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Optimizing factors affecting development and propagation of *Bletilla striata* in a temporary immersion bioreactor system

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ABSTRACT

Culturing factors regarding to B. striata plantlets growth and pseudobulb development through temporary immersion bioreactor system (TIBS) were studied. Immersion frequency, sucrose concentration and inoculant amount were analyzed for their influence on seedling development. An immersion frequency of 3 min per 2 h was found to substantially promote the development of pseudobulbs. Seedling growth and development were also affected by the immersion frequency. The best seedling development was observed among those cultures that were immersed at a rate of 3 min per 6 h. Similarly, sucrose concentration was identified to affect the development of seedlings and pseudobulb formation obviously, with the best development recorded at 40 g/L weight ratio. A prominent seedling development, in terms of stem diameter, plant height and leaf width, were observed when 300 explants was loaded in the TIBS tank. The development of pseudobulbs was also found to be obviously enhanced at this explants loadings. The current findings verified the optimal immersion frequency, sucrose concentration and explant loads for improved seedling growth and pseudobulb development strategy for *B. striata*.

1. Introduction

Bletilla striata (Thunb.) Teichb. F., a perennial herb that belongs to Orchidaceae family, has been commonly used as traditional Chinese medicine for over 1500 years (Diao et al., 2008). It is one of the most economically important orchids, while the current harvest is lack of surging market demands. Seed germination of *B. striata* is difficult under natural conditions and hence, the plant is usually cultivated through division propagation techniques (Guan et al., 2010; Li et al., 2015). Generally speaking, *B. striata* division propagation has a low yields and the seedlings has poor resistance to filed challenges (Yang et al., 2002). Since the destruction of natural habitat and over-harvesting, *B. striata* is currently threatened with extinction (Zhang et al., 2016). Plant tissue culture techniques are now becoming a promising strategy for the rapid propagation of various endangered orchid species (Islam et al., 2015).

With the development of plant tissue culture technology, propagation of *B. striata* with traditional solid culture has been widely employed (Gao et al., 2017; Wang et al., 2016; Zou et al., 2013). Temporary immersion bioreactor system (TIBS), displays significant advantages compared to the traditional solid culture techniques (Mallon et al., 2012; Othmani et al., 2009; Park et al., 2015). The system combines the advantages of both solid and liquid culture in culturing plant tissues. Particularly, gentle air exchange in TIBS allows an adequate assimilation of nutrients in liquid medium (Albarran et al., 2005; McAlister et al., 2005). TIBS were specially designed for plant tissue culture (Niemenak et al., 2008) and was successfully employed to different species, such as *Xanthosoma sagittifolium* L. Schott (Niemenak et al., 2013), *Ananas comosus var.* Comosus (Scherer et al., 2013) and *Tectona grandis* L. (teak) (Quiala et al., 2012). Furthermore, plants via TIBS were found to be more adaptive to acclimatization and the following photoautotrophic phase.

The capability of pseudobulbs to store water, mineral and carbohydrates involves a serious of physiological processes that are important for growth and survival in nutrient limited epiphytic biotope (Shu et al., 2012). A rapid development of *B. striata* pseudobulb is of great significance for large-scale cultivation in industry (Zhao et al., 2017). The aim of the present study is to establish an economically

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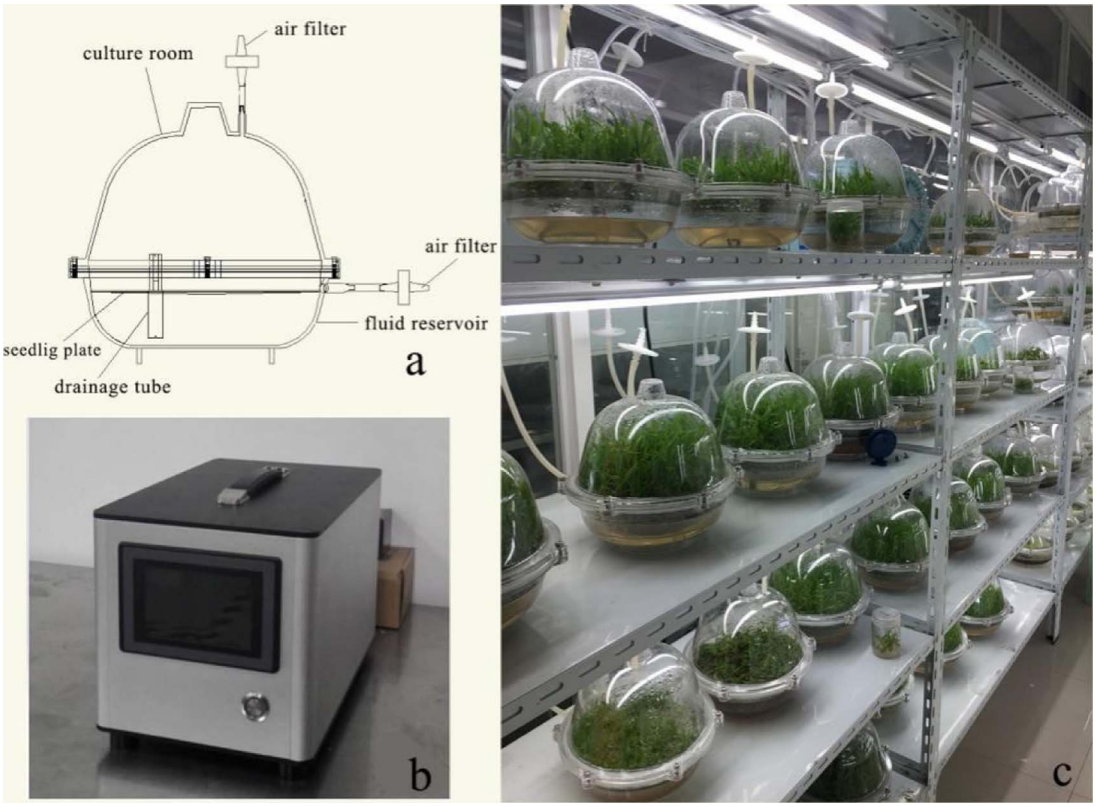

Fig. 1. (a) culture tank. (b) bioreactor controller. (c) a typical culturing array.

competitive and efficient protocol for the improvement of *B. striata* micro-propagation and pseudobulbs through TIBS.

2. Materials and methods

2.1. Bletilla striata

B. striata was collected from Hunan Province, China. It was phylogenetically identified as per our previous study (Song et al. 2017 unpublished data).

2.2. Bioreactor culture system

The temporary immersion bioreactor system (TIBS) (BFJX-IV, 6.6 L volume) was a courtesy by Biofunction Co. Ltd. (Nanjing, China). The system includes culture tank, bioreactor controller, air filter equipped with filter papers (0.22 μm) and so on (Fig. 1).

2.3. Preparation methods

The *B. striata* plants were grown in greenhouse and seed capsules were acquired by pollination at Nanjing Tech University, China. Matured but intact capsules were thoroughly flushed with running water for 10 min and then were surface sterilized by immersion in 75% (v/v) for 30 s. After rinsing twice by distilled sterile water, the ethanol sterilized capsules were immersed in 0.1% mercuric chloride ($HgCl_2$) for 10 min and 4 times washing in sterile distilled water for 2 min in a laminar flow cabinet. These materials were firstly dried on sterile filter papers and were seeded on 1/2 MS medium (pH5.8) supplemented with 1.0 mg/L NAA and 6.5 g/L agar (Shanghai Yuhan Bio-tech Co. Ltd) (Zhang et al., 2009). Seeds grow in a dark chamber for 7 days, and were transferred into a photoperiod of 12 h light/12 h dark at 24 ± 1 °C in tissue culture bottles under an illuminance of 2000 Lux (ZPZ-32, Shanghai Yuhan Bio-tech Co. Ltd). In this study, only approximate 6 mm seedlings were chosen for individual experiments. The experiments were replicated three times.

2.4. Immersion frequency

Four immersion frequencies, 3/2, 3/4, 3/6 and 3/8, were compared for seedling development in TIBS. Specifically, "3/2", for example, means the explants were immersed in liquid culture for 3 min per 2 h interval. Bioreactor tanks were inoculated with 300 explants and kept with a photoperiod of 12 h light/12 h dark at 24 ± 1 °C.

2.5. Sucrose concentration

B. striata explants were placed and grown for 2 months. 1 L 1/2MS medium, which was supplemented with 0.5 mg/L NAA, 60 g/L potato extracts and 4 individual sucrose concentrations (20, 30, 40 and 50 g/L), was filled in the bioreactor tank. A total of 300 explants were inoculated into each bioreactor tank and were immersed in liquid medium for 3 min per 4 h interval.

2.6. Inoculant amount

4 Different inoculum densities (100, 300, 500 and 700) of explants were performed for seedling pseudobulb induction in TIBS. Explants with uniform quality were placed in the bioreactor tank with the immersion frequency of 3 min per 4 h interval. All cultures were kept with a photoperiod of 12 h light/12 h dark at 24 ± 1 °C.

2.7. Comparison of TIBS and solid tissue culture

120 explants, which were 6 weeks of post-germination, were cultured into 20 bottles of solid medium. Each bottle was inoculated with 6 explants and kept for 2 months in a photoperiod of 12 h light/12 h dark at 24 ± 1 °C. Solid medium was formulated with 1/2 MS medium,