Research Article

# Effect of Growth Regulators on Callus Induction and Plant Regeneration in Potato (*Solanum tuberosum* L.) Explants

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**Abstract** | The main objective of this study was to find a best protocol for callus induction and plant regeneration of potato (*Solanum tuberosum* L.). The experiment was carried out in complete randomized design (CRD). Leaves and internodes of potato cv. Desiree were used as explant for callus induction and plant regeneration. Two types of callus induction media (CIM) were used, i.e. CIM1 and CIM2 with concentrations of 2, 4-D 2mg/lit and 3 mg/lit respectively. Among the two different callus induction media (CIM), CIM1 with 2mg/lit auxin (2, 4-D) showed the best results. All the internodal explants placed on CIM1 produced 100% callus while CIM2 produced 70% callus. No callus was observed from leaves on CIM1 and CIM2. After callus induction, four different types of media were used for plant regeneration, having different concentrations of cytokinin (BAP and Zeatin) i.e. RZ1.5, RZ2, RB1 and RB2. The most efficient regeneration media was RZ2, having Zeatin 2mg/lit showed early and maximum shoot formation. On average, 05 shoots per explant were observed on RZ2. No shoot formation was observed with regeneration media having BAP, i.e. RB1 and RB2. From the research work, it is concluded that auxin (2, 4-D) @ 2mg/lit and cytokinin (zeatin) @ 2mg/lit proved to be the optimum quantity for callus induction and plant regeneration in potato respectively.

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## Introduction

Potato (*Solanum tuberosum* L.) is worldwide considered as one of the most economically important annual vegetable crops of Solanaceae family (Solmon and Baker, 2001). Potato is a good and cheap source of carbohydrates, proteins, some important vitamins and minerals (Dhaka and Nailwal, 2015). Many important minerals such as potassium, magnesium and phosphorus are also present in potato (Debnath and Jain, 2015). Potato is an important cash crop and is cultivated almost in every part of the world including Pakistan. Pakistan has an area of about 187.2 thousand hectares with an annual production of 3853.9 thousand tonnes (Pakistan Bureau of Statistics, 2017-18). Although

production of potato in Pakistan has increased but it's per hectare yield is much less than other countries of the world (Abbasi *et al*., 2014). Potato production in Pakistan is considerably low because of several biotic and abiotic factors (Farooq *et al*., 2009).

Potato is vegetatively propagated crop thus creates many problems for plant breeders. Conventional method of breeding for induction of resistant cultivar involves the process of hybridization (Jansky *et al*., 2009). This method is not very efficient in potato because of its complex genetic makeup, i.e. autotetraploid nature and partial or total sexual incompatibility. These problems can be avoided by using alternative approaches like different biotechnological techniques (Birchler and Veitia, 2007). *In vitro* tissue culture techniques like callus induction (calliclones), may exploit or induce natural variation that can be used in crop improvement (Brar and Jain, 1998).

Callus induction and plant regeneration from explants on culture media require the presence of proper concentrations and combinations of plant growth regulators. Different scientists worked to optimize the concentrations of growth regulators for callus induction and potato regeneration (Ehsanpour and Jones, 2000; Fiegert *et al*., 2000; Yasmin *et al*., 2003; Shirin *et al*., 2007). This study was conducted to identify the best hormonal combination for callus induction and plant regeneration in potato that can be utilize as a valuable tool in subsequent tissue culture based on potato improvement programs.

## Materials and Methods

Four weeks old potato plants (*Solanum tuberosum* L. cv. Desiree) in test tubes were collected from Hazara Agriculture Research Station (HARS), Abbottabad. These plants were shifted to growth chamber having 25°C temperature and 16 hours light and 8 hours dark conditions. These in-vitro grown plants were further propagated by nodal cutting on MS medium. Data was collected on several parameters for callus induction and plant regeneration. Data was statistically analyzed through MS Excel software.

*Preparation of callus induction media*

Initially 500 mL MS medium was prepared by dissolving 2.22 g MS media powder in 400 mL distilled water along with 15 g sucrose. The pH was set to 5.6 using 0.5 M KOH as the pH adjustment buffer and distilled water was used to make the final volume 500mL. Three grams (3g) phyto agar was added to the medium and the solution was kept in autoclave machine for 20 minutes at 121°C. When the media cooled, it was divided into two parts. In one part, 2 mL solution from 2, 4-D stock (CIM1) and in other 3 mL solution from 2, 4-D stock was added under the laminar flow hood (Table 1). The media was stirred well and about 25 mL media was poured in each autoclaved petri plates. These plates were kept for some hours to allow the media to settle. The plates were then wrapped well and stored at 4 °C.

**Table 1:** *Callus induction media with different auxin concentrations.*

| Callus induction media (CIM) | Auxin (2,4-D) Concentration |
|---|---|
| CIM1 | 2mg/l |
| CIM2 | 3mg/l |

**CIM1: Callus Induction media 1; *CIM2: Callus induction media 2.*

**Table 2:** *Regeneration media with different hormones concentrations.*

| Regeneration media | Composition |
|---|---|
| RZ1.5 | MS media, 0.01mg/l IAA + 0.1mg/l GA3 + 1.5mg/l Zeatin |
| RZ2 | MS media, 0.01mg/l IAA + 0.1mg/l GA3 + 2mg/l Zeatin |
| RB1 | MS media, 0.01mg/l IAA + 0.1mg/l GA3 + 1mg/l BAP |
| RB2 | MS media, 0.01mg/l IAA + 0.1mg/l GA3 + 2mg/l BAP |

**IAA: Indole acetic acid; GA3: Gibberillic acid; BAP: Benzylaminopurine.*

*Preparation of regeneration media*

Four different regeneration media were prepared each with a varying concentration of cytokinins. The cytokinins used for the study included Zeatin and Benzyl Amino purine (BAP) whereas the auxin used for regeneration was Indole acetic acid (IAA) and the gibberellin used was gibberillic acid (GA3). The working concentration of IAA and GA3 was kept constant for all regeneration media i.e. 0.1 mg/L GA3 and 0.01 mg/L IAA (Table 2). For first type of regeneration media (RZ1.5), the cytokinin used was Zeatin with a working concentration of 1mg/L whereas 2mg/L Zeatin was used in second type of regeneration media (RZ2). Third and fourth type of regeneration media, i.e. RB1 and RB2 contained BAP

with working concentration of 1mg/L and 2mg/L, respectively (Table 2).

Internodal and leaf explants of cv. Desiree were cultured on both types of callus induction media, i.e. CIM1 and CIM2) for a period of two weeks. 12-15 explants were put on each plate. The plates were put in dark by covering in aluminium foil and put at 25°C in the growth chamber for three weeks. The main callus produced by both types of media was sub cultured after every two to three weeks in the same media. The long term (14-20 weeks) callus cultured were shifted on regeneration media. 5-6 calluses were put on each plate. The plates were properly wrapped, labeled and put in the growth chamber having 25°C temperature and a photo period of 16 hours. The regenerated shoots were shifted to fresh media after 2 weeks.

## Results and Discussion

It was observed that variation in concentration of auxin (2, 4-D) in both types of Callus induction media (CIM), i.e. (2mg/lit and 3mg/lit) greatly effected induction of callus. Data was recorded for number of days to callus initiation, callus type, color of callus, percentage of explants that formed callus and degree of callus formation.

*Callus induction*

Two different Callus Induction Media (CIM) having different concentrations of auxin were used which showed different effects on callus induction in potato.

*Days to callus initiation*

It was observed that the callus developed only by internodes and no callus formation was observed by leaves in both Callus induction media, i.e. CIM1 and CIM2. The process of callus formation started after one week at the cutting edges of internodes but it covers the whole explant after some days. CIM2 showed early callus formation, i.e. 21 days, as compared to CIM1 which produced callus in 24 days (Table 3).

*Percentage of explant formed callus*

The internodes cultured on both types of media, i.e. CIM1 and CIM2 behaved differently in term of callus induction. CIM1 proved to be more efficient in callus induction as 100% internodes produced callus whereas 70% internodes produced callus on CIM2 (Table 3).

*Degree of callus formation*

The results showed that the internodes culture on CIM1 produced massive callus whereas internodes cultured on CIM2 produced moderate callus (Table 3). It was observed that rooty callus was produced by CIM2 which might be due to more concentration of auxin.

*Type and color of callus*

It was witnessed that both types of media, i.e. CIM1 and CIM2 produced friable (soft) type of callus. Both types of media produced same greenish white callus from internodes (Table 3). It was notable that callus produced by both types of media changed its nature and color after sub-culturing. After two sub-culturing, the calli became greenish and compact for both types of media.

*Potato regeneration*

Different regeneration media showed different pattern of regeneration for potato. Data was recorded for number of days to regeneration, percentage of regeneration, shooting or rooting media, number of shoots per callus and number of roots per callus.

*Number of days and explant percentage to regeneration*

Different types of regeneration media revealed different results. Callus started to turn green after a week in all types of regeneration media, i.e. RB1, RB2, RZ1.5 and RZ2. But Shoot formation from green callus was different in different types of media. RB1 and RB2 proved to be rooting media and just produced roots rather than shoots. RZ1.5 and RZ2 showed good shoot formation and RB1.5 took 20 days to regenerate while RB2 took 15 days (Table 4). The results revealed that the percentage of explants regeneration for RZ1.5 and RZ2 is quite different. Shoot formation was observed in 70% of explants placed on RZ1.5 whereas 90% explants placed on RZ2 showed shoot formation (Table 4).

*Number of roots/shoots per callus*

It was observed that RB1 and RB2 was just rooting media and didn't produce any shoots. Figure 2 shows that callus placed on RB1 and RB2 produced long and thick roots. On average, RM1 and RM2 produced 3 roots per callus while RZ1.5 and RZ2 produced more shoots with very thin roots. RZ1.5 was good for regeneration as it produced 3 shoots per callus. On the other hand, RZ2 performed better regarding shoots per callus (Table 4).

**Table 3:** *Effect of different concentrations of auxin (2, 4-D) on callus induction in potato.*

| Callus induction media (CIM) | Composition of media | Explant | Number of days to callus initiation | % of explant formed callus | Degree of callus formation | Type of callus | Color of callus |
|---|---|---|---|---|---|---|---|
| CIM 1 | MS media, 2, 4-D (2mg/lit) | Internode | 24 days | 100% | Massive | Friable | Greenish white |
| CIM1 | MS media, 2, 4-D (2mg/lit) | Leaf | -- | -- | -- | -- | -- |
| CIM2 | MS media, 2, 4-D (3mg/lit) | Internode | 21 days | 70% | Moderate | Friable | Greenish white |
| CIM2 | MS media, 2, 4-D (3mg/lit) | Leaf | -- | -- | -- | -- | -- |

*--: No callus.*

**Table 4:** *Effect of Different regeneration media on callus regeneration of potato.*

| Regeneration media (RM) | Composition of media | Number of days to regenerate | Percentage of regeneration | Shooting/rooting media | Average number of shoots per callus |
|---|---|---|---|---|---|
| RB1 | BAP 1mg/lit, $Ga_3$ 0.1mg/lit IAA 0.01 mg/lit | -- | -- | Rooting media | -- |
| RB2 | BAP 2mg/lit, $Ga_3$ 0.1mg/lit IAA 0.01 mg/lit | -- | -- | Rooting media | -- |
| RZ1.5 | Zeatin 1.5 mg/lit, $Ga_3$ 0.1mg/lit IAA 0.01 mg/lit | 20 days | 70% | Both rooting and Shooting media | 03 |
| RZ2 | Zeatin 2mg/lit, $Ga_3$ 0.1mg/lit IAA 0.01 mg/lit | 15 days | 90% | Both rooting and Shooting media | 05 |

*--: No regeneration.*

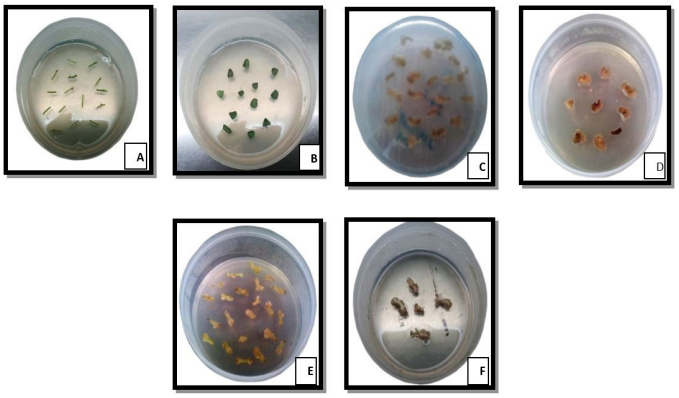


**Figure 1:** *Callus induction of explants on different callus induction media (CIM).*
*(A) Internodal Explants; (B) Leaves explant; (C) Callus induction on CIM1; (D) Subculturing of callus on CIM1; (E) Callus induction on CIM2; (F) Subculturing of callus on CIM2.*

The present experiment was conducted to optimize the best protocol for induction of Callogenesis and regeneration in potato cv. Desiree. In our study, different concentrations of auxin (2, 4-D) were used to investigate the maximum amount of callus induction. Results revealed that the callus induction was affected by different auxin concentrations and explants. Callus development was found to be high with nodal segments while leaves produced no callus.

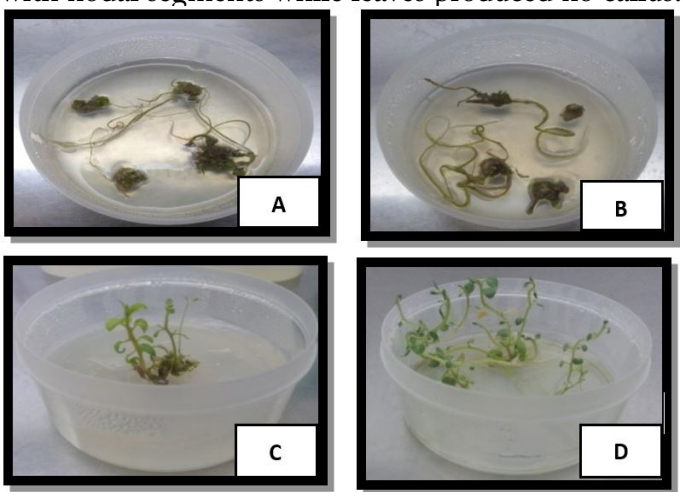


**Figure 2:** *Response of callus on different regeneration media. (A) Callus on RB1; (B) Callus on RB2 (C).*

Callus induction media1 (CIM1) having 2mg/lit 2, 4-D showed maximum callus initiation as compared to callus induction media 2 (CIM2) with 3mg/lit 2, 4-D. All the explants (100%) showed massive callus proliferation on CIM1 while 70% explants produced callus on CIM2. These results are in accordance with Munir *et al.* (2011), who reported that nodal

segments are proved to be better than leaves in term of callus development. They also reported more callus (80%) with 2mg/lit of 2, 4-D while 3mg/lit of 2, 4-D produced less callus (70%). Sherkar and Chavan (2014) also reported in his study that all the explants of potato produced massive callus on media with 2mg/lit 2, 4-D. It was observed in the present study that the explants placed on media with 3mg/lit 2, 4-D (CIM2) produced earlier callus than CIM1 as shown in Table 3. CIM2 produced callus after 21 days while explants placed on media with 2mg/lit 2, 4-D (CIM2) showed callus after 24 days. Similar results in potato were also reported by Shahab-ud-Din *et al.* (2011) which observed the earlier callus induction on media with 3mg/lit 2, 4-D as compared to 2mg/lit 2, 4-D. They observed that, the increase in concentration of 2, 4-D resulted in earlier callus initiation. These results revealed that increase in concentration of 2, 4-D has positive correlation with number of days to callus initiation. It was also observed that the callus produced by CIM2 were rooty in nature. Al-Hussaini *et al.* (2015) also observed that explants placed on media with 2, 4-D produced rooty callus in different varieties of potato. It may be due to increase in concentration of auxin (2, 4-D) which induces roots in plants.

Our results showed that the callus produced on both types of callus induction media (CIM), i.e. CIM1 and CIM2 produced whitish green and friable callus. These results are in agreement with Al-Hussaini *et al.* (2015) who also observed that the all explants placed on media supplemented with 2mg/lit 2, 4-D produced whitish green and friable callus in different varieties of potato. After successive sub-culturing, the calli became greenish and compact. This green color is due to the mature chlorophyll molecule that resulted in efficient regeneration.

Although regeneration protocols for potato plant have been set up, yet in the current study, the effect of different cytokinin on callus regeneration was assessed. Explants were exposed to four types of regeneration media (RM). Results revealed that the regeneration media supplemented with BAP didn't show any profound effect on shoot formation from callus. Regeneration media supplemented with BAP, i.e. 1 mg/lit (RB1) and 2mg/lit (RB2) didn't produce any shoot. Similar results were also reported by Sherkar *et al.* (2014) in his study. They reported that the regeneration media with 1mg/lit BAP produced no shoot and only 12% callus placed on media with 2mg/lit BAP produced shoots after 60 days. RB1 and RB2 proved to be better rooting media rather than shooting media.

Regeneration media with 1.5mg/lit zeatin (RZ1.5) and 2mg/lit (RZ2) proved to be better in term of shoot formation. Results proved that RZ2 was better than RZ1.5 regarding shoot formation. 90% explants placed on RZ2 produced shoots whereas 70% explants on RZ1.5 produced shoots. Doo and Boe (2001) conducted the generic experiment and they also quoted uniform results. They observed that MS medium supplemented with 3.5 mg/lit IAA and 4 mg/lit zeatin produced good shoots in potato. Early shoot formation was observed in RZ2 with 15 days and RZ1.5 showed shoot formation after 20 days. Molla *et al.* (2011) also found the same results in potato. He observed the earliest shoot formation with 2mg/lit and 3mg/lit zeatin as compared to TDZ and BAP. These results suggested the zeatin as best regeneration hormone in potato.

Our results revealed that RZ2 produced average 05 shoots per callus as compared to RZ1.5 that produced average 03 shoots per callus. Our results are in agreement with Zel and Mlakar (1999) who reported in their study that different explants of potato cv. Igor showed better shoot formation with different concentrations of zeatin. They observed that increase in concentration of zeatin has positive effect on number and length of shoots. Results concluded that increasing concentration of zeatin has positive correlation with number and height of shoot per callus.

## Conclusions and Recommendations

Different concentrations of auxin (2, 4-D) behaved differently for callus induction. Results revealed that the most suitable amount of auxin (2, 4-D) was 2mg/lit on which all the explants formed massive callus. Internodes proved to be the most suitable explant for callus induction in potato as compared to leaves. Results showed that the more efficient cytokinin for plant regeneration was zeatin (2mg/lit).

## Novelty Statement

It was observed that the different types of growth regulators and different explants behaved differently for callus induction and plant regeneration in potato.

## Author's Contribution

**Muhammad Adeel Qureshi:** Performed experiment, data collection and wrote paper.
**Raza Ahmad:** Conceived the idea and technical support.
**Bilal Ahmed:** Reviewed the paper.
**Tanzim Ullah Khan:** Incorporated the reviewer comments.

*Conflict of interest*
The authors have declared no conflict of interest.

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