Influence of Node Number and Orientation of Explants on *in Vitro* Growth and Development of Potato

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Summary Potato (*Solanum tuberosum*) is one of the most important food crops in the world which can be used in tissue culture techniques, ranging from *in vitro* propagation via shoot cultures to regeneration of whole plants from protoplasts. The study was undertaken to determine the influence of node number (single or double) and its orientation (horizontal or vertical) on different growth parameters of potato varieties (Kufri Bahar, Kufri Jyoti, Kufri Lauvkar, Kufri Sindhuri and Kufri Chandramukhi). Six to eight cuttings of double node and single node were inserted vertically and horizontally per bottle at an equilateral distance in MS media without any growth regulators. The study revealed that node number, explants orientation, potato varieties, and their interaction had significant effect on different morphological characters viz., microplant height, number of leaves, nodes, roots as well as dry weight.

Key words *In vitro*, Potato, Explants, Orientation, Micropropagation, *Solanum*.

Potatoes (*Solanum tuberosum* L.) are one of the essential food crops in the world and are grown in more than 100 countries under temperate, subtropical, and tropical conditions. India has seen a revolution in potato production during recent years (Scott and Suarez 2011). Potatoes are served for either table consumption or as processed products. In addition to being a source of carbohydrates, potatoes also contain high content of protein, vitamin C, fiber, minerals, and have a low fat content (Navarre *et al.* 2009). The potato is amenable to a number of tissue culture techniques, ranging from *in vitro* propagation via shoot cultures to regeneration of whole plants from protoplasts. Tissue culture-based hi-tech seed potato production is spreading very fast in the country. Micropropagation at faster rates and free from cultural contamination is an integral component for the success of *in vitro* mass multiplication of potatoes (Buckseth *et al.* 2016). In general terms, these all involve the growth of plants, cells, tissues, and organs in sterile conditions, supported by an appropriate culture medium. Media normally contain a mixture of major and minor salts, vitamins, sugar (as a carbon source), and plant growth regulators (Karp *et al.* 1987). The most widely used formulations are based on that of Murashige and Skoog (1962) which is available commercially. The success of regeneration through plant tissue culture depends upon the explants chosen and the way the explants are placed on the culture medium (Kozak *et al.* 2013).

Thus the aim of the present study was to evaluate the influence of node number (single or double) and its orientation (horizontal or vertical) on growth and development of five different potato varieties.

Materials and methods

The study was carried out at ICAR-Central Potato Research Institute, Shimla. Five different varieties of potato viz. Kufri Bahar, Kufri Jyoti, Kufri Lauvkar, Kufri Sindhuri, and Kufri Chandramukhi (K.C.M) were used. The basic Murashige and Skoog medium without growth regulators was poured in bottles and autoclaved at 121°C for 22 min. Six to eight cuttings of double nodes were inserted vertically and horizontally (Fig. 1a, c) per bottle at an equilateral distance. These bottles were marked as double node vertical (DNV) and double node horizontal (DNH). Another six to eight cuttings of a single node were inserted vertically and horizontally (Fig. 1b, d) per bottle at an equilateral distance. These bottles were marked as single node vertical (SNV) and single node horizontal (SNH). The culture tubes were kept for 21 days in a culture room with 16h light (irradiance of 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and 8h dark photoperiodism at 22 $\pm$ 1°C. Various growth parameters viz. microplant height, root length, number of nodes, leaves and roots, fresh weight as well as dry weight was recorded after 21 days of incubation. In case of roots, as there was secondary branching, only primary roots were counted. Root length was recorded for the longest root in each plant. Fresh and dry weight was taken for all three plantlets along with the

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root. Microplants from each test tube were dried at 80°C for 48 h in the hot air oven and dry weight was recorded after bringing it to room temperature. The experiment was conducted in a completely randomized design. Each treatment comprised four replicates, each replicate consists of four bottles having six to eight plantlets. The analysis of variance was done using the software AGRES and means were separated according to the least significant differences at 0.05 level of probability.

Results and discussion

The number of nodes (single or double) and its orientation (horizontal or vertical) on the culture medium showed a high influence on the growth and development of different potato varieties. The experimental results

Fig. 1. (a) Single Node Vertical (SNV), (b) Double Node Vertical (DNV), (c) Single Node Horizontal (SNH) and (d) Double Node Horizontal (DNH).

showed that in Kufri Bahar, there was a significant increase in microplant height (11.35 cm) when compared to the other varieties (Fig. 2) whereas in Kufri Sindhuri and K.C.M plants, DNV (Fig. 2) showed increased growth (6.2 and 8.6 cm). For Kufri Bahar, Kufri Jyoti, and Kufri Lauvkar, the microplant height (13.2, 9.1, and 5.3 cm, respectively) was on the higher side in SNV. The frequency of shoot regeneration from vertical explants was significantly higher than those of horizontal explants as reported by Tiwari *et al.* (2016) in Brahmi. The number of leaves was significantly higher in Kufri Bahar (5.8) in comparison to the other varieties. DNV enhanced the number of leaves in Kufri Bahar (6.1) and K.C.M (6.1). However, similar results were observed with SNV in Kufri Sindhuri (4.7). In Kufri Lauvkar, the number of leaves (5.1) was on the higher side with DNH. But the overall experimental results of DNV and SNV were on the higher side as compared to DNH and SNH (Fig. 2). This might be due to the direct uptake and flow of nutrients from the culture media by the cell tissues. For the number of leaves, the data analyzed showed a significant difference among all the varieties (Table 1).

It was observed that in Kufri Bahar, number of nodes (5.8) was significantly higher than the other four varieties (Fig. 2). For Kufri Bahar, Kufri Jyoti, and K.C.M, DNV showed enhanced growth in number of nodes (6.2, 4.2, and 5.7). The number of nodes in Kufri Lauvkar (5.1) was significantly higher with DNH which was in agreement with the studies of Kozak *et al.* (2013) concluding that the horizontal orientation of non-defoliated and defoliated shoot tips and nodes of *Cosmos* sp.

Fig. 2. (a) Double Node Horizontal, (b) Double Node Vertical, (c) Single Node Horizontal, (d) Single Node Vertical of five varieties i.e., Kufri Bahar, Kufri Lauvkar, Kufri Jyoti, Kufri Sindhuri.

Table 1. Effect of number of nodes and its orientation on Microplant Height and No. of Leaves of different potato.

Variety	Microplant height (cm)					No. of leaves				
	DNV	DNH	SNV	SNH	Mean	DNV	DNH	SNV	SNH	Mean
K. Bahar	12.700	8.767	13.233	10.733	11.358	6.100	5.533	5.900	5.900	5.858
K. Jyoti	5.867	3.867	9.133	6.667	6.383	4.433	4.000	4.367	3.433	4.058
K. Lauvkar	4.100	4.333	5.367	4.000	4.450	3.667	5.100	3.667	3.100	3.883
K. Sindhuri	6.200	3.633	5.900	4.900	5.158	4.433	3.100	4.767	4.000	4.075
K.C.M	8.633	7.400	7.133	7.400	7.642	6.133	4.333	4.000	4.567	4.758
Mean	7.500	5.600	8.153	6.740		4.953	4.413	4.540	4.200	
Variety	MH	V×MH				Variety	No. of L	V×L		
CD (P-0.5%)	0.956	0.855	1.912				0.631	NS	1.263	
SE(d)	0.471	0.421	0.942				0.311	0.278	0.623	

slightly increased the number of regenerated shoots. An increase in shoot numbers was observed when shoots were placed on the medium horizontally (Orlikowska *et al.* 2000, Debnath 2005, Rajeswari and Palival 2008) or vertically in an inverted position with the shoot tip down (Ziv *et al.* 1970, Seabrook *et al.* 1976, Kozak 1991, Orlikowska *et al.* 2000). There was an increase in the number of roots in Kufri Jyoti (10.8) in comparison to the other four varieties. SNV enhanced the number of roots in Kufri Bahar (7.2), Kufri Jyoti (14.8), and Kufri Lauvkar (7.9). However, in Kufri Sindhuri and K.C.M, DNV increased the number of roots (10.5 and 5.2). The overall results for the number of roots showed that the performance of DNH and SNH were not satisfactory than that of DNV and SNV. The frequency of root regeneration was highest in case of node explants, either placed vertically or horizontally followed by leaves and internode explants (Tiwarei *et al.* 2016). The present study attributed significant differences in case of number of roots and the orientation of nodal cuttings among all the varieties (Table 2).

In three varieties of potato *viz.*, Kufri Lauvkar, Kufri Jyoti and Kufri Sindhuri, SNH showed enhanced root growth (8.6, 9.3, and 11.3 cm, respectively). However, in Kufri Bahar and K.C.M., root length was increased with

DNV (9.7 and 10.6 cm). Sharma *et al.* reported that root length was significantly enhanced by a single node in Kufri Bahar and double nodes in Kufri Chandramukhi. The present study explains that the genotype is sole responsible to confer the microplants behavior to the kind and position of explants but most of the genotypes respond very well to the vertical orientation. Fresh and dry weight of Kufri Jyoti (352 and 35 mg) was the highest followed by Kufri Bahar (301 and 24 mg) and Kufri Lauvkar (194 and 18 mg). All the tested varieties did not vary significantly for root length and fresh weight respectively (Table 3). Kozak *et al.* reported that in *Cosmos atrosanguineus*, the shoots regenerated from defoliated shoot tips placed in an inverted position in relation to the natural orientation were characterized by the highest fresh weight. The performance of DNV and SNV in all the varieties was on the higher side in comparison to SNH and DNH.

There are many reports about the beneficial effect of the horizontal orientation of explants on the regeneration ability of explants: *Myrtus communis* (Nobre 1994), *Codiaeum variegatum* (Orlikowska *et al.* 2000) *Vaccinium vitis-idaea* (Debnath 2005), and *Albizia odoratissima* (Rajeswari and Paliwal 2008). But, in case of the potato this is the first report on the influence of node number

Table 2. Effect of number of nodes and its orientation on No. of nodes and No. of roots of varieties.

Variety	No. of nodes					No. of roots				
	DNV	DNH	SNV	SNH	Mean	DNV	DNH	SNV	SNH	Mean
K. Bahar	6.233	5.533	5.900	5.900	5.892	6.433	6.333	7.233	5.233	6.308
K. Jyoti	4.233	4.000	3.900	2.900	3.758	12.233	6.433	14.800	10.000	10.867
K. Lauvkar	3.800	5.100	3.333	2.900	3.783	5.767	4.133	7.900	6.433	6.058
K. Sindhuri	4.533	3.100	4.767	4.000	4.100	10.533	4.667	6.800	4.567	6.642
K.C.M	5.767	4.533	3.967	4.900	4.792	5.200	4.533	4.667	4.000	4.600
Mean	4.913	4.453	4.373	4.120		8.033	5.220	8.280	6.047	
	Variety	Nodes	V×N			Variety	Roots	V×R		
CD (<i>p</i> -0.5%)	0.573	0.512	1.146			1.553	1.389	3.107		
SE(d)	0.282	0.253	0.565			0.766	0.685	1.531		

Table 3. Effect of number of nodes and its orientation on root length, fresh weight and dry weight of varieties.

Variety	Orientation	K. Bahar	K. Jyoti	K. Lauvkar	K. Sindhuri	K.C.M	Mean	CD (<i>p</i> -0.5%)	SE(d)
Root length (cm)	DNV	9.733	6.633	8.267	8.933	10.633	8.840	Variety	NS
	DNH	9.067	5.633	6.833	7.067	8.633	7.447	RL	NS
	SNV	9.333	7.200	8.167	10.333	7.667	8.540	V×RL	NS
	SNH	8.333	9.367	8.667	11.333	8.433	9.227		
	Mean	9.117	7.208	7.983	9.417	8.842			
Fresh weight (mg)	DNV	395.600	402.800	213.533	195.933	124.933	266.560	Variety	46.756
	DNH	236.267	285.933	136.100	111.967	195.533	198.560	FW	41.820
	SNV	317.033	370.867	214.700	134.767	217.933	251.060	V×FW	NS
	SNH	255.867	351.133	194.500	122.000	132.733	211.247		
	Mean	301.192	352.683	196.458	141.167	167.783			
Dry weight (mg)	DNV	29.633	45.267	20.567	16.400	10.100	24.393	Variety	4.595
	DNH	26.133	33.600	17.500	17.467	21.933	23.327	DW	4.110
	SNV	19.867	31.200	18.867	13.000	12.700	19.127	V×DW	NS
	SNH	22.333	33.900	17.367	11.000	10.633	19.047		
	Mean	24.492	35.992	18.575	14.467	13.842			

and orientation of explants on *in vitro* growth and development suggesting that the vertical orientation with single or double nodes are successful for rapid multiplication of healthy potato microplants.

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