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Callus Induction and Plant Regeneration from Internodal and Leaf Explants of Four Potato (*Solanum tuberosum* L.) Cultivars

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Abstract: The present investigation was conducted to develop a protocol for rapid callus induction and plant regeneration of potato. The internodal and leaf explants of four potato (*Solanum tuberosum* L.) cultivars *viz.* Diamant, Multa, Atlus and Lalpakri were cultured for callus induction and plant regeneration. The explants were cultured onto MS media supplemented with different concentrations and combinations of 2, 4-D, NAA alone and NAA with BA for callus induction. The highest 80% internodal explants of Atlus induced to develop callus in medium containing 3.0 mg L^{-1} 2, 4-D. Of all cultivars the callusing response of both types of explants was the best in 3.0 mg L^{-1} 2, 4-D containing MS media. MS medium supplemented with different concentrations and combinations of BA, NAA and KIN were employed for shoot regeneration. MS medium containing 4.0 mg L^{-1} KIN + 0.5 mg L^{-1} NAA was the best for maximum shoot regeneration from the internode and leaf derived calli in most of the cultivars. The regenerated shoots were rooted in MS_0 medium and successfully transplanted to the field.

Key words: Callus induction · plant regeneration · cultivar · potato

INTRODUCTION

Potato *(Solanum tuberosum)* is one of the most economically important annual vegetable crop of Solanaceae family. It is the fourth most cultivated food crop in the world after wheat, rice, maize and the most important dicotyledonous tuber crop [1]. In respect to land under cultivation potato is the third major subsistence crop in Bangladesh [2]. To make Bangladesh sufficient in food, extensive research work has been performed for the improvement of economically important crops that exists in the country. *In vitro* plant regeneration has become a popular and useful technique and being applied to solve the problems of many agricultural crops.

Now a day, plant cell and tissue culture techniques are being applied for rapid clonal propagation of potato. The callus formation was first reported in 1951 [3] leading to plant regeneration in 1975 [4]. Successful *in vitro* plant regeneration has been achieved from explants of different organs and tissues of potato such as leaf [5]; stem [6, 7]; tuber discs [8, 9] and unripe zygotic embryos [10]. Plant regeneration in potato has progressed a lot in recent years [11, 12]. It is important to standardize the protocol for plant regeneration through callus of potato varieties grown in Bangladesh. Because plants regenerated through callus culture are genetically identical to the source material, free from pathogens and it is possible to produce a huge number of plantlets in a very short period of time. But the response of explants for callus induction and regeneration are not same in different media concentrations. Therefore, the aim of the present study was to establish an effective protocol for rapid plant regeneration from internodes and leaf explants derived callus of potato cultivars cultivated to a great extent in Bangladesh.

MATERIALS AND METHODS

The research work was carried out during the period from December, 2004 to November 2005 at the Plant Breeding and Gene Engineering Laboratory, Department of Botany, University of Rajshahi, Bangladesh to evaluate the response of potato genotypes cultivated widely throughout the country. Internode and leaf segments of four *in vitro* grown potato cultivars *viz* Diamant, Multa,

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Atlus and Lalpakri were used for callus induction and plant regeneration. MS [13] semi-solid media with different concentration and combination of auxin (2, 4-D, NAA) and cytokinin (BA, kinetin) were used for this purpose. In each treatment 10 explants were inoculated. Explants were cultured in culture tubes containing MS medium (30 g L^{-1} sucrose, 7 g L^{-1} agar). The pH of the medium was adjusted to 5.8 by using 1N NaOH. The media were autoclaved at 121°C for 20 min after adjusting the pH. The explants were incubated on callus induction medium at 25±2°C for 3-6 weeks. The calli were transferred to the fresh callus inducing medium about 21 days interval for further proliferation and maintenance. After 40-60 days of incubation in the dark, the callus induction frequency was determined and the well developed calli were selected and sub-cultured on regeneration media. MS medium was supplemented with different concentration and combination of KIN, BA and NAA for shoot regeneration. The cultures were incubated at 25±2°C under white light (2500-3000 lux). After 4-6 weeks differentiation shoot formation was observed. The shoots were excised and transferred to MS_0 (without growth regulators) medium for root induction. Percentage of explants induced callus proliferation, callus color, degree of callus formation, percentage of calli regenerated shoots and No. of shoots per callus were recorded to investigate the effect of different treatments and response of different genotypes.

RESULTS AND DISCUSSION

The leaf and internodal explants of four potato cultivars were cultured on MS media containing different concentrations of 2, 4-D, NAA alone and in combinations of NAA with BA. Data were analyzed after six weeks of culture and the analysis showed that the percentage of explants induced to develop callus, callus color and degree of callus formation varied with culture media formulations and potato genotype (Table 1). Among all concentrations and combinations, 3.0 mg L^{-1} 2, 4-D was found to be most effective auxin concentration for callus induction in all cultivars. With this concentration the highest callusing rate from internodal explants was 80% recorded for cultivar Atlus and Diamant (Fig. 1A & B). However, for the leaf explants the rate of callus formation was very poor. The highest 50% callusing rate was found in Diamant on 2.5 mg L^{-1} 2, 4-D. The same percentage was found in Atlus and Lalpakri on 3.0 mg L^{-1} 2, 4-D. Among different concentrations of NAA the highest callusing rate from internodal explants recorded for cultivar Diamant was 40% in the medium containing 3.0 mg L^{-1} NAA. Where a combination of NAA and BA were applied, the highest callusing rate of 50% was observed for the internodal explants on all the cultivars in the medium containing 1.0 mg L^{-1} NAA + 1.0 mg L^{-1} BA. When leaf explants were used, the highest percentage of callus formation was 40% for the cultivars Diamant and Lalpakri with the same concentration of Plant Growth regulators. calli color varied from white, light brown to light green. Many researchers observed 2, 4-D as the best auxin for callus induction as common as in monocot and even in dicot [14-18]. In the present piece of work 2, 4-D alone showed better effect for callus inducton in potato. This result is an agreement with Sultana [19] who used 2, 4-D alone for callus induction from internode and leaf explants of potato in cultivars Diamant and Cardinal and obtained similar results. Khatun *et al.* [20] used 2, 4-D alone for callus induction and also found the best results in potato cultivar Diamant for callus induction but the concentration of 2, 4-D was 2.5 mg L^{-1}.

When different concentrations of BA with NAA and KIN with NAA were introduced to the medium for shoot proliferation the results showed that of the two combinations KIN + NAA was found to be more effective than BA+NAA for shoot regeneration from internodal explant derived calli for all cultivars (Table 2). However, regeneration from leaf explants derived calli BA+NAA proved to be more effective. Among the different concentrations and combinations of BA with NAA, the highest 40% internodal derived calli were induced to develop shoots in Diamant in 3.0 mg L^{-1} BA+1.0 mg L^{-1} NAA. The highest No. of shoots per callus was also found in this concentration which was 4.5. On the other hand, for shoot regeneration from the leaf explants derived calli the highest shoot regeneration observed for Diamant and Lalpakri was 40% in medium containing 3.0 mg L^{-1} BA +0.1 mg L^{-1} NAA. The highest No. of shoots per callus was also found in same concentration which was 5.4 in Diamant and 6.2 in Lalpakri. Khatun *et al.* [20] also found the same results in potato which is an agreement with Sultana [19] who found the same result in potato cultivars. However, for most potato cultivars maximum shoot regeneration from both types of explants was found to be best in 4.0 mg L^{-1} KIN+0.5 mg L^{-1} NAA containing MS media. On this concentration the highest shoot regeneration rate was 50% in Diamant and Multa from internodal explants derived calli (Fig. 1C). The highest No. of shoots per callus was also found in same concentration. These results are similar to Nasrin *et al.* [21]. Sultana [19] used KIN with NAA in MS medium for shoot regeneration from callus in *Solanum tuberosum* and

Table 1: Effects of different concentrations of 2, 4-D, NAA and combinations of NAA with BA in MS medium on callus induction from internodal and leaf explants of different potato cultivars. In each treatment 10 explants were inoculated. Data were recorded after six weeks of culture

		Cultivars											
		Diamant			Multa			Atlus			Lalpakri		
Type of explants	Growth regulators mg l^{-1}	% of explants induced callus	Callus colour	Degree of callus formation	% of explants induced callus	Callus colour	Degree of callus formation	% of explants induced callus	Callus colour	Degree of callus formation	% of explants induced callus	Callus colour	Degree of callus formation
Internode	2, 4-D												
	1.0	10	C	+	-	-	-	10	W	+	-	-	-
	1.5	20	B	+	20	LG	+	40	W	+	-	-	-
	2.0	40	LB	++	40	LG	+	30	W	+	10	LB	+
	2.5	50	LB	++	50	LG	+	50	B	++	30	LB	+
	3.0	80	B	+++	60	LG	++	80	B	+++	60	B	+++
	3.5	60	B	++	40	LG	+	70	B	+++	40	LB	++
	4.0	50	B	+	40	LG	+	60	B	++	20	LB	+
	NAA												
	1.0	-	-	-	-	-	-	-	-	-	-	-	-
	1.5	10	W	+	-	-	-	10	W	+	-	-	-
	2.0	30	LY	+	20	LG	+	30	W	+	30	LB	+
	2.5	20	W	+	30	LG	+	20	W	+	10	LB	+
	3.0	40	LY	++	10	LG	+	10	W	+	10	W	+
	NAA+BA												
	0.5+0.5	20	W	+	20	LG	+	10	W	+	-	-	-
	0.5+1.0	20	W	+	40	LG	+	10	W	+	10	LG	+
	1.0+0.5	40	W	++	40	B	++	20	W	+	30	LG	+
	1.0+1.0	50	W	+++	50	B	++	50	LB	++	50	LG	+
	1.5+0.5	30	W	+	30	B	+	20	W	+	10	LG	+
	1.5+1.0	20	W	+	10	B	+	10	W	+	10	LG	+
Leaf	2, 4-D												
	1.0	-	-	-	-	-	-	10	LB	+	-	-	-
	1.5	20	LB	+	10	LG	+	20	LB	+	10	LB	+
	2.0	30	LB	+	20	LG	+	30	LB	+	10	LB	+
	2.5	50	LB	+++	20	LG	+	30	LB	+	30	LB	+
	3.0	40	B	++	40	LG	++	50	LB	+++	50	LB	+++
	3.5	30	B	+	30	LG	+	40	LB	++	30	LB	+
	4.0	20	B	+	10	LG	+	30	LB	+	20	LB	+
	NAA+BA												
	0.5+0.5	-	-	-	-	-	-	10	LG	+	-	-	-
	0.5+1.0	10	LG	+	10	LG	+	20	LG	+	-	-	-
	1.0+0.5	20	LG	+	10	LG	+	20	LG	+	20	LG	+
	1.0+1.0	40	LB	++	20	LG	+	30	LG	+	40	LG	++
	1.5+0.5	30	LB	+	-	-	-	20	LG	+	20	LG	+
	1.5+1.0	20	LB	+	-	-	-	10	LG	+	10	LG	+

B = Brown, LB = Light Brown, C = Creamy, Gr B = Greenish Brown, LG = Light Green, W = White, Y = Yellow, LY = Light Yellow, - = No Callus, + = Slight Callus, ++ = Moderate Callus, +++ = Massive Callus

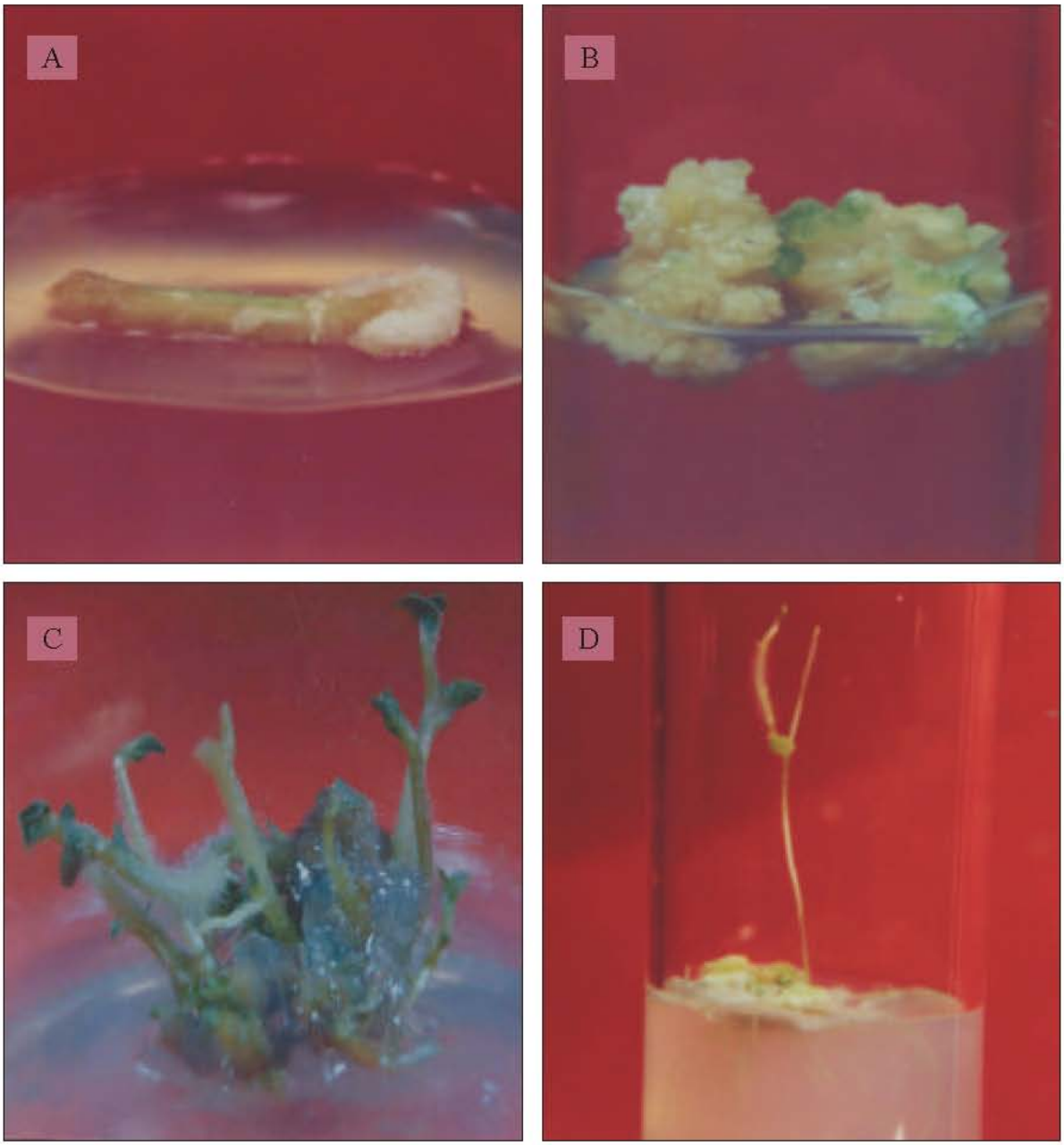

Fig. 1: A-D: Callus induction and plant regeneration from internodal explants in potato cv. Diamant. (A) Callus formation in MS + 3.0 L^{-1} 2, 4-D after three weeks of culture; (B) Callus formation in MS + 3.0 L^{-1} 2, 4-D after six weeks of culture; (C) Shoot regeneration in MS + 4.0 L^{-1} KIN + 0.5 L^{-1} NAA after seven weeks of subculture; (D) Rooting of regenerated shoots in MS_0

found similar results. KIN with NAA in MS medium also stimulated proliferation and elongation of shoots in garlic (*Allium sativa* L.) mentioned by earlier researchers [22].

In this investigation it was observed that auxin alone and in combination with cytokinin can produce callus but 2, 4-D was the most effective for callus induction and proliferation. Rapid shoot regeneration was achieved when the calli were sub-cultured in KIN with NAA supplemented MS medium. The regenerated shoots were excised and individually transferred onto semi solid MS_0 rooting medium. Cent percent shoots were induced to develop root within 2 weeks of culture (Fig. 1D). The

Table 2: Effects of different concentrations and combinations of BA with NAA and KIN with NAA in MS medium on shoot regeneration from internodal and leaf explants derived calli of four potato cultivars. In each treatment 10 explants were inoculated. Data were recorded four weeks after culture

		Cultivars							
		Diamant		Multa		Atlus		Lalpakri	
Callus type	Growth regulators mg l^{-1}	% of calli regenerated shoots	No. of shoots/callus	% of calli regenerated shoots	No. of shoots/callus	% of calli regenerated shoots	No. of shoots/callus	% of calli regenerated shoots	No. of shoots/callus
Internodal	BA+NAA								
explants	2.0+1.0	10	2.0	10	1.0	10	2.0	10	2.2
derived	2.0+1.5	20	2.8	10	2.0	10	1.2	20	2.0
calli	3.0+0.1	10	2.3	30	3.1	30	3.8	20	2.5
	3.0+0.5	20	2.5	20	1.4	20	3.7	30	3.1
	3.0+1.0	40	4.5	20	1.5	20	5.0	10	2.1
	3.0+1.5	30	2.1	10	1.0	10	2.1	10	2.0
	KIN+NAA								
	3.0+0.1	10	2.0	10	1.8	10	2.0	10	1.1
	3.0+0.5	20	2.3	20	2.6	20	2.2	20	2.5
	3.0+1.0	20	2.0	30	3.7	20	2.5	20	2.5
	4.0+0.5	50	5.8	50	6.8	40	3.5	30	4.2
	4.0+1.0	30	4.5	40	3.2	50	6.8	50	7.2
	4.0+1.5	30	3.5	30	4.6	20	2.4	40	4.0
Leaf	BA+NAA								
segments	2.0+1.0	10	1.6	-	-	10	1.2	10	2.0
derived	2.0+1.5	10	3.0	10	1.4	10	1.2	10	2.5
calli	3.0+0.1	40	5.4	30	4.0	30	6.6	40	6.2
	3.0+0.5	30	4.1	40	4.8	20	5.0	20	4.0
	3.0+1.0	20	3.0	20	2.7	20	3.0	10	4.1
	3.0+1.5	10	2.1	-	-	10	2.0	10	2.5
	KIN+NAA								
	3.0+0.1	10	2.3	-	-	10	1.2	10	2.4
	3.0+0.5	10	2.5	10	1.2	10	1.0	10	2.1
	3.0+1.0	10	3.0	20	2.0	10	1.0	10	2.0
	4.0+0.5	30	5.7	30	3.0	20	2.4	20	3.5
	4.0+1.0	20	3.0	20	1.4	-	-	20	1.5
	4.0+1.5	20	4.0	10	1.4	-	-	10	3.2

plantlets were finally and successfully transferred to the field and grown to maturity. The present study describes an efficient method for *in vitro* regeneration of potato cultivars which could be considered for large scale multiplication and propagation of this important vegetable crop.

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