IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 3, Issue 4 (Jul. – Aug. 2017), PP 31-34
www.iosrjournals.org

Direct and Indirect Regeneration of Potato Cultivar Kufri Jyoti

Tara Singh Rawat[1], Krishnaprasad B.T.[2*], Veena S Anil[1]
[1.] *Department of Plant Biotechnology, GKVK, University of Agricultural Sciences, Bangalore 560065 India*
[2.] *Department of Agri. Biotechnology, College of Agriculture, Hassan 573225, India*
**Corresponding author: Krishnaprasad B.T.*

Abstract: *Potato crop improvement requires genetic transformation methods and also regeneration of somaclonal variants derived from callus. Prerequisite for both the approach is a simple reliable tissue culture mediated direct and indirect plant regeneration protocol. In this study effect of combination of growth regulators to regenerate potato plants from internodes and leaf explants is examined. It was found that internodes are the best explants to regenerate the plants by direct and indirect regeneration. Callus induction from internodes was found to be best with growth regulator concentration of 4 mg/L BAP and 1 mg/L NAA. Indirect regeneration from the callus and direct regeneration from the internodes is achieved by placing them in MS medium containing 3 mg/L BAP and 1 mg/L GA_3. Combination of growth regulators 0.1 mg/L GA_3 and 0.1 mg/L NAA in MS medium gave rooting of shoots derived from direct regeneration.*

Keywords: *Potato, Direct regeneration, Indirect regeneration, Kufri Jyoti, Tissue culture*

Date of Submission: 19-07-2017 Date of acceptance: 28-07-2017

I. Introduction

Potato is the third most important food crop in the world after rice and wheat in terms of human consumption. Presently, more than half of global potato production comes from developing countries [1]. In order to achieve new potato varieties with superior agronomic traits and product diversification it is essential to perform targeted genetic improvement. Genetic transformation of potato is becoming inevitable to realize enhanced nutritional quality such as higher protein content, improved amino acid content, additional vitamins, modified starch composition to suit industrial needs. Genetic improvement to pests and disease particularly to late blight disease caused by *Phytophthora infestance* is key to accomplish higher yield. *In vitro* regeneration of plants achieved by tissue culture is the fundamental tool required for crop improvement through genetic transformation. The development of a reliable, rapid and efficient system of tissue culture for plant regeneration has been a foremost prerequisite. *In vitro* regeneration response is generally species and often cultivar specific. Various combinations of growth regulator and explants for the regeneration of various cultivars of potato are available in the literature. Huge differences in the shoot formation efficiency among commercial cultivars of potatoes have been reported [2],[3],[4]. Ganapathi et. al., 2007 [5] used direct regeneration approach from internodal segments for regeneration of transformed potato plant. Yee et. Al., 2001 [6] developed efficient regeneration protocol using petiole with intact leaf as explants. They found regeneration in MS medium supplemented with 1mg/L BAP, 1mg/L GA_3 and 1mg/L IAA. Sarker and Mustafa 2002 [7] observed maximum shoot regeneration in medium containing 1mg/L BAP and 0.1mg/L GA_3. In the present study effect of growth regulators for direct and indirect regeneration of potato cultivar Kufri Jyoti from internodes and leaf explants was studied. Direct regeneration method found in this paper can be used for *Agrobacterium* mediated transformation of potato and indirect method of regeneration is useful to regenerate potato plants from somaclonal variants obtained through callus.

II. Materials And Method

Tubers of potato variety Kufri Jyoti were obtained from CPRI, Shimla. Nodes of potato were grown and maintained in a test tube containing Murashige and Skoog [8] medium supplemented with 0.1 mg/L NAA and 0.1 mg/L GA_3. Leaf and internodes of *in vitro* grown plants were used as explants. For direct regeneration five different combination of medium differing in the concentration of BAP, GA_3, NAA and IAA were used. For indirect regeneration callus is transferred to MS medium containing three concentration of BAP (3, 4, and 5mg/L) in combination with four concentrations of GA_3 were used. Observations on number of explants responding, days taken for callus induction and shoot initiation shoots per explants, callus induction frequency and regeneration frequency were recorded. To induce rooting, well developed shoots (3-8 cm in length) were excised carefully from the indirect regeneration and transferred to root inducing media containing different levels of sucrose and growth regulators. Days to root initiation, root length and number of roots per plant were recorded. All the experiments were repeated three times. The number of days to initiate root was noted when

root primordia was induced in 50% shoots and number of roots and root length were recorded two weeks after transferring the shoots to rooting media

III. Results And Discussion

Callus induction

Callus induction response was observed shortest period both in leaf and internode explants in medium containing BAP and NAA (Table-1). Combination of BAP and NAA induced callusing both in leaf and internode explants. Variation was observed in terms of percentage callus induction and days taken for callus induction. Similar results were also observed by Onamu et al., 2012[9], Yasmin et al. 2003 [10], Beaujean et al., 1998[11] and Martel and Carcia (1992) [12], that combination of BAP with NAA are efficient combination for induction of callus in potato. It was found that combination of 4 mg/L of BAP and 1 mg/L of NAA is the best for callus induction in Kufri Jyoti.

Table-1: Effect of callus induction medium and explant type on callogenesis

Hormones Used(mg/L)				Number of days taken for callus Initiation		Callus induction frequency	
BAP	GA_3	NAA	IAA	*Internode*	*Leaf*	*Internode*	*Leaf*
1.0	0.1	-	-	24.30	*	66.67^{b}	*
2.0	-	2.5	-	9.50	12.33	100.00^{a}	73.34^{b}
3.0	1.0	-	-	12.00	15.00	100.00^{a}	100.00^{a}
4.0	-	-	1.0	22.34	*	33.33^{c}	*
4.0	-	1.0	-	7.16	17.41	100.00^{a}	100.00^{a}
CD @ 5%						5.39	8.38
SEM						1.03	1.10

(Each value is the mean of four replicates. Any two means having a common alphabet are not significantly different at p = 0.05 using CRD)

Direct regeneration

Shoot induction in the internodes was observed in all the media containing BAP with small quantity of GA_3. BAP 3mg/L with 1 mg/L GA_3 found to be best for fast shoot induction. This media gave highest number of shoots as well as shoot number per explants (Table-2). If auxin is included in the medium it did not support shoot induction in Kufri Jyoti cultivar of potato. Media with 1 mg/L BAP and 1 mg/L GA_3 and SIM5 (1 mg/L BAP + 0.1 mg/L GA_3) were able to induce shoots with regeneration frequency of 94.10 directly from internodal segments. These observations are parallel to the previous observations that GA_3 is essential hormone for the production of shoots from potato. Farhatullah and Abbas (2007) [13] also have reported that dosage of 0.248 mg/L of GA_3 in the MS medium boosted all morphological characters in *in vitro* raised potato plantlets. Ullah *et al*. (2012)[14] also reported that GA_3 is involved in cell elongation and its addition in MS medium enhanced shoot growth in *in vitro* propagated plants of potato variety Desiree. Kufri Jyoti cultivar of potato, 3 mg/L of BAP with 1mg/L of GA_3 found to be best for direct regeneration of shoots.

Indirect regeneration

Indirect regeneration protocol is important to regenerate potato somaclonal variants obtained from callus. Best quality callus was obtained from the internode kept in the medium containing 4 mg/L BAP with 1 mg/L of NAA (Table-3). These calluses were transferred shoot inducing medium with combination of BAP and GA_3. It was found that 5 mg/L BAP with GA_3 is the best combination for shoot regeneration for potato *var.* Kufri Jyoti with regeneration frequency of 80.0 percent. Callus derived from the internodes gave higher regeneration of shoot than callus derived from the leaf. The above results are also supported by the findings of Dhaka *et al.*, (2015) [15] that combination of BAP and GA_3 is must for shoot induction from callus. They used 8.88 µM BAP and 1.00 µM GA_3 in shoot inducing media which gave significantly high average number of shoots, shoot length and number of leaves per explant. Similar results were also observed by Onamu *et al.,* (2012)[9], Saker *et al.,* (2012) [16] in different varieties of potato.

Table -2: Effect of shoot initiation medium and explant type on caulogenesis and shoot morphology

Hormones Used (mg/L)				Number of days taken for shoot initiation		Regeneration frequency (%)		No. of shoots per callus		Shoot height (cm)	
BAP	*GA_3*	*NAA*	*IAA*	*Internode*	*Leaf*	*Internode*	*Leaf*	*Internode*	*Leaf*	*Internode*	*Leaf*
1.0	0.1	-	-	15.00	-	62.38^{b}	0.00	1.00^{bc}	0.00	2.10^{c}	0.00
2.0	5.0		-	22.33	-	68.03^{b}	0.00	2.06^{b}	0.00	5.50^{b}	0.00
2.0	-	2.5	-	-	-	0.00	0.00	0.00	0.00	0.00	0.00
3.0	1.0	-	-	7.00	10.00	94.10^{a}	87.66^{a}	11.93^{a}	18.57^{a}	10.00^{a}	10.20^{a}
4.0	-	-	1.0	-	-	0.00	0.00	0.00	0.00	0.00	0.00
CD @ 5%				-	-	7.86	1.82	1.15	0.95	0.41	0.40
SEM				-	-	17.29	14.66	1.92	3.10	1.67	1.70

(Each value is the mean of four replicates. Any two means having a common alphabet are not significantly different at p = 0.05 using CRD, SAS 9.21)

Root initiation

It was evident that all the five media significantly differed from each other in terms of number of roots, length of roots and number of days to root formation (Table-4). The results showed that the highest mean number of roots per regenerated shoots was 46.06 on media containing 1 mg/L IBA. The best quality of shoots and roots were observed on media containing 0.1 mg/L NAA with 0.1 mg/L GA_3. Root initiation took place in a short period of time of 5.66 days with this combination of hormones. Representative picture of direct and indirect regeneration of potato plant cultivar Kufri Jyoti is given in (Plate-1).

Table-3: Effect of different concentrations of the BAP and GA_3 on caulogenesis from internode and leaf derived callus.

Hormone concentration		Days to shooting		Regeneration frequency (%)		No. of shoots per callus		Shoot height (cm)	
BAP (mg/L)	**GA_3 (mg/L)**	*Internode*	*Leaf*	*Internode*	*Leaf*	*Internode*	*Leaf*	*Internode*	*Leaf*
-	-	-	-	0.00^{g}	0.00^{e}	0.00^{f}	0.00^{e}	0.00^{h}	0.00^{g}
3.0	0.3	24.66	24.66	21.42^{f}	7.87^{de}	0.57^{ef}	0.11^{de}	3.76^{fg}	2.00^{def}
4.0	0.3	27.33	27.00	24.44^{ef}	7.03^{de}	1.38^{de}	0.10^{de}	6.80^{cd}	5.00^{ab}
5.0	0.3	16.00	29.01	78.25^{ab}	46.29^{a}	9.00^{a}	0.75^{b}	7.93^{ab}	5.66^{a}
3.0	0.6	19.66	22.02	29.36^{ef}	15.37^{cd}	0.80^{ef}	0.34^{cd}	4.26^{f}	3.50^{bcd}
4.0	0.6	25.66	30.66	16.19^{f}	17.39^{cd}	0.45^{ef}	0.21^{de}	6.40^{d}	3.40^{cd}
5.0	0.6	25.33	26.33	50.95^{cd}	28.24^{bc}	1.52^{de}	0.52^{bc}	5.30^{e}	3.93^{bc}
3.0	1.0	22.66	23.66	63.69^{bc}	39.62^{ab}	2.54^{d}	0.78^{b}	6.33^{d}	2.26^{de}
4.0	1.0	24.00	27.00	37.30^{de}	7.407^{de}	$0.73^{ef.}$	0.14^{de}	$3.60f^{g}$	1.60^{ef}
5.0	1.0	15.66	21.33	80.09^{a}	37.96^{ab}	4.76^{c}	0.75^{b}	$7.46b^{c}$	4.83^{abc}
3.0	3.0	22.33	24.33	24.07^{ef}	3.703^{de}	0.49^{ef}	0.07^{de}	3.26^{g}	0.66^{fg}
4.0	3.0	26.66	29.66	24.52^{f}	7.870^{de}	0.53^{ef}	0.15^{de}	3.93^{fg}	1.66^{ef}
5.0	3.0	15.33	19.66	78.57^{a}	46.82^{a}	6.88^{b}	2.23^{a}	8.43^{a}	4.76^{abc}
CD @ 5%		-	-	14.75	14.14	1.20	0.29	0.79	1.50
SEM		-	-	7.42	4.73	0.75	0.16	0.65	0.49

(Each value is the mean of three replicates. Any two means having a common alphabet are not significantly different at p = 0.05 using CRD)

Table-4: Effect of different rooting media on rhizogenesis and root morphology

Media	No. of days taken for rooting	No. of roots per plant	Root length at 15^{th} day	Root phenotype
MS media	6.66	5.66^{d}	3.83^{b}	Slender and long
MS media with 1.8% sucrose	5.33	11.10^{c}	5.13^{a}	Thick and long
MS media with 0.5 mg/L IBA	8.20	39.26^{b}	1.03^{d}	Thick and short
MS media with 1.0 mg/L IBA	8.66	46.20^{a}	2.26^{c}	Thick and short
MS media with 0.1mg/L NAA and 0.1mg/L GA_3	5.66	38.43^{b}	3.03^{b}	Thick and long
CD @ 5%	-	3.33	0.40	-
CD@ 1%	-	4.73	0.57	-
SEM	-	8.24	0.66	-

(Each value is the mean of four replicates. Any two means having a common alphabet are not significantly different at p = 0.05 using CRD)

IV. Conclusion

Combination of hormones to achieve direct and indirect regeneration of Kufri Jyoti cultivar of potatao from internodes and leaf explants was examined. It was found that callus induction was achieved in shortest period of time with 4, 1 mg/L of BAP and NAA respectively. For direct regeneration of shoot from internode MS media with 3 mg/L BAP and 1mg/L GA_3 found to be best. Indirect regeneration of callus derived from internode was achieved with 5, 1 mg/L BAP and GA_3 respectively. Rooting of *in vitro* derived shoots is possible with 0.1 mg/L of NAA and GA_3.

Acknowledgement

We thank CPRI, Shimla, for providing the potato cultivar Kufri Jyoti used in this study

REFERENCES

[1] https://cipotato.org/potato/facts/

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

[3] Y. P. S. Bajaj Regeneration of plants from potato meristems freeze preserved for 24 months. Euphytica, 1981 **30**(1): 141-145.

[4] P. R. Miller, L. Amirouche, T. Stuchbury and S. Mathews The use of plant growth regulators in micropropagation of slow-growing potato cultivars. Potato Res., 1985, **28**: 479-486.

[5] T. R. Ganapathi, S. B. Ghosh, N. H. S. Laxmi and V. A Bapat Expression of an antimicrobial peptide (MSI-99) confer enhanced resistance to Aspergillus niger in transgenic potato. Ind. J. Biotech. 2007 **6**: 63-67.

[6] S.Yee, B.Stevens, S.Coleman, J. Seabrook and Q. Li,, High efficiency regeneration in vitro from potato petioles from intact leaflets. Am. J. Potato Res., 2001**7 8**: 151-157

[7] R. H. Sarker and B. M. Mustafa Regeneration and Agrobacterium-mediated genetic transformation of two indigenous potato varieties of Bangladesh. Plant Tiss. Cult., 2002,**12**(1): 69-77.

[8] T. Murashige and F. Skoog A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 1962 **15**: 473-497.

[9] R.Onamu, P. L., Juan, C. S. Jaime, L. R. Jose and N. P. Joel In vitro regeneration and agrobacterium-mediated transformation of potato (Solanum tuberosum L.) cultivars grown in Mexico. Plant Tissue Cult. & Biotech., 2012 **22**(2): 93-105.

[10] S.Yasmin, , K. M. Nasiruddin, R. Begum and S. K. Talukder, Regeneration and establishment of potato plantlets through callus formation with BAP and NAA. Asian J. Plant Sci. 2003 **2**(12): 936-940.

[11] R.Onamu, P. L.Juan, C. S. Jaime, , L. R. Jose and N. P. Joel In vitro regeneration and agrobacterium-mediated transformation of potato (Solanum tuberosum L.) cultivars grown in Mexico. Plant Tissue Cult. & Biotech. 2012 **22**(2): 93-105

[12] A. Martel and E. Carcia In vitro formation of adventitious shoots on discs of potato (Solanum tuberosum L. cv. Sebago) tubers. Phyton Buenos Aires 1992 **53**: 57-64.

[13] Z. A. Farhatullah, And J. S. Abbas, In vitro effects of gibberellic acid on morphogenesis of potato explant. Int. J. Agric. Biol. 2007 **9**: 200

[14] I.Ullah, M. Jadoon, A. Rehman, T. Zeb and K. Khan Effect of different GA_3 concentration on in vitro propagation of potato variety Desiree. Asian J. Agric. Sci. 2012 **4**(2):108-109.

[15] M. Dhaka, and T. K. Nailwal High efficiency macropropagation of potato (Solanum tuberosum L.) cv. Kufri Jyoti in Kumaun Hills. J. Plant Breed. Crop Sci.. 2015 **7**: 203-210.

[16] M. M. Saker, A. A. Tarek, N. Z. H. Moussa, H. A. Amany, E. Abo and R. M. H. Abdel-Rahman, Selection of an efficient in vitro micro-propagation and regeneration system for potato (Solanum tuberosum L.) cultivar Desiree. Afr. J. Biotech., 2012 **11**(98): 16388-16404

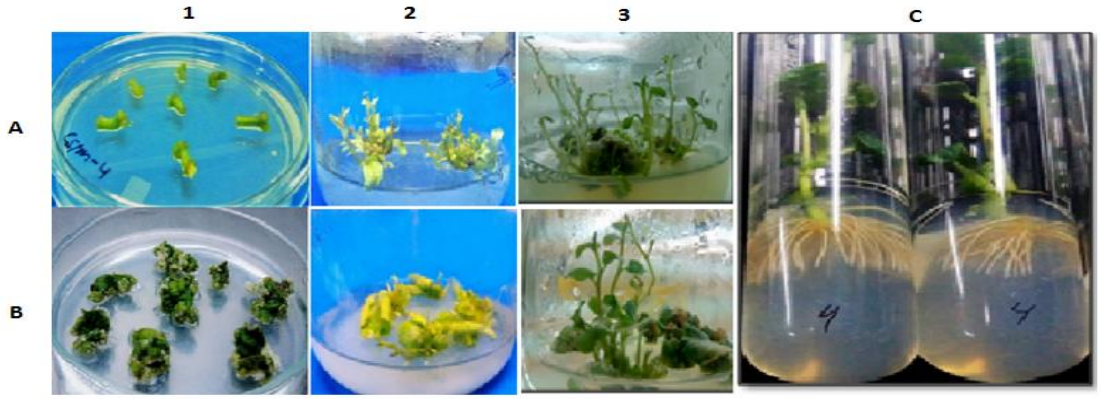

PLATE-1: Representation of different stages of regeneration of potato cultivar Kufri Jyoti from Internode (A) and Leaf (B) as explants. 1. Callus induction 2. Direct shoot induction 3. Indirect shoot induction. C. Rooting of shoot derived from callus. Pictures of best treatments mentioned in the text are given in the plate.

IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) is UGC approved Journal with Sl. No. 4033, Journal no. 44202.

Tara Singh Rawat. "Direct and Indirect Regeneration of Potato Cultivar Kufri Jyoti." IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) 3.4 (2017): 31-34.