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In vitro Propagation and Genetic Assessment by using Markers in Potato (*Solanum tuberosum* L.) cv Kufri Ganga

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ABSTRACT

The purpose of this study was to determine the best types and concentrations of plant growth regulators to use during different stages of micropropagation, as well as to assess the genetic fidelity of tissue culture raised plants of a cv Kufri Ganga using reliable markers. According to the data on shoot bud initiation, a combination of 0.25 mgl^{-1} BAP and 0.5 mgl^{-1} Kinetin results in the greatest number of shoots per explant (3.4±0.11) in the shortest amount of time (3.2±0.09) days. The maximum number of *in vitro* shoots per shootlet (4.7±0.15) was recorded when auxins were combined with cytokinin (MS medium + 0.25 mgl^{-1} BAP + 0.01 mgl^{-1} IAA) for shoot proliferation. Root initiation was observed in (2.2±0.10) days on MS medium fortified with 1.5 mgl^{-1} NAA. The maximum number of *in vitro* roots per shoot (14.2±0.31) was observed when MS media was fortified with 2.0 mgl^{-1} IBA. Molecular markers were used to assess genetic fidelity in plants grown *in vitro*. Monomorphic DNA banding patterns were found in all tissue culture raised plants and mother plants, indicating true to type planting material. This protocol for tissue culture propagation, as well as testing its genetic fidelity, may be beneficial for the successful application of better conservation of germplasm.

Keywords *In vitro,* Shootets, Auxins, Cytokinins, *Solanum tuberosum* L.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the significant industrial crops amply produced all over the world, owing to its enumerative price and wide consumption as staple food item (Singh *et al.* 2021). Potato contains good amounts of carbohydrates (mainly starch), superior quality proteins, dietary fiber and negligible amounts of fat (Kaur and Aggarwal 2016) and is a good supplement to the cereal diets. To fully capitalize on modern production techniques in crops like potato, such as high-yielding varieties and quality planting material, certain challenges need to be addressed. These challenges arise due to

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the genetic characteristics, propagation methods and susceptibility to diseases and pests associated with vegetatively propagated crops like potato. Potato seed production faces inherent limitations such as a slow multiplication rate, high seed requirement, progressive accumulation of degenerative viral diseases, perishability and bulkiness of the seed. These factors contribute to a scarcity of sufficient quantities of high-quality planting material available at an affordable price. Consequently, the inadequacy of affordable, quality planting material and the high seed rate translate to a significant portion, approximately 40-50%, of the overall cultivation costs being allocated solely to potato seed. Therefore, to build up ample seed stocks, the initial disease-free tuber material needs to be field multiplied for several years. Over time, as potato seeds are multiplied, viral diseases progressively accumulate, leading to the degradation or depletion of seed stocks. To address these issues, various measures have been developed and incorporated into conventional potato seed production programs. These include techniques like tuber indexing to ensure virus-free status, implementing multiple stages of seed multiplication and establishing certification standards for seed quality. These modifications aim to overcome some of the challenges associated with potato seed production and ensure the availability of healthier and more reliable planting material (Naik and Buckseth 2018). Researchers are always interested in improvement of tissue culture techniques of potato to increase high-quality pathogen-free plantlets production with consistent genetic and physiological traits that are highly efficient in terms of photosynthesis (Pruski *et al.* 2001, Mohapatra and Batra 2017, De Morais *et al.* 2018). *In vitro* techniques could also be employed to stimulate the strength and improvement of potato breeding programs and to overcome negative effects related to conventional breeding methods (Tazeb 2017). Previous reports suggest that the regeneration of potato is highly dependent on explant, PGR combinations and cultivar gown (Hussain *et al.* 2005, Kumlay 2014, Naqvi *et al.* 2019). Under appropriate growth conditions, potato shoots, roots and stem explants with nodes have ability to regenerate, even without any plant growth regulators (PGRs) (Kolachevskaya *et al.* 2019). However, regeneration of plantlets without PGRs is time extensive inducing low number of shoots, leaves and roots per explants (Kumlay 2014). Consequently, the introduction of exogenous plant growth regulators (PGRs) serves as a viable method to expedite the regeneration of potato plantlets and shorten the growth cycle in *in vitro* micropropagation (Mendel *et al.* 2020).

MATERIALS AND METHODS

The present study on "*In vitro* propagation and genetic fidelity testing in potato (*Solanum tuberosum* L.) cv Kufri Ganga" was carried out at Center for Plant Biotechnology (CPB), Department of Science and Technology, Haryana, Chaudhary Charan Singh Haryana Agricultural University, New campus, Hisar. Kufri Ganga is a high yielding, medium maturing, white tuber cultivar with late blight resistance and outstanding keeping quality under ambient storage conditions that is appropriate for cultivation in the country's plateau regions. Kufri Ganga is also notable for its consistent tuber size and compatibility for early planting heat stress circumstances. This cultivar yields a lot of medium-sized tubers, which is a desirable trait. The promising results were obtained by using shoot tips as explants in our study. To obtain a sufficient number of *in vitro* shoots, we cultivated the shoot tips explants on a modified MS basal medium (Murashige and Skoog 1962) containing MS inorganic salt (Myo-inositol, vitamins, and table sugars), as well as various quantities and combinations of BAP and KIN. After 2-3 weeks, the *in vitro* shootlets were removed from axenic cultures and cultivated on multiplication medium containing MS basal salt enriched with various amounts and combinations of growth hormones such as GA_3, BAP, KIN, NAA, IBA, and IAA under aseptic environmental conditions.

In vitro establishment of explants

The sterilized shoot tip explants of the cultivar Kufri Ganga were inoculated on various MS media having different concentrations and combinations of cytokinins. The cultures were incubated in a growth environment with a temperature of 25±1°C and fluorescent tubes that supplied a light intensity of 1000 lux. A 16-hour light/eight-hour dark photoperiod was provided.

In vitro multiplication of potato cultivar Kufri Ganga

In vitro proliferated shoots obtained on shoot bud initiation media were inoculated on different diffs multiplication medium having MS medium fortified with different concentrations and combinations of growth hormones like GA_3, BAP, KIN, NAA, IBA and IAA for multiple shoot formation. The inoculated cultures were kept in culture room under the same conditions (as above).

In vitro rooting of potato cultivar Kufri Ganga

The proliferated shootlets obtained were separated aseptically and were transferred on MS basal medium supplemented with different concentrations of auxins (IBA, NAA) after 28 days for rooting. The same conditions (as above) were maintained for the inoculated cultures kept in culture room.

Rooting % regeneration

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

Hardening of potato cultivar Kufri Ganga

The *in vitro* plants of the cultivar were obtained after few days and washed under running tap water to remove sticking gel from the plants. Different potting mixtures (sand, FYM, soil, vermicompost, peat moss, cocopeat, perlite and vermiculite) were used for growing in vitro raised potato plants under greenhouse conditions.

DNA isolation and genetic fidelity testing by using RAPD primers

In the experiment, the genetic fidelity/stability of *in vitro* raised plants of potato cultivar (Kufri Ganga) was tested by using 20 RAPD (10-mer) primers (Table 1). DNA was isolated from the young leaves of mother plants and 150 randomly selected *in vitro* raised plants of potato. Genomic DNA was isolated from a sample of 5 g young leaves of i*n vitro* raised plants and mother plants of potato cultivar Kufri Ganga following modified Cetyl Trimethyl Ammonium Bromide (CTAB) extraction method modified by Sagai-Maroof *et al.* (1984) and Xu *et al.* (1994). 20 µl reaction volume containing 2 µl of 20 ng/µl template DNA, 2.5 µl of 250 µM dNTPs mix, 2.0 µl of 10 µM primer, 0.3µl of 1X *Taq* DNA polymerase buffer and 0.3 µl of 1.6 Units/µL *Taq* DNA polymerase was taken to perform PCR. 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 thermal cycler. For complete separation of bands, the PCR products were then run on 1.5% agarose gel at 70V for 2h. Gel Doc System was used for scanning the gel.

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

Data analysis

The data related to various characters for shoot bud initiation, shoot multiplication and rooting were recorded in replicated form using Complete Random-

ized 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 were recorded for number of *in vitro* shoots formed after fixed interval of time i.e. 7th, 14th, 21st and 28th day of inoculation. The number of responding explants per treatment, shoots per shootlet and roots per shoot were counted and the results were examined sophisticatedly. The whole experiment was performed thrice. 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.

RESULTS

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

Surface sterilized explants of the cultivar of potato Kufri Ganga were inoculated on MS medium fortified with different concentrations and combinations of growth regulators. The data for *in vitro* establishment of Kufri Ganga for shoot bud initiation and number of days taken for bud initiation were recorded. Results obtained for shoot bud initiation and number of days taken for bud initiation for the cultivar is presented in Table 2.

Table 2. Effect of different concentrations growth regulators on *in vitro* establishment of shoot tip explants of potato cv Kufri Ganga: Number of days taken for bud initiation and number of buds per explant.

Medium code *PM	Concentration of growth regulators (mgl^{-1})	Average number of buds per explant	Average number of days taken for bud initiation
PM_0	MS medium	2.2±0.05	4.9±0.14
PM_1	BAP 0.25	3.0 ±0.09	3.5±0.10
PM_2	BAP 0.50	2.7±0.17	3.8±0.09
PM_3	KIN 0.25	2.5±0.10	3.7±0.13
PM_4	KIN 0.50	2.4±0.04	3.9±0.17
PM_5	0.25 BAP + 0.25 KIN	2.8±0.13	3.5±0.10
PM_6	0.25 BAP + 0.50 KIN	3.4±0.11	3.2±0.09

*PM-Potato medium.

The Table 2 reveals that the maximum buds (3.4) were reported on shoot tip explants of cv Kufri Ganga on medium PM_6 (MS medium + 0.25 BAP + 0.50 KIN mgl^{-1}) in minimum 3.2 days followed by medium PM_1 (MS medium + BAP 0.25 mgl^{-1}) with 3.0 buds in 3.5 days. The least number of buds (2.4) were observed on medium PM_4 (MS medium + KIN 0.50 mgl^{-1}) in 3.9 days. It was found that the BAP was found to be more effective than kinetin at the concentration of 0.25 mgl^{-1} for shoot bud initiation in cv Kufri Ganga. The medium PM_6 (MS medium + 0.25 BAP + 0.50 KIN mgl^{-1}) containing BAP and kinetin was found most effective amongst the array of media used for *in vitro* establishment of shoot tip

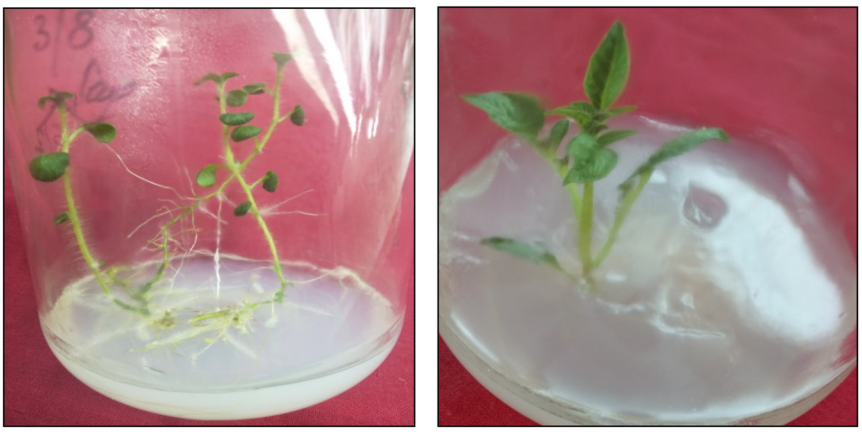

Fig. 1. *In vitro* establishment of shoot tips of potato cv Kufri Ganga on medium PM_6 (MS medium + 0.25 mg/l BAP + 0.50 mg/l KIN).

Table 3. Effect of different concentrations of growth regulators on *in vitro* multiplication of potato cv Kufri Ganga: Number of shoots per explant after fix interval of time.

Medium code *PM	Concentration of growth regulators (mg/l)	Average number of shootlets 7th day	14th day	21st day	28th day
PM_0	MS medium	1.2±0.11	1.5±0.10	1.9±0.13	2.3±0.17
PM_1	BAP 0.25	1.4±0.11	1.5±0.10	1.7±0.13	2.4±0.29
PM_2	BAP 0.5	1.0±0.00	1.2±0.10	1.4±0.10	2.3±0.17
PM_3	BAP 0.75	1.1±0.10	1.3±0.17	1.5±0.23	2.7±0.29
PM_4	BAP 1.0	1.3±0.00	1.5±0.10	1.6±0.20	2.3±0.33
PM_5	BAP 1.25	1.2±0.10	1.5±0.10	1.8±0.23	3.1±0.23
PM_6	KIN 0.25	1.1±0.10	1.4±0.10	1.6±0.20	2.8±0.23
PM_7	KIN 0.5	1.1±0.10	1.4±0.10	1.6±0.00	3.0±0.20
PM_8	KIN 0.75	1.4±0.10	1.5±0.10	1.7±0.13	3.3±0.00
PM_9	KIN 1.0	1.1±0.10	1.4±0.10	1.7±0.13	3.2±0.10
PM_{10}	KIN 1.25	1.1±0.10	1.2±0.10	1.7±0.13	2.7±0.13
PM_{11}	0.25 BAP + 0.25 KIN	1.4±0.10	1.9±0.13	2.2±0.10	3.9±0.13
PM_{12}	0.25 BAP + 0.01 NAA	1.6±0.00	1.9±0.13	2.2±0.10	4.1±0.29
PM_{13}	0.25 BAP + 0.01 IAA	1.4±0.10	2.1±0.10	2.9±0.13	4.7±0.15
PM_{14}	0.25 BAP + 0.01 IBA	1.3±0.00	2.4±0.10	2.8±0.23	3.6±0.20
PM_{15}	0.25 KIN + 0.01 NAA	2.2±0.10	2.4±0.10	3.8±0.20	4.4±0.20
PM_{16}	0.25 KIN + 0.01 IAA	1.3±0.17	1.5±0.29	2.7±0.13	3.4±0.20
PM_{17}	0.25 KIN + 0.01 IBA	1.9±0.13	2.4±0.10	3.2±0.29	4.3±0.17
PM_{18}	0.01 BAP + 0.25 GA_3 + 0.01 NAA	2.2±0.10	2.2±0.10	2.9±0.13	4.2±0.32
PM_{19}	0.25 BAP + 0.25 GA_3 + 0.01NAA	1.9±0.13	2.1±0.10	2.6±0.00	4.2±0.49
PM_{20}	0.25 BAP + 0.25 GA_3 + 0.01 IAA	1.4±0.10	2.2±0.10	2.5±0.29	3.6±0.20
PM_{21}	0.25 BAP + 0.25 GA_3 + 0.01 IBA	1.2±0.10	1.5±0.10	2.9±0.13	4.2±0.10
PM_{22}	0.25 BAP + 0.25 GA_3 + 0.02 NAA	1.4±0.10	1.5±0.10	1.9±0.13	3.8±0.23
PM_{23}	0.25 BAP + 0.25 GA_3 + 0.02 IAA	1.7±0.13	2.1±0.10	2.4±0.10	4.1±0.23
PM_{24}	0.25 BAP + 0.25 GA_3 + 0.02 IBA	1.3±0.17	1.4±0.20	1.9±0.13	3.6±0.20

*PM-Potato medium.

explant of cv Kufri Ganga (Fig. 1). This may be due to the synergetic effect of growth regulators.

In vitro shoot multiplication response

The regenerated shoots produced from explants of both the cultivars were further cultured on MS medium fortified with different concentration of growth regulators alone or in combination. The data were recorded for number of shoots per explant after fixed interval of time i.e., 7th, 14th, 21st and 28th day of inoculation (Table 3).

The data recorded (Table 3) reveals that the maximum number of shoots (4.7) were observed on medium PMT_3 (MS medium + 0.25 mgl^{-1} BAP + 0.01 mgl^{-1} IAA) on 28th day of subculture followed by medium PM_{15} (MS medium + 0.25 mg/l KIN + 0.01 mg/l NAA) with 4.4 shoots on 28th day of subculture. The minimum shoots (2.3) were observed on medium PM_2 (MS medium + 0.5mgl^{-1} BAP) and on PM_4 (MS medium + 1.0 mgl^{-1} BAP) on 28th day of subculture. The table clearly demonstrates that the use of MS medium supplemented with various concentrations of growth regulators in combination proved to be more effective for the *in vitro* multiplication of potato cv Kufri Ganga compared to using individual concentrations of BAP and kinetin. This enhanced effectiveness can be attributed to the synergistic effect of the growth regulators (Fig. 2). Therefore, it is recommended to utilize the combination of BAP and kinetin for the *in vitro* multiplication of potato cv Kufri Ganga.

In vitro rooting

After twenty eight days, the elongated shoots of cultivar Kufri Ganga were transferred to MS medium supplemented with different concentrations of auxins

Fig. 2. *In vitro* multiplication of potato cv Kufri Ganga on medium PM_{13} (MS medium + 0.25 mgl^{-1} BAP + 0.01 mgl^{-1} IAA).

for rooting. Data were recorded for rooting percentage, number of days required for root initiation and number of roots formed per shoot (Table 4).

In the present investigation it can be revealed that 100% rooting was achieved on all media used (Table 4). On 21st day of subculturing, the maximum number of roots per shoot (14.2) in potato cv Kufri Ganga were reported on medium PR_4 (MS medium + 2.0 mgl^{-1}IBA) followed by medium PR_9 (MS medium + 2.0 mgl^{-1}NAA) with 13.2 roots and least number of roots (9.7) were observed on medium PR_1 (MS medium + 0.5 mgl^{-1}IBA). The medium PR_8 (MS medium + 1.5 mgl^{-1} NAA) takes least number of days (2.2) for root initiation. It was observed that the IBA at the concentration of 2.0 mgl-1was more effective for *in vitro* rooting in potato cv Kufri Ganga (Fig. 3).

Survival percentage of regenerated plantlets

After successful *in vitro* rooting, the rooted plantlets were transferred to pot containing different potting mixtures.

Table 4. Effect of different concentrations of auxins on *in vitro* rooting of potato cv Kufri Ganga: Number of days required for root initiation and number of roots formed per shoot (on 21th day).

Sl. No.	Medium code (mgl^{-1}) *PR	Rooting percentage	Days required for root initiation	Average number of roots per shoot (on 21st day)
1	PR1 (IBA 0.5)	100	3.4±0.1	9.7±0.33
2	PR2 (IBA 1.0)	100	3.0±0.14	10.5±0.43
3	PR3 (IBA 1.5)	100	2.7±0.13	10.7±0.33
4	PR4 (IBA 2.0)	100	2.3±0.10	14.2±0.31
5	PR5 (IBA 2.5)	100	2.8±0.15	10.5±0.22
6	PR6 (NAA 0.5)	100	2.8±0.15	10.3±0.42
7	PR7 (NAA 1.0)	100	3.0±0.13	10.8±0.40
8	PR8 (NAA 1.5)	100	2.2±0.10	10.3±0.21
9	PR9 (NAA 2.0)	100	2.3±0.11	13.2±0.31
10	PR10(NAA 2.5)	100	2.4±0.08	10.7±0.49

*PR-Potato rooting.

Fig. 3. *In vitro* rooting of potato cv Kufri Ganga on medium PR_4 (MS medium + 2.0 mg/l IBA).

Fig. 4. Hardened plants of potato cv Kufri Ganga.

The Table 5 depicts the effect of different potting mixture on survival percentage of *in vitro* raised plants of cv. Kufri Ganga. Different potting mixture i.e. sand, soil, FYM and vermicompost were used alone and in combinations. Further, cocopeat, vermiculite and perlite in definite proportion and peat moss separately were also used (Fig. 4). Maximum survival percentage (100%) was recorded on potting mixture POM_7 (coco peat + vermiculite + perlite in 3:1:1) in both cultivars of potato. At par results were obtained on potting mixture POM_6 (peat moss) in both the cultivars. However, survival percentage was further decreased when FYM was used alone in both the cultivars. Minimum survival percentage of

Table 5. Effect of different potting mixture on acclimatization of potato cultivar Kufri Ganga.

Sl. No.	Potting mixture (*POM)	Per cent survival of Kufri Ganga
1	POM_1 (sand)	65.0
2	POM_2 (sand + FYM in 1:1)	75.0
3	POM_3 (sand + soil+ FYM in 1:1:1)	85.0
4	POM_4 (sand + soil+ vermicompost in 1:1:1)	85.0
5	POM_5 (sand + soil+ FYM+ vermicompost in 1:1:1:1)	90.0
6	POM_6 (peat moss)	95.0
7	POM_7 (coco peat + vermiculite + perlite in 3:1:1)	100.0

*POM-Potting mixture.

Table 6. List of RAPD primers showing amplification in cv Kufri Ganga.

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

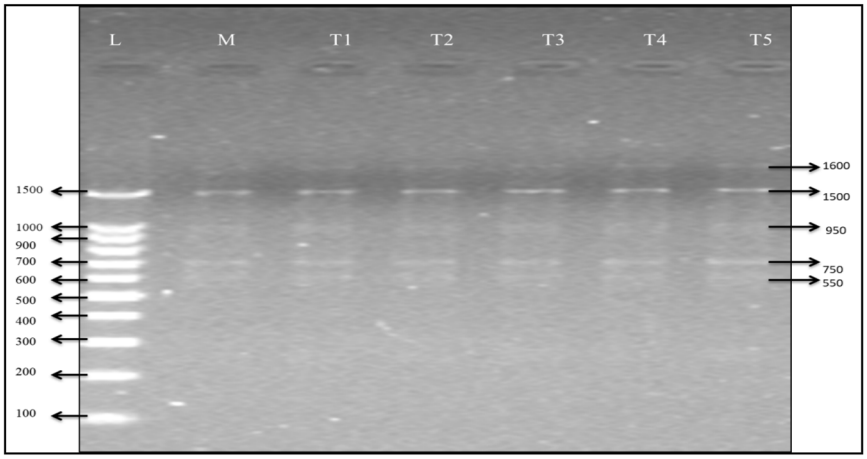

Fig. 5. RAPD profiles of mother plant and *in vitro* raised plants of potato cv Kufri Ganga using primer OPB-04.
L: Ladder (100 bp), M: Mother plant DNA, T1: 1st Tissue culture plant DNA, T2: 2nd Tissue culture plant DNA, T3: 3rd Tissue culture plant DNA, T4: 4th Tissue culture plant DNA, T5: 5th Tissue culture plant DNA.

regenerated plantlets (65%) was recorded in potting mixture POM_1 (Sand).

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

To study the genetic fidelity the DNA was isolated from mother plant of cv Kufri Ganga and 150 tissue culture raised plants were randomly selected from hardened plants of the cultivar. Total 20 Random Amplified Polymorphic DNA (RAPD) primers were screened for genetic fidelity testing. Out of 20 primers screened, four primers produced amplification (Table 6) in the cultivar Kufri Ganga. DNA banding patterns of all tissue culture raised plants and mother plants were found similar and monomorphic which shows that all the plants raised through tissue culture using shoot tips were true to type and were genetically identical to the mother plant. Hence it confirms the genetic stability of tissue culture raised plants in the present study.

Table 6 shows the genetic fidelity testing of potato cultivar Kufri Ganga using RAPD primers. The maximum five bands were reported in primer OPB-12 (Fig. 5), four in OPB-04, and one band in both primers OPB-01 and OPB-11. The banding pattern of mother plant and five tissue culture raised plants were compared. It was found that all bands were monomorphic (Fig. 5). Hence, the plants produced using the present tissue culture protocols were true to type in nature.

DISCUSSION

For large-scale production of homogeneous and identical potato seed material, *in vitro* propagation is preferable to conventional propagation. Explant is critical in their response to the *in vitro* propagation system for shoot regeneration protocol. For micropropagation and germplasm conservation, various explants such as axillary buds, shoot tips, and nodal stems are commonly used (Zhang *et al.* 2017). Because of their rapid mitotic activity, highly meristematic tissue of a virus-infected plant generally remains virus-free. Plantlets derived from shoot tips are easily accepted for international exchange by the quarantine authority without any checks. So, in order to conserve germplasm, we used young shoot tips as explants in our experiment. In this experiment, the *in vitro* propagation cycle took about 6-8 weeks to complete, compared to 8-10 weeks in other conventional methods. Sucrose accounts for 34% of the total production cost (Demo *et al*. 2008). We replaced sucrose in plant propagation medium with table sugar, which reduced the cost of medium by 70-80%. Furthermore, more than 12 shootlets can be produced from a single shoot tip, yielding about 2-3 kg from a single plantlet in the field. As a result, the protocol described in the study is an improved

protocol for an efficient plant regeneration system for potato cv Kufri Ganga. Plant growth regulators (PGRs) are essential for potato shoot regeneration and proliferation (Fabian *et al.* 2000). When compared to regeneration on control medium, our results showed that PGR applications were effective at shortening the days to shoot bud initiation. The results of the current experiment agreed with those of Sarder (2010), who found that combining BAP and KIN comprised medium produced better results, emphasising that this application was superior to using BAP or KIN alone for multiple shoot induction. The findings were not consistent with those of Bhuiyan (2013) and Hajare *et al.* (2021). When compared to the other PGR treatments, Bhuiyan (2013) discovered that explants grown in a medium supplemented with BAP (0.5-1.0 mg l^{-1}) provided the best results for *in vitro* establishment. The most shoots were obtained by Hajare *et al.* (2021) in MS medium supplemented with NAA and BAP. The findings are consistent with those of Salem and Hassanein (2017), who demonstrated that BAP and KIN had significant effects on shoot bud induction in *in vitro* grown potato explants. Because the increased number of shoots can be easily multiplied for further shoot multiplication, the number of shoots per explant is an important parameter to consider when studying the growth rate of explants. The current study's findings are consistent with those of Emaraa *et al.* (2017), who obtained the greatest number of shoots per plant by cultivating the explant on MS medium supplemented with NAA and KIN. The findings did not agree with those of Rabbani *et al.* (2001), Hussain *et al.* (2005), and Hajare *et al.* (2021). Rabbani *et al.* (2001) discovered that using 2.0 mg l^{-1} BAP resulted in the most shoots being formed in the potato variety Desiree. Hussain *et al.* (2005) observed the highest number of shoots in cultivars Altamash and Diamond when nodal cuttings were grown on 2.0 mg l^{-1} BAP medium containing MS medium. In the MS medium containing 2.5 mg l^{-1} KIN, Hajare *et al.* (2021) obtained the highest number of multiple shoots. Furthermore, some researchers discovered that BAP, KIN, and ascorbic acid provide the best results for apical shoot regeneration or multiple shoot formation (Rout *et al.* 2001). The results may vary due to seasonal effects, explant type, and environmental influence of explant sources. The ability of IBA regenerated shoots to induce a higher number of roots and improved rooting efficiency suggests that IBA supplemented medium prepares plantlets for easy acclimatization to *ex vitro* conditions and increases their chances of survival under greenhouse conditions. The findings broadly support those of Badawi *et al. (*1996), Rabbani *et al.* (2001) and Khadiga *et al.* (2009). They demonstrated that the best rooting response in potatoes occurred when IBA concentration was higher than NAA and IAA in a combination of the two. Mohapatra *et al.* (2016), on the other hand, demonstrated that after 10 days, maximum *in vitro* rooting was observed in Kufri Frysona on MS basal medium fortified with 2.0 mg l^{-1} NAA. The differences in PGR combinations for in vitro rooting are most likely due to cultivar differences in physiological conditions. Maintaining the genetic stability of micropropagated plants and establishing the trustworthiness of molecular markers for monitoring the genetic fidelity of tissue culture-derived plants are of utmost importance (Debnath and Arigundam 2020). Tissue culture raised plants are genetically identical to the mother plant, according to RAPD primer analysis, and no polymorphic bands were found. This study found no evidence of morphological variation.

CONCLUSION

An efficient micropropagation via shoot tips as initial explants along with its genetic fidelity was described for potato cv Kufri Ganga. The studies could also be expanded for the successful application of gene transfer technique and better conservation of germplasm. However, this work will contribute to the global demand to produce high-quality virus-free potato planting material, to enhance the yield and productivity of the crop.

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