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GIBBERELIC ACID (GA₃) ALONE AND IN COMBINATION WITH INDOLE 3 BUTYRIC ACID (IBA) MODULATION DURING *IN VITRO* PROPAGATION OF POTATO (*SOLANUM TUBEROSUM* L.) MICROPLANTS

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ABSTRACT

Growth hormones can alter the properties of the plants at physiological and cellular level. Gibberellic acid plays an important role in shoot proliferation. IBA is the synthetic auxin and effect on the plant root. This study was done in Central Potato Research Institute Modipuram, Meerut U.P. India. The aim of this research to find out the effect of gibberellic acid (GA₃) alone and in combination with indole 3 butyric acid (IBA) on potato cultivar Kufri Bahar and Kufri Surya micro plants *in vitro*. The effect of 6 concentrations (0.02 mg/l, 0.04 mg/l, 0.05 mg/l, 0.1 mg/l, 0.2 mg/l and 0.3 mg/l) of GA₃ and the 6 concentration (0.05 mg GA₃/l +0.005 mg IBA/l, 0.1 mg GA₃/l +0.01 mg IBA /l, 0.2 mg GA₃/l+0.01 mg IBA /l, 0.1 mg GA₃/l +0.02 mg IBA /l, 0.2 GA₃mg/l + 0.02 mg IBA /l and 0.4 mg GA₃/l +0.04 mg IBA/l) of GA₃ and IBA in combination on micro plants root, shoot growth, number of node, internode, leaves and fresh weight of shoot were investigated. The best medium formulas suitable for potato micro-planting were 0.3 mg/l GA₃ and 0.4 mg/l GA₃ +0.04 mg/l IBA. Farmer can use the breeder seed for commercial purpose.

Keywords: Growth hormones, GA₃, IBA, Microplants, *In-vitro*, MS Media, Plant tissue Culture, Kufri Bahar, Kufri Surya and Potato variety.

Introduction

Potato plant belongs to the family Solanaceae and this family is also known as night shade family. In the year of 2022, the volume of potato produced across India was estimated to be around 53.58 million metric tons. The highest Indian potato production are recorded in Northern states of India specially in Uttar Pradesh (Statista Research Department 2022). In India the potato production is second-largest after China (Hill *et al.*, 2021). The potato (*Solanum tuberosum* L.) is the third most important food crop after wheat and rice. India is the second-largest producer country of potatoes globally after China (Hill *et al.*, 2021). Therefore, careful nutrient management is very important to get maximum yield. Potato tubers are formed via swelling of the underground stem into stolon, subsequently growing as an inflated structure like tuber due to storage of starch, also has chances of direct uptake of nanoparticles from medium, thus manipulating the tuberization. Indole 3 Butyric acid is the synthetic hormone of auxin. This is the elicited hormone and used for promote the root initiation, stem bending, and leaf epinasty (Zimmerman and Wilcoxon, 1935). IBA is also a endogenous compound which examined the plant species (Korasick *at el.*, 2013) and it is the conversion of IAA (Strader and Bartel, 2011). Since the effect of GA₃ and IBA alone or in combination has been established, in this experiment, influence the six concentrations of IBA and GA₃ on qualitative, quality of microplants were investigated.

Material and Methods

The investigations, lab and field experiments were planned and conducted during autumn season at Central Potato Research Institute Campus, Modipuram, Meerut (UP) India. The site is situated at 29° 05' 19" N latitude, 77° 41' 50" E longitude, and 237 metres above mean sea level in a semi-arid and sub-tropical environment. The Central Potato Research Institute in Shimla provided verified virus-free microplants of the potato (*Solanum tuberosum* L.). Nodal cuttings of both cultivars of potatoes were cultivated for microplant multiplication in test tubes (25 mm) containing 15 ml of solidified (0.8 percent agar) Supplemental nutrition (SN) medium, Murashige and Skoog (1962), the calcium pantothenate (2 mg/l) and growth hormones GA₃ (0.02 mg/L, 0.04 mg/L, 0.05 mg/L, 0.1 mg/L, 0.2 mg/L and 0.3 mg/L) and GA₃+IBA in combinations concentration (0.05mg/L+0.005 mg/L, 0.1 mg/L+0.01 mg/L, 0.2 mg/L+0.01 mg/L, 0.1 mg/L+0.02 mg/L, 0.2 mg/L+0.02 mg/L and 0.4 mg/L+0.04 mg/L) were added. Before autoclaving at 121°C for 20 minutes, the medium pH was adjusted to 5.8. Cultures were incubated at 25±1°C under 16h photoperiod (fluorescent, 100 µmole/m²/s) (Fig. 1 and 2). After 21 days, the shoot length, number of nodes, number of leaves, internode length, and root length and shoot fresh weight were measured. Five replications of the experiment were used in the Completely Randomized Block Design (C.R.B.D.) layout. IRRISTAT software was used for data analysis. After 21 days, the roots of the microplants were gently washed to remove the agar medium. The root zone of mass micro propagated plants

from culture tube was dipped in ordinary tap water to wash out adhering liquid medium. These were then planted in plastic trays with compost mixture of farmyard manure, sand and soil (1:1:1) and maintained at 20°C under high humidity under shade (Fig-5 and 6). IRRISTAT software was used for data analysis.

Results and Discussion

Shoot length

The study on shoot length of Kufri Bahar and Kufri Surya after 21 days of inoculation showed significant increasing trend with increasing GA₃ and the combination of GA₃+IBA. The maximum and superior shoot length (12.3 cm) was same in both varieties with 0.3 mg/l. The minimum shoot length was observed in control. In another treatment the maximum shoot length (8.81 cm) was recorded with 0.4 mg/l+0.04 mg/l in GA₃+IBA. Moreover, this was significant form 0.1 mg/l+0.02 mg/l, 0.2 mg/l+0.01 mg/l, 0.1 mg/l+0.01 mg/l, 0.05 mg/l+0.005 mg/l and control in variety Kufri Bahar. In Kufri Surya maximum plant height per microplant (8.53 cm) was recorded with 0.4 mg/l+0.04 mg/l and it was at par with 0.2 mg/l+0.02 mg/l, 0.1 mg/l+0.02 mg/l, 0.2 mg/l+0.01 mg/l, 0.1 mg/l+0.01 mg/l, 0.05 mg/l+0.005 mg/l and control in GA₃+IBA. The minimum shoot length was observed in control. In case of GA₃, the results were in agreement with the findings of Makau *et al.*, 2022. According to Al-Taleb *et al.* (2011) IBA gave maximum shoot length with 0.5 mg/l.

Number of nodes

Like shoot length, number of nodes per microplant increased with the concentration of GA₃ and control treatment showed the least number of nodes. Number of nodes per microplant significantly increased with increasing concentration of GA₃ in both the varieties. The maximum number of nodes (9.75) was observed with 0.3 mg/l treatment in Kufri Bahar. However, it was at par with 0.05 mg/l, 0.04 mg/l, 0.02 mg/l and control. Similarly maximum number of nodes (9.89) was observed in 0.3 mg/l treatment in Kufri Surya and it was at par with 0.1 mg/l, 0.05 mg/l, 0.04 mg/l, 0.02 mg/l and control. Minimum number of nodes per microplant after 21 days of inoculation was recorded in control.

Different levels of GA₃+IBA concentrations significantly influenced the number of nodes per microplant. The maximum number of nodes (7.62) was recorded with 0.4 mg/l+0.04 mg/l in Kufri Bahar. However, it was at par with 0.1 mg/l+0.02 mg/l, 0.2 mg/l+0.01 mg/l, 0.1 mg/l+0.01 mg/l, 0.05mg/l+0.005 mg/l and control. Similarly, the maximum number of nodes (7.29) was recorded with 0.4 mg/l+0.04 mg/l in Kufri Surya and it was at par with 0.1 mg/l+0.02 mg/l, 0.2 mg/l+0.01 mg/l, 0.1 mg/l+0.01 mg/l, 0.05 mg/l+0.005 mg/l and control. Minimum number of nodes (5.59 and 5.52) in both the varieties was recorded in control. IBA, NAA or IAA with or without GA₃ also stimulates the number of node and number of shoots (Shibli *et al.*, 2002). Shibli *et al.*, 2002 found that maximum number of nodes (10.2) with 1 mg/l GA₃+2 mg/l IBA.

Internode length

Maximum internode length was found with 0.3 mgGA₃/l and 0.4 mg GA₃/l+0.04 mg IBA/l in both variety of Kufri Bahar and Kufri Surya (Table 2). GA₃ significantly

influenced the internode length per microplant. Significantly maximum internode length (1.28 cm) was recorded at 0.3 mg/l treatment which was at parwith 0.04 mg/l, 0.02 mg/l and control in Kufri Bahar. In Kufri Surya significantly maximum internode length (1.31 cm) was also observed in 0.3 mg/l treatment. However, this was atpar with 0.05 mg/l, 0.04 mg/l, 0.02 mg/l and control. Minimum internode length in both the varieties was recorded in control. Different levels of GA₃ + IBA concentrations significantly influenced the internode length per microplant. The maximum internode length (1.13 cm) was recorded with 0.4 mg/l+0.04 mg/l in Kufri Bahar. However, there were no significant differences. In Kufri Surya significantly maximum internode length (1.18 cm) was recorded with 0.4 mg/l+0.04 mg/l and it was at par with control. The minimum internode length was observed in control (Table 2).The result was similar with molla *et al.* (2011) who recorded that IBA at 05 mg/l and GA₃ at 0.2 mg/l gave the batter result for internode length and for root.

Number of leaves

The data presented in Table 3 also revealed that the number of leaves increasedwith increasing concentration of GA₃ in both the varieties. Significantly maximum number of leaves (9.95) was recorded with 0.3 mg/l treatment in Kufri Bahar. However, it was at par with 0.05 mg/l, 0.04 mg/l, 0.02 mg/l and control in Kufri Bahar. In Kufri Surya maximum number of leaves (10.1) per plant was recorded with 0.3 mg/l treatment; it was at par with 0.1 mg/l, 0.05 mg/l, 0.04 mg/l, 0.02 mg/l and control. Minimum number of leaves (5.77 and 5.61) was recorded in control.

The data presented in Table 4 also revealed that the number of leaves increased with increasing GA₃+IBA concentration in both the varieties during both the years of study. Maximum number of leaves (7.79) was recorded with 0.4 mg/l+0.04 mg/l in Kufri Bahar. However, it was at par with 0.1 mg/l+0.02 mg/l, 0.2 mg/l+0.01 mg/l, 0.1 mg/l+0.01 mg/l, 0.05mg/l+0.005 mg/l and control in Kufri Bahar. In Kufri Surya maximum number of leaves (7.52) per plant was recorded in 0.4 mg/l+0.04 mg/l; it was at par with 0.1 mg/l+0.02 mg/l, 0.2 mg/l+0.01 mg/l, 0.1 mg/l+0.01 mg/l, 0.05 mg/l+0.005 mg/l and control. Minimum number of leaves in both the varieties were recorded in control. Similar response of GA₃ concentration were observed by Makau *et al.*, 2022 and Fatima *et al.*, 2005.

Root length

Different concentration of GA₃ markedly influenced root length. Treatment 0.3 mg/l gave maximum mean root length (6.48 cm) which was at par with 0.1 mg/l, 0.05 mg/l, 0.04 mg/l, 0.02 mg/l and control in Kufri Bahar (Table 1B). Similarly, significantly maximum root length (6.29 cm) was also noted with 0.3 mg/l treatment in Kufri Surya and, it was at par with 0.05 mg/l, 0.04 mg/l, 0.02 mg/l and control. The mean root length (4.43 cm and 5.04 cm) per microplant was recorded in control in Kufri Bahar and Kufri Surya respectively (Table 2).

Significant variations were found among the varieties in respect of root length per microplant. During GA₃+ IBA treatments, the highest mean length of root per microplant (7.51 cm and 7.47 cm) was recorded with 0.4 mg/l+0.04 mg/l in Kufri Bahar and in Kufri Surya (Table 4). However, it was at par with 0.1 mg/l+0.02 mg/l, 0.2 mg/l+0.01 mg/l, 0.1 mg/l+0.01 mg/l, 0.05mg/l+0.005 mg/l and control. According

to Ahmed (2022). Danu *et al.* (2015) showed that the combination of GA₃ and IBA increased the sprouting and rooting percentage in *Paris polyphylla* but at high concentration.

Fresh weight of shoot

Shoot fresh weight used to estimate the total plant biomass yield in potato. Shoot fresh weight recorded just after the harvested of the microplant. The data presented in Table 3 also revealed that fresh weight of shoot increased with increasing GA₃ concentration in both the varieties. Significantly maximum fresh weight of shoot (746 mg, 741 mg) was recorded with 0.3 mg/l in Kufri Bahar and in Kufri Surya and it was at par with 0.2 mg/l, 0.1 mg/l, 0.05 mg/l, 0.04 mg/l, 0.02 mg/l and control. Minimum fresh weight of shoot (172 mg and 173 mg) was recorded under control in both the varieties.

The data presented in Table 4 also revealed that fresh weight of shoot increased with increasing GA₃+IBA concentration in both the varieties under study period. Maximum fresh weight of shoot (493 mg and 534 mg) was recorded in 0.4 mg/l+0.04 mg/l in Kufri Bahar and in Kufri Surya. It was at par with 0.2 mg/l+0.02 mg/l, 0.1 mg/l+0.02 mg/l, 0.2 mg/l+0.01 mg/l, 0.1 mg/l+0.01 mg/l, 0.05 mg/l +0.005 mg/l and control. Minimum fresh weights of shoot in both the varieties were recorded under control. This result is similar with Parika *et al.* (2020) and Sillu *et al.* (2012) with GA₃+IBA. Haida *et al.* (2020) and Makau *et al.*, 2022 observed the highest leaf fresh weight with GA₃ and sucrose, might be possible GA₃ increase the fresh weight of shoot also.

Table 1 : Effect of GA₃ on shoot length, number of nodes and internode length of varieties Kufri Bahar (KB) and Kufri Surya (KS) microplants at 21 days after inoculation *in vitro*.

Treatments GA ₃ in mg/L	Length of shoot per microplant (cm)				Mean		Number of nodes per microplant				Mean		Internode length per microplant (cm)				Mean	
	1st year		2nd year				1st year		2nd year				1st year		2nd year			
	K.B	K.S	K.B	K.S	K.B	K.S	K.B	K.S	K.B	K.S	K.B	K.S	K.B	K.S	K.B	K.S	K.B	K.S
0.02	8.89	8.43	8.85	8.68	8.87	8.55	7.64	7.90	7.66	8.08	7.65	7.99	1.12	1.09	1.10	1.08	1.11	1.08
0.04	9.70	9.20	9.74	9.30	9.72	9.25	8.06	8.36	7.86	8.26	7.96	8.31	1.19	1.10	1.18	1.11	1.18	1.10
0.05	10.50	10.30	10.90	10.80	10.70	10.50	8.62	8.28	8.70	8.34	8.66	8.31	1.20	1.19	1.21	1.22	1.20	1.21
0.1	11.10	11.20	11.00	11.30	11.00	11.20	9.42	8.50	9.36	8.40	9.39	8.45	1.21	1.24	1.21	1.26	1.21	1.25
0.2	12.10	11.80	12.10	11.90	12.10	11.80	9.62	9.48	9.52	9.56	9.57	9.52	1.26	1.30	1.25	1.30	1.25	1.30
0.3	12.40	12.20	12.30	12.30	12.30	12.30	9.80	9.96	9.70	9.82	9.75	9.89	1.28	1.32	1.27	1.31	1.28	1.31
Control	5.86	5.94	5.92	5.98	5.89	5.96	5.60	5.54	5.58	5.50	5.59	5.52	1.03	1.01	1.04	1.07	1.03	1.04
S Em ±	0.30	0.11	0.46	0.56	0.27	0.29	0.30	0.36	0.43	0.38	0.26	0.28	0.04	0.04	0.03	0.03	0.03	0.02
CD (P=0.05%)	0.88	0.32	1.34	1.63	0.79	0.84	0.88	1.07	1.24	1.10	0.77	0.83	0.1	0.13	0.11	0.1	0.10	0.08
CV	20.6	20.65	20.3	20.49	20.32	20.55	16.28	15.78	16.16	15.68	16.21	15.71	6.68	9.13	6.47	8.09	6.688	8.62

Table 2 : Effect of GA₃+IBA on shoot length, number of nodes and internode length of varieties Kufri Bahar (KB) and Kufri Surya (KS) microplants at 21 days after inoculation *in vitro*.

Treatments GA ₃ +IBA in mg/L	Length of shoot per microplant (cm)				Mean		Number of nodes per microplant				Mean		Internode length per microplant (cm)				Mean	
	1st year		2nd year				1st year		2nd year				1st year		2nd year			
	KB	KS	KB	KS	KB	KS	KB	KS	KB	KS	KB	KS	KB	KS	KB	KS	KB	KS
0.05+0.005	6.01	6.00	6.00	6.74	6.00	6.37	5.80	5.62	5.64	5.54	5.72	5.58	1.06	1.07	1.05	1.08	1.05	1.07
0.1+0.01	6.32	6.58	6.31	6.50	6.31	6.54	5.86	5.74	5.82	5.82	5.84	5.78	1.07	1.09	1.07	1.09	1.07	1.09
0.2+0.01	6.50	6.74	6.48	7.22	6.49	6.98	6.04	6.16	5.94	6.22	5.99	6.19	1.08	1.11	1.10	1.10	1.09	1.10
0.1+0.02	7.16	7.68	7.13	7.69	7.14	7.68	6.94	6.82	7.04	6.70	6.99	6.76	1.09	1.13	1.11	1.13	1.10	1.13
0.2 +0.02	8.17	7.88	8.28	7.84	8.22	7.86	7.40	6.92	7.42	6.94	7.41	6.93	1.11	1.15	1.12	1.14	1.11	1.14
0.4 +0.04	8.74	8.47	8.88	8.60	8.81	8.53	7.56	7.30	7.68	7.28	7.62	7.29	1.15	1.16	1.12	1.20	1.13	1.18
Control	5.86	5.94	5.92	5.98	5.89	5.96	5.60	5.54	5.58	5.50	5.59	5.52	1.03	1.01	1.04	1.07	1.03	1.04
S Em ±	0.28	0.23	0.30	0.19	0.22	0.17	0.21	0.15	0.39	0.26	0.19	0.16	0.03	0.03	0.03	0.03	0.02	0.02
CD (P=0.05%)	0.83	0.68	0.88	0.57	0.64	0.49	0.63	0.45	1.15	0.75	0.57	0.49	NS	NS	NS	NS	NS	0.08
CV	14.77	12.88	15.37	11.46	15.07	11.98	11.76	10.44	12.94	10.39	12.34	10.40	3.26	4.35	2.85	3.74	2.99	3.91

Table 3 : Effect of GA₃ on number of leaves, root length and fresh weight of shoot of varieties Kufri Bahar (KB) and Kufri Surya (KS) microplants at 21 days after inoculation *in vitro*.

Treatments GA ₃ in mg/L	Number of leaves per microplants				Mean		Length of root per microplants				Mean		Fresh weight of shoot				Mean	
	1st year		2nd year				1st year		2nd year				1st year		2nd year			
	K.B	K.S	K.B	K.S	K.B	K.S	K.B	K.S	K.B	K.S	K.B	K.S	K.B	K.S	K.B	K.S	K.B	K.S
0.02	7.84	8.1	7.86	8.24	7.85	8.17	5.6	5.42	5.58	5.54	5.59	5.48	401	403	402	404	401	404
0.04	8.24	8.56	8.02	8.54	8.13	8.55	5.67	5.74	5.63	5.77	5.65	5.76	426	425	427	426	427	426
0.05	8.74	8.82	8.86	8.94	8.8	8.88	5.72	5.85	5.85	5.84	5.78	5.84	479	476	478	475	479	475
0.1	9.54	9.06	9.68	9.12	9.61	9.09	6.13	5.97	6.04	5.98	6.08	5.98	517	519	516	520	517	519
0.2	9.8	9.7	9.74	9.76	9.77	9.73	6.19	6.02	6.23	6.00	6.21	6.01	656	657	655	658	655	657
0.3	10	10.1	9.9	10.2	9.95	10.1	6.44	6.22	6.53	6.36	6.48	6.29	746	740	746	741	746	741
Control	5.8	5.68	5.74	5.54	5.77	5.61	4.39	5.02	4.48	5.07	4.43	5.04	172	172	173	173	172	173
S Em ±	0.29	0.39	0.43	0.42	0.26	0.32	0.04	0.09	0.25	0.2	0.12	0.11	2.04	1.24	1.95	1.79	1.53	0.77
CD (P=0.05%)	0.86	1.15	1.27	1.23	0.77	0.94	0.13	0.27	0.74	0.58	0.37	0.32	5.98	3.6	5.65	5.2	4.43	2.25
CV	15.81	15.58	16.11	16.28	15.95	15.85	10.81	6.56	10.55	6.49	10.67	6.53	35.43	35.24	35.32	35.19	35.39	35.18

Table 4 : Effect of GA₃+IBA on number of leaves, root length and fresh weight of shoot of varieties Kufri Bahar (KB) and Kufri Surya (KS) microplants at 21 days after inoculation *in vitro*.

Treatments GA ₃ +IBA in mg/L	Number of leaves per microplants				Mean		Length of root per microplants				Mean		Fresh weight of shoot				Mean	
	1st year		2nd year				1st year		2nd year				1st year		2nd year			
	KB	KS	KB	KS	KB	KS	KB	KS	KB	KS	KB	KS	KB	KS	KB	KS	KB	KS
0.05+0.005	5.96	5.78	5.84	5.76	5.9	5.77	5.3	5.58	5.37	5.56	5.33	5.57	309	343	308	344	308	344
0.1+0.01	5.98	5.96	6.02	5.98	6	5.97	5.54	5.78	5.74	5.89	5.64	5.83	323	383	324	384	323	384
0.2+0.01	6.2	6.4	6.14	6.42	6.17	6.41	6.34	6.23	6.4	6.3	6.37	6.26	344	405	343	404	343	404
0.1+0.02	7.1	7.02	7.2	6.98	7.15	7	7.18	6.52	7.06	6.52	7.12	6.52	424	412	425	413	424	412
0.2+0.02	7.62	7.16	7.61	7.14	7.61	7.15	7.47	7.29	7.38	7.23	7.42	7.26	482	513	483	514	482	513
0.4+0.04	7.8	7.56	7.78	7.48	7.79	7.52	7.47	7.48	7.56	7.47	7.51	7.47	492	534	494	534	493	534
Control	5.8	5.68	5.74	5.54	5.77	5.61	4.39	5.02	4.48	5.07	4.43	5.04	172	172	173	173	172	173
S Em ±	0.26	0.17	0.37	0.24	0.21	0.16	0.13	0.12	0.1	0.11	0.09	0.09	1.66	1.38	1.5	1.53	1.33	0.88
CD (P=0.05%)	0.76	0.5	1.07	0.72	0.62	0.48	0.4	0.35	0.3	0.34	0.26	0.26	4.81	4	4.35	4.45	3.87	2.56
CV	50.09	51.37	48.24	49.94	51.05	51.52	54.41	53.05	54.67	53.00	55.12	53.60	61.97	61.81	62.06	61.71	62.13	61.90

Fig-1 Microplants of Kufri bahar (GA₃+IBA)Fig-2 Microplants of Kufri surya (GA₃+IBA)Fig-3 Microplants of Kufri bahar (GA₃)Fig-4 Microplants of Kufri surya (GA₃)

Fig-5 Microplants of Kufri bahar transferred in a plastic tray for 7 days



Fig-6 Microplants of Kufri surya transferred in a plastic tray for 7 days

Conclusion

Potato is a good source of food, because it has carbohydrate, minerals, vitamins and traces elements. The growth hormone GA₃ treatment 0.3 mg/l and the combination of GA₃+IBA treatment 0.4 mg/l +0.04 mg/l were found to be best for shoot, root length, number of nodes, internode length, number of leaves and fresh weight of shoot. Variety Kufri Bahar showed the superior root length when applied GA₃. In case of combination GA₃+IBA Kufri Surya showed the best shoot length, number of nodes, number of leaves and root length. Tissue culture used to produce disease-free healthy potato plants and also give significantly improvement to the food system for marginal farmers and also provide nutritive and affordable food for urban consumers.

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