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Micropropagation of an orchid Dendrobium strongylanthum Rchb.f.

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Summary: A simple and reliable procedure for *in vitro* propagation of an orchid *Dendrobium strongylanthum* Rchb.f. was studied. Protochorm was induced from seed explants on 1/2 MS medium supplemented with 0.2 mg/L NAA. A mass of protochorm could be multiplied on proliferated medium of 1/2 Ms containing 0.5 mg/L V-6-BA. And bud differentiation of green global body was cultured in the same media, 2–2.5 cm shoots were formed after 30 day of culture. Addition of mashed banana and 0.5 mg/L NAA to 1/2 MS medium promoted root formation and vigorous growth. The plantlets were acclimatized and transplanted to compound materials of humus:sawdust (1:1) in greenhouse, the survival rate was more than 98%.

Key words: *Dendrobium strongylanthum*, propagation, protochorm

Abbreviations: MS (*Murashige & Skoog*, 1962), B_5 (*Gamborg* et al., 1968), N_6 (*Chu* et al., 1975), 1/2 MS (Half strength MS medium), 1/4 MS (Quarter strength MS medium), Tween 80 (Polyoxyethylene sorbitan monooleate), NAA (1-Naphthaleneacetic acid [86-87-3]), 6-BA (N^6-Benzyladenine [1214-39-7]), KOH (Potassium hydroxide), CM (Coconut milk), KH_2PO_4 (Potassium dihydrogen phosphate)

Introduction

The *Dendrobium* is the second genus of *Orchidaceae*, which is composed of approximately 1500 species scattered in the world, and quarter of these is used as ornamentals due to beautiful flowers (*Chen & Ji*, 1998). In these species, there are more than 75 species in China, and about 40 species have been widely utilized in folk medicine (*Li & Xu*, 1991). *Dendrobium strongylanthum* Rchb.f. is a kind of *Dendrobium* genus plant, which is mainly distributed in Yunnan province of China, Northeast of India, Burma (*Bao* et al., 2001). It is one of the so-called jewel orchids, which are planted originally for its beautiful flower as well as could be used as medicine against digestive, respiratory and ophthalmic diseases in many countries especially in China (*Chen & Guo*, 2003). Its capsule is small, and seed is powdery. The seedlings are conventionally propagated by seeds, however the germination rate is less than 5%. Nowadays this orchid is facing the threat of extinction owing to over collection from natural resources (*Zhang* et al., 2000).

A tissue culture procedure for clonal propagation of *Dendrobium sp.* was developed by Morel in 1960. But this valuable information fails to give a detailed introduction on micropropagation of *Dendrobium sp.* Moreover, for mass propagation of *Orchideaceae*, protochorm propagation from seed and/or nodal explants of adult plants was an effective method, and it was superior to shoot tip on account of an exponential propagation rate (*Wang* et al., 1995). To promote industrialization development of *D. strongylanthum* and lessen conflict of supply and demand for it in medicine, we will introduce a simple and fast *in vitro* regeneration system for the propagation of this species.

Materials and methods

Plants of *D. strongylanthum* had been collected in Yunnan province China and were used as a source of explants. The capsules were disinfected with 70% ethanol for 30 sec followed by surface sterilization with 3% sodium hypochlorite (supplemented 2–3 drops of Tween 80 each 500 ml) for 20 min and then washed for 4–5 times in sterile distilled water. The capsules were cut apart on sterilized trays, and seeds diluted with sterile water were homogeneously cultured on the surface of medium.

Five different basal media of MS (Murashige-Skoog medium, *Murashige & Skoog*, 1962), 1/2 MS (half strength of MS), 1/4 MS (quarter strength of MS), B_5 (Gamborg's medium, *Gamborg* et al., 1968) and N_6 (Chu's medium, Chu *et al.*, 1975) were used in whole experiment. For different abduction and multiplication growth, different media were supplemented with different concentration and combination of NAA (0.2 and 0.5 mg/L), 6-BA (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 mg/L), natural organic compounds (mashed banana, potato extract and

CM) relying on the aims of the experiment. The pH of all media was adjusted to 5.8 with 0.1 M KOH before sterilization. All media with 0.7% agar and 2% sucrose added in the present experiment were autoclaved at 121 °C for 20 min.

Plant explants were cultured in 220 ml glass jars containing 30 ml medium, which were closed with semipermeable plastic caps. All the cultures were kept in the culture room at 26 °C and 10/14h photoperiod under 1000-2000 lux light intensity.

Regenerating shoots of 2–2.5 cm in height were separated and cultured onto solid rooting medium for vigorous growth and rooting. After 30 day of culture, these opened bottles holding well-rooted shoots were transferred to a greenhouse of 25 °C and shade with low natural light, the relative humidity was 95%, and these plantlets would be acclimated outside conditions for 15 day. So the rooted plantlets (8–10 cm in height) were washed with water to remove residual media, and dipped into 50% 800×carbendazim for 15 min, then directly transplanted to three different materials of vermiculite, sawdust and humus:sawdust (1:1) under high relative humidity. Survival rate was recorded after 30 day of transplantation.

Experiments were designed thoroughly randomized and repeated three times. Every treatment had three replications. Observations on the number of roots and plantlets height were recorded after 30 day of culture, data were the mean of totals, and histograms were completed by Excel.

Table 1 Results of inducing protochorm from seeds on different media

Basal media	Phytohormone concentration (mg/L)		Length of occurring protocorm (d)	Characters of induced protochorm
	6-BA	NAA		
MS	0.4	0.2	29	White, small and much
	–	0.2	28	Yellow-white, small and little
1/2 MS	0.4	0.2	27	Yellow-white, small and much
	–	0.2	25	Kelly, mulberry shape, big
B5	0.4	0.2	35–38	White, small and much
	–	0.2	35	Yellow-white, small and little
N6	0.4	0.2	31	White, small and much
	–	0.2	30	Yellow-white, small and little

Figure 1 Induced protochorm after 30 d of culture

Results and discussion

Protochorm could be induced from mature embryo culturing on 10 sorts of media (*Table 1*), length of occurring protochorm was different, the shortest was 25 day and longest was 38 day. Of the four basal media (MS, 1/2 MS, B_5 and N_6) tested, 1/2 MS was found more conformable to seeds inducement culture of *D. strongylanthum* than other three basal media, frequently used for growth of another orchid (*Zhang* et al.,1992). The supplement of phytohormone 6-BA in media was not befitting for shoots differentiation but for protochorm proliferation, because plantlets cultured on medium adding 6-BA were easily found to be vitrification during subsequent serials of rooting experiments. Thus, according to occurring time and growth responses of protochorm, the basal medium of 1/2 MS supplemented with an optimal concentration of NAA 0.2 mg/L was fitting to seeds inducement culture of *D. strongylanthum* (*Figure 1*). The color of induced protochorm on 1/2 MS containing 0.2 mg/L NAA medium was green, and differentiation capacity was much better than that of protochorm on other media. To shorten culturing procedure, the induced protochorm could be transferred onto the above selected medium, and 2–2.5 cm shoots could be formed after 30 day of culture.

At stage of proliferated culture, the protochorm induced from seeds was transferred onto 8 kinds of proliferated media (1/2 Ms containing different concentrations of 6-BA), a great deal of protochorm were largely multiplied, and 6-BA was essential for multiple protochorm formation. After 10 day of culture, the protochorm started to be in differentiation, and those gradually became green with extension of culturing time. During of 0.1–0.5 mg/L 6-BA, the multiplication rate of

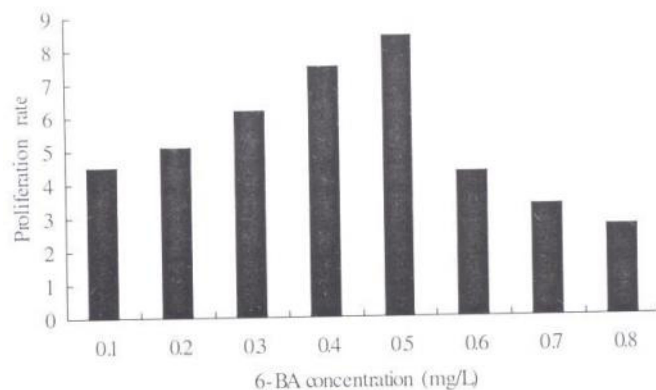

Figure 2 Effect of different concentration of 6-BA on protochorm multiplication

Table 2 Rooting and vigorous growth of *D. strongylanthum* seedlings after 30 d of culture

Basal media	Natural organic compounds			NAA (mg/L)	Length of roots (cm)	Plant height (cm)	Number of roots (strip)
	Mashed banana (g/L)	Potato extract (g/L)	CM (ml/L)				
1/2 MS	100	–	–	–	5.27	7.14	10
	100	–	–	0.5	7.24	8.67	14
	–	200	–	0.5	5.35	6.97	9
	–	–	200	0.5	7.22	6.78	12
1/4 MS	100	–	–	–	5.23	6.69	6
	100	–	–	0.5	6.28	7.34	12
	–	200	–	0.5	5.43	6.56	8
	–	–	200	0.5	6.25	6.11	11

protochorm was increasing with rising 6-BA concentration, but higher concentration of 0.5–0.8 mg/L 6-BA reduced the proliferation rate. Among different concentrations of 6-BA tested, maximum proliferation rate of protochorm was recorded at 0.5 mg/L 6-BA (*Figure 2*). Similar result in another *Dendrobium* was also found by *Hou* et al. (2006).

Different basal medium and natural organic compounds were used with the aim to enhance *in vitro* vigorous shoot and root development. According to *Table 2*, the auxin NAA and natural organic compounds could promote shoots rooting and vigorous growth of *D. strongylanthum*. The best result was obtained when 1/2 MS, mashed banana and NAA 0.5 mg/L were used as the rooting and vigor medium. The effect of mashed banana on plantlets growth in *D. strongylanthum* was similar with other plants such as in *D. candium*, *D. loddigesi*, *D. waggi* and *D. moniliforme* (*Zeng* et al. 1998; *Shiau* et al. 2005) where plantlets growth rate increased with addition of banana in the medium. These observations also were supported by others (*Huang* et al. 2001; *Jiang* et al.2003). And the additive of CM to culture medium resulted in more vigorous growth and roots than on medium containing potato extract, the effect of CM and mashed banana on plantlet growth was almost the same. The same action of CM enhancing vigorous growth of *Dendrobium* was found in another orchid (*De* et al., 2006). But it was necessary that banana was used to mass propagation of *D. strongylanthum*, because the banana is cheaper than coconut and commercially feasible.

The transplantation was an important step of plant tissue culture. Acclimatized shoots with well-developed roots (*Figure 3*) were transferred to different materials and kept in a greenhouse with 25 °C and 90% relative humidity. After two weeks the liquid fertilizer of 2‰ KH_2PO_4 sprayed on leaves of plantlets was a kind of foliar nutriment for *D. strongylanthum*. The survival percentage was recorded after 30 day of transplantation, and it was different due to plantlets transplanted in different materials. From *Figure 4*, it could be showed that the survival rate of plantlets of *D. strongylanthum* transplanting to medium of humus:sawdust (1:1) was high and reached more than 98% in the greenhouse.

Figure 3 Well-rooted plantlets of *D. strongylanthum*

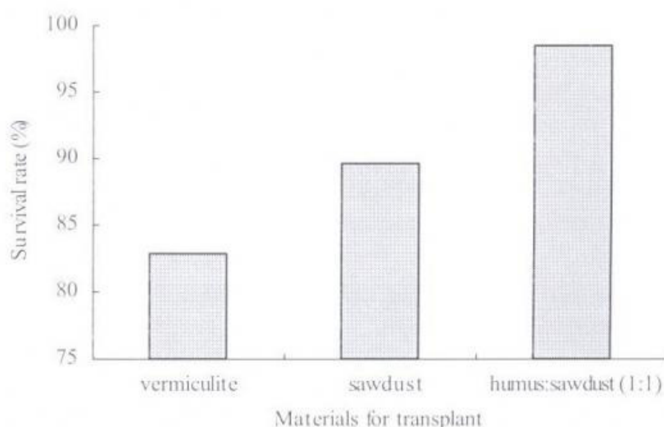

Figure 4 Influence of materials for transplant on survival rate of *D. strongylanthum*

Through a series of experiments, the system of rapid and efficient protochorm proliferation rate, rooting and successful transplant of plantlets to the greenhouse was established, and it made this procedure fitting for large scale multiplication as well as *in vivo* conservation of this important herbal orchid.

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