX (2004) Photosynthetic responses of four *Hosta* cultivars to shade treatments. Photosynthetica. 42:213-218.

Zhang M, Yin FJ, Chen SJ (2001) Researches on morphology and anatomy of root system of *Dendrobium* nobile Lindl. Chin J Chin Mater Med. 26:384-393.

Zhao P, Wang W, Feng FS, Wu F, Yang ZQ, Wang WJ (2007) High frequency shoot regeneration through transverse thin cell layer culture in *Dendrobium Candidum* Wall. *Ex* Lindl. Plant Cell Tiss Organ Cult. 90:131-139.

Zheng YP, JiangW, Liao FL, Lu HF (2012a) Optimization of light quality for production of alkaloid and polysaccharide in *Dendrobium candidum* Wall. Ex Lindl. J Med Plants Res. 6:560-565.

Zheng YP, Jiang W, Silva EN, Mao L, Hannaway DB, Lu H (2012b) Optimization of shade condition and harvest time for *Dendrobium candidum* plants based on leaf gas exchange, alkaloids and polysaccharides contents. Plant Omics J. 5:253-260.