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In vitro production of potato microtubers in liquid medium using temporary immersion

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Summary

CIRAD developed a new apparatus for plant tissue culture, using temporary immersion in a liquid medium. This apparatus was adapted to the microtuber production in potato. The procedure is as follows: single node cultivation on MS medium containing 30 g/l sucrose in the light for 2 weeks, induction of microtuberisation with 80 g/l sucrose over a 2 week period in the light, followed by a further 6 weeks in the dark. All experiments were performed at 20 °C. The basic vessel had a capacity of approximately 1 l; 30 nodes were cultivated per vessel. Depending on the cultivars tested (Bintje, Ostara and Désirée) 47 to 115 microtubers were harvested per vessel. Between 30 and 60% of the microtubers weighted over 0.5 g and between 10 and 40% over 0.8 g. Sprouting is still under investigation. Preliminary results indicate that the dormancy period was relatively short and several stems were obtained per microtuber. These results seem to be better than those usually reported. Only one simple protocol has been tested and further improvements are probably easy to obtain.

Introduction

The use of liquid medium for in vitro culture simplifies handling, reduces production costs and is considered to be the best way to achieve automation (Aitken-Christie, 1991). It may also have disadvantages including suboptimal tuberisation (Vreugdenhil & Struik, 1989). Some physiological disorders such as hyperhydricity and vitrification can also appear. To avoid these problems different procedures have been developed: a raft to support plants over stationary liquid (Connor & Meredith, 1984; Hamilton et al., 1985), adding liquid medium to established cultures on agar (Maene & Debergh, 1985; Aitken-Christie & Davies, 1988) and mist culture (Wheathers & Giles, 1988). Over the past ten years we developed a procedure using temporary immersion of cultured tissues, organs and/or plantlets in a liquid medium. The system was first tested with meristem proliferation of pineapple and banana (Alvard et al., 1993) and was so successful that experiments were extended to microcutting and to somatic embryogenesis of banana (Escalant et al., 1994), citrus (Cabasson et al., 1997), coffee (Teisson et al., 1995) and rubber tree (Etienne et al., 1997). The frequency and duration of the immersion which produced the best results were found to be surprisingly low. To adapt the procedure a new vessel was designed and a culture protocol developed for the production of potato microtubers.

Materials and methods

Description of the apparatus. The vessel, named RITA, is made of two compartments, an upper one which contains the plants and a lower one with the liquid medium (Fig. 1). These two compartments are linked in such a manner that overpressure applied in the lower compartment pushes the medium up in the upper one. Plants are immersed as long as the overpressure is applied. During the immersion period a flow of air aerates the medium, agitates plants and replaces the atmosphere inside the culture vessel as the overpressure escapes through an outlet at the top of the apparatus. When pressure drops, the medium returns to the lower compartment by gravity. The frequency and duration of immersions are easily controlled by programming overpressure application with a solenoid valve and a timer switch. To prevent contamination the air flows through 0.2 μ hydrophobic filters (Gelmann, Bacterial Air Vent ref. 4210).

The vessel is made up of re-usable and autoclavable plastic (polysulfone) on sale at Vitropic S.A. (34270 St. Mathieu de Traviers, France). Particular attention was paid to facilitate the handling (assembling the apparatus, changing the medium, manipulating the plants), to reduce the risks of contamination (only one vessel with one lid) and to limit the costs. The container can also be used for mass propagation through somatic embryogenesis.

The basic vessel has a capacity of about 1 liter, and is well adapted to maturation and germination of somatic embryos (Teisson & Alvard, 1994). As potato microtuber production depends on the size of the plantlet, we increased the volume of cultivation

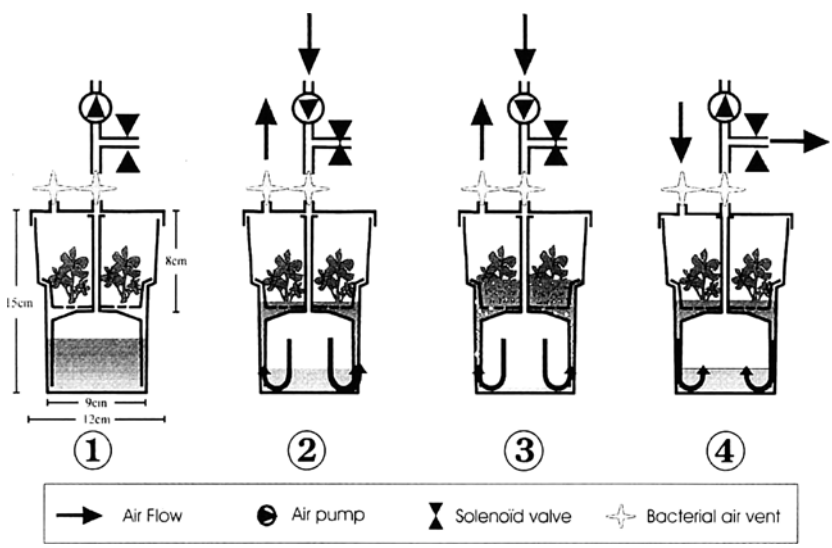

Fig. 1. RITA: Mode of operation.

in the following way. Two RITA containers were connected via the central outlet on their lids. The internal parts of RITA were discarded and the central tube was extended to the bottom of the vessel. In the vessel containing the plants the central tube was connected to a disk of polyurethane foam (stamped out of domestic foam), which breaks the air flow and supports the plants. Liquid medium was transferred from one vessel to the other by alternative application of overpressure (Fig. 2).

Plant material and culture conditions. Stock plants of potato cultivars Bintje, Désirée and Ostara were maintained in vitro as microcuttings by subculturing of single nodes on a modified MS semi-solid medium in test tubes under light (27 μmol m^{-2} s^{-1} photoperiod of 12 h). Thirty single nodal shoot segments were transferred to each container, a single or double RITA, containing 200 ml MS medium with 30 g/l sucrose, to facilitate shoot growth for two weeks. The medium was then replaced by MS medium containing 80 g/l sucrose and the cultures were kept for two weeks under light, and then for six more weeks under darkness. In the case of double RITA, the complete immersion of shoots was achieved by increasing the volume of the medium to 600 ml during the last two steps. Immersion took place four times a day for one minute during the first step, and four times a day for one hour during the subsequent

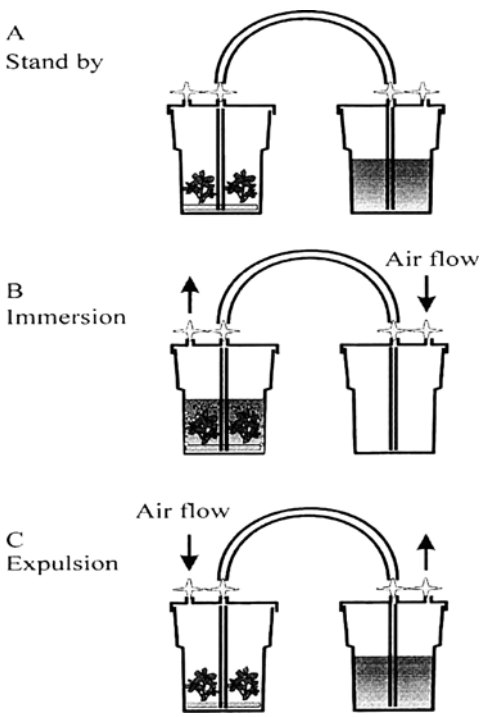

Fig. 2. Double RITA.

stages, following the recommendations outlined by Akita & Takayarna (1994). All cultures were performed at 20 °C. See Fig. 3.

At the end of each experiment (10 weeks after inoculation), microtubers were harvested and tuber number and weights of individual tubers recorded. Statistical analysis was performed using the Newman-Keuls test (Newman, 1939).

Results and discussion

During preliminary experiments (data not shown), semi-solid medium (same composition), was found to be very limiting and produced poor results (number of nodes, internode length, number of microtubers) and was no longer used as a control. Comparison between single and double RITA is illustrated in Table 1: increasing the

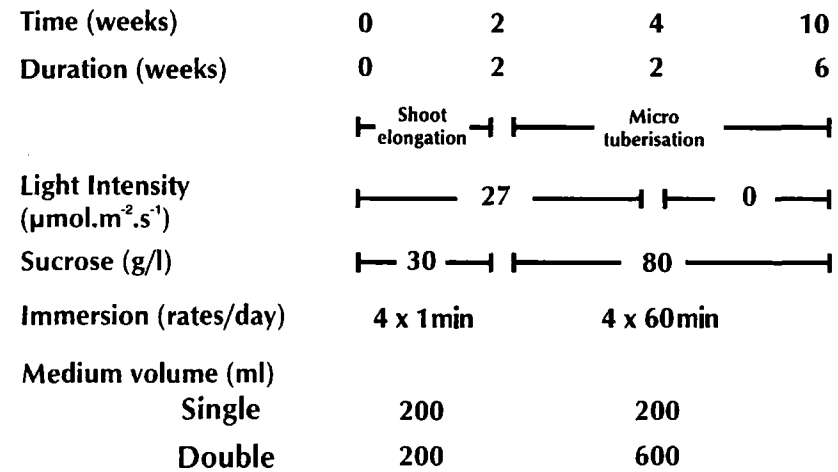

Fig. 3. Conditions for the production of potato microtubers in temporary immersion.

Table 1. Comparison of the Bintje microtubers production in a single and double RITA.

	Single RITA	Double RITA
Total volume	1 liter	1 liter x 2
No. microtubers / node	1.7	3.0
No. microtubers / vessel	50.8	90.1
Weight (g) microtubers / vessel	20.6	51.5
% Size of microtubers		
0 - 0.25 g	31.2	24.8
0.25 - 0.5 g	34.2	27.7
0.5 - 0.75 g	25.8	20.6
0.75 - 1 g	5.8	18.9
> 1 g	3.1	8.0

volume of the vessel led to an increase in the number and weight of the microtubers. Results in a double RITA with three different cultivars are given in Table 2. The number of microtubers obtained in ten weeks ranged from 48 for Désirée to 90 for Bintje. For the latter variety this means that three microtubers could be harvested per initial single node. As previously described by other authors (Akita & Takayama, 1994) microtubers were mainly located at the top of the stem. The most interesting result concerns the size of the microtubers, as over 50% of them were heavier than 0.5 g (Table 2). Microbers of Désirée were very big, some of them weighing 6 grams.

Sprouting of the microtubers was very efficient and germination in a temporary immersion system led to the rapid development of several stems from one single tuber. The results we obtained are only preliminary and must be repeated. The number and the individual weight of microtubers obtained, only ten weeks after transfer of a single nodal section, are better than those usually described in the literature on semi-solid medium, in liquid medium, in large fermentors or with a raft system (Alchanatis et al., 1994; Levin, 1995). As was previously the case in meristem proliferation and somatic embryogenesis it is assumed that the success obtained is linked to the physical conditions in culture vessels induced by temporary immersion, i.e.:

- a better supply of nutrient elements by renewed direct contact with the medium (Debergh, 1983);
- low disruption of gas exchanges between the plant and the atmosphere by short immersion periods;
- dessication prevented by a capillary film of medium during immersion periods;
- complete renewal of the atmosphere inside the vessel at regular intervals.

RITA is very simple to use. Medium changes are very easy and the procedure may be tested to improve the procedure: use of growth regulators (Vreugdenhil & Struik, 1989) and extension of duration of culture. The use of overpressure to displace the medium is a way to test the efficiency of controlled atmosphere as autotrophy has been clearly demonstrated to be feasible for potato micropropagation (Kozai et al., 1988).

Table 2. Production of potato microtubers in a double RITA.

RITA	Bintje	Ostara	Désirée
No. microtubers / node	1.5	2.3	1.4
No. micortubers / vessel	52	68	48
Weight (g) microtubers / vessel	20.6	35.5	39.9
% Size of microtubers			
0 - 0.25 g	31.8	29.5	21.2
0.25 - 0.5 g	38.8	28.7	21.0
0.5 - 0.75 g	18.2	19.4	18.2
0.75 - 1 g	8.0	12.6	17.8
> 1 g	3.2	8.8	21.8

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