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Full length Article

Effect of 2,4-D; BAP and TDZ on Callus Induction and Shoot regeneration in Potato

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ABSTRACT

The main objective of the study was to develop a protocol for callus induction and shoot regeneration in potato (*Solanum tuberosum* L.). The sprouts of potato tubers were used as starter explant to establish initial culture. The explants were cultured on basal Murashige and Skoog medium supplemented with different hormonal concentrations and combinations were studied. Ten explants were cultured in each combination. The highest degree of callus formation on MS media supplemented with 2.0-4.0 mg/l of 2,4-D was observed. The regeneration of shoots observed when calli were sub cultured on MS media supplemented with 1-4mg/l of TDZ and 1-4 mg/l of BAP.

Key words: Callus Induction, 2,4-D, Bap, TDZ, Regeneration, Potato

INTRODUCTION

Potato (*Solanum tuberosum* L.) belongs to family solanaceae and is one of the staple food crop grown all over the world (Salmon and Baker 2001.)The edible part of plant is tuber used as cheap food, industrial raw material, animal feed and seed tuber (Feustel, 1987). Potato is cultivated as a vegetable crop produces largest quantity of carbohydrates per day per unit area among food crops (Zaag and Harton, 1983). Potato production with seed tuber is constrained by the accumulation of pathogen, physiological decline and low multiplication rates. These constrains can be overcome by using tissue culture techniques for rapid multiplication of elite plantlets. The regeneration of plants from tissue culture have potential not only to improve the existing cultivars but also for the generation of novel plants in a comparatively short time compared to conventional breeding (khadiga *et al.,* 2009). Great progress has been made in potato for plant regeneration in recent years (Ehsanpour & Jones, 2000; Feegert *et al.,* 2000; Ahn *et al.,* 2001). Remarkable work has been done on plant regeneration with different explants and growth regulators.

Both callus induction and plant regeneration from explants require the presence of appropriate combination and concentration of plant growth regulator in the culture media. The callus induction and plant regeneration is also dependant on type of explants, media components, growth conditions and crop and variety. Many author work to standardize the optimum concentration of BAP and NAA for regeneration of potato (Dobranszki *et al.,* 1999; Hansen *et al.,* 1999; Zel *et al.,* 1999; Fomenko *et al.,* 2000; Asthma *et al.,* 2001).

Although potato regeneration protocol was established in many laboratories in the world but still there is very less information regarding the studies on secondary metabolites in regenerated plants. Therefore, the present study was carried out to find best concentration of hormone and shoot regeneration of potato.

MATERIALS AND METHODS

The present study was carried out in the plant tissue culture laboratory, H.U.Gugle Biotech, Jamkhed, Dist Ahmednagar in 2010. The potato tubers cv. *Kufri pukhraj* were collected from Rhizo Biotech, Ludhiana, Punjab. The tubers were treated with 20 ppm GA3 and at room temperature for sprouting.

Explant surface sterilization

For surface sterilization, elongated sprouts of potato were cut sharply and treated with 0.2% bavistin to avoid the load of microbe for half an hour and carried to laminar air flow for further treatment. The sprouts were surface sterilized first with 70% alcohol for 30sec and then with 0.1% HgCl2 for 3 min and then rinsed three times with sterile distilled water to remove all the traces of alcohol and HgCl2

MS media preparation

MS medium having all major, minor elements with Fe EDTA and organic constituents, vitamin and 2 mgs/l IBA was prepared in advance. Agaragar used as gelling agent. The pH of medium was adjusted to 5.8 and then media was autoclaved at 121.c for 20 min.

Explant growing

To establish invitro cultures the potato tuber sprouts were inoculated on MS medium with 3% sucrose after surface sterilization. The cultures were allowed to grow in growth room and incubated at 28 ±2°c having 8 hours photoperiod under white light intensity (3000 lux). The cultures were observed for growth of explants and microbial contamination.

Callus induction and regeneration

One month old in vitro grown potato shoots were used as a source of explants for callus induction and regeneration study. For the induction of callus leaf segments and node internodes and shoot tips explants were used and cultured on basal MS media supplemented with different concentration of 2,4-D in mgs per liter (1.0,1.5,2.0,3.0,4.0) for 4 to 6 weeks. The callus multiplication was done by transferring calli on fresh callus inducing media for every 20 days interval. The data were recorded for percent of explant formed callus, days to callus induction, callus texture, callus colour and degree of callus formation. Ten explants were placed in each treatment.

Well developed calli were selected as an explant for shoot regeneration study. The calli were sub cultured and inoculated on regeneration media. The basal MS media was supplemented with different concentrations of BAP (0.0, 1.0, 2.0, 3.0, 4.0 mgs/l) and TDZ (0.0, 1.0, 2.0, 3.0, 4.0) for shoot regeneration. The cultures were incubated at 28±2°c with 8 hours photoperiod. The data were recorded after 60 days of incubation for percent of callus with shoot regeneration and number of shoot per callus and average shoot length.

RESULTS AND DISCUSSION

Callus formation

The effect of various concentrations of 2,4-D on callus induction have been presented in table 1. The shoot tip leaf explants from invitro established potato shoots were cultured on basal MS media having different various concentration of 2,4-D. Ten explants were inoculated per bottle in each treatment. The data was recorded after four weeks of the inoculation of the explants. The data were studied and analyzed for days to callus induction, the percentage of explants that formed callus, days to callus initiation, callus colour and degree of callus formation (Table-1). The callus initiation of invitro cultured explants observed at all concentrations of 2,4-D after two weeks. The explants did not show any callus formation on MS media without 2,4-D. 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. The percentage of explants that formed callus at this concentration was 100% (table-1). These results are in support of (khadiga *et al.,* 2009; shirin *et al.,* 2007, castllo *et al.,* 1998). They reported that 2,4-D alone or incombination with cytokinin has been widely used to enhance callus induction and maintenance. The above results are in convention with (shirin *et al.,* 2007) in which 2,4-D was used for induction of callus from leaf and internodes explants. Moreover, many researchers observed 2,4-D as the best auxin for callus induction as common as in monocot and even dicot (Evans *et al.,* 1989; Ho and Vasil 1983; Jaiswal and Naryan, 1985; Chee, 1990; Manun *et al.,* 1998).

Table 1: Effect of different concentrations of 2,4-D on Callus induction of potato variety *Kufri pukhraj*.

Sr. no.	2,4-D (in mgs)	% explants formation of callus	Texture of callus	Callus color	Degree of callus formation
1	0.0	--	--	--	--
2	1.0	60	Compact	Y	+
3	1.5	87	Loose	YG	++
4	2.0	100	Loose Watery	G	+++
5	2.5	100	Loose Watery	G	+++
6	3.0	100	Loose Watery	GW	+++

--=no callus, +=slight callus, ++=moderate callus, +++=massive callus, Y-yellowish, G-greenish, GW-greenish white, W- whitish, YW- yellowish white

Table 2: Effect of leaf explants on callus induction by using 2,4-D in potato

Sr. no	2,4-D (in mgs)	Type /texture of callus	Callus weight(in gms)
1	0.0	--	--
2	1.0	Compact	0.67
3	1.5	Compact	0.72
4	2.0	Loose	0.64
5	2.5	Loose Watery	0.77
6	3.0	Loose Watery	0.81

Table 4: Effect of internode explants on callus induction by using 2,4-D in potato.

Sr. no.	**2,4-D (in mgs**	**Type /texture of callus**	**Callus weight (in gms)**
1	--	--	--
2	1.0	Compact	0.61
3	1.5	Loose	0.44
4	2.0	Loose Watery	0.54
5	2.5	Loose Watery	0.57
6	3.0	Loose Watery	0.41

Effect of explants

Various supplements showed significant variation in shoot regeneration ability. The highest callus induction was observed when leaf explants used followed by internodes explants (Table 2 and 3). It was also found that leaf was always more responsive explant than internodes segments. Yadav *et al.,* (1998); Alphonse *et al.,* (1998); and Hamdi *et al.,* (1998) observed that leaf was the best explant for callus induction and regeneration.

Regeneration of plants from callus

Effect of different concentrations of BAP-

After 60 days of inoculation the highest percentage (37%) of shoot regeneration was recorded in 3 mgs /liter BAP and the least shoot regeneration was found in 2 mgs/liter BAP whereas in hormone free control MS medium, inoculated calli did not show any shoot regeneration. Maximum shoot length of regenerated shoots was observed in medium supplemented with 3 mgs /liter BAP, maximum number of shoots per calli was also observed in same media. Whereas least number of shoot per calli were found in medium supplemented with 2 mgs/l BAP.

Table 5: Effect of BAP on shoot regeneration from callus in potato

Sr. no.	BAP (in mgs	Days of shoot initiation	Shoot formation %	No of shoot per calli	Shoot length (mm)
1	0.0	--	--	--	--
2	1.0	--	--	--	--
3	2.0	60	12	1	3
4	3.0	60	37	1.6	4
5	4.0	60	20	1.4	3

(The data were recorded after 60 days after inoculation. 10 explants were placed in each treatment)

Table 6: Effect of TDZ on shoot regeneration from callus in potato

Sr. no.	TDZ (in mgs	Days of shoot initiation	Shoot formation percent	No of shoot per calli	Shoot length (mm)
1	0.0	--	--	--	--
2	1.0	60	65	1.0	4
3	2.0	60	90	1.6	5
4	3.0	50	90	1.3	5
5	4.0	50	85	1.4	6

(The data were recorded after 30 days after inoculation.10 explants were placed in each treatment)

Table 7: Effects of IBA on in vitro rooting of Potato *(K. pukhraj)*

Conc. of IBA(mgs/l)	Rooting response %	Adventitious rooting %	Av. Height of shoot m(mm)	Av. no of roots
0.0	100%	0.00%	33	2
0.5	100%	0.00%	34	3
1.0	100%	30%	44	7
2.0	100%	95%	47	8
3.0	100%	100%	39	13

(Av. Of 10 plantlets in each treatment) after 60 days of inoculation

Effect of different concentrations of TDZ-

After the callus induction using different explants the calli were multiplied on basal MS media supplemented with 3mgs 2,4-D. these calli were subcultured on MS media supplemented with different concentrations of BAP (Table 5 and TDZ (Table 6). The requirement of cytokinin BAP and TDZ for shoot regeneration is well documented (Beck and Caponetti, 1983) and Evans *et al.,* 1984). The highest percentage of regenerated shoots (90) and mean number of shoots per calli (1.6) were recorded on MS medium supplemented with 2mgs/liter TDZ. Huetteman and Preece, 1993 reported that TDZ is considered to be one of the most active cytokinin for shoot induction in plant tissue culture. Other reports suggested that TDZ induces shoot regeneration better than other cytokinin (Thomas *et al.,* 2003). There was no shoot regeneration without growth regulator.

In vitro rooting of Regenerated shoots

The shoots developed were transferred for rooting on MS media containing different concentrations of IBA.

There was 100% rooting response in all the media combinations as well as media without IBA (Table 7). However, the best rooting number was found in MS media containing 1.0mgs/l IBA but the best shoot growth was observed in media having 2.0 mgs IBA. Increase in IBA concentration results in adventitious rooting from internodes and leaves. The plantlets in media having 3.0mgs/l IBA shows 100% adventitious roots followed by media having 2.0 mgs/l IBA. A medium devoid of IBA do not show any adventitious roots. Well developed shoots having profuse roots were transplanted in portrays having cocopeat for hardening. After one month of hardening plantlets were potted in earthen pots.

CONCLUSION

The callus formation and shoot regeneration was observed in both leaf and internode explants. The maximum shoot regeneration was observed on medium containing 3mgs/l BAP and 2mgs/l TDZ. The factors influenced shoot regeneration were explants source, growing media, duration of culture, and invitro growth conditions.

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