

## Micro and mini-tubers formation and *in vitro* shoot regeneration from bud sprout of potato (*Solanum tuberosum* L.)

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**Abstract:** The present study was carried out to develop micropropagation protocol of potato using sprout as an explants. Explants were cultured on in Murashige and Skoog's (MS; 1962) media supplemented with eleven different combination of Indole-3-butyric acid (IBA), Kinetin, 1-Naphthaleneacetic acid (NAA) and 2,4-Dichlorophenoxyacetic acid (2,4-D) for initiation of cultures. The treatment involving a combination of IBA, Kinetin, NAA and 2,4-D gave good response for growth of shoot. The regenerated shoots were sub-cultured further using nodal cutting as explants in same media for further multiplication. The developed plantlets were acclimatized in a green house. Acclimatized plants were transplanted in the soil for further growth and development which yielded 3-17 healthy mini-tubers . For microtuber production, high level of sucrose (8%) found promising results than low level of sucrose (3%).

**Keywords:** *Kufri Badshah*, explants, IBA, Kinetin, NAA, microtuber

### Introduction

Potato (*Solanum tuberosum* L.) is a very popular and one of major vegetable crop of India. *S. tuberosum* L. belong to family *Solanaceae* and is native to South America. Micropropagation allows rapid multiplication of clones in a short duration under disease free, controlled environment and on a year round basis (Singh *et al.*, 2010; Rai *et al.*, 2012; Tripathi *et al.*, 2012). Micropropagated potato plants, when cultured under suitable conditions, produce *in vitro* micro-tubers (Copeland, 1982; Espinoza *et al.*, 1986; Wang and Hu, 1982). Although a few may also be formed in the medium, micro-tubers of 2 to 10 mm diameter generally

originate as aerial structures from the micro-stems. The use of sucrose at 8%, as compared with 4% or 12%, advanced the initiation higher number of tuber formation of larger micro-tubers (Garner and Jennet, 1989) Micro-tubers, when grown in soil, produce mini-tubers 5 to 25 mm diameter. Alternatively, Micropropagated plants can be grown directly in soil to produce mini-tubers. The difference between micro- and mini-tubers is not only in their size but also in the way they are produced. Although, some large sized micro-tubers may be of the same size or bigger than small mini-tubers , micro-tubers are produced *in vitro* from Micropropagated plants, whereas mini-tubers are produced by growing Micropropagated plants or micro-tubers in soil. Looking to the requirement of potato planting material tissue culture regenerated plants could be alternative propagating material in potato. Therefore the experiment was planned using sprouts of *Kufri Badshah* variety of potato. *Kufri Badshah* is one of the popular, medium maturing, blight resistant potato variety with round to oblong tubers, yellowish skin, shallow eye and white pulp.

### Materials and Methods

The research was conducted at Biotechnology laboratory of Department of Genetics and Plant Breeding, C.P. College of Agriculture, S. D. Agricultural University, Sardarkrushinagar, North Gujarat during the 2009-11. An indigenous elite potato (*Solanum tuberosum* L.) cultivar, *K. Badshah* were collected from Main Potato Research Station, S.D. Agricultural University, Deesa (North Gujarat)

**Culture media:** To study the shooting-rooting and microtuber formation of Potato in culture, Murashige and Skoog's (1962) media was used. This media contained the basal salts (macro and micro) and vitamins.

**Tuber sprouting:** The tubers of mother potato were washed with water followed by treatment with 0.3%

GA<sub>3</sub> and then packed in craft paper bags which were persevered in dark at 21°C. Development of sprout took 3-4 weeks (Fig. 1). Sprouts of 2-3 cm were excised from tubers to be used as explants.

**Shoot culture:** The sprouts were cut into a 0.4 to 0.5 cm containing one bud in each explant. explants were then washed with tap water, followed by a 70% ethanol rinse, treated with 0.1% HgCl<sub>2</sub> (Mercury chloride) for 30 seconds and lastly washed with sterile distilled water. The explants were cultured in MS media supplemented with different combination and concentration of plant growth regulators *i.e.* IBA, Kinetin, NAA and 2-4-D. The cultures were incubated at 25 + 2°C under 16 hours photoperiod.

**Sub culturing nodes:** Shoot cuttings having a node were subculture further for development of plantlets in jar containing MS salts with different levels of kinetin, IBA, NAA and 2,4-D (Table 1).

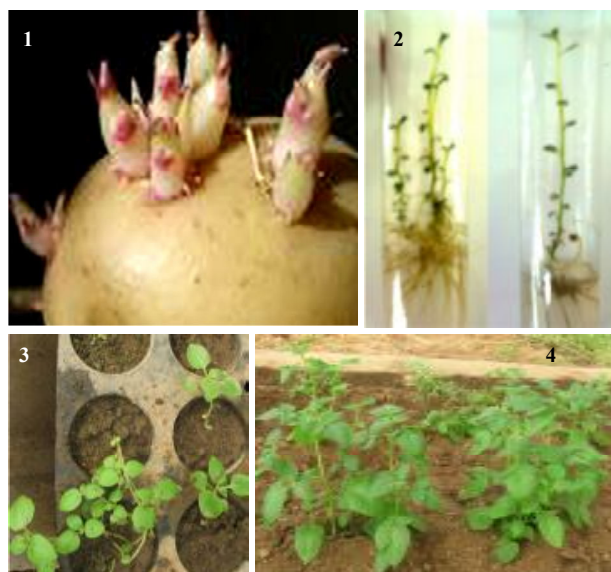
**Table 1:** Concentration and combination of different growth hormones.

Treatments	Growth hormones			
	IBA (mg l <sup>-1</sup> )	Kinetin (mg l <sup>-1</sup> )	NAA (mg l <sup>-1</sup> )	2 4-D (mg l <sup>-1</sup> )
T <sub>1</sub>	1.0	1.0	1.0	0.0
T <sub>2</sub>	1.0	1.0	2.0	0.0
T <sub>3</sub>	1.0	2.0	1.0	1.0
T <sub>4</sub>	1.0	1.0	2.0	0.0
T <sub>5</sub>	1.0	1.0	1.0	1.0
T <sub>6</sub>	2.0	2.0	2.0	1.0
T <sub>7</sub>	2.0	2.0	1.0	1.0
T <sub>8</sub>	2.0	1.0	1.0	1.0
T <sub>9</sub>	2.0	2.0	0.0	0.0
T <sub>10</sub>	2.0	2.0	1.0	0.0
T <sub>11</sub>	2.0	2.0	2.0	1.0

**Micro tuber culture:** The nodal shoots were cut in to 1-2 cm and inoculated in culture media containing half strength MS basal media supplemented with different level of sucrose (Table 4). Subcultures were incubated at 18°C to 20°C in dark room.

## Results and Discussion

The present study was under taken to establish the protocol for production of micro tuber in local cultivar of potato using MS medium supplemented with different concentration of IBA, kinetin, NAA and 2,4-D. Eleven combination were tested for initiation and multiplication of shoots. The shoots formations were started after one week of inoculation. Four kinds of results were observed among different combination and concentration of growth hormones *viz.*, single shoots, single shoot with branches, multiple shoots and both shoots as well as roots. The results were differed



according to combination of treatment were IBA 1.0 mg l<sup>-1</sup> and NAA 1.0 mg l<sup>-1</sup> + Kinetin 2.0 mg l<sup>-1</sup> single shoot were observed. IBA 1.0 mg l<sup>-1</sup> + NAA 2.0 mg l<sup>-1</sup> + Kinetin 1.0 mg l<sup>-1</sup> single shoot as well as branches were observed (Fig. 2). IBA 1.0 mg l<sup>-1</sup> + NAA 1.0 mg l<sup>-1</sup> + Kinetin 1.0 mg l<sup>-1</sup> + 2 4-D 1.0 mg l<sup>-1</sup> and same combination except 2,4-D 1 mg l<sup>-1</sup> combination multiple shoots were observed. IBA 1.0 mg l<sup>-1</sup> + NAA 1.0 mg l<sup>-1</sup> + Kinetin 2.0 mg l<sup>-1</sup> and 2.0 mg l<sup>-1</sup> IBA + 2.0 mg l<sup>-1</sup> kinetin + 2.0 mg l<sup>-1</sup> NAA + 1.0 mg l<sup>-1</sup> 2-4-D mg l<sup>-1</sup> combination multiple shoots as well as roots were observed (Fig. 2 and Table 3) Shoot and root formation were found to be better in combine treatment of IBA and Kinetin than single treatment of IBA or Kinetin were

**Table 2:** Percentage of shooting from sprouts of potato (*S. tuberosum*) in different concentration and combinations. (2009-10)

Treatments	No. of explants kept	No. of explants Shooting	Shooting (%)
T <sub>1</sub>	06	05	83.33
T <sub>2</sub>	06	04	66.66
T <sub>3</sub>	06	04	66.66
T <sub>4</sub>	08	06	75.00
T <sub>5</sub>	06	05	83.33
T <sub>6</sub>	09	07	88.88
T <sub>7</sub>	09	08	77.77

**Table 3:** Treatments used for shoot and root proliferation (2010-11)

Treatments	No. of explants kept	No. of plantlets/ explants/ (Range)	Number of nodes/ shoot	Plantlets survived for hardening (%)	Minituber/ plant (Range)
T <sub>4</sub>	36	2-3	2-3	62	3-17
T <sub>6</sub>	36	2-3	2-3	78	3-17

**Table 4:** Percentage of micro-tuber development from shoots of potato (*S. tuberosum*) in different sucrose level. (2009-10)

Sucrose level (g/l)	No. of explants kept	Tuber developed	Percentage Tuber developed
8	06	04	80
7	06	02	40
6	06	02	40
5	06	01	20
4	06	01	20
3	06	00	00

reported by Khuri and Moorby (1996). Observation for shoot regeneration was recorded from 36 explants. Results showed in Table 3 indicates that treatment T<sub>6</sub> was superior as it gave three to five shoots per explants in 4 weeks and number of nodes per shoot were three to four. These shoots were sub-cultured (Fig. 3) for further multiplication in same media using nodal cuttings. The frequency of regeneration of shoots was recorded 78% in three week in treatment T<sub>6</sub> (Fig. 4). These shoots having may be used for further nodal cutting or may be allowed to rooting. The shoots having 5-6 nodes with leaf and sufficient amount of root mass were shifted in green house for hardening (Fig. 4). After seven days of hardening these were transplanted in soil for further growth and development. The plantlets yielded 3-17 healthy mini-tubers. The micro-tubers were developed from the one month old shoots which were cuts in small pieces (1-2 cm including nodes). It subcultured in combination of half strength MS media supplemented with different level of sucrose *viz.*, 80 g l<sup>-1</sup> (8% sucrose), 70 g l<sup>-1</sup> (7% sucrose), 60 g l<sup>-1</sup> (6% sucrose), 50 g l<sup>-1</sup> (5% sucrose), 40 g l<sup>-1</sup> (4% sucrose) and 30 g l<sup>-1</sup> (3% sucrose); (Table 4) the Microtuber was appeared after 3-4 week of inoculation. The morphology of the microtuber is appearing after 6<sup>th</sup> days of culture. Similarly Desire (1995a) & Desire (1995) reported from the 12<sup>th</sup> days. The sessile microtuber becomes round in shape with diameter of 2-3 mm., thereafter with the growth of cornical cells and the high accumulation of starch and protein, the final size is reached (4-5 mm.). In present study results found that in half strength MS supplemented with 8% sucrose media were developed tubers whereas media 3% sucrose were not developed any tuber (Table 4). These results are supported with the findings of (Uddin and Khulna, 2006) that presence of high level of sucrose (8%) was beneficial and slightly larger microtuber produced and higher yield. Similarly number and weight of microtuber, formation of shoots, shoots length found superior when sugar concentration

at 8% in medium as also reported and significantly slower microtuber growth rates was observed when sugar concentration was 4% instead of 8% were accordance with Yu *et al.* (2000).

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