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Microtuberization, minitubers formation and *in vitro* shoot regeneration from bud sprout of potato *(Solanum tuberosum* L.) cultivar *K. badshah*

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Kufri badshah **is one of the important medium maturing, blight resistant potato varieties with round to oblong tubers; it has yellowish skin, shallow eye and white pulp. This variety is popular among farmers. The study on development of tissue culture protocol was carried out using sprout as an explant for initiation of culture in MS media supplemented with eleven different combinations of growth hormones: indole butyric acid (IBA), kinetin, naphthalene acetic acid (NAA) and 2,4-dichlorophenoxy acetic acid (2,4-D). The response for growth proliferation was observed. The treatment involving a combination of IBA, kinetin, NAA and 2,4-D gave good response for growth of shoot. The resultant shoots were sub-cultured further using nodal cutting as explants in same media for further multiplication. The developed plantlets were hardened in green house. Hardened plants were transplanted in the soil for further growth and development. The plants yielded 3-17 healthy minitubers. For microtuber production, high level of sucrose (8%) gave promising results than low level of sucrose (3%).**

Key words: *Kufri badshah*, explants, indole butyric acid (IBA), kinetin, naphthalene acetic acid (NAA), 2,4-dichlorophenoxy acetic acid (2,4-D) and microtuber.

INTRODUCTION

Potato *(Solanum tuberosum* L.) is a popular and major vegetable crop in India. *S. tuberosum* L. belongs to the family, *Solanaceae* and is South America native. It is the most widely cultivated food crop after wheat, rice and maize, hence it is considered as the most important dicotyledonous and tuber crop (World book, Potato, 2000). Potato is normally propagated by planting the bud or 'eyes' present on the tubers. Micro propagation allows rapid multiplication of clones in a short duration under disease free, controlled environment on yearly basis. Potato can be easily micro propagated (Copeland, 1982; Espinoza et al., 1986). Micro propagated plants, when cultured under suitable conditions, produce *in vitro* micro-tubers (Wang and Hu, 1982). Micro-tubers are 2 to 10 mm diameter and originate as aerial structures from the micro-stems; although a few may also be formed in the medium. The use of 8% sucrose compared to 4 or 12% advanced the initiation of tuberization and gave more and larger micro-tuber (Garner and Jennet, 1989). Microtubers have become an important mode of rapid multiplication for pre basic stock in seed tuber multiplication as well as germplasm exchange (Zakaria et al. 2008).

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Abbreviations: GA_3, Gibberellic acid; **IBA,** indole butyric acid; **NAA,** naphthalene acetic acid; **2,4-D,** 2,4-dichlorophenoxy acetic acid.

Tuberization in potato highly complex development process, regulated by many factors amongst carbon source to nutrient media is the most important factor (Altindal and Karadogan, 2010). Micro-tubers, when grown in soil, produce mini-tubers of 5 to 25 mm diameter. Alternatively, micro propagated 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 minitubers, micro-tubers are produced in *vitro* from micro propagated plants; whereas mini-tubers are produced by growing micro propagated plants or micro-tubers in soil. Looking at the requirement of potato seed, alternative propagating material in potato can be tissue culture product. Therefore, the present experiment was planned.

MATERIALS AND METHODS

The research was conducted at Biotechnology laboratory Department of Genetics and Plant Breeding, C.P. College of Agriculture, S. D. Agricultural University, Sardarkrushinagar, North Gujarat in November 2009 to 2011. An indigenous elite potato (*Solanum tuberosum* L.) cultivar, *K. badshah* was collected from Main Potato Research Station, S. D. Agricultural University, Deesa (North Gujarat).

Culture media

To study the shoot-rooting and micro-tuber formation of potato in culture, Murashige and Skoog (1962) medium was used. This medium contained basal salts (macro and micro) and vitamins.

Tuber sprouting

The cultivars of mother potato were washed with water and treated with 0.3% gibberellic acid (GA_3). They were then packed in craft paper bags which were persevered in the dark at 21°C. Development of sprout took three to four weeks (Figure 1). 2 to 3 mm sprouts were excised from tubers and used as explants.

Shoot culture

The sprouts were cut into a 0.4 to 0.5 cm containing one bud in each explant. The explants were washed with tap water and then rinsed in 70% ethanol. They were treated with 0.1% $Hgcl_2$ (Mercury chloride) for 30 s and then washed with sterile distilled water. The explants were cultured in MS media (Murashige and Skoog, 1962), supplemented with different combination and concentration of growth hormones, indole butyric acid (IBA), kinetin, naphthalene acetic acid (NAA) and 2,4-dichlorophenoxy acetic acid (2,4-D). The cultures were incubated at 25 + 2°C under 16 h light period.

Sub-culturing nods

Cuts of shoots having nod were subcultured further for developed plantlets having shoots and roots placed in jar containing MS salts with different levels of kinetin, IBA, NAA and 2,4-D (Table 1).

Minituber production

The plantlets having 5-6 nodes with leaf, root mass were transferred to pot tray containing mixture of sand: vermin: compost: cocopit in ratio of 1:1:1 v/v and drenched with fungicide (Bavistin) under green house. Three to four mist irrigation was given to keep soil moist and to maintain the humidity for initial one week. (Figure 5). 8-10 days hardened plantlets were transplanted to normal fertile soil minitubers production.

Micro tuber culture

The nodal shoots were cut into 1 to 2 cm and inoculated in culture media containing half strength MS basal media supplemented with different levels of sucrose (Table 4). Subcultures were incubated at 18 to 20°C in dark room/condition.

RESULTS AND DISCUSSION

The present study was undertaken to establish the protocol for producing micro tuber in cultivar of potato using MS medium supplemented with different concentrations of IBA, kinetin, NAA and 2,4-D. Eleven combinations were tested for initiation and multiplication of shoots (Table 2). The shoots formations were started after one week of inoculation. Four kinds of results were observed among different combinations and concentrations of growth hormones viz.: single shoots, single shoot with branches, multiple shoots and both shoots as well as roots. The results differed according to the combinations of treatment: IBA 1.0 mg l^{-1} and NAA 1.0 mg l^{-1} + kinetin 2.0 mg l^{-1} were observed for single shoot. IBA 1.0 mg l^{-1} + NAA 2.0 mg l^{-1} + kinetin 1.0 mg l^{-1} were observed for single shoot as well as branches (Figure 2a). IBA 1.0 mg l^{-1} + NAA 1.0 mg l^{-1} + kinetin 1.0 mg l^{-1} + 2 4-D 1.0 mg l^{-1} and same combination except 2 4-D 1 mg l^{-1} were observed for combination multiple shoots. IBA 1.0 mg l^{-1} + NAA 1.0 mg l^{-1} + kinetin 2.0 mg l^{-1} and 2.0 mg l^{-1} IBA + 2.0 mg l^{-1} kinetin + 2.0 mg l^{-1} NAA + 1.0 mg l^{-1} 2,4-D mg l^{-1} were observed for combination multiple shoots as well as roots (Figure 2b and Table 3). Shoot and root formation were found to be better in combined treatment of IBA and kinetin than in single treatment of IBA or kinetin (Khuri and Moorby, 1996).

Observation of shoot regeneration was recorded from 36 explants. Results in Table 3 indicate that treatment T_6 was superior as it gave three to five shoots per explants in 4 weeks and number of nodes per shoot was three to four. These shoots were sub-cultured (Figure 3) for further multiplication in same media using nodal cuttings. The frequency of regeneration of shoots was recorded (78%) in three weeks in treatment T_6 (Figure 4). These shoots may be used for further nodal cutting or may be allowed to root. The shoots having 5-6 nodes with leaf and sufficient amount of root mass were shifted in green house for hardening (Figure 5). After seven days of hardening, these were transplanted into soil for further growth and development (Figures 6 and 7). The plantlets

Figure 1. 3-4 weeks old etiolated sprouts emerging from tubers cultivar *K. badshah* after treated of 0.3% GA_3 and persevered in dark at 21°C.

Table 1. Concentration and combination of different growth hormones.

Treatment	Growth hormone			
	IBA (mg l^{-1})	Kinetin (mg l^{-1})	NAA (mg l^{-1})	2 4-D (mg l^{-1})
T_1	1.0	1.0	1.0	0.0
T_2	1.0	1.0	2.0	0.0
T_3	1.0	2.0	1.0	1.0
T_4	1.0	1.0	2.0	0.0
T_5	1.0	1.0	1.0	1.0
T_6	2.0	2.0	2.0	1.0
T_7	2.0	2.0	1.0	1.0
T_8	2.0	1.0	1.0	1.0
T_9	2.0	2.0	0.0	0.0
T_{10}	2.0	2.0	1.0	0.0
T_{11}	2.0	2.0	2.0	1.0

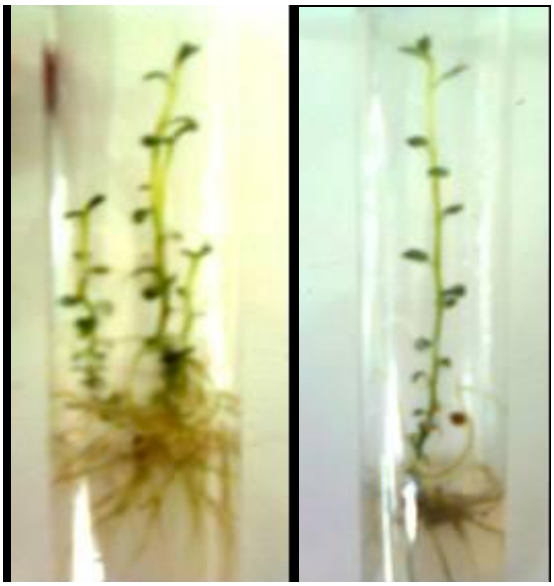
Table 2. Percentage of shooting from sprouts of potato (*S. tuberosum*) in different concentration and combinations (2009-2010).

Treatment	No. of explants kept	No. of explants shooting	Shooting (%)
T_1	06	05	83.33
T_2	06	04	66.66
T_3	06	04	66.66
T_4	08	06	75.00
T_5	06	05	83.33
T_6	09	07	88.88
T_7	09	08	77.77

yielded 3 to 17 healthy minitubers (Figure 8).

The microtubers were developed from the one month old shoots which were cuts in small pieces (1-2 cm including nodes). It was subcultured in combination with half strength MS media supplemented with different levels of sucrose viz.: 8 gl^{-1} (8% sucrose), 7 g l^{-1} (7% sucrose), 6 g l^{-1} (6% sucrose), 5 g l^{-1} (5% sucrose), 4 g l^{-1} (4% sucrose) and 3 g l^{-1} (3% sucrose) (Table 4). Microtuber appeared after three to four weeks of inoculation. The morphology of the microtuber appeared after 6th days of culture (Figure 9). Similarly, Desire (1995a, b) reported that from the 12th day sessile microtuber becomes round in shape with diameter of 2 to 3 mm (Figure 9); thereafter with the growth of cornical cells and high accumulation of starch and protein, the final size becomes 4-5 mm. In the present study, results

a



b

Figure 2. a, Shoot development with branching (IBA 1.0 mg l^{-1} + NAA 2.0 mg l^{-1} + kinetin 1.0 mg l^{-1}); b, multishoots and root development (IBA 1.0 mg l^{-1} + NAA 1.0 mg l^{-1} + kinetin 2.0 mg l^{-1} and 2.0 mg l^{-1} IBA + 2.0 mg l^{-1} kinetin + 2.0 mg l^{-1} NAA + 1.0 mg l^{-1} 2,4-D mg l^{-1}).

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

Treatment	No. of explants kept	No. of plantlets/ explants (range)	Number of nodes/ shoot	Plantlets survived for hardening (%)	Minituber/ plant (range)
T4	36	2-3	2-3	62	3-17
T6	36	2-3	2-3	78	3-17

Table 4. Percentage of microtuber development from shoots of potato (*S. tuberosum*) in different sucrose level (2009-2010).

S/N	Sucrose level (g/l)	No. of explants kept	Tuber developed	Percentage tuber developed
1	8	06	04	80
2	7	06	02	40
3	6	06	02	40
4	5	06	01	20
5	4	06	01	20
6	3	06	00	00

Figure 3. Nodal sub culturing from shoot.

Figure 4. Nodal cutting developed into plantlets.

Figure 5. The shoots having 5-6 nodes with leaf and sufficient amount of root mass shifted to green house for hardening.

Figure 6. Minituber transplanted to soil for further growth and development after seven days of hardening.

Figure 7. Normal development and growth of plantlets in field condition.

Figure 8. 3-17 healthy minitubers yielded by the plantlets under normal field condition.

Figure 9. Microtuber formation.

show that half strength MS supplemented with 8% sucrose media developed tuber, whereas 3% sucrose media did not develop any tuber (Table 4). These results are supported by the findings of Uddin (2006), which showed that the presence of high level sucrose (8%) was beneficial and led to the production of slightly larger microtuber and higher yield. Similarly, number and weight of microtuber, formation of shoots, shoots length were found superior at sugar concentration of 8%, which was also reported by Fatima et al., (2005). And significantly, slower microtuber growth rates were observed when sugar concentration was 4% instead of 8%; this is in line with Yu et al. (2000). From the present investigation it can be concluded that low level (3%) of sucrose was not found suitable for the microtuber production under invitro conditions. Similar results were also supported by El-sawy et al. 2007 and Hoque, 2010.

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