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Effect of Culture Medium on Direct Organogenesis from Different Explants of Various Potato Genotypes

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Abstract: Stem segments (about 1 cm in length and without axillary buds) of four potato genotypes, i.e. two cultivars of *Solanum tuberosum*, Desiree and Maris Piper and of two wild species, *S. commersonii* and *S. acaule* and tubers explants of the two cultivars of *S. tuberosum* were cultured on three different regeneration media, as developed by Jarret *et al.*[1], Iapichino *et al.*[2] and Ahloowalia[3]. The medium of Iapichino *et al.*[2] took less time to initiate shoots from stem explants, while shoot regeneration from tuber explants was earlier on the medium of Ahloowalia[3]. Among the genotypes, shoot regeneration (when occurred) was in general quicker in Maris Piper. Shoots from the stem explants of *S. commersonii* were only regenerated on the two media i.e., of Iapichino *et al.*[2] and Ahloowalia[3], while the explants of *S. acaule* failed even to survive on either of the media tested. Regeneration frequency (%) and number of shoots regenerated in both, stem and tuber explants, were higher on the medium of Iapichino *et al.*[2] and in *S. tuberosum* Cv. Maris Piper.

Key words: *In vitro* culture, plantlet regeneration, *Solanum*, shoot initiation

INTRODUCTION

The *Solanum*, like other Solanaceous genera, shows considerable regeneration activity in culture and adventitious shoots can be produced both directly from organ cultures and indirectly via a callus phase under appropriate conditions. Plants regenerated through direct organogenesis (directly from organs) are considered as true to type and don't exhibit somaclonal variation. Organogenesis is highly dependent on the interaction between naturally occurring endogenous growth hormones and exogenous growth regulators added to the culture medium. Depending upon the genotype, the origin of the explant and the culture conditions, it is often necessary to alter the composition and/or concentration of growth regulators in the culture medium. Generally a low ratio of auxin to cytokinin is required for adventitious shoot development. Multiple shoot regeneration from tuber discs was first achieved by Lam[4] on modified MS medium containing kinetin and BAP (6-benzylaminopurine). In a later experiment, he found that the addition of zeatin in the medium resulted in the formation of fully developed shoots[5]. Jarret *et al.*[1] initiated adventitious shoots from tuber discs of 8 out of 10 cultivars tested on modified MS medium supplemented with NAA (γ-naphthalene acetic acid), BAP and GA_3 (gibberellic acid). Later, Kikuta and Okazawa[6] also obtained shoots from tuber explants on modified White's medium supplemented with IAA (indole-3-acetic acid) and zeatin.

Maroti *et al.*[7] reported plantlet regeneration from shoot segments of four potato cultivars; Bintje, Desiree, Gracia and Ostara. The highest number of plantlets was developed from the explants cultured on MS medium supplemented with NAA and kinetin. Later, many workers[8-11] regenerated plantlets from various explants (leaf, rachis, stem pieces) of dihaploid and tetraploid clones using sequential methods. Plants have also been regenerated from stem and leaf explants of wild *Solanum* species. Kaburu M'Ribu and Veilleux[12] regenerated plants of various *in vitro* anther-derived monoploids (2n = x = 12) of *Solanum phureja* using stem and leaf explants under various environmental conditions. Iapichino *et al.*[2] examined the influence of growth regulators on plant regeneration from leaf and stem explants of *S. commersonii*. Various auxins and cytokinins in different combinations were used and nearly all explants produced shoots with an average of 12 shoots per explant when IAA and zeatin were used in the medium.

Merja *et al.*[13] summarized experimental results of their experiment concerning the regeneration, rapid propagation of potatoes through meristem. From five

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different media used, better results for plantlet regeneration were obtained with NAA, IAA and kinetin, especially for Cvs. Premiere, Diamant and Escort. Zaman *et al.*[14] evaluated the effect of three different auxins viz, NAA, IAA and IBA (indole-3-butyric acid) each at four levels (0, 0.1, 0.5 and 1.0 mg L^{-1}) on meristem culture of potato (*S. tuberosum*) for production of virus-free plantlets. Maximum plantlet height (8.3 cm), largest number of nodes/plantlet (7.3) and highest number of leaves/plantlet (8.9) were recorded at 0.5 mg L^{-1} of NAA followed by IBA at 1 mg L^{-1}. Ghaffoor *et al.*[15] also successfully regenerated plantlets from meristem cultures of potato Cv. Desiree on MS medium supplemented with various concentrations (0.0, 0.05, 0.15, 0.25 and 0.35 mg L^{-1}) of NAA, IAA and IBA. These results indicate that the organogenesis in potato is highly dependent on the genotype, origin of the explant, growth regulators added to the culture medium and culture conditions. In the present study, three already developed media were used to evaluate their efficacy on shoot regeneration directly from stem and tuber explants of different potato genotypes.

MATERIALS AND METHODS

In vitro shoot cultures of four potato genotypes, i.e. two cultivars of *Solanum tuberosum*, Desiree and Maris Piper and of two wild species, *S. commersonii* and *S. acaule*, were established as described previously[16]. Stem segments (without axillary buds), about 1 cm in length, were prepared from *in vitro* growing shoots and cultured in petri dishes (Ø 90 mm) containing different regeneration media. Three different media, as developed by Jarret *et al.*[1], Iapichino *et al.*[2] and Ahloowalia[3], were used to regenerate the shoots (Table 1). Five explants were cultured in each petri dish. Twenty explants of each genotype were cultured on each medium and the experiment was repeated four times. Petri dishes were sealed with Parafilm and incubated at 25±1°C in a 16 h photoperiod under a light intensity of 1000 lux.

Tubers of *S. commersonii* and *S. acaule* were not available and cultures were established from seed. Therefore, for shoot regeneration from tubers explants only two cultivars of *S. tuberosum* i.e. Desiree and Maris Piper were used. Explants were prepared from virus-free tubers and sterilized as described previously[17]. Explants were cultured in jars (60 mm in height) containing regeneration medium. Three different regeneration media, as developed by Jarret *et al.*[1], Iapichino *et al.*[2] and Ahloowalia[3], were used. Only one explant was cultured in each culture jar. Ten explants of each genotype were cultured on each medium and the experiment was repeated four times. The culture jars were capped and incubated at 25±1 °C under approximately 1000 lux light intensity in a 16 h photoperiod.

Table 1: Composition (mg L^{-1}) of different media used for shoot regeneration from stem and tuber explants of potato genotypes

Constituents	Jarret *et al.*[1]	Iapichino *et al.*[2]	Ahloowalia[3]
Macronutrients			
NH_4NO_3	1650.0	1650.0	825.0
KNO_3	1900.0	1900.0	950.0
$CaCl_2.2H_2O$	440.0	440.0	220.0
KH_2PO_4	170.0	170.0	85.0
$MgSO_4.7H_2O$	370.0	370.0	185.0
Micronutrients			
$MnSO_4.4H_2O$	22.3	22.3	11.1
$ZnSO_4.7H_2O$	8.6	8.6	4.3
H_3BO_3	6.2	6.2	3.1
KI	0.83	0.83	0.41
$Na_2MoO_4.2H_2O$	0.25	0.25	0.12
$CuSO_4.5H_2O$	0.025	0.025	0.012
$CoCl_2.6H_2O$	0.025	0.025	0.012
$Na_2.EDTA.2H_2O$	37.3	37.3	18.6
$FeSO_4.7H_2O$	27.8	27.8	13.9
Vitamins			
Myo-inositol	100.0	10.0	50.0
Thiamine-HCl	0.1	0.1	0.05
Pyridoxine-HCl	0.5	0.5	0.25
Glycine	2.0	4.0	1.0
Nicotinic acid	0.5	0.5	0.25
Folic acid	0.5	-	-
D-biotin	0.05	-	-
Growth regulators			
IAA	-	1.0	-
2,4-D	-	-	0.5
NAA 0.03	-	-	
BAP	3.0	-	-
GA_3	0.5	-	-
Zeatin	-	2.0	1.0
Supplements			
Adenine sulphate	-	40.0	-
Casein hydrolysate	1000.0	1000.0	-
Sucrose	25000.0	20000.0	10000
Agar	9000.0	10000.0	5000
pH adjusted	5.6±0.1	5.7±0.1	5.9±0.1

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 appearance (days), 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[18].

RESULTS AND DISCUSSION

Plantlet regeneration from stem explants: On medium of Jarret *et al.*[1], after one week of incubation, callus initiation was observed from *S. tuberosum* Cvs. Desiree and Maris Piper and *S. commersonii* and *S. acaule* stem explants, which continued to grow. Shoot initiation was observed after 49 days of culture in Maris Piper and 57 days in Desiree. In *S. commersonii*, explants produced callus but no shoot regeneration occurred. However, some roots were produced. In *S. acaule*, after 42 days of culture, explants became necrotic and after 84 days almost all the explants were dead (Fig. 1).

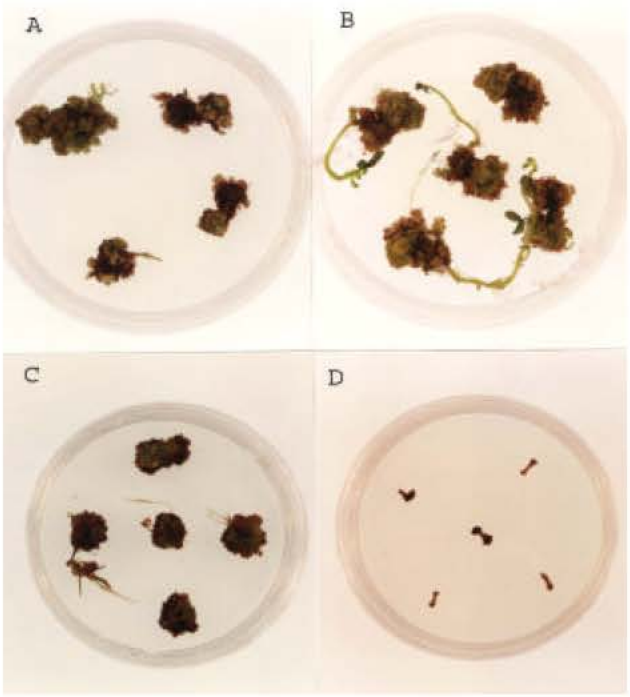


Fig. 1: Shoot regeneration from stem explants (without axillary buds) cultured on Jarret *et al.*[1] medium. Photographed after 70 days of culture (Petri dish Ø 90 mm).
A = Desiree B = Maris piper C = *S. commersonii* D = *S. acaule*

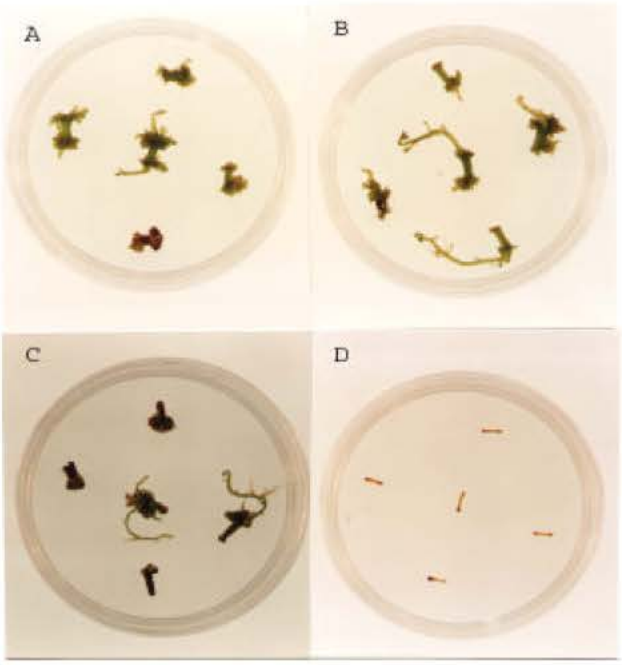
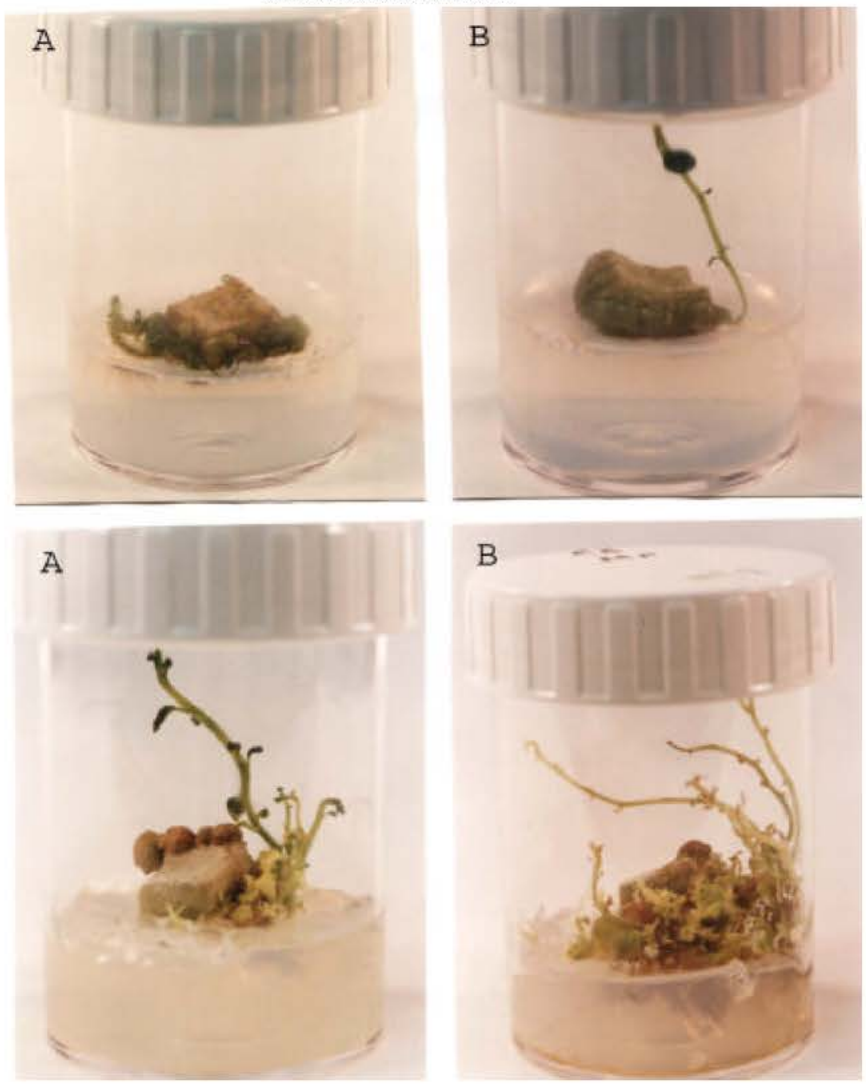


Fig. 2: Shoot regeneration from stem explants (without axillary buds) cultured on the medium of Iapichino *et al.*[2]. Photographed after 56 days of culture (Petri dish Ø 90 mm).
A = Desiree B = Maris piper C = *S. commersonii* D = *S. acaule*

Medium of Ahloowalia[3]



Medium of Iapichino *et al.*[2]

Fig. 3: Shoot regeneration from tuber explants cultured on Ahloowalia[3] and Iapichino *et al.*[2] media. Photographed after 84 days. A = Desiree B = Maris Piper

Table 2: Plant regeneration from stem explants (without axillary buds) of potato genotypes

Culture medium used	Desiree	Maris Piper	*S. commersonii*	*S. acaule*
Time required for shoot appearance (days)				
Jarret *et al.*[1]	57.00±5.48	49.00±6.32	n.a.*	n.a.
Iapichino *et al.*[2]	34.00±5.24	21.50±2.69	41.50±5.72	n.a.
Ahloowalia[3]	41.50±4.15	34.50±4.03	42.25±4.87	n.a.
Frequency of explants producing shoots (%)				
Jarret *et al.*[1]	70.00±14.58	77.50±11.46	0.00±0.00	0.00±0.00
Iapichino *et al.*[2]	78.75±9.60	88.75±8.93	80.00±7.91	0.00±0.00
Ahloowalia[3]	76.25±10.83	82.50±11.46	65.00±9.35	0.00±0.00
Number of shoots regenerated per explant				
Jarret *et al.*[1]	2.00±0.32	1.05±0.23	-	-
Iapichino *et al.*[2]	3.10±0.79	5.05±0.72	2.25±0.40	-
Ahloowalia[3]	2.20±0.31	2.15±0.32	1.10±0.32	-

*n.a. = Shoots did not appear even after 84 days of culture.

Table 3: Plant regeneration from tuber explants of two potato (*S. tuberosum*) cultivars

Culture medium used	Desiree	Maris Piper
Time required for shoot appearance (days)		
Jarret *et al.*[1]	n.a.*	n.a.
Iapichino *et al.*[2]	70.75±4.92	69.75±6.46
Ahloowalia[3]	49.00±4.74	48.50±5.72
Frequency of explants producing shoots (%)		
Jarret *et al.*[1]	0.00±0.00	0.00±0.00
Iapichino *et al.*[2]	87.50±8.29	92.50±8.29
Ahloowalia[3]	80.00±12.25	75.00±11.18
Number of shoots regenerated per explant		
Jarret *et al.*[1]	-	-
Iapichino *et al.*[2]	7.35 ± 1.09	13.75±2.51
Ahloowalia[3]	4.00 ± 0.62	3.05±0.69

*n.a. = Shoots did not appear even after 84 days of culture.

On medium of Iapichino *et al.*[2], swelling at the wound sites of explants appeared after 7 days of culture and multiple shoots with minimal callus appeared after 21 days in Maris Piper. In Desiree, shoots appeared after 34 days and in *S. commersonii* after 41 days. In *S. acaule*, no shoot regeneration occurred and almost all explant were dead after 28 days of culture (Fig. 2).

On the medium of Ahloowalia[3], swellings or protuberances also appeared on cut ends of the explants after 7 days of culture in all the genotypes except *S. acaule*. Multiple shoots were initiated after 34 days in Maris Piper and after about 42 days in Desiree and *S. commersonii*. However, in *S. acaule* there were no signs of shoots initiation and most of the explants were dead within 28 days of culture.

The main difference in shoot regeneration on these media was that on medium of Jarret *et al.*[1], explants first produced callus and then differentiated into shoots, while on medium of Iapichino *et al.*[2] and that of Ahloowalia[3], very little callus was produced and shoots arose from swellings or protuberances on the cut sites of explants. Medium of Iapichino *et al.*[2] also took less time to initiate shoots from explants of the potato genotypes compared with other two media tested.

Stem explants (internodal) cultured on the medium of Jarret *et al.*[1], containing 3 mg L^{-1} BAP, 0.03 mg L^{-1} NAA and 0.5 mg L^{-1} GA_3, first produced callus and shoots were later generated (in *S. tuberosum* only) from that callus. On the other hand, on the medium of Iapichino *et al.*[2] and that of Ahloowalia[3], containing 2 mg L^{-1} zeatin + 1 mg L^{-1} IAA and 1 mg L^{-1} zeatin + 0.5 mg L^{-1} 2, 4-D, respectively, multiple shoots arose from swellings on tissues with a minimum amount of callus formation at the cut edges of the explants. Shoot regeneration (when this occurred) from internodal explants was 1-4 weeks earlier on the medium of Iapichino *et al.*[2] than on the other two media. Several workers have reported BAP to be an effective growth regulator in stimulating organogenesis in different *S. tuberosum* cultivars[4,19]. In the present study, media containing zeatin were more effective than that containing BAP. Similar findings have been reported on stem and leaf explants of dihaploid clones of *S. tuberosum*[20] and of *S. commersonii*[2].

Among the genotypes, shoot regeneration was in general quicker in Maris Piper on all the media tested (Table 2). Shoots from the explants of *S. commersonii* were regenerated on the two media i.e., of Iapichino *et al.*[2] and Ahloowalia[3], while the explants of *S. acaule* failed even to survive on either of the media tested. Therefore, a satisfactory protocol should have to be developed before any experimental work could be performed with *S. acaule*. Several workers have already reported genotypic differences for regeneration ability from explant cultures of potato clones[9, 11, 12].

Frequency of explants producing shoots (%) and number of shoots produced per explant of potato genotypes on different media is given in Table 2. Regeneration frequency was higher in Maris Piper and on the medium of Iapichino *et al.*[2]. Maximum number of shoots per explant was regenerated on the medium of Iapichino *et al.*[2] and minimum on the medium of Jarret *et al.*[1]. Number of shoots produced was greater in the cultivars of *S. tuberosum* as compared to *S. commersonii*.

Plantlet regeneration from tuber explants: After 14 days of culture, explants prepared from Desiree and Maris Piper virus-free tubers turned from creamy white to light green in colour, indicating chlorophyll content. After 28 days of culture, small compact green protuberances, possibly meristemoid bodies appeared along the outer edges of explants and after 42 days, shoot primordia were visible under the dissecting microscope on the explants cultured on medium of Ahloowalia[3]. After 49 days shoots started appearing from the explants (Fig. 3). Shoots appeared after 70 days of culture from explants cultured on medium of Iapichino *et al.*[2] (Fig. 3), but no shoot regeneration occurred from explants cultured on medium of Jarret *et al.*[1]. Frequency of explants producing shoots was higher on medium of Iapichino *et al.*[2]. After 84 days of culture shoot regeneration efficiency was assessed on the basis of the number of shoots per explant. On the medium of Jarret *et al.*[1], no shoots were produced from explants of either Desiree or Maris Piper. On the medium of Iapichino *et al.*[2] on averages of 7.35 and 13.75 shoots per explant were produced in Desiree and in Maris Piper, respectively. While, on the medium of Ahloowalia[3], 4 shoots per explant in Desiree and 3 shoots per explant in Maris Piper were produced (Table 3). Regenerated shoots were removed and transferred to shoot culture medium of Espinoza *et al.*[21] for rooting.

Shoots arose from small protuberances of tissue formed around the edges of the explant 28 days after transfer but the time required for shoot initiation varied with the medium used. Although shoot regeneration was earlier on the medium of Ahloowalia[3], the frequency of explants producing shoots and number of shoots produced were higher on the medium of Iapichino *et al.*[2]. No significant difference in the time required for shoot initiation were observed between the explants of Desiree and Maris Piper (Table 3). The medium of Jarret *et al.*[1], which was developed for shoot regeneration from tuber explants, proved unsuccessful in the present study, while the media of Iapichino *et al.*[2] and Ahloowalia[3], developed for shoot regeneration from internodal explants and callus respectively, proved to be successful. Similar results have already been reported by Wheeler *et al.*[9].

In the present study, both media containing zeatin[2,3] proved successful, while the medium containing BAP[1] failed to generate shoots from tuber explants, which indicates that zeatin is more effective than BAP. Similar results have been reported by Shermann and Bevan[22] in tuber explants of *S. tuberosum* cultivars. Although, Lam[4] induced adventitious shoots from cultured tuber explants on a medium containing 0.8 mg L^{-1} kinetin and 0.4 mg L^{-1} BAP, but in a later experiment, he found that the addition of zeatin in the medium was more effective[5]. Kikuta and Okazawa[6] also obtained shoots from tuber explants using 0.5 mg L^{-1} zeatin and 0.1 mg L^{-1} IAA in the medium.

Genotypic differences for regeneration ability from explant cultures of potato clones have also been reported by other workers[9,11,12]. Due to their morphological differences, the explants do not represent identical tissues and therefore a direct comparison of explants (tuber and stem) may not be appropriate. It is, however, important that in potato various tissues i.e. stem pieces and tuber discs can be used as explants for shoot regeneration directly.

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