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ESTABLISHMENT OF PLANTLETS REGENERATION OF SOME POTATO (*SOLANUM TUBEROSUM* L.) CULTIVARS THROUGH CALLUS FORMATION

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ABSTRACT: *The leaf segments from three in vivo grown potato cultivars namely Desiree, Lady Rossita and Spunta were cultured for callus induction and regeneration on MS medium supplemented with different concentrations of 2,4-D with Kintin and NAA with BAP and GA_3. For regeneration MS modified medium supplemented with 10mg/L GA_3 and 2.24mg/L BAP was used. The best media for callus formation and led to regeneration was modified MS supplemented with 0.2mg/L NAA/L ,2.24mg/L BAP and 10mg/L GA_3. Desiree cultivar gave the highest plant regeneration percentage (77.03%) followed by Spunta (53.5%) and Lady Rosetta (28.45%). The regenerated plantlets were rooted on MS supplemented with 10mg/L GA_3 and 2% sucrose.*

Key words: *Potato (Solanum tuberosum L) cultivars, callus, regeneration, plantlet.*

INTRODUCTION

The tetraploid cultivated potato (*Solanum tuberosum* L.) is the fourth important crop of the world with annual production approaching 300 million tons. The tuber, the most important part of the plant, is an excellent source of complex carbohydrates, proteins, and vitamins (Anjan *et al.,* 2006). Another important export product are varieties of potato seed (Ronald *et al.,* 2002). Also it is one of the most important vegetable crops in Egypt with total production up to two million tons annually (David *et al.,* 2000).

Potato plantlets have successfully been regenerated from callus tissue induced from leaves. Callus induction, subsequent plant regeneration and microtuberization are somehow dependent on the type of explants, media components, growth conditions and varieties(Fiegert *et al.*, 2000).

The highest percentage and minimum time (~8.0 days) of callus formation (95%) was induced with 2.5 mg/L NAA+2 mg/L BAP (Yasmin *et al.,* 2003). The maximum shoot regeneration in two indigenous potato (*Solanum tuberosum* L.) namely Lal Pakri and Jam Alu was observed on MS semi-solid medium supplemented with 1.0 mg/L BAP and 0.1 mg/L GA_3.In addition, it was found

that half strength of MS containing 0.1 mg/L IAA is the best medium for root induction from the excised shoots (Sarker and Barkat, 2002). On the other hand, Khatun *et al.* (2003) cultured the nodal segments from *in vitro* grown plantlets of potato cv. Diamant for callus induction and regeneration. MS semisolid media supplemented with different concentrations of 2,4-D, NAA, BAP separately and NAA in combination with BAP were used . Highest callus formation (90.0%) was observed in MS+2.5 mg/L 2,4-D. The second highest callus induction (83.33%) was recorded in MS+5.0 mg/L BAP. Maximum percentage (70.00%) of calli-induced shoots were observed in MS medium fortified with 5.0 mg/L BAP+0.1 mg/L IBA. The regenerated shoots were rooted on MS and ½MS medium containing different concentrations of IBA. Maximum rooting response was achieved in ½ MS+1.0 mg/L IBA. Regenerated plants were successfully established in soil after acclimatization.

The objective of this study is to detect the best medium to establish a potato tissue culture technique of regeneration from callus. We started tissue culturing using leaves. We used three potato (*Solanum tuberosum* L.) cultivars, Desiree, lady Rosetta and Spunta .

MATERIALS AND METHODS

Potato cultivars:

In the present study three different cultivars of potato (*Solanum tuberosum* L.) namely, Desiree, Lady Rossita and Spunta were used. The cultivars were obtained from Agriculture Research Center (ARC)-Cairo-Egypt.

Callus formation:

Leaves from 15-30 day old plant were selected from *in vivo* plants for initiation and maintenance of callus tissue. The explant sections were washing in soapy water supplemented with drops of tween 20 (polyoxyethlene sorbitan monolaurate) as a wetting agent, dipped in 70% alcohol, sterilized in 0.1% $HgCl_2$ for 5 min, and rinsed three times in sterile distilled water using laminar air flow cabinet and sterilized instruments. The burned tissues were removed. The explants prepared by cutting out both ends, and using the middle section only. Murashige and Skoog (MS) (1962) powder medium (DUCHEFA) supplemented with 1ml MS vitamin (1mg/L Thiamin-HCl, 0.5mg/L Nicotanic acid) and with different kinds and concentrations of plant growth regulators was used. Media composition and modification are presented in (Tables 1 and 2).

The five different media were supplemented with 3% sucrose, 100mg/L Myo-inositol, 1ml MS vitamins (1mg/L Thiamin-HCl, 0.5mg/L Nicotanic acid) and 8.5g TC Agar/L. The pH was adjusted to 5.6-5.7.

Table (1): Composition of basal medium and modified basal medium of Murashige and Skoog (1962).

Constituent	Concentration of basal medium (mg/l)	Concentration of modified basal medium (mg/l)
Macro - nutrients:		
$NH4NO_3$	1650	1650
KNO_3	1900	1900
$CaCl_2.2\ H_2O$	440	440
$MgSO_4.7\ H_2O$	370	370
KH_2PO_4	170	170
Micro-nutrients:		
$MnSO_4.4\ H_2O$	22.3	22.3
$ZnSO_4.4\ H_2O$	8.6	8.6
H_3Bo_3	6.2	6.2
KI	0.83	0.83
$NaMoO_4.2\ H_2O$	0.25	0.25
$CuSO_4.5\ H_2O$	0.025	0.025
$CoCl_2.6\ H_2O$	0.025	0.025
Iron:		
Na_2EDTA	37.25	37.25
$FeSO_4.7\ H_2O$	37.85	37.85
Vitamins:		
Nicotinic acid	0.5	0.5
Pyridoxine-HCL	0.5	0
Thiamin-HCL	0.1	1
Myo-inositol	100	100
Amino acid:		
Glycin	2	0

Table (2).MS media with different growth regulators.

Medium No.	Concentration (mg/L)				
	2,4-D	NAA	6BAP	GA_3	Kin
1	5	-	-	-	1
2	2.5	-	-	-	1
3	1.3	-	-	-	1
4 (MS only)	-	-	-	-	-
5(MS modified)	-	0.2	2.24	10	-

Regeneration culture:

Callus were transferred as clusters onto the surface of regeneration medium containing MS modified medium (Table 2) with 100 mg/1 myo-inositol, 1ml MS vitamin (1mg/L Thiamin-HCl, 0.500mg/L Nicotanic acid), 3% sucrose, 10mg/1 Gibberellic acid (GA_3), 2.24mg/L 6-benzylamiriopurine (BA) and 8.5gm TC Agar per liter.

The callus clusters were incubated at 25±1°C under a photoperiod of 16-hours/day and 3000 Lux for 60 days.

Rooting of regenerated shoots

When the regenerated shoots reached 2-3cm in length (after 70 days from culture) they were transferred to root regeneration medium. Media were composed of MS modified supplemented with 2% sucrose, 100mg/L myo-inositol, 10mg/1 GA_3, 1ml/l Thiamin-HCl, 0.5mg/L Nicotinic acid and 8gm TC Agar per liter. The pH was adjusted to 5.6-5.7. Medium was dispensed into screw top glass containers, plugged with polypropylene closure caps and autoclaved at 121°C (15psi) for 20 min.

RESULTS AND DISCUSSION

This investigation was carried out for callus induction and subsequent regeneration from *in vivo* young healthy leaves of Desire, Lady Rossita and Spunta cultivars of potato (*Solanum tuberosum* L).

Callus formation:

Callus formation from leaf segments was tested on MS medium containing different concentrations of the growth regulators within 10-30 days depending upon the concentrations and combinations of hormone. Table (3) and Fig. (1) illustrate that, among all the other treatments, the highest percentage of callus induction was observed on medium containing

Table (3): Callus initiation of the three tested potato *Solanum tuberosum* L cultivars Desiree, Lady Rossita and Spunta

Cultivar	Desiree				Lady Rosetta				Spunta			
Medium	Total No of used leaf discs explants	Callus mean weight (mg)	No. of initiated callus	Percentage of initiated callus %	Total No of used explants	Callus weight (mg)	No. of initiated callus	Percentage of initiated callus %	Total NO of used explants	Callus weight (mg)	NO. of initiated callus	Percentage of initiated callus %
MS+5mg/l2,4-D+10mg/l GA_3	130	700± 61.39*	78± 6.84*	60 ± 5.26*	105	286± 27.91*	37± 3.61*	35.2± 2.83*	127	200± 17.74*	78± 6.92*	61.4± 4.43*
MS+2.5mg 2,4-D+ 10ml GA_3		802± 70.34*	95± 8.33*	73 ± 6.40*		640± 62.45*	45± 4.39*	42.8± 3.45*		610± 54.12*	95 ± 8.42*	74.8± 5.23*
MS+2.25mg/l 2,4-D+10mg/l GA_3		528± 46.30*	75± 6.57*	57.7 ± 4.38*		300± 29.27*	12± 1.17*	11.4± 0.91*		450± 39.93 *	75 ±	59± 2.61*
MS		0	0	0		0	0	0		0	0	0
MS modified +0.2mg/lNAA ,2.24mg/lBAP and 10mg/l GA_3	135	225± 19.36*	115± 9.89*	85.1± 7.32*	116	195± 18.10*	58± 5.38*	50 ± 4.64*	129	128± 11.26*	93± 8.18	72.1± 6.34*

* Standard Error

Fig.(1): Callus initiation observed on the edgesof the explants of cv. Lady Rossita .

MS modified +0.2mg/L NAA, 2.24mg/L BAP and 10mg/L GA_3. The percentages of callus formation ranged from 50% for Lady Rossita to 85.1% for Desiree. In fact data presented in table (3) showed some what resemblance between two tested cultivars Desiree and Spunta. This was clear through with slight differences in overall. Moreover, MS media failed to support growth of any tested cultivars. Out of our data, it seams that callus mean weight has an important on percentage of callus initiation. This is clear for Desiree cultivar compared with Lady Rossita. Spunta cultivar showed highest percentage (74.8%) of initiated callus on MS + 2.5mg 2,4-D+10ml GA_3 whereas Desiree cultivar gave 73% on the same medium. The callus formation were produced after 15-20 days for Desiree and Lady Rossita respectively. (Table 4). Data presented in table (4) clearly demonstrated the expected variations between the three tested cultivars. Desiree cultivar showed the least time for callus initiation, followed by Spunta and Lady Rossita (15, 18 and 20 days) respectively.

Table(4): Time comparison between cultivars to regenerate

cultivar	Time to callus initiation in days	Time to shoot regeneration in days	Time to produce root in days
Desiree	15±1.3*	23±1.98*	50±4.3
Lady Rosetta	20±1.8*	35±3.2*	70±6.4*
Spunta	18±1.58*	30±2.64*	60 ±5.28*

* Standard Error

Some trends of variations is pronounced regarding shoot and root regeneration. In fact, our obtained results come in agreement with what have been puplishrd befor by several authors (Alphonse *et al.* 1998, Hamdi *et al.* 1998, Wendent *et al.*, 2001 and Yasmin *et al.*, 2003). Where they indicated that callus induction and plant regeneration from explants is dependent somehow on the plant varieties, type of explants, media composition and the presence of appropriate combination and concentrations of plant growth regulators in the culture media.

Regeneration through callus:

The initiated callus were transferred to regeneration medium containing MS modified medium supplemented with 2.24mg/L 6BAP ,10mg/L GA_3. Subculturing was carried out every 15 days for multiplication. Shoots differentiation was observed after 23-35 days from culturing (Table 4 and Fig. 2). Data presented in table (5) show the percentage of obtained plantlets of the tested potato cultivars. Desiree cultivar realized the highest percentage (77.03%). Whereas, Spunta regeneration scored 5305%. Lady Rossita however, came to the last in regeneration ability as it showed 28.45%.

As one might expected, this mainly dependet on variation if potato cultivars (Yasmin *et al.*, 2003).

Fig. (2): Callus regeneration and shoots differentiation in cv.Desiree.

Table (5) : Plant regeneration percentage of Desire, Lady Rosetta and Spunta potato cultivars.

cultivar	No of explants formed callus(a)	No. of obtained callus	No. of regenerated callus culture (b)	regeneration percentage(b/ax100)
Desiree	135	115	104	77.03
Lady Rosetta	116	58	33	28.45
Spunta	129	93	69	53.5

Rooting of regenerated shoots:

After multiplication stage, shoots were dissected and transferred to rooting medium containing MS modified media supplemented with 2% sucrose and 10mg/L GA_3. Regenerated Roots were observed after 50-70 days from culturing of leaf explants (Table 4 and Fig. 3).

(Jayasree e*t al.,* 2001) indicated that various combination of hormonal composition of the media showed significant variation in regeneration ability. In our study the regeneration was observed on MS modified medium supplemented with 2.24mg/L BAP and 10mg/L GA_3. This agree with Sarker and Barkat (2002) who indicated that the maximum shoot regeneration in two indigenous potato was observed on MS medium supplemented with 1.0 mg/L BAP and 1.0 mg/L GA_3. Also, the importance of BAP for shoot regeneration in potato was confirmed by Khatun *et al.* (2003) who found that maximum shoot regeneration was observed on MS medium fortified with 5mg/L BAP+0.1mg/L IBA. The results in the present study support the finding of Abdel-Halim (2006), who demonstrated that the efficiency of callus growth rate and shoot formation to be in part genotype dependent. Also he revealed that, the MS medium in combination with GA_3, BAP and NAA for callus induction, and supplemented with BAP and GA_3 for shoot formation, appeared to be suitable for regeneration plantlets from leaf explants.

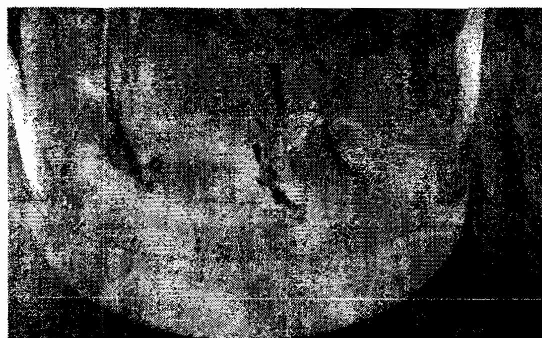

Fig. (3): Rooting of the dissected shoots in cv. Spunta.

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تحديد ظروف تكشف البادرات في بعض أصناف البطاطس من خلال تكوين كالوس.

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الملخص العربى

تم استخدام أجزاء من أوراق نباتات البطاطس أصناف ديزيريه و سبونتا و ليدى روزيتا المزروعة *in vivo* لاستحداث كالس و كذلك عمل تكشف لهذا الكالس. ولقد تم دراسة استحداث الكالس على بيئة MSمزودة بتركيزات مختلفة من 4-D مع Kintin و كذلك كل من NAA و BAP وGA_3 معا. ولقد تم تكشف للكالس الناتج على بيئة MS محورة مزودة ب ١٠مجم /لتر GA_3 و. ٢٤. ٢مجم / لترBAP و٠,٠٢مجم / لترNAA. أما أفضل بيئة لتكون الكالس و ساعدت علي تكشفه لنبيتات فكانت بيئة MS محورة و مزودة ب ١٠مجم /لتر GA_3 و. ٢٤. ٢مجم / لترBAP و٠,٢ مجم / لترNAA .

و لقد أعطي صنف ديزيريه اعلي نسبة تكشف الكالس إلى نبيتات حيث كانت هذه النسبة (٧٧,٠٣%) تلاه صنف سبونتا بنسبة (٥٠,٥٣ %) ثم صنف ليدى روزيتا بنسبة(٢٨,٤٥%).أما النباتات المتكشفة فتم دفعها لعمل جذور علي بيئة MS مزودة ب ١٠مجم /لتر GA_3 و ٢% / لتر سكروز.