Original Research Article

IN VITRO REGENERATION OF POTATO *(Solanum tuberosum* L.) USING SPROUT EXPLANT

ABSTRACT

In vitro regeneration ability of four varieties of potato (*Solanum tuberosum*) was evaluated for plantlet production ~~under~~ applying different hormone concentrations and combinations. The highest plantlet production was observed in MS+ 2, 4- D (2 mg/l) + BAP (2 mg/l). The genotype chollisha was the best for plantlet production. Among different hormonal combinations, MS + 2,4-D (2 mg/l) was more effective in the case of higher root generation and MS+BAP (2 mg/l) was more effective in the case of higher shoot generation. Among the four varieties successfully established in the soil, the survival rate was 64% in the Cchollisha variety of potatoes.

Keywords: *i~~I~~n v~~V~~itro*, p~~P~~lantlet, ~~R~~regeneration, potato

INTRODUCTION

In Bangladesh, potato represents about 53% of the total edible vegetables. In terms of total production, it ranks first among vegetables (BBS, 2020). Potato has a great demand throughout the year, but its production is concentrated from January to March in Bangladesh. During the lean period of vegetables ~~in Bangladesh~~, potatoes play a vital role. The popularity of potatoes is increasing for its various preparations like chips and ~~French fry~~french fries in different processing industries and fast food. It is the most important non-cereal food crop and fourth in total global food production after maize, wheat, and rice (Chakraborty *et al.*, 2000).

The production of plantlets from tissue culture is an important and essential component for virus-free seed potato production. Pruski and Krzyszt *et al.* (2002) proposed that micropropagation (*in vitro* propagation) was introduced to seed potato production programs more than two decades ago. Generally, true potato seed is not economically viable for successful tuber production. A higher frequency of viral contamination reduces yield and affects quality potato production. Therefore, the production of virus-free plantlets via tissue culture is an important tool for the micropropagation of potatoes and the production of virus-free seed potatoes. Numerous *in vitro* ~~micro~~propagation protocols have been reported by many researchers in the last decade. Zaman *et al.* (2015) *in vitro* Plantlets' formation potentiality of potatoes was investigated to establish a disease-free plantlet system in potatoes. The study was carried out in *in -vitro* Preservation Lab, Plant Genetic Resource Institute (PGRI), National Agriculture Research Centre (NARC) Islamabad, from January to February 2014. The germplasm of exotic potatoes is routinely maintained *in vitro* laboratory on MS media. The study aimed to investigate the effects of the hormone on *in vitro* virus-free potato plantlets. MS medium was supplemented with 3 mg/L of BAP and showed (5.79 cm) shoots fine performance in respect of multiple shoot regeneration in 6.5 days and (8.5) in MS+ BAP lmg/l. Shoot length (4.0 cm) was observed in MS + 3mg/L BAP; Simple MS medium formed the lowest number of shoots (2.14) per plant. MS + 6% sucrose + 3 mg/L BAP combination of treatment shows (20) shoots with a length (of 6.5cm) in (25.5) days for

Formatted: Font: Italic

Comment [OB1]: Citation is missing from the reference list. Please complete!

Comment [OB2]: Citation is missing from the reference list. Please insert it!

Comment [OB3]: Add this citation to the reference list!

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Comment [OB4]: Add citation to the reference list!

in vitro virus-free plantlets. The present investigation elucidates the efficiency of potato ? plantlet production of selected cultivars in different hormonal combinations.

MATERIALS AND METHODS

The experiment was conducted in the Tissue Culture Laboratory of the Department of Genetics and Plant Breeding, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, from July 2019 to June 2020. Tubers of four varieties of potato (*Solanum tuberosum*), namely Astrix, Diamont, Jolpai, and Chollisha, were used in the present investigation to see their *in vitro* regeneration ability. Tubers were used to grow sprouts in aseptic conditions. Tubers of collected varieties were washed in running tap water, and dirt and ~~the~~ inert matter were cleaned properly. Deformed and contaminated tubers were discarded. For one minute, healthy tubers were rinsed in 70% ethanol and washed with sterile water. Finally, surface disinfection was done with 0.1% $HgCl_2$ solution for 20 minutes. The tubers were ~~washed~~ rinsed 5 times with sterilized water to remove the sterilant. The sterilized tubers were then placed in sterile condition and sprayed with 2 ppm GA_3 for higher and quicker sprouting ~~and quicker sprouting~~. In a study, Liljana *et al*. (2012) evaluated the effectiveness of the auxin and cytokinin combination. For rapid sprouting, clean Potato tubers were *in vitro* treated with 2 ppm GA_3. The tubers were incubated in the dark till they sprouted. After sprouting, seven days old sprouts were used as explants for plantlet production.

Attempts have been taken for shoot and root generation using sprout in MS medium supplemented with different hormones T_0(MS), T_1{MS+ 2,4-D (2 mg/l)}, T_2 {MS+ BAP (2 mg/l)} and T_3 {MS+ 2, 4- D (2 mg/l) + BAP (2mg/l)}. Sprouts were incubated in a culture tube. The culture tubes containing explants were placed under fluorescence light in a room with a controlled temperature until plantlet regeneration. The plantlets with sufficient root systems were removed from the culture tube, washed gently in tap water, and transplanted into small pots containing 50% cocopeat + 25% compost + 25% garden soil. Immediately after transplanting the plants, the pots were covered with a moist polythene bag to prevent desiccation. To reduce sudden shock, the pots were kept in a controlled environment in the growth room. At the same time, plantlets were also nourished with Hoaglandhoglands solution. After 2-3 days, the polythene bags were perforated to expose the plants to the natural environment. The polythene bags were completely removed after 7-10 days. The plantlets at this stage were placed in the natural environment for 3-10 hours daily. Finally, after 10-15 days, they were transferred to ~~the~~ field condition.

RESULTS AND DISCUSSION

Data on different explants for plantlet regeneration in four potato genotypes were analyzed for variance and corresponding interacting components. There were significant variations among the treatments for plantlet regeneration. The interaction between variety and treatment showed significant variations in plantlet regeneration. Sa~~r~~ker and Mustafa (2010~~0~~2) reported the regeneration of two indigenous potato varieties of Bangladesh, lal pakhri and jam alu.

After 15 DAI in the case of shoot, length treatment was found to be highly significant, (Table-1) highest Shoot length observed (2.22 cm) for T_3 treatment in Chollisha, Lowest (1.28 cm) for T_0 treatment in Astrix.

Statistically, significant variation was recorded for different morphological parameters in different varieties of potato plantlet regenerated under *in vitro* conditions. Data revealed that after 15 DAI in the case of root length treatment found to be highly significant(Table-2).

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Subscript

After 15 DAI highest root length was observed (1.7 cm) for T_3 treatment in Diamont, Lowest (0.62 cm) for T_2 treatment in Astrix.

Statistically, significant variation was recorded for different morphological parameters in different varieties of potato plantlet regenerated under *in vitro* conditions(Table-3).

In 15 DAI highest Number of Shoot observed (4.8) for T_2 treatment in Diamont, Lowest (2.4) for T_1 treatment in Chollisha.

Statistically, significant variation was recorded for different morphological parameters in different varieties of potato plantlet regenerated under *in vitro* conditions(Table-4).

In 15 DAI, the highest number of roots was observed (8) for T1 treatment in Astrix and the lowest (5.8) for T_3 treatment in Diamont and Jolpai.

In the case of shoot length, the Chollisha variety exhibited superior performance in the T_3 combination, for root length diamond showed better performance in the T_3 combination. In contrast, Chollisha shows better performance in the T_1 combination. Diamont showed superior performance in the case of the number of shoots in the T_2 combination. The number of root Astrix shows better performance in the T_1 combination(Table-5).

Kaur et al. (2015) showed the effect of growth regulators on in vitro micropropagation of four potato cultivars (Solanum tuberosum) evaluated. MS+ BAP (1.0 mg/l) gave maximum shoot generation out of four media combinations. Bakul et al. (2005) reported the regeneration of potato plantlets and micro tuberization of Cardinal, Diamont, and Patrones varieties. BAP has been found to enhance plantlet regeneration mostly.

Comment [OB5]: add citation to the reference list

Table 1: Analysis of variance of shoot length

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	3	0.3254	0.10846	3.2514	0.02654
Treatment	3	6.7444	2.24813	67.3941	< 2e-16 ***
Residuals	73	2.4351	0.03336		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 2: Analysis of variance of root length

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	3	0.081	0.0270	1.1635	0.3296
Treatment	3	13.055	4.3517	187.5275	<2e-16 ***
Residuals	73	1.694	0.0232		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 3: Analysis of variance of Number of shoot

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	3	6.25	2.0833	2.4431	0.0709
Treatment	3	23.05	7.6833	9.0102	3.76e-05 ***
Residuals	73	62.25	0.8527		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 4: Analysis of variance of Number of root

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	3	5.2	1.7333	1.6222	0.1916
Treatment	3	49.6	16.5333	15.4735	6.912e-08 ***
Residuals	73	78.0	1.0685		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 5: Effect of Genotype × treatment interaction on Shoot length of Potato Sprout Culture

Genotype	Combination	Particulars			
		Shoot Length (cm) ± SE	**Root Length (cm) ±SE**	**Number of Shoot (cm)** ±SE	**Number of Root (cm)** ±SE
Astrix	T_0	1.28 ±0.037	0.94±0.074	3.4±0.50	6.8±0.37
	T_1	1.34 ±0.081	1.58±0.086	3.4±0.50	8.0±0.44
	T_2	1.80 ±0.207	0.62±0.066	4.4±0.50	7.2±0.37
	T_3	2.10 ±0.054	1.64±0.050	4.2±0.58	6.0±0.54
Diamont	T_0	1.30 ±0.044	0.96±0.081	3.6±0.24	6.6±0.67
	T_1	1.32 ±0.058	1.60±0.070	3.2±0.58	7.8±0.37
	T_2	1.82 ±0.086	0.66±0.092	4.8±0.58	7.0±0.44
	T_3	1.80 ±0.044	1.70±0.070	4.0±0.44	5.8±0.37
Jolpai	T_0	1.32 ±0.058	1.00±0.070	3.8±0.37	6.4±0.50
	T_1	1.36 ±0.06	1.50±0.094	2.8±0.20	7.4±0.50
	T_2	1.86 ±0.067	0.68±0.058	4.6±0.40	6.6±0.50
	T_3	1.78 ±0.037	1.62±0.066	3.8±0.48	5.8±0.37
Chollisha	T_0	1.36 ±0.050	1.18±0.037	3.0±0.31	7.0±0.70
	T_1	1.42 ±0.086	1.56±0.050	2.4±0.24	7.6±0.50
	T_2	1.90 ±0.070	0.70±0.044	3.8±0.37	6.8±0.37
	T_3	2.22 ±0.037	1.66±0.06	3.6±0.24	5.4±0.50
LSD		0.20**	0.10	0.90	0.82
CV		20.28	13.41	39.27	19.64

Plate-1: Potato tuber with sprout

Plate-2: Shoot and root growth in test tube

Plate-3: Pot culture of plantlet

CONCLUSION

This study showed that chollisha variety of potato exhibited superior performance among themselves. T_3 {MS+ 2, 4- D (2 mg/l) + BAP (2 mg/l)} hormonal combination showed high effectiveness for *in vitro* plantlet regeneration. Therefore this protocol might be used in future micropropagation of ~~in~~ potato for large scale potato plantlet production.

REFERENCES

1. Aryakia, E. and Hamidoghli, Y. 2010. Comparison of kinetin and 6-banzyl amino purine effect on potato (*Solanum tuberosum* L). American Eurasian J. Agri. Environ. Sci.8(6):710-714.

2. Badoni, A. and Chauhan, J.S. 2009. Effect of growth regulators on meristem-tip development and *in vitro* multiplication of potato cultivar 'kufri himalini'. Nature and Science,7(9):31-34.

3. Hoque, M.E. 2010. *In vitro* tuberization in potato (*Solanum tuberosum* L). Plant Omics J. 3(1):7-11.

4. Kaur, M., Kaur, R., Sharma, C, Kaur, N. and Kaur, A. 2015. Effect of growth regulators on micropropagation of potato cultivars. African J. Crop Sci. 3 (5): 162-164.

5. Khadiga, Elaleem, G.A., Rasheid and Khalafalla, M. 2009. Effect of cultivar and growth regulator on *in vitro* micropropagation of potato (*Solanum tuberosum* L). American-Eurasian *J. Sustain. Agri*. 3(3): 487-492.

6. Khalafalla, M,M., Abd Elaleem, K.G, and Modawi, R.S. 2010. Callus formation and organogenesis of potato (*solanum tuberosum*) cultivar almera. *J. Phytology*. 2(5): 40-46.

7. Liljana, K.G., Mitrev, S., Fidanka, T. and Ilievski, M.I. 2012. Micropropagation of potato *Solanum tuberosum* L. Electronic J. Biol. 8(3): 45-49.

8. Mohapatra, P.P. and Batra, V.K. 2017. Tissue Culture of Potato (*Solanum tuberosum* L.) Intentional J. Current Microbial. Appl. Sci. 6(4): 489-495.

9. Saker, M.M., Moussa, T.A.A., Heikal, N.Z., Ellil, A.H.A.A. and Rahman, R.M.H.A. 2012. Selection of an efficient *in vitro* micropropagation and regeneration system for potato (*Solanum tuberosum* L.) cultivar desirée. African J. Biotech. 11 (98): 16388-16404.

10. Shahab-ud-din, M., Sultan, N., Kakar, M.A., Yousafzai, A. and Sattar, F.A. 2011. The Effects of Different Concentrations and Combinations of Growth Regulators on the Callus Formation of Potato (*Solanum tubrosum*) Explants. Current Res. J. Biol. Sci.3(5): 499-503.

11. Srivastava, A.K., Diengdoh, L.c., Rai, R., Bag, T.K. and Singh, B.P. 2012. *In vitro* micropropagation and micro-tuberization potential of selected potato varieties. Indian J. Hill Fann. 25(2):14-17

Formatted: Font color: Red

Comment [OB6]: Insert these references In the text!

Formatted: Font color: Red

Formatted: Font color: Red

Comment [OB7]: insert reference into the text

Formatted: Font color: Red

Formatted: Font color: Red

Comment [OB8]: citation is missing from the text

Formatted: Font color: Red

Formatted: Font color: Red

Comment [OB9]:

Comment [OB10]: citations are missing from the text

Formatted: Font color: Red

12. Zobayed, S.M.A., Armstrong, J. and Armstrong, W. 2001. Micropropagation of potato: evaluation of closed, diffusive and forced ventilation on growth and tuberization. Annals Bot. 87: 53-59.