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Full Length Research Paper

Optimization of light quality for production of alkaloid and polysaccharide in *Dendrobium candidum* Wall. *ex* Lindl.

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The main bioactive substances of traditional medicine *Dendrobium candidum* are alkaloids and polysaccharides. A strategy is described for optimizing light quality to maximize the production of alkaloids and polysaccharides in *D. candidum*, so as to provide the basis for industrial production. Concentration of alkaloids and polysaccharides were studied by acid dye colorimetry and vitriol-phenol colorimetry, respectively, after culturing the *D. candidum* fewer than three light qualities: red light, blue light and white light. Production of alkaloids and polysaccharides were increasing with the growth extension under all light treatments. Explants cultured under red light contained significantly more alkaloids and polysaccharides than explants cultured under blue light, followed by white light, exhibiting that the most effective light was red light. But, upon the onset of continuous red light and blue light irradiation, growth of *D. candidum* decreased in a continuous manner. These observations suggested that among different light qualities, a mixture of red and nature light would be necessary for normal plant and had the potential to replace others for the large-scale production.

Key words: *Dendrobium candidum*, alkaloids, polysaccharides, light quality.

INTRODUCTION

Dendrobium candidum Wall. *ex* Lindl. is one of the most well-known traditional medicines, and is classified as *Dendrobium* (Orchidaceae) (Commission of Chinese Pharmacopoeia, 2005). The stem has been used to treat throat, lung, and ophthalmic disorders. The plant is epiphytic on cliffs or trunks commonly covered with moss and humus at altitudes of about 800 to 1500 m. In addition, seed germination of this species requires a symbiotic fungus, because the seeds are extremely small and lack endosperm and nutrient substances. Thus, it is difficult to grow intact plantlets, and investigations in the wild have indicated that many populations of *D. candidum* recorded previously have been extirpated (Su and Yang, 2006; Sun et al., 2006). *In vitro* culture could offer uniform and large quantities of original material in a finite time with a strictly controlled environment (Shen et al., 2007). Since the first mass propagation of orchids was reported by Morel (1960), plant regeneration in the *Dendrobium* system has proved particularly efficient for conserving germplasm and enabling further investigations (Puchooa et al., 2004; Roy et al., 2007; Zhao et al., 2007; Zhao et al., 2008; Shiau et al., 2005). Among the secondary metabolites produced by plants, alkaloids feature as an outstanding class of defensive compounds (Fattorusso and Scafati, 2007) and polysaccharides have exhibited immuno-modulatory and anti-oxidant activities (Liu et al., 2007; Paulsen, 2001). The main bioactive substances of *D. candidum* are alkaloids and polysaccharides (Chen and Wang, 2005).

Recent studies found that alkaloids from genus *Dendrobium*, such as dendrobine, increased antioxidant capacity and showed anti-cancer activity through improvement of human immunity (Bulpitt et al., 2007). Polysaccharides isolated from this genus also possessed immunological activity (Hsieh et al., 2008). Most of the studies into polysaccharides and alkaloids of this species, just as for other plants in this genus, have focused on

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medium optimization (Wei et al., 2007; Zha et al., 2007), component analysis (Yang et al., 2006; Ye et al., 2002) and pharmacological action (Zhao et al., 2001). Growth and development of *D. candidum* are closely related to the surrounding environment. Adapted plasticity is accomplished by a complicated integration of multiple environmental factors, in which light signals supply plants with seasonal, spatial and time information (Whitelam and Halliday, 2007).

The influence of light quality on the formation of primary and secondary metabolites has been reported in *Cistanche deserticola* (Jie et al., 2003) and *Perilla* plants (Nishimura et al., 2009). There has also been research into light quality induction of polysaccharide content in *Gelidium sesquipedale* (Torres et al., 1995; Carmona et al., 1998) and *Porphyridium cruentum* (You and Barnett, 2004). However, no studies have reported the optimum light quality for maximizing production of alkaloids and polysaccharides in *D. candidum*. Thus, in this investigation, *in vitro* cultures of *D. candidum* were studied to investigate the variation in alkaloids concentration (AC) and polysaccharides concentration (PC), determined under red light (RL), blue light (BL) and white light (WL). This research identified the optimal light quality for enhancing the production of alkaloids and polysaccharides by inducing protocorm-like bodies (PLBs) and regenerating plantlets of this species.

MATERIALS AND METHODS

Plant material and culture condition

Seeds of *D. candidum* were collected in the laboratory of Zhejiang Normal University and transferred to a basal Murashige and Skoog (MS) medium for inducing PLBs and proliferation (Murashige and Skoog, 1962). Experiments were carried out under controlled laboratory conditions. PLBs of *D. candidum* were cultured onto 1/2-MS basal media (0.3 $mg \cdot L^{-1}$ 1-naphthaleneacetic acid (NAA), 30 $g \cdot L^{-1}$ sugar, 10 $g \cdot L^{-1}$ agar) adjusted to pH 5.8 at 27°C, 1500 lx light intensity and a 12/12 h cycle of light/dark photoperiods. The cultured seedlings were then transplanted to a greenhouse at Zhejiang Senyu Company Limited, China. After seedlings were approximately 4 cm in height, plants with the same provenance and clonal composition were transferred to different qualities of RL, BL and WL, for periods of 10, 20 and 30 days and the AC and the PC were then determined. Three sets of samples were taken for each treatment period and each light treatment (RL, BL and WL), giving a total of nine groups, with each of groups comprising of 20 flasks. The experiment was carried out in a thermostatically controlled room (27°C) and external refrigerated recirculation was used to maintain the temperature of the experiment at 27 ± 2°C.

Determination of Ratio of dry weight (DR)

DR of 9 groups of *D. candidum* plantlets was measured.

$$\text{Raito of dry weight} = \text{Dry weight} / \text{Fresh weight} \times 100\%$$

(The dry weight was measured after the PLBs had been dried to constant weight in an oven at 60°C).

Determination of AC

Materials were dried in an oven at 105°C for 30 min and then dried at 60°C until a constant weight was obtained. Triturated powders (400 mg) were used for the determination of AC. The powder was moistened with ammonia solution and extracted with chloroform (10 mL) in a 100 mL conical flask. The flask was weighed and placed in a water bath at 65°C for 2 h. The flask was then cooled and brought back to the original weight by adding fresh chloroform. Filtered sample volumes of 2.0 mL were injected into 8 mL of chloroform. 4.0 mL of the diluted solutions were poured in a separating funnel and mixed with 6.0 mL chloroform, 5.0 mL buffer at pH 4.6, and 1.0 mL of 0.04% bromocresol green solution. A 6.0 mL sample was combined with 1.0 mL of 0.01 mol/L NaOH as the final solution for analysis. Absorbance was measured at 630 nm wavelength (A standard sample was obtained from Zhejiang Senyu Company Limited China).

Determination of PC

For extraction of polysaccharides, a residue extracted from PLB powder using 80% ethanol (v/v) was dried and then extracted twice with 3 mL distilled water for 30 min under ultrasound. After filtration and extraction with chloroform three times, the supernatants were concentrated and ethanol added to a final concentration of 80% (v/v). Samples were kept overnight at 4°C, the polysaccharide precipitate was then collected and washed with absolute ethanol, acetone, ether and dried under vacuum to obtain crude polysaccharides. PC was determined by the phenol-sulfuric acid method (Dubois et al, 1956).

Statistical analysis

Statistical significance between mean values was assessed by Duncan's multiple range test (P = 5% level).

RESULTS AND DISCUSSION

Fresh weight, dry weight and DR of *D. candidum*

The biosynthesis of active substances in plant cells involves multiple internal and external factors, and light quality is the most important factor (Xie et al., 2000). As reflected in the values of total DR, which got to the highest under BL 30 days whereas it was of the lowest value under RL 20 days and WL 20 days with 11.26±0.076% and 11.26±0.217%, respectively (Table 1). DR in different phases of 10, 20 and 30 days were presented in Figure 1C, in which showed the trend of high - low – high. That was, 10 days treatment had higher DR than 20 days treatment, and maximum under 30 days treatment (except for DR under WL 30 days treatment, which is less than under WL 10 days treatment). Irradiated with the different light qualities also resulted in distinct DR. Totally, the growth in response to BL was similar to that of WL, and two of all achieved higher DR than RL.

Significant difference between the DR under RL 30 day's treatment, WL 30 day's treatment and BL 30 day's treatment was detected, indicating that BL 30 day's

Table 1. Fresh weight, dry weight, and ratio of dry weight of *D. candidum* under three different light qualities.

Light treatment	Fresh weight (g)	Dry weight (g)	Ratio of dry weight (%)
BL 30 days	7.87±0.020	1.08±0.010	13.72±0.090
WL 10 days	12.03±0.017	1.58±0.010	13.13±0.071
Initial (0 day)	10.56±0.026	1.38±0.010	13.10±0.121
WL 30 days	12.49±0.010	1.63±0.026	13.05±0.202
RL 30 days	10.30±0.036	1.30±0.017	12.62±0.142
BL 10 days	12.94±0.053	1.55±0.053	11.98±0.355
BL 20 days	10.51±0.026	1.23±0.046	11.70±0.411
RL 10 days	10.62±0.026	1.20±0.026	11.30±0.229
WL 20 days	10.92±0.026	1.23±0.026	11.26±0.217
RL 20 days	10.92±0.017	1.23±0.010	11.26±0.076

Values represent means ± SD.

treatment made higher DR visually than WL 30 day's treatment and RL 30 day's treatment to conclude that BL was favorable for raising production of *D. candidum* in some sense. However, taking further analysis of fresh weight (Figure 1A) and dry weight (Figure 1B), different light irradiations significantly affected the growth of *D. candidum*. Gradual significantly decrease in fresh weight and dry weight was observed in the plants cultivated under BL light treatment. Though DR was optimum in plants that were grown under BL as compared to those with other light treatments, it is because of fresh weight decreased faster than dry weight. RL slighter inhibited the growth of *D. candidum* than BL. So homogeneous light, such as BL and RL, could not satisfy growth demand of *D. candidum*.

AC and PC of *D. candidum* under three different light qualities

Figure 2 showed the difference of the total AC of *D. candidum* seedling, and it was on the rise during 30 days under three light qualities. At just the beginning (0 day), AC was the lowest of 0.0218%. The highest yielded AC was achieved after 30 days treatments; AC was 0.0314, 0.0265 and 0.0235% under RL, BL and WL, respectively, which was much higher than that of *D. candidum* in wild (0.02%). AC of *D. candidum* increased more sharply under RL compared with under BL while there was no apparent increase under WL, indicating that the most effective light on the total of alkaloid content was RL, followed by BL and WL. Our result was in accordance with "the RL is more suitable for alkaloid synthesis than BL" (Lu et al., 2006). PC under three light qualities was shown in Figure 3. There was significant difference among the treatments and the amount of PC ranged from 14.30±1.099 to 20.906±3.289%. PC under WL 0 day was lowest at 14.30±1.099%, compared with 14.909±1.855% under WL 30 days, which showed increase but no significant change in the total. PC changed significantly greater under RL and BL treatments than WL treatment.

The first phase (during 0 day and 10 days), a large increase of the PC was observed, with 19.076±0.352 and 16.739±1.524% under RL 10 days and BL 10 days, respectively. During the second and third phase, there was no significant increase of PC under RL and BL. PC reached its highest value of 20.906±3.289% under RL 30 days, similar to PC of *D. candidum* in wild (22%) (Xu et al., 2005). PC extracted from the WL- grown plants yielded similar values in the total compared to that of the initial treatment. In contrast, all the other light treatments induced an increase in PC, especially RL and significant difference between RL treatment and BL treatment ($p < 0.05$) was detected, which indicated that RL was optimum used to increase production of polysaccharides. This result also confirms that the optimum light quality for the accumulation of photosynthate was RL (Zheng et al., 2008). It was also reported that both field light quality and greenhouse light quality experiment can drive the conclusion that salidroside content was significantly higher under RL (Yan et al., 2003; Yan et al., 2004). In addition, Kowallik (1982) reported higher carbohydrate content in crops cultured in RL.

Conclusion

Light quality is a major mode of plant growth, morphological structure, photosynthesis and metabolism. And most biosynthesis can be modulated by light quality (Shen et al., 1999). Our results clearly demonstrated that RL and BL significantly reduced the growth of *D. candidum*, but improved accumulation of alkaloids and polysaccharides. Based on our study, it appears that a mixture of red and nature light will be necessary for normal plant development in *D. candidum* and if the most appropriate radiation of red is used, improvement of its production and quality is possible. The similar AC and PC of *D. candidum in vitro* and wild were obtained. Therefore, facing with gradually reducing of wild

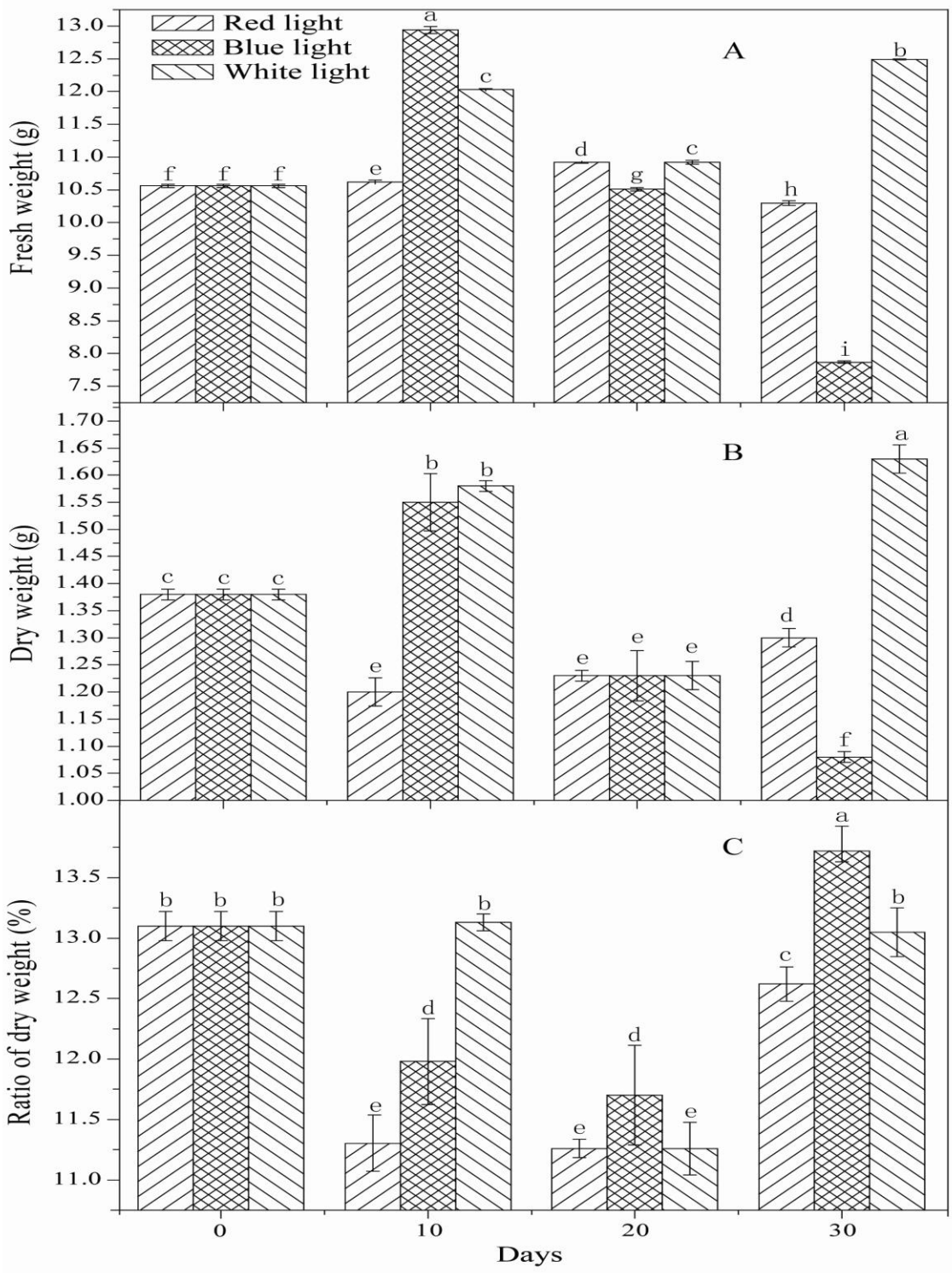

Figure 1. Effects of three light qualities on Fresh weight, Dry weight, and Ratio of dry weight. The dry weight was measured after the PLBs had been dried to constant weight in an oven at 60℃. Raito of dry weight=Dry weight/Fresh weight × 100%. Note: Error bars indicate 1 SD; different letters be notability difference (P <*0.05*).

D. candidum resources, current results will contribute substantially to appealing prospect of using plantlets of *D.candidum* in place of wild resources, to future efforts in the field of offsetting medicinal herbs scarcity,

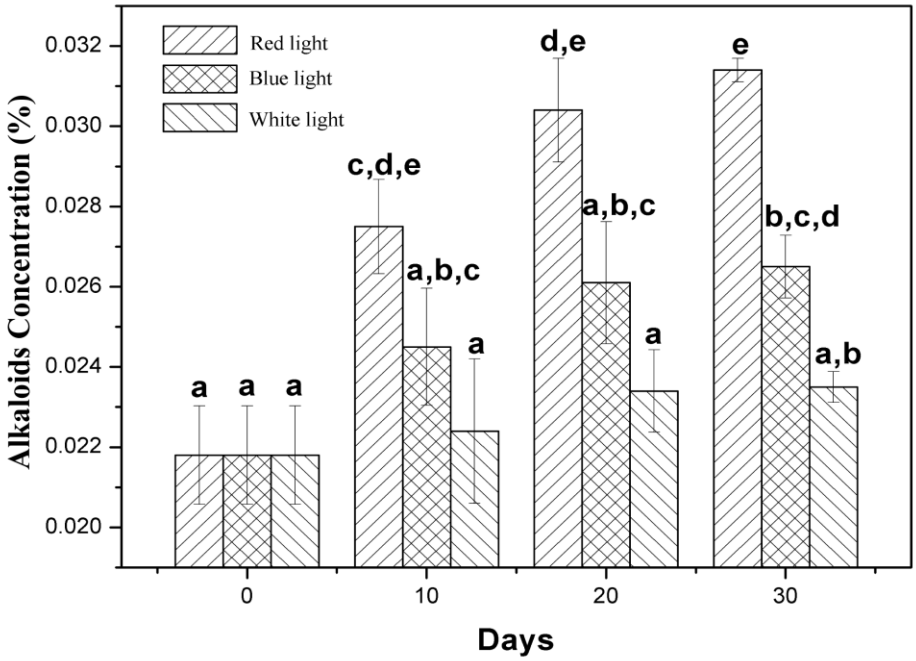

Figure 2. Analysis of accumulated AC of *D. candidum* treated with RL, BL and WL. When *in vitro* seedlings were approximately 4cm in height, subsequently, plants with the same provenance and clonal composition were transferred to different light qualities of RL, BL and WL, for each light treatment experimental period of 10, 20 and 30days. The experiment was carried out in a thermostated room (27°C) and an extra refrigerated recirculator was used to maintain the temperature of the experiment at 27±2°C. Note: Error bars indicate 1 SD; different letters be notability difference (P <*0.05*).

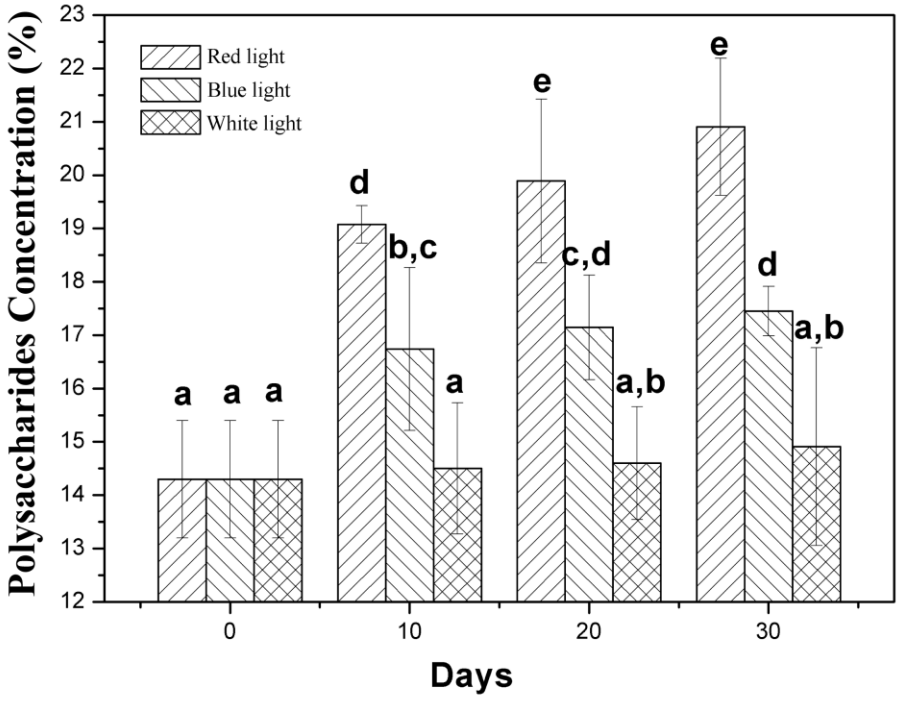

Figure 3. Analysis of accumulated PC of *D. candidum* treated with RL, BL and WL. Note: Error bars indicate 1 SD; different letters be notability difference (P <*0.05*).

environment protection and commercial benefits.

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