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*Article*

# Effect of growth regulators on *in vitro* multiplication of potato (*Solanum tuberosum* L.) cv. diamant

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**Abstract:** Shoot tip and nodal segment explants from field grown plants were used as experimental materials in this investigation. All explants were cultured on MS medium supplemented with various plant growth regulators. For surface sterilization of explants, $HgCl_2$ (0.1%) for 2 minutes was found to be most effective for complete killing of surface pathogens and getting healthy tissues. Shoot regeneration was observed from both shoot tips and nodal explants for the studied plant. Various concentrations of BAP (0.1, 0.2, 0.3mg/l) and $GA_3$ (0.1, 0.2, 0.3mg/l) were used for shoot multiplication. In case of BAP, the highest length of shoot was recorded 4 cm and the highest percentage of shoot multiplication (73%) was noticed in MS+0.2mg/l BAP. And in case of $GA_3$, the highest response for shoot multiplication (82%) was noticed in MS+ 0.1 mg/l $GA_3$.But among all of the media formulations used in this experiment, the highest response for shoot multiplication (95%) within7-10 days was noticed in hormone free MS media. In case of root regeneration, the highest percentage (96%) of root induction was recorded in MS medium supplemented with no hormone.

**Keywords:** potato; shoot tips; micropropagation; BAP; GA3

## 1. Introduction

The potato (*Solanum tuberosum* L.) one of the important vegetable crop in Bangladesh is a staple food crop in many countries of the world as well. It ranks fourth in production among crop plants grown for human consumption. It is an annual herbaceous plant, which is vegetatively propagated by the tuber. Potato is the 2nd largest food crop in Bangladesh and occupying 2nd position after rice. According to the FAO report, Bangladesh was raised to the rank 6th just behind the German in the scenario of world potato production. Many researchers used different growth regulators for *in vitro* induction of microtuber in potato (Hossain and Sultana, 1998). It is grown in 180 countries worldwide. Meristem culture was possibly the first biotechnological approach used to eliminate viruses from systemically infected potato clones. Over the years, this technique has been successfully combined with micro propagation to produce disease free potato seed. Plant tissue culture is a specialized technology used for plant propagation. It operates on the principle of growing disease free plant tissues under sterile conditions in artificial plant growth medium. Through tissue culture vary large numbers of identical plantlets can be derived from one mother plantlet. This technology and the resulting plantlets now form the basis of many plant nursery and flower trade industries. Throughout the world, thousands of laboratories apply plant tissue culture technologies to crops, ornamental plants and endangered plant species. In the late 1970's, the technology for large scale tissue culture was refined for potato production. Today, almost all seed potato production systems incorporate this technology in some way. At present, there are thirty plant tissue culture laboratories established in a different part of the country chiefly targeted for seed potato production. In addition about twelve thousands small farmers have been integrated with seed potato production by using disease

indexed plantlets purchased from private companies (Hossain and Islam, 2013). The objectives of the present investigation was to develop a reproducible cost effective protocol for large scale production of *Solanum tuberosum* L. plantlets from selectively better clones through plant *in vitro* propagation methods; selection of growth regulators for proper multiple shoots regeneration, elongation and root induction; to produced genetically uniform plantlets and to obtain a large number of plantlets within a short period of time.

## 2. Materials and Methods

The present research was conducted at Genetic Engineering and Biotechnology central lab in Jessore University of Science and Technology, Bangladesh from January, 2014 to June, 2014.

### 2.1. Plant materials

In the present investigation *Solanum tuberosum* (potato) one of the important plants of Bangladesh was used as explants source for initial experiment. Shoot tips and nodal segments were used for micro propagation of *Solanum tuberosum* (potato).

### 2.2. Chemicals

**Plant growth regulators**

Different types of plant growth regulators and additives were used for this experiment. They are presented in the following Table 1:

**Table 1. Plant growth regulators.**

| | |
|---|---|
| **Cytokinins** | |
| 6- benzyl amino purine | BAP |
| Kinetin | KIN |
| **Gibberellins** | |
| Gibberellic acid | $GA_3$ |
| **Auxin** | |
| Indole-3 acetic acid | IAA |

**Nutrient basal salts**

For plants nutrient basal salts were used which contains macro, micro nutrients and vitamins.

(a) **Macronutrients:** $MgSO_4.7H_2O$, $KH_2PO_4$, $NaH_2PO_4.H_2O$, $KNO_3$, $NH_4NO_3$, $CaCl_2.2H_2O$, $(NH_4)_2SO_4$.

(b) **Micronutrients:** $H_3BO_3$, $MnSO_4.H_2O$, $ZnSO_4.7H_2O$, $NaMoO_4.2H_2O$, $CuSO_4.5H_2O$, $CoCl_2.6H_2O$, KI, $FeSO_4$, $Na_2$.EDTA.

(c) **Vitamins:** Thiamine. HCL, Pyrodoxine.HCL, Nicotinic acid, Myo-inositol.

### 2.3. Media used for micro propagation

In the present experiment different culture media with various growth regulators and additives were used for shoot tip and nodal segment culture:

i) MS (Murashige and Skoog, 1962) medium with different concentrations and combinations of BAP, $GA_3$ were used for shoot induction. For carbon source 3% sugar was used and the medium was solidified with agar.

ii) Full strength of medium with different concentration of IAA and KIN were used for root induction.

### 2.4. Preparation of 1 litter MS medium

Following steps were done for the preparation of 1 litter MS medium

**a. Assembling of the medium components**

For the preparation of 1 litter of MS medium, 20 ml of stock solution-I, 10 ml stock solution-II, 10 ml stock solution-III, 10 ml stock solution-IV, and 10 ml stock solution MS-V , 10 ml stock solution VI, 10 ml stock solution VII, were added in 1 liter flask containing 500 ml distilled water and mixed well.

**b. Sucrose**

Sucrose (30 gm/l) was added and final volume of the mixture was made 1 litter by adding distilled water.

**c. Addition of growth regulators**

Stock solution of growth regulators were added in appropriate concentration and combination in above solution

and were mixed well.

**d. Adjust pH of the medium**

In all tests the pH of the medium was adjusted to 5.7 using a digital pH meter with the help of 0.1N HCl, or 0.1 N NaOH (where necessary) before addition of agar.

**e. Addition of agar**

After adjusting pH Agar (6.5 g/l) was added. Then the medium was heated for 5 minutes in a microwaven to melt agar completely.

**f. Medium dispensing to culture vessels**

The prepared melted medium was disposed into culture vessels such as test tube (13X25 mm) or conical flasks (250 ml), through separating funnel. The culture vessels were plugged with cotton-plugs, warped with cheesecloth, which were inserted tightly at the mouth of vessels. The culture vessels were marked to designate specific hormonal supplement.

**g. Sterilization**

Finally the culture vessels containing the medium were autoclaved at 15 id/inch$^2$ pressures and at the temperature of 120°C- 121°C for 20 min to insure sterilization. Then the vessels with the medium were allowed to cool as vertically and then marked with a glass marker pen to indicate specific hormonal supplements and stored in the culture room for ready use.

### 2.5. Culture technique for shoot tip and nodal segment explants

Following methods were employed in the present experiment for the establishment of shoot tip and nodal maintenance culture.

#### 2.5.1. Collection and preparation of plant materials

Terminal shoot tips with immature leaves from field grown plants were collected. The excess unnecessary parts like mature shoots and leaves were removed from the collected materials and the remaining part of shoot segments were cut into nodal segments with convenient size (4-5 cm in length) were collected in separated conical flask. Both the materials were washed thoroughly under running tap water for several times to reduce the dust and surface contaminants and then taken in conical flask containing distilled water 30 minutes then adding with a few drops of savlon and washed for 4-5 minutes with constant shaking. Second washing was accomplished with 70% (v/v) ethanol for 30 seconds. Gradual changing of distilled water until removing all traces of above chemicals then followed it.

#### 2.5.2. Surface sterilization

The procedure of surface sterilization was carried out inside of laminar airflow cabinet. The above materials were taken into sterile flask and suspended in 0.1% $HgCl_2$ solution for different period to ensure contaminant free culture. The sterilized materials were washed 7-8 times with sterile distilled water immediately to remove all the traces of $HgCl_2$. The surface sterilized explants were sized to 1.0-1.5 cm in length.

#### 2.5.3. Inoculation of explants

Prepared explants were carefully inoculated in culture vessels (especially test tube) containing agar gelled nutrient medium supplemented with different concentration of hormones. The cotton plugs of the culture vessels were removed inside laminar airflow cabinet in presence of spirit lamp flame. During inoculation, special cares were taken that the explants must touch on the medium equally and not dip into the medium. After inoculation the mouth of culture vessels were tightly plugged and marked by glass marker with inoculation date.

#### 2.5.4. Incubation

The inoculated culture vessels were incubated in a growth chamber providing a special culture environment. All culture vessels were grown in the growth chamber illuminated by 40 watts white fluorescent tubes fitted at a distance at 30-40 cm from the cool fluorescent light intensity varied from 2000-3000 lux. The photoperiod was maintained generally 16 hours light and 8 hours dark. The culture vessels were checked daily to note the response.

#### 2.5.5. Acclimatization and transplantation of plantlets

Micro propagated plantlets with well-established root system were washed carefully to remove media and then transferred to pots containing sterile fertile garden soil. Plantlets (5-7 cm high) were transferred to soil and each pot was enclosed in a polyethylene bag. Bags were progressively opened weekly. After 3 weeks, when

acclimatization was completed, then plantlets were transferred to large pots for further growth in the field.

## 3. Results

### 3.1. Direct regeneration

Different growth regulators including BAP and $GA_3$ were used in different concentrations for induction of direct shoot buds from nodal explants and shoot tips of potato. Nodal segments and shoot tips responded for direct organogenesis in the MS media having different concentrations and combinations of growth regulators.

#### 3.1.1. Effects of BAP

In consideration of BAP, three different concentrations (0.1, 0.2 and 0.3 mg/l) were used to test their effects on multiple shoot induction from shoot tips and nodal segments. The highest percentage of shoot multiplication (73%) was noticed in MS+0.2mg/l BAP (Table 2). The highest length of shoot was recorded 4 cm. Lowest percentage of shoot multiplication was 56% and length of shoot was 3 cm was obtained in MS+ 0.1 mg/l BAP within 15-20 days.

**Table 2. Effects of different concentration of cytokinin (BAP) on multiple shoot regeneration from shoot tips and nodal segments. Data were recorded after five weeks.**

| Hormone supplement used in MS medium mg/l BAP | No. of explants inoculated | % of explants responded | Days to shoot formation | Highest length of shoots in cm (M±S.E.) |
|---|---|---|---|---|
| 0. 1 | 20 | 56 | 15-20 | 3±0.29 |
| 0.2 | 20 | 73 | 10-15 | 4±0.58 |
| 0.3 | 20 | 65 | 12-18 | 3.5±0.29 |

#### 3.1.2. Effects of $GA_3$

Different concentration of $GA_3$ (0.1, 0.2 and 0.3mg/l) were tested to find out their effects on multiple shoot induction from shoot tips and nodal segments. The highest percentage of shoot multiplication (82%) was observed in MS+ 0.1 mg/l $GA_3$ (Table 3).The highest length of shoot was recorded 4.5 cm.A gradual decline in shoot induction was observed when $GA_3$ concentration was increased above 0.1 mg/l. The lowest percentage of shoot multiplication was 63% and length was 3 cm obtained in MS +0.3 mg/l $GA_3$ within 10-15 days (Figure 1, 2 and 3).

**Table 3. Effects of different concentration of gibberellic acid ($GA_3$) on multiple shoot regeneration from shoot tips and nodal segments. Data were recorded after five weeks.**

| Hormone supplement used in MS medium mg/l GA3 | No. of explants inoculated | %of explants responded | Days to shoot formation | Highest length of shoots in cm (M±S.E.) |
|---|---|---|---|---|
| 0.1 | 20 | 82 | 9-12 | 4.5±0.29 |
| 0.2 | 20 | 75 | 9-14 | 4±0.14 |
| 0.3 | 20 | 63 | 10-15 | 3±0.43 |
| MS0 | 20 | 95 | 7-10 | 5±0.58 |

Here, **M= Mean; S.E. =Standard Error**

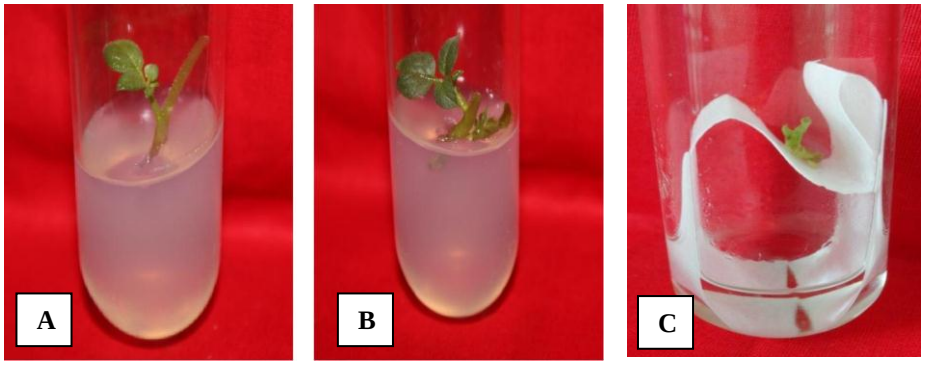


**Figure 1.Various explants (A, B and C) inoculated into the media.**

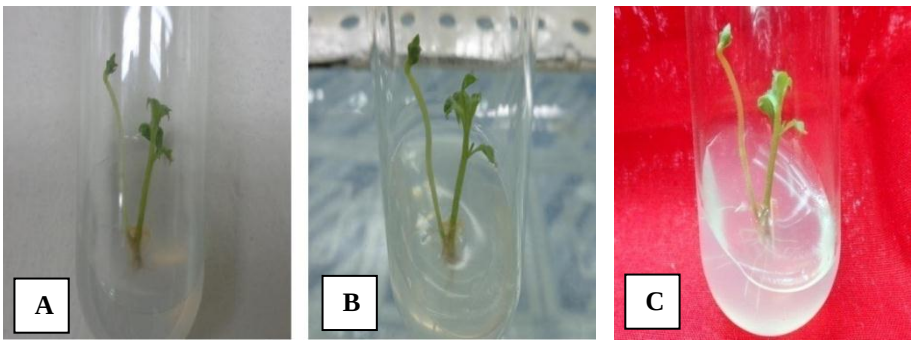


**Figure 2. Different stages (A, B and C) of multiple shooting.**

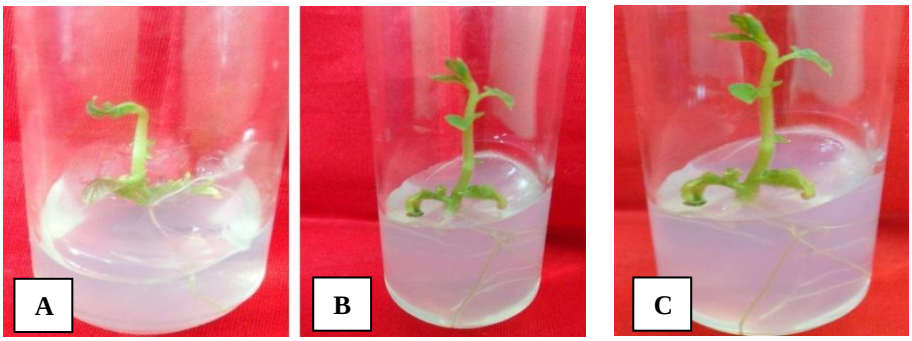


**Figure 3. Gradual stages (A, B and C) of shoot and root regeneration.**

### 3.1.3. Effects of $MS_0$

MS media with no hormone was also tested to find out its effect on multiple shoot induction from shoot tips and nodal segments. The maximum (95%) explants responded within 7-10 days (Table 3). The highest length of the shoot was found 5 cm. It was observed from the data analysis that shoot regeneration in $MS_0$ media was higher than the media supplemented with BAP and $GA_3$.

### 3.1.4. Root induction

Micro shoots were inoculated in MS media supplemented with growth regulators. Shoots were excised from *in vitro* grown cultures and sub-cultured in MS medium supplemented with different combinations (0.5+0.5,1+1,1.5+1.5 and 2+2 mg/l) of IAA and KIN. The highest percentage of root induction (96%) was recorded in MS medium supplemented with no hormone (Table 4). Higher concentration of hormone showed negative effect on root induction.

**Table 4. Effects of different concentrations of IAA and KIN in MS medium on root induction from regenerated shoots.**

| Hormonal supplement for rooting (mg/l) | No. of shoot sub-Cultured | Shoot derived from the explants of mature plants | | | |
|---|---|---|---|---|---|
| | | % of rooting | Days to root generation | Average no. of roots | Average root length(cm) |
| IAA+KIN | | | | | |
| 0.5+0.5 | 20 | 50 | 15-20 | 6.00 | 1.75 |
| 1.0+1.0 | 20 | 65 | 12-15 | 5.25 | 2.00 |
| 1.5+1.5 | 20 | 40 | 14-18 | 4.25 | 1.50 |
| 2.0+2.0 | 20 | 72 | 10-12 | 5.00 | 2.25 |
| MS0 | 20 | 96 | 7-10 | 6.45 | 2.50 |

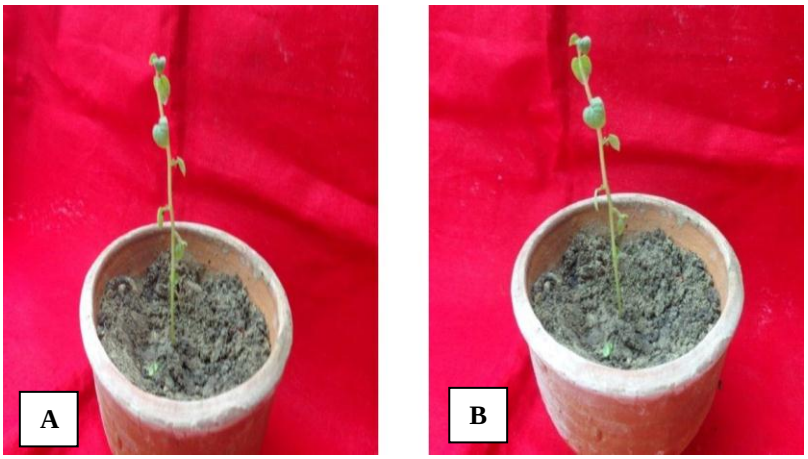


**Figure 4. Plantlets (A and B) after acclimatization.**

### 3.1.5. Acclimatization

When the regenerated plantlets formed well developed root systems they were transferred to the soil (Figure 4). Before transplantation, the individual rooted plantlet was brought out of the test tube and its root system was made agar gel free thronging continuous flowing of sterilized distilled water with taking care not to damage the root system. The plantlets then made ready for transplantation. *In vitro* rooted plantlets were initially planted in especially made plastic trays and later in small pots containing garden soil, compost and sand at the ration of (2:2:1) or a mixture of sterile sand, soil and farmyard manure (1:1:1). Each pot was enclosed with a polythene bag after watering and maintained in growth chamber. After three weeks of acclimatization, plantlets were transferred to large pots for further growth. These were then transferred to the field. No morphological variation was noticed in these plantlets when compared to field grown plants.

## 4. Discussion

### 4.1. Surface sterilization of explants

For many kind of *in vitro* experiments, surface sterilization is essential to free the culture from microbial contamination. For surface sterilization of explants, many workers used many type of sterilizing agents with different concentrations. The treatment may include 1% solution of sodium hypochloride, 70% alcohol, 0.1% $HgCl_2$ solution, 1% silver nitrate solution. There are also many other reports of using $HgCl_2$ (Bhojwani, 1990; Razdan, 1983; Boxus, 1974) for surface sterilization of the explants. Druart and Gruselle (1986) described that concentrations of disinfectants and suspended times are adjusted according to the sensitivity of explants to sterilants. From this investigation, it was observed that explants were contamination free with no tissue damage when treated with 0.1% $HgCl_2$ solution for 2 minutes, was considered to be the most effective and suitable for shoot multiplication.

### 4.2. Shoot multiplication from shoot tip and nodal segment

In recent study, shoot tip and nodal segment of explants were taken from young, newly formed of the plants for shoots multiplication. In BAP concentration, the highest percentage of culture (73%) was found in medium

containing 0.2 mg/l BAP. Earlierreports are available on role of BAP in promoting the number of lateral shoot (Uddin, 2002; Hussain *et al.,* 2005; Azar *et al.,* 2011). Similar results were also reported by Sarkerand Mustafa (2002) that the BAP showed better response in terms of shoot per explants, shoot length, number of nodes and leaves in potato varieties LalPari and JamAlu. Similar behavior was also noticed in varietiesDiamont, Altamash and Cardinal. The results also coincide with the reports of Hoque *et al.* (1996a, 1996b) and Mila (1991) for other potato varieties. Hussain *et al.* (2005) obtained maximum regeneration percentage from nodal explants of potato on MS basal medium with 2.0 mg/l BAP and 0.5 mg/l IAA. Molla *et al.* (2011) also studied the effect of growth regulators on direct regeneration of potato. In the present investigation, for shoot regeneration different concentration of $GA_3$ (0.1, 0.2, 0.3 mg/l) were also used**.** But among all the media formulations used in this experiment, the best result for shoot regeneration (95%) was obtained in media supplemented with no hormone and this finding will minimize the cost of hormones.

### 4.3. Root induction of regenerated shoot

Rooting of regenerated shoots is especially important for establishing tissue culture derived plantets. Although in most of the cases regenerated shoots produced roots spontaneously. In this experiment, the highest percentage of root induction was 96% recorded in MS medium supplemented with no hormone. These results are in agreement with Vinterhalter *et al.* (1997) who reported that potato is an easy to root species and nodal explants do not require exogenous hormone forrooting. After rooting the regenerated plantlets were adapted to the natural environment through the acclimatization process and finally transferred to the field.

## 5. Conclusions

A large number of plantlets of potato can be raised from very small size explants within a short span of time by using this protocol. This protocolalsoprovides reliable and economical method of maintaining pathogen free plantlets in a state that can allow rapid multiplication and also facilitate the exchange of germplasm and its transportation. This can be also used for commercial purpose in medicinal industries especially in off season.

## Conflict of interest

None to declare.

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