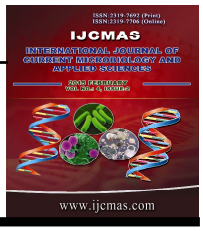
Int.J.Curr.Microbiol.App.Sci (2015) 4(2): 267-276

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 2 (2015) pp. 267-276**
http://www.ijcmas.com



Original Research Article

In vitro Organogenesis of (*Solanum tuberosum L.*) Plant Cultivar Alpha through Tuber Segment Explants Callus

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A B S T R A C T

The aim of this study is to establish a protocol for callus induction, plant regeneration and root induction of potato plants (*Solanum tuberosum* L.) cultivars Alpha. The tuber segment explants was cultured for callus induction, plant regeneration and *in vitro* rooting. Highest callus degree (4.7±0.4[a]) was observed on Murashige and Skoog media (MS) supplemented with 2.0 mg/l 2,4-dichlorophenoxy acetic acid (2, 4-D). MS medium supplemented with 6-Benzylaminopurine (BAP) and Thidiazuron (TDZ) singly or TDZ and BAP in combination withindole-3-butyricacid (IBA) and 2,4- D were employed for shoot regeneration. Highest shoot number/callus (1.6 ± 0.5) was observed on MS media supplemented with 5.0 mg/l BAP alone or 5.0 mg/l BAP in combination with 1.0 mg/l IBA. The effect MS media at full and half salt strength supplemented with different concentrations of IBA was evaluated for root induction, highest number of root obtained (42.5±3.4) root/micro plant on half MS media verified with 2.0 mg/l IBA and 1.5 mg/l IBA. Well *in vitro* rooted micro plants acclimatized and gradually transferred to greenhouse. The data obtained in present study describes protocol for *in vitro* regeneration of *(Solanum tuberosum* L.) Plant Cultivar Alpha.

Keywords

Tuber segment explants, Callus induction, *Solanum tuberosum L*, Rooting

Introduction

The potato (*Solanum tuberosum* L.) belongs to the family *Solanaceae* which includes tomato, eggplant, and peppers (Haque *et al.*, 1996). It is an annual herbaceous and short duration crop that produces large amount of calories in a short period of time (Vrolijk, 1994). 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. It is the most important cultivated food after wheat, rice and maize, and the most important dicotyledonous crop (Khatun *et al.*, 2003).

Conventionally, potato in Sudan is propagated through tubers. This propagation method is characterized by low multiplication rate that ranges from 1:4 to 1:15 (Rabbani *et al.*, 2001). Farmers usually use previous harvest as seed tubers.

The high cost of certified seed tubers is considered as one of the major constraints facing potato production in Sudan. It accounts for 75% of the total cost of potato production (Khalafalla, 2001). Therefore, farmers in Sudan usually cut the seed tuber into small eye-pieces mainly to economize on seed cost. The subdivision of the seed tubers into small seed pieces might result in seed-piece decay and also transmission of several kinds of diseases with the cutting knives (Khalafalla, 2001).

Biotechnology can contribute to solution of these problems and provide great benefits 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 short period of time compared to conventional breeding methods. The recent advancement in tissue culture, especially micro propagation allows for alternative methods of propagation through *in vitro* techniques (Tovar and Dodds, 1986). *In vitro* micro propagation either through direct or indirect methods in potato is generally used to bulk up new cultivars and breeding lines, for germ plasm storage, transport and production of minitubers which are easy to store, transfer, distribute, for production of disease- free plants within a short period of time all year around and production of transgenic plants (Jones, 1988). *In vitro* micropropagation of potato by the serial culture of axillary shoots on separated nodes has been reported by a number of researchers, and is now becoming established as an effective means of rapidly multiplying new or existing cultivars in disease-free conditions (Hussey and Stacey, 1981). In many countries these techniques have boosted first multiplication steps in seed production programs by using *in vitro* produced micro tubers (Bizarri and Ranalli, 1995) or minitubers (Hussey and Stacey, 1981).

Organogenesis

Potato plants can be regenerated indirectly via callus under certain environmental conditions and growth regulators types and concentration. Organogenesis is highly dependent on genotype, origin of explants, and the interaction between endogenous and exogenous hormones. It is difficult to generalize the method of indirect organogenesis, because this process may vary among species, cultivars, and explants.

Shoot regeneration from tuber discs was first reported by Lam (1975), on modified MS medium supplemented with Kin and BAP. Lam (1977) reported shoot regeneration from tuber discs explants using zeatin. Jarret *et al.* (1980) regenerated shoots from tuber discs explants of eight cultivars on MS medium supplemented with 0.03 mg/l NAA, 3.0 mg/l BAP and 0.5 mg/l GA_3. Many research was carried out for indirect regeneration, Nasrin *et al.* (2003) regenerated plantlets from nodal and intermodal explants of the potato cultivar Diamant and Multa, Khatun *et al.* (2003) recorded plantlet regeneration on MS medium fortified with 5.0 mg/l BAP+0.1 mg/l IBA from a potato cultivar, Shrin *et al.* (2007) regenerated plants from intermodal and leaf explants callus of four potato cultivars using MS medium supplemented with different concentrations and combination of BA, NAA and Khadiga *et al.* (2009) regenerated plant from tuber segment explants of cv. Diamant using 2,4 D, BAP and TDZ, Vijay Kumar *et al.* (2014) studied Callus Induction and Plant Regeneration in *Solanum tuberosum L.* cultivars (Kufri Chipsona 3 and MP-97/644) via Leaf, Sherkar and Chavan (2014) studied the

effect of 2,4-D; BAP and TDZ on callus induction and shoot regeneration in Potato and reported, the concentrations of 2,4-D (3.0 mg/l) was found to be the most effective for callus induction in all the explants that formed callus (Table 1).

The aim of this research was to establish an effective protocol for callus induction from potato (*Solanum tuberosum L.*) cultivar Alpha, indirect regeneration and rhizogenesis under *in vitro* conditions,

Materials and Methods

Plant material

Certified seeds of potato cultivar Alpha used in this study were obtained from the Horticulture Sector, Ministry of Agriculture and Forestry, and the Potato Committee Sudanand used as a source for explants throughout the experiments..

Sterilization of explants

Tubers were surface sterilized first by washing under running tap water and 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.

Tuber segments about 1-2 cm^2 were treated with 70% alcohol for 1min, rinsed three time with sterile distilled water, then sterilized for 15 min in25% Clorox solution (containing 5.25% of sodium hypochlorite) with few drops of liquid soap (tween twenty). Finally explants were rinsed five times with sterile distilled water, disinfested tuber pieces were put on sterilized paper tissue in sterilized Petri dishes as explants ready for inoculation.

Preparation of Media

MS media was used in standard component. Media were prepared by adding MS basal medium component + 30g/l sucrose (Murashige and Skoog, 1962). The PH of media was set at 5.8±0.02, and then agar was added at concentration of 0.6% and then melted and dispensed in the tissue culture containers. The containers were then autoclaved at 121ºC for 15 minutes at 15 psi, and stored at incubator till use.

Preparation of Growth regulators and inoculation of potato explants:

Cytokinins, auxin, and their combinations were used for callus induction, plant regeneration and rhizogenesis. These PGR include thidiazuron (TDZ), 6-benzylaminopurine (BAP), 2, 4, -dichlorophenoxy acetic acid (2, 4-D), and indole butyric acid (IBA).

Callus induction

Tuber segments were aseptically placed horizontally on the solidified medium in jars containing about 25 ml of MS medium with different concentrations of growth regulator for the initiation of callus, four segments per jar. The cultures were grown at 25°C ± 2 temperatures, under continuous darkness for eight weeks. Data recorded included: day of callus initiation, percentage of callus formation, type of callus, callus colors and callus degree.

Shoot regeneration from callus

To induce indirect shoot from callus well-developed callus obtained from tuber segments were transferred to MS medium free of hormones (control) or supplemented with different growth regulators. Data recorded were: day of shoot initiation, % of

callus with shoot, number of shoots /callus and shoot length.

Rooting of *in vitro* induced shoots

Regenerated shoots were excised from calli and transferred to MS and 1/2 MS media with different concentration of IBA (0.0, 0.5, 1.5 or 2.0 mg/l) for rooting. All the media used in this study were supplemented with 3% (w/v) sucrose, solidified with 0.6% (w/v) agar and the PH was adjusted to 5.8 ± 0.1 with 1 M NaOH before autoclaving at 121°C and 15 psi for 15 min.

The effect of auxins was evaluated on the number of shoots rooted, number of roots /shoot, root length and rooting percentage.

Culture incubation conditions

Cultures were maintained in a growth room at 25 ºC under cool white fluorescent light lamp (1000 lux) under a photoperiod regime of 16 h light and 8 h dark for regeneration, rooting and 24h dark for callusing.

Hardening of *in vitro* plantlets

The rooted plantlets were first transferred to pots containing 50% moist peat moss and 50%vermicompost as potting mix and kept in plastic cover for two week to provide humidity. Then plastic cover were taken away plantlets were transferred to polybags containing (soil: sand) 1:1 proportion and kept in net house. About 95% plants survived.

Results and Discussion

In the present study callus induction, regeneration and root induction of potato plant was successfully achieved from *tuber* explants of *S. tuberosum* cultivar Alpha (Figure 1, 2 and 3).

Callus initiation on cut ends of *in vitro* cultured explants could be observed in all hormone combinations after 8 - 30 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).

Data showed that all callus parameter are varied with different growth regulator. Among all treatment 2.0 mg/l 2, 4-D was found to be most effective concentration for callus degree, the finding is in agreement with Khadiga *et al.* (2009) reported that auxin 2,4-D by itself at 3.0 mg/l or in combination with BA each at 2.0mg/l has been widely used to enhance callus induction and maintenance.

In this study highest callus degree (4.7 ± 0.4^{a}) obtaining at 2.0 mg/l 2,4-D, (Figure 1). The 100% callusing was obtained among most of treatment with different level on callus degree. Callus color and texture are varied from white, yellow, light yellow to brown for callus color and variable, watery to compact for callus texture. It is well known that various combination and concentration of auxins and cytokinins are effective for callus induction. These results are in line with the results of Khatun *et al.* (2003; Jelenic *et al.,* 2001).

Most research improved that 2, 4-D is most effective auxin for callus induction on potato plant alone or in combination with cytokinins. The present findings were also similar to Vijay Kumar *et al.* (2014), whom induce creamy and light yellowish in colorcallus from *S. tuberosum* cv. Kufri Chipsona 3 on MS media containing high concentration of 2,4-D (3.0 mg/l) in combination with low concentration of cytokinin, kinetin (1.0 mg/l).

Figure.1 Callus formation in MS medium supplemented with 2.0 mg/l 2,4-D after four weeks of culture

Figure.2 Shoot regeneration on MS media supplemented with 5.0 mg/l BAP after six weeks of sub-culture

Figure.3 Regenerated plantlets with well-developed roots induced on ½ MS + 0.5 mg/l IBA.

Table.1 Effects of different concentrations of 2, 4-D and NAA on callus induction from tuber segments of potato cultivar Alpha. Data were recorded after six weeks of culture on MS medium

Growth regulators mg/l	Day of callusing	%of callusing	Texture of callus	Callus color	Degree of callus
2,4 D (0.0)	-	-	-	-	0.0±0.0d
2,4-D1.0	12	100	watery	Yellow	2.0±0.0c
2,4-D 1.5	11	100	watery	Yellow	2.0±0.0c
2,4-D 2.0	11	100	watery	Yellow	4.7±0.4a
2,4-D 3.0	11	100	variable	White	4.1±0.2ab
2,4-D 4.0	10	100	variable	White	4.0±0.0ab
2,4-D 5.0	8	100	variable	Light yellow	4.0±0.0ab
NAA (0.0)		-	-	-	0.0±0.0 d
NAA 0.5	30	100	compact	Light yellow	0.9±0.0d
NAA1.0	26	100	compact	Light yellow	1.0±0.c
NAA 1.5	26	100	compact	Brown	1.0±0.0c
NAA 2.0	20	100	watery	Light yellow	2.0±0.0c
NAA 3.0	20	100	watery	Light yellow	2.0±0.0c
NAA 4.0	18	100	variable	Yellow	4.0±0.0ab
NAA 5.0	18	100	variable	White	4.0±0.0ab

Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P=0.05)(DMRT) (Duncan, 1955).

Table.2 Effect of BAP and TDZ singly and TDZ and BAP in combination withIBA and 2,4- D on plant regeneration from tuber segments callus of potato cultivar Alpha after twelve weeks of culture on MS medium

Growth regulators mg/l	Day of shoot initiation	% of callus with shoot	No of shoot /callus	Shoot length
MS (control)	Callus	0	0.0±0.0d	0.0±0.0g
TDZ1	Callus	0	0.0±0.0d	0.0±0.0g
TDZ1.5	Callus	0	0.0±0.0d	0.0±0.0g
TDZ2	callus	0	0.0±0.0d	0.0±0.0g
TDZ3	80	25	0.3±0.1d	0.1±0.0fg
TDZ4	80	25	0.3±0.1d	0.1±0.1efg
TDZ5	70	25	0.3±0.2d	0.4±0.2efg
BA1	callus	0	0.0±0.0d	0.0±0.0g
BA1.5	callus	0	0.0±0.0d	0.0±0.0g
BA2	callus	0	0.0±0.0d	0.0±0.0g
BA3	70	25	0.3±0.1d	0.9±0.4abcd
BA4	65	25	0.3±0.2d	1.1±0.5abc
BA5	52	31	0.4±0.2d	1.5±0.6a
TDZ3 IBA0.5	53	68	1.1±0.2bc	1.0±0.3abcd
TDZ3 IBA1	48	48	1.2±0.4abc	0.7±0.2bcdef
TDZ3,2,4D0.5	54	48	1.1±0.4bc	1.1±0.4abc
TDZ3,2,4D 1	57	35	0.2±0.1d	0.6±0.3cdefg
TDZ5IBA0.5	52	73	1.4±0.3abc	1.1±0.3abc
TDZ5 IBA1	46	48	1.5±0.4ab	0.8± 0.3bcd
TDZ5,2,4D0.5	53	48	1.1±0.3bc	1.3±0.4ab
TDZ5,2,4D 1	56	36	0.4±0.2d	0.7±0.3bcdef
BA3IBA0.5	53	48	1.0±0.2c	1.0±0.3abcd
BA3 IBA1	48	68	0.0±0.0d	0.7±0.2bcdef
BA3,2,4D0.5	callus	0.0	0.0±0.0d	0.0±0.0g
BA3,2,4D 1	callus	0.0	0.0±0.0d	0.0±0.0g
BA5IBA0.5	46	48	1.5±0.3ab	0.9±0.3bcd
BA5 IBA1	52	73	1.6±0.5a	0.7±0.2bcdef
BA5,2,4D0.5	callus	0.0	0.0±0.0d	0.0±0.0g
BA5,2,4D 1	callus	0.0	0.0±0.0d	0.0±0.0g

Means followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test (P=0.05)(DMRT) (Duncan, 1955).

Table.3 Effect of different concentrations of IBA and MS salt strength on rooting of indirectly regenerated potato shoots of two potato cultivar Alpha after five weeks of culture

Growth Regulators mg/l	Basal media strength	Number of root/shoot	Root length	Root %
IBA0	MS	1.6±0.3c	4.0±1.3b	100
IBA.5	Ms	1.8±0.4c	0.9±0.2c	100
IBA1	MS	3.8±1.6c	0.4±0.1c	100
IBA1.5	MS	4.0±1.1c	0.7±0.1c	100
IBA2	MS	5.1±0.8c	0.3±0.0c	100
IBA0	1/2MS	10.0±1.0c	8.3±1.0a	100
IBA.5	1/2MS	28.6±4.3b	3.6±0.5b	100
IBA1	1/2MS	23.8±6.3b	3.3±0.4b	100
IBA1.5	1/2MS	38.8±1.6a	4.3±0.2b	100
IBA2	1/2MS	42.5±3.4a	3.4±0.3b	100

Means followed by the same letter(s) in each column are not significantly different according to Duncan's Multiple Range Test (P=0.05) (DMRT) (Duncan, 1955).

The present findings were also similar to (Sherkar and Chavan, 2014; Khadiga *et al.*, 2009; Shrin *et al.*, 2007; Vijay Kumar *et al.*, 2014; Khatun *et al.*, 2003) in using 2,4-D alone or in combination with cytokinin to enhance callus induction and maintenance. On the other hand these findings are disagree with Nasrin *et al.* (2003) who regenerated plantlets from nodal and intermodal explants of the potato cultivar Diamant and Multa on MS medium containing 3.0 mg/l kin with 1.5 mg/l NAA. This may be due to variation on cultivar and explants.

In other plant species *Trigonella foenum-graecum*, higher concentration of 2,4-D (2.0 mg/l) and NAA (2.0 mg/l) is useful to induce callus from cotyledons and hypocotyls explants (Khadiga *et al.*, 2014)

Indirect regeneration

After sufficient callus induction, the callus were initiated subsequent organogenesis when sub-cultured on MS medium supplemented with different concentrations of BAP and TDZ each alone or in combination with IBA and 2,4- D (Table 2).

The best result of mean numbers of shoot number/callus (1.6 ± 0.5) was observed on MS media supplemented with 5.0 mg/l BAP alone or and BAP (5.0 mg/l) with IBA (1.0 mg/l) in combination where mean number was (1.6 ± 0.5) shoots/callus (Figure 2).

Shoot initiation in MS media supplemented with TDZ alone or in combination with IBA,2,4 D, and NAA from potato tuber callus are new finding, whereas regeneration of plantlet from potato callus with BAP in combination with IBA is in agreement with Lam (1975), Lam (1977), Jarret *et al.* (1980) Kikuta and Okazawa (1982) and Ahloowalia (1982).

A similar effect of BAP and IBA was also observed by (Khatun *et al.*, 2003) MS medium containing 5.0 mg/BAP alone or in combination with 1.0 mg/l IBA was the best for maximum shoot regeneration from potato cultivar Alpha, this agree with Shrin *et al.* (2007).

Rooting

Well-developed shoots were transferred for rooting on full and half salt strength of MS

medium supplemented with IBA in different concentration (0.0, 0.5, 1.0, 1.5 and 2.0 mg/l).

There was 100% rooting response in all the media combinations as well as media without IBA. The best results for number of root per shoot (42.5±3.4) were obtained by using half strength MS medium supplemented with IBA at 2.0 mg/l (Table 3 and Figure 3). The beneficial effect of using IBA and half-strength MS for *in vitro* rooting has already reported for potato by (Khatun *et al.,* 2003).

The present study describes an efficient and easy to handle protocol for *in vitro* regeneration of *Solanum tuberosum L.* Plant Cultivar Alpha which could be considered for large scale multiplication and propagation of this important plant.

Acknowledgements

The first author did this work as part of her doctoral studies at Elneelain University, Khartoum, Sudan. The authors are grateful to the Sudan Potato Committee, Faculty of Agriculture, University of Khartoum, Sudan, for providing potato tuber seeds. We are also grateful to the Administration of Commission for Biotechnology and Genetic Engineering, National Centre for Research, Sudan, for providing laboratory facilities.

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