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# Callus Induction and Regeneration from Nodal Segment of Potato Cultivar Diamant

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**Abstract:** The nodal segments of Diamant cultivar of potato from ***in vitro*** grown plantlets were cultured for callus induction and regeneration on MS semisolid medium supplemented with different concentrations of 2,4-D, NAA, BAP alone and NAA with BAP. Highest 90.00% of callus formation was observed in MS+2.5 $mgl^{-1}$2,4-D. The second highest 83.33% callus induction was recorded in MS+5.0 $mgl^{-1}$ BAP. Maximum percentages (70.00%) of calli-induced shoots were observed in MS medium fortified with 5.0 $mgl^{-1}$ BAP+0.1 $mgl^{-1}$IBA. The regenerated shoots were rooted on MS and ½MS medium containing different concentrations of IBA and maximum rooting response was achieved in ½ MS +1.0 $mgl^{-1}$IBA. Regenerated plants were successfully established in soil after acclimatization.

**Key words:** Potato, diamant, node, explant, callus, induction and regeneration

## Introduction

The potato (***Solanum tuberosum*** L.) is a vegetable crop of major economical importance world -wide. It is the further most cultivated food crop after wheat, rice and maize and therefore, the most important dicotyledonous and tuber crop (Jones, 1994) As such potato growers produce about 325 million tons of potato annually (World book, 2000). At present In Bangladesh potato ranks third after rice and wheat in regards to land area under cultivation. A number of improve varieties are now cultivated in Bangladesh but the local cultivars are still occupy a significant coverage of total area of cultivation. Several modern varieties (HYV) are now cultivated in Bangladesh but the traditional varieties still occupy about 35% of the total potato production area (Ilangantileke ***et al.***, 2001). Area under potato cultivation has been increasing over the past several years with an enhanced rate of production (BBS). In Bangladesh potato production is being seriously hampered due to the attack of virus, fungus and bacterial diseases causing 30%-100% loss in cultivation during cultivation and storage. Meristem culture by ***in vitro*** techniques provides a novel new venture for developing virus free potato varieties.

Somaclonal variation has been possible through callus culture producing disease free plants caused by the pathogen bacteria, fungus but not virus. Somaclonal variation is an appropriate method fungus, bacteria elimination and genetic improvement. It has been suggested that somaclonal variation may be a way of generating useful genetic variation and selection for desired trails could be performed ***in vitro***.

Somaclonal variation may be powerful tool for the production of regenerates tolerant to environmental stresses (Nabors and Dykes, 1985) and pathogens. The potato somaclones we are also screened for both late and early blight resistance (De, 1992). In addition, several other disease resistant varieties were recovered and such variant are registrant to early blight and to multiple races of phytophthpra infestans (De, 1992).

**Materials and Methods**

Shoot tips of potato Diamant cultivar were collected from 20-25 days old field grown plants and wash thoroughly under running tap water, than treated with 1.0% savlon and 1-2 drops of tween-80 for about 20 min. This followed by successive three washing with distilled water to make the material free from savlon. Surface sterilization was carried out with 0.1% $HgCl_2$ for 2 min. followed by gentle shaking. Then the sterilized shoot tips were washed at least five times with sterile distilled water immediately to remove all traces of $HgCl_2$. After sterilization, the explants were laid on the sterile like using sterile forceps. Shoot tips was hold in one hand under stereomicroscope with the held of a pair forceps and the immature leaves and primordial leaves were snapped with slight pressure from the needle. Then the exposed meristem tips that appeared as a shiny dome were gentle isolated with a sharp blade. A single excised meristem tip was carefully placed on the filter paper bridge of the culture tubes containing MS (Murashige and Skoog, 1962) liquid medium with the growth regulator of KIN and $GA_3$. The culture tubes were placed in the growth chamber under 16/8 hrs dark/ light cycle at $20\pm2^0$c. After 20-25 days the culture tube transferred on MS semi solid medium with the growth regulator (KIN + $GA_3$) after four weeks the plantlets attained a highest of 9-10 cm micro propagation was started. The plants were removed from the test tube over a sterile tile using a pair of forceps. On the tile the leaves from the stem were carefully remove and cut into single node segment The node were cultured on to agar solidified MS medium supplemented with different concentrations and combinations of growth regulators 2, 4-D, NAA, BAP and NAA with BAP in order to find out the most suitable culture media formulation to induce the explants to develop maximum callus. The cultured test tubes were incubated in dark condition at $20\pm2^\circ$C for 50 to 60 days. Callus induction started within 30 to 40 days and continues to grow 50 to 60 days. After 60 days the calli were sub cultured in the same callus induction medium. The selected calli were transferred on MS regenerate medium supplemented with different combination 5.0 $mgl^{-1}$ BAP +0.1 $mgl^{-1}$ IBA for shoot induction. The cultured tubes were incubated in the growth chamber under 16/8 hrs light/dark cycles at $20\pm2^0$C. Data on shoot proliferation efficiency were recorded after two weeks of culture. Proliferated shoots were transferred to MS and ½ MS basal media with different concentrations of IBA for adventitious root formation.

**Results and Discussion**

This investigation was carried for callus induction and subsequent regeneration from nodal explants of Diamant cultivar of potato. Callus induction was observed on MS medium containing different concentration of 2,4-D, NAA, BAP alone and NAA in combination with BAP within 30-70 days of incubation of nodal segments depending upon the concentrations and combinations of

Table 1: Effect of 2, 4-D, NAA, BAP and NAA with BAP in MS semi solid medium on callus induction from nodal explants of the Diamant cultivar of potato after twelve weeks of culture

| Growth regulators ($mgl^{-1}$) | Days to callus initiation | % of callus formation | Texture of callus | Callus colour | Degree of callus initiation |
|---|---|---|---|---|---|
| 2, 4-D 1.0 | 50-60 | 20.00 | non friable | creamy | + |
| 2, 4-D 1.5 | 50-60 | 40.00 | non friable | creamy | + |
| 2, 4-D 2.0 | 45-60 | 70.00 | friable | light brown | ++ |
| 2, 4-D 2.5 | 45-60 | 90.00 | friable | light brown | +++ |
| 2, 4-D 3.0 | 45-60 | 80.00 | non friable | brown | +++ |
| 2, 4-D 3.5 | 50-65 | 50.00 | non friable | brown | + |
| 2, 4-D 4.0 | 50-70 | 40.00 | non friable | brown | + |
| NAA 1.0 | 40-60 | 10.00 | watery | white | + |
| NAA 1.5 | 40-60 | 30.00 | watery | white | + |
| NAA 2.0 | 40-60 | 30.00 | watery | light yellowish | + |
| NAA 2.5 | 40-55 | 50.00 | soft | light yellowish | ++ |
| NAA 3.0 | 40-55 | 56.00 | soft | light yellowish | ++ |
| NAA 3.5 | 40-60 | 40.00 | watery | light yellowish | + |
| NAA 4.0 | 40-60 | 20.00 | watery | white | + |
| BA 1.0 | - | - | - | - | - |
| BA 2.0 | - | - | - | - | - |
| BA 3.0 | - | - | - | - | - |
| BA 4.0 | 50-60 | 50.00 | hard | green | ++ |
| BA 5.0 | 30-40 | 83.33 | hard | green | +++ |
| BA 6.0 | 50-60 | 66.00 | hard | green | +++ |
| BA 7.0 | 50-60 | 33.33 | hard | green | + |
| BA 8.0 | - | - | - | - | - |
| NAA 0.5+BA 0.5 | 50-60 | 20.00 | cottony | white | + |
| NAA 0.5+BA 1.0 | 40-60 | 30.00 | cottony | light green | + |
| NAA 1.0+BA 0.5 | 40-60 | 30.00 | cottony | light green | + |
| NAA 1.0+BA 1.0 | 30-45 | 60.00 | cottony | light brown | +++ |
| NAA 1.5+BA 0.5 | 30-60 | 40.00 | cottony | light brown | + |
| NAA 1.5+BA 1.0 | 30-60 | 30.00 | cottony | white | + |

Massive = +++, Moderate = ++, Slight = +

Table 2: Effect of BAP in combination with IBA in MS medium on shoot regeneration from node derived callus of the Diamant cultivar of potato after eight weeks of culture

| Growth regulators ($mgl^{-1}$) | Days to shoot initiation | % of calli with shoots | Number of shoots/ callus | Shoot length after (cm) | |
|---|---|---|---|---|---|
| | | | | 15 days | 30 days |
| BAP 5.0 + IBA 0.1 | 15-20 | 70.00 | 8.60 | 1.90 | 4.20 |
| BAP 5.0 + IBA 0.5 | 17-30 | 50.00 | 5.90 | 1.30 | 2.90 |
| BAP 5.0 + IBA 1.0 | 20-30 | 30.00 | 5.00 | 0.70 | 1.90 |
| BAP 5.0 + IBA 1.5 | 20-30 | 10.00 | 4.90 | 0.50 | 1.60 |

hormone. Callus induction was noticed in most of the media formulations. But there was a wide rang of variation in days to callus initiation, percentage of callus formulation, texture of callus and colour of callus. Among all the treatments the highest percentage of callus induction (90.00) was observed on medium containing 2.5 $mgl^{-1}$2, 4-D within 45-60 days and this was followed by

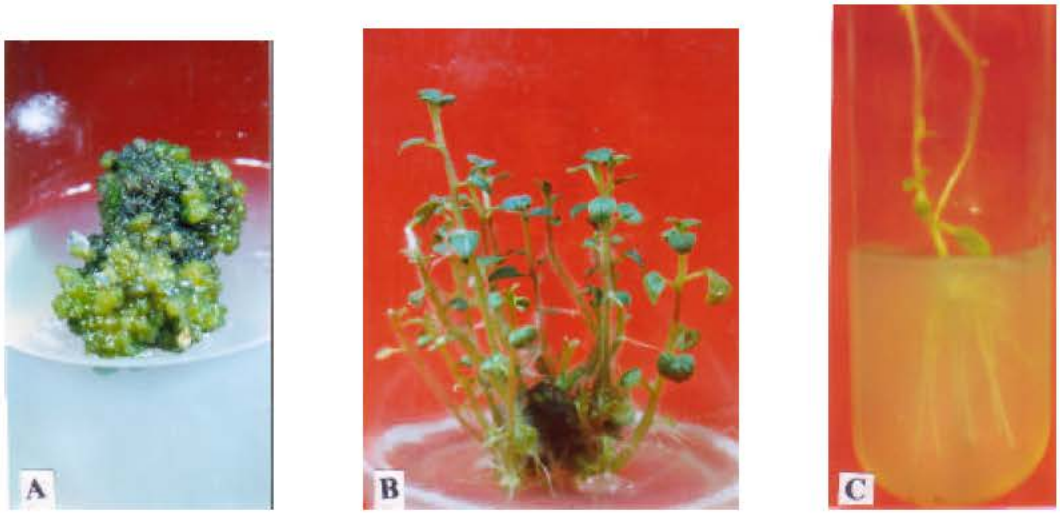


Fig: 1: Callus induction and shoot proliferation from node explants of Diamant cultivar of potato

A. Induction of green callus from nodal segment on MS + 5.0 $mgl^{-1}$ BAP after 8 weeks of culture

B. Multiple shoots produced from nodal segment derived callus on MS+5.0 $mgl^{-1}$ lBAP+0.1 $mgl^{-1}$IBA

C. Rooting of regenerated shoot

83.33 percentages in 5.0 $mgl^{-1}$BAP (Table 1). Colours of calli were mostly green, light green, brown and yellowish. Many workers observed 2, 4-D as the best auxin for callus induction as common as in monocot and even dicot (Evans *et al*., 1981; Ho and Vasil, 1983; Jaiswal and Naryan, 1985; Chee, 1990). In the present investigation 2, 4-D alone showed effect for callus induction in potato. This finding is in agreement with that of Kamat and Rao (1987) and Mamun *et al*. (1996) who reported that 2, 4-D also effective when used alone for other species. In Diamant cultivar maximum callusing rate 90.00% were found at 2.5 $mgl^{-1}$2, 4-D from nodal explants. The results corroborate with that of Malamug *et al*. (1991) who also observed highest callus induction from nodal explants of potato.

In the present investigation it was observed that auxin (2,4-D, NAA) cytokinin (BAP) along and auxin with cytokinin (NAA + BAP) in combination could induce callus but 2,4-D or BAP alone produced callus better than other growth regulators and it was also observed that usually callus proliferation was started from the cut surface of the explants and finally covered the explants. For shoot differentiation, calli induced in 5.0 $mgl^{-1}$BAP were sub-cultured on MS medium supplemented with 5.0 $mgl^{-1}$ BAP with different concentrations of IBA. The maximum 70.00% of shoot regeneration was observed in 5.0 $mgl^{-1}$ of BAP and 0.1 $mgl^{-1}$IBA and number of shoots per callus was 8.60 and this was followed by 50.00% in 5.0 $mgl^{-1}$ of BAP with 0.5 $mgl^{-1}$ of IBA and number of shoots per callus was 5.90 (Table 2). It was noticed node derived calli in potato under the present investigation when transferred to medium containing 5.0 $mgl^{-1}$ BAP and 0.1 $mgl^{-1}$ IBA

shoot proliferation started within 15 to 20 days of subculture similar results also been reported by Islam and Riazuddin (1993) when the calli were transferred to medium containing BAP (2-10 $mgl^{-1}$) and IAA (0.1-1.0 $mgl^{-1}$) shoot proliferation was started within two weeks days of subculture. In the present study it has been observed that length of shoot decreased with the increased in shoot number. Similar result observation was made earlier (Karth ***et al.***, 1981; Rao and Chopra, 1989). For adventitious root formation, plantlets were sub cultured on MS and ½ MS medium supplemented with different concentrations of IBA. It was observed that 1.0 $mgl^{-1}$ IBA in ½ MS medium was the most effective for rooting of shoots in Diamant cultivar of potato (Data were not shown).

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