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Article

In vitro plant regeneration of potato (*Solanum tuberosum* L.) at the rate of different hormonal concentration

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Abstract: One of the goals of the experiment is to standardization of $HgCl_2$ treatment for explants sterilization. The objectives also include developing a reproducible cost effective protocol for large scale production of *Solanum tuberosum* of Cardinal variety 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 produce genetically uniform plantlets within a short time capable surviving in natural condition raised in *in vitro* environment. Shoot tip and nodal segment explants from field grown plants were used as experimental materials in this investigation. All explants were cultured on Murashige and Skoog 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 destroying of surface pathogens and getting healthy tissues. Shoot regeneration was observed from both shoot tips and nodal explants for the studied plant. Maximum number of shoot per culture (17) was recorded and it also obtained the highest average length of the shoot (5cm) in Murashige and Skoog medium containing no hormone. On the other hand 6-benzyl amino purine (0.2mg/l) in 3 media showed the highest rate of shoot multiplication (73%) and the highest average length (4cm). In case of Gibberellic acid (0.1mg/l) in Murashige and Skoog media showed its highest rate of shoot regeneration (82%) and the highest average length (4.5cm). From the overall experiment it was observed that shoot tips are more responsive for micro propagation. In root induction Murashige and Skoog medium supplemented with different concentration (0.5, 1, 1.5 and 2mg/l) of indol-3-acetic acid and kinetin. Indol-3-acetic acid and kinetin (1.5+1.5 mg/l) showed its lowest rate of root regeneration (40%) and the average length of the root (1.5 cm). On the contrary Murashige and Skoog medium with no hormone showed the rate of root regeneration (96%) and the highest average length of the root (2.5 cm). The supplemented Murashige and Skoog media with no hormone showed the best performance for root regeneration.

Keywords: regeneration; potato; indole acetic acid (IAA); kinetin (KIN)

1. Introduction

Potato (*Solanum tuberosum* L.) *is* a tuber-bearing tetraploid species belonging to the Solanaceae family. The cultivated potato has 4 sets of chromosomes; however, there are approximately 150 species of "potatoes" or Solanum which are tuber-bearing tetraploids, triploids, diploids, etc. Potato belongs to the family which includes about 90 genera and 2500 species. Although the family is found through the world, it is specially

concentrated in the tropical regions of Latin America (Cearley and Bolyard, 1997).Potato is not only an important vegetable crop but is also a substitute food crop next to rice and wheat in Bangladesh.The potato tuber is a modified stem. Its composition is about 70-75% water, 25-30% dry matter; high levels of starch (20% more or less); some sugars (<3%); there is 5-8% protein on dry weight basis.

The potato produces nearly twice the amount of calories per hectare than rice or wheat (Trujillo *et. al.*, 2001). This crop is 83% more efficient than rice in producing protein. The tubers are one of the richest sources of B complex group of vitamins such as pyridoxine (vitamin B_6) thiamin, niacin, pantothenic acid and folates. Fresh potato is a good source of antioxidant; vitamin-C.

The objectives of the experiment include standardization of $HgCl_2$ treatment for explants sterilization. To develop a reproducible cost effective protocol for large scale production of *Solanum tuberosum* of Cardinal variety 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. To obtain a large number of plantlets within a short time. To establish the *in vitro* raised plantlets in the natural conditions.

2. Materials and Methods

The experiment was conducted at the Department of Genetic Engineering and Biotechnology, Jessore University of Science and Technology, Bangladesh.

Shoot tips and nodal segments were used for micro propagation of *Solanum tuberosum* (potato) a cultivar of cardinal. The surface sterilized explants were sized 1.0-1.5 cm in length.$HgCl_2$(0.1%) was used as surface sterilizing agent at different time duration ranging from one to five minutes then washed thoroughly under running tap water and then treated with two drop of Tween-20 (wetting agent) for 5-6 minutes and then the materials were washed several times with distil water. Then savlon (ACI Ltd. BD) was used as detergent and surfactant. For plants nutrient basal salts were used which contains macro, micro nutrients and vitamins. In the present experiment different culture media with various growth regulators and additive were used for shoot tip and nodal segment culture: (a) Murashige and Skoog (Murashige and Skoog, 1962) medium with different concentrations of 6-benzyl amino purine, Gibberellic acid singly was used for shoot induction .For carbon source 3% sugar was used and the medium was solidified with 6-6.2% agar.(b) Half and full strength of medium with different concentration of indol-3-acetic acid and kinetin were used for root induction.

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 inoculated on callus induction medium at 25 ± 2^{0} C for 3-6 weeks under white light (2500-3000 lux). The photoperiod was maintained generally 16 hours and 8 hours dark. The culture vessels such as test tube, bottle conical flask, measuring cylinders, glass rods, beakers, pipette pumps, parafilm, cotton plug, rubber bands, filter paper, aluminum foils, forceps, fire box, marker pen, spirit lam, needle, sharp blade, stereomicroscope, electronic balance, autoclave, pH meter, magnet stirrer, laminar airflow machine etc. were used as the present investigation. Data were recorded on the following parameters, different time duration for surface sterilization of explants, days to shoot regeneration, number of shoot per explants, days to root induction, number of root per explants.

3. Results

This study deals with effects of different concentration of $HgCl_2$ with different duration on surface sterilization of explants, micro propagation and establishment of the obtained plantlets in nature. A number of experiments were conducted with different types of explants viz.; nodal segments, shoot tips. The explants were used for the induction of shoots and roots to the *in vitro* regenerated shoots.

Standardization for surface sterilization was carried out by trial and error experiments. Surface sterilization was carried out by 0.1%$HgCl_2$ solution at different time duration ranges from one to five minutes. The effects of different treatment duration on surface sterilization of explants are summarized in Table1.

When the explants were treated with 0.1% $HgCl_2$ solution for 1 minute, contamination was occurred as the treatments failed to kill the microorganism Murashige and Skoog attached to the explants.80% of explants found contamination free when treated for 1.5 minutes and 90% of nodal segments were found contamination free with healthy tissue when the explants were treated for 2 minutes. No contamination was found but partial and complete tissue killing was observed when explants were treated for long time (5 minutes).

When lower concentrations of $HgCl_2$ were used in short duration they fail to kill the microorganism Murashige and Skoog attached to the surface of the explants. As a result, all cultures were contaminated within 4-6 days of

inoculation. When higher concentrations of $HgCl_2$ were used in short duration, it showed some efficiency but when those higher concentrations were applied in long duration tissue killing occurred.
Surface standardization of explants, treated with 0.1% $HgCl_2$ solution for 2 minutes was found most effective.

Table 1. Effects of different time duration 0.1% $HgCl_2$ for surface sterilization of explants.

Treating period in Minutes $Hgcl_2$	No. of different explants	Contamination rate after days					% of survival explants
		3	5	7	10	12	
1	20	2	5	6	8	10	0.0
1.5	20	-	-	1	1	2	80
2	20	-	-	-	-	1	90
3	20	*	*	*	*	*	42
4	20	**	**	**	**	**	10
5	20	***	***	***	***	***	0.0

Note: - = No contamination
*= Partial tissue killing
**=Moderate tissue killing
***=Complete tissue killing

Different growth regulators including 6-benzyl amino purine and gibberellic acid were used in different concentration for induction of direct shoot buds from nodal explants and shoot tips of potato (Figure 1). Nodal segments and shoot tips underwent direct organogenesis in the Murashige and Skoog media having different concentration and combinations of growth regulators.*In vitro* regeneration of intertnodal segment was found (Figure2).
In consideration of 6-benzyl amino purine, four different concentrations (0.1, 0.2 and 0.3) were used to test their effects on multiple shoot induction from shoot tips and nodal segments. Results of this study have been presented in Table 2. The highest percentage of shoot multiplication (73%) was noticed in Murashige and Skoog +0.2mg/l 6-benzyl amino purine. The maximum numbers of shoots per culture were obtained 12 in this concentration within 10-15 days. The highest length of shoot was recorded 4 cm.
The lowest percentage of shoot multiplication was 56% and length of shoot was 3 cm was obtained in Murashige and Skoog + 0.1 mg/l 6-benzyl amino purine within 15-20 days.

Table 2. Effects of different concentration of cytokinetinin (6-benzyl amino purine) on multiple shoot regeneration from shoot tips and nodal segments.

Hormone supplement used in MS medium mg/l	No. of explants inoculated	% of explants responded	Days to shoot formation	No. of shoots per culture	Highest length of shoots in cm (M±S.E.)
BAP					
0. 1	20	56	15-20	9	3±0.29
0.2	20	73	10-15	12	4±0.58
0.3	20	65	12-18	10	3.5±0.29
MS_0	20	95	9-14	17	5.0±0.58

Different concentration of gibberellic acid (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. Results are shown in Table 3. The highest percentage of response of shoot multiplication (82%) was noted in Murashige and Skoog + 0.1 mg/l gibberellic acid. The maximum numbers of shoots per culture were obtained 15 in this concentration within 9-12 days. The highest length of shoot was recorded 4.5 cm.
A gradual decline in shoot induction was observed when gibberellic acid concentration was increased above 0.1 mg/l. The lowest percentage of shoot multiplication was 63% and length was 3 cm obtained in Murashige and Skoog +0.3 mg/l gibberellic acid within 10-15` days.
Murashige and Skoog media with no hormone was also tested to find out its effect on multiple shoot induction from shoot tips and nodal segments. Results are shown in Table 3. Ten explants were inoculated into the Murashige and Skoog hormone free media . 95% of explants responded within 7-10 days and the number of the shoots per culture was 17. The highest length of the shoot was found 5 cm. it is observed from the data analysis

that shoot regeneration in Murashige and Skoog media without hormone was higher than the response in 6-benzyl amino purine and gibberellic acid supplemented media.
3 cm obtained in Murashige and Skoog +0.3 mg/l gibberellic acid within 10-15` days.
Murashige and Skoog media with no hormone was also tested to find out its effect on multiple shoot induction from shoot tips and nodal segments. Results are shown in Table 3. Ten explants were inoculated into the Murashige and Skoog hormone free media. 95% of explants responded within 7-10 days and the number of the shoots per culture was 17. The highest length of the shoot was found 5 cm. it is observed from the data analysis that shoot regeneration in Murashige and Skoog media without hormone was higher than the response in 6-benzyl amino purine and gibberellic acid supplemented media. Multiple shooting and rooting in a hormone free media also showed the best performance (Figure 3).

Table 3. Effects of different concentration of gibberellic acid on multiple shoot regeneration from shoot tips and nodal segments.

Hormone supplement used in MS medium mg/l	No. of explants inoculated	% of explants responded	Days to shoot formation	No. of shoots per culture	Highest length of shoots in cm (M±S.E.)
GA_3					
0.1	20	82	9-12	15	4.5±0.29
0.2	20	75	9-14	13	4±0.14
0.3	20	63	10-15	10	3±0.43
MS_0	20	95	7-10	17	5±0.58

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

Figure 1. Inoculation of potato explants (Shoot tips).

Figure 2. *in vitro* plant regeneration from (Inter nodal segment).

Micro shoots were inoculated in Murashige and Skoog and (½)Murashige and Skoog media supplemented with growth regulators. In case of indol-3-acetic acid and kinetin combination, shoots were excised from *in vitro* grown cultures and sub-cultured in both full and half strength of Murashige and Skoog medium supplemented with different concentration(0.5,1,1.5 and 2mg/l) of indol-3-acetic acid and kinetin. In root induction *in vitro* regenerated shoots half Murashige and Skoog medium was found to be the much longer than those in full Murashige and Skoog for *in vitro* rooting. But in full Murashige and Skoog medium, most of the culture produced healthy root system with little bit basal callus formation. The lowest percentage of root induction was 40% recorded in Murashige and Skoog medium supplemented with 1.5mg/l (indol-3-acetic acid+kinetin). The lowest mean number of root (4.25) and mean root length (1.5 cm) were found. The highest percentage (96%) was found in the MS_0 media and mean root number was 6.25 and the mean root length was 2.5 cm. Higher concentration of hormone showed negative effect on root induction.
Plants propagated *in vitro* are not transferred readily to an open soil environment. A weanling stage is usually used in which the plants transferred from *in vitro* condition in to humid conditions of green house and the humidity is then reduced gradually over three to four weeks to that of the open soil environment. In the present finding different attempts were made to establish plantlets and the survival rate was found too high (85%) in the soil condition. When the regenerated plantlets formed well developed root system in Murashige and Skoog they were transferred to the soil. Before transplantation the individual rooted plantlet was brought out of the test

tube and its root system was made agar gel free thronging continuous flowering of sterilized distilled water with taking care not to damage the root system. The plantlet 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. Bags are progressively opened weekly. After three weeks of indoor condition acclimatization have been completed, plantlets were transferred to large pots for further growth. These were further transferred to the field. No morphological variation was noticed on these plants when compared to field grown plants.

Table 4. Effects of different concentrations of auxins (indol-3-acetic acid and α-napthalene acetic acid) in Murashige and Skoog 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+NAA					
0.5+0.5	20	50	15-20	6.00	1.75
1.0+1.0	20	65	12-15	7.25	2.00
1.5+1.5	20	40	14-18	4.25	1.50
2.0+2.0	20	72	10-12	8.00	2.25
MS_0	20	96	7-10	6.25	2.50

Figure 3. Multiple shooting and rooting of the explants.

4. Discussion

The present research was conducted with a view to developing a reproducible protocol for rapid propagation of potato. *In vitro* technique provides a viable alternative technique of mass production of healthy plants with uniform characteristic methods (Lim and Kong, 1985). The techniques are becoming popular as an alternative means of vegetative propagation for commercially important plants. With the help of tissue culture it is possible to propagate a huge number of plantlets from single explants within shorter span of time (Bajaj *et. al*., 1981). There have been many reports on using $HgCl_2$ for surface sterilization of explants from field grown plants (Boxus, 1974). In other research work Druart and Gruselle, (1986) described that concentrations of disinfectants and suspended times are adjusted according the sensitivity of explants to sterilants.

In the present study plant materials from field grown plants were used as explants for primarily establishment of culture. The results obtained in the present investigation are discussed in the following paragraph with an endeavor to justify them.

For many Kinds 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 concentration. The treatment may include 1% solution of sodium hypochloride, 70% alcohol, 0.1% $HgCl_2$ solution, 1% silver nitrate solution (Hoque, 2010). There are also many other reports of using $HgCl_2$ (Bhowani and Razdan, 1983) for surface sterilization of the explants. $HgCl_2$ (0.1%) Was used for three minutes in different crops (Shirin *et.al*., 2007)

From the result it is observed that about 90% 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.
In recent study, shoot tip and nodal segment of explants were taken from young, newly formed portions of the plants for shoots multiplication. This plant highly responded in Murashige and Skoog hormone free medium. In 6-benzyl amino purine concentration, the highest percentage of culture responded (73) was found in medium containing 0.2 mg/l 6-benzyl amino purine. The maximum numbers of shoots per culture were obtained 15 in this concentration within 9-12 days.
The shoot development as well as the rooting of generated shoots is especially important for establishing tissue culture derived shoots. Although in most of the cases regenerated shoots produced roots spontaneously. The highest percentage of root induction was 96% recorded in Murashige and Skoog medium where mean number of root (10.45) and mean root length (2.5 cm) were found. The lowest percentage (40%) was found in the Murashige and Skoog medium supplemented with (indol-3-acetic acid+ kinetin) 1.5 mg/l and mean root number was (4.25) and the mean root length was 1.5 cm. The root organogenesis in shooting medium is not common and usually shoot organogenesis occurs on regeneration media (Struik and Wiersema, 1999). The rooting of the shoots occurred on (¼) strength of Murashige and Skoog medium supplemented with indol-3-butaric acid or α-napthalene acetic acid. The use of low salt Murashige and Skoog medium for rooting of the in vitro induced shoots is a very common practice (Hossain and Islam, 2013).
From the result, it is concluded that a large number of plants can be raised from even small size explants within a short span of time for potato. It also provides reliable and economical method of maintaining pathogen free plants in a state that can allow rapid multiplication and also facilitates exchange of germplasm and its transportation and also can be used for commercial purpose in medicinal industries specially in off season.

5. Conclusions

The result of this study set a standard of $HgCl_2$ treatment for explants sterilization of *Solanum tuberosum*. More importantly the study provides a suitable result in selection of growth regulators for proper multiple shoots regeneration, elongation and root induction in case of producing genetically uniform plantlets. The techniques are becoming popular as an alternative means of vegetative propagation for commercially important plants like potato. The *in vitro* technique of propagation has a number of advantages over conventional method and it can be widely used for commercial micro propagation.

Conflict of interest

None to declare.

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