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Potato Research 30 (1987) 111–116 SHORT COMMUNICATION

In vitro mass tuberisation as a contribution to potato micropropagation

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Summary

Mass tuberisation was obtained on plantlets growing from nodal potato cuttings cultured in vitro on Murashige-Skoog (MS) medium under continuous light of 5000 lux at 24–25 °C. Tuber formation was stimulated by transferring the plantlets to MS or White-Nitsch-Morel (WhNM) liquid or solid media supplemented with 8% sucrose, 2 mg l^{-1} benzyladenine (BAP), 2 mg l^{-1} naphthalenacetic acid (NAA) and/or 100 mg l^{-1} (2-chloroethyl) trimethylammonium chloride (CCC). Liquid media invariably induced heavier minitubers (390–790 mg each). The process to tuberisation took only two months. Minitubers always produced normal growing plants after dormancy break.

Introduction

Potatoes can be micropropagated rapidly and on a large scale by meristem and shoot-tip culture (Escalada & García, 1982; Goodwin et al., 1980; Henshaw & Roca, 1976; Morel, 1975; Murashige, 1974; Roca, 1975; Roca et al., 1978), proliferation by axillary shoots developed from in vitro cultured nodal cuttings (Espinoza et al., 1984; Hussey & Stacey, 1981; Nozeran et al., 1977; Roca et al., 1978), and in vitro mass tuberisation (Hussey & Stacey, 1984; Wang & Hu, 1982).

These methods facilitate the production and conservation of potato germplasm in controlled, disease-free conditions, using space very efficiently (Espinoza et al., 1984, Hussey & Stacey, 1981, 1984). Minitubers are very convenient to store, transport, handle and plant disease-free material (Espinoza et al., 1984; Hussey & Stacey, 1984; Wang & Hu, 1982).

The aim of this work was to obtain rapidly many minitubers of suitable size from plantlets grown in vitro.

Materials and methods

The cv. Spunta, which dominates potato production in Argentina, was used in all experiments except where stated otherwise. Virus-free stock was kindly supplied by the International Potato Center (CIP), Lima, Peru. Plantlets growing from nodal cuttings with one axillary bud were used in culture techniques following Roca et al. (1978) and Hussey & Stacey (1981). Our techniques involved two steps: (a) production of plants from nodal cuttings in liquid or solid media under continuous light; (b)

production of tubers in continuous darkness after transfer of those plants growing in liquid media alone to liquid or solid tuberisation media.

Nutrient media

The media for the nodal cuttings contained Murashige-Skoog (MS) mineral salts (1962) and were either solid, in 0.8 % bacteriological agar, or liquid. They were supplemented, per litre, with 30 g sucrose, 100 mg inositol, 5 mg nicotinic acid, 2.5 mg calcimum panthothenate, 1 mg pyridoxine-HCl and 0.5 mg thiamine-HCl. Sixty ml of liquid or 40 ml of solid media were placed in 360-ml metal screw-capped flasks. The pH was adjusted to 5.6 for liquid and 5.7 for solid media. The flasks were autoclaved at 121 °C for 20 minutes.

Nodal cutting culture

Under aseptic conditions nodal cutting with a single axillary bud were prepared from 8 to 10 node plantlets. Ten explants were placed in each flask and the flasks were then sealed with 50-μm Resinite AF50 (Borden) sheets.

Incubation conditions

The cultures were incubated at 24–25 °C under continuous light of 5000 lux from 40 W standard fluorescent and 40 W Gro-Lux Sylvania tubes in a ratio of 2:1.

Plantlet proliferation

During multiplication the plantlets were transferred to fresh medium every 22–28 days until there were sufficient multinodal shoots for mass tuberisation. At the final transfer four nodal cuttings were placed in each flask in liquid medium.

Mass tuberisation experiments

Two culture media were evaluated: (a) the MS (1962) liquid or solid media described above; (b) White (1943) macronutrient salts containing twice the phosphate concentration, supplemented with Nitsch (1951) micronutrient mixture (N) and Morel (1948) vitamin complex (M) also as liquid or solid media; Tizio (1979) successfully used this mixture to study the hormonal mechanism of tuberisation. These two basic media were variously supplemented with 2 mg l^{-1} naphthalenacetic acid (NAA), 2 mg l^{-1} benzylaminopurine (BAP) and 100 mg l^{-1} (2-chloroethyl)trimethylammonium chloride (CCC) to form 14 treatments shown in Table 1.

Table 1. Composition of media and treatments for mass tuber production.

Composition of the media	Treatments													
	S1	S2	S3	S4	S5	S6	L1	L2	L3	L4	L5	L6	MS-S5	MS-L5
WhNM	+	+	+	+	+	+	+	+	+	+	+	+		
MS													+	+
NAA		+	+			+		+	+			+		
BAP				+	+	+				+	+	+	+	+
CCC			+		+				+		+		+	+

References: S = solid and L = liquid medium; WhNM = White-Nitsch-Morel medium; MS = Murashige-Skoog medium.

All media were supplemented with 8 % sucrose and the pH adjusted as above.

Four plantlets, 4 weeks old, were transferred to the liquid media with their root systems intertwining. For solid cultures the roots were first excised and the cut end of the stem inserted into the medium up to the third shoot. Each treatment consisted of ten 360-ml glass flasks containing 40–50 ml of liquid or solid media, sealed with Resinite and kept in continuous darkness at 20–22 °C throughout the experiments.

All minitubers were harvested when shoot senescence began. The significance of differences between treatments was tested by the analysis of variance.

Results and discussion

Growth of in vitro plantlets

Nodes of initial plantlets placed in liquid MS nutrient medium produced from the axillary bud an upright shoot, which was much more vigorous and twice as high as those produced on solid medium, and which had a well developed root system.

After 3–4 weeks the shoots were 70–100 mm high, each having 8–12 nodes. They were then much easier to separate into individual nodes than those growing on solid medium.

Continuous culture in liquid media tended to increase the proliferation of hyperhydric-like structures in growing shoots. These disappeared when the shoots were transferred to solid medium. The cause of such abnormalities is unknown.

Development of in vitro tubers

The tuberisation curves were similar for solid or liquid media (MSL5–MSS5;

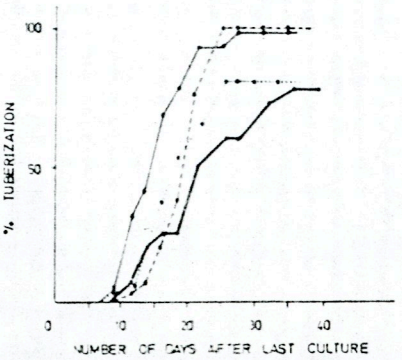

Fig. 1. Evolution of in vitro tuber formation in: S6 (———), White-Nitsch-Morel solid medium supplemented with 2 mg l^{-1} BAP and 2 mg l^{-1} NAA; L5 (– – –) as S6 but in liquid condition; MS-S5 (. . . .), solid Murashige-Skoog medium supplemented with 2 mg l^{-1} BAP and 100 mg l^{-1} CCC, MS-L5 (———), as MS-S5 but in liquid condition.

Table 2. Effect of medium on five measures of tuberisation in plantlets derived from nodal cuttings.

	Treatments													
	L1	L2	L3	L4	L5	L6	S1	S2	S3	S4	S5	S6	MS-S5	MS-L5
Mean tuber weight (mg)	442	328	372	359	343	430	201	167	173	203	159	215	225	790
			A							B				C
Number of days to reach 50 % tuberised plantlets	46	41	42	41	40	45	43	44	43	39	38	40	42	47
Number of days to tuber harvest	65	63	63	63	60	63	63	60	60	58	53	60	67	63
Mean tuber number per flask	3.0	4.3	4.4	4.0	4.0	4.0	3.8	3.9	3.8	3.9	3.9	4.0	3.2	3.3
	D						E						D	
% of tuberised plantlets	80	100	97	100	97	100	90	97	95	95	100	98	80	77

References: S = solid medium; L = liquid medium; S1...S6 and L1...L6 is White-Nitsch-Morel medium; MS-S5 = Murashige-Skoog solid medium; MS-L5 = Murashige-Skoog liquid medium.
Treatments with different letters are statistically different at $P<0.01$.

L6–S6) of comparable composition (Fig. 1); the same was true for the other treatments not shown. Full tuberisation (from 75–100 % of cultured plantlets) was attained 20–30 days after transferring the plantlets to the tuber forming media. In MS media supplemented with BAP and CCC tuber formation was slower and never reached the potential of 100 % tuberised plantlets (Fig. 1).

Behaviour of minitubers after dormancy break and planting

Eighty minitubers grown in L4 and MSL5 media were kept for one year in darkness at 20–22 °C in aseptic conditions. All minitubers produced only one apical sprout 8–10 cm in length. Then, they were exposed to continuous light (5000 lux) during ten days. These sprouts were removed and excised in nodal cuttings as before. The cuttings and the minitubers deprived of apical sprouts were transfered to a solid MS basal medium at 25 °C under continuous light of 5000 lux. They all gave rise to new normal plantlets.

In another experiment minitubers produced in S4 and L4 media were kept under continuous light of 1000 lux at 25 °C for 6 months. Sprouted tubers showing strong apical dominance were then planted in pots containing a mixture of equal parts of sandy soil and vermiculite. They soon produced normal plants. No differences in vigour were observed between plants from S4 minitubers (203 mg mean weight) and those from L4 minitubers (359 mg mean weight).

WhNM liquid medium supplemented with BAP, NAA and/or CCC seemed to be more suitable for rapid, mass tuberisation than the MS liquid medium. In our experiments only about 2 months elapsed between the culture of nodal cuttings and harvest of minitubers, half the time reported by Wang & Hu (1982) whose tubers were lighter. They obtained 30–50 minitubers weighting up to 200–300 mg each per 100 elongated shoots.

Hussey & Stacey (1984) reported that the most effective way of obtaining minitubers of the cultivars Red Craigs Royal and Ulster Sceptre was to transfer nodes to MS solid medium containing 2 mg l^{-1} BAP and to culture them in daylength of 8 hours. Most of their subcultured nodes developed into minitubers within 6–8 weeks and continued to swell to a diameter of 5 mm or more after 3–4 months. They also found that the addition of high concentrations (500 mg l^{-1}) of CCC produced minitubers 3–4 mm in diameter after 4–6 weeks. We discarded such small minitubers.

The growth of minitubers after storage and planting suggests that they are the ideal end product of potato micropropagation for storage, transport and planting of healthy germplasm.

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Potato Research 30 (1987) 117–120 SHORT COMMUNICATION

Rapid seed multiplication by planting into beds micro tubers and in vitro plants

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Summary

Micro tubers of 3 weight categories with means of 0.63 g, 1.25 g or 2.50 g and in vitro plants were planted in nursery beds at densities of 24 and 48 plants per m². The average tuber numbers and tuber weights were, respectively, 38 % and 17 % higher at 48 than at 24 plants per m². In vitro plants produced significantly more but smaller tubers than did plants grown from micro tubers. Plants grown from the larger micro tubers produced a similar number of tubers but larger ones than did plants grown from small micro tubers. Tuber yields and multiplication rates are discussed.

Introduction

Micro tubers and in vitro plants are being increasingly used in seed multiplication programmes (Wang & Hu, 1982; Tovar et al., 1985). These delicate planting stocks require special growing conditions and cannot usually be multiplied in the field. They are usually free of pathogens and should be multiplied in a disease-free environment. High-density planting in nursery beds, where plants can be effectively protected from diseases, is a satisfactory method of producing good-quality seed tubers from true potato seed (Wiersema, 1986). This paper reports the use of this method for the production of seed tubers from micro tubers and in vitro plants.

Materials and methods

Experiments were carried out at the La Molina Experimental Station of the International Potato Center, located in the coastal desert of Peru. Micro tubers, produced from pathogen-tested in vitro plants of the clones LT-1 and DTO-33, were size-graded into groups with mean weights of 0.63 g, 1.25 g and 2.50 g. Tubers in each size grade did not deviate by more than 12.5 % from the mean weights. Tubers were stored at 4 °C and presprouted in diffused light, at an average temperature of 18 °C, for 3 weeks before planting. In vitro plants were produced from the same clones and removed from the test tubes at the 6–8 node stage. Both micro tubers and in vitro plants were planted in 1-m wide nursery beds filled with a 1:1 mix of sand:shredded

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