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Efficient Propagation by Bioreactor System of Korean Native Seosanjong in Ginger

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ABSTRACT

For the purpose of establishing an efficient propagation through airlift bioreactor system of *Zingiber officinale* Rosc. Korean native Seosanjong, the effect of different factors and bioreactor on cultured plantlets were investigated. The highest number of plantlets, fresh weight per plant was obtained from explants when cultured in MS liquid medium including 0.3 mg/L NAA and 2.0 mg/L kinetin for 40 days. A 10 L bottle type bubble bioreactor, compared with 250 mL Erlenmeyer flask, was more efficient, producing 4.7 plantlets or from 1.5 to 1.6 times more than did the Erlenmeyer flask. The results demonstrate the rapid mass propagation of airlift bioreactor to produce normal ginger.

Key Words : bioreactor, suspension culture, *Zingiber officinale* Rosc.

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) is one of the most important perenial herb crop, has been cultivated in the world for its medicinal using as well as a food and a spice. It is propagated vegetatively by rhizomes including 3 to 4 new emerging buds. Since the mass propagation is difficult and entirely no culture system is established. Thus, the application of bioreactor system for culture system of ginger is to be considered. The airlift bioreactor system for ginger is important in the mass propagation, yet only a few reports (Akita and Takayama, 1993, 1994) have dealt with its bioreactor system. Although it has been many reports on the micropagation through various explants of ginger (Wang, 1989; Malamag et al., 1991; Kackar et al., 1993), it seems necessary to make researches by bioreactor system of the mass propagation in ginger. Therefore, we investigated that the efficient propagation of ginger by bioreactor system when compared with traditional suspension culture of Korean native Seosanjong in ginger.

MATERIALS AND METHODS

Plant materials

Plant materials used in this experiment were explants of plantlets (ca. 220 mg), obtained from *in vitro* regenerated plantlet through shoot tip culture of Korean native Seosanjong of ginger.

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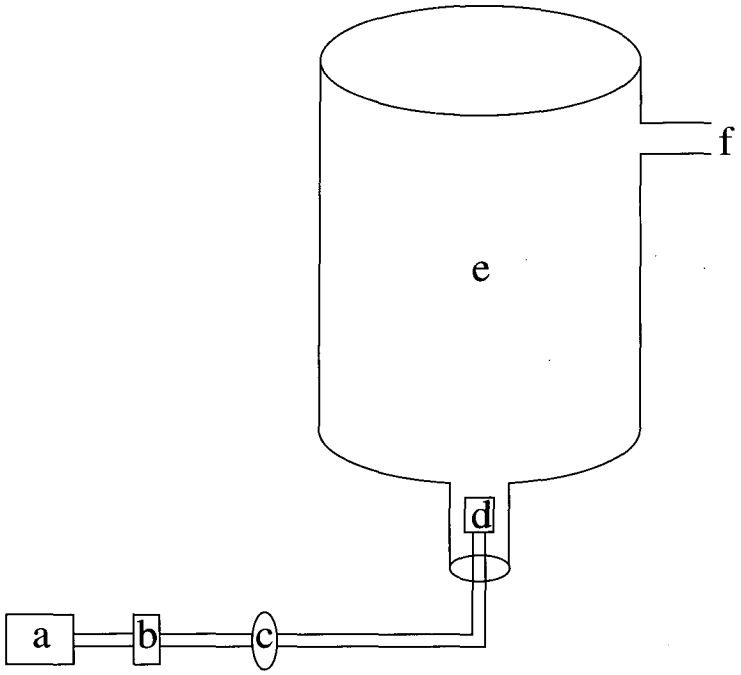

Fig. 1. Configuration of 10 L sized bottle type bubble bioreactor (BTBB) system used for mass propagation of Korean native Seosanjong in ginger. a, air pump (220V, 3W); b, flowmeter (Dwyer instruments, INC., USA); c, membrane filter (0.2 ㎛ PTFE); d, sparger of porous glass; e, body of bottle type bubble bioreactor; f, vent.

Culture medium

Explants were cultured in MS (Murashige and Skoog, 1962) liquid medium containing 30 g/L sucrose, 0.3 ㎎/L NAA and three kinetin levels (0.2, 2.0, and 5 ㎎/L) were prepared, pH adjusted to 5.8. The medium was autoclaved for 15 min at 1.2㎏/㎠, and then 40 mL, and 3 L was dispensed into 250 mL Erlenmeyer flasks, and 10 L bottle type bubble bioreactor (BTBB), respectively.

Culture conditions

Suspension clture was taken by inoculating three g fresh weight into 250 mL Erlenmeyer flask containing 40 mL of previously described liquid medium and agitated at 100 rpm with gyratory shaker. A 10 L BTBB (airlift type) was used (Figure 1). Airlift bioreactor was cultured by inoculating thirty g fresh weight into 10 L BTBB including 3 L of earlier described liquid medium. Each BTBB was agitated 1,500 cc/min for 3 L medium with sterilized air during culture.

The culture condition was maintained at 25±2℃ air temperature, a 60μmol · m^{-2} · s^{-1} in Erlenmeyer flask suspension culture for 60 days, and in BTBB culture for 40 days, photosynthetic photon flux(PPF) with a 16 h photoperiod using white fluorescent lamps.

RESULTS AND DISCUSSION

The result of plantlets through airlift bioreactor system and suspension culture is presented in Table 1. The analysis of variances of NAA and kinetin concentrations on plantlet development revealed that did not have significant treatments effects for any of these variables. When the explants of plantlets were suspension cultured on MS medium including NAA and

kinetin combinations, they produced about 3 new plantlets. The fresh weight per plant ranged from 320.5 mg to 381.1 mg. The number of plantlets, and fresh weight per plant using BTBB culture was higher than that of suspension culture using an Erlenmeyer flasks. Rapid multiplication of ginger plantlets in vitro was conducted by inoculating plantlets into BTBB including liquid MS medium containing 0.3 mg/L NAA and 2.0 mg/L kinetin. The best number of plantlets, and the highest weight of fresh per plant in complete regenerated plantlets from BTBB were cultured for 40 days. The weight of fresh per plant ranged from 500.0 mg to 730.0 mg.

The plant growth regulators did not affect the number of plantlets and mean fresh weight, but there were significant differences in culture vessel types. The BTBB culture was dramatically rapid and greater than the GS suspension culture on the number of plantlets, and fresh weight per plant. The reason for the rapid growth performance seemed partly due to high gas exchange capacity of the culture vessel (Hulscher et al, 1996). Therefore, our method appears to provide stable multiplicaton system for ginger, and regenerated plants can be obtained with normal morphology. As a whole, there seemed no problems in the mass production of ginger entire plants, however, more intensive researches on an inlet/outlet ports in this model are still needed. It has previously succeeded to mass propagation of potato (Akita and Takayama, 1994; Son et al., 1999). We think that the agricultural application of the ginger multiplication method should be attempted using bioreactor system.

The advantages of our multiplication method for ginger are as follows. 1) BTBB culture is very simple, and economical and stable system. The method is also rapid and then regenerated ginger can be obtained in 40 days of culture. 2) Detectable variations among regenerated plants are minimal. 3) The method is applicable to many crops. Further the investigation is seems to the condition that is rapid mass production for the stable improvement by bioreactor system in culture.

Table 1. Effect of bioreactor system on rapid mass production of Korean native Seosanjong in *Zingiber officinale* Rosc.

Cultured periods (days)	Type of culture[z]	Plant growth regulators (mg/L)		Number of plantlets	Fresh weight (mg/plant)
		NAA	Kinetin		
40	BTBB	0.3	0.2	3.7a[y]	500.0a
		0.3	2.0	4.7a	730.0a
		0.3	5.0	4.2a	650.0a
60	GS	0.3	0.2	2.8b	320.5b
		0.3	2.0	2.9b	381.1b
		0.3	5.0	3.1b	350.4b
Significance					
Plant growth regulators (A)				ns	ns
Type of culture vessels (B)				**	***
Interaction (A × B)				ns	ns

[z]Explantlets were produced in above described liquid medium of 3 L in 10 L sized bottle type bubble bioreactor (BTBB), 40 mL in 250 mL sized Erlenmeyer flask by gyratory shaker(GS).

[y]Values are means of five replications and mean separation within columns is by Duncan' s multiple range test, 5 % level.

ns, **, ***Nonsignificant or significant at $P \leq 0.01$, or 0.001, respectively, by analysis of variance.

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