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Efficient microtuber production of potato in modified nutrient spray bioreactor system

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ABSTRACT

A laboratory scale bioreactor system has been developed using nutrient spray technology for in vitro mass production of potato microtubers. Its effectiveness on the production of microtubers was investigated and compared with conventional liquid and semi-solid culture systems through bioreactor. Optimal culture conditions such as spray intervals, varying concentrations of 6-benzylaminopurine (BAP) and explants density were determined for the NSB. In order to determine optimal spray intervals, liquid medium was sprayed inside the NSB at different intervals (½, 1–4 h) of which the 1 h interval resulted in the highest number of shoots (3.47) and length (8.99 cm). Number of microtubers produced (5.13) was highest with 1 h intervals and fresh weight of microtubers (0.90 g) was highest for ½ h interval. Different concentrations of BAP (0.5, 1.0 and 1.5 mg/L) were used to evaluate its effect on microtuberization. It was observed that number and diameter of microtubers were increased (5.31 and 0.96 cm) when 0.5 mg/L BAP was supplemented in MS medium. We found fresh weight of microtubers (0.97 g) was increased when 1.0 mg/L BAP were added to the medium. In order to determine suitable explants density, single nodes grouped into five categories e.g 30, 45, 60, 75, and 90 and placed in the NSB system. A density of 60 explants resulted in increases in shoot length (17.5 cm) number of internodes (12.5) and with highest amount of chlorophyll (40.2 mg/g) as well as with highest number and fresh weight of microtubers (4.43 and 0.89 g, respectively). Out of the three culture systems, the NSB performed best where 1.5–2.0 fold increases in shoot growth and microtuberization without hyperhydration. The NSB also produced the highest number (4.67), fresh weight (0.86 g) and diameter (0.78 cm) of microtubers. From this study we may conclude that the NSB system has good potential for commercial mass production of potato micro-tuber.

1. Introduction

Potato (*Solanum tuberosum* L.) is grown in a wide range of climatic conditions and no other crops in the world can match the potato in energy value (Asghari-Zakaria et al., 2009). In vitro grown microtubers are genetically identical, high quality, and pathogen-free small-sized seed potatoes (about 0.5–1.5 cm diameter) which do not need acclimatization similar to the field-grown seed tubers (Srivastava et al., 2012). Potato plants derived from microtubers are normal and strong, and can be used in the production of original seed (Perez et al., 2007). The handling and shipping of microtubers is also more convenient which facilitate its commercialization (Imani et al., 2010). In a commercial laboratory, microtubers are produced round the year in a conventional semi-solid nutrient medium. However, this method usually produces 1.0–1.5 microtubers per plantlet, with an average diameter of <0.5 mm, thus limiting the success rate of direct plant to field conditions (Struik and Wiersema, 1999).

Bioreactor systems are used mostly for secondary metabolite production from cell and root cultures in order to achieve rapid and efficient growth and multiplication of high quality plant propagules at low costs (Ziv, 2005). Various bioreactor systems such as recipient with automatized temporary immersion (RITA), bubble column bioreactor (BCB), and balloon type bubble bioreactor (BTBB) have been developed for plant micropropagation (Takayama 1991;

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Alvard et al., 1993; Shohael and Paek, 2013). They offer several benefits including better control of culture conditions, optimal supply of nutrients and growth regulators, renewal of culture atmosphere, changing of the medium during the culture period according to developmental stage, filtration of the medium for exudates, contamination control and production of clusters of buds or somatic embryos (Ziv, 2005).

Several studies have reported the potential of different bioreactor systems for the mass propagation of potato microtubers (Donnelly et al., 2003). Liquid cultures together with various temporary immersion techniques, ebb and flood, Rita™ system, twin-flask system, plastic bag cultivations as well as tilting rocker system have also been evaluated for the production of potato microtubers (Estrada et al., 1986; Akita and Takayama, 1994; Teisson and Alvard, 1999; Jimenez et al., 1999; Grigoriadou and Leventakis, 2003; Piao et al., 2003; Kämäräinen-Karppinen et al., 2010). Most of these techniques, however, involve the periodical immersion of plant propagules in liquid media. However, Weathers and Giles (1988) suggested that a different bioreactor system, wherein plant materials were cultured in nutrient mist created by ultrasonic transducers, might be more effective for plant micropropagation. They cultured various plants in nutrient mist bioreactor systems and reported improved growth (Hao et al., 1998). As plant propagules were directly exposed to the nutrient mist, composed of medium micro-particles and gas, both nutrient absorption and gas exchange of the plant tissue improved, resulting in enhanced plant growth (Hao et al., 1998). Although nutrient mist bioreactors are mainly used for hairy root cultures, other plant propagules may also be cultured in such systems (Weathers et al., 2008). Hao et al. (1998) and Kurata et al. (1991) reported improved shoot growth and microtuberization of potato in a nutrient mist bioreactor. A nutrient spray bioreactor (NSB) functions on a principle similar to that of a nutrient mist bioreactor, except that in the former the liquid medium is sprayed over the plant materials by a spray nozzle rather than by ultrasonic transducers.

In recent years, the farming of crops particularly seed-potato development through tissue culture has silently revolutionized in agricultural sector of Bangladesh and also in the world. Quality seeds of different crops including potato are produced in commercial laboratories in Bangladesh using conventional micropropagation techniques such as semi-solid and liquid cultures. These laboratories have standardized suitable protocols of their own with the standard potato cultivars of the country by incorporating different factors like culture media, light, temperature, explants, etc for maximizing microtuber production (Hossain, 2005; Hoque, 2010). However, little work has been done on microtuberization in bioreactor systems. In this study a nutrient spray bioreactor (NSB) was developed at the laboratory scale and its potential for application in potato micropropagation was evaluated.

The aim of this study was to develop and optimize a suitable system for potato shoot growth and microtuberization by investigating the effect of different nutrient spray intervals, BAP concentrations and inoculation densities on various aspects of microtuber production. It also aimed to compare microtuber productivity of the NSB system with conventional semi-solid and liquid culture systems.

2. Materials and methods

2.1. Plant materials

Nodal cuttings (with one leaf) of potato plantlets were cultured in 0.6% agar solidified MS (Murashige and Skoog, 1962) medium containing 30 g/L sucrose (pH 5.8); incubated at 25 °C and 50–55% relative humidity, and subjected to 50 μ mol m^{-2} s^{-1} light intensity with 16 h photoperiod. After 3 weeks of culture, each shoot was divided into nodal segments, each containing one node, and subcultured in a similar medium.

2.2. Culture systems

The nutrient spray bioreactor (NSB): The NSB system has developed by us consists of two vessels, each constructed from 1 L glass reagent bottles – an upper culture chamber, and a lower medium reservoir (Fig. 1a). The culture chamber is 12 cm in height with 9.5 cm inner diameter, and has 2.5 cm wide rims at the top and bottom. It has a lid (diameter: 14.5 cm) with a brass spray nozzle (diameter: 0.8 cm, length: 3.7 cm, spray orifice: 0.5 mm) attached. The medium reservoir is 10 cm in height and 9.5 cm in diameter, and has a 2.5 cm wide rim at the top, an air inlet, and a medium outlet, which is connected to the spray nozzle by a silicone tube (ID 0.6 mm). Nutrient spray was created by releasing compressed air into the medium reservoir via a hydrophobic membrane filter (0.22 μm) connected to the air inlet. There is a one-way check valve at the bottom of the culture chamber, which allows the sprayed liquid to return to the medium reservoir but prevents reverse flow of liquid or air from the medium reservoir to the culture chamber.

The semi-solid and liquid culture systems: For the semi-solid culture system (Fig. 1b) the protocol described in Piao et al. (2003) was followed. For the liquid culture system (Fig. 1c) a combined support of stainless steel net and filter paper (Whatman No. 1) was used and for sterile air exchange the caps of the culture vessels were fitted with 0.22 μm hydrophobic filters.

2.3. Culture medium and growth conditions

Liquid MS medium was used in the NSB and liquid culture systems. For the study on shoot growth 30 g/L sucrose solution was added to the medium and the pH was adjusted to 5.8 before autoclaving (121 °C, 15 psi). Then, the culture systems were incubated under 50 μmol m^{-2} s^{-1} light intensity for 3 weeks at 25 ± 1 °C in 16/8 h day/night cycles. For the microtuber development study fully grown plantlets were transferred into similar culture vessels in which the MS medium was supplemented with 80 g/L sucrose solution and various concentrations of BAP (0.5, 1.0 and 1.5 mg/L). Cultures were incubated in the dark at 20 ± 1 °C for 10 weeks.

2.4. Optimization of nutrient spray interval, concentration of BAP and explants density in NSB systems

In order to determine the optimum nutrient spray interval, liquid nutrient medium was sprayed at five different intervals e.g ½, 1–4 h and controlled by a solenoid valve, regulated by an electronic timer. Compressed air was supplied at 0.4 MPa, which created a liquid nutrient spray at the rate of 0.2 L/min in the NSB system. For the study investigating the effect of growth regulators on microtuberization, the MS medium was supplemented with different concentrations of BAP (0.5, 1.0 and 1.5 mg/L). Sixty explants were inoculated in each culture vessel and nutrient medium was sprayed at 1 h intervals. Then the NSB system was incubated in darkness at 20 ± 1 °C for 10 weeks. To optimize explants density in the NSB, explants (single nodes) were grouped into five categories e.g 30, 45, 60, 75, and 90 and placed in the culture vessel (9.5 cm in diameter) with 250 mL of liquid medium in the reservoir. Nutrient medium was sprayed at 1 h intervals and the NSB system was incubated for shoot growth and microtuber induction in the above-described culture conditions.