Effect of Kinetin on Growth and Development of Microtubers of Potato (*Solanum Tuberosum* L.)

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Abstract:

The present study was conducted at Central Research Potato Institute, Campus, Modipuram, India to assess the effect of kinetin on the growth and development of microtubers formation in vitro in two potato varieties Kufri Bahar and Kufri Surya. The lower concentration of kinetin (0.75 mg/l) showed decreased parameters like the number of microtubers, fresh weight of microtubers, eyes per microtuber. Higher concentration of kinetin (1.5 mg/l and 2.25 mg/l) led to increasing parameters as compared to control but decreased shoot length in both the varieties but kinetin at (2.25 mg/l) concentration gave best results for* in vitro *microtuberization for quality and yield parameters.

Keywords: ***Plantlets, potato-varieties, kinetin, Microtubers, Kufri-Bahar, Kufri-Surya, Growth-attributes,***

Introduction:

Potato is the third most important food crop in the world after rice and wheat, which is consumed by other than a billion people worldwide. According to CPRI vision, 2030 India is the second-largest potato production in the world after China; with the uppermost potato productivity among the top four potato production in the world.

India has the second-largest producer of potatoes in the worlds, after China. Together, both the countries contribute 38 percent to the world's total potato production (''Potato production rises 51% in Over a decade: agriculture minister. d. 2021).
Growth regulators are usually used in in vitro multiplication. Some combinations, like 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). Plant growth regulators play vital tasks in the growth and development of the plants, by altering various important physiological and biochemical processes required by plants when exposed to heavy metal toxicity (Chauhan *et al.*, 2019).
Tissue culture is an important method of plant propagation available to growers. This technique is used for the mass production of potato plants in a very short period. *In vitro* microtuber formation potentiate of potato was investigated by Houqe (2010) to establish a rapid disease-free seed production system in potato.

Cytokinin stimulated the transition of axillary buds into stolons, which could be useful in tuberization *In Vitro* but not useful for shoot cultures (Vinterhalter *et al*., 1997). The main effect of cytokinin is on cell division (Vreugdenhil, 2004) but cytokinin also affect cell enlargement (Arteca, 1996). cytokinin stimulated the transition of axillary buds into stolons, which could be useful in tuberization. Kinetin increases the tuber initiation of potato and stimulates the enzyme activity precociously in the growth and in earlier tuber initiation (Kefi *et al.*, 2000). Hemberg (1985) reported that exogenous cytokinins can break the dormancy of potato tubers, and the level of endogenous cytokinins increases before the termination of dormancy. More than one form of cytokinins is found in potato tubers. Cytokinins such as 6-Benzylaminopurine (BA), Zeatin, Kinetin, or Cytokinin like PGR such as Thidiazuron (TDZ) and Forchlorfenuron (CPPU) can be added to the tissue culture medium (Ahmad et al., 2018).
Science the effect of kinetin on potato micro tuberization has been established, in this experiment, the 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, the weight of microtubers and eyes of microtuber of potato were investigated.

Materials and Methods

The research was conducted at the Central Potato Research Institute (CPRI), Modipuram, Meerut Campus, and India. During the season of 2012-2013 and 2013-2014. Disease free certified micro

plants of potato (Solanum tuberosum L.) were collected from (CPRI) Modipuram, Meerut. Two cultivars namely kufri ahar and kufri Surya were opted for tissue culture studies under lab conditions. For plantlets proliferation, node cuttings of potato both cultivars were cultured in test tubes (25×150 mm) containing 15 ml of solidified (0.8% agar) Murashige and Skooge MS (Murashige and Skoog, 1962)

Macronutrients Amount in g/L

[1] KNO_3-38.00 g/L, NH_4NO_3-33.00 g/L, $MgSO_4.7H_2O$-7.4 g/L, KH_2PO_4-3.4 g/L and $CaCl_2.2H_2O$- 8.8 g/l. KNO_3, NH_4NO_3, $MgSO_4.7H_2O$ and KH_2PO_4 were mixed with 1000 ml double distilled (DD) water. $CaCl_2.2H_2O$ was mixed with 500 ml double distilled (DD) water. After that, both the solutions were mixed together and make volume to 2000 ml with DD water. The complete prepared solution was stored at 4°C (Figure 1, Table-1).

Micronutrients Amount in mg/L

For micronutrients taken $MnSO_4.4H2O$- 2.230 mg/L, $ZnSO_4.7H_2O$-860 mg/L, H_3BO_3–620 mg/L, KI-83 mg/L, $Na_2MOO_4.2H_2O$-25 mg/L, $CuSO_4.5H_2O$-2.5 mg/L and $COCl_2.6H_2O$-2.5 mg/L compounds. All the micronutrients were dissolved in DD water and volume was made with 1000 ml and then stored at 4°C (Figure 2, Table-1).

Vitamins Amount of mg/L Vitamins like 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 were dissolved in DD water and make volume to 1000 ml Store below 0°C (Figure 3, Table-1).

Iron Source Amount of g/L Iron compounds like $Na_2EDTA.2H_2O$-3.73 g/L and $FeSO_4.7H_2O$-2.78 g/L $FeSO_4.7H_2O$ and $Na_2EDTA._2H_2O$ were dissolved individually in 400 ml/L of double distilled water. $Na_2EDTA.2H_2O$ was heated until it dissolved. $FeSO_4.7H_2O$ was added to the warmed Na_2 EDTA solution with continuous stirring. The solution was a slight yellowish tinge. The volume was also accommodated to 1000 ml with double distilled water. This stock was stored in a coloured bottle at 4°C. The pH of the medium was accommodated in 5.8 with newly prepared 1 N HCl or 1 N KOH (Figure 4, Table-1).

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 for 20 min. Cultures were incubated at 25 1°C for 16 h photoperiod (fluorescent light of 100 µmole/m /s). After 21 days when sufficient numbers of plantlets were obtained on solid medium in test tube, they were cut with three nodes cutting and inoculated with 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 Kinetin concentrations (0.75q , 1.5 and 2.25 mg/L). These flasks were then incubated at 18 ± 2°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).

[1]Meenakshi Kumari (2020). Modulation in growth and development of potato (*Solanum tuberosum* L.) microtubers by different concentration of 6-benzyl aminopurine. African Journal of plant Science. **14 (2):** 102-107.



Results and Discussion

In vitro tuberization in potato was studied in Kufri Bahar and Kufri Surya cultivars. To assess the physical characteristics connate as number of microtubers, weight of mircotubers and growth rate like number of eyes and tuber initiations were opted as indicators.

Microtuber Initiation

The data presented in Table 2 revealed that there was significant variation over control with respect to microtuber initiation. MS media supplemented with different concentration of kinetin were used for microtuber initiation. Days required for tuber initiation decreased with increasing concentration of KIN in both the varieties studied. Significantly maximum days for tuber initiation (20.0 and 20.4) were recorded in control in Kufri Bahar and Kufri Surya. However, it was at par with T3 in Kufri Bahar and T3, T2, T1 in Kufri Surya. Significantly minimum days for tuber initiation (12.5 and 12.7) were recorded in T3 in both the varieties respectively. The present finding is in conformity with the earlier findings (Kotkas & Peter, 1998; Shibli *et al.,* 2001; Kumley *et al.,* 2021). Kumley *et al.,* 2021 reported that root initiation and shoot initiation increase at 2 mg/l of Kinetin. Aksenova *et al.,* (2000) also reported that cytokinin and sucrose at high concentration stimulated tuber initiation. The earlier finding revealed that MS media supplemented with 2.25 mg/l of kinetin showed the best result on tuber induction.

Number of Microtubers

Different concentration of KIN significantly influenced the number of microtubers. Significantly maximum microtubers number (9.15) was recorded with T3. However, it was at par with T2 and T1 in Kufri Bahar. Significantly the maximum microtubers number (9.16) was also recorded with T3 in Kufri Surya. However, it was at par with control in Kufri Surya. Minimum numbers of microtubers (5.60 and 5.30) in both the varieties and in both the year under study were recorded under control (Table 2). These results agree with Romanov *et al.,* 2000 and Kefi *et al.,* 2000 who found that the higher mean number of microtubers was achieved by the combined effect of cytokinin and sucrose promotes *In vitro* microtuberization. Kinetin influenced the microtuber initiation, therefore microtuber number increased; besides, effect of phytohormones on microtuberization parameters depends on plant genotype and the amount of sucrose in media. Astarini et al., 2021 reported that 2 g L^{-1} phytagel + 10 mg L^{-1} kinetin treatment (T4) was an effective treatment to enhance microtuber growth.

Fresh Weight of Microtubers

The perusal of the data presented in Table 3 also revealed that different concentration of KIN significantly influenced the fresh weight of microtubers per flask. Significantly maximum mean fresh weight (2.76g) was recorded with concentration of T3 in Kufri Bahar. However, it was at par with T2, T1 and control. In Kufri Surya maximum fresh weight of microtubers (2.87g) was also recorded with T3 and it was at par with T2, T1 and control. The minimum fresh weight of microtubers (0.47g and 0.51g) was observed in control. Our study agrees with Aslam and Iqbal (2010) who reported that 2.5 mg/l of kinetin with high concentrations of sucrose gave better response for tuber induction, mean number and mean fresh weight of microtubers per multinode explants. The result was also similar with Wu *et al.,* 2021, they find out that cytokinin promotes cell division and increase cell expansion during the proliferation and expansion stages of leaf cell development.

Number of yes

The data presented in Table 3 revealed that there was a significant difference over control with respect to the number of eyes per microtuber. Significantly maximum number of eyes per microtuber (3.07) was reported with concentration of T3 in Kufri Bahar. The mean number of eyes per microtuber was at par with control. Alike trend was found with Kufri Surya, where a significantly highest number of eyes per microtuber (3.28) was observed with T3 and it was at

par with control. The fewest number of eyes (1.88 and 1.93) per microtuber in both the cultivars was recorded in control. Our findings with relevance to effect of Kinetin on microtuberization agree with antecedent studies (Palmer and Smith, 1970; Chandra *et al.* 1988; Alchanatis *et al.* 1994; Wrobel *et al.*, 2017). cytokinin stimulated transition of axillary buds into stolons, which could be applied in tuberization.

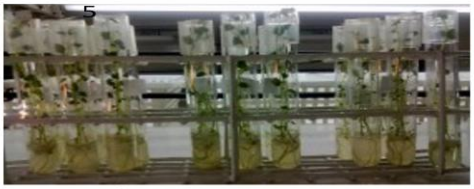


Fig-5 Kufri Bahar



Fig-6 Kufri Surya

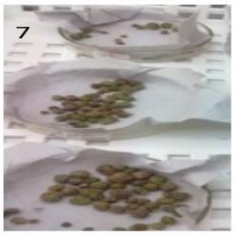


Fig-7 Kufri Bahar

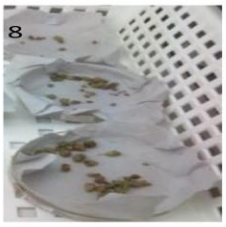


Fig-8 Kufri Surya

CONCLUSIONS

From the results, it appears that kinetin in combination with sucrose promote *in vitro* microtuber induction and development. The kinetin is stimulatory to starch metabolizing enzymes, thus creating a strong metabolic sink. As a result, subsequent accumulation of starch occurred which is seen as the swelling of the microtuber. This combined ability can be termed as an excessive substrate (sucrose) and stimulus (Kinetin) that triggers the enzymatic activity in the tuberization processes. The results suggested the need of developing genotype specific protocols to maximize *In Vitro* performance for microtuberization and in vivo minitubers performance.

On the basis of this experiment, we can conclude that the kinetin 2.25 mg/l may be exploited to enhance tuber quality as well as crop growth under *in vitro* conditions farmer level and industrial level. So that for future scope Potato has a good source for food energy. Cytokinin enhance tuber quality and tuber growth so that farmers can improve the income and nutritional value related potato production.

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Macronutrients	Amount in g/l	Micronutrients	Amount in mg/l	Vitamins	Amount in mg/l	Iron source	Amount in g/l
		$MnSO_4.4H_2O$	2.230				
KNO_3	38.00	$ZnSO_4.7H_2O$	860	Myo-inositol	10,000		
NH_4NO_3	33.00	H_3BO_3	620	Thiamine-HCl	10		
$MgSO_4.7H_2O$	7.4	KI	83	Nicotinic acid	50	$Na_2EDTA.2H_2O$	3.73
KH_2PO_4	3.4	$Na_2MOO_4.2H_2O$	25	Pyridoxine-HCl	50	$FeSO_4.7H_2O$	2.78
$CaCl_2.2H_2O$	8.8	$CuSO_4.5H_2O$	2.5	Glycine	200		
		$COCl_2.6H_2O$	2.5				

Table-1 Macronutrient, micronutrient, vitamins and iron source compositions

Treatment	Microtuber initiation				Mean		Number of microtubers per flask				Mean	
KIN	1st Year		2nd Year				1st Year		2nd Year			
	KB	KS	KB	KS	KB	KS	KB	KS	KB	KS	KB	KS
0.75 mg/L	16.0	16.6	16.6	16.3	16.3	16.4	7.20	7.14	7.16	7.24	7.18	7.19
1.5 mg/L	15.4	14.2	15.6	14.0	15.5	14.1	7.70	7.80	7.64	7.90	7.67	7.85
2.25 mg/L	**12.6**	**12.6**	**12.4**	**12.8**	**12.5**	**12.7**	**9.30**	**9.12**	**9.00**	**9.20**	**9.15**	**9.16**
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±	2.12	1.30	2.47	1.23	1.53	1.06	0.46	0.84	0.54	1.15	0.30	0.74
CD or LSD (P = 0.05%)	NS	3.90	NS	3.70	4.60	3.19	1.38	2.54	1.62	NS	0.90	2.23
CV	16.09	17.99	17.17	18.69	16.62	18.33	18.79	19.32	15.45	18.43	17.12	18.87

Table-2 *Effect of KIN on microtuber initiation and number of microtubes per flask of 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	
KIN	1st Year		2nd Year				1st Year		2nd Year			
	KB	KS	KB	KS	KB	KS	KB	KS	KB	KS	KB	KS
0.75 mg/L	2.00	2.08	1.92	2.00	1.96	2.04	2.60	2.64	2.80	2.76	2.70	2.70
1.5 mg/L	2.12	2.14	2.14	2.22	2.13	2.18	2.76	2.80	2.72	2.84	2.74	2.82
2.25 mg/L	**2.86**	**2.86**	**2.66**	**2.88**	**2.76**	**2.87**	**3.04**	**3.36**	**3.10**	**3.20**	**3.07**	**3.28**
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±	0.17	0.21	0.23	0.25	0.12	0.14	0.33	0.26	0.30	0.22	0.23	0.19
CD or LSD (P = 0.05%)	0.52	0.65	0.71	0.76	0.37	0.43	NS	0.79	NS	0.68	0.69	0.57
CV	46.64	45.2	44.89	45.28	45.89	45.35	18.06	17.94	15.88	18.51	16.87	18.09

Table-3: *Effect of KIN 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.*

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