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Effect of Culture Medium on Shoot Initiation from Calluses of Different Origin in Potato (*Solanum tuberosum* L.)

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Abstract: Attempts were made to regenerate shoots from calluses of different origin in potato on different media. Shoot initiation from growing tuber calluses on the medium of Iapichino *et al.*[1] was about 45 days earlier than on the medium of Ahloowalia[2] in both the potato cultivars i.e. Maris Piper and Desiree. Frequency of calluses producing shoots and number of shoots produced per callus were higher on the medium of Iapichino *et al.*[1] as compared to that of Ahloowalia[2] and in Cv. Maris Piper than the Cv. Desiree. Attempts to regenerate shoots on the medium of Jarret *et al.*[3] proved unsuccessful. Shoot regeneration from leaf calluses of both Desiree and Maris Piper was achieved on all the three media tested. Shoots were initiated earlier from the calluses subcultured on the medium of Iapichino *et al.*[1] followed by that of Lam[4], while it took the longest time on the medium of Ahloowalia[2]. The frequency of calluses producing shoots and number of shoots produced per callus were higher on the medium of Iapichino *et al.*[1] and the lowest on the medium of Ahloowalia[2]. The performance of Cv. Maris Piper was better because regeneration frequency was higher and also more number of shoots per callus was regenerated in this cultivar. Shoot regeneration from calluses derived from suspension-cultured cells of stem origin was 10-15 days earlier from the calluses subcultured on the medium of Bokelmann and Roes[5] than from those subcultured on the medium of Lam[4] in both Desiree and Maris Piper. Frequency of calluses producing shoots was higher in Cv. Maris Piper. The number of shoots regenerated per callus was also higher on the medium of Bokelmann and Roest[5] than on the medium of Lam[4], probably because these media contained different growth regulators in different concentrations.

Key words: *In vitro* culture, organogenesis, plantlet regeneration, *Solanum*, tissue culture

INTRODUCTION

Potato plantlets have successfully been regenerated from unorganised callus tissue and suspension-cultured cells of different origins. It is very difficult to generalize the method of inducing adventitious shoots and regeneration *in vitro* because the process of organogenesis may vary among species, cultivars (clones) and especially the donor tissue. In past, regeneration of plantlets from callus proved to be quite difficult and was not achieved for many years. Wang and Huang[6] regenerated plantlets from stem and shoot tip-derived callus of Cv. Norina No. 1 using kinetin and IAA in MS medium. Patrascu[7] used zeatin alone in the modified MS medium to induce shoot formation, while Ahloowalia[2] used zeatin with 2, 4-D in a half-strength MS medium and obtained multiple shoot primordia in the proliferating calluses that stayed regenerative even after routine subculturing during more than 3 years. These shoot primordia did not develop into shoots until they were transferred to hormone-free medium. Al-Wareh *et al.*[8] transferred calluses of potato Cvs., Red LaSoda, Norgold"M" and Viking to different shoot inducing media, regeneration occurred only from Red LaSoda leaf callus cultured on MS medium supplemented with IAA, BA and GA_3. Only 30% of the calluses transferred produced one to three shoots per callus.

Plants have also been regenerated from single, isolated, suspension-cultured potato cells. Lam[4] regenerated plantlets from isolated single cells of tuber tissue origin. The frequency of shoot initiation varied from 10% in *Solanum tuberosum* to 70% in *S. demissum*. Austin and Cassells[9] regenerated shoots from individual calluses produced from separated cells of potato stem callus. The procedure developed by Lam[4] to regenerate plantlets from suspension-cultured cells was used by several workers. Lindeque *et al.*[10] established suspension cultures by inoculating friable callus, initiated from stem

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internodal sections of potato Cv. BP1, into a MS liquid containing 2,4-D, NAA and kinetin. They also regenerated plantlets from these suspension-cultured cells and reported phenotypic variation among regenerated plants. The literature indicates quite confusing results and necessitates to conduct experiments with locally used cultivars and to refine media for tissue and cells of different origin. Therefore, in the present study attempts were made to regenerate shoots from calluses of different origin on different media in two cultivars of potato.

MATERIALS AND METHODS

Calluses were initiated from tuber and leaf explants of *Solanum tuberosum* L. Cvs. Desiree and Maris Piper[11]. For plantlet regeneration calluses were transferred onto different media. For tuber-derived callus, three media as developed by Jarret *et al.*[3], Iapichino *et al.*[1] and Ahloowalia[2] were used, while for leaf-derived callus the media of Iapichino *et al.*[1], Ahloowalia[2] and Lam[4] were used (Table 1). All the cultures were kept at 25±1 °C and a light intensity of 1000 lux with a 16 h photoperiod. After every four weeks, calluses were subcultured onto the fresh media.

Callus was also initiated from stem (internodal) segments and cell suspensions were prepared as described previously[12] and plated onto the surface of Lam[4] cell plating medium at a plating density of $0.5x10^5$ cells mL^{-1}. Petri dishes were sealed with Parafilm and incubated at 25±1 °C in 16 h photoperiod with low light intensity of about 250 lux. After 4 weeks, when calluses reached the size of 2-3 mm in diameter, the light intensity was increased to 1000 lux. After 2 weeks, when calluses had become light green, they were transferred singly either to the shoot regeneration medium developed by Lam[4], or to the medium developed by Bokelmann and Roest[5] (Table 1) and incubated in 16 h photoperiod with approx. 3000-4000 lux illumination provided by cool-white fluorescent lights at 20±1 °C.

Table 1: Composition (mg L^{-1}) of different media used for shoot regeneration

Constituents	Jarret *et al.*[3]	Iapichino *et al.*[1]	Ahloowalia[2]	Lam[4]	Bokelmann and Roest[5]
Macronutrients					
NH_4NO_3	1650.0	1650.0	825.0	1650.0	1650.0
KNO_3	1900.0	1900.0	950.0	1900.0	1900.0
$CaCl_2.2H_2O$	440.0	440.0	220.0	440.0	440.0
KH_2PO_4	170.0	170.0	85.0	170.0	170.0
$MgSO_4.7H_2O$	370.0	370.0	185.0	370.0	370.0
Micronutrients					
$MnSO_4.4H_2O$	22.3	22.3	11.1	22.3	22.3
$ZnSO_4.7H_2O$	8.6	8.6	4.3	8.6	8.6
H_3BO_3	6.2	6.2	3.1	6.2	6.2
KI	0.83	0.83	0.41	0.83	0.83
$Na_2MoO_4.2H_2O$	0.25	0.25	0.12	0.25	0.25
$CuSO_4.5H_2O$	0.025	0.025	0.012	0.025	0.025
$CoCl_2.6H_2O$	0.025	0.025	0.012	0.025	0.025
$Na_2.EDTA.2H_2O$	37.3	37.3	18.6	37.3	37.3
$FeSO_4.7H_2O$	27.8	27.8	13.9	27.8	27.8
Vitamins					
Myo-inositol	100.0	100.0	50.0	100.0	100
Thiamine-HCl	0.1	0.1	0.05	0.5	0.1
Pyridoxine-HCl	0.5	0.5	0.25	0.5	0.5
Glycine	2.0	4.0	1.0	2.0	2
Nicotinic acid	0.5	0.5	0.25	5.0	0.5
Folic acid	0.5	-	-	0.5	-
D-biotin	0.05	-	-	0.05	-
Growth regulators					
IAA	-	1	-	0.05	-
2,4-D	-	-	0.5	-	-
NAA	0.03	-	-	0.1	0.01
GA_3	0.5	-	-	0.2	0.01
BAP	3	-	-	0.5	-
Zeatin/Z. riboside*	-	2	1	0.5	1.0*
Kinetin	-	-	-	0.2	-
Supplements					
Adenine sulphate	-	40	-	-	-
Casein hydrolysate	1000	1000	-	1000	-
Sucrose	25000	20000	10000	10000	30000
Glucose	-	-	-	10000	-
D-Mannitol	-	-	-	40000	-
Agar	9000	10000	5000	10000	8000

pH adjusted to = 5.7±0.2

The pH of all the media used was adjusted to 5.7±0.2. The experiments were laid out as factorials with Completely Randomised Design, having two factors (genotypes and culture media) and repeated four times. In both the experiments, data were collected on; time required for shoot initiation (days), regeneration frequency i.e. frequency of explants producing shoots (%) and number of shoots regenerated per explant. The SD (standard deviation) for each parameter was computed by the method of Steel and Torrie[13].

RESULTS AND DISCUSSION

From tuber-derived calluses: Attempts to regenerate plantlets from growing tuber calluses on the medium of Jarret *et al.*[3] proved unsuccessful. The calluses of both Desiree and Maris Piper failed to produce shoots even after 25 weeks of culture, and remained creamy white or turned brown instead of green, without any indication of shoot regeneration. On the medium of Iapichino *et al.*[1] calluses changed to a light green colour within 30 days of transferring to this medium. Shoot initiation started from the edges of calluses after 75 days in Maris Piper and 88 days in Desiree. However, shoot development on the medium of Ahloowalia[2] was delayed and shoots appeared after 120 days of culture in Maris Piper and after 132 days in Desiree (Table 2).

Frequency of calluses producing shoots was greater on the medium of Iapichino *et al.*[1], where 72.25% of calluses of Maris Piper and 67.50% calluses of Desiree produced shoots. Regeneration frequencies were 56.25 and 48.50% in Maris Piper and Desiree cultivars, respectively on the medium of Ahloowalia[2]. Maximum number of shoots per callus (5.75) was regenerated in Cv. Maris Piper on the medium of Iapichino *et al.*[1], followed by in Cv. Desiree (4.20) on the same medium. The medium of Ahloowalia[2] resulted in significantly low number of shoot per callus (2.65 – 2.70) in both the cultivars tested as compared to Iapichino *et al.*[1] medium (Table 2).

The medium of Jarret *et al.*[3], which was developed for shoot regeneration from tuber explants, proved unsuccessful in the present work, while the media of Iapichino *et al.*[1] and Ahloowalia[2], developed for shoot regeneration from internodal explants and callus, respectively, proved to be successful. Shoot regeneration was earlier on the medium of Iapichino *et al.*[1] and from the calluses of Cv. Maris Piper. Frequency of calluses producing shoots was higher and more number of shoots was produced on the medium of Iapichino *et al.*[1] as compared to that of Ahloowalia[2]. Regeneration frequency and number of shoots regenerated per callus was also higher in Cv. Maris Piper than the Cv. Desiree. Several workers have reported BAP to be an effective growth regulator in stimulating organogenesis from tuber discs and leaf explants in different *S. tuberosum* cultivars[14,15]. In the present study, media containing zeatin i.e. of Iapichino *et al.*[1] and Ahloowalia[2], were more effective than that containing BAP i.e. of Jarret *et al.*[3] for shoot regeneration from tuber-derived calluses. Similar findings have been reported on stem and leaf explants of dihaploid clones of *S. tuberosum*[16] and of *S. commersonii*[1].

Table 2: Plant regeneration from tuber-derived callus of two potato cultivars

Culture medium used	Desiree	Maris piper
Time required for shoot appearance (days)		
Jarret *et al.*[3]	n.a.*	n.a.
Iapichino *et al.*[1]	88.25±8.45	75.00±7.23
Ahloowalia[2]	132.00 ±9.95	120.50±8.75
Frequency of calluses producing shoots (%)		
Jarret *et al.*[3]	-	-
Iapichino *et al.*[1]	67.50±6.67	72.25±7.88
Ahloowalia[2]	48.50±5.81	56.25±8.93
Number of shoots regenerated per callus		
Jarret *et al.*[3]	-	-
Iapichino *et al.*[1]	4.20±0.92	5.75±0.78
Ahloowalia[2]	2.70±0.37	2.65±0.41

* = Shoots did not appear even after 175 days of culture.

Table 3: Plant regeneration from leaf-derived callus of two potato cultivars

Culture medium used	Desiree	Maris piper
Time required for shoot appearance (days)		
Iapichino *et al.*[1]	76.75±6.42	63.25±5.97
Ahloowalia[2]	120.00±10.21	112.50±8.66
Lam[4]	98.00±8.83	91.25±9.43
Frequency of calluses producing shoots (%)		
Iapichino *et al.*[1]	87.50±10.11	90.25±9.73
Ahloowalia[2]	52.50±6.95	60.75±7.88
Lam[4]	75.00±10.37	80.50±9.67
Number of shoots regenerated per callus		
Iapichino *et al.*[1]	5.00±2.29	9.25±3.33
Ahloowalia[2]	2.25±1.11	3.75±2.01
Lam[4]	3.50±0.77	4.00±1.85

Table 4: Plant regeneration from stem callus-derived suspension-cultured cells of two potato cultivars

Culture medium used	Desiree	Maris piper
Time required for shoot appearance (days)		
Lam[4]	58.50±5.99	55.25±4.84
Bokelmann and Roest[5]	43.50±4.55	45.00±4.02
Frequency of calluses producing shoots (%)		
Lam[4]	88.25±7.33	92.75±5.29
Bokelmann and Roest[5]	88.50±8.85	95.00±4.13
Number of shoots regenerated per callus		
Lam[4]	3.75±1.17	3.50±1.00
Bokelmann and Roest[5]	4.25±0.72	4.75±0.63

From leaf-derived calluses: As the medium of Jarret *et al.*[3] proved unsuccessful to regenerate shoots from tuber-derived calluses, therefore, to regenerate shoots from leaf-derived callus, the medium was omitted and the medium of Lam[4] was included in this experiment. The calluses changed to a light green colour within 28 days of transferring to all the regeneration media used. Shoot initiation started from the edges of calluses after

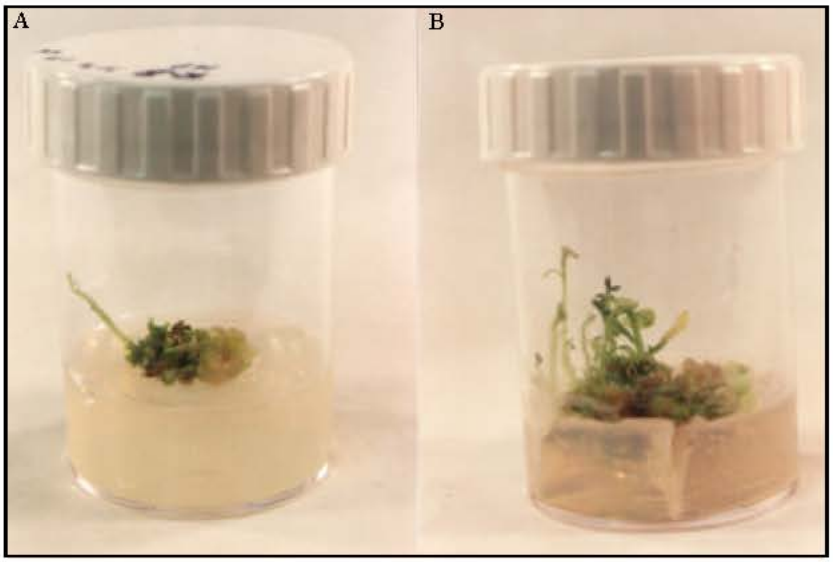

Fig. 1: Multiple shoot development from leaf-derived calluses cultured on lapichino *et al.*[1] medium. Photographed after 90 days of culture. A = Desiree B = Maris Piper

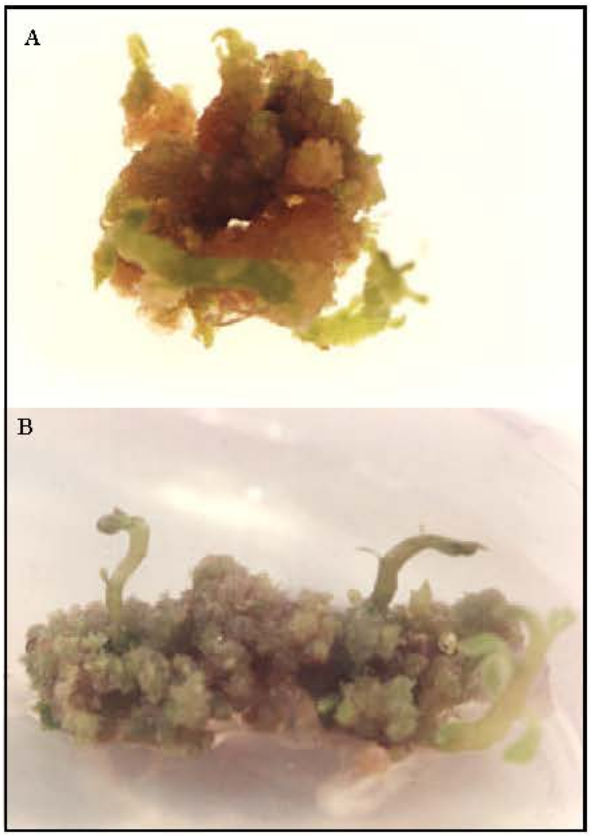

Fig. 2: Shoot regeneration from leaf-derived calluses cultured on the medium of Lam[4]. Photographed after 120 days of culture. A = Desiree B = Maris Piper

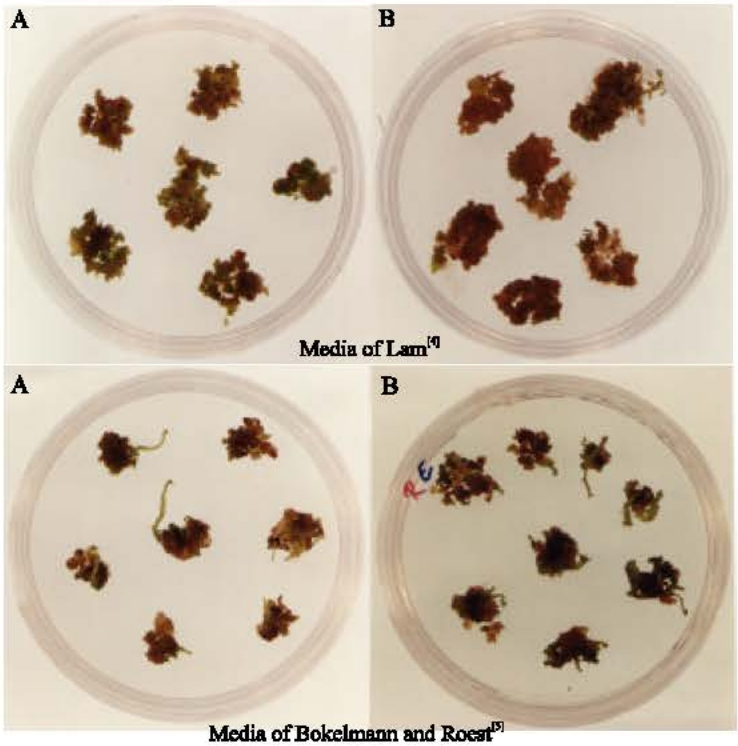

Fig. 3: Shoot regeneration from calluses produced from stem-derived suspension-cultured cells cultured on Lam[4] and Bokelmann and Roest[5] media. Photographed after 70 days. A = Desiree B = Maris Piper

63 days in Maris Piper and 77 days in Desiree on medium of Iapichino *et al.*[1] (Fig. 1). No shoot differentiation was observed on medium of Lam[4] until the 80th day when shoot primordia appeared. Shoots were visible after 91 days in Maris Piper and 98 days in Desiree (Fig. 2). Shoot development on medium of Ahloowalia[2] was achieved after 112 days in Maris Piper and 120 days in Desiree (Table 3).

Frequency of calluses producing shoots and the number of shoots regenerated per callus were higher on the medium of Iapichino *et al.*[1] followed by that of Lam[4], while these were the lowest on the medium of Ahloowalia[2]. As far as the cultivars are concerned, the performance of Cv. Maris Piper was better because regeneration frequency was high and also more number of shoots per callus was regenerated in this cultivar (Table 3).

Shoot regeneration from leaf calluses of both Desiree and Maris Piper was achieved on all the three media tested. Shoots were initiated earlier from the calluses subcultured on the medium of Iapichino *et al.*[1] followed by that of Lam[4], while it took the longest time on the medium of Ahloowalia[2]. The frequency of calluses producing shoots and number of shoots produced per callus was also higher in the same sequence. This was probably due to the concentrations of cytokinin used, which were 2 mg L^{-1} zeatin; 0.2 mg L^{-1} kinetin+0.5 mg L^{-1} BAP+0.5 mg L^{-1} zeatin and 1 mg L^{-1} zeatin in a different auxin to cytokinin ratio, respectively in the above media. Shoot regeneration from leaf calluses was also 1-2 weeks earlier from Maris Piper calluses than from those of Desiree, which indicates that cultivars may vary in their response to shoot regeneration. These results are in accordance with the findings of Lam[4] and Al-Wareh *et al.*[8].

From stem callus-derived suspension-cultured cells: Cell suspensions were plated onto the cell plating medium of Lam[4] to regenerate the calluses from cultured cells. After 4 weeks, when calluses were 2 - 3 mm in diameter, instead of transferring them to a second medium with a reduced concentration of NAA for greening[4,9,10], these were kept on the same medium but the light intensity was increased from 250 lux to 1000 lux. This proved quite useful and calluses became light green, and were then transferred to other media for shoot regeneration. The media chosen for use in present work were; a) medium of Lam[4], developed for shoot regeneration from tuber callus-derived cell suspensions of Cv. Superior and b) medium of Bokelmann and Roest[5], developed for shoot regeneration from protoplast-derived calluses of Cv. Bintje.

Shoot regeneration started 43-45 days after transferring calluses to medium of Bokelmann and Roest[5] and 55-58 days to medium of Lam[4] in both Desiree and Maris Piper (Fig. 3). Frequency of calluses producing shoots was almost same on both the media tested, however, it was higher in Cv. Maris Piper as compared to in Cv. Desiree. On the other hand, number of shoots regenerated per callus was almost similar in both the cultivars tested, however, it was higher on the medium of Bokelmann and Roest[5] than on Lam[4] medium (Table 4).

Regenerated adventitious shoots about 1-2 cm long were excised and transferred to MS medium containing 30 g L^{-1} sucrose+8 g L^{-1} agar+2 mg L^{-1} Ca-pantothenic acid+0.25 mg L^{-1} GA_3 for root formation. Root formation was observed in more than 90% shoots. The rooted plantlets were then transferred to plastic pots containing sterilized compost under high humidity. After 1-2 weeks plants were transferred to pots containing soil mix and moved to a greenhouse under non-sterile conditions, where more than 80% rooted plantlets survived.

To regenerate shoot from calluses derived from suspension-cultured cells of stem origin, both the media used in the present study, were found to be efficient. The medium of Lam[4] has been successfully used by some workers[9,10] for shoot regeneration from stem callus-derived cell suspensions. However, shoot regeneration was 10-15 days earlier from the calluses subcultured on the medium of Bokelmann and Roest[5] than from those subcultured on the medium of Lam[4]. Number of shoots regenerated per callus was also higher on this medium, probably because these media contained different growth regulators in different concentrations. Cytokinins could possibly act through transfer RNA and the medium of Bokelmann and Roest[5] contained zeatin riboside, which was probably readily available to the plant cells.

The regenerated shoots produced roots when transferred to cytokinin-free medium and developed into complete plants when shifted to pots under non-sterile conditions. These results indicate that complete plants can be produced in potato from calluses of different origin depending upon the concentrations and nature of growth regulators in the medium used.

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