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Effects of Culture Type and Inoculation Quantity in Bioreactor on Production of Potato Plantlets

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Abstract. Potato (*Solanum tuberosum* 'Dejima') plantlets were investigated on culture type and initial quantity of inoculation in bioreactor and survival rate by hydroponics for mass production. Node stems (1 to 1.5 cm in length) of potato plantlets multiplied *in vitro* were grown for 3 weeks in liquid Murashige and Skoog (MS) medium with sucrose 30 g L^{-1}. When plantlets (80-node inoculation) were raised in 10 L balloon type bubble (BB) bioreactor, the healthiest growth of plantlets was obtained from explants cultured in ebb & flow culture with medium supplied periodically 12 times per day. The suitable inoculation quantity of 20 L BB bioreactor was 120 pieces of stem segments (mean 2.2 g fresh weight) in ebb & flow culture. Number of nodal shoot was eight on the average. In controlled culture room, survival rate of plantlets at 7 days after stem cutting was above 70% when they were acclimatized by hydroponics grown in deep flow and solid medium culture. The highest survival rate of the stem cutting plantlets was in nutrient solution adjusted to EC 1.4 $dS \cdot m^{-1}$. Stem cutting plantlets through one culture could be obtained 670~900, when plantlets were grown in ebb & flow culture during 3 weeks using a 20 L bioreactor with initial 120 pieces of nodal segments. It is possible to do mass production of seedlings cultured in bioreactor and hydroponics.

Key words : ebb & flow culture, hydroponics, mass production, stem cutting, *Solanum tuberosum* 'Dejima'

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Introduction

Recently, the rapid development of plant biotechnology has made it possible to produce germ free plant propagules on a large scale in a short culture period. The use of the liquid media is ideal technique in micro propagation for reducing plantlet production costs and automation (Aitken-Christie, 1991). Indeed, liquid culture conditions, the media can easily be renewed without changing the container, sterilization is possible by micro filtration and container cleaning after a culture period is much easier. The advantages of *in vitro* culture in a liquid medium are often counterbalanced by technical problems such as asphyxia, hyperhydricity, shear forces and the need for complex equipment. Several methods have been proposed to avoid these problems, one being the twin flasks system or temporary immersion of the explants (Escalona et al., 1999). Bioreactors using a liquid media have been used for large- scale cultured plant tissue such as lily, ginseng and potato (Jo et al., 2001; Son et al., 1999).

The potato is a very important crop in the world agriculture. But the rate of seed potato deterioration is high due to the physiological and pathological disadvantage, various methods for the mass production of seed potatoes have been researched and developed by Akita and Takayama (1993) and Joung (1989) et al.

Potato shoots can be easily propagated and multiplied from single nodal segments and such a multiple shoot have numerous buds on which tubers can be induced (Akita and Takayama, 1993). The stem cuttings method is used for the rapid multiplication of seed potatoes 30% in North America and 25% in Europe of the total seed potatoes (Jones, 1991).

This experiment was conducted to reasonability for mass production of potato plantlets using a bioreactor.

The suitable culture type and inoculation quantity of potato plantlets in bioreactor culture were determined. And survival rate of plantlets after cutting stems were investigated to select the culture type by hydroponics for stable production.

Material and Methods

Virus-free *in vitro* potato (*Solanum tuberosum* 'Dejima') seedlings were provided from the Chungchongnam-do Agricultural Research & Extension Services. After multiplication the shoots in MS medium containing 0.89 mol BA, each shoot was elongated in MS medium without plant growth regulator until the height and diameter of the shoot reached to 7~8 cm and 0.3~0.4 cm, respectively in hexahedron glass vessel (8.5×8.5×14 cm). Explants were cultured in MS liquid medium without PGR added 30 $g \cdot L^{-1}$ sucrose, pH adjusted to 5.8. Before inoculating mentioned above shoots cultures into BB bioreactor, apical parts, some expanded large leaves, and root system were removed. Stem explants having small leaves were divided into 1cm length and transferred into the BB bioreactor aseptically.

To select the most efficient method of liquid culture, plantlets were grown by three different types, ebb & flow, immersion and mixed culture combined ebb& flow and immersion culture. Plantlets inoculated with 80 pieces of stem segments were placed on the plastic net sieve (pore size in 0.1×0.1 mm) installed inside the 10L BB bioreactor. To determine the optimum frequency of medium supply in ebb & flow culture, medium was periodically supplied and drained with 12, 24 and 48 cycles per day with immersion period about 2 min by the timer and solenoid valve. In immersion culture, BB bioreactor was continuously aerated through inlet airflow 100~200 $ml \cdot min^{-1}$. Mixed culture had two types, immersion or ebb & flow for initial one week and reversed for later two weeks. Medium was supplied 24 times per day during ebb & flow culture in mixed culture.

To obtain the suitable inoculation quantity, 100, 120, and 160 pieces of stem nodal segments were inoculated into the 20 L BB bioreactor. They were cultured the ebb & flow culture.

The culture room was maintained at 24±2°C at a 16 photoperiod using white fluorescent lamp, and supplemented by installing round fluorescent lamp upside to the bioreactor. Growth characteristics of potato plantlets were measured after the 3 weeks from the inoculated day.

Potato shoots were cut with one or two nodes, transferred the controlled culture room, and then acclimated by the hydroponics. Survival rate was measured in immersion and solid medium culture filled with artificial soil (perlite: vermiculite=7:3 v/v). Immersion culture had two types, transferred in nutrient solution directly and after plantlet was grown in distilled water for 3 days. The concentration of nutrient solution was contained 17.3 N, 4 P, 8 K, 8 Ca, 4 Mg $me \cdot L^{-1}$, respectively. EC of nutrient solution was 0.6 $dS \cdot m^{-1}$ in immersion culture. Survival rate was measured by different EC levels, 0.6, 1.0 and 1.4 $dS \cdot m^{-1}$.

Results and Discussion

Ebb & flow culture with medium periodically supplied with 12 cycles per day was the most suitable for the growth (Table 1). The plantlet growth lowered in immer-

Table 1. Effects of various culture types on growth of potato plantlets cultured 10 L balloon type bioreactor during 3 weeks.

Culture type	Supply frequency of medium per day	Shoot length (cm)	No. of number/Shoot	Fresh shoot wt.(g)	Total shoot wt.(g)/Bioreactor
Ebb& flow culture	12	20.2±2.8[z]	8.4±0.5	1.93±0.4	160.3
	24	16.3±0.4	8.4±1.1	1.27±0.5	90.1
	48	20.0±3.5	7.8±1.3	3.12±0.4	264.8
Immersion culture	Continuous	15.0±1.6	7.4±1.1	1.11±0.3	60.1
Mixed culture[y]	24 → Continuous	15.8±1.3	7.8±1.1	1.25±0.3	103.9
	Continuous → 24	16.6±0.9	7.8±0.4	0.82±0.2	64.3

[z]Data are average of 5 replications
[y]Medium supplied on ebb & flow or immersion for initial one week and reversed for later two weeks.

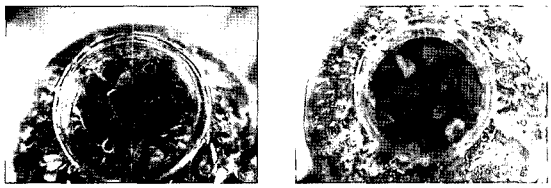

Fig. 1. States of plantlets after cultured in immersion (left) and ebb & flow (right) with medium supplied 12 cycles per day during 3 weeks using a 20 L balloon type bioreactor.

sion or mixed culture, especially initial culture started in immersion. Temporary immersion or ebb and flow bioreactor was described as a better aeration system through the periodic immersion and exposure to the gaseous phase (Etienne et al., 1997). According to Etienne and Berthouly (2002), temporary immersion generally improved plant material quality, besides the gain in production. Plantlets were observed slightly epinasty and excessive lateral bud induction in immersion and ebb & flow with medium supplied 48 times per day in spite of the highest growth of plantlets. Potato plantlets in immersion culture showed similar hyperhydricity symptoms, i.e., translucent, curled and thickened leaves and stems (Fig. 1). Necrosis of plantlets was observed only in immersion and mixed culture. Micropropagation in liquid culture media increases nutrient uptake and promotes growth, but continuous contact of plant tissues with liquid medium is the source of hyperhydricity (Ziv et al., 1983). Akita and Takayama (1988) reported growth of potato was suppressed and the plants were slightly swollen, when they grown continuously submerged conditions in the control culture.

Shoot length of potato plantlets reached to 15-19 cm and potato plantlets almost filled with working space in bioreactor when they were cultured for 3 weeks, regardless of inoculation quantity treatment (Table 2). Number of nodal shoot was eight on the average. Shoot length was enhanced by high inoculation quantity. The highest growth (mean 2.2 g fresh weight) was a piece of 120 stem segments in BB bioreactor (20 L). When chrysanthemum plantlets were cultured in bioreactor, their shoot length increased with increasing numbers of single nodes, but stem diameters and the numbers of branches were reduced (Hahn and Paek, 2005). On the other hands, when potato plantlets filled with working space in bioreactor, necrosis of plantlets slightly observed in apical parts. When their growth was rapid after two week culture, pH of liquid medium was observed to decrease bellow 4.2 in all treatments (data not shown). Jo et al. (2001) reported potato plantlets were significantly more vigorous in bioreactor than traditional suspension culture of 100 ml Erlenmeyer flasks. Kozai et al. (1995) reported that the growth and development of potato plantlets *in vitro* could be affected by directly on the culture medium and microclimate in the vessel and indirectly on the vessel environment. In this experiment, their necrosis considered vigorous growth of potato plantlets in bioreactor system induced partly due to low gas exchange capacity of the culture vessel.

Under the 30% shade in controlled culture room, survival rate of plantlets at 7 days after stem cutting was above 70% when they were acclimatized by hydroponics grown in immersion and solid medium culture (Table 3). The highest of survival rate was observed when nodal cutting was grown in distilled water during 3 days and transferred into EC 0.6 $dS \cdot m^{-1}$ nutrient solution. The highest survival rate of the stem cutting plantlets were in nutrient solution adjusted to EC 1.4 $dS \cdot m^{-1}$ (Table 4). Optimum solution EC vary with plant species, season, growth stage and the quality of water. Schwarz (1995) suggested low strength nutrient solution (1.3 $dS \cdot m^{-1}$) for rooting and seedling and high strength nutrient solution (1.8 to 2.5 $dS \cdot m^{-1}$) for vegetable stage. Fonteno (1996)

Table 2. Effect of inoculation quantity on growth of potato plantlets cultured 20 L balloon type bioreactor during 3 weeks.

Inoculum node number	Shoot length (cm)	No. of node / Shoot	Fresh shoot wt. (g)	Total fresh shoot wt.(g)/Bioreactor
100	16.0±1.0[z]	8.0±0.3	1.37±0.3	171.8
120	16.5±2.8	8.0±0.7	2.22±0.5	257.0
160	17.6±0.9	7.8±1.1	1.40±0.4	248.7

[z]data are average of 5 replications

Table 3. Effect of culture type on survival rate of potato plantlet at 7 days after stem Cutting.

Culture type	Survival rate (%)
Immersion I	72.3±1.9[z]
Immersion II[y]	92.1±4.5
Solid	80.0±3.2

[z]data are average of 5 replications
[y]Plantlet was grown in distilled water for 3 days and transferred EC 0.6 $dS \cdot m^{-1}$ in nutrient solution.

Table 4. Effect of EC of nutrient solution on survival rate of potato plantlet at 2 weeks after stem cutting.

EC ($dS \cdot m^{-1}$)	Survival rate (%)
0.6	75±3.8[z]
1.0	63.9±4.8
1.4	86.1±3.2

[z]data are average of 5 replications

also recommended low nutrient content at seedling stage so that sensitive young plants were not damaged by excessive nutrient uptake. The results from our experiment showed the single-node stems could grow to plantlets more vigorously at relative high EC level (1.4 $dS \cdot m^{-1}$) than low levels (0.6 $dS \cdot m^{-1}$). It is possible to do mass production of seedlings through successive hydroponics. Cutting shoot number seedlings through one culture could be obtained 670~900, when plantlets were grown in ebb & flow culture during 3 weeks using a 20 L bioreactor with initial 120~160 pieces of stem segments.

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