Single Node Callus Culture: Improvement for Micropropagation of *Solanum tuberosum (cv. Kufri Himalini)*

Anoop Badoni * and J. S. Chauhan **

***** Research Scholar, Young Scientist (UCOST), Department of Seed Science and Technology, Faculty of Agriculture, H. N. B. Garhwal University (CHURAS CAMPUS), Srinagar- 246 174, Uttrakhand, India, *For correspondence: annabadoni@yahoo.co.in

** Assoc. Professor, Head of Department, Seed Science and Technology, Faculty of Agriculture, H. N. B. Garhwal University (CHURAS CAMPUS), Srinagar- 246 174, Uttrakhand, India, jaisinghchauhan@gmial.com

Abstract:

In the present study MS media supplemented with different growth regulators such as BAP and Kn was used for callus induction and shoot regeneration. Shoots regenerated from callus were shifted to MS media supplemented with different combinations of NAA and Kinetin for shoot elongation and root formation. Percentage response to callus induction was best (60%) on medium with the combination of 0.6 mg/l BAP + 0.6 mg/l Kn. Shoot height (6.4 ± 0.6), node number (5.0 ± 0.7), root length (8.2 ± 0.5) and rooting percentage (80%), was reported highest on medium with 0.1 mg/l NAA + 0.01 mg/l Kn. After rooting on shoots the plantlets were shifted to sterile soil field pots for acclimatization. . The plantlets were survived well as about 70-80%. [Nature and Science. 2009;7(3):99-103]. (ISSN: 1545-0740).

Key words: *Kufri himalini*, growth regulators, callus induction, shoot regeneration, rooting and acclimatization

Introduction:

As a crop of high biological value for its protein and a substantial amount of vitamins, minerals and trace elements, it is undoubtedly a very important crop in many countries (Gebre and Sathyanarayana 2001). Potato is a semi-perishable crop susceptible to many diseases and insect pests. Production of quality planting material is essential not only for improving domestic potato productivity but also to ensure minimum commercial quality. Shortage of good quality seed has been recognized as the single most important factor limiting potato productivity in the developing countries. The availability of tissue culture technology for rapid multiplication of disease-free planting material has facilitated potato seed production to a great extent (Dodds 1988). The recent advancement in tissue culture and the flexibility of organ development in potato allows alternative methods of propagation through *in vitro* techniques.

Much work has been carried out on callus induction and growth in potatoes. This has resulted in a range of protocols and procedures being established by researchers since tissue culture gained an importance in plant propagation, conservation and breeding (Ahloowalia 1982; Wareh et al., 1989). Callus is used for most of these transformation methods such as particle gun (McCabe et al., 1998) and *Agrobacterium tumefaciens*-mediated transformation (Stiekema et al., 1988) as well as initiation of cell culture. A callus from an explant tissue occurs as a result of dramatic changes in the appearance and metabolism of the cells (Aitchison et al., 1978). Induction of callus, physical disorganization of cultured cells, is thought as result of the breakdown of intercellular physical and chemical communication (Lindsey and Jones, 1992). It has been already an established fact from the earlier findings in which the callus culture showed higher multiplication rate in comparison to other methods of *in vitro* culture, as in nodal culture the major factors limiting the rates of multiplication, short height of the plantlets and the low number of nodes on the plantlets (Gebre and Sathyanarayana 2001). Improvements have been made possible by callus culture and addition of growth regulators to the medium. Keep these points on view the present study was done using nodal explants for callus culture to reduce the losses due to conventional propagation methods of potato.

Material and Methods:

The nodal segments as explants were taken from the plants of *Kufri Himalini* and washed thoroughly in running tap water and surface sterilized with Tween-20 for 10 minutes. Sterilized explants were rinsed with sterile double distilled water for 3-4 times. These explants were treated with 0.5% (4%

concentrated sodium hypochloride, qualigence) for 5 minutes and finally rinsed with sterilized double distilled water for 3-4 times to remove the traces of sterilants.

For callus induction, surface sterilized nodal segments were transferred to full strength MS media (Murashige and Skoog, 1962) supplemented with different growth regulators such as BAP and Kn and incubated at 25 ^{0}C up to 16 hr photoperiod. These hormones were used separately and with combinations of each other at different concentrations (0.2, 0.4, 0.6, and 0.8 mg/l). After 35 days of culturing, calli induced were analyzed and scored depending on growth, texture and colour.

Shoots regenerated from callus were shifted to MS media supplemented with different combinations of NAA (0.1 mg/l) and Kinetin (0.01 mg/l, 0.001 mg/l, 0.1 mg/l) for shoot elongation and root formation. After rooting the complete plantlets were transferred to sterilized soil field pots for acclimatization. The mixture of soil, sand and vermi compost was used for hardening in the ratio of 2:1:1.

Results:

Callus induction: All the concentrations of growth regulators (BAP and Kn) induced callus on nodal explants when cultured on MS media with varying degrees of success (Table-1). Direct regeneration of shoots from callus (Plate.1-a and b) was observed with 60% response on medium containing the combination of BAP and Kn (0.6 + 0.6 mg/l). Concentrations of 0.6 mg/l BAP induced good amount of compact, light green callus with 40% shoot regeneration. Kn with 0.4 mg/l concentration was also sowed very good compact callus but poor shoot regeneration was occurred in this concentration.

Shoot elongation, Rooting of shoots and Hardening: The results for shoot elongation and rooting indicate (Table-2 and 3; Plate1-c and d) that in MS media with 0.1 mg/l NAA + 0.01 mg/l Kn showed higher growth of shoots and rooting percentage. Shoot height reached 6.4 cm. (±0.6) with 5.0 (±0.7) nods in MS+0.1 mg/l NAA + 0.01 mg/l Kinetin media and 5.3 cm. (±1.2) with 4.2 (±0.8) nods and 4.0 cm. (±0.6) with 2.7 (±0.7) nods in other media. The MSKN2 (0.1 mg/l NAA + 0.001 mg/l Kn) having low concentration of Kinetin and NAA and MSKN3 (0.1 mg/l NAA + 0.1 mg/l Kn) combinations having higher concentration of Kinetin and low concentration of NAA, responded the least mean Shoot height and number of nodes. Low concentration of Auxin (0.1 mg/l NAA) plus moderate concentration of Cytokinine (0.01 mg/l Kinetin) showed good development of complete plantlets from nodal segments. Hardening of the well rooted plantlets was done in the potting mixtures of soil, sand and vermi compost (2:1:1) and kept under poly house condition for survival and growth. The plantlets were survived well as about 70-80%.

Table-1 Effect of BAP and Kn on callus induction and shoot regeneration using nodal explants

Hormones concentrations with MS media (mg/l)		Callus formation	Kind of callus	Callus color	Shoot regeneration percentage
BAP	Kn				
Control		-	-	-	0
0.2	-	+	Friable	White	0
0.4	-	+	Friable	White	0
0.6	-	+++	Compact	Light Green	40
0.8	-	++	Compact	Light Green	20
-	0.2	-	-	-	0
-	0.4	+++	Compact	Greenish White	20
-	0.6	++	Compact	Light Green	10
-	0.8	+	Friable	White	0
0.2	0.2	+	Friable	White	0
0.4	0.4	+	Friable	White	0
0.6	0.6	+++	Compact	Greenish White	60
0.8	0.8	++	Compact	Greenish Brown	20

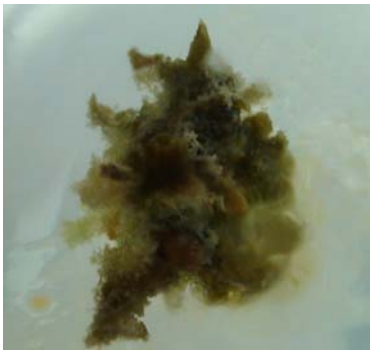
Observations were recorded after 35 days of culture: (-, No callus production; +, poor callus; ++ good callus; +++ very good callus)

Table-2: Effect of different hormonal combinations with MS media on shoot height, node number, and root length after 35-40 days of culture:

Growth regulators (mg/l)			Shoot height (cm)	Node number	Rooting Percentage	Root length (cm)
NAA	Kn	Symbol used				
0.1	0.01	MSKN 1	6.4 ± 0.6	5.0 ± 0.7	80	8.2 ± 0.5
0.1	0.001	MSKN 2	5.3 ± 1.2	4.2 ± 0.8	60	6.8 ± 0.8
0.1	0.1	MSKN 3	4.0 ± 0.6	2.7 ± 0.7	50	5.3 ± 0.9

Table-3: Effect of different hormonal combinations with MS media on shoot and root fresh weight and root: shoot ratio after 35-40 days of culture:

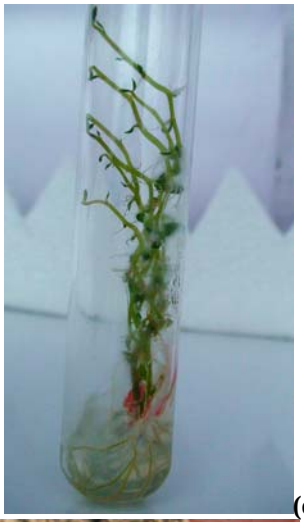
Hormonal Combination	Shoot fresh weight	Root fresh weight	Root: Shoot ratio
MSKN 1	0.226 ± 0.01	0.144 ± 0.01	1.57 ± 0.1
MSKN 2	0.171 ± 0.03	0.120 ± 0.07	1.42 ± 0.2
MSKN 3	0.141 ± 0.05	0.112 ± 0.08	1.27 ± 0.5



(a)

(b)


(c)


(d)


(e)

Plate 1 (a-e): Callus induction, shoot regeneration and rooting in potato cv. *Kufri Himalini*
(a) Callus formation on 0.6 mg/l BAP+0.6 mg/l Kn MS media **(b)** Shoot regeneration **(c-d)** Shoot Multiplication, elongation and rooting on 0.1 mg/l NAA + 0.01 mg/l Kinetin MS media **(e)** Hardening of plantlet to the mixture of sterile soil, sand and vermi compost

Discussion:

The present study was carried out with an aim to *in vitro* culture of potato cv. *Kufri Himalini* through callus culture to get disease free, uniform plantlets and for mass multiplication of cultures. This was undertaken to standardize the micropropagation technique of potato cv. *Kufri Himalini* using callus culture. *Kufri Himalini* is a medium maturing late blight resistance variety released for hills by ICAR (Anonymous, 2005). Potato, like other Solanaceous crops, shows considerable regenerative ability in culture, producing adventitious shoots both directly from organ tissue and from callus under appropriate conditions (Wang and Huang, 1975; Skirvin *et al.* 1975; Roest and Bokelmann, 1976). Through *in vitro* propagation of potato by serial culture of axillaries shoots has been reported by a number of workers and has become established as an effective means of rapidly multiplying new or existing cultivars in diseases-free conditions (Nozeran *et al.*, 1977; Goodwin *et al.*, 1980; Hussey and Stacey, 1981).

The explants selection was the most critical feature in callus induction. The texture and type of callus depend on the concentrations of growth regulators. The callus formed in media containing 0.2 and 0.4 mg/l BAP and 0.8 mg/l Kn showed friable and white callus while the callus produce in media containing 0.6 mg/l BAP and the combination of BAP (0.6 mg/l) and KN (0.6 mg/l) were compact and greenish white in colour. Higher shoot regeneration was observed in the media containing 0.6 mg/l BAP + 0.6 mg/l Kn. For shoot regeneration several workers have used various hormonal combinations of different

hormones with MS salts. Similarly as the observations of the present study Morozova *et al.* (1977) obtained largest number of regenerated plants from the early variety Izobilie using low concentration of kinetin (0.001 mg/l) while midlate variety Istrinskii gave best results under higher concentration (0.01 mg/l). This could attribute the fact that the regeneration of shoots is not totally dependent on hormonal combinations but the variety is also responsible for *in vitro* shoot proliferation. The combined effect of NAA and kinetin was reported to be better in cvs. Bintje, Desiree, Gracia and Ostara that developed the largest number of plantlets on a medium containing 0.5 mg/l NAA, 0.4 mg/l kinetinand 0.7 mg/l thiamine (Maroti *et al.*, 1982). The result is also supported by the findings of Ruzic *et al.*, (1997), in which they reported 0.01 mg/l NAA, 0.1 mg/l GA and 1 mg/l kinetin proved in production of prolific and healthy shoots and good root development.

References:

Anonymous, The Hindu, 29th May, New Delhi, India, 2005

Ahloowalia B. S. Plant regeneration from callus culture in potato, Euphytica 1982; 31: 755-759

Aitchison P.A., MacLeod AJ, Yeoman MM Growth patterns in tissue (callus) cultures In HE Street, ed, Plant Tissue and Cell Culture, Blackwell Sci. Pub. Oxford 1978; 267-306

Dodds, J.H. Tissue culture technology: practical application of sophisticated methods. Am. Potato J. 1988; 65: 167-180

Gebre, Enadale and Sathyanarayana Tapioca- A new and cheaper alternative to agar for direct *in vitro* shoot regeneration and microtuber production from nodal cultures of potato. Afri. Cr. Sci. J. 2001; 9 (1): 1-8

Goodwin, P. B. Kim, Y. C. and Adisarwanto T. Propagation of potato by shoot tip culture. Potato Res. 1980; 23: 9-18

Hussey, G. and Stacey N. J. *In vitro* propagation of potato (*Solanum tuberosum* L.) Ann. Bot. 1981; 48(6): 787-796

Lindsey K, Jones MGK. Plant Biotechnol. in Agric. John Willey and Sons Ltd., England 1992: 241

Maroti, M., Rudolf J., Bognar J. and Pozsar B. I. *In vitro* plantlets from potato shoot segments. Acta Bot. Acad. Sci. Hung. 1982; 28(1-2): 127-132

Morozova, S.E. and Melik-Sarkisov, O.S. Induction of stem formation in explants of potato by culture in a liquid medium. Doklady Vesoujnoi-Ordene. 1977; 5: 14-15

Murashige, T. and Skoog F. A revised medium for rapid growth and bio-assays with tobacco tissue cultures, Physiol. Plant, 1962; 15: 473-497

Nozeran, R. B. andilho, Rossignol L. and Glenan S. Nouvelles possibilities etde multiplication rapie de clones sains de pomme de erre (*Solanum tuberosum* L.). C.R. Acad Sci. 1977; 285(1): 37-40

Roest, S. and Bokelmann, G.S. Vegetative propagation of *Solanum tuberosum* L. *in vitro.* Potato Res. 1976; 19: 173-178

Ruzic, D. Milinkovic, M. and Milosevic, D. *In vitro*propagation of potato. Acta Hort. 1997; 462: 959-964

Skirvin, R.M. Lam, S.L. and Janick, J. Plantlet formation from potato callus *in vitro.* Hort. Sci, 1975; 10: 413

Stiekema WJ, Heidekamp F, Louwerse JD, Verhoeven HA, Dijkhuis P. Introduction of foreign genes into potato cultivars Bintje and Desiree using an Agrobacterium tumefaciens binary vector. Plant Cell Reports 1988; 7: 47-50

Wang, P.J. and Huang, L.S.. Callus culture from potato tissue and exclusion of virus X from plants regenerated from stem tips. Can. J. Bot. 1975; 53: 2565-2567

Wareh HA, Trolinder NL, Gooding J.R. Callus initiation, shoot regeneration, and micropropagation of three potato cultivars. Hortsci. 1989; 24(4): 680-682.

2/17/2009