EFFECTS OF GENOTYPES, EXPLANTS AND MEDIA ON POTATO CALLUS INDUCTION AND PLANTLETS REGENERATION

By

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ABSTRACT

This study was conducted to evaluate the genetic diversity of some tetraploid potatos (*Solanum tuberosum*) on callus induction, callus weight and plant regeneration. Five genotypes (cultivars) i.e., Arinda, Diamont, King Edward, Nicola and HPS 1/67 were used as a sourse of three explants (stem segments , root-tips and leaf discs). Four media were used for callus induction using MS medium supplemented with different growth regulators, whereas four regeneration media were adapted to investigate the ability of producing regenerates from callus.

Results showed that the potato variety Nicola gave the highest vigorous callus (100%) than other cultivars on CM_4 using stem segments. The same cultivar recorded the highest callus fresh weight (790.0 mg) using stem explants on CM_2. On the other hand, Diamont gave the highest regeneration percentage (67.1%) when callai were cultured on RM_1 compared with King Edward cultivar which yielded 13.5% on RM_3. Meanwhile, no response of Arinda cultivar was recorded using any of the four regeneration media. The results also indicated that Diamont and Nicola stem explants were the efficient genotypes for callus induction on CM_1 for the first and CM_4 for the later.

INTRODUCTION

Potato (*Solanum tuberosum* L.) belongs to the family Solanacea. It is one of the most important and widely grown food crops in the world (Hawkes, 1978). This crop is an integral part of the diet of a large proportion of the world's population. Its production ranks the fifth among major food crops behind wheat, rice, maize and barely (Hooker, 1981).

Potato supplies at least 12 essential vitamins and minerals including an extremely high density of vitamin C; (Thornton and Sieczka, 1980). Potato also provides a significant amount of protein, carbohydrate and iron (Gray and Hughes, 1978).

Potato is a tetraploid, vegetatively propagated crop and therefore, poses several problems for plant breeders. This includes a high level of

heterozygosity and the common occurrence of pollen sterility (Howard. 1978). The autotetraploid and tetrasomic inheritance of common potato (*Solanum tuberosum* spp. tuberosum) 2n = 4x = 48 make the genetic studies and breeding work difficult (Cardi *et al.*, 1992).

The present study aimed to evaluate the tissue culture response characters and the regeneration potentiality of some potato genotypes in order to determine the best conditions for somaclones production.

MATERIALS AND METHODS

I. Materials:

I.A. Potato cultivars:

Five genotypes (cultivars) of potato tubers (*Solanum tuberosum* L.); Arinda, Diamond, King Edward, Nicola and HPS1/67 were kindly obtained from International Potato Center (CIP); Region IV North Africa and Middle East, Kafr El-Zyatt, Egypt.

I.B. Media and reagents:

MS medium pH 5.7 (Murashige and Skoog, 1962) was used and supplemented with different combinations of plant growth regulators (PGRs); i.e. (2, 4-D), (NAA), (BA), (IAA), (GA_3), (Zea) and (KIN) for the following purposes

1-Establishment of *in vitro* micropropagation:

MS, free hormones (Hussey and Stacey, 1981).

2-Callus induction and subculturing media (CM):

Four media were used for callus induction (MS supplemented with different plant growth regulators).

a- CM_1: (Chandra *et al.*, 1981) with some modification in KIN and NAA concentrations (MS + 2.4 mg/L KIN + 0.8 mg/L NAA)

b- CM_2: (Tavazza *et al.*, 1988).

c- CM_3: (Sabbah and Tal, 1990) with some modification in KIN and NAA concentration.

d- CM_4: (Carputo *et al.*, 1995).

3-Regeneration (shooting) media (RM):

Four (RM) media were used to investigate the ability of producing regenerants from the callus.

a. RM_1: (Hassan *et al.*, 1989 and Carputo *et al.*, 1995.

b. RM_2: (Sebastiani *et al.*, 1994).

c. RM_3: (Austin and Cassells, 1983).

d. RM_4 (Wei *et al.*, 1986).

4-Rooting media:

a- MS-free hormones (Sebastiani *et al.*, 1994).

b- MS + 2 mg/L IAA (El-Aref *et al.*, 1996).

Media pH was adjusted to 5.7 and 0.8% agar was added, then the media were sterilized by autoclaving at 121°C for 20 minutes. All supplementations were filter sterilized and added to the media just before solidification.

II. Methods:

II.A. Establishment *in vitro* micropropagation of potato:

The function of this stage is to establish a sterile explant in culture.

1. Tuber pretreatment:

Potato tubers (*Solanum tuberosum* L.) of the five cultivars were maintained in dark condition at 25 ± 1°C for 10-15 days until sprouting. If the tuber was in the dormancy stage, breaking this dormancy was done by soaking the tuber for one hour in 100 mg/l GA_3 and maintained in dark for 10-15 days at 25 ± 1°C until sprouting (Michael, 1996).

2. Sterilization and micopropagation:

The explants were sterilized according to the method of Michael (1996). The sterile sprouts were cultured on MS free hormones as reported by Hussey and Stacey (1981).

II.B. Callus induction:

From *in vitro* cultures, 4 weeks old, stem segments and root-tips (2-5 mm) as well as leaf discs from field grown plants were cut into 0.5 cm^2 with cork borer. All explants were cultured on four callus induction media. Two replicates with five explants for each treatment according to Park *et al.* (1995) were used. Cultures were incubated at 25 ± 1°C in complete darkness for 15 days after that they were incubated at 16 hr photoperiod. Callus induction was recorded after 4 weeks; photographed; weighted and subcultured.

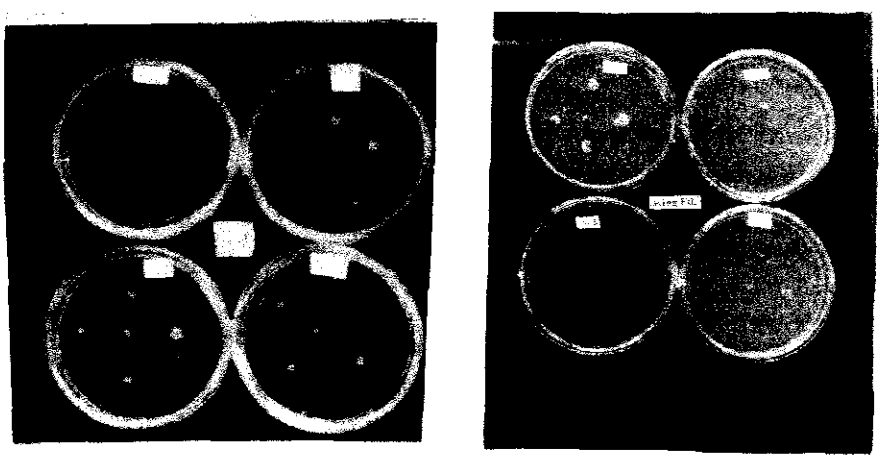
II.c. Plant regeneration:

To test the best media for calli regeneration; 0.5-1.5 cm^2 calli were transferred to the four regeneration media (RM). All cultures were incubated at 25 ± 1°C and 24hr Photoperiod for 2-3 months. The following parameters were recorded; regeneration percentage, number of shoots and albino to green plants. Afterwards, the regenerated shoots were transferred to MS basal medium without growth regulators for rooting (Sebastiani *et al.*, 1994).

RESULTS AND DISCUSSION

1. Effect of genotypes, explants and media on callus induction:

Table (1) presents the callus induction percentages obtained from three different explants of the five potato cultivars cultured on four callus induction media. The data in Table (1) showed that the majority of the stem segment explants (S) [over all tested genotypes] consistently produced higher amounts of calli, using all the different supplemented MS media, with percentages obviously higher than that obtained from either root (R) or leaf discs (L) explants. However, the genotype/callus induction media combinations showed variable trends since Nicola cultivar produced 100% calli from stem segments on CM_4 (Figure 1-a), followed by Diamont (90%) on CM_1 and HPS 1/67 (80%) on CM_4. Generally, the stem explant from Diamont cultivar exhibited the highest mean percentage of callus induction (70.0 ± 7.1), followed by Nicola (67.5 ± 11.8) and HPS 1/67 (60.0 ± 9.1). The lowest two genotypes for callus induction from stem segments were King Edward and Arinda genotypes, since they produced 50.0 ± 9.1 and 40.0 ± 9.1 mean percentages, respectively.



a b

Fig. (1). a- Differential response to callus induction by Nicola stem-explants on different callus induction media
b- Differential response to callus induction by king Edward leaf discs on different callus induction media.

Table (1). Callus induction percentages of the five *Solanum tuberosum* genotypes cultured on four media using three different explants.

Cultivars	Arinda			Diamont			King-Edward			Nicola			HPS 1/67			Media
Explants / Media	S*	R**	L***	S	R	L	S	R	L	S	R	L	S	R	L	$\bar{x}$
CM_1	50.0	10.0	70.0	90.0	60 0	50.0	30.0	10.0	90.0	50.0	10.0	10.0	40.0	60.0	70.0	46.7
CM_2	30.0	0.0	0.0	60.0	20.0	0.0	60.0	40.0	50.0	50.0	20.0	0.0	50.0	40.0	10.0	28.7
CM_3	20.0	0.0	30.0	60.0	0.0	20.0	40.0	40.0	80.0	70.0	0.0	10.0	70.0	70.0	60.0	38.0
CM_4	60.0	0.0	70.0	70.0	30.0	10.0	70.0	30.0	60.0	100.0	30.0	60.0	80.0	90.0	60.0	54.7
$\bar{x} \pm$ S.E.	40.0±9.1	2.5±2.5	42.5±17.0	70.0±7.1	27.5±12.5	20.0±10.8	50.0±9.1	30.0±7.1	70.0±9.1	67.5±11.8	15.0±6.4	20.0±13.5	60.0±9.1	65.0±10.4	50.0±13.5	
Cultivar $\bar{x}$	28.33			39.16			50			34.16			58.336			
Explant $\bar{x}$	S = 57.5						R = 28.0			L = 40.5						

Table (2). Callus fresh weight (mg) of the five *Solanum tuberosum* genotypes cultured on four media using three different explants.

Cultivars	Arinda			Diamont			King-Edward			Nicola			HPS 1/67			Media
Explant / Media	S*	R**	L***	S	R	L	S	R	L	S	R	L	S	R	L	$\bar{x}$
CM_1	37.5	0.5	185.0	336.5	126.5	5.5	15.0	0.5	30.0	610.0	0.5	0.5	105.0	190.0	437.5	138.7
CM_2	98.0	0.0	0.0	120.0	90.0	0.0	85.0	1.0	115.0	790.0	0.5	0.0	370.0	15.0	265.0	129.9
CM_3	91.0	0.0	15.0	176.0	0.0	0.5	85.0	40.0	145.0	610.0	0.0	3.5	265.0	70.0	497.5	33.2
CM_4	127.5	0.0	256.5	185.0	1.5	3.0	178.0	92.5	195.0	741.0	8.0	160.0	406.0	169.0	330.5	190.2
$\bar{x} \pm$ S.E.	88.5±17.0	0.1±0.1	114.1±63.3	204.4±46.3	54.5±31.9	2.25±1.2	90.7±33.3	33.5±21.7	121.2±34.6	687.8±45.9	0.45±0.2	41.0±39.6	286.5±67.5	111 0±41.3	382.6±52.2	
Cultivar $\bar{x}$	67.6			87.1			81.8			243.1			260.0			
Explant $\bar{x}$	S = 271.6						R = 39.9			L = 132.2						

* S : Stem explant ** R : Root explant *** L : Leaf explant

When the root-tips were used as explants of the tested genotypes, no calli were obtained from Arinda cultivar at CM_2, CM_3 and CM_4. The same finding was obtained from Diamont and Nicola cultivars when cultured on CM_3 (Table 1).

On the contrary, HPS 1/67 root-tips proved to be the highest efficient explant to produce calli when compared with the majority of the genotype /media combinations. Regarding the mean percentages of the callus induction from root-tip explants, HPS 1/76 was the superior one (65.0 ± 10.4) followed by King Edward, Diamont, Nicola and Arinda (30.0 ± 7.1, 27.5 ± 12.5, 15.0 ± 6.4 and 2.5 + 2.5, respectively).

With regard to the four tested media, callus induction medium (CM_1) exhibited the highest effect on leaf disc explants from all tested potato genotypes, except Nicola which produced the highest callus percentage on CM_4. Meanwhile, CM_3 exhibited the second efficiency with the same explant from Diamont, King Edward and HPS 1/76 genotypes. The results in Table (1) showed also that the leaf disc explants of King Edward cultivar proved to be the highest calli producer using all four tested media except on CM_4 (Figure 1-b). No calli could be obtained from Arinda, Diamont and Nicola leaf discs when it was cultured on CM_2. The mean percentage of the produced calli from the leaf discs was highest for King Edward cultivar (70.0 ± 9.1) followed by HPS 1/67 (50.0 ± 13.5), Arinda (42.3 ± 17.0. Similar values were obtained for Diamont and Nicola cultivars (20.0 ± 10.8 and 20.0 ± 13.5, respectively).

Out of the above recorded results, it could be concluded that the mean values of callus induction percentages varied erratically between genotype/explant/callus induction media; however stem segments (S) proved to be superior explants at 80 percent of combinations over root-tips (R) and at 70 percent of combinations over leaf disc (L) explants. Moreover, CM_4 proved to be the highest effective medium for callus induction from stem explants descended from Nicola (100%). On the other hand, CM_1 exhibited the highest callus induction percentage from Diamont stem explants (90%) (Table 1). These results are in good harmony with those obtained by Bolwell (1985), Sabbah and Tal (1990), Annenkov and Beluga (1991) and Carputo *et al.* (1995) .They reported that the callus induction from potato explants depended largely on cultivar explant, hormones and their concentrations.

Moreover, the present results confirmed those of Dhingra *et al.* (1987) who found that potato genotypes cultured on medium containing NAA and KIN produced more calli.

2. Effect of genotype, explant and media on callus fresh weight:

The obtained calli from each explant on MS media supplemented with different growth regulators exhibited clear noticeable different sizes. Data in Table (2) showed that the recorded fresh weight of calli obtained from Nicola stem explants was highest on all tested media compared with all other genotypes stem explants. At the same time, the over all mean weight of Nicola stem explant calli in mg. (687.8 ± 45.9) was the highest followed by those belonging to HPS 1/67 (286.5 ± 67.5, Diamont (204.4 ± 46.3); King Edward (90.7 ± 33.3) and Arinda (88.5 ± 18.7). With regard to the different callus induction media, CM_4 produced the heaviest calli from stem segments of HPS 1/67, King Edward and Arinda genotypes. Meanwhile, Diamont and Nicola potato cultivars produced their heaviest calli when each respective stem explant was cultured on CM_1 and CM_2, respectively.

Regarding, root-tips as explants, HPS 1/67 cultivar produced the heaviest calli on CM_1, CM_3 and CM_4, while calli produced from Diamont root-tips were the heaviest from cultivation on CM_2. Moreover, CM_1 produced the highest fresh weight of the produced calli from HPS 1/67 and Diamont root-tips explants followed by CM_4 for HPS 1/67, King Edward and Nicola genotypes, respectively. It is clearly noticed from Table (2) that the potato cultivar HPS 1/67 exhibited the highest mean of callus fresh weight (111.0 ± 41.3 mg) followed by Diamont (54.5 ± 31.9 mg); King Edward (33.5 ± 21.7 mg), Nicola (0.45 ± 0.2 mg) and the lowest weight was from Arinda root-tips (0.1 ± 0.1 mg).

When leaf discs were applied as potato genotype explants, it produced the heaviest calli fresh weight on CM_4 for Arinda, King Edward and Nicola genotypes, i.e., 256.5, 195.0 and 160.0 mg, respectively. However, the remained two genotypes, i.e., HPS 1/67 and Diamont showed their heaviest calli weight on CM_3 and CM_1, respectively. The descending order of the mean weights ± S.E of callus fresh weight from leaf explants were as follows, HPS 1/67, King Edward, Arinda, Nicola and Diamont potato genotypes.

These results confirmed that cultivars, media, explants and their combinations play an important role on callus growth (callus weight). This finding supports previous reports on tetraploid potato by Tavazza *et al.* (1988) who reported that the growth response of the callus obtained was influenced by the different media. Also, Carputo *et al.* (1995) evaluated the response of various wild and cultivated *Solanum* and they found that it was strongly influenced by genotype, explant source and media utilized. They also found high correlations between different tissue culture responses, suggesting linkage and/or pleiotropic effect of genes.

Furthermore, Abe and Futsuhora (1984 and 1986) reported that the existence of significant variations among genotypes in the abilities for callus induction and callus growth (weight) suggested that the callus growth was under genetic control.

3. Effect of genotypes, explants and media on plantlet regeneration:

To study the ability of calli obtained from each explant (stem, root-tips and leaf discs) to regenerate into potato plantlets, they were cultured on different regeneration media. Table (3) presents the percentages of the regenerants from each potato cultivar explant on each regeneration media.

Although no albino plantlets were obtained, however only seven out of 60 different combinations produced green regenerated plantlets. Data in Table (3) clearly showed that no regenerants could be obtained from root-tip calli on any regeneration medium for any potato genotype. The same finding was detected for calli produced from leaf disc explants and for all genotypes with the exception of Diamont genotype leaf discs when cultured on RM_1 since 43.85% of its calli proved to have the ability of development to green regenerants. On the other hand, calli produced from stem segment explants and for all tested genotypes, except Arinda, had developed to green regenerate plantlets on variant MS supplemented media. Diamont stem calli produced green plantlets on RM_1 (Figure 3) with the highest percentage (67.10) and RM_2 with percentage of 54.20. HPS 1/67 genotype stem calli succeeded to regenerate on both RM_2 and RM_4 with 46.15 and 23.05 percentages, respectively. Each of Nicola and King Edward stem calli produced plantlets on either RM_1 or RM_3 with 27.65 and 13.50 percentages, respectively.

Fig. (2): Plant regeneration from Diamont genotype on RM_1

Table (3). Plant regeneration percentages of the five *Solanum tuberosum* genotypes cultured on four media using three different explants.

Cultivars	Arinda			Diamont			King-Edward			Nicola			HPS 1/67			Media
Explant / Media	S*	R**	L***	S	R	L	S	R	L	S	R	L	S	R	L	$\bar{X}$
RM_1	0.00	0.00	0.00	67.10	0.00	43.85	0.00	0.00	0.00	27.65	0.00	0.00	0.00	0.00	0.00	9.24
RM_2	0.00	0.00	0.00	54.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	46.15	0.00	0.00	6.69
RM_3	0.00	0.00	0.00	0.00	0.00	0.00	13.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.90
RM_4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	23.05	0.00	0.00	1.54
$\bar{x} \pm$ S.E	0.00±0.00	0.00±0.00	0.00±0.00	30.32±17.7	0.00±0.00	10.96±10.96	3.37±3.37	0.00±0.00	0.00±0.00	6.91±6.91	0.00±0.00	0.00±0.00	17.30±1.04	0.00±0.00	0.00±0.00	
Cultivar $\bar{x}$	0.00			13.76			1.12			2.3			5.7			
Explant $\bar{x}$	S = 11.58 R = 0.00 L = 2.19															

* S : Stem explant ** R : Root explant *** L : Leaf explant

Since regeneration of potato plantlets from its calli is considered a problem for a long time as reported by Steward and Caplin (1951) and Okazawa *et al.* (1967), however, the obtained plantlets from this study confirmed those previously reported by Lam, (1975) who found that the MS medium supplemented with GA_3, IAA, KIN and BA promotes the regeneration of potato callus to plantlets. This finding is considered a successful step towards using the modern techniques for desirable potato cultivars selection, specially for pathogen resistance. This supports the previous results of Narayanaswamy (1977) who reported that regeneration occurs in a simple medium, containing organic and inorganic elements and phytohormones. He also added that the phytohormones are also dependent upon a number of other factors, such as source of the callus origin; its genotype and age, hormones level and various physical factors.

The obtained results indicated that the regeneration of potato calli is depending largely on cultivar, explant source and media as reported by Chandara *et al.* (1985), Wheeler *et al.* (1985) and Juned *et al.* (1991) on different cultivars from those obtained in the present study.

Furthermore, Carputo *et al.* (1995) found that shoot regeneration was strongly influenced by the genotype, explant source and medium utilized. Moreover, there was also an interaction between the particular regeneration protocol used. Hulme *et al.* (1992) also found that the regeneration of dihaploid potato plants (derived from cultivars Cara and Pentland Crown) depended on using a two-step regeneration method, controlled by heterozygous genes in the tetraploid parents. Ohki *et al.* (1978) found that beside the genotype-hormone interaction, the type of explant and also developmental stage of tomato tissue have an effect on subsequent shoot regeneration and allowed the direct study of gene products.

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الملخص العربى

على أبو شوشة ــ حسن جلال ــ اسماعيل خطاب ــ جمعة الفاضلى
قسم الوراثة ــ كلية الزراعة كفر الشيخ ــ جامعة طنطا

أجريت هذه الدراسة بغرض التقييم الوراثى لبعض أصناف البطاطس رباعية التضاعف لصفات الاستجابة لزراعة الأنسجة (تكون الكالس واعادة التكشف) لخمسة أصناف وهى ارينـــدا وديامنت وكينج ادوارد ونيكولا و HPS1/67 باستخدام ثلاثة بادئات نباتية (مستقطعات من الساق ــ وقمم الجذور والورق) بزراعتها على بيئة موراشيجى وسكوج مضافا إليها أنواع وتركـــيزات مختلفة من منظمات النمو.

وكانت النتائج المتحصل عليها هى:

١. أعطى الصنف نيكولا أعلى نسبة تكوين كالس بنسبة ١٠٠% على بيئة استحداث الكـــالس رقم ٤ باستخدام أجزاء من الساق. وكذلك أعطى نفس الصنف أعلى وزن للكالس على بيئة كالس رقم ٢ (٧٩٠ مجم) باستخدام أجزاء من الساق.

٢. أعطى الصنف ديامنت أعلى نسبة تكوين كالس بنسبة ٩٠% على بيئـــة الكـــالس رقـــم ١ وكذلك أعلى نسبة إعادة تكشف على بيئة رقم ١ بنسبة ٦٧,١% وأعطـــى الصنـــف كنـــج إدوارد ١٣,٥% ولكن الصنف ارينداً لم يعطى أى نسبة لاعادة التكشف.

٣. وبذلك يعتبر الصنف نيكولا على بيئة الكالس رقم ٤ تركيب وراثى فعال فى تكوين الكـــالس والصنف ديامنت على بيئة الكالس رقم ١ تركيب وراثى فعال فى إعادة التكشف على بيئـــة اعادة التكشف رقم ١.