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Full length Research Paper

Callus induction and regeneration of potato from shoot tip culture

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Two laboratory experiments were performed with potato cv. Granula to investigate callus induction ability and regeneration capability from shoot explants and to study the influence of BAP, IAA and GA_3 on plantlet regeneration from callus. In the first experiment, MS medium with 2.0 mg/l 2,4-D were used to identify the highest callus formation (95.00%) within a minimum number of days (10.00), number of calli /culture (4.75) and weight of callus (0.074 g). However, the second experiment was done for both of shoot and root formation. In case of shoot regeneration, combination of 1.0 mg/l BAP and 0.5 mg/l GA3 showed the highest number of shoots per explants (4.67), shoot initiation (93.33%) at required days (15.00), number of leaves per plantlet (3.67) and longest shoot (5.33 cm). On the other hand, the combination of 1.0 mg/l IAA and 1.0 mg/l GA3 showed the highest number of roots per shoot (3.33) within a minimum number of days (12.33) and longest root (6.33 and 7.33 cm) after 20 and 28 days, respectively. Therefore, it was concluded that the hormonal concentration to effect the callus formation and regeneration. Further attempts have been used for production of disease free potato variety from this study.

Keywords: Callus, culture medium, in vitro, regeneration, shoot tip.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fourth most cultivated food crop after wheat, rice and maize (Moeinil *et al.,* 2011). It is a native of South America; in sixteenth century Spanish explorers introduced it in Europe and later it became an important food crop of the world (Khoso, 1988). It is an economically important vegetable crop in Bangladesh. Potato is the fourth most important crop by volume of production; it is high yielding, having a high nutritive value and gives high returns to farmers. Moreover, antioxidants are considered as a good source of potato (Chen *et al.,* 2007).

Traditional breeding of potato is very difficult due to its vegetative propagation ability, heterozygosity and tetraploidity (Solmon-Blackburn and Baker, 2001). On the other hand the productivity of potato is very low. There are several reasons for this low productivity, the major one being the non-availability of disease free and certified seed of high yielding potato varieties resistant to pests and diseases for different ecological zones. Yield losses are directly proportional to the intensity of virus infection. Per hectare yield could be easily doubled by the use of healthy and sound seed (F.C. Bawden and B. Kassanis, 1965). Biotechnology could be used to solve this problem and realize great benefit to potato farmers.

Plant regeneration from cell and tissue culture represent an essential component of biotechnology which is used to improve not only the existing cultivars, but also for the generation of novel plants in a comparatively short time than conventional breeding (G.A.E., Khadiga, S.M. Rasheid and M.M. Khalafalla,2009). Tissue culture may causes variation is either problematic or useful for horticulturists and plant breeders and frequency of adventitious plant regeneration or long-term callus culture

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is so high (Kaeppler, S. M., H. F. Kaepler and Y. Rhee, 2000). Several researchers studied the progress which has been made in callus induction and plant regeneration of potato (G.A.E., Khadiga, S.M. Rasheid and M.M. Khalafalla,2009; Shirin, F., M. Hossain, M. F. Kabir, M. Roy and S. R. Sarker, 2007; Khalafalla, M., G. A. Khadiga and S. M. Rasheid, 2010; Shahab-ud-din I., N. Sultan, M. A. Kakar, A. Yousafzai, I. F., A. Sattar, F. Ahmmad, M. Ibrahim, M. Hassanullah and B. Arif, 2011). Tissue culture systems are capable of creating genetic variability and producing plants with novel characters. This suggests, tissue culture application could be the viable alternatives in developing new cultivars apart from generating virus free planting stocks and ameliorating heterozygous segregates. The objectives of this study was to investigate the efficiency of callus induction and plant regeneration of potato from shoot tip culture and also to detect the different hormonal concentration on callus induction (2, 4-D) as well as plant regeneration (*BAP*, IAA and GA_3) from embryogenic calli.

MATERIALS AND METHODS

The research work was conducted at the Biotechnology Laboratory of the Department of Biotechnology, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Plant materials

Potato tubers of variety Granula were collected from Bangladesh Agricultural research Institute (BARI), Joydebpur, Gazipur. Disease-free potato tubers were sprouted in the dark condition at room temperature in the Laboratory. The sprouted tubers were then inoculated into MS medium for growing as micro plants. One month old *in- vitro* grown micro plants were used as the source of explants. Under aseptic condition, one month old micro plants were taken out from the test-tube and placed on a sterilized petridish. The shoot tips were cut into small pieces and carefully sterilized to make the microbes free. Firstly, the explants were thoroughly washed with distilled water for 3 times. Then inside the Laminar Air Flow Cabinet the explants were washed with 70% ethanol for surface sterilization followed by the treatment with 0.1% $HgCl_2$ in addition with few drops of Tween-20 as a surfactant for inner surface sterilization. Finally, the explants were washed with sterile distilled water for several times to remove all the sterilizing agents and then in the laminar air flow cabinet, the shoot tip explants were cut into small pieces ranging in size from 0.1-1.0 mm by sterile surgical blade. The explants were then inoculated aseptically into the media.

Media preparation

The composition of MS medium (Murashige T and Skoog F, 1962) was adjusted by the addition of the premade stock solutions containing macronutrients, micronutrients, organic supplements and vitamins. Different concentration of 2, 4-D was used for callus induction. For the regeneration of plantlets from calli different concentration of BAP and GA_3 for shooting as well as IAA and GA_3 for rooting were used with the MS medium. The pH was adjusted to 5.8–6.0 with 1 mol L^{-1} NaOH or HCl for MS media. For the solidification of the media 0.7% (w/v) agar was added to the MS media prior to autoclaving at 121 ºC for 15 min.

Explants culture

Attempts have been made for the induction of organogenesis using shoot tip explants in MS medium supplemented with 2, 4-D (0.0, 0.2, 2.0 mg/l). Four to five explants were directly inoculated to each glass vial containing 20 ml of MS medium supplemented with different hormone concentrations as per treatments, the vial were covered and sealed with cork. The prepared cultures were kept in a growth room on the shelves. All the cultures were kept at 25±2°C illuminated with 1.83-m fluorescent tubes (4.83 ft C84 TDFL/Phillips). These tubes gave broad spectrum of light, especially in the red wavelength. The room was illuminated 16 h daily with a light intensity of 1500 lux.

Subculture of the callus for shoot regeneration

For shoot initiation, the attained convenient sized calli 28 days after inoculation of explants were placed for sub culturing on petridishes containing MS medium supplemented with different concentration of BAP (0.0, 0.5, 1.0 and 1.5 mg/l) and GA_3 (0.0, 0.5, 1.0 and 1.5 mg/l) maintaining sterilized conditions in Laminar Air Flow Cabinet. The sub cultured vials were then incubated at 22±2°C with 16h photoperiod.

Subculture of the regeneration shoot for root initiation

The sub cultured calli contained proliferated and differentiated shoots. When these shoot grew about 2-5 cm in length, rescued aseptically from the cultured vial and separated from each other and cultured on another vials with freshly prepared root induction medium with IAA (0.0, 1.0 and 2.0 mg/l) and GA_3 (0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l). Vials containing plantlets were incubated under continuous light. Day to day intended observations were recorded in response.

Hardening and Acclimatization

When the regenerated plantlets were of 30 days aged with well developed shoots and roots, they were taken out from culture vessel with the help of fine forceps. The medium attached with the roots was washed with tap water carefully. The regenerated plantlets were successf-

Table 1. Effect of 2, 4-D on callus formation (%), days to callus initiation, number of calli/culture and weight of callus (g).

2,4-D (mg/L)	Callus formation (%)	Callus initiation/days	Number of calli/culture	Weight of callus (g)
0 (control)	0 c	0	0 c	0 c
0.2	90.00 b	11.55	4.500 b	0.049 b
2.0	95.00 a	10.00	4.750 a	0.074 a
CV (%)	**14.30**	**11.04**	**14.30**	**37.36**

N.B: Similar letter indicates identical and different letter means they are not same.
(**= Significant at 1% level of probability
*= Significant at 5% level of probability
ns= not significant)

ully established *ex vitro* on sand, soil and cow dung mixture (1:2:1). To reduce sudden shock, the pots were kept in the controlled environment in the laboratory. When the plantlets appeared to be self sustainable they were then transferred to the field.

DATA COLLECTION AND ANALYSIS

The experiment was laid out in the Completely Randomized Design (CRD) with 4 replications. The data recorded above were subjected to analysis as per the design used. Analysis of variance for different records above was performed and means were compared by the Duncan's Multiple Range Test (DMRT). Visual observation of cultures was made every week and the data were recorded after 2 weeks. Three replications were used for all the treatments. At final stage all the data were analyzed critically and arithmetic mean of all treatments for each replication was calculated to find out the best treatment:

$$\% \text{ of callus formed} = \frac{\text{No. of cultures formed callus}}{\text{Total No. of culutres inoculated}} \times 100$$

$$\text{Per cent shoot formation} = \frac{\text{Number of calli showing shoot}}{\text{Number of calli inoculated}} \times 100$$

RESULTS

The experiment was conducted to assess the performance of potato variety Granula on *in vitro* callus formation with associated traits, and shoot and root formation with the phenotypic characters.

Callus induction and subculture

Profound callus were obtained from the MS medium **(Plate 1a & 1b)** were supplemented with various concentration of 2, 4-D. Per cent of callus initiation had shown significant differences between the different concentration of 2, 4-D used in culture media. No callus formation occurred in the control treatment. 2, 4-D concentration 0.2 mg/l had taken significantly the maximum days (11.55) while in contrast 2.0 mg/l had taken minimum days (10.00). The highest number of callus/culture (4.750) was observed in the concentration of 2.0 mg/l 2, 4-D. The effect of different concentration of 2, 4-D on callus weight had shown significant differences. 2, 4-D at 2.0 mg/l had highest weight of callus (0.074g). The lowest weight of callus (0.049g) was found for 0.2 mg/l2, 4-D (Table 1).

Effect of BAP and GA_3 on in vitro shoot regeneration of potato

The combined effects between different concentrations of BAP and GA_3 has shown significant differences on a number of shoots/explants, per cent shoot initiation, days required for shoot initiation, number of leaves/plantlet and shoot length.

The maximum number of shoots (4.67) was produced by 1.0 mg/l BAP and 0.5 mg/l GA3 concentration which was followed by 1.0 mg/l BAP and 1.0 mg/l GA3 (4.33). In contrast, MS media without BAP and 0.5 mg/l GA3 was produced the minimum number of shoots/explants (0.67) **(Table 2).**

The highest shoot percentage (93.33%) was observed with the combination of 1.0 mg/l BAP + 0.5 mg/l GA3 which was followed by 1.0 mg/l BAP and 1.0 mg/l GA3 (86.67). In contrast, the lowest percentage (13.33%) appeared in without BAP+0.5 mg/l GA3 (**Table 2).**

The maximum days (21.00) required to shoot initiation was found for 0.0 mg/l BAP + 0.5 mg/l GA3 which was followed by 19 days for 0.0 mg/l BAP + 1.0 mg/l$GA_3$and 18.33 days for 1.5 mg/l BAP + 1.5 mg/lGA_3. In contrast, minimum number of days (15.00) was required for shoot initiation was in 1.0 mg/l BAP + 0.0 mg/l GA3 **(Table 2 and Plate 1c).**

Table 2. Effect of BAP and GA_3 on *in vitro* shoot regeneration of potato.

BAP (mg/L)	GA_3 (mg/L)	No. of shoots/ explants	Shoot initiation (%)	Shoot initiation (days)	No. of leaves/ plantlet	Shoot length (cm)
0	0	0.00 f	0.00 f	0.00	0.00 e	0.00 f
	0.5	0.67 ef	13.33 ef	21.00	0.33 ef	1.67 e
	1.0	1.33 de	26.67 de	19.00	0.67 def	2.00 de
	1.5	2.33 cd	46.67 cd	17.67	1.33 cde	2.67 cde
0.5	0	1.33 de	26.67 de	18.67	0.67 def	2.00 de
	0.5	2.33 cd	46.67 cd	16.67	1.33 cde	2.67 cde
	1.0	3.33 c	66.67 c	16.33	2.33 bc	4.00 abc
	1.5	2.67 c	53.33 c	15.67	1.67 cd	3.67 abcd
1.0	0	2.67 c	53.33 c	15.00	2.33 bc	3.33 bcde
	0.5	4.67 a	93.33 ab	15.00	3.67 a	5.33 a
	1.0	4.33 ab	86.67 ab	15.33	3.33 ab	5.00 ab
	1.5	3.33 bc	66.67 bc	16.67	2.33 bc	4.00 abc
1.5	0	2.67 c	53.33 c	17.33	1.67 cd	3.33 bcde
	0.5	3.33 bc	66.67 bc	16.33	2.33 bc	4.00 abc
	1.0	3.00 c	60.00 c	15.33	1.67 cd	3.33 bcde
	1.5	1.33 de	26.67 de	18.33	0.33 ef	2.00 de
CV (%)		**21.97**	**21.97**	**4.89**	**34.40**	**29.05**

N.B: Similar letter indicates identical and different letter means they are not same.
(**= Significant at 1% level of probability
*= Significant at 5% level

The highest number of leaves/plantlet (3.67) was produced by the combination of 1.0 mg/l BAP +0.5 mg/l GA3 which was followed by 1.0 mg/l BAP +1.0 mg/l GA3 (3.33), while the lowest number of leaves (1.667) was produced with 0.0 mg/l BAP + 0.5 mg/l GA3 **(Table 2 and Plate 1d).**

The highest shoot length (5.33cm) was produced by the combination of 1.0 mg/l BAP +0.5 mg/l GA3 which was followed by 1.0 mg/l BAP +1.0 mg/l GA3 (5.00 cm), while the lowest shoot length (1.67 cm) was produced with 0.0 mg/l BAP + 0.5 mg/l GA3 **(Table 2 and Plate 1e & 1f).**

Effect of IAA and GA_3 on in vitro root regeneration of potato

The combined effects between different concentrations of IAA and GA_3 showed significant differences on number of roots/shoot, days required for root initiation, root length after 20 days.

The highest number of roots/shoot (3.33) was observed in 1.0 mg/lIAA+1.0 mg/l GA3 which was followed by 0.0 mg/lIAA+3.0mg/l GA3 (2.33), while the lowest number of roots (0.67) was observed with the combination of 0.0 mg/l IAA + 1.0 mg/l GA3 and 2.0 mg/l IAA + 5.0 mg/l GA3 **(Table 3).**

Table 3 Effect of IAA and GA_3 on *in vitro* root regeneration of potato

IAA (mg/L)	GA_3 (mg/L)	No. of roots/shoot	Days to root initiation	Length of root	
				20 Days per initiation (DAI)	28 Days per initiation (DAI)
0	0	0.00 e	0.00	0.00 e	0.00 e
	1.0	0.67 de	18.67	3.67 d	4.67 cd
	2.0	1.00 cde	17.33	4.00 cd	5.00 cd
	3.0	2.33 abc	15.67	5.33 abc	6.00 abcd
	4.0	1.67 bcd	16.33	4.67 bcd	5.67 abcd
	5.0	1.33 cde	18.33	4.33 cd	5.33 bcd
1.0	0	1.67 bcd	16.67	4.67 bcd	5.67 abcd
	1.0	3.33 a	12.33	6.33 a	7.33 a
	2.0	3.00 ab	13.67	6.00 ab	7.00 ab
	3.0	2.33 abc	14.00	5.33 abc	6.33 abc
	4.0	1.67 bcd	15.33	4.67 bcd	5.33 bcd
	5.0	1.33 cde	16.67	4.33 cd	5.33 bcd
2.0	0	2.33 abc	13.67	5.33 abc	6.33 abc
	1.0	3.00 ab	13.67	6.00 ab	7.00 ab
	2.0	2.33 abc	14.33	5.33 abc	6.00 abcd
	3.0	2.00 abcd	15.67	5.00 abcd	6.00 abcd
	4.0	1.33 cde	16.67	4.33 cd	5.00 cd
	5.0	0.67 de	19.00	3.67 d	4.33 d
CV (%)		**38.27**	**4.48**	**14.76**	**15.15**

N.B: Similar letter indicates identical and different letter means they are not same.
(**= Significant at 1% level of probability
*= Significant at 5% level of probability
ns= not significant)

The maximum number of days (18.67) was required for root initiation from shoot tip with the combination of 2.0 mg/l IAA and 5.0 mg/l GA3 which was followed by 0.0 mg/lIAA+3.0mg/l GA3 (18.33), while the lowest number of days (12.33) was with the treatment combination of 0.0 mg/lIAA+1.0 mg/l GA3 **(Table 3).**

The highest length (6.33 and 7.33 cm) of root was observed with 1.0 mg/l IAA + 1.0 mg/treatment combination 20 and 28 days after initiation respectively. The lowest (3.67 and 4.67 cm) was observed with the treatments combination of 0.0 mg/l IAA + 1.0 mg/l GA3 after 20 and 28 days from initiation respectively **(Table 3 and Plate 1g & 1h).**

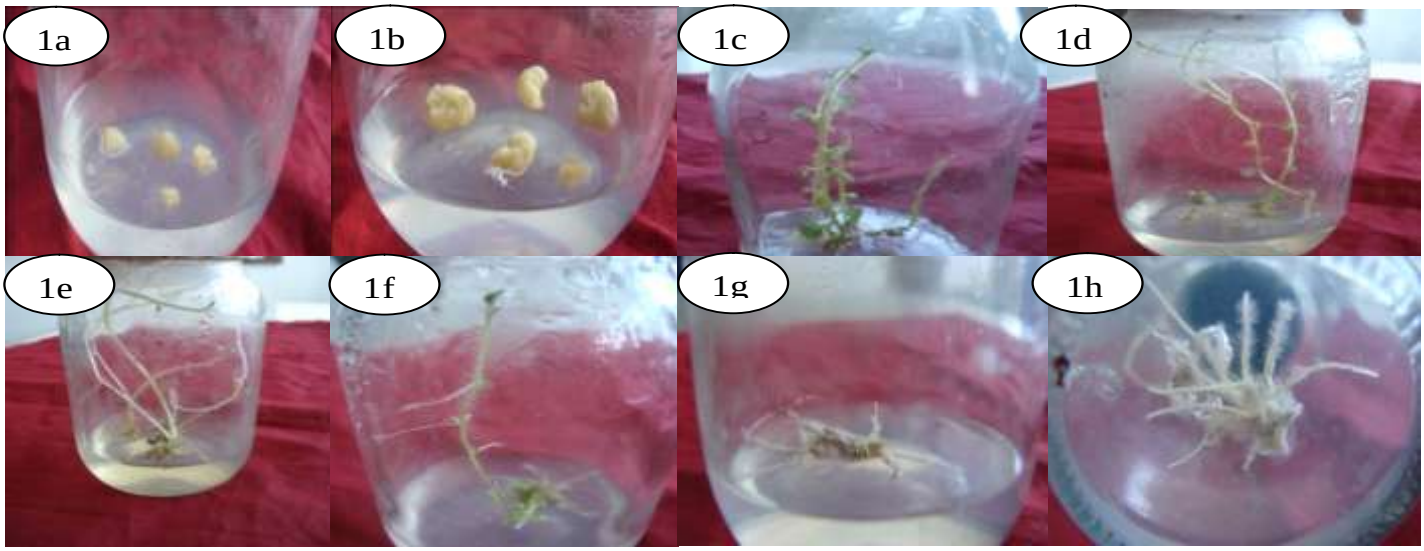

Plate 1 Callus induction and regeneration of potato viz., (1a) calli after 10 days of culture at 2.0 mg/l of 2, 4-D, and (1b) calli after 12 days of culture at 0.2 mg/l2, 4-D, (1c) shoot initiation at 0.0 mg/l BAP+0.5 GA_3 after 21 days, (1d) highest number of leaves at 1.0 mg/l BAP + 0.5 mg/l GA_3 after 28 days, (1e) highest length of shoots at 1.0 mg/l BAP + 0.5 mg/l GA_3 after 27 days, (1f) highest number of leaves at 1.0 mg/l BAP + 0.5 mg/l GA_3, (1g) highest root length (cm) at the 0.0 mg/l IAA + 1.0 mg/l GA_3 after 20 days and (1h) highest root length (cm) at 1.0 mg/l IAA + 1.0 mg/l GA_3 after 28 day.

DISCUSSION

For callus induction, different concentrations of 2, 4-D (0.2 mg/land 2.0mg/l) had shown better callus percentage (95.00%) in 2.0 mg/l 2, 4-D within a minimum number of days (10.00). Maximum number (4.75) of calli/culture in 2.0 mg/l2, 4-D. Weight of callus is also better performance (0.074 g) in 2.0 mg/l2, 4-D. Among the all parameter did not show any results in control treatment (without 2, 4-D). Many researchers observed 2, 4-D as the best auxin for callus induction as common as in monocot and even in dicot (V. S. Jaiswal and P. Narayan, 1985). This result is an agreement with (Sultana R. S., 2000) that used 2, 4-D for callus induction of cultivar Diamante and cardinal potato variety and was obtained similar result. (N. Khatun, M. A. Bari, R. Islam, S. Huda, N. A. Siddque Rahman, M. A. and M. U. Mullah, 2003) used 2, 4-D alone for callus induction but the concentration of 2,4-D was 2.5 mg/L.

For regeneration, maximum number (3.75) of shoots/explants and highest shoot initiation (75.0%) was observed within a number of days (15.50) in 1.0 mg/l BAP. BAP at 1.0 mg/l also showed maximum number (2.92) of leaves/plantlet and longest shoot (4.42 cm). In control treatment (without BAP), both of the highest number (1.08) and initiation (21.67%) of shoots/explants had found within 14.42 days. The main effect of GA_3 had shown better performance at 1.0 mg/l. The number (3.00) of shoots/explants and shoot initiation (60.00%) were shown at days 16.50. Maximum number (2.00) of shoots/explants and longest shoot (3.58 cm) were also found in 1.0 mg/lGA_3. In case of interaction effect of BAP and GA_3 all the parameters for shoot regeneration showed significant differences. Maximum number (4.67) of shoot/explants, the highest shoot percentage (93.33%), the highest number (3.67) of leaves/plantlet, the longest shoot (5.33 cm) were observed with 1.0 mg/l BAP + 0.5 mg/l GA3 within a minimum number of days (15.00 days) for shoot initiation. These results are similar with (Resende R. D. O. and Paiva M, 1986). The maximum days (21.00) required in 0.0 mg/l BAP + 0.5 mg/lGA_3. Minimum number of days (15.00) was required for shoot initiation in 1.0 mg/l BAP + 0.0 mg/lGA_3. This result is confirmed earlier by (A. Martel and E. Carcia,1992).

On the other hand the root initiation was done by the effect of different concentrations of IAA. Maximum number (2.22) of roots/shoot within a number of days (14.78), the longest (5.22 and 6.62cm) root was observed with 1.0 mg/l IAA after 20 and 28 DAI, respectively. The main effect of GA_3, maximum number of roots/shoot (2.33) within a number of days (14.89), the longest root after 20 DAI (5.33 cm) and after 28 DAI (6.33 cm) were observed with 1.0 mg/lGA_3. The combined effect of IAA and GA_3, maximum number (3.33) of roots/shoot within a minimum number of days (12.33), the longest root at 20 DAI (6.33 cm) and at 28 DAI (7.33 cm) were observed with 1.0 mg/l IAA + 1.0 mg/lGA_3. The present study is similar with (J. M. Amezqueta, C. A. Mingo and E., 1989) who also observed that the highest regenerated plants and fastest growth rates from shoot tip explants with IAA and GA_3.

CONCLUSION

It can be concluded from this study that the MS media with 2.0 mg/l 2, 4-D is a good option for studying callogenesis in potato Granula variety. Because of the polyploidy, sexual reproduction in potato is difficult demanding an alternative to introduce variation in existing potato cultivars. Callus culture is one such option. From the above result, it can be concluded that the best hormonal combination for shoot tip explants of Granula variety is 1.0 mg/l BAP + 0.5 mg/l GA3 and 1.0 mg/l IAA + 1.0 mg/lGA_3. The protocol developed from the present experiment may be useful for large scale production of healthy and disease-free planting materials of potato commercially. Also the findings of the study may be used for genetic transformation for the improvement of potato using biotechnological approach.

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