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REGULAR ARTICLE

CALLUS FORMATION AND ORGANOGENESIS OF POTATO (*SOLANUM TUBEROSUM* L.) CULTIVAR ALMERA

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SUMMARY

A procedure for plant regeneration from callus culture of potato, *Solanum tuberosum* L. is described. Calli were induced from 1.0 cm^2 tuber segment of potato cultivar Almera on Murashige and Skoog's medium (MS) supplemented with different levels (1.0-5.0 mg/l) of 2, 4-dichlorophenoxy acetic acid (2, 4-D). The highest degree of callus formation (3.0) and hundred percent (100%) of explants produced nodular calli on MS medium within 7-12 days when supplemented with 2.0-5.0 mg/l of 2, 4-D. Calli were differentiated into shoot-primordia when subcultured on MS medium supplemented with 1.5 -5.0 mg/l of thidiazuron (TDZ) and 2.0-5.0 mg/l of benzyladenine (BA). The best result for number of shoot per callus (3.3 ± 0.3) and longest shoot (0.8 ± 0.1) were obtained by using TDZ at 5.0 mg/l. Callus derived shoots were rooted most effectively in full-strength MS medium containing 1.0 mg L^{-1} 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: Almera, *Solanum tuberosum*, Callus induction, 2, 4-D, TDZ, Organogenesis

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1. Introduction

Potato (*Solanum tuberosum* L.) is a crop of high biological value for its protein and a substantial amount of vitamins, minerals and trace elements. [1]. It is the fourth most cultivated food crop exceeded only by wheat, rice, and maize [2 and 3]. The edible part of the plant is the tuber, which is formed at the end of underground stems called stolon. It is used as cheap food, industrial raw material, animal feed, and seed tuber. Potato produced more protein and calories per unit area per unit time and per unit of water than any other major plant food [4].

In Sudan potato is a relatively new crop as it was introduced in late 1930's [5]. Its importance in the Sudan agricultural economy significantly increased in terms of area and productivity during the past two decades. 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 [6]. Potato is prone to several fungal, viral and bacterial pathogens and causes heavy economic loss every year during the cultivation and storage. Conventional breeding technique has a boundary of limitations. In the case of inbreeding, the progress in the improvement is hampered because of lacking of genetic variability.

Potatoes breeding programs can highly benefit of biotechnological tools, which are capable of surpassing some limitations found by traditional plant breeding methods and open new avenues for crop improvement. The success of plant biotechnology relies on several factors which include an efficient tissue culture system for regeneration of plants from cultured cells and tissues [7].

Shoot generation and rooting are important in the realization of the potential of the cell and tissue culture techniques for plant improvement [8]. Plant regeneration in

potato has been progressed a lot in recent years [9]. Successful *in vitro* plant regeneration has been achieved from explants of different organs and tissues of potato such as leaf [10], stem [11, 12], tuber discs [13, 14] and unripe zygotic embryos [15].

The aim of the present study was to establish an effective protocol for callus induction and rapid plant regeneration from tuber segment explants of potato cultivar Almera one of commercial crop in Sudan.

2. Materials and Methods

This study was carried out in the Laboratory of Plant Cell and Tissue Culture, Commission for Biotechnology and Genetic Engineering, National Center for Research.

Plant material

Certified seeds of potato cultivar Almera used in this study were obtained from the Horticulture Sector, Ministry of Agriculture and Forestry, Sudan. Seeds were 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 cm2 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 [16] basal media supplemented with different concentrations of 2, 4-D 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 and 1/2 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).

3. Result and Discussion

Reliable callus induction and regeneration of viable plants considered as a limiting steps to the successful use of modern

techniques in genetic improvement of the major crops [17]. Here in this study in order to induce callus, tuber segment explants of potato cultivar Almera were cultured on MS media containing different concentrations of 2, 4-D. Data were analyzed after six weeks of culture and the result showed that, the tuber segment explants induced callus on MS medium supplemented with 2, 4-D. On the other hand failed to produced callus on MS medium without 2, 4-D, this declared that, the presence of 2, 4-D. was capable to inducing callus. The auxin 2, 4-D, by itself or in combination with cytokinins, has been widely used to enhance callus induction and maintenance [18]. Moreover, many researchers observed 2, 4-D as the best auxin for callus induction as common as in monocot and even in dicot [19, 20, 21, 22, and 23]. Depending on 2, 4-D concentration there was a range of variations in days to callus initiation, percentage of explants developed callus, callus texture, callus color and degree of callus formation (Table 1). Callus initiation on cut ends of *in vitro* cultured explants could be observed in all 2, 4-D levels after 7-17 days. Similar findings were reported by [24, 25 and 26]. The result showed that, when the MS media were supplemented with the highest concentration (2.0 -5.0 mg/l) of 2, 4-D, 100% of the explants were formed yellow, and watery callus and recorded the highest degree (3.0 ± 0.0) for callus formation (Table 1, fig.1a). This result is in agreement with [9] who used 2, 4-D for callus induction from internodes and leaf explants of four potato cultivars and found that among all concentrations, only 2, 4-D at high concentration (3.0 mg/l) was found to be the most effective concentration for callus induction in all cultivars used.

Figure 1. Callus induction and plantlet generation from tuber segment explants of potato *(Solanum tuberosum* L.) cultivar Almera. 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 - Potato plant established in soil under green house conditions.

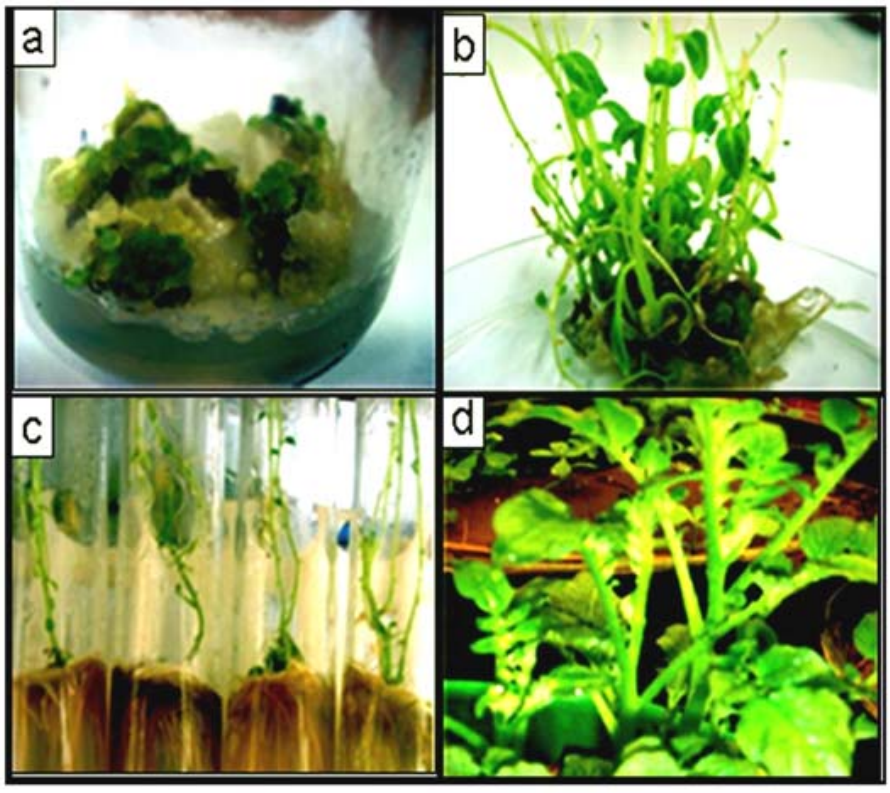

Table 1. Effect of different concentrations of 2, 4-dichlorophenoxy acetic acid (2, 4-D) on the callus induction of potato cultivar Almera

2,4-D (mg/l)	Days to callus initiation	% of explants formed callus	Callus texture	Callus color	Degree of callus formation
0.0	-	-	-	-	0.0 ± 0.0
1.0	17	66	watery	Brown	0.7 ± 0.1
1.5	15	88	watery	Yellow	0.7 ± 0.1
2.0	12	100	watery	Yellow	3.0 ± 0.0
3.0	11	100	watery	Yellow	3.0 ± 0.0
4.0	7	100	watery	Yellow	3.0 ± 0.0
5.0	7	100	watery	Yellow	3.0 ± 0.0

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

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 2). The necessity of cytokinin for shoot initiation is well documented [27, 28]. The best result for the percentage of callus with shoot (100%) and mean number of shoots per callus (3.3±0.03) were recorded on MS medium supplemented with 5.0 mg/l TDZ (Fig. 1b). [29], reported that TDZ, a synthetic phenylurea, is considered to be one of the most active cytokinins for shoot induction in plant tissue culture. Other reports suggested that TDZ induces shoot regeneration better than other cytokinins [30, 31 and 32].

Table 2: Effects of benzyl adenine (BA) and thidiazuron (TDZ) on shoot regeneration from tuber segments callus of potato plant cultivars Almera after twelve weeks of culture on MS medium.

Growth regulators (mg/l)	Days of shoot initiation	% of callus with shoot	No of shoot /callus	Shoot length (cm)
TDZ 0.0	- (callus)	0	0.0 ± 0.0	0.0 ± 0.0
1.0	- (callus)	0	0.0 ± 0.0	0.0 ± 0.0
1.5	90	75	0.3 ± 0.1	0.2 ± 0.1
2.0	90	75	0.3 ± 0.1	0.2 ± 0.1
3.0	70	88	1.8 ± 0.3	0.2 ± 0.1
4.0	70	100	1.9 ± 0.2	0.5 ± 0.1
5.0	60	100	3.3 ± 0.3	0.8 ± 0.1
BA 1.0	- (callus)	0	0.0 ± 0.0	0.0 ± 0.0
1.5	- (callus)	0	0.0 ± 0.0	0.0 ± 0.0
2.0	- (callus)	0	0.0 ± 0.0	0.0 ± 0.0
3.0	70	25	0.8 ± 0.3	0.5 ± 0.3
4.0	65	75	0.3 ± 0.1	0.5 ± 0.3
5.0	65	75	0.4 ± 0.1	0.7 ± 0.3

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

In vitro induction of roots in potato regenerated shoots was studied by using various concentrations of IBA with different MS medium strengths (Table 3). The rooting response varied with basal media strength and IBA concentrations. All four concentrations of IBA with both MS and half-MS strengths were capable to induce 100% rooting in the regenerated plantlets. However, the root formation was not observed when shoots were cultured on a medium lacking auxin. In the present experiment the highest number of root per shoot (35.6±0.6) was induced on full MS - strength supplemented with 1.0 mg/l IBA, while the longest root was obtained on the same medium containing 0.5 mg/l IBA (Fig. 1c). The usage of auxin IBA for *in vitro* rooting has already reported for potato by [3], who declared that 1.0 mg/l IBA was the most effective for *in vitro* rooting of shoots in Diamant cultivar of potato.

Table 3: Effect of different concentrations of IBA and MS salt strength on rooting of indirectly regenerated potato shoots of potato plant cultivar Almera after five weeks of culture

IBA (mg/l)	Basal media strength	Number of root/shoot	Root length (cm)	Rooting %
0.0	MS	0.0 ± 0.0	0.0 ± 0.0	0.0
0 .5	Ms	20.4 ± 2.1	4.9 ± 1.1	100
1.0	MS	35.6 ± 0.6	2.1 ± 0.1	100
1.5	MS	20.0 ± 2.7	0.6 ± 0.0	100
2.0	MS	5.3 ± 0.6	0.4 ± 0.0	100
0.5	1/2MS	17.8 ± 3.0	2.8 ± 0.2	100
1.0	1/2MS	21.5 ± 2.1	2.7 ± 0.3	100
1.5	1/2MS	16.8 ± 3.0	1.9 ± 0.3	100
2.0	1/2MS	8.8 ± 0.8	1.7 ± 0.2	100

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

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. Thereafter, the plantlets were transferred to green house, where the plants appeared morphologically uniform with normal leaf form, shape and growth pattern. Similar procedure for potato regenerants acclimatization was done in our laboratory [6] (Fig. 1d).

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

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