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SHORT NOTE

POTATO MICROPROPAGATION IN A MEDIUM DEVOID OF ANY PLANT GROWTH REGULATORS

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ABSTRACT: The MS media without any growth regulator is being used for the potato micropropagation. An analysis demonstrated that microplants developed without the growth regulators proved to have equivalent potential of increasing the multiplication rate of *in vitro* potato varieties. There was no significant difference in the growth and morphological parameters of potato cultivars regenerated with and without the use of growth regulators. The same cost effective protocol has been shared with the seed entrepreneurs and they are successfully following it in their labs.

KEYWORDS: Virus free, *in vitro* plantlets, Plant Growth Regulators (PGRs), MS media

Potato is commonly grown by vegetative propagation through tubers. But for commercial potato production, the availability of quality seeds is a major constraint especially in developing countries like India (Kumar *et al*., 2007). There are severe yield losses resulting in lower productivity of potato crops due to the degeneration of seed stocks over the years. To overcome this problem, conventional methods, rapid multiplication techniques and hi-tech seed production systems like micro-propagation and aeroponics are being used at the commercial level for producing enough quantity of healthy seed tubers in a less time period (Buckseth *et al.,* 2022). Since potato is highly amenable to tissue culture, micropropagation is one of the most commonly used methods for the rapid multiplication of true-to-type genotypes (Tiwari *et al*., 2013). Moreover, tissue culture-based hi-tech seed potato production is a commercially used technology in potato-growing countries including India (Buckseth *et al*., 2016). As per expectations, the clonally propagated plantlets ought to be the exact copy of the mother plant but, a contrasting behavior can be seen when the material involving PGR experiences unfavorable environmental conditions and seasonal cycles. Epigenetic changes are often temporary and plants may revert back to the original phenotype quite effectively, yet some can be long lasting and may even be transferred during sexual propagation (Jullien, 2010). The principle goal of raising potato plantlets is to produce high quality uniform tubers both for seed and table purpose. Any kind of distortion whether genetic or phenotypic, diminishes the market value of potato and raises issues on its usage. Retaining in born strength of in vitro proliferating material is one of the real concerns for analysts (Zilberman and Henikoff, 2007). Unexpectedly, during our routine micropropagation, we observed the tuber shape variation (elongated) in one of the most popular variety Kufri Bahar in the tuber produce of tissue-culture raised plants which were originated from the mother tuber having round shape. These elongated tubers with

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sugary tapering end are highly undesirable and also unsuitable for commercial seed production, and results in rottage and early decay of seed stocks during storage. To achieve healthy and quality seed tubers, the maintenance of the genetic identity of tissue culture-derived plants is very crucial in potato (Tiwari *et al*., 2013). Later, considering the growth regulator as a culprit for tuber shape variation, a decision of exclusive use of hormone –free media for potato tissue culture was taken (Tiwari *et al.,* 2021). Keeping the above factors in view a study was conducted to investigate whether the growth regulators are essential for appropriate development and improvement of potato varieties under controlled conditions.

In the present study (2018-19 & 2019-20), 29 commercial potato varieties were used. MS media with (100 ul/L GA3 and 10ul/L NAA) and without growth regulators supplemented with sucrose (300 g/L) and gelrite (2g/L) was used. The pH was adjusted to 5.8 before autoclaving. The cultures were grown in culture room at 21±2°C temperature under 16/8 h (day/night) photoperiod with 50–60 µmol m^{-2} s^{-1} light intensity. Three explants with single node cuttings (SNC's) were aseptically inoculated in each culture tube (25 × 150 mm) containing MS media. Individual variety was replicated thrice and each replicate consisted of five test tubes and was allowed to regenerate under controlled conditions for 21 days. After twenty-one days of culturing, observations were recorded on morphological parameters such as micro-plant height (cm); number of green leaves, nodes and roots; inter-nodal and root length (cm); fresh as well as dry mass (mg) as described by Naik and Buckseth, 2018.

As the experiment was conducted twice, data were pooled over individual experiments. The analysis of variance was done using the software AGRES and means were separated according to the least significant differences (LSD) at 0.05 level of probability. Significant means were reported as a sum of means and standard errors (±SE). No significant difference was recorded for plant height among the treatments (**Figure 1**, **Table 1**). The maximum plant height was recorded in variety K. Lauvkar in both GR (17.22±0.98) as well as WGR (17.42±0.70) thereby indicating the use of hormone-free media for the *in vitro* mass multiplication. Surprisingly, the minimum plant height was recorded in variety K. Lima in both GR (7.18±0.89) as well as WGR (7.21±1.03) respectively. However, most of the study reported only the significant use of GA3 (1 ppm) and NAA (100ppm) to increase the plant height which indicates the superiority of these chemicals in bringing about enhanced growth.

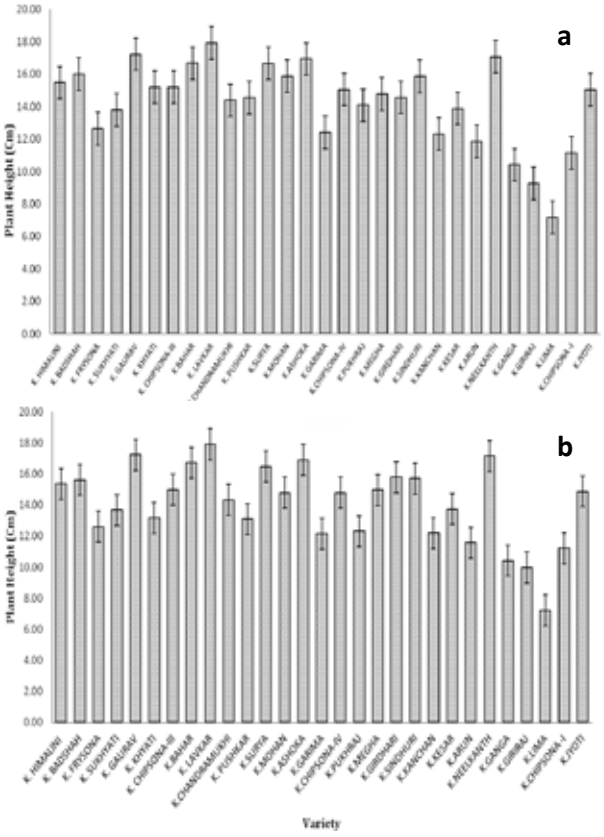


Figure 1: Microplant height of 29 potato varieties a) GR, b) WGR

Table 1: Morphological assessment of *in vitro* propagated potato varieties

Varieties	Microplant height (cm)		Root Length (cm)		No. of Leaves		No. of Nodes		No. of Roots		Fresh Weight (mg)		Dry Weight (mg)	
	GR	WGR	GR	WGR	GR	WGR	GR	WGR	GR	WGR	GR	WGR	GR	WGR
K. Himalini	15.47±0.54	15.37±0.51	6.06±0.40	6.03±0.43	5.33±0.29	5.67±0.35	4.89±0.31	5.00±0.50	15.00±1.83	14.89±1.47	0.98±0.22	1.54±0.44	0.07±0.01	0.07±0.08
K. Badshah	16.01±0.51	16.01±0.48	6.47±0.79	6.54±0.95	4.33±0.37	4.22±0.63	4.22±0.55	3.78±0.99	12.22±4.08	12.33±3.70	1.27±0.03	1.33±0.04	0.17±0.07	0.10±0.03
K. Frysona	12.63±1.96	12.61±1.44	8.18±1.13	7.21±1.80	4.11±0.72	3.89±1.59	4.22±1.02	3.67±1.66	10.00±2.72	9.56±2.61	1.26±0.19	1.24±0.29	0.08±0.02	0.09±0.09
K. Sukhyati	13.80±0.79	13.69±0.63	5.46±0.36	5.38±0.71	7.67±0.87	7.56±1.35	6.67±0.78	7.11±1.18	11.00±2.73	10.78±2.62	1.43±0.23	1.42±0.27	0.07±0.03	0.06±0.16
K. Gaurav	17.22±0.98	17.24±0.70	5.71±0.42	5.12±0.79	6.33±0.76	6.33±1.12	6.11±0.89	6.22±0.95	12.33±2.21	12.33±1.81	1.45±0.11	1.46±0.21	0.08±0.01	0.08±0.02
K. Khyati	15.20±1.34	13.84±0.66	5.61±0.63	5.29±0.90	5.00±0.55	4.78±1.07	4.56±0.73	4.33±1.22	17.11±2.41	17.33±1.59	1.26±0.05	1.24±0.06	0.09±0.00	0.09±0.03
K. Chipsona-3	15.20±1.34	15.00±1.42	7.52±0.76	7.23±1.08	5.89±0.75	5.67±1.15	5.44±0.97	5.00±1.63	7.11±1.18	6.33±1.60	1.06±0.09	1.07±0.22	0.09±0.02	0.08±0.13
K.Bahar	16.67±1.16	16.74±0.90	8.11±0.66	7.36±1.03	4.78±0.40	4.56±0.55	4.89±0.42	4.56±0.55	7.89±2.06	7.67±2.40	1.46±0.14	1.44±0.24	0.09±0.01	0.08±0.04
K. Lauvkar	17.91±0.94	17.92±0.78	6.26±0.60	5.79±0.76	6.89±0.82	6.89±1.17	7.00±0.90	6.89±1.17	8.22±1.08	8.33±1.34	1.48±0.12	1.51±0.23	0.19±0.11	0.09±0.03
K.Chandramukhi	14.39±0.73	13.67±0.61	7.02±0.73	6.80±0.86	3.56±0.56	3.22±0.49	3.56±0.60	3.22±0.49	8.56±1.88	8.78±2.07	1.13±0.09	1.13±0.17	0.08±0.01	0.08±0.02
K. Pushkar	14.54±0.99	14.20±1.42	7.73±0.69	7.20±0.67	3.89±0.59	3.67±1.06	3.78±0.55	3.67±1.06	8.00±1.89	7.67±2.14	1.63±0.26	1.61±0.26	0.08±0.01	0.09±0.02
K.Surya	16.66±1.34	16.49±0.96	6.12±0.63	5.82±1.01	4.89±0.61	5.44±0.79	5.44±0.71	5.33±0.83	7.44±1.55	7.11±1.81	1.60±0.26	1.52±0.42	0.19±0.10	0.09±0.10
K.Mohan	15.87±1.22	16.36±1.17	5.33±0.71	5.40±1.00	5.33±0.65	5.22±1.05	5.22±0.66	5.11±1.07	13.33±2.96	13.22±2.40	1.35±0.09	1.42±0.13	0.15±0.08	0.08±0.05
K.Ashoka	16.93±0.90	16.91±0.61	6.28±0.57	6.22±0.82	6.67±0.75	6.33±0.73	6.67±0.65	6.33±0.73	10.89±2.30	10.78±1.98	1.48±0.06	1.52±0.08	0.15±0.07	0.09±0.04
K.Garima	12.41±1.09	12.16±1.14	4.73±0.73	4.52±1.70	4.33±0.53	4.11±0.69	3.89±0.45	4.00±0.71	9.44±1.56	9.44±1.45	1.62±0.20	1.63±0.28	0.11±0.00	0.11±0.05
K.Chipsona-4	15.04±0.63	14.80±0.41	5.80±0.52	6.10±0.51	5.67±0.33	5.56±0.39	5.67±0.33	5.67±0.35	9.67±1.21	9.78±1.16	1.00±0.07	0.95±0.13	0.08±0.01	0.08±0.04
K.Pukhraj	14.09±1.65	13.98±2.39	6.37±0.54	5.11±1.33	5.67±0.87	5.44±1.81	5.11±0.79	5.22±1.80	8.33±2.89	8.11±3.20	1.19±0.21	1.23±0.46	0.13±0.04	0.09±0.07
K.Megha	14.77±1.47	14.97±1.30	6.37±0.69	6.32±0.92	4.78±0.55	4.56±0.75	4.44±0.71	4.00±0.78	11.44±1.68	11.33±1.57	0.97±0.07	1.00±0.20	0.08±0.01	0.07±0.06
K.Girdhari	14.56±1.31	15.79±1.00	4.97±0.55	4.59±1.74	6.00±0.44	5.89±0.52	5.89±0.39	5.89±0.72	8.67±1.47	8.56±1.64	1.01±0.04	0.99±0.10	0.07±0.01	0.07±0.06
K.Sindhuri	15.86±0.52	16.04±0.50	7.06±0.68	7.17±0.77	6.22±0.57	5.89±0.73	6.00±0.53	5.89±0.73	9.22±1.16	9.33±1.16	0.78±0.08	0.79±0.21	0.05±0.01	0.05±0.10
K.Kanchan	12.31±0.83	12.20±0.95	6.16±0.67	6.23±0.94	4.67±0.29	4.56±0.57	4.89±0.39	4.56±0.57	5.33±0.87	5.22±1.21	0.56±0.07	0.54±0.13	0.04±0.00	0.04±0.00
K.Kesar	13.89±0.96	13.74±1.11	5.72±0.26	5.83±0.47	5.00±0.44	4.67±0.76	4.44±0.41	4.33±0.87	8.11±1.71	8.22±1.70	1.08±0.14	1.19±0.28	0.10±0.01	0.09±0.08
K.Arun	11.84±0.62	11.58±0.57	5.63±0.39	5.53±0.44	4.00±0.33	3.67±0.39	4.00±0.24	3.67±0.39	11.56±1.82	11.56±1.72	1.44±0.09	1.50±0.20	0.06±0.02	0.09±0.05
K.Neelkanth	17.07±0.86	17.16±0.59	5.06±0.36	4.71±0.51	5.89±0.31	5.56±0.41	5.22±0.32	5.56±0.41	11.89±2.08	11.89±1.92	1.05±0.12	1.11±0.20	0.04±0.02	0.06±0.02
K.Ganga	10.41±0.88	10.42±1.00	4.54±0.79	3.33±0.65	4.11±0.45	4.00±0.65	4.11±0.42	4.00±0.65	6.67±1.20	6.44±1.60	0.58±0.04	0.62±0.08	0.05±0.01	0.04±0.00
K.Giriraj	9.27±0.58	9.18±0.49	3.96±0.28	3.76±0.37	2.89±0.35	2.56±0.42	2.67±0.24	2.44±0.42	7.44±0.71	7.33±0.80	0.37±0.09	0.38±0.28	0.04±0.00	0.04±0.03
K.Lima	7.18±0.89	7.21±1.03	2.47±0.29	2.20±0.73	2.44±0.24	2.44±0.51	2.56±0.24	2.56±0.51	5.67±1.56	5.33±2.08	0.35±0.09	0.35±0.28	0.03±0.01	0.04±0.10
K.Chipsona -1	11.13±0.41	11.37±0.51	5.63±0.37	5.79±0.46	4.67±0.50	4.67±1.06	4.22±0.46	4.89±0.73	7.56±0.69	7.44±0.76	1.31±0.12	1.29±0.19	0.13±0.01	0.13±0.05
K.Jyoti	15.03±0.14	14.88±0.15	6.97±0.19	7.21±0.22	5.78±0.46	5.56±0.46	5.11±0.48	5.33±0.65	11.78±1.38	11.67±1.23	0.80±0.10	0.80±0.14	0.05±0.01	0.06±0.06

Significant difference among the treatments were recorded for root length and number of roots (**Table 1**). The maximum root length was observed in variety K. Frysona with GR (8.18±1.13) and in K. Bahar WGR (7.36±1.03). More number of primary and secondary roots were recorded in variety K. Khyati in both WGR (17.31±1.59) and GR (17.11±2.41). In case of number of roots both primary and secondary roots were counted. The maximum roots per plant was observed for K. Khyati in WGR (17.33±1.59) and GR (17.11±2.41) which indicates that the genotype is sole responsible to confer the microplants sensitivity as well as its tolerance limit to different growth hormones (Venkataslam *et al*., 2017).

Easy to root species, (e.g. potato) usually regenerate roots upon subculture on hormone-free medium (Pruski *et al*., 2003). Interestingly, more number of leaves were recorded in variety K. Sukhyati in GR (7.67±0.97) and WGR (7.56±1.35) and, least in variety K. Lima in both the treatments (GR 2.44±0.82; WGR 2.44±0.51) respectively. In the clonal regeneration, sub-culturing cycles influenced morphological characters in the various cultivars differently. In general, 10-12 cycles of sub-culturing is safe to attain or maintain the true-to-type nature of the *in vitro* propagated variety (Buckseth *et al.,* 2017). Genetically controlled in vitro response has already been reported in potato previously (Gopal, 2004). No morphological differences were observed by the additional supplementation of growth regulators in the MS medium by Venkataslam *et al*., 2016. More the number of nodes, more will be the multiplication rate. The results revealed non-significant difference for the node numbers. The highest number of nodes per plant was recorded in K. Sukhyati WGR (7.11±1.18) whereas, GR supplemented media recorded maximum no of nodes per plantlet (7.00±0.90) in K. Lauvkar which otherwise recorded (6.89±1.17) in the absence of GRs. The minimum number of nodes were observed in variety K. Giriraj both in GR (2.67±0.24) and WGR (2.44±0.42). Non-significant differences were observed for fresh weight and dry weight. The data (**Table 1**) indicated that there was no significant change in the weight of all cultivars subjected to the application of GRs compared to the one where no GRs were used. A question frequently placed in connection to the potato shoot polymorphism is the PGR balance required to maintain coherent potato body architecture. This question is subject to extensive research and data analysis. However, one thing is already clear, the green, leafy potato shoot is the main (first, starting, default) shoot type. With such a position, it has the unique

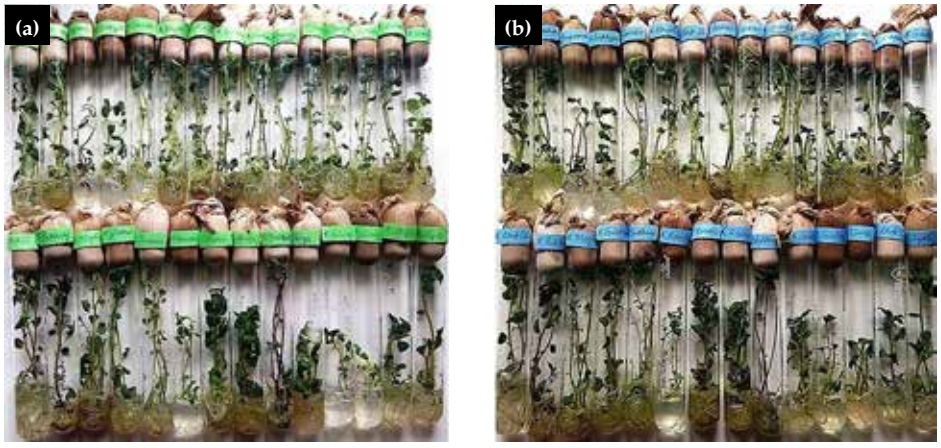


Figure 2. 29 potato varieties grown in MS media a. With GR'S; b. Without GR'S

privilege of needing no external PGRs for its growth. Therefore, the leafy potato shoot cultures can complete the whole propagation protocol including the rooting stage on a simple, hormone-free medium. These results are in conformity with Vinterhalter D (2008) (**Figure 2**). The study is simple though informative with lots of practical utility.

The study concluded and signifies that microplants developed without growth regulators proved to have the equivalent potential of increasing the multiplication rate of *in vitro* potato varieties. The same cost-effective protocol has been shared with the seed entrepreneurs and they are successfully following it in their labs.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

ETHICAL STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors

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