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Optimization of an Efficient Micropropagation Protocol and the Assessment of Genetic Fidelity of *Solanum tuberosum* L. cv Kufri Neelkanth

Bichhinna Maitri Rout, A.K. Bhatia, Lila Bora, Shohaib Sheikh Ayub Chauhan, Love Sapra, Tejinder Singh

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ABSTRACT

The aim of our experiment was to determine the optimal types and concentrations of plant growth regulators used during different stages of micropropagation and assess the genetic fidelity of tissue culture raised plants of a purple colored cv Kufri Neelkanth by using reliable markers. According to the shoot bud initiation data, it was found that utilizing a combination of 0.25 mg l^{-1} BAP and 0.5 mg l^{-1} Kinetin resulted in the greatest number of shoots per explant (3.5±0.12) within a timeframe of (3.3±0.08) days. The maximum number of *in vitro* shoots per shootlet (10.4±0.59) were recorded when auxins were used in combination with NAA (0.01 mg l^{-1} NAA) and Kinetin (0.25 mg l^{-1}) for shoot proliferation. *In vitro* root initiation was observed in (2.1±0.07) days on MS medium fortified with 2.5 mg l^{-1} NAA. The maximum number of *in vitro* roots per shoots (13.0±0.55) were observed when MS media fortified with 2.5 mg l^{-1} IBA. Maximum 100% rooting was observed in all MS media supplemented with different concentrations of auxins. *In vitro* raised plants were assessed for genetic fidelity by using twenty RAPD primers (genetic markers). Out of twenty primers used only four primers produced amplification. DNA banding patterns of all tissue culture raised plants and mother plants were monomorphic showing true to type planting material. This protocol for tissue culture propagation along with testing its genetic fidelity could be useful for better conservation of germplasm and genetic transformation studies in potato.

Keywords Shoot proliferation, Micropropagation, *Solanum tuberosum* L., Genetic markers.

Bichhinna Maitri Rout[1], A.K. Bhatia[2], Lila Bora[3], Shohaib Sheikh Ayub Chauhan[4], Love Sapra[5], Tejinder Singh[6]*

[1,4,5,6]PhD Scholar, [2]Principal Scientist, [3]Assistant Scientist
[1,4]Department of Vegetable Science, ICAR-IARI, Pusa, New Delhi 110012, India
[2,3,5,6]Department of Vegetable Science, CCS Haryana Agricultural University, Hisar, Haryana 125004, India

Email : tejinderror26@gmail.com
*Corresponding author

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important temperate tuber crops grown worldwide. It is one of the valuable staple foods which plays an integral role in nutritional security, poverty alleviation, and income generation (Hajare *et al.* 2021). Potato is vegetatively propagated by means of tubers and conventional propagation of potato through true

potato seed resulted in low multiplication rates and virus accumulation in different progenies. In various regions worldwide, the expense attributed solely to seed potatoes represents approximately 40 to 60% of the overall production costs. In developing countries, the scarcity of high-quality planting material has been recognized as a significant hindrance in potato cultivation. The utilization of "cut potato" has been found to yield unsatisfactory results (Nielson *et al.* 1989, Rykbost and Lockeil 1999), increasing the risk of virus proliferation, ultimately resulting in economic losses for the growers. Adaptation is difficult as cuts transmit more viruses. 40% yield can be reduced by some viruses independently and the loss may reach up to 90% when combined with other viruses (Siddiqui *et al.* 1996). Insufficient maintenance or infrequent replacement of seed stock can lead to a significant infiltration of viruses, reaching up to 100% in 3-4 consecutive crop seasons. This infiltration can result in yield reductions of nearly half or one-third (Biniam and Tadesse 2008). To address this issue, micropropagation can be employed within the potato production chain. It offers efficient propagation of desired materials, facilitates the maintenance of germplasm banks, enables genetic exchange, supports research on the species and its interactions with biotic and abiotic factors, and allows to produce genetically modified plants and pathogen-free seed potatoes (de Morais *et al.* 2018).

Micropropagation has proven valuable in the identification and selection of desirable traits, as well as in reducing the space needed for field trials. It enables the production of true-to-type plants regardless of the season or crop within a specific timeframe (George and Manuel 2013). Nodal cuttings are typically used for one cycle of multiplication, which typically takes approximately four weeks (Asakaviciute 2011, Milinkovic *et al.* 2012). On average, 3-5 new cuttings can be established from a single plantlet (Ranalli 1997), ensuring efficient propagation. Efficient plant regeneration depends upon various factors including the composition of culture media, concentrations of growth hormones and especially the genotype of the explants (Madhu and Savithramma 2014).

When it comes to clonal regeneration, preserving the genetic stability of *in vitro* propagated material (Zilberman and Henikoff 2007) and establishing effective conservation programs (Butiuc *et al*. 2016) are of utmost importance. Detecting changes resulting from *in vitro* procedures using morphological and biochemical criteria is not feasible (Roca *et al.* 1978). The possibility of developing somaclonal variations among the sub-clones' parental lines arises due to several key factors such as genotypes used, composition of growth regulators, expression of chromosomal mosaicism, spontaneous mutation. The use of some antibiotics like tetracycline and chloramphenicol in the culture medium adversely affects the *in vitro* development of potato explants (Pereira and Fortes 2003). Previous researchers also faced problems in their techniques, such as the delay in the differentiation of the meristematic tissue in the culture medium and explant survival difficulty due to its small size (De Morais *et al.* 2018).

Molecular markers are employed to examine somaclonal variation, confirm the genetic fidelity of micropropagated plants, and identify genotypes that exhibit the desired response to *in vitro* culture conditions. It also reduces the chances for inclusion of variable genotypes. DNA markers have developed into many systems based on different polymorphism detecting techniques or methods such as RFLP, AFLP, RAPD, SSR, SNP, (Farokhzadeh and Fakheri 2014). For evaluating the genetic fidelity of *in vitro* propagated clones, RAPD markers are considered the most reliable method. These are very simple, fast, cost effective, highly discriminative, and accessible and exclude the use of any radioactive probes as in RFLP. So, the efficient production of *in vitro* plants can be enhanced by detecting the true to type plants with the help of RAPD markers.

As a result, in this study, we have successfully formulated a highly efficient protocol for the micropropagation of the significant cultivar Kufri Neelkanth. Furthermore, the genetic fidelity of *in vitro* raised plants was also investigated by using the reliable RAPD markers.

MATERIALS AND METHODS

In the present study, we used a newly developed

purple colored potato cultivar Kufri Neelkanth which is an anthocyanin rich table purpose variety. This cultivar does not catch the scorching disease, so there is no need for any kind of chemical spray and hence reducing the cost of cultivation. The yield of Kufri Neelkanth is 35–38 t ha^{-1}. We obtained this planting material from the research field of Department of Vegetable Science, Chaudhary Charan Singh Haryana Agricultural University and green house of Center for Plant Biotechnology, Haryana, India. The promising results were obtained by using shoot tips as explants in our study. Therefore, to get enough *in vitro* shoots, we cultured the shoot tips explants onto modified MS basal medium consisting of MS inorganic salts (Myo-inositol, vitamins and table sugars) (Murashige and Skoog 1962) and different concentrations and combinations of BAP and KIN respectively. Following a period of 2-3 weeks, the *in vitro* shootlets were carefully removed from sterile cultures in a controlled environment and placed onto a multiplication medium. This medium consisted of MS basal salt supplemented with various concentrations and combinations of growth hormones such as GA3, BAP, KIN, NAA, IBA, and IAA.

Shoot bud initiation and *in vitro* shoot proliferation media

In experiment 1, the shoot tip explants were surface sterilized with 0.2% bavistin+0.4% streptocyclin for 45 minutes and with 0.1% $HgCl_2$ for 35 seconds followed by rinsing with double distilled water for 3-4 times. The sterilized shoot tips were inoculated on various MS basal media having different concentrations and combinations of cytokinins viz., BAP, KIN (0.25 or 0.50 mg l^{-1}) and both BAP and KIN (0.25 or 0.50 and 0.25 or 0.50 mg l^{-1}) respectively. In experiment 2, the *in vitro* shootlets obtained were inoculated on different multiplication medium having MS basal salt supplemented with different concentrations and combinations of growth hormones like GA_3, BAP, KIN, NAA, IBA and IAA for multiple *in vitro* shoot formation. The pH of all media was adjusted to 5.8 using 1N NaOH and 1N HCl and 0.8% agar was added to solidify the media. The media were sterilized in autoclave at 121°C at 15 psi for 15 minutes followed by storage at room temperature at 25±1°C for 3-4 days, then used for inoculation or subculture.

***In vitro* root formation media**

In experiment 3, the proliferated *in vitro* shoots obtained were separated aseptically and were transferred on MS basal medium supplemented with different concentrations of auxins (IBA, NAA) after 35 days for rooting. Number of *in vitro* shoots showing root formation, days to root initiation and number of roots per shoot were determined. Rooting percentage regeneration was calculated by using the formula :

Rooting % regeneration

$$\frac{\text{Number of } \textit{in vitro} \text{ rooted adventitious shoots regenerated}}{\text{Total number of inoculations}} \times 100$$

Aseptic culture conditions

In all the stages of experiment, the cultures were kept in growth room where temperature was maintained at 25±2°C and light intensity of 1000 lux was provided using florescent tubes. Photoperiod of 16 hrs/8 hrs of light and dark was provided.

Acclimatization of regenerated *in vitro* plantlets

The *in vitro* plantlets of the cultivar were obtained after few days and washed under running tap water to remove adhering gel from the plants. The *in vitro* plantlets were subsequently transplanted into various potting mixtures, including sand, FYM, soil, vermicompost, peat moss, cocopeat, perlite and vermiculite. These potted plantlets were then cultivated under greenhouse conditions with a temperature of 24±2°C, relative humidity of 80%, and a photoperiod of 16 hrs.

Isolation of DNA and genetic fidelity testing by using RAPD primers

In the last experiment, the genetic fidelity/stability of *in vitro* raised plants of Potato cultivar (Kufri Neelkanth) was tested by using 20 RAPD (10-mer) primers (Table 1). DNA was isolated from the young leaves of mother plants and 50 randomly selected *in vitro* raised plants of potato following (CTAB) extraction method of Murray and Thompson (1980), modified by

Sagai-Maroof *et al.* (1984) and Xu *et al*. (1994). PCR reaction was carried out using 20 µl reaction volume containing 2 µl of 20 ng μl^{-1} template DNA, 2.5 µl of 10 mM dNTPs mix, 2.0 µl of primer, 0.3µl of 10X Taq DNA polymerase buffer and 0.3 µl of 5 Units μl^{-1} Taq DNA polymerase. The standardization of reagent concentration was done for PCR reaction condition, which includes template DNA, deoxynucleotide triphosphate, primers, $MgCl_2$, Taq buffer and Taq DNA polymerase. The amplification cycle includes following steps, denaturation step at 94°C, followed by 35 cycles of denaturation at 94°C, annealing and extension at 72°C. For primers, standardization of annealing temperature was done and amplification cycle was run in an Eppendorf™, Mastercycler™ Nexus Thermal Cycler (Eppendorf AG, Germany). For complete separation of bands, the PCR products were then run on 1.5% (m/v) agarose gel containing ethdium bromide (0.3 µg cm^{-3}) in 1 × TBE at 70V for 2 hrs. Gel Doc System (Bio-Rad Laboratories, Inc, Berkley, California) was used for scanning the gel and the banding patterns were observed.

Data analysis

The data related to various characters for shoot bud initiation, shoot multiplication and rooting were recorded in replicated form using Complete Randomized Design (CRD). 3 replicates per experiment were taken in this study. The basal medium without containing any growth regulator was taken as a control in all the experiments for better interpretation of the results. The cultures were observed periodically and morphological changes were recorded at fix intervals. In *in vitro* shoot multiplication, data was recorded for number of *in vitro* shoots formed after fixed interval of time i.e., 7th, 14th, 21st, 28th and 35th day of inoculation. The experiment involved assessing the number of responsive explants per treatment, shoots per shootlet, and roots per shoot, with the results being meticulously analyzed. The entire experiment was conducted three times for accuracy. The results are presented as means ± SE. Data were analyzed statistically using one-way ANOVA (OPSTAT software on CCS HAU website) and significant differences were calculated at $p<0.05$ by Duncan's multiple range test.

Table 1. Different RAPD primers along with their sequence used for testing of genetic fidelity.

Sl. No.	Primers	Sequence 5'-3'
1	OPB-01	GTTTCGCTCC
2	OPB-02	TGATCCCTGG
3	OPB-03	CATCCCCCTG
4	OPB-04	GGACTGGAGT
5	OPB-05	TGCGCCCTTC
6	OPB-06	TGCTCTGCCC
7	OPB-07	GGTGACGCAG
8	OPB-08	GTCCACACGG
9	OPB-09	TGGGGGACTC
10	OPB-10	CTGCTGGGAC
11	OPB-11	GTAGACCCGT
12	OPB-12	CCTTGACGCA
13	OPB-13	TTCCCCCGCT
14	OPB-14	TCCGCTCTGG
15	OPB-15	GGAGGGTGTT
16	OPB-16	TTTGCCCGGA
17	OPB-17	AGGGAACGAG
18	OPB-18	CCACAGCAGT
19	OPB-19	ACCCCCGAAG
20	OPB-20	GGACCCTTAC

RESULTS

Effect of growth regulators on *in vitro* shoot bud initiation

The effect of different growth regulators and their concentrations on *in vitro* shoot bud initiation is summarized in Table 2. The *in vitro* shoots were obtained from explants on medium containing different concentration and combination of growth regulators. The shoot buds were initiated after 3-6 days depending on the type of media. In this study, initiation of shoot buds occurred directly from the explants surpassing an intermediate callus stage (Fig. 1A).

In this study, the type of response was dependent on concentration and combinations of growth regulators in induction medium. Among different concentrations and combinations of growth regulators studied, medium supplemented with 0.25 BAP and 0.5 KIN mg l^{-1} showed the highest number of shoots per explants (3.5±0.12) and minimum number of days (3.3±0.08) for shoot bud initiation. However, the number of shoots increased as the concentration of BAP and KIN increased from 0.25-0.5 mg l^{-1} when taken separately but the individual effect of KIN showed better response than BAP. The concen-

tration of KIN was kept more than BAP when used in combination as the dominant effect of BAP over KIN reduce its effectiveness. The good results were observed when KIN and BAP are used in combination as compared to separate concentrations.

Effect of growth regulators on *in vitro* shoot multiplication

In this study, the efficacy of *in vitro* shoot multiplication was improved with the addition of different growth regulators onto the medium and is summarized in Table 3. The *in vitro* shoots were formed from each shootlet inoculated onto the medium. In addition to, all the media showed response with regard to *in vitro* shoot multiplication. The *in vitro* shoots were elongated after 5-6 days of culturing and the number of *in vitro* shoots increased simultaneously after fix interval. After 28 days the cultures showed robust growth with lush green small leaves and indefinite number of shoot hairs (Fig. 1B). Furthermore, among different combinations used, MS basal medium fortified with 0.25 mg l^{-1} KIN and 0.01 mg l^{-1} NAA (SM_{15}) showed best results (10.4±0.59) on 35th day of culture. Thus, according to data presented in the above Table it is concluded that for *in vitro* multiplication of *Solanum tuberosum* MS medium fortified with lower concentration of KIN along with auxins found to be better than other concentrations of growth regulators used.

Effect of auxins on *in vitro* rooting

The *in vitro* roots were initiated from cultured *in vitro* shoots on different concentration and combinations of auxins used. The efficiency increased when we used MS basal medium supplemented with 2.5 mg l^{-1} IBA (IR_5) (Table 4). The best results were observed on this medium (13.0±0.55) in comparison to other medium. On the contrary, the quick response on root initiation (2.1±0.07) was observed on MS basal medium supplemented with 2.5 mg l^{-1} NAA (IR_{10}). The initiation of roots occurred after 3-5 days of culturing. The well-developed root system along with fully expanded leaves were observed on 7-10 days after initiation of *in vitro* roots (Fig. 1C). Finally, the plantlets were transferred to pots containing nutrient growth media (Fig. 1D). However, these were hardened under controlled conditions in greenhouse which was required for the growth and development of the plantlets.

Table 2. Effect of different concentrations of growth regulators on number of days taken for shoot bud initiation and number of shoots proliferated per explant in cv Kufri Neelkanth. *SR-Shoot regeneration. Results are presented as mean±SE, and with different letters in the same column indicate significant differences at $p<0.05$ analyzed by Duncan's multiple range test.

Medium code SR*	Concentration of growth regulators (mg l^{-1})	Average number of days taken for bud initiation	Average number of shoots per explant
SR_0	MS Basal	5.1±0.12^{g}	2.4±0.07^{abc}
SR_1	BAP 0.25	3.4±0.11^{b}	3.1 ±0.07^{bdc}
SR_2	BAP 0.5	3.9±0.09^{e}	2.8±0.11^{cde}
SR_3	KIN 0.25	3.8±0.11^{d}	2.8±0.07^{cd}
SR_4	KIN 0.5	4.0±0.14^{f}	2.4±0.05^{ab}
SR_5	0.25 BAP + 0.25 KIN	3.7±0.10^{c}	2.7±0.12^{cdf}
SR_6	0.25 BAP + 0.5 KIN	3.3±0.08^{a}	3.5±0.12^{acd}

Effect of different potting mixture on survival of plantlets

Survival percentage obtained on different potting mixture after hardening has been shown in Table 5. Maximum survival percentage (100%) was recorded in PHM_7 (coco peat + vermiculite + perlite in 3:1:1) in the potato cultivar Kufri Neelkanth (Fig. 1E). The survival percentage was further decreased when FYM was used alone in the cultivar. Minimum survival percentage of regenerated plantlets (55%) was recorded in potting mixture PHM_1 (Sand).

Genetic assessment of *in vitro* raised plants by using RAPD markers

Plantlets obtained from meristems could be more genetically stable than plantlets derived through other *in vitro* procedure - leaf discs, (Slack 1980). RAPD analysis was performed to test genetic fidelity of plants randomly selected from regenerated populations of *in vitro* plants and mother plants. In cv Kufri Neelkanth four primers produced amplification (Table 6) while rest sixteen primers did not show any amplification. DNA banding patterns of all tissue culture raised plants and mother plants were found similar showing monomorphism explaining that all

Table 3. Effect of different growth regulators on number of *in vitro* shoots formed after fix interval of time after subculturing in cv Kufri Neelkanth. *SM-Shoot multiplication. Results are presented as mean±SE, and with different letters in the same column indicate significant differences at $p<0.05$ analyzed by Duncan's multiple range test.

Medium code *SM	Concentration of growth regulators (mg/l)	Average number of shootlets on 7th day	14th day	21st day	28th day	35th day
SM_0	MS Basal	1.3±0.00^{u}	1.5±0.10^{uv}	1.9±0.13^{ts}	2.1±0.29^{uvw}	4.6±0.20^{bt}
SM_1	BAP 0.25	1.4±0.10^{u}	1.7±0.13^{vt}	1.5±0.13^{rw}	2.3±0.17^{ux}	4.9±0.20^{bs}
SM_2	BAP 0.5	1.0±0.00^{v}	1.5±0.10^{uv}	1.5±0.10^{rw}	2.2±0.10^{ux}	4.9±0.23^{bs}
SM_3	BAP 0.75	1.1±0.10^{v}	1.2±0.10^{tu}	1.4±0.10^{tr}	2.6±0.20^{dr}	4.5±0.10^{ys}
SM_4	BAP 1.0	1.2±0.10^{u}	1.3±0.00^{tu}	1.4±0.10^{tr}	2.1±0.10^{er}	5.4±0.10^{szy}
SM_5	BAP 1.25	1.1±0.10^{v}	1.3±0.17^{ur}	1.6±0.20^{rw}	3.0±0.20^{dr}	5.9±0.20^{dz}
SM_6	KIN 0.25	1.1±0.10^{v}	1.3±0.00^{tu}	1.4±0.10^{tr}	2.7±0.13^{dr}	5.4±0.10^{szy}
SM_7	KIN 0.5	1.0±0.00^{v}	1.2±0.10^{tu}	1.4±0.10^{tr}	2.9±0.13^{dr}	4.6±0.20^{ys}
SM_8	KIN 0.75	1.2±0.10^{u}	1.5±0.10^{uv}	1.6±0.00^{rw}	3.2±0.10^{rz}	6.1±0.10^{ds}
SM_9	KIN 1.0	1.1±0.10^{v}	1.3±0.17^{ur}	1.5±0.10^{rw}	3.0±0.20^{dr}	4.5±0.10^{ys}
SM_{10}	KIN 1.25	1.2±0.10^{u}	1.4±0.10^{tu}	1.7±0.13^{rw}	2.4±0.10^{ux}	4.4±0.20^{ys}
SM_{11}	0.25 BAP + 0.25 KIN	1.4±0.10^{u}	1.7±0.13^{vt}	2.1±0.10^{ql}	3.7±0.29^{qz}	6.5±0.10^{zg}
SM_{12}	0.25 BAP + 0.01 NAA	1.7±0.13^{x}	2.0±0.00^{wx}	2.2±0.20^{ql}	3.8±0.23^{qz}	6.6±0.20^{zg}
SM_{13}	0.25 BAP + 0.01 IAA	1.3±0.00^{u}	1.7±0.13^{vt}	2.7±0.13^{rv}	4.5±0.10^{qz}	10.2±0.66^{tuw}
SM_{14}	0.25 BAP + 0.01 IBA	1.4±0.10^{u}	2.1±0.23^{vx}	2.9±0.13^{rv}	3.9±0.13^{qz}	8.6±0.20^{tzx}
SM_{15}	0.25 KIN + 0.01 NAA	2.2±0.10^{w}	2.3±0.17^{vw}	3.7±0.13^{zy}	4.3±0.33^{qz}	10.4±0.59^{tvu}
SM_{16}	0.25 KIN + 0.01 IAA	1.3±0.17^{y}	1.5±0.10^{uv}	2.7±0.13^{rv}	3.3±0.17^{rz}	5.6±0.38^{szu}
SM_{17}	0.25 KIN + 0.01 IBA	2.1±0.10^{s}	2.2±0.10^{vx}	3.1±0.29^{vs}	4.2±0.10^{utr}	7.7±0.59^{zwx}
SM_{18}	0.01 BAP + 0.25 GA_3 + 0.01 NAA	2.3±0.17^{t}	2.5±0.10^{zu}	3.3±0.17^{wf}	4.3±0.33^{qz}	8.7±0.29^{tzx}
SM_{19}	0.25 BAP + 0.25 GA_3 + 0.01NAA	1.8±0.13^{r}	2.1±0.1^{wx}	2.6±0.00^{us}	4.1±0.39^{zyx}	8.6±0.20^{tzx}
SM_{20}	0.25 BAP + 0.25 GA_3 + 0.01 IAA	1.5±0.10^{z}	1.9±0.20^{vx}	2.3±0.17^{zu}	3.5±0.10^{uyr}	7.7±0.13^{xwy}
SM_{21}	0.25 BAP + 0.25 GA_3 + 0.01 IBA	1.2±0.10^{u}	1.6±0.00^{uv}	2.8±0.23^{us}	4.1±0.10^{qz}	8.4±0.10^{yzw}
SM_{22}	0.25 BAP + 0.25 GA_3 + 0.02 NAA	1.3±0.00^{u}	1.4±0.10^{tu}	1.7±0.13^{rw}	3.7±0.13^{tuy}	6.9±0.49^{zg}
SM_{23}	0.25 BAP + 0.25 GA_3 + 0.02 IAA	1.6±0.00^{z}	1.9±0.20^{vx}	2.3±0.17^{zu}	4.0±0.20^{tuy}	7.4±0.59^{zg}
SM_{24}	0.25 BAP + 0.25 GA_3 + 0.02 IBA	1.4±0.10^{u}	1.5±0.10^{uv}	1.7±0.13^{rw}	3.4±0.29^{rz}	6.6±0.38^{ds}

the plants raised through tissue culture using shoot tips were true to type or genetically identical to the mother plant (Fig. 2). Consequently, this affirms the genetic stability of plants raised through tissue culture. Evaluating the genetic fidelity or variability of *in vitro* plants is of utmost importance in order to establish appropriate conservation programs (Butiuc-Keul *et al.* 2016).

DISCUSSION

In vitro propagation is the better option over conventional propagation of potato for extensive production of homogeneous and identical seed material of potato. Explant plays a vital role in their response to *in vitro* propagation system for shoot regeneration protocol. Various types of explants, including axillary buds, shoot tips and nodal stems, are frequently employed in micropropagation and germplasm conservation practices (Zhang *et al*. 2017). Generally, highly meristematic tissue of a virus infected plant remains free from virus due to their fast mitotic activity. Plantlets derived from shoot tip are easily by the quarantine authority for international exchange without any checking. So, we have used young shoot tips as explant in our experiment for better conservation of germplasm. In contrast to previous studies that

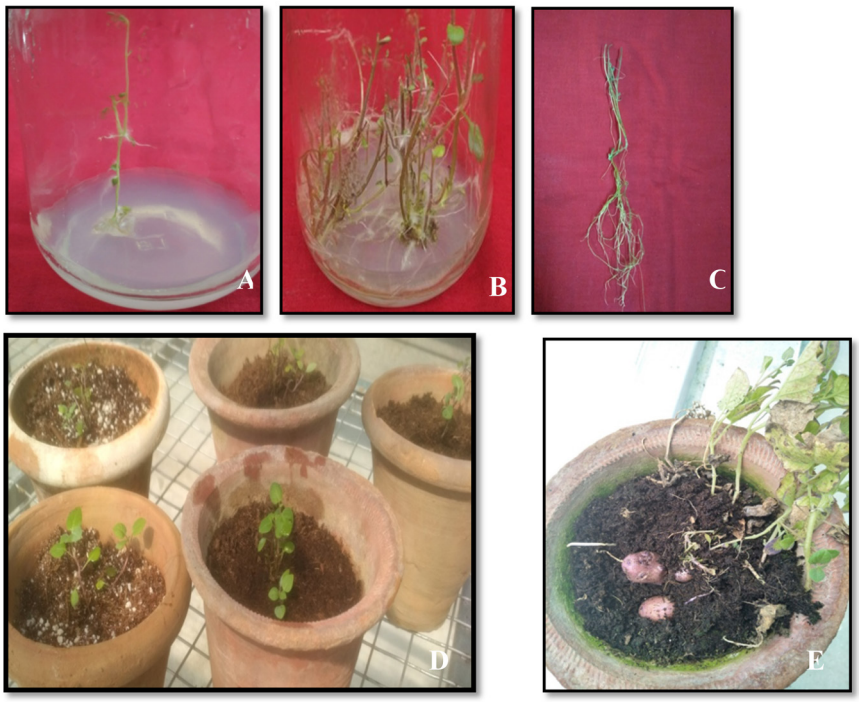

Fig. 1. Micropropagation in potato cultivar Kufri Neelkanth. A- *In vitro* establishment of shoot tips of potato cv Kufri Neelkanth. B- *In vitro* multiplication of potato cv Kufri Neelkanth. C- *In vitro* rooting of potato cv Kufri Neelkanth. D- Hardened plants of potato cv Kufri Neelkanth. E- Tubers of potato cv Kufri Neelkanth formed from tissue culture raised plants.

utilized nodal segments for shoot regeneration (Shah Zaman *et al.* 2001, Badoni and Chauhan 2010, El Dessoky *et al.* 2016), it was observed that shoot tips were more effective in producing early regenerants. In this experiment, about 6-8 weeks were required to complete *in vitro* propagation cycle rather than 8-10 weeks in other conventional methods. Sucrose alone accounts 34% of the production cost. Additionally, utilizing a single shoot tip allowed us to generate over 12 shootlets, resulting in a yield of approximately 2-3 kg per plantlet under field conditions. Therefore, the protocol described in the study can be considered an improved protocol for efficient plant regeneration system for potato cv Kufri Neelkanth.

Plant growth regulators (PGRs) play a critical role in shoot regeneration and proliferation of potato (Fabian *et al*. 2000). Our results reflected that the PGR applications were efficient to shorten days to shoot bud initiation compared to regeneration on control medium. The findings of this study align with the observations made by Salem and Hassanein (2017) regarding the substantial effects of BAP and KIN on shoot bud initiation in potato explants cultured

Table 4. Effect of different growth regulators on number of days required for root initiation and number of roots formed per shoot in cv Kufri Neelkanth. *IR-*In vitro* rooting. Results are presented as mean±SE and with different letters in the same column indicate significant differences at $p<0.05$ analyzed by Duncan's multiple range test.

Medium code IR*	Concentration of growth regulators (mg l^{-1})	Rooting percentage	Days to be required for rooting	Average number of roots per shoot
IR_1	IBA 0.5	100	3.2±0.11pqr	10.0±0.32asd
IR_2	IBA 1.0	100	2.8±0.10prq	10.6±0.25afh
IR_3	IBA 1.5	100	2.9±0.21pqr	11.4±0.51jyg
IR_4	IBA 2.0	100	2.8±0.12prq	12.0±0.71plm
IR_5	IBA 2.5	100	2.3±0.11pqe	13.0±0.55uhn
IR_6	NAA 0.5	100	2.8±0.15prq	11.4±0.40yrt
IR_7	NAA 1.0	100	2.9±0.13vsy	11.2±0.51upn
IR_8	NAA 1.5	100	3.0±0.15pqr	10.8±0.37asd
IR_9	NAA 2.0	100	2.5±0.16prq	10.8±0.37asd
IR_{10}	NAA 2.5	100	2.1±0.07mnp	11.4±0.87yrt

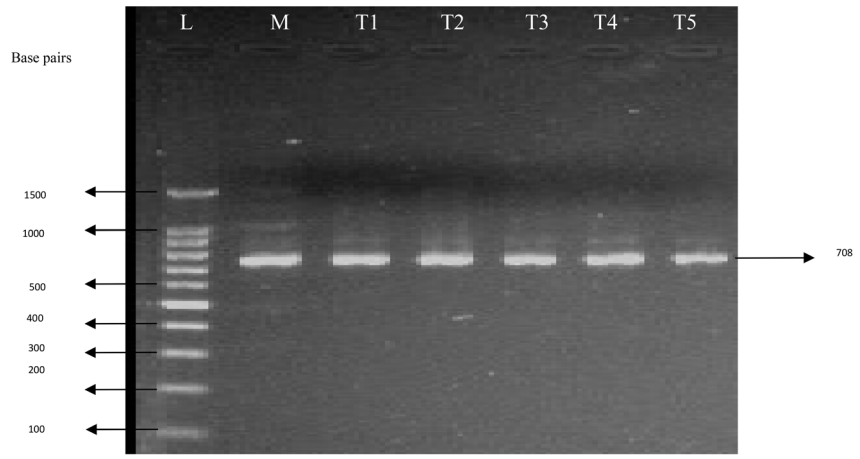

Fig. 2. RAPD profiles of mother plant and *in vitro* raised plants of Kufri Neelkanth using primer OPB-11.
L: Ladder, M: Mother plant DNA, T_1: 1st Tissue culture plant DNA, T_2: 2nd Tissue culture plant DNA, T_3: 3rd Tissue culture plant DNA, T_4: 4th Tissue culture plant DNA, T_5: 5th Tissue culture plant DNA.

in vitro. The ability to easily multiply the increased number of shoots for further multiplication makes the number of shoots per explant an important parameter for assessing explant growth rate. The outcomes of the present experiment were in consensus with the findings of Sarder (2010), who regenerated the better results with the combination of BAP and KIN comprised medium, and emphasized this application was superior to using BAP or KIN alone for multiple shoot induction. The outcomes of our study differed from the findings reported by Bhuiyan (2013) and Hajare *et al.* (2021). Bhuiyan (2013) observed that the use of BAP (0.5–1.0 mg l^{-1}) in the growth medium yielded the most favorable results for *in vitro* establishment compared to other treatments involving plant growth regulators (PGRs). Hajare *et al.* (2021) obtained highest number of shoots in MS medium supplemented with NAA and BAP. However, the results may vary due to influence of various conditions like cultivar, type of explant, PGR concentration and combinations.

Integration between auxins and cytokinins influenced the rate of endogenous auxin through preventing the oxidation of excess NAA to keep up the optimum level for inducing shoot morphogenesis (Emaraa *et al.* 2017). The data of the current research are in complete harmony with Emaraa *et al.* (2017) who obtained the maximum number of shoots per plant from culturing the explant on MS medium supplemented with NAA and KIN. The results were not similar to the findings of Rabbani *et al.* (2001), Hussain *et al.* (2005) and Hajare *et al.* (2021). Rabbani *et al.* (2001) found that the maximum number

Table 5. Effect of different potting mixture on survival percentage of *in vitro* raised plantlets of potato cv Kufri Neelkanth. *PHM-Potato hardening mixture.

Sl. No.	Potato hardening mixture (PHM*)	Percent survival of Kufri Neelkanth
1	PHM_1 (sand)	55.0
2	PHM_2 (sand+FYM in 1:1)	65.0
3	PHM_3 (soil+sand+FYM in 1:1:1)	75.0
4	PHM_4 (soil+sand+vermicompost in 1:1:1)	90.0
5	PHM_5 (sand + soil+ FYM+ vermicompost in 1:1:1:1)	85.0
6	PHM_6 (peat moss)	95.0
7	PHM_7 (coco peat + vermiculite + perlite in 3:1:1)	100.0

Table 6. Random primers showing amplification in cv Kufri Neelkanth.

Sl. No.	Primer sequence (5'-3')
1	OPB-01 (GTTTCGCTCC)
2	OPB-04 (GGACTGGAGT)
3	OPB-11 (GTAGACCCGT)
4	OPB-12 (CCTTGACGCA)

of shoots were formed in potato variety desiree when 2.0 mg l^{-1} BAP was used. Hussain *et al.* (2005) found that the cultivars Altamash and Diamond exhibited the highest number of shoots when using nodal cuttings on a medium containing 2.0 mg l^{-1} of BAP in combination with MS medium. Hajare *et al.* (2021) achieved the highest number of multiple shoots by incorporating 2.5 mg l^{-1} of KIN in the MS medium. Additionally, other researchers have also reported that the combination of BAP, KIN and ascorbic acid produces favorable results for regeneration or the formation of multiple shoots from apical shoots (Rout *et al.* 2001). The results may be different because of seasonal effect, type of explants, environmental influence of explants sources.

The results of this study showed edge of IBA regenerated shoots to induce higher number of roots and enhanced rooting efficiency indicate that IBA supplemented medium prepares plantlets for easy acclimatization to *ex vitro* conditions and increases their chance to survival under greenhouse conditions. The results largely support the findings of Badawi *et al.* (1996), Rabbani *et al.* (2001) and Khadiga *et al.* (2009). According to their findings, the optimal rooting response in potatoes was observed when the concentration of IBA was higher compared to NAA and IAA in a combination of the two. On the contrary, Mohapatra *et al.* (2016) demonstrated that maximum *in vitro* rooting was observed on MS basal medium fortified with 2.0 mg l^{-1} NAA after 10 days in Kufri Frysona. The differences in combinations of PGRs for *in vitro* rooting are most likely due to different cultivars with different physiological conditions.

It is pertinent to ensure that the genetic stability of micropropagated plants and molecular markers are reliable for monitoring the genetic fidelity of tissue culture raised plants (Debnath and Arigundam 2020). Based on the assessment of genetic fidelity through RAPD primers, tissue culture raised plants are genetically identical to the mother plant and did not detect any polymorphic bands. No evidence of morphological variation has been found in this study.

CONCLUSION

The regenerants have been grown in open field conditions after acclimatization. An efficient micropropagation via shoot tips as initial explants was described for potato cv Kufri Neelkanth. The studies could also be further expanded by investigating the factors that affect microtuberization in micropropagated potato plantlets. Furthermore, it is helpful for genetic transformation studies in potato. However, this work will contribute to the national demand for the production of high-quality virus-free potato planting material (cv Kufri Neelkanth), to enhance the yield and productivity of the crop.

REFERENCES

Asakaviciute R (2011) Meristem culture for potato seed production in Lithuania. In: Kuusiene S, Ziauka J, Masalaite R (eds), Advances in plant biotechnology in Baltic sea region, Lithuanian Research Center for Agriculture and Forestry, Kaunas, Lithuanian, pp 65—67.

Badawi MA, El-Sayed SF, Edriss NH, El-Barkouki TM (1996) Factors affecting production of potato plantlets from nodal cutting. *Egyptian J Horticult* 22 : 117—125.

Badoni A, Chauhan JS (2010) *In vitro* Sterilization Protocol for Micropropagation of *Solanum tuberosum* cv 'Kufri Himalini'. *Academia Arena* 2 (4) : 24—27.

Bhuiyan FR (2013) *In vitro* Meristem Culture and Regeneration of Three Potato Varieties of Bangladesh. *Res in Biotechnol* 4 (3) : 29—37.

Biniam T, Tadesse M (2008) A survey of viral status on potatoes grown in *Eritrea* and *in vitro* virus elimination of a local variety 'Tsaeda embaba'. *African J Biotechnol* 7(4) : 397—403.

Butiuc-Keul A, Farkas A, Cristea V (2016) Genetic Stability Assessment of *in vitro* Plants by Molecular Markers. *Studia Universitatis Babes-Bolyai, Biologia* 61 (1) : 107—114.

Debnath SC, Arigundam U (2020) *In vitro* Propagation Strategies of Medicinally Important Berry Crop, Lingonberry (*Vaccinium vitis-idaea* L.). *Agronomy* 10 (5) : 744.

De Morais TP, Asmar SA, de Jesus Silva HF, Luz J MQ, de Melo B (2018) Application of tissue culture techniques in potato. *Biosci J* 34 (4) : In press.

El Dessoky S, Attia AO, Ismail IA, El-Hallous EI (2016) *In vitro* Propagation of Potato under Different Hormonal Combinations. *Internat J* 4 (1) : 684—689.

Emaraa HA, Hamza EM, Fekry WA (2017) *In vitro* propagation and microtuber formation of potato in relation to different concentrations of some growth regulators and sucrose. *Middle East J Agric Res* 6 (4) : 1029—1037.

Fabian T, Lorbiecke R, Umeda M, Sauter M (2000) The cell cycle genes cycA1, 1 and cdc2Os-3 are coordinately regulated by gibberellin in planta. *Planta* 211 : 376—383.

Farokhzadeh SARA, Fakheri BA (2014) Marker-assisted selection for disease resistance : Applications in breeding. *Internat J Agric Crop Sci (IJACS)* 7(14) : 1392—1405.

George P, Manuel J (2013) Low cost tissue culture technology for the regeneration of some economically important plants for

developing countries. *Internat J Agriculture, Environ Biotechnol* 6 (4S) : 703.

Hajare ST, Chauhan NM, Kassa G (2021) Effect of growth regulators on *In vitro* Micropropagation of Potato (*Solanum tuberosum* L.) Gudiene and Belete Varieties from Ethiopia. *The Scientific World Journal*.

Hussain IQBAL, Muhammad AISH, Chaudhry Z, Naqvi RASMS, Rashid H (2005) Morphogenic potential of three potato (*Solanum tuberosum*) cultivars from diverse explants, a prerequisite in genetic manipulation. *Pak J Bot* 37(4) : 889.

Khadiga G, Rasheid SM, Mutasim MK (2009) Effect of cultivar and growth regulator on *in vitro* micropropagation of potato (*Solanum tuberosum* L). *American-Eurasian J Sustainable Agric* 3(3) : 487—492.

Madhu SV, Savithramma DL (2014) Standardization of high efficient and rapid regeneration protocol for Agrobacterium mediated transformation of tomato (*Solanum lycopersicum* L.) cv Pusa Ruby, Vaibhav and ArkaMeghali. *Internat J Agron Agricult Res* 5 : 161—169.

Milinkovic M, Horstra CB, Rodoni BC, Nicolas ME (2012) Effects of age and pretreatment of tissue cultured potato plants on subsequent mini-tuber production. *Potato Research* 55 : 15—25.

Mohapatra PP, Batra VK, Kajla S, Poonia AK, Kumar NM (2016) Effect of different growth regulators on *in vitro* micro-propagation of Kufri Frysona. *J Appl Natural Sci* 8 (2) : 535—540.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco cultures. *J Pl Physiol* 15 : 473—479.

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res* 8 : 4321—4325.

Nielson M, Iritani WM, Weiler LD (1989) Potato seed productivity: Factors influencing eye number per seed piece and subsequent performance. *American Potato J* 66 (3) : 151—160.

Pereira JES, Fortes GRDL (2003) Protocolo para produção de material propagativo de batata em meio líquido. *Pesquisa Agropecuária Brasileira* 38 (9) : 1035—1043.

Rabbani ASMA, Askari BEENISH, Abbasi NA, Bhatti MUSSARAT, Quraishi AZRA (2001) Effect of growth regulators on *in vitro* multiplication of potato. *Internat J Agricult Biol* 3 (2) : 181—182.

Ranalli P (1997) Innovative propagation methods in seed tuber multiplication programmes. *Potato Res* 40 (4) : 439—453.

Roca WM, Espinoza NO, Roca MR, Bryan JE (1978) A tissue culture method for rapid propagation of potatoes. *American Potato J* 55 (12) : 691—701.

Rout GR, Palai SK, Samantaray S, Das P (2001) Effect of growth regulator and culture conditions on shoot multiplica tion and rhizome formation in ginger (*Zingiber officinale* Rosc.) *in vitro*. *In vitro Cellular Developmental Biology-Plant* 37 (6) : 814—819.

Rykbost KA, Lockeil KA (1999) Effect of seed piece size on performance of three potato varieties in the Klamath Basin of Oregon. *American Journal Potato Research* 76 (2) : 75—82.

Sagai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley : Mendelian inheritance, chromosomal location and population dynamics. *Proceedings of the National Academy Sci* 81 : 8014—8018.

Salem J, Hassanein AM (2017) *In vitro* propagation, microtuberization and molecular characterization of three potato cultivars. *Biologia Plantarum* 61 (3) : 427—437.

Sarder NU (2010) *In vitro* propagation of elite indigenous potato (*Solanum tuberosum* L. var. Indurkani) of Bangladesh. *J Pl Sci* 5 (3) : 293—297.

Shah Zaman M, Quraishi A, Hassan G, Sardar R, Khabir A, Gul N (2001) Meristem Culture of Potato (*Solanum tuberosum* L.) for Production of Virus-Free Plantlets. *On Line Journal of Biological Sciences* 1 (10) : 898—899.

Siddiqui SU, Chaudharay MF, Anwar R (1996) *In vitro* preservation of potato (*Solanum tuberosum* L.) germplasm. *Pakistan Journal Botany* 28 : 37—40.

Slack SA (1980) Pathogen-free plants by meristem-tip culture. *Plant Disease* 64 (1) : 14—17.

Xu GW, Magill CW, Schertz KF, Hart GE (1994) An RFLP linkage map of *Sorghum bicolor* (L.). Moench. *Theoretical Applied Genetics* 89 : 139—145.

Zhang Y, Sun D, Hu S (2017) *In vitro* propagation of medicinal and ornamental plant *Lysimachia davurica*. *European J Horticult Sci* 82 (1) : 54—60.

Zilberman D, Henikoff S (2007) Genome-wide analysis of DNA methylation patterns. *Development* 134 : 3959—3965.