

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 different hormone concentrations and combinations. The highest plantlet production was observed in MS+ 2, 4- D (2 mg/l) + BAP (2mg/l). The genotype chollisha was the best for plantlet production. Among different hormonal combinations, MS + 2,4-D (2mg/l) was more effective in the case of higher root generation and MS+BAP (2mg/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 chollisha variety of potatoes.

Keywords:In Vitro, Plantlet, 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 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 Krzysztof *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 micropropagation 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 1mg/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 *in vitro* virus-free plantlets. The present investigation elucidates the efficiency of 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% HgCl₂ solution for 20 minutes. The tubers were washed 5 times with sterilized water to remove the sterilant. The sterilized tubers were then placed in sterile condition and sprayed 2ppm GA₃ for higher 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 GA₃. 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 T₀(MS), T₁{MS+ 2,4-D (2 mg/l)}, T₂ {MS+ BAP (2 mg/l)} and T₃ {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 hoglands 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. Sarker and Mustafa (2002) 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 T₃ treatment in Chollisha, Lowest (1.28 cm) for T₀ 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). After 15 DAI highest root length was observed (1.7 cm) for T₃ treatment in Diamont, Lowest (0.62 cm) for T₂ 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 T₂ treatment in Diamont, Lowest (2.4) for T₁ 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 T₃ treatment in Diamont and Jolpai.

In the case of shoot length, the Chollisha variety exhibited superior performance in the T₃ combination, for root length diamond showed better performance in the T₃ combination. In contrast, Chollisha shows better performance in the T₁ combination. Diamont showed superior performance in the case of the number of shoots in the T₂ combination. The number of root Astrix shows better performance in the T₁ 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.

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

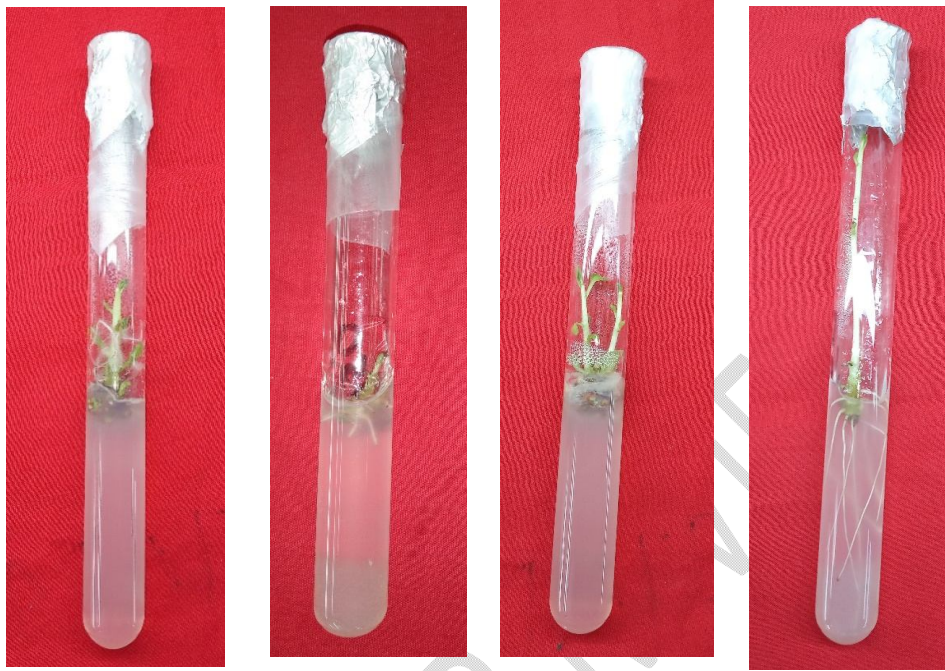


Plate-2: Shoot and root growth in test tube



Plate-3: Pot culture of plantlet

CONCLUSION

This study showed that chollisha variety of potato exhibited superior performance among themselves. T₃ {MS+ 2, 4- D (2 mg/l) + BAP (2mg/l)} hormonal combination showed high effectiveness for *in vitro* plantlet regeneration. Therefore this protocol might used in future micropropagation in potato for large scale potato plantlet production.

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