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A SIMPLE TWO-PHASE SYSTEM FOR EFFICIENT IN VITRO TUBERIZATION IN POTATO

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In vitro tuberization was induced by simply overlayering the solid propagation medium with a liquid induction medium. Nodal cuttings were grown for three weeks in hormone-free Murashige-Skoog (MS) medium, then a liquid modified MS-medium with 10% sucrose, 10 mg/l benzyladenine (BA) and 1.2 ml/l commercial cycocel was added. During tuber induction, the incubation vessels were placed at 20°C, 8 h day 40 $\mu Em^{-2}s^{-1}$. Almost full tuberization was achieved within four weeks for all cultivars tested, and after ten weeks, a tuber size of 200-400 mg was obtained.

Key words: *Solanum tuberosum* L., tissue culture, tuberization.

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In vitro propagation of potato is an efficient way of rapidly multiplying new or existing cultivars in disease-free conditions. Propagation may be achieved by serial culture of nodal cuttings (Hussey and Stacey 1981, Rosell et al. 1987). The in vitro grown shoots can be induced to produce tubers (Hussey and Stacey 1984, Wang and Hu 1982, Tovar et al. 1985), which are very convenient for storing, transporting and handling. In vitro tubers are suggested to be the ideal end product of in vitro propagation (Rosell et al. 1987). The process of in vitro tuberization has been studied by several investigators, and there is general agreement on several factors which favor tuberization. High sucrose concentrations, cytokinins, and the gibberellin biosynthesis inhibitor chlorocholine chloride generally promote tuberization. These substances are usually added together (Hussey and Stacey 1984, Tovar et al. 1985, Rosell et al. 1987), although this is not strictly necessary for all cultivars. Temperatures at 25° to 30°C are clearly inhibitory and a temperature of 18° or 20°C is commonly used (Koda and Okazawa 1983, Wang and Hu 1982). There is evidence that short days also promote in vitro tuberization (Hussey and Stacey 1984, Wang and Hu 1982). Both a solid induction medium and a liquid medium which generally give larger minitubers have been used (Rosell et al. 1987). Based on these generally accepted effects of media components and environmental factors we have developed a very simple, quick and reliable method for producing in vitro tubers in a solid/liquid two-phase system.

MATERIALS AND METHODS

Plant material

The materials used were: The commercial cv. Beate propagated by taking nodal cuttings after initial meristem culture. The breeding line F x Aq (good resistance to late blight), N73-20-262 (highly resistant to gangrene) and the two pollen sterile lines N-80-37-34 (nematode resistant, highly resistant to gangrene) and CT-81-22-25 (complex hybrid, highly resistant to late blight) were propagated from tuber sprouts after surface sterilization. N-80-21-135 x 'Rosamunda' (later referred to as xRosamunda) was propagated from one true seedling. *Solanum acaule* (primitive, non-tuber producing) propagated from one true seedling was also used in some of the experiments.

Media and incubation conditions

The plants were grown either in Magenta boxes (350 ml) or, as in one experiment, in glass jars (350 ml). The medium routinely used was the TM5-medium of Shahin (1985), which is a modified half-strength MS medium with 1% sucrose and no hormones. Shoot tips or nodal cuttings were transferred to fresh medium about once a month. The vessels were incubated at 20°C, 12 h day 120 $_{\mu}$Em-^{2}s-1. Regular hormone-free MS medium with 3% sucrose was also used for propagation, and was always the medium chosen prior to induction of tubers.

Two different media for tuberization were investigated. 1) A solid modified MS medium after Tovar et al. (1985) with 8% sucrose, 5.0 mg/l benzyladenine (BA) and 0.6 ml/l cycocel (American Cyanamid 320 g/l cholinechloride, 460 g/l chloromequatchloride) (CCC). 2) Liquid MS medium with 10% sucrose, 10 mg/l BA and 1.2 ml/l CCC. Vessels with plantlets for induction of tubers were exposed to 8 h daylengths at 40 $_{\mu}$Em-^{2}s-1 and 20°C.

RESULTS

Each top shoot with 2-3 leaves was cut from 4-week-old plants and transferred to the solid induction medium (Table 1). Although tuberization was efficiently induced, the tubers were smaller than reported by others (Tovar et al. 1985,

Table 1. Number of tubers per shoot and weight of tubers after three different tuber induction treatments. Apical shoots with 2-3 or 6 leaves were transferred to Magenta boxes with solid induction medium, or liquid induction medium was added to the solid propagation medium. The number and the fresh weight of tubers were scored 10 weeks after transfer to or addition of induction medium. On average, there were 14 shoots per treatment

Plant material	Shoots with 2-3 leaves solid medium		Shoots with 6 leaves solid medium		Shoots with 6 leaves solid/liquid medium	
	Tuber/shoot	weight/tuber mg	Tuber/shoot	weight/tuber mg	Tuber/shoot	weight/tuber mg
Beate	0.7	56	1.1	96	1.0	209
xRosamunda	0.7	45	1.5	50	1.7	337
FxAq	1.4	67	1.1	72	1.6	306
CT-81-22	1.1	28	1.0	52	1.3	142
N-80-37-34	1.0	46	NA	NA	1.1	336
S. acaule	1.1	10	NA	NA	1.2	33
Mean	1.0	42	1.2	68	1.3	227

(NA = not available)

Table 2.The effect of various amounts of solid and liquid medium on tuberization. One shoot was cut into one top shoot with 2-3 leaves and six nodal segments each with one leaf and axillary bud. The cuttings were placed in Magenta boxes with 10 or 20 ml hormone-free MS-medium. After 3 weeks, 10, 20 or 40 ml induction medium was added. There were 14 cuttings per treatment. The number of tubers was scored after 4 weeks and 10 weeks, and fresh weight was measured after 10 weeks

Plant material	Solid propagation medium ml	Liquid induction medium ml	Tubers/shoot after 4 weeks	Tubers/shoot after 10 weeks	Weight/tubers ml
Beate	10	10	0.9	1.0	162
	10	20	1.0	1.0	245
	20	20	0.9	1.0	312
	20	40	0.9	0.9	374
xRosamunda	10	10	0.8	1.0	88
	10	20	0.9	1.3	159
	20	20	0.9	1.3	288
	20	40	0	1.8	230

Hussey and Stacey 1984). When shoots with 6 leaves were transferred to the induction medium, the result was an increase in tuber weight (Table 1). However, in both cases, growth was inhibited on the induction media; this was probably due to the lack of a root system to absorb minerals and sugar. Therefore, in the next experiments the top shoots with 2-3 leaves were transferred to MS medium with 3% sucrose and no growth regulators. After three weeks the shoots had grown to about the 6th leaf stage and had a well-developed root system. At this stage 20 ml liquid MS medium with 10% sucrose, 10 mg/l BA and 1.2 ml/l CCC was poured into the vessels, and the vessels transferred to 8 h day, low light intensity. This treatment efficiently induced tuberization and the average weight of *S. tuberosum* tubers was high (Table 1, Fig. 1). Also *S. acaule*, which usually does not produce tubers in the field, produced tubers in vitro, demonstrating that the conditions chosen were highly inductive.

In the experiment presented in Table 2, shoots with 7-8 leaves were cut into nodal segments and placed in vessels with 10 or 20 ml MS-medium. After 3 weeks, 10, 20 or 40 ml liquid induction medium was added. This experiment showed that 20 ml of solid and 20 or 40 ml of liquid medium was suitable, and both nodal segments and top segments could be used.

The type of vessel or sealing used was important to good growth and tuberization. Figure 2 shows tuberized plants about 7 weeks after the liquid induction medium was added to plants of the cultivar Beate grown in glass jars sealed with parafilm and for plants grown in Magenta boxes. The plants in the glass had thick stems and poorly-developed leaves, typical effects of ethylene, whereas the plants in the Magenta boxes had well-developed leaves and also produced larger tubers.

DISCUSSION

The experiments showed that the in vitro tubers obtained when shoots were transferred to solid induction medium were rather small. The reason for this poor development was probably the lack of a root system for efficient absorption of nutrients. Roots did not develop due to the high concentration of BA in the medium. Such tubers are probably too small for good production of tubers in the next step, and dormancy breakage may

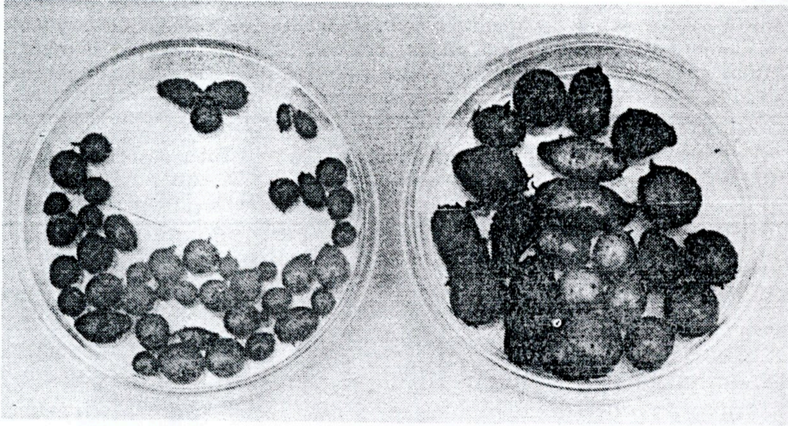

Fig 1. In vitro tubers of 'Beate' produced on a solid (left) a solid/liquid (right) medium. The scale bar is 10 mm

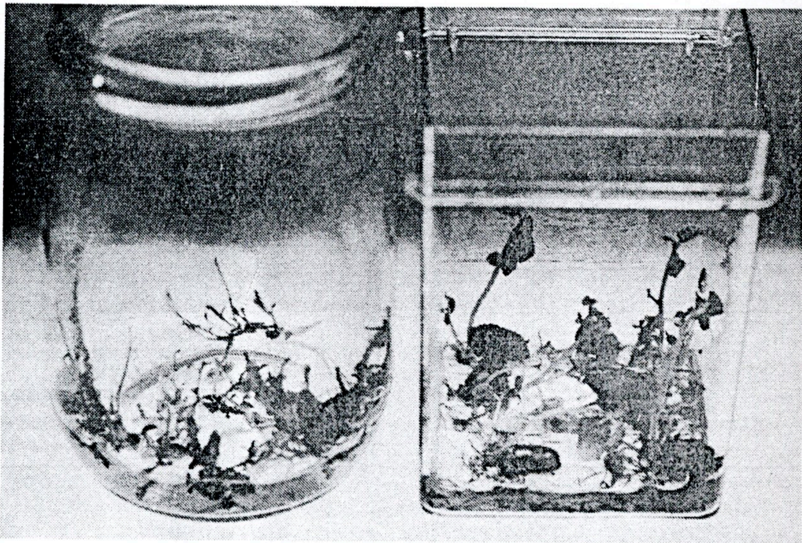

Fig 2. Tuberized plants grown in glass jars and sealed with parafilm (left) and Magenta boxes (right)

also be a problem (Tovar et al. 1985). However, full tuberization was obtained by simply overlayering the solid propagation medium with a liquid induction medium when the plants already had a welldeveloped root system. The high sucrose concentration (10%) of the induction medium caused dehydration of the solid medium and the roots were progressively exposed to higher sucrose concentrations, a situation which is probably beneficial to the tuberization process. Concentrations of BA and CCC were doubled compared with the protocol of Tovar et al. (1975), since these substances have to diffuse into the solid

medium before they can be taken up by the roots. The tubers produced by this method were about 5 times larger than those produced when solid medium was used only. These tubers were also larger than the tuber sizes reported in other investigations (Hussey and Stacey 1984, Tovar et al. 1985). This method could easily be combined with mass propagation by serial nodal cuttings or layering of detopped shoots on the solid medium. Tuberization could then be induced at any time by adding the liquid induction medium and transferring the plants to short days and low light intensity. Studies of dormancy breakage and further applications of the in vitro tubers are in progress.

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