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## Characterization of Potato (*Solanum tuberosum* L.) adquirido de Centro Internacional de la Papa

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*In vitro* Plantlets formation potentiality of potato was investigated to establish a disease free plantlet system in potato. 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 potato is routinely maintained in *In Vitro* laboratory on MS media. Aim of the study was to investigate the effects of hormone on *In Vitro* virus free plantlet of potato. MS medium supplemented with 3 mg/L of BAP showed (5.79 cm) shoots fine performance in respect of multiple shoot regeneration in 6.5 days and (8.5) in MS+1mg/L BAP. Shoot length (4.0 cm) was observed in MS + 3mg/L BAP. Simple MS medium formed lowest number of shoot (2.14) per plant. MS + 6% sucrose + 3 mg/BAP combination of treatment show (20) shoots with length (6.5cm) in (25.5) days for *in vitro* virus free plantlets.

**Key words** Potato, benzyl-amino-purine (BAP), sucrose; *In vitro*; lux, CIP

### Introduction

Potato (*Solanum tuberosum* L.) is the world's most important non-grain food crop after wheat, rice and maize (Moeinil et al. 2011). Potato is used worldwide for human and animal consumption, and as raw material for starch and alcohol production. In Pakistan, during the year 2008 its production was 2.5 million metric tons (FAO, 2010). The most important aspect in potato is the production of virus free plants globally (Badoni et al. 2010). Many researchers used different growth regulators for *in vitro* induction of microtuber in potato (Hossain et al. 1998). Many researchers reported the production of virus free

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plantlets and tuberization of potato through *invitro* techniques (Rahman et al. 2010; Yousef et al. 2001). The potential value of tissue culture in potato production has been widely recognized. This technology has been used for disease free seed production in many countries (Wang and Hu,1982). This technology has ensured greater availability of diseases free seed for cultivation, which ultimately helps in boosting overall potato production in the country. In view of the above, a protocol have been developed for sterilization of explants and found the suitable hormonal combination with MS medium for *in vitro* nodal and shoot regeneration. Use Indole butyric acid (IBA) for production of virus free plantlets. Therefore, the experiment was designed to find out the virus free plantlets.

## Materials and Methods

The present 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 potato is routinely maintained in *In Vitro* laboratory on MS media. Aim of the study was to investigate the effects of hormone on *In Vitro* virus free plantlet of potato. For *In Vitro* plantlet, single nodal cuttings were cut from twelve weeks old *In Vitro* plantlets of the (CIP-25) potato germplasm acquire from International Potato Centre Peru Latin America. Cuttings were cultured and incubated. Murashige and Skoog medium (MS) was used for routine multiplication of the potato plants as it is the most suitable and commonly used basic tissue culture medium for plant regeneration. It was developed by Toshio Murashige and Folke K. Skoog in 1968. MS media were supplemented with 1, 3 mg/L of bezylaminopurine (BAP). All the tools needed for inoculation were surface sterilized in an autoclave at 121°C temperature and 15 PSI Pressure for 1hour.under aseptic condition young and tender plantlets were taken out a sterile plate, with the help of sterile forceps. These plantlets were not sterilized as these were already being maintained under in vitro condition. Using sterile scalpel, the root of these plantlets was cut. The leaves were removed and finally shoot part was cut into small segment. Each segment retaining at least one node. Maintaining the correct polarity of the cut segment, these were individually inoculated in the in the culture medium in test tubes (size 25×190mm, containing 10ml of solidified media).After inoculation, explants cultures were incubated at 25°C under the light of white fluorescent tubes for 3 weeks. the cultures were always incubated at 25±1 °C under 16 hours light (2,000 lux) with white florescent tube .the PH of the medium was set at 5.8 and then agar was added to it at concentration of 0.8 % and then melted and

dispensed in the tissue culture (size 25×190mm, containing 10ml of MS broth). These test tubes were autoclaved at 121°C for 7min at 15PSI. Inoculation was carried out day by day to check contamination problem. Data of shoot formation, number of shoot per plants, shoot length were recorded.

## Results and Discussions

### *In vitro shoot and virus free plantlets formation*

MS media supplemented with different concentration of BAP were used for virus free plantlets production. The data are presented in Table 1. It was revealed that, simple MS media had the ability to produce shoot under *in vitro* condition. But days required for shoot regeneration was highest (13.5days) in control condition. The minimum time (6.5) was required in MS + 3 mg/L BAP treatment. The maximum time (13.5) was recorded in simple MS. between the minimum and maximum time the data was recorded days of formation (8.5) in MS+1mg/L BAP. The time variation between two treatments was very less. Shoot per plant was the highest (5.79) in MS + 3 mg/L BAP and the second highest (4.05) in 1 mg/L BAP. The simple MS medium formed lowest number of shoot (2.14) per plant. Highest shoot length (4.0 cm) was observed in MS + 3mg/L BAP. The second highest data was recorded (3.21) in simple MS. the lowest shoot length was recorded (1.06) in MS+1mg/L. Our results are in agreement with that of (Shibbi *et al.* 2001) with a little variation.

**Table 1:** Effect of BAP on virus free plantlets.

Media	No. of plantlets	Days to shoot formation	No. of shoot per plant	Shoot length (cm)
Simple MS	22	13.5	2.14	3.21
MS + 1 mg/l BAP	22	8.5	4.05	1.06
MS + 3mg/l BAP	22	6.5	5.79	4.0

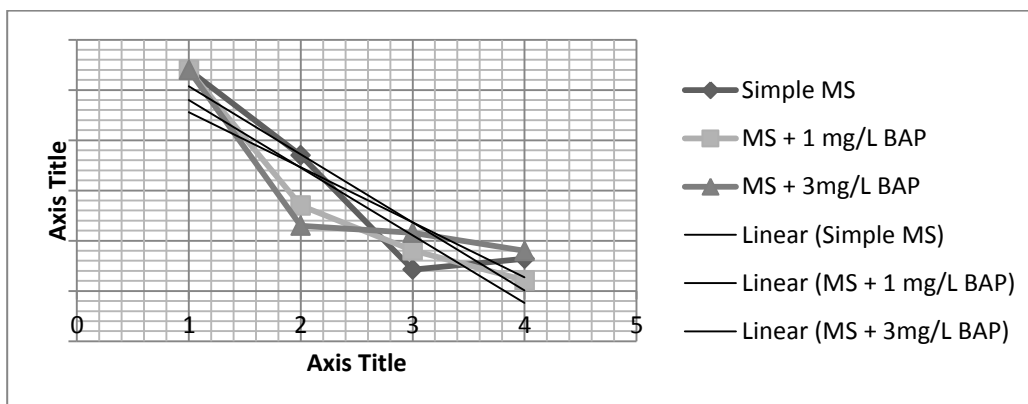


Fig: 1 Graph shows the Effect of BAP concentration on virus free plantlets



Fig: 1.2 Effect of different hormone concentration on virus free plantlets.

### ***Role of sucrose on in vitro virus free plantlets***

In table 3 the effect of two different concentration of sucrose were applied with hormone or without hormone. It showed that 3% of sucrose without any hormone can produce (7) Plantlets with length (4.0cm) in 55.5 days. but 6% sucrose show (10) plantlets in 45.5 days with (4.5cm) length. As compare to 3% and 6% sucrose without any hormone the 3% sucrose with MS+1mg/L show fine result (15) shoots in 35.5 days for virus free plantlets and (5.0cm) length. The MS+3mg/L show very superior results as compare to other concentration, 6% sucrose show (20) shoots with length(6.5cm) in minimum (25.5) days. The similar phenomenon was reported by (Nagar and Enas, 2012).

The other researchers (El-Sawy *et al.* 2007) observed that the highest shoot and tuber formation was achieved when 12% sucrose was added to culture media.

**Table 2:** Role of sucrose on *in vitro* virus free plantlets

Media	Sucrose Concentration	Total plantlets	Days to Virus free Plantlets	No. of shoot per plants	Highest Shoot length (cm)
MS Simple	3%	22	55.5	7	4.0
MS Simple	6%	22	45.5	10	4.5
MS+1mg/L BAP	3%	22	35.5	15	5.0
MS+3mg/L BAP	6%	22	25.5	20	6.5

Fig. 2.1 Graph shows the role of sucrose on *in vitro* virus free plantlets

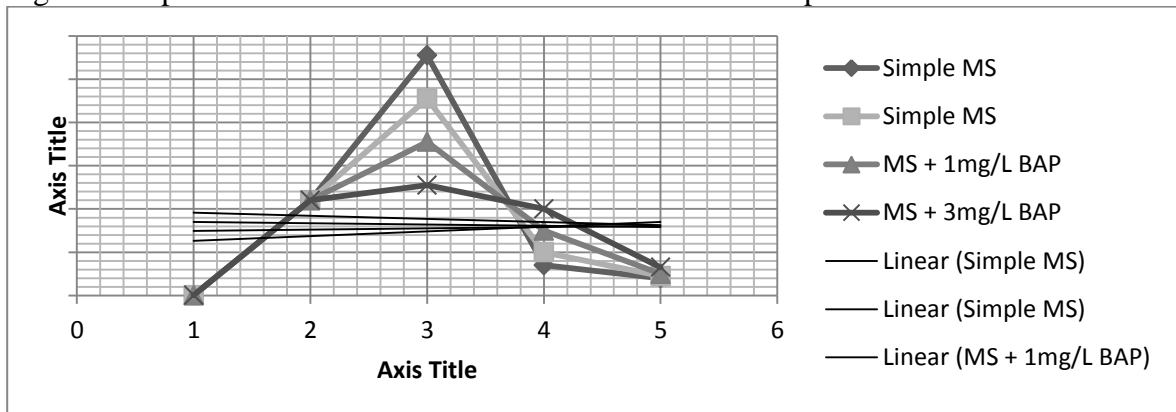




Fig: 2.2 Role of sucrose concentration on virus free plantlets.

## Conclusion

*In vitro* virus free plantlet was studied on MS medium supplemented with different concentration of BAP in potato germplasm (CIP-25) acquire from International Potato centre Peru Latin America. Nodal cutting is used as plantlet. And different concentration of sucrose is used for healthy plantlets. It resulted that the MS+3Mg/L BAP and 6% sucrose response is excellent for all the parameters.

Thus: MS+ 6% sucrose+3mg/L BAP produced maximum (20) shoots (6.5cm) length in 25.5 days.

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