

Full Length Research Paper

Modulation in growth and development of potato (*Solanum tuberosum* L.) microtubers by different concentration of 6-benzyl aminopurine

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Two potato varieties namely, *kufri bahar* and *kufri surya* were tested for *in vitro* tuberization response to a same culture medium supplemented with three levels of 6-benzyl aminopurine (0.75, 1.5 and 2.25 mg/L) in a completely randomized block design. The study was conducted in the Central Potato Research Institute (CPRI), Modipuram, Meerut Campus, and India during the period of 2012 to 2013 and 2013-2014. The objective was to determine optimum concentration of 6-benzyl aminopurine for tuberization. Both varieties, exhibited a better response when the culture medium supplemented with 2.25 mg/L of 6-benzyl aminopurine than the other concentrations and control for mean values of number of days for tuber initiation microtuber number, fresh weight of microtubers and number of eyes per microtuber was found optimum and it may be useful to enhance tuber quality as well as crop growth under *in vitro* conditions at farmer and industrial levels.

Key words: *In vitro* plantlets, potato cultivars, 6- benzyl aminopurine, *in vitro* plants.

INTRODUCTION

In India, potato is an important commercial crop (As per the final estimates of 2017-2018, horticulture production stood at record 311.7 MT, which is 3.7% higher than the previous year and 10% higher than the past five years average production area (<https://m.economictimes.com/news>). India is second largest producer of potatoes in the world after China. India showed tremendous growth in potato production during last one and half decade (Rana and Anwer, 2018). The crop is damaged by many pests and diseases (like late blight, Bacterial wilt etc), so to provides and cultivate diseases-free plants and enhances genetic manipulation

to improve the existing cultivars and to generate of novel plants a good procedure is require. Approximately 15% of the total area under potato cultivation around the world is used for the production of tuber seeds (Amina et al., 2006). However, tubers formed through these conventional conditions are susceptible to pathogen infections, thereby resulting in poor quality and yield and are difficult to transport and store due to their large size (Nhut et al., 2006). Tissue culture is a reliable technique can eliminate viruses in during tuber seed production programs and microtuber is one of the strategies in this perspective (Wang and Hu, 1982). The work on potato

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Figure 1. Macronutrients Solution.



Figure 2. Micronutrients Solution.

plant has been conducted as *in vitro* growth of potato plant (*in vitro* tuberization) with the main purpose of initiation of potato plant culture *in vitro*, micro propagation of potato plant, production of microtubers and hardening and transfer of plant to the field (soil) (Joseph et al., 2015). Microtubers are very small tubers produced by allowing micro plants to grow under tuber inducing conditions.

Microtubers have some advantages over *in vitro* micro plants. They are very convenient and easy to transport

and store. Production of microtubers is possible throughout the year. They have the same health status as *in vitro* micro plants, and unlike micro plants they do not require hardening (Struik and Wiersema, 1999). The quality *in vitro* micro plants and micro tubers can be affected by a combination of growth regulators and environmental conditions (Salimi et al., 2010; Yeasmin et al., 2011).

Growth regulators are commonly used in *in vitro* multiplication. Several compounds, including gibberellic acid (GA3), cytokinin (CK), jasmonic acid, auxin, abscisic acid and sucrose have been reported to participate in the regulation of tuber formation (Rodriguez-Falcon et al., 2006). Kinetin, 6- benzylaminopurine (BAP) and Chloro choline chloride (CCC) have extensively been used in tissue culture medium to promote the micro tuberization (Hussey and Stacey, 1981). BAP at concentration (below 8 mg/L) used for microtuber production average number, weight and eyes, while with the increasing concentration of BAP (up to 10 mg/L) inhibits the average number, weight and eyes number of micro tubers (Badoni and Chauhan, 2010). Since the effect of BAP on potato micro tuberization has been established, in this experiment, influence of three concentrations (0.75, 1.5 and 2.25 mg/L) of 6-benzyl aminopurine on physical characteristics of microtubers such as microtuber initiation, number of microtubers, weight of microtubers and eyes of microtuber of potato were investigated.

MATERIALS AND METHODS

The study was conducted in the Central Potato Research Institute (CPRI), Modipuram, Meerut Campus, and India during the period of 2012-2013 and 2013-2014. Virus free certified micro plants of potato (*Solanum tuberosum* L.) were collected from (CPRI) Modipuram, Meerut. Two varieties namely *kufri bahar* and *kufri surya* were selected for tissue culture studies under lab conditions. For plantlets multiplication, nodal cuttings of potato both varieties were cultured in test tubes (25x150 mm) containing 15 ml of solidified (0.8% agar) Murashige and Skoog MS (Murashige and Skoog, 1962)

Macronutrients amount in g/L

KNO_3 -38.00 g/L, NH_4NO_3 -33.00 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ -7.4 g/L, KH_2PO_4 -3.4 g/L and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ - 8.8 g/l. KNO_3 , NH_4NO_3 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and KH_2PO_4 were dissolved in 1000 ml double distilled (DD) water. $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was dissolved in 500 ml double distilled (DD) water. After that both the solutions were mixed together and make volume to 2000 ml with DD water. The prepared solution was stored at 4°C (Figure 1 and Table 1).

Micronutrients amount in mg/L

$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ - 2.230 mg/L, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ -860 mg/L, H_3BO_3 – 620 mg/L, KI-83 mg/L, $\text{Na}_2\text{MOO}_4 \cdot 2\text{H}_2\text{O}$ -25 mg/L, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ -2.5 mg/L and $\text{COCl}_2 \cdot 6\text{H}_2\text{O}$ -2.5 mg/L. All the micronutrients were dissolved in DD water and make volume to 1000 ml and were stored at 4°C (Figure 2 and Table 2).

Table 1. Macronutrients amount in g/l

Macronutrients	Amount in g/L
KNO ₃	38
NH ₄ NO ₃	33
MgSO ₄ .7H ₂ O	7.4
KH ₂ PO ₄	3.4
CaCl ₂ .2H ₂ O	8.8

Table 2. Micronutrient amount in mg/L

Micronutrient	Amount in mg/L
MnSO ₄ .4H ₂ O	2.23
ZnSO ₄ .7H ₂ O	860
H ₃ BO ₃	620
KI	83
Na ₂ MOO ₄ .2H ₂ O	25
CuSO ₄ .5H ₂ O	2.5
COCl ₂ .6H ₂ O	2.5

**Figure 3.** Vitamins Solution.**Vitamins amount of mg/L**

Myo-inositol- 10,000 mg/L, Thiamine-HCl- 10 mg/l, Nicotinic acid-50 mg/L, Pyridoxine-HCl-50 mg/L and Glycine- 200 mg/L. All the vitamins were dissolved in DD water and make volume to 1000 ml Store below 0°C (Figure 3 and Table 3).

Table 3. Vitamins amount in mg/L.

Vitamin	Amount in mg/L
Myo-inositol	10,000
Thiamine-HCl	10
Nicotinic acid	50
Pyridoxine-HCl	50
Glycine	200

**Figure 4.** Iron solution.**Table 4.** Iron source amount in g/L.

Iron source	Amount in g/L
Na ₂ EDTA.2H ₂ O	3.73
FeSO ₄ .7H ₂ O	2.78

Iron source Amount of g/L

Na₂EDTA.2H₂O-3.73 g/L and FeSO₄.7H₂O-2.78 g/L FeSO₄.7H₂O and Na₂EDTA.2H₂O were dissolved separately in 400 ml/L of double distilled water. Na₂EDTA.2H₂O was heated until it dissolved completely. FeSO₄.7H₂O was added to the warm Na₂ EDTA solution with continuous stirring. The solution was slight yellowish tinge. The volume was then adjusted to 1000 ml with double distilled water. This stock was stored in coloured bottle at 4°C. The pH of the medium was adjusted to 5.8 with freshly prepared 1 N HCl or 1 N KOH (Figure 4 and Table 4).

100 ml of macronutrients and 10 ml each of solution micronutrients, vitamins and iron source were taken and mixed together to prepare Murashige and Skoog medium (1962). The pH of the medium was adjusted to 5.8 before autoclaving at 121°C



Figure 5. Microplants of Kufri Bahar

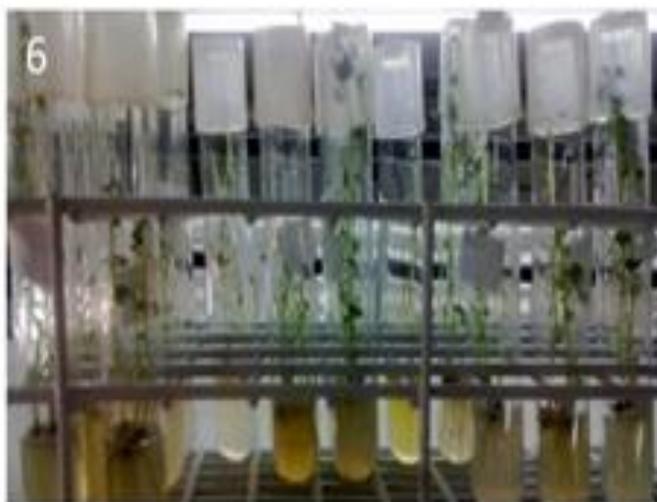


Figure 6. Microplants of Kufri Surya.

for 20 min. Cultures were incubated at 25 °C for 16 h photoperiod (fluorescent light of 100 $\mu\text{mole/m}^2/\text{s}$). After 21 days when sufficient numbers of plantlets were formed on solid medium in test tube, they were cut with three node cutting and inoculated in 20 ml of liquid media in 250 ml Erlenmeyer flask. Composition of the liquid medium was the same as that of solid medium except agar which was not added (Figures 5 and 6). For micro tuberization, after 20 days the liquid propagation medium was replaced by tuber induction medium containing MS salt, vitamins, sucrose (8%) and supplemented with different BAP concentrations (0.75, 1.5 and 2.25 mg/L). These flasks were then incubated at $18 \pm 2^\circ\text{C}$ under complete dark condition. After 75 days microtuber were harvested and physical (Number of microtuber and fresh weight of microtubers) and growth parameters (Number of eyes and Days to microtuber initiation) analyzed. The pool data obtained were statistically analyzed using completely randomized design with the software IRRISTAT (IRRI, 1999).

RESULTS AND DISCUSSION

In vitro tuberization in potato was studied in *kufri bahar* and *kufri surya* varieties. To evaluate the physical characteristics such as number of microtubers, weight of microtubers and growth rate including number of eyes and tuber initiations were selected as indicators.

Microtuber initiation

MS media supplemented with different concentration of 6- benzyl aminopurine were used for micro tuber initiation. The data presented in Table 5 revealed that there was significant variation over control with respect to microtuber initiation. MS media supplemented with different concentration of 6- benzyl aminopurine were used for microtuber initiation. Maximum days 20.0 (± 1.53) days were recorded for microtuber initiation for the control (no BAP added) *kufri bahar*.

However, there were no significant differences. In *kufri surya* the maximum days for microtubers initiation 20.4 (± 1.06) days were recorded for microtubers initiation with the control. However, it was at par with 2.25 and 1.5 mg/L in *kufri surya*. Significantly minimum days for microtuber initiation were recorded in 2.25 mg/L. The findings are in agreement with the studies made by Yong et al. (1996) who observed that 6- benzyl aminopurine promoted initiation and growth of microtubers.

Number of microtubers

Effects of 6- benzyl aminopurine on physiological and growth parameter were studied. The number of microtubers increased with increasing 6- benzyl aminopurine concentration in both the varieties during both the years of study (Table 5). Significant maximum number of microtubers 8.24 ± 0.33 was recorded with 2.25 mg /L of BAP in *kufri bahar*. It was significantly at par with 1.5 and 0.75 mg /L and control. In *kufri surya* maximum number of microtubers 8.83 ± 0.44 was recorded with 2.25 mg /L and it was at par with 1.5 and 0.75 mg/L and control. Significantly minimum number of microtubers (5.60 and 5.30) was recorded in control. These results agree with Naqvi et al. (2019) who found that the higher mean number of microtubers was achieved by the effect of BAP which promotes *in vitro* microtuberization (Figures 7 and 8).

Weight of microtubers

Significant variation was found between concentration of BAP for the fresh weight of microtubers per flask in both varieties during the two years of study (Table 6). Significantly maximum microtubers mean fresh weight (3.13 g) was recorded with 2.25 mg /L in *kufri bahar*.

Table 5. Effect of BAP on microtuber initiation and number of microtubers per flask of varieties.

Treatment	Microtuber initiation (days)				Mean	Number of microtubers per flask				Mean		
	1st year		2nd year			1st year		2nd year				
	KB (A1)	KS (B1)	KB (A2)	KS (B2)		KB (A1)	KS (B1)	KB (A2)	KS (B2)			
0.75 mg/L	17.4	17.2	17.5	17.4	17.4	17.3	6.32	6.66	6.44	6.60	6.38	6.63
1.5 mg/L	15.6	15.5	15.5	15.6	15.5	15.5	6.66	6.78	6.58	6.80	6.62	6.79
2.25 mg/L	12.9	12.4	12.7	12.6	12.8	12.5	8.22	8.82	8.26	8.84	8.24	8.83
Control	19.8	20.2	20.2	20.6	20.0	20.4	5.40	5.20	5.80	5.40	5.60	5.30
S Em ±	1.82	1.77	2.16	1.79	1.70	1.55	0.71	0.50	0.48	0.57	0.33	0.44
CD or LSD(P=0.05%)	NS	5.32	NS	5.37	NS	4.67	NS	1.51	1.44	1.73	1.00	1.34
CV	24.8	24.3	29.3	24.3	23.1	21.2	24.1	16.5	15.9	18.8	11.2	14.6

kufri bahar (KB) and *kufri surya* (KS) *in vitro* condition. KB- *kufri bahar*, KS- *kufri surya*, NS-non significant .

**Figure 7.** Microtubers of Kufri Bahar**Figure 8.** Microtubers of Kufri Surya

However, it was at par with 1.5 and 0.75 mg/L and control. In *kufri surya* significantly maximum mean fresh weight of microtubers (3.04 g) was also observed with 2.25 mg /L and the result was at par with 1.5 and 0.75 mg /L and the control. Minimum mean fresh weight of

microtubers (0.47 and 0.51 g) was recorded for the control. The findings are in agreement with the studies carried by Hossain et al. (2015) who observed that at [5 mg/L of BAP] and 9% sucrose, increase in the number and fresh weight of microtubers. Aryakia and Hamidoghi

Table 6. Effect of BAP on fresh weight of microtubers per flask and number of eyes per microtuber of potato varieties *kufri bahar* (KB) and *Kufri Surya* (KS) *in vitro* condition.

Treatment	Fresh weight of microtuber per flask (g)				Mean		Number of eyes per microtuber				Mean	
	1st year		2nd year				1st year		2nd year			
	KB	KS	KB	KS	KB	KS	KB	KS	KB	KS	KB	KS
0.75 mg/l	0.55	0.58	0.58	0.57	0.56	0.57	3.38	3.12	3.30	3.00	3.34	3.06
1.5 mg/l	0.95	0.90	0.96	0.92	0.95	0.91	4.90	5.00	4.98	5.04	4.94	5.02
2.25 mg/l	3.12	3.02	3.14	3.06	3.13	3.04	5.06	5.32	5.14	5.34	5.10	5.33
Control	0.47	0.51	0.48	0.52	0.47	0.51	1.80	2.00	1.96	1.86	1.88	1.93
S Em ±	0.35	0.29	0.31	0.26	0.12	0.13	0.24	0.34	0.21	0.42	0.16	0.32
CD or LSD(P=0.05%)	1.07	0.89	0.94	0.80	0.38	0.41	0.74	1.04	0.62	1.27	0.49	0.96
CV	62.9	53.1	54.4	47.1	22.4	24.7	14.6	20.3	12.2	24.9	9.7	18.8

KB- *kufri bahar*, KS- *kufri surya*, NS-non significant.

(2010) also observed, at high concentrations (0.75 and 1 mg/L) of BAP showed incremental effect on weight and size of microtubers.

Number of eyes

Different concentrations of BAP markedly influenced the number of eyes per microtuber. BAP at 2.25 mg/L gave significant maximum number of eyes per microtuber (5.10) which was at par with 0.75 mg /L and the control whereas it was significantly minimum (1.88) in control in *kufri bahar* (Table 6). Similarly, maximum number of eyes per microtuber (5.33) was also noted with 2.25 mg/L in *kufri surya*. However, it was at par with 0.75 mg/L and the control. Significant mean number of eyes (1.93) per plant was lowest in control in *kufri surya* (Table 6). The findings are in agreement with the studies carried by Badoni and Chauhan (2010) who observed that the lower concentration of BAP (below 8 mg/L) increases the number of eyes per microtuber, while increased concentration of BAP (up to 10 mg/L) inhibits the production of eyes by micro tubers. So that economically and qualitatively both varieties of potato are be useful in enhancing tuber quality as well as crop growth.

Conclusion

The BAP 2.25 mg/L should be useful to enhance tuber quality as well as crop growth under *in vitro* conditions at farmer and industrial levels. The results suggested the need of developing genotype specific protocols to maximize *in vitro* performance for microtuberization and *in vivo* minitubers performance.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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