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Full Length Research Paper

Effect of plant growth regulators on callus induction and plant regeneration in tuber segment culture of potato (*Solanum tuberosum* L.) cultivar Diamant

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The present study was conducted to investigate the effects of different concentrations and combinations of growth regulators on callus induction and plant regeneration of potato (*Solanum tuberosum* L.) cultivar Diamant. The tuber segments were used as explants and cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of α-naphthalene acetic acid (NAA), 2,4-Dichlorophenoxy acetic acid (2,4-D), benzyl adenine (BA) and thidiazeron (TDZ) alone and 2,4-D in combinations with BA for callus induction. The best degree for callus formation (6.0) was obtained on MS medium supplemented with 2,4-D alone at 3.0 mg/l or 2,4-D in combination with BA both at 2.0 mg/l. MS media supplemented with different levels of BA and TDZ were employed for shoot regeneration. MS medium containing 5.0 mg/l TDZ was the best for days to shoot initiation, the highest percentage of callus with shoot (81%) and highest number of shoot per callus (3.4). Callus derived shoots were rooted most effectively in half-strength MS medium containing 0.5 mg/l IBA. The success of plant tissue culture for *in vitro* culture of potato was encouraged by acclimatization of the plantlets in the greenhouse conditions. Regenerated plants were morphologically uniform with normal leaf shape and growth pattern.

Key words: Potato, 2,4-D, callus, regeneration, acclimatization.

INTRODUCTION

Cultivated potato (*Solanum tuberosum* L.) is one of the most important vegetable crops in the world (Solmon-Blackburn and Baker, 2001). It is the fourth most cultivated food crop exceeded only by wheat, rice and maize in world production for human consumption (Ross, 1986). Potato tubers give an exceptionally high yield per acre many times that of any grain crop (Burton, 1969) and are used in a wide variety of table, processed, livestock feed and industrial uses (Feustel, 1987).

In Sudan potato is grown as a winter crop. It is a relatively new crop to Sudan as it was introduced in late 1930's (El-hassan, 1989). Its production started in Khartoum state and later spread to other part of the country (Aisha, 2007).The area under potato cultivation in Sudan has been increasing over the past several years with enhanced production. In 1989 potato production area was about 3,000 ha with an average yield of 5 - 12 tons/ha. In 2004 the area under potato cultivation became about 20,000 ha with an average yield of 15 tons per ha (Aisha, 2007). Despite the tremendous increases in the average yield and area under cultivation still potato production in Sudan lag behind that of the world average. In Sudan potato production is seriously hampered due to the attack of fungus and bacterial diseases causing huge loss in crop during the cultivation and storage.

Biotechnology can contribute to solution of these problems and realize great benefit to potato farmers. The regeneration of plants from cell and tissue culture represent an essential component of biotechnology and have the potential not only to improve the existing cultivars, but also for the generation of novel plants in a comparatively

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short time compared to conventional breeding.

Much works has been carried out on potato to enhance callus induction, improve the frequency of plant regeneration from the callus and investigate the factors affecting plant regeneration. Both callus induction and plant regeneration from explants require the presence of appropriate concentrations and combinations of plant growth regulators in the culture media. Many researchers work to standardize the optimum concentrations of growth regulators for regeneration of potato and consequently great progress has been made in potato callus induction and plant regeneration (Ahloowalia, 1982; Dobranszki et al., 1999; Hansen et al., 1999; Ehsanpour and Jones, 2000; Fiegert et al., 2000; Khutan et al., 2003; Yasmin et al., 2003; Shirin et al., 2007).

This study was undertaken to standardize the protocol for callus induction and plant regeneration for the Diamant cultivar of potato widely grown in the Sudan, which would be efficient and suitable for the investigation of induced somaclonal variation and successful application of gene transfer technique. Based on the study the influence of auxins and cytokinins on the callus induction and subsequent plant regeneration was observed and dis-cussed.

MATERIALS AND METHODS

Plant material

Tubers of potato cultivar Diamant were obtained from Horticulture Sector and Ministry of Agriculture. Sudan kept under culture room conditions and used as a source for explants throughout the experiment.

Explants and surface sterilization

Tubers were surface sterilized first by washing under running tap water and laundry bleach for 20 min. then sprayed with 70% alcohol and cleaned with a clean towel before transfer to a laminar flow. Under a laminar flow tubers were cut into pieces and surface sterilized by immersing in 70% alcohol for 1 min, washed three times with sterilized distilled water to remove the trace of alcohol then immersed in 25% (v/v) sodium hypochlorite solution supplemented with 2 drops of liquid soap for 20 min and finally rinsed five times with sterilized distilled water. Disinfested tuber pieces were cut to 1.0 cm^2 segments then put on sterilized paper tissue in sterilized Petri dishes as explants ready for inoculation.

Inoculation, callus induction and regeneration

Explants were cultured in culture bottles containing MS (Murashige and Skoog, 1962) basal media supplemented with different concentrations of auxins (2,4-D, NAA and 1BA), cytokinins (TDZ and BAP) and combinations of 2,4-D and BAP for callus induction. The explants were inoculated on callus induction media for 4 - 6 weeks. The calli were transferred to the fresh callus inducing media every 21 days interval for further proliferation and maintenance. After 6 - 8 weeks of incubation in the dark, the callus induction frequency was determined and well developed calli were selected and sub-cultured on regeneration media. MS medium was supplemented with different concentrations (0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 5.0 mg/l) of BA and TDZ for shoot regeneration at 25 ± 2°C with a 16 h photoperiod.

Rooting of *in vitro* induced shoots

Regenerated shoots were excised from calli and transferred to MS,1/2 MS and ¼ MS media with different concentration (0.25, 0.5 or 1.0 mg/l) of IBA for rooting. All the media used in this study were supplemented with 3% (w/v) sucrose, solidified with 0.8% (w/v) agar and the pH was adjusted to 5.8 ± 0.1 with 1 M NaOH before autoclaving at 121°C and 15 lb psi for 15 min.

Acclimatization

In vitro rooted plants were removed from rooting medium and washed to remove adhering gel and transplanted to plastic pots containing autoclaved garden soil and sand at 3:1 ratio and covered with bottle. Plants were kept under culture room conditions for 15 days then transferred to green house and placed under shade until growth was observed.

Statistical analysis

Results on days to callus initiation, percentage of explants formed callus, callus texture, callus color and degree of callus formation were recorded in callus induction experiment. For shoot regeneration, Data on days for shoot initiation, percentage of callus with shoot, number of shoot per callus and the shoot length were recorded. The parameters recorded for rooting are number of shoot rooted, number of root per shoot, root length and rooting percentage. Data were collected at regular intervals from three independent experiments and subjected to analysis of variance and presented as average ± standard error (SE).

RESULTS AND DISCUSSION

Tuber segment explants of Diamant cultivar of potato were cultured on MS media containing different concentrations of 2,4-D, NAA, BA and TDZ alone and 2,4-D in combinations with BA. Data were analyzed after six weeks of culture and the result showed that there was a wide range of variations in days to callus initiation, percentage of explants developed callus, callus texture, callus color and degree of callus formation depending on culture media formulations (Tables 1 and 2). Callus initiation on cut ends of *in vitro* cultured explants could be observed in all hormone combinations after 7 - 20 days.

However, the explants cultured on MS medium without growth regulators did not produce any callus. These results are in support of the results obtained by Fiegert et al. (2000), Jayasree et al. (2001) and Yasmin et al. (2003). Among all the growth regulators used 2,4-D was found to be the most effective growth regulator for potato callus induction either when used a lone or in combinations with cytokinins (BA). Castillo et al. (1998) reported that auxin 2,4-D by itself or in combination with cytokinins has been widely used to enhance callus induction and maintenance. Moreover, many researchers observed 2,4-

Table 1. Effect of different concentrations of α-naphthalene acetic acid (NAA), 2,4-dichlorophenoxy acetic acid (2,4-D), benzyladenine (BA) and thidiazeron (TDZ) on the callus induction of potato.

Growth regulator (mg/l)	Days to callus initiation	% of explants formed callus	Callus texture	Callus color	Degree of callus formation
NAA					
0.0	-	0.0	-	-	0.00 ± 0.00
0.5	-	0.0	-	-	0.00 ± 0.00
1.0	19	100	friable	light green	2.50 ± 0.13
1.5	19	100	friable	green	1.94 ± 0.27
2.0	19	100	friable	light green	3.19 ± 0.28
3.0	15	100	friable	light green	4.44 ± 0.26
4.0	12	100	friable	light green	4.50 ± 0.22
5.0	12	100	friable	light green	5.81 ± 0.10
2,4-D					
0.5	19	100	watery	yellow	2.89 ± 0.28
1.0	16	100	watery	yellow	3.20 ± 0.00
1.5	16	100	watery	yellow	4.50 ± 0.10
2.0	15	100	watery	yellow	5.50 ± 0.10
3.0	15	100	friable	yellow	6.00 ± 0.00
4.0	15	100	friable	yellow	4.00 ± 0.00
5.0	12	100	friable	yellow	4.00 ± 0.00
BA					
1.0	15	100	hard	green	2.00 ± 0.10
2.0	17	100	hard	green	2.30 ± 0.10
3.0	18	100	hard	green	2.20 ± 0.10
4.0	18	100	hard	green	2.40 ± 0.10
5.0	18	100	hard	green	3.10 ± 0.00
TDZ					
1.0	14	100	hard	green	1.90 ± 0.00
2.0	15	100	hard	green	2.50 ± 0.10
3.0	17	100	hard	green	3.00 ± 0.00
4.0	15	100	hard	green	2.60 ± 0.10
5.0	13	100	hard	green	3.10 ± 0.00 Each

Value represents the mean ± standard error (S.E.) of ten replicates per treatment in three repeated experiments.

D as the best auxin for callus induction as common as in monocot and even in dicot (Evans et al., 1981; Ho and Vasil, 1983; Jaiswal and Naryan, 1985; Chee, 1990; Mamun et al., 1996).

Within the different concentrations of 2,4-D when used alone, the highest degree (6.0) for friable yellow callus formation from the tuber segment was recorded in the MS medium supplemented with 3.0 mg/l (Table 1 and Figure 1a). This result is in agreement with Shirin et al. (2007) who used 2,4-D for callus induction from internodal and leaf explants obtained from four potato cultivars including Diamant and found that among all concentrations and combinations 2,4-D at 3.0 mg/l was found to be the most effective auxin concentration for callus induction in all cultivars.

Cytokinins, such as BA and kinetin, at low concentrations, in combination with auxins were often used in plant species to promote callus initiation (Chai and Mariam, 1998). In order to evaluate the effect of BA on callus induction, the results were recorded for tuber segments cultured on MS medium supplemented with different combinations of BA and 2,4-D. Callus initiation was observed between 7-20 days depending on concentrations and combinations. The best result (6.0) for degree of callus formation was obtained when 2,4-D at 2.0 mg/l was used in combination with BA at the same concentration (Table 2).

In this investigation it was observed that 2,4-D when used in combination with BA produced friable green callus in shorter period (Shortened by one week) than when it used a lone (Tables 1 and 2). After sufficient callus induction, the explants were initiated subsequent organogenesis when were sub-cultured on MS medium supplemented with different concentrations of BAP and TDZ (Table 3). The necessity of cytokinins for shoot initiation is well documented (Beck and Coponetti, 1983;

Table 2. Effects of 2,4-dichlorophenoxy acetic acid (2,4-D) in combination with benzyladenine (BA) on callus induction of potato.

Growth regulator (mg/l)		Day to callus initiation	% of explants formed callus	Callus texture	Callus color	Degree of callus formation
2,4-D	BA					
0.5	0.5	10	100	watery	yellow	1.50 ± 0.2
0.5	1.0	8	100	watery	yellow	2.90 ± 0.1
0.5	2.0	7	100	watery	yellow	3.60 ± 0.1
0.5	5.0	20	100	hard	green	3.50 ± 0.1
1.0	0.5	8	100	watery	yellow	3.80 ± 0.1
1.0	1.0	9	100	watery	yellow	4.00 ± 0.1
1.0	2.0	10	100	hard	green	4.10 ± 0.1
1.0	5.0	17	100	hard	green	4.00 ± 0.2
1.5	0.5	7	100	friable	light yellow	4.40 ± 0.1
1.5	1.0	8	100	friable	light yellow	4.90 ± 0.2
1.5	2.0	8	100	friable	yellowish	5.80 ± 0.1
1.5	5.0	15	100	friable	green	5.10 ± 0.2
2.0	0.5	8	100	friable	yellowish	5.90 ± 0.1
2.0	1.0	8	100	friable	yellowish	5.90 ± 0.1
2.0	2.0	8	100	friable	green	6.00 ± 0.0
2.0	5.0	10	100	friable	green	5.00 ± 0.3
3.0	0.5	8	100	friable	yellowish	5.30 ± 0.2
3.0	1.0	9	100	friable	yellowish	5.40 ± 0.1
3.0	2.0	10	100	friable	green	5.30 ± 0.1
3.0	5.0	10	100	friable	green	5.00 ± 0.3

Each value represents the mean ± standard error (S.E.) of ten replicates per treatment in three repeated experiments.

Table 3. The effect of different concentrations of benzyladenine (BA) and thidiazeron (TDZ) on potato's callus regenerations.

Growth regulator (mg/l)		Day to shoot initiation	% of callus with shoot	Number of shoot per callus	Shoot length (cm)
BA	TDZ				
1.0	0.0	- (callus)	0	0.0 ± 0.0	0.0 ± 0.0
1.5	0.0	- (callus)	0	0.0 ± 0.0	0.0 ± 0.0
2.0	0.0	56	25	0.4 ± 0.2	0.5 ± 0.3
3.0	0.0	68	25	0.8 ± 0.3	0.5 ± 0.3
4.0	0.0	54	45	2.9 ± 0.6	1.7 ± 0.3
5.0	0.0	52	50	1.9 ± 0.8	1.5 ± 0.3
0.0	1.0	38	25	0.1 ± 0.1	0.1 ± 0.1
0.0	1.5	21	49	1.0 ± 0.4	0.5 ± 0.2
0.0	2.0	20	50	2.5 ± 0.6	0.7 ± 0.2
0.0	3.0	20	50	1.6 ± 0.3	0.9 ± 0.2
0.0	4.0	19	66	1.3 ± 0.3	0.5 ± 0.2
0.0	5.0	15	81	3.4 ± 0.9	1.4 ± 0.3

Evans et al., 1984). The best result for the percentage of callus with shoot (81%) and mean numbers of shoots per callus (3.4 ± 0.09) were recorded on MS medium supplemented with 5.0 mg/l TDZ (Figure 1b).

In vitro induction of roots in potato regenerated shoots was studied by using various concentrations of IBA with different MS medium salt strengths (Table 4). The rooting response varied with basal media strength and IBA concentrations. All four concentrations of IBA with the three MS strength were capable to induce roots in the regenerated plantlets along with the induction of some callus. However, the root formation was not observed when shoots were cultured on a medium lacking auxin. In the present experiment the usage of IBA at 0.5 mg/l was found to be capable to induce 100% rooting of shoots cultured in all basal media strengths used.

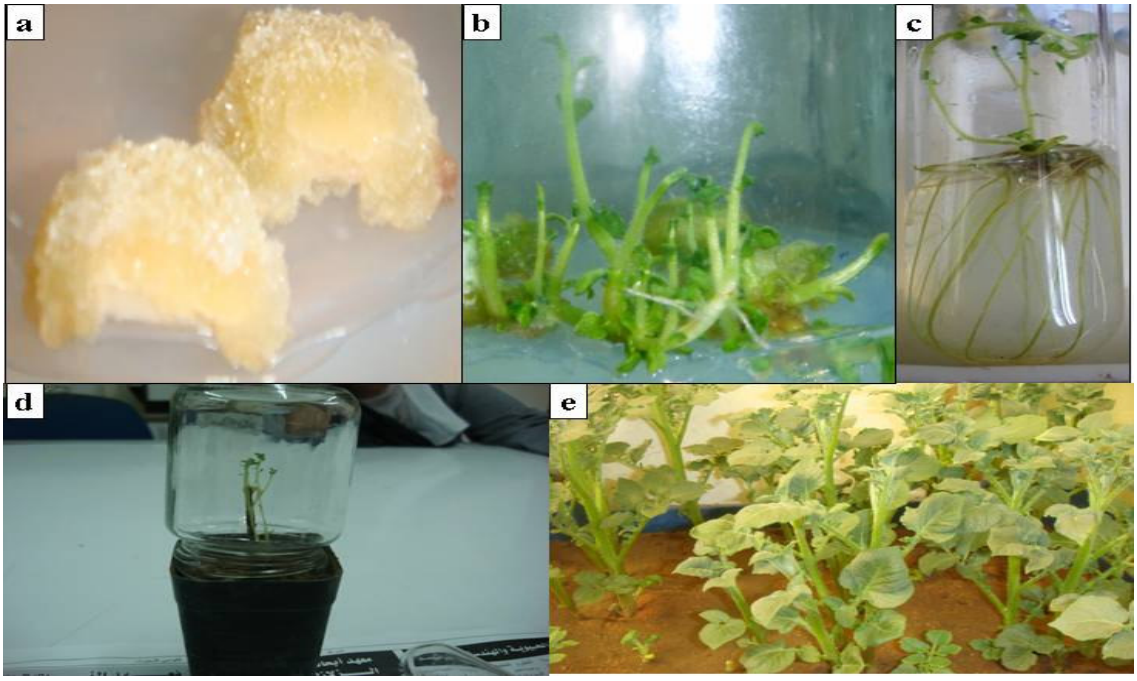

Figure 1. Callus induction and plantlet generation from tuber segment explants of potato *(Solanum tuberosum* L.) cultivar Diamant. a - Callus formation in MS medium supplemented with 3.0 mg/l 2,4-D after four weeks of culture. B - Shoot regeneration in MS + 5.0 mg/l TDZ after six weeks of sub-culture. C - Regenerated plantlets with well developed roots induced on ½ MS+ 0.5 mg/l IBA. d - Acclimatization of plantlet under culture room conditions. e- Potato plant established in soil under green house conditions.

Table 4. Effect of different concentrations of IBA and MS salt strength on rooting percentage, number of root per shoot and root length in potato.

Auxin (IBA) (mg/L)	Basal media strength	Rooting percentage	Number of root per shoot	Root length (cm)
0.0	MS	0.0	0.0	0.0
0.5	1/4MS	100	12.3	5.7
1.0	1/4MS	100	9.3	7.2
1.5	1/4MS	80	7.0	1.8
2.0	1/4MS	80	3.3	1.6
0.5	1/2MS	100	34.1	13.7
1.0	1/2MS	80	21.8	4.5
1.5	1/2MS	90	17.9	1.6
2.0	1/2MS	90	24.1	2.3
0.5	MS	100	19.8	12.3
1.0	MS	100	18.6	6.4
1.5	MS	80	15.8	2.3
2.0	MS	80	12.5	2.0

However, the best results for number of root per shoot (34.1) and root length (13.7) were obtained by using half-strength MS medium supplemented with IBA at 0.5mg/l (Table 4 and Figure 1c). The beneficial effect of using IBA and half-strength MS for *in vitro* rooting has already reported for potato by Khatun et al. (2003).

For acclimatization, the rooted shoots were removed from the culture bottle, washed thoroughly to remove remnants of agar from roots and transplanted to small pots containing garden soil and sand (1:1, v/v). Plants were covered with bottle to ensure high humidity while irrigating regularly and kept for three weeks under culture room conditions (Figure 1d). Thereafter, the plantlets were transferred to green house, where the plants ap-

peared morphologically uniform with normal leaf form, shape and growth pattern (Figure 1e).

In conclusion, the system established in the present study for tissue culture of potato can get enough callus and plant regeneration efficiency to perform transgenic operation. Moreover, as the potentiality of shoot multiplication from callus continued for a long time, regenerates may be characterized by somaclonal variation and giving birth to traits of agronomic importance.

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