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A simple method for mass production of potato microtubers using a bioreactor system

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A simple protocol for mass propagation of potato microtubers was developed using an automated low-cost bioreactor system. Microtubers of potato were induced by a two-step culture method. In the first step (step A), the stock plants were inoculated in the bioreactor for growth and multiplication of plantlets. After four weeks, the medium was replaced with a new one to proceed to step B for microtuber induction. Comparative studies between solid and bioreactor culture (continuous immersion [with net or without net] and temporary immersion in liquid medium using ebb and flood) revealed that shoot multiplication and growth were more efficient in continuous immersion (with net) bioreactor. We also studied the effect of inoculation density on potato micropropagation during bioreactor culture and maximum responses were recorded when there were 50 nodal explants per bioreactor. After shoot proliferation, the culture medium was replaced with one containing a higher concentration of sucrose, with or without 6-benzylaminopurine (BAP) and kept under dark conditions. The analysis of tuber classification according to size showed that addition of BAP

in the culture medium influenced the formation of microtubers larger than 1.1 g. It has also been observed that there is a strong influence of medium renewal on individual microtuber growth during bioreactor culture of potato. The results indicate that our system could be applied for mass propagation of potato tubers at low cost.

AUTOMATION of organogenesis in a bioreactor has been advanced as a possible way of reducing cost of micropropagation^{1–4}. Organogenic plant propagules are intensively cultivated in bioreactors to produce transplants for mass production. Intensive cultivation of potato microtubers and bulblets of lily is another strategy for producing propagules, which can be handled for direct planting in the field, thus facilitating commercialization. The main problems associated with microtuber production in conventional containers are the low yield of tubers and small tuber size that limits direct transplanting to field conditions. Recently, the adaptation of air-lift, bubble column, ebb and flow-type bioreactor (EFBR), and temporary immersion bioreactors for propagation of shoots and bud-clusters has provided a workable means for improving tuber quality and the number of tubers per plant^{5–7}. Potato microtubers were produced in jar fermentors by semi-continuous liquid medium surface-level control⁵. Hulscher *et al.*⁷ reported that 1600–1700 potato tubers can be produced by using an EFBR system, with a 10 l culture vessel in 18 weeks. Such systems are expensive, thus increasing the cost per propagule unit. One of the approaches to solve these problems is to simplify the culture system.

The aim of the present study was to establish an automated low-cost production system for microtubers and to investigate the different bioreactor systems (temporary immersion system using ebb and flood, continuous immersion system without net or with net), inoculation density, growth regulators and number of medium exchanges on the quality of the plants, and number and size of the tubers during potato shoot multiplication and tuber induction stages.

The meristem culture of potato ‘Atlantic’ was maintained onto MS solid medium⁸ (3% sucrose + 2.4 g l⁻¹ gelrite without phytohormone) and kept at 25°C, 70% relative humidity and a 50 µmol m⁻² s⁻¹ PPF (16 h/day). After four weeks of culture, nodal explants with one leaf were used for the experiments.

Potato microtubers were induced by the two-step culture method. In step A, the stock plants were inoculated in the bioreactor for growth and multiplication of plantlets. After three weeks, the medium was replaced with a new one to proceed to step B for microtuber induction.

Solid culture: Nodal explants (6 nodes per culture vessel) were inoculated to cylindrical culture vessels (0.5 l capacity) containing 150 ml MS medium containing 3% sucrose and 0.24% gelrite. The pH of the medium was

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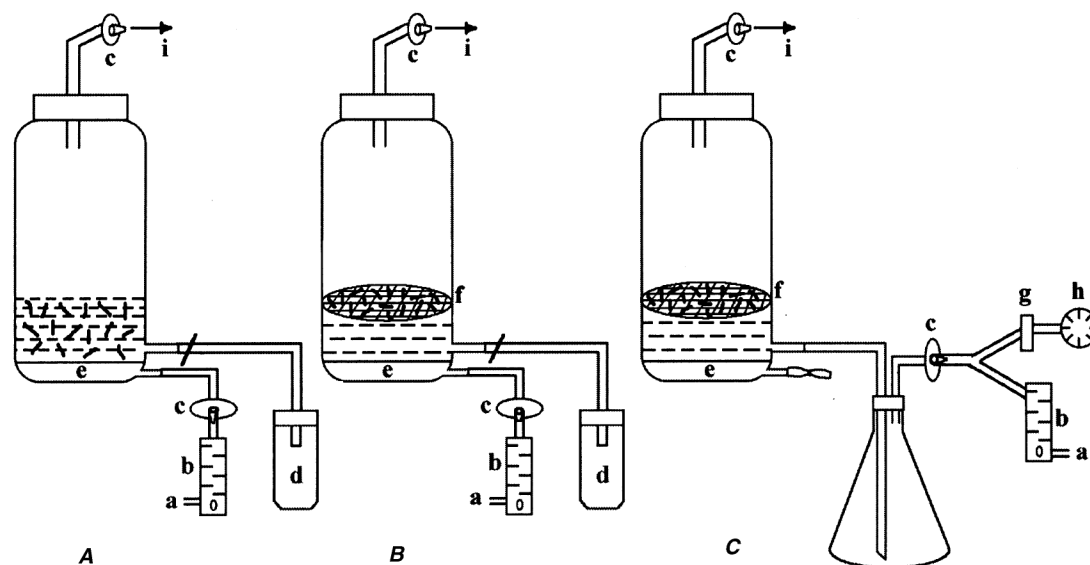


Figure 1. Layout of a continuous immersion [without net (*A*) or with net (*B*)] and temporary immersion bioreactor using ebb and flood (*C*). a, Air inlet; b, Airflow meter; c, Membrane filter; d, Sampling port; e, Sparger; f, Supporter (net); g, Solenoid valve; h, Timer; i, Air outlet.

adjusted to 5.8 before autoclaving (at 121°C and 1.2 kg cm⁻² pressure for 15 min).

Bioreactor culture: Two types of culture system, temporary immersion (ebb and flood) and continuous immersion (with and without net) (Figure 1), were used to select a suitable method for growth of potato plantlets. Nodal segments (50 nodal segments per bioreactor) were transferred to 10 l column-type bioreactor with 1.5 l MS liquid medium supplemented with 3% sucrose. The pH of the medium was adjusted to 5.8 before autoclaving (at 121°C and 1.2 kg cm⁻² pressure for 40 min). In the immersion (without net)-type bioreactor, plantlets were submerged in liquid during the whole period, whereas in the case of immersion with net, a supporting net was used to hold the plant material in order to avoid complete submersion of explants in the liquid medium. The volume of input air was adjusted to 0.1 vvm (air volume/culture volume, min). The ebb and flood system was programmed to immerse the plantlets in the medium 12 times per day and 60 min each time. All the bioreactors and culture vessels were maintained at 25°C under 100 µmol m⁻² s⁻¹ PPF under 16 h photoperiod per day. Studies were also done to investigate the effect of inoculation density, viz. 20, 40, 50, 60 and 80 nodal cuttings per bioreactor, on potato micropropagation during immersion-type (with net) bioreactor culture. Other conditions were the same as described above. The growth and multiplication of shoots were recorded after three weeks of culture. Dry weight was determined after drying the plantlets for 24 h at 80°C.

After four weeks, the medium was replaced with 1.5 l of the tuber-inducing medium containing 8% sucrose,

with or without 4.44 µM 6-benzylaminopurine (BAP) (step B). Cultures were maintained at 25°C under dark conditions for microtuber formation and harvested after eight weeks of dark culture. To investigate the effect of medium exchange on microtuber formation during step-B culture, the old medium was replaced with a new medium after four weeks of dark culture. The percentage of microtuber formation, number of microtubers per vessel and the fresh mass of microtubers were investigated after eight weeks of culture.

All experiments were repeated three times with three replicates. Data were subjected to Duncan's multiple range test using SAS program (Version 6.12, SAS Institute Inc, Cary, USA).

Comparative studies between solid and bioreactor culture (continuous immersion [with net or without net] and temporary immersion in liquid medium using ebb and flood) revealed that shoot multiplication and growth were more efficient in continuous immersion (with net) bioreactor culture (Table 1). In the case of immersion system without net, nodal explants were continuously immersed into the liquid medium and did not grow rapidly, probably due to lack of oxygen. In the case of immersion system with net, the basal part of the shoots was continuously in contact with the medium which enabled a constant supply of nutrients as well as aeration to explants. This led to plantlet growth and multiplication. The ebb and flood bioreactor system, functioning on the principle of temporary contact between the plants and the liquid medium rather than permanent contact, was also found to be an efficient means for bioreactor culture of potato plantlets. But such system includes pneumatic

Table 1. Effect of culture methods on growth of potato var. 'Atlantic' plantlets after three weeks of culture (Immersion A: Continuous immersion without supporting net; Immersion B: Continuous immersion with supporting net) in a bioreactor

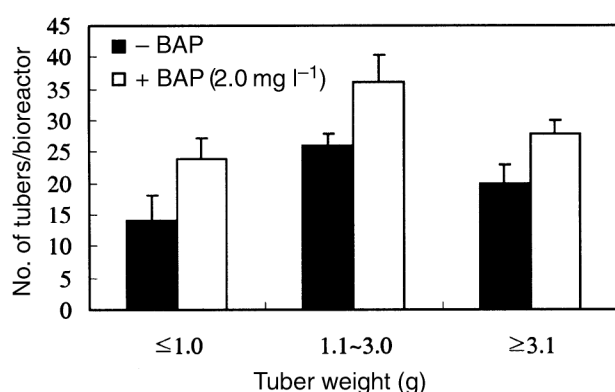
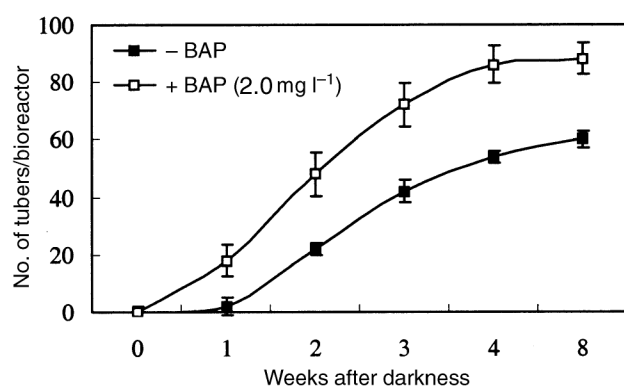
Culture method	No. of nodes		Shoot length (cm)	Shoot diameter (mm)	Shoot fresh wt. (g/plantlet)
	Per plantlet	Per vessel			
Solid	8.2b*	65.6d	8.6c	1.1c	0.6c
Immersion A	12.6a	315.0c	15.9b	1.6b	3.1b
Immersion B	14.0a	532.0a	18.3a	1.5b	3.0b
Ebb and flood	13.2a	409.2b	16.2b	2.1a	4.2a

*Mean separation within columns by Duncan's multiple range test at 5% level.

Table 2. Effect of inoculation density of nodal cuttings on growth of potato var. 'Atlantic' plantlets after three weeks of culture

Nodal cuttings per 10 l volume of bioreactor culture	No. of nodes/plantlet	Shoot length (cm)	Shoot wt./plantlet (mg)		Root fresh wt./plantlet (mg)
			Fresh	Dry	
20	9.4c*	13.5d	1085.2e	43.3c	269.2a
40	11.3b	15.6c	1785.4b	77.0b	223.5b
50	14.0a	18.3a	3005.2a	90.4a	235.9b
60	11.5b	17.3b	2238.4a	85.5a	246.4b
80	10.0c	15.2c	1443.4cd	51.9c	276.5a

*Mean separation within columns by Duncan's multiple range test at 5% level.

**Figure 2.** Distribution of tuber weight in potato var. 'Atlantic' after 12 weeks of bioreactor culture.**Figure 3.** Tuber formation of potato var. 'Atlantic' as affected by BAP treatment during 12 weeks of bioreactor culture.

transfer of the medium from a tank to the container holding the explants by a solenoid valve connected to a programmable plug, and thereby increasing the production cost. We also studied the effect of inoculation density on potato micropropagation during bioreactor culture. Number of shoots/nodal explants, shoot fresh and dry weight, and shoot length increased linearly as the inoculation density increased. Maximum responses were observed when there were 50 nodal explants per bioreactor (Table 2). The need for an optimum inoculation density for faster microplant growth during micropropagation has been reported in a number of crop species⁹⁻¹².

The culture medium was replaced to proceed to step B with the same MS medium as in step A, but the concentration of sucrose was increased to 80 g l⁻¹ with or without BAP and kept under darkness. Tubers were harvested after eight weeks of culture. Figures 2-4 show the efficiency of microtuber formation in this culture system. Tuberization was clearly stimulated by adding BAP in the medium. The analysis of tuber classification according to size showed that addition of BAP in the culture medium influenced the formation of microtubers larger than 1.1 g (Figures 2 and 3). Tubers of this size are the most desirable for commercial production, because they can be planted in the field without an acclimatization stage and stored for longer periods without detrimental loss of weight¹³. Effect of BAP on microtuber formation in potato plantlets *in vitro* has also been reported by Badawi *et al.*¹⁴. They suggested that dark conditions were more favourable for BAP at the time of tuber induction.

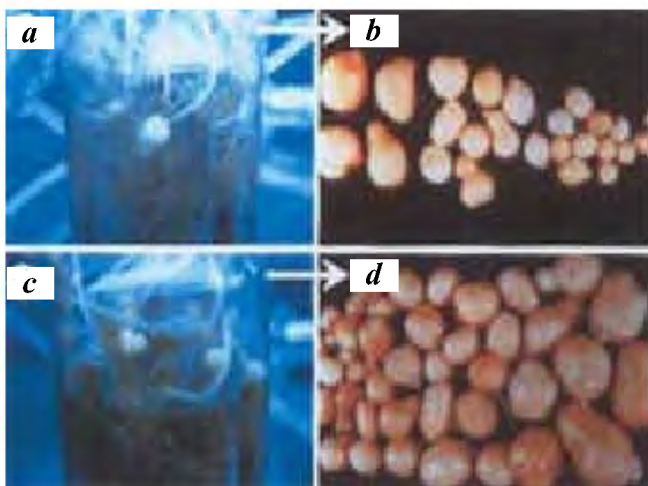


Figure 4. Microtuberization of potato var. 'Atlantic' in medium without BAP (a) inside bioreactor; (b) after harvest, and with 4.44 µM BAP, (c) inside bioreactor and (d), after harvest, after 12 weeks of culture.

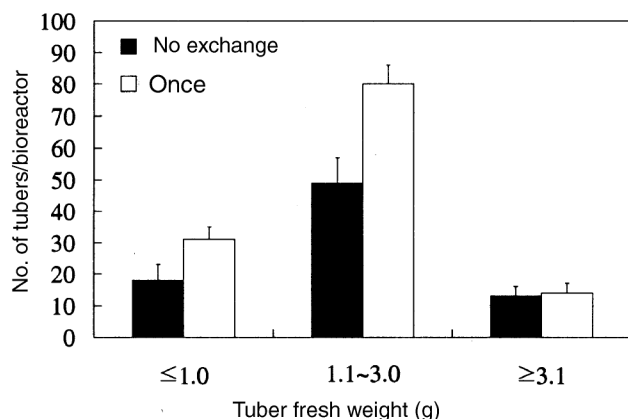


Figure 5. Effect of medium exchange on microtuber growth of potato var. 'Atlantic' after 12 weeks of bioreactor culture.

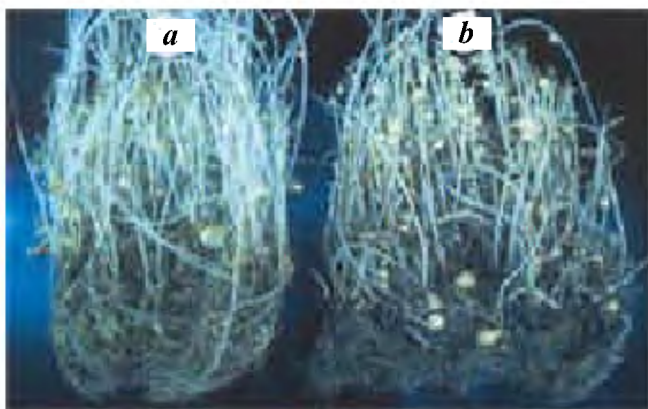


Figure 6. Effect of medium exchange on microtuber of potato 'Atlantic' after 12 weeks of bioreactor culture; a, No exchange; b, One-time medium exchange.

The total fresh weight of shoots as well as tubers increased markedly, with at least one-time medium repl-

nishment during immersion (with net)-type bioreactor culture (Figures 5 and 6). There is a strong influence of medium renewal on individual microtuber growth during bioreactor culture of potato. This indicates that proper medium supply is essential for continued microtuber growth and appears to be a key factor for achieving large microtubers. Similar results were observed by Lian *et al.*¹⁵. They reported that the total sugars and mineral nutrients in the medium steadily decreased as growth of bulblets proceeded during bioreactor culture of *Lilium* bulblets.

Immersion-type bioreactor (with net) system is a valuable option for potato microtuber production. The procedure described in this communication not only induced more tubers per plant than the solid medium, but also increased the size and weight of the tubers. This system can also be used for shoot multiplication during the planting season, when *in vitro* plants can be immediately acclimatized and transplanted.

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