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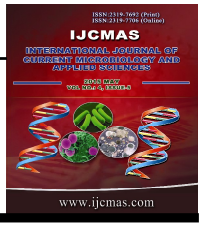

Original Research Article

Effect of Different Medium on Callus Induction and Regeneration in Potato Cultivars

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A B S T R A C T

The present study was undertaken to develop an effective protocol for optimum callus induction and plant regeneration in 4 potato varieties; Arnova, Burren, Provento and Riviera. Different combinations of hormones (2mg/l BA +2.5mg/l NAA; 2mg/l BA+ 2mg/l 2,4-D ; 2mg/l 2,4-D) with control treatment (hormone free) were tested for callus induction. After first, second and third subculture, callus were transferred to regeneration media that contained different combinations of hormones (2.5mg/l BA+5mg/l GA3; 3mg/l BA+0.5mg/l GA3+ 0.03mg/l NAA; 0.22mg/l TDZ+ 0.49mg/l NAA; 5 mg/l TDZ) with control treatment (without hormone). Data of % callus induction, number of days required for callus induction, callus morphology, callus fresh weight, number of days required for regeneration, % regeneration, number of shoots/callus clump, shoot length, number of nodes/shoot and number of leaves/shoot were taken. Stem segments of one clone from Provento, Burren and Riviera were planted on tuberization medium to study the effect of varieties on microtuber induction potential. Results showed that there were significant differences among varieties; Burren and Riviera had the highest % callus induction, fresh weight and number of days for callus induction. A medium containing 2 mg/l BA + 2.0 mg/l 2,4-D and 2 mg/l 2,4-D alone gave good response and a good callus proliferation. The results concerning with regeneration revealed that when callus transferred to regeneration media after first subculture, excellent regeneration was observed in a medium with 3mg/l BA+ 0.5mg/l GA_3 + 0.03 mg/l NAA in Burren , Provento and Riviera, while in Arnova variety shoot regenerated only on media containing 0.22mg/l TDZ+ 0.49mg/l NAA. Shoot formation completely failed when callus after second and third subculture transferred to regeneration media. For microtubrizaition, differences were detected among the cultivars in all characteristics studied except tuber number.

Keywords

Callus, Hormones, Potato, Regeneration, Tuberizaion

Introduction

In tissue culture technique, callus has a great potential due to produced genetic variability which is very important in breeding program. The first attempt to initiate callus was achieved by Chapman (1955) followed by many studies that employed the technique on cells mutant by using mutation and somaclonal variation (Larkin and Scowcroft, 1981; Van Harten *et al*., 1981). The success of callus induction and regeneration is dependent on genotype, the composition of the culture medium and the presence of appropriate combinations and concentration of hormones in the culture media. Many studies have been reported that *in vitro* callus induction was not only dependent on plant species but also on type of explants, light, temperature and explant age (Salehi *et al*., 2008; Mohebodini *et al*., 2011; Shirin *et al*., 2007). In potato, callus has been successfully induced from numerous explant, including leaf, stem segments including 1 or 2 node or without node (internode) and tuber (Khalafalla *et al*., 2010**;** Omidi and Shahpiri, 2003; Shirin *et al*., 2007; Sajid, 2010**;** Yasmin *et al*., 2003). According to the effect of hormones and their concentration on callus induction and regeneration, Khalafalla *et al.* (2010) founded the highest percentage of callus induction (100%) was at 2.0 -5.0 mg/l 2,4-D, Abd Elaleem *et al*. (2009) mentioned that the best degree for callus formation was obtained with 3.0 mg/l 2,4-D or 2.0 mg/l 2,4-D +2.0 mg/l BA, while Yasmin *et al.* (2003) reported that the highest % callus (95%), % regeneration (80%) and number of plantlets (16 plantlets/callus) with shortest period of time to shoot initiation (25.80 day) were obtained with 2.5 mg/l NAA + 2 mg /l BAP. Recent studies have focused on the role of Thidiazuron (TDZ) to establish a high efficiency shoot regeneration system. Sajid (2010) observed that the combinations of 2.46 µM NAA + 1.0 µM TDZ was the best choice to get highest percentage of regeneration in Cardinal and Desiree potato varieties. Abd Elaleem *et al*. (2009) reported that TDZ at 5 mg/l gave the highest % regeneration (81%) and number of shoot per callus (3.4 shoot) in shortest period for shoot formation (15 days).

In Iraq, potato is an important commercial crop. The crop is damaged by many pests and diseases, making it an important to candidate a technique that provide diseases free plants and produce genetic manipulation to improve the existing cultivars and to generate of novel plants. Somaclonal variation through callus culture and in vitro selection has been possible to producing diseases free plants and may be a way of generating useful genetic variation for desired traits. However, few studies have been carried out on local potato cultivars to enhance callus induction and no one of these studies tested the regeneration of plantlets from callus and the factors that affecting callus induction and plant regeneration. So, the present study was undertaken to investigate the *in vitro* callus induction and regeneration with optimum combinations and concentration of plant regulators for four local potato varieties.

Materials and Methods

Potato tubers of Arnova, Burren, Provento and Riviera were brooked the dormancy and sprout. The sprouts were cleaned and sterilized by dipping in 2% sodium hypochlorite for 10 min (Al-Taweel *et al.*, 2004). Shoot tips at 0.1- 0.3 mm with leaf primordial were excised and placed on tube (20x2.5 cm) containing MS semi-solid medium (Murashige and Skoog, 1962). The cultures were incubated in growth room chamber at 25°C±2 under 16 h light and 8 h dark. For further proliferation and

maintenance, plantlets were fragmented and cultured on semi-solid propagation medium (MS salt supplemented with mg/l of 0.4 Thiamine –HCL, 100 Inositol, 2 Glycine, 2 Nicotinic Acid and 1 Indole Acetic Acid and 30 gm/l sucrose). After 3 subculture, the internode cuttings (stem segments approximately 1- 1.5 cm size, without node) from in vitro plantlets were used for callus induction. Intermodal segments inoculated on vessel (15x5 cm) containing MS semi-solid medium supplemented with different combinations of hormones [control treatment (hormone free medium), 2mg/l 2,4-D, 2mg/l BA+2.5mg/l NAA and 2mg/l BA+ 2mg/l 2,4-D]. Calluses were subcultured on selected media every 30 days interval. After first, second and third subculture, callus were transferred to regeneration media that contained different combinations of hormones [control treatment (hormone free medium), 2.5mg/l BA+5mg/l GA3, 3mg/l BA+0.5mg/l GA3+ 0.03mg/l NAA and 0.22mg/l TDZ+ 0.49mg/l NAA; 5 mg/l TDZ]. All cultures were placed in a growth room chamber under the same light and environmental conditions as previously stated. Data of % callus induction, number of days required for callus induction, callus morphology, callus fresh weight, number of days required for regeneration, %regeneration, number of shoots/callus clump, shoot length, number of nodes/shoot and number of leaves/shoot were taken. One clone from each cultivars were fragmented (Figure 2G) and cultured on semi-solid propagation medium for further proliferation and maintenance. For tuberization, stem segments (with 2-3 axillary buds along their length) of one clone from Provento, Burren and Riviera were planted on tube (20x2.5 cm) containing 20ml of semi-solid tuberization medium (MS salt supplemented with mg/l of 0.4 Thiamine –HCL, 100 Inositol, 2 Glycine, 2 Nicotinic Acid, 1 Indole Acetic Acid, 4 Kintein and 80 gm/l sucrose).

All cultures were placed in a growth room chamber at 25±2 °C under 16 h light and 8 h dark photoperiod, after 10 days the cultures were placed in a growth room chamber at 18±2 °C with darkness until microtubers harvest. Data of % tuberization, number, diameter and weight of microtubers were taken after 90 days.

The data was subjected to ANOVA (Analysis of Variance) testing and the mean values were separated by least significance difference (LSD) using standard statistical GenSTAT software.

Results and Discussion

Callus induction

The results showed (Table 1) that explant of all varieties failed to produce callus on hormone free medium tested for callus induction. Callus induction occurred at the cut edges of the explants then callus extension to progressively cover the whole explant, good callus formation was obtained on medium containing 2 mg/l 2,4-D + 2 mg/l BA. A medium containing 2 mg/l 2,4-D alone also gave good response and a good callus proliferation. Those media took less time to initiate callus from stem explant, while the longest days required for callus induction in all varieties were in medium containing 2.5mg/l NAA+2 mg/l BA. Callus induction was noticed with all the genotypes tested, Burren and Riviera gave the highest %callus induction (100%) followed by Provento (88.90%) and Arnova (27.80%) on medium containing 2 mg/l 2,4-D + 2 mg/l BA. The lowest %callus induction in Provento (27.80%), Riviera (11.1%) and Burren (16.70%) were on medium containing 2.5mg/ l NAA+2 mg/ l

BA, while the lowest %callus induction in Arnova (11.1%) was in medium containing 2 mg/ l 2,4-D.

Effect of callus induction medium and varieties on callus fresh weight and callus morphology were presented in table (2). The results showed that the medium containing 2.5mg/l NAA+2mg/l BA looks like retarder for callus formation compared with other media, callus fresh weight reached 55.30, 72.50, 14.40 and 47.20 mg for Arnova, Proveto, Riviera and Burren respectively.

The response of explants were better in concentration of 2 mg/l 2,4-D + 2 mg/l BA, callus fresh weight in this combinations reached 114.1, 302.6, 404 and 548 mg for Arnova, Proveto, Riviera and Burren respectively. From other hand, there was a wide range of variation in callus fresh weight among varieties, the highest callus fresh weight was observed with Burren which was statistically different from other varieties. Differential genotypic ability for callus induction is in agreement with other results published by different authors (Carputo *et al*., 1995; Iqbal *et al*., 2014; Shirin *et al*., 2007; Ud-din *et al*., 2011), this may be due that tissue culture response being under polygenic control (Jia *et al*., 2009).

The data regarding callus morphology shows different colors and texture of callus at different treatments and varieties, most calli were greenish white and green although yellowish white, yellowish green and yellow callus also appeared (Table 2). Most calli textures were compact and it is worth noting that most of the calli were friable with root on medium contained 2 mg/l 2,4-D alone.

In this investigation it was observed that the auxin 2,4-D alone and in combinations with BA was the most effective for callus induction and proliferation. These results agreed with many authors (Abd Elaleem *et al*., 2009; Shirin *et al*., 2007; Ud-din *et al*., 2011), the reason might be due the influence of 2,4-D on cell enlargement and cell divisions are more efficiently than other growth regulators**.** Medium supplemented with 2mg/l 2,4-D+ 2mg/l BA and 2mg/l 2,4-D alone were adopted on further culturing.

Regeneration (shoot formation)

The experiment concerning with the number of subcultures of callus showed a critical step for shoot formation. Second and third subcultures of callus completely failed to have any shoot, while transferred the callus after first subcultures to regeneration medium excellent shoot formation was observed (Table3), this is probably due to morphogenesis *in vitro* is a complex process affected by several endogenous and external factors with cumulative effects (Delporte *et al*., 2014). It seems clear that callus induction medium is very critical for shoot regeneration which was obtained afterward onto regeneration medium (Table 3, Figure 2-3), all cultivars except Riviera failed to form shoots in all interaction regeneration media with callus induction medium containing 2 mg/l 2,4-D while callus induction medium that contained 2mg/l of both 2,4-D and BA showed superiority and proved to be the favorable medium that acts as complementary factor with hormones in regeneration medium for production of the highest shoot regeneration characteristics. From other hand all cultivars failed to form shoot in all interaction of callus induction media and hormone free medium or medium containing 5mg/l TDZ.

Table 1. Effect of different combinations of hormones on % callus induction and number of days required for callus induction.

hormones (mg/l)	% Callus induction				Number of days required for callus induction			
	Arnova	Burren	Riviera	Provento	Arnova	Burren	Riviera	Provento
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2.5 NAA+2 BA	16.70	16.70	11.10	27.80	14.52	14.65	13.76	13.98
2 2,4-D + 2 BA	27.80	100.0	100.0	88.90	11.01	10.67	10.11	10.39
2 2,4-D	11.10	83.30	50.00	88.90	11.91	10.89	9.88	10.78
LSD 0.05	18.77				0.45			

Table 2. Effect of different combinations of hormones on callus fresh weight and callus morphology of potato varieties.

Hormones (mg/l)	Fresh weight (mg)				Callus morphology (color-texture)			
	Arnova	Burren	Riviera	Provento	Arnova	Burren	Riviera	Provento
2.5 NAA+2 BA	55.30	47.20	14.40	72.50	green - compact	greenish white – compact	yello wish white - compact	yellow - compact
2 2,4-D + 2 BA	114.1	548.0	404.0	302.6	greenish white- compact	green - compact	green - compact	greenish white with little purple area, compact
2 2,4-D	36.4	387.7	211.9	316.7	green- friable with roots	whitish green- friable with roots	yellowis h green- friable with roots	greenish yellow with purple area – very friable
LSD 0.05	86.59							

Table.3 Effect of varieties and interactions of callus induction and regeneration media on plant regeneration characteristics.

Hormone concentrations (mg/l)		varieties	% regeneration	No. days required for regeneration	No. shoot/ callus clump	Shoot length (cm)	No. nods/ shoot	No. leaves /shoot
callus induction media	regeneration media							
2 2,4-D + 2 BA	0.00	Arnova	0.00	0.00	0.00	0.00	0.00	0.00
		Provento	0.00	0.00	0.00	0.00	0.00	0.00
		Riviera	0.00	0.00	0.00	0.00	0.00	0.00
		Burren	0.00	0.00	0.00	0.00	0.00	0.00
	2.5 BA+5 GA3	Arnova	0.00	0.00	0.00	0.00	0.00	0.00
		Provento	0.00	0.00	0.00	0.00	0.00	0.00
		Riviera	25.00	35.00	0.33	0.42	0.57	0.73
		Burren	25.00	46.67	1.00	0.75	1.00	1.00
	3 BA+0.5 GA3+ 0.03 NAA	Arnova	0.00	0.00	0.00	0.00	0.00	0.00
		Provento	83.33	36.33	2.17	2.50	2.17	2.50
		Riviera	100.00	21.00	5.92	1.51	2.16	2.63
		Burren	66.67	36.00	2.00	1.83	1.67	2.33
	0.22 TDZ+ 0.49 NAA	Arnova	25.00	46.67	1.00	0.75	1.00	1.00
		Provento	0.00	0.00	0.00	0.00	0.00	0.00
		Riviera	25.00	35.00	0.33	0.37	0.58	0.75
		Burren	0.00	0.00	0.00	0.00	0.00	0.00
	5 TDZ	Arnova	0.00	0.00	0.00	0.00	0.00	0.00
		Provento	0.00	0.00	0.00	0.00	0.00	0.00
		Riviera	0.00	0.00	0.00	0.00	0.00	0.00
		Burren	0.00	0.00	0.00	0.00	0.00	0.00
2 2,4-D	0.0	Arnova	0.00	0.00	0.00	0.00	0.00	0.00
		Provento	0.00	0.00	0.00	0.00	0.00	0.00
		Riviera	0.00	0.00	0.00	0.00	0.00	0.00
		Burren	0.00	0.00	0.00	0.00	0.00	0.00
	2.5 BA+5 GA3	Arnova	0.00	0.00	0.00	0.00	0.00	0.00
		Provento	0.00	0.00	0.00	0.00	0.00	0.00
		Riviera	25.00	35.00	0.30	0.37	0.57	0.70
		Burren	0.00	0.00	0.00	0.00	0.00	0.00
	3 BA+0.5 GA3+ 0.03 NAA	Arnova	0.00	0.00	0.00	0.00	0.00	0.00
		Provento	0.00	0.00	0.00	0.00	0.00	0.00
		Riviera	25.00	30.00	0.33	0.42	0.58	0.75
		Burren	0.00	0.00	0.00	0.00	0.00	0.00
	0.22 TDZ+ 0.49 NAA	Arnova	0.00	0.00	0.00	0.00	0.00	0.00
		Provento	0.00	0.00	0.00	0.00	0.00	0.00
		Riviera	0.00	0.00	0.00	0.00	0.00	0.00
		Burren	0.00	0.00	0.00	0.00	0.00	0.00
	5 TDZ	Arnova	0.00	0.00	0.00	0.00	0.00	0.00
		Provento	0.00	0.00	0.00	0.00	0.00	0.00
		Riviera	0.00	0.00	0.00	0.00	0.00	0.00
		Burren	0.00	0.00	0.00	0.00	0.00	0.00
LSD 0.05			10.63	2.11	0.73	0.28	0.22	0.38

Table.4 Microtubers characters of potato varieties**.**

Varieties	% Tuberization	Tuber numbers	Tuber diameters (cm)	Tuber weight (gm)
Riviera	95.0	1.50	0.59	0.52
Provento	75.0	1.21	0.47	0.38
Burren	73.8	1.05	0.37	0.32
LSD 0.05	19.14	n.s	0.15	0.09

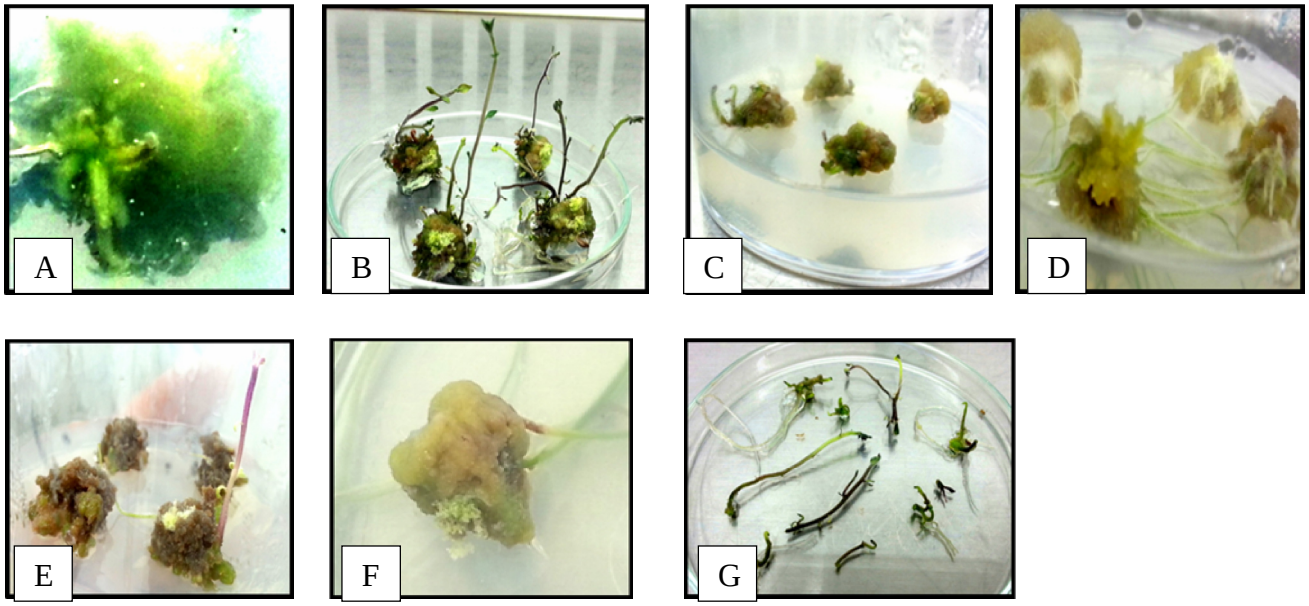

Figure.2 Shoot formation (succeed and failed) for Riviera cultivar; (A) callus medium: 2mg/l BA+ 2 mg/l 2,4-D + regeneration medium: 2.5 mg/l BA+ 5mg/l GA3, (B) callus medium: 2mg/l BA+2 mg/l 2,4-D + regeneration medium: 3mg/l BA+ 0.5 mg/l GA3 + 0.03mg/l NAA, (C) callus medium: 2 mg/l 2,4-D + regeneration medium: 0.22mg/l TDZ+0.49mg/l NAA, (D) callus medium: 2mg/l BA+2 mg/l 2,4-D + regeneration medium: 5mg/l TDZ, (E) callus medium: 2 mg/l 2,4-D+ regeneration medium: 2.5 mg/l BA+ 5mg/l GA3, (F) callus medium: 2mg/l BA+2 mg/l 2,4-D + hormone free medium, (G) fragmented shoots for micro propagating

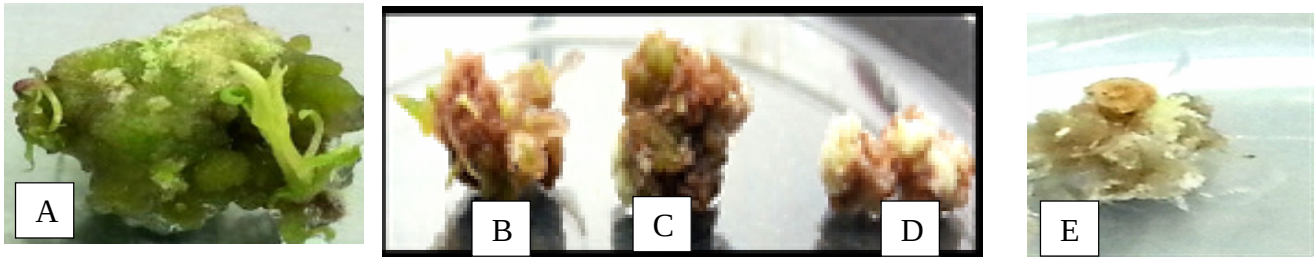

Figure.3 Shoot formation (succeed and failed) for Provento cultivar, (A) callus medium: 2mg/l BA+2 mg/l 2,4-D + regeneration medium: 3mg/l BA+ 0.5 mg/l GA3 + 0.03mg/l NAA, (B) callus medium: 2mg/l BA+2 mg/l 2,4-D + regeneration medium: 2.5 mg/l BA+ 5mg/l GA3, (C) callus medium: 2 mg/l 2,4-D + regeneration medium: 0.22mg/l TDZ+0.49mg/l NAA, (D) callus medium: 2 mg/l 2,4-D + regeneration medium: 5mg/l TDZ, (E) callus medium: 2mg/l BA+2 mg/l 2,4-D + hormone free medium.

Significant differences observed among cultivars in all the tested characters and the best medium for regeneration in all varieties except Arnova was in the interaction between callus induction medium containing 2mg/l of both 2,4-D and BA and the regeneration medium containing 3 mg/l BA+ 0.5 mg/l GA_3 + 0.03 mg/l NAA. Riviera significantly surpassed in %regeneration (100%), number of days required for regeneration (21.00 days), number of shoots/ callus clump (5.92 shoot) and number of leaves/shoot (2.63 leaf), while Provento surpassed in length of shoot (2.50 cm) and number of nodes/shoot (2.17 node). Arnova gave shoot formation only on medium containing 0.22mg/l TDZ+ 0.49mg/l NAA. The differences in response of varieties depends on regeneration media could be due to the kind of endogenous hormonal in cells which control many circumstances expressed by cells (El Far *et al*., 2009). The combinationss of external growth regulators (cytokinins with auxins) are essential requirement to stimulate shoots formation from callus (Anjum and Ali, 2004; Khatun *et al*., 2003; Sajid, 2010). Schmülling *et al*. (1997) mentioned that gene expression can be markedly altered in response to cytokinins, cytokinin target genes are frequently regulated by additional stimuli, such as auxin and the products of the regulated genes play a role in diverse biological processes, such as cell division and chloroplast development. In the present study, GA3 appeared to promote shoot development and elongation, GA3 induce elongation through activation of cell division in the meristem with increased cell wall plastisty (Abdool, 1987).

Microtuberization

Plantlets from regeneration step succeeded to grow and development in all cultivars except Arnova. It was observed that there were significantly differences among the cultivars Burren, Porvento and Riviera in all studied traits except tuber number (Table 4). Riviera surpassed in %tuberization (95%), tuber diameter (0.95 cm) and tuber weight (0.52 g). The differences in response of varieties is in accordance with previous findings of different scientists who reported large variations in the efficiency of microtuber formation between different potato genotypes under the same cultural conditions (Gopal *et al.*, 1998; Srivastava *et al.*, 2012) which seemed to pertain to inherent genetic potential of the genotype

(Bachem *et al.*, 2000; Gargantini *et al.*, 2009).

The present study has established a protocol for callus induction and regeneration. Many factors including the choice of growth regulators in medium for callus induction and regeneration and the subculture were responsible for shoot formation.

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