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

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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 cv. Kufri Pushkar by using reliable markers. The data of shoot bud initiation indicated that combination of 0.25 mg l^{-1} BAP and 0.5 mg l^{-1}. Kinetin leads to highest number of shoots per explant (3.6±0.07) in (3.3±0.08) days. The maximum number of *in vitro* shoots per shoot let (4.4±0.15) were recorded when auxins were used in combination with cytokinin (MS medium + 0.25 mg/l BAP + 0.01 mg/l IAA) for shoot proliferation. *In vitro* root initiation was observed in (2.3±0.10) days on MS medium fortified with 1.5 mg l^{-1} NAA. The maximum number of *in vitro* roots per shoots (14.0±0.37) were observed when MS media fortified with 2.0 mg l^{-1} IBA. *In vitro* raised plants were assessed for genetic fidelity by using molecular markers. 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 successful application of gene transfer technique.

Keywords: Shoot proliferation, micropropagation, *Solanum tuberosum* L., genetic markers.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most widely grown tuber crops in the world, belonging to the Solanaceae family (Solomon- Blackburn and Barker 2001). Potato is a semi-perishable crop that is vegetatively propagated, extremely heterozygous, and tetraploid (4X) (Naik and Buckseuth 2018). Potato tubers have the highest output per hectare of any crop (Burton, 1969) and are widely employed in a wide range of table, processed, livestock feed, and industrial applications (Feustel, 1987). Tubers are the conventional planting material for potato (potato seed). During clonal propagations, all traditional potato seed production technologies have a low multiplication rate and a steady accumulation of degenerative viral infections. A key barrier in potato production is the lack of quality seed at an affordable price at the correct time. As a result, growers are frequently obliged to use locally produced seed despite considerable yield losses (Singh, 2003). The problem is exacerbated by the high seed rate, which means that the cost of seed potatoes alone accounts for 40 to 60% of the overall production cost, putting the crop out of reach for impoverished farmers. Tubers are prone to build up and transmit pathogens (bacterial, fungal, and viral illnesses) to the following generation, which reduces the plant's output capacity (Rolot and Seutin 1999). Potato is susceptible to 40 distinct viral species (Valkonen, 2007), the majority of which are transmitted mechanically with the exception of a few, such as PLRV (Mayo *et al.*, 2000). Nielson *et al.* (1989); Rykbost and Lockeil (1999) show that the poor performance of "cut potato" increases the risk of viral replication, resulting in a significant economic loss for the grower. In the current circumstances, state and central seed production organisations in India are only able to meet roughly 20–25 percent of the demand for high-quality potato seed. The situation is almost similar to the other developing countries. So, in an effort to overwhelm the above constraints in potato production through conventional propagation we adopted micropropagation as an alternate method. Nowadays, micropropagation has become a common method of the seed production system providing disease/virus free plant in a short duration throughout the year. In addition, for large-scale production of healthy seed tubers, large-scale combination of traditional and micropropagation is required (Pandey, 2006). The ability to maintain virus-free plants for an indefinite period of time under

controlled conditions and multiply them in artificial media supplemented with various growth regulators under sterile conditions in the laboratory throughout the year has revolutionised seed production in the potato industry around the world (Naik and Buckseth 2018).

Plant growth regulators (PGRs) are chemical substances that regulate a variety of physiological and biochemical processes in plants. PGRs have the potential to provide opportunities for potato growth and development. Cytokinins play an effective role in potato tuber formation (Sembiring *et al.*, 2020). The addition of GA_3 to the culture media has been shown to promote shoot growth and development, allowing for faster multiplication (Miller and Lipschutz 1984). In a study using nodal fragments from in vitro grown potato plants, Ahmed *et al*. (1993) found that culture media with 4.5 mg L^{-1} GA_3 produced better results. Similarly, BAP boosts the growth of potato plants (Mumtaz and Quraishi 1989). Three to four nodal cuttings (size 1.0–1.5 cm) were taken from each meristem derived in vitro plant and inoculated in fresh culture tubes (three cuttings/tube) or magenta boxes (ten cuttings/tube) containing MS medium supplemented with different growth regulators and incubated under a 16-hour photoperiod (50–60 µmol $m^{-2}s^{-1}$ light intensity) at 24°C in micropropagation of potato under aseptic conditions Within three weeks, the cuttings' axillary/apical buds develop into complete plants, and they can be subcultured on fresh media at a 25-day interval. As a result, from a single virus-free microplant, 315 (14.3 million) microplants can be created in a year. The deployment of liquid culture method leads to an increment of multiplication rates many folds (Naik and Buckseth 2018).

The goal of our study was to create an efficient rapid multiplication system using removed shoot tips of an important cultivar, Kufri Pushkar, in vitro. Plantlets grown in vitro can be utilised for rapid multiplication, microtuber generation (in vitro), minituber creation in the greenhouse (Struik and Lommen 1999), or transplant production followed by field transfer. By propagating virus-tested stem cuttings, 800-900 plants can be grown from a single clone in three years using the traditional approach (Hussey and Stacey 1981). *In vitro* propagation, on the other hand, can generate thousands of plants from a single clone in a single year, albeit at least one generation in the field is required before usage. This technology is a viable option for meeting the ever-increasing demand for high-quality potato seed since it provides for year-round production of disease-free, high-quality seed at the appropriate time.

Many researchers have found de-differentiation and subsequent organogenesis/embryogenesis with associated genetic alterations, hence in vitro propagation methods including meristem tips, nodal cuttings, and micro tubers aid in retaining genetic integrity of multiple clones (Wang and Hu 1982). Using molecular markers to observe the degree of genetic fidelity among in vitro plants is useful to identify variance based on plant proteins, which are expressed from specific areas of DNA, or DNA polymorphism, which helps to reduce the likelihood of variable genotypes being included (Farokhzadeh *et al*., 2014). Because they are very simple, fast, cost effective, highly discriminative, and reliable, RAPD (Random Amplified Polymorphic DNA) markers are the most reliable for assessing the genetic fidelity of in vitro raised clones. They do not require any radioactive probes like restriction fragment length polymorphism.

This study was undertaken to standardize the protocol for *in vitro* propagation and genetic fidelity for the Kufri Pushkar cultivar of potato, which would be efficient and suitable for the successful application of gene transfer technique and better conservation of germplasm. Based on the study, the influence of different concentrations and combinations of auxins, gibberellins and cytokinins on *in vitro* propagation and subsequent genetic fidelity was observed and discussed.

MATERIALS AND METHODS

The present study on "*In vitro* propagation and genetic fidelity testing in potato (*Solanum tuberosum* L.)" was carried out at Centre for Plant Biotechnology (C.P.B.), Department of Science and Technology, Haryana, Chaudhary Charan Singh Haryana Agricultural University, New campus, Hisar. Kufri Pushkar (JW 160) 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 Pushkar 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 (Kang *et al*., 2007). 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 GA3, BAP, KIN, NAA, IBA, and IAA under aseptic environmental conditions.

A. In vitro establishment of explants

The sterilized shoot tip explants of the cultivar Kufri Pushkar were inoculated on various MS media having different concentrations and combinations of cytokinins (Table 2). 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.

B. In vitro multiplication of potato cultivar Kufri Pushkar

In vitro proliferated shoots obtained on shoot bud initiation media were inoculated on different 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 (Table 3). The inoculated cultures were kept in culture room under the same conditions (as above).

***In vitro* rooting of potato cultivar Kufri Pushkar.** 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 (Table 4). The same conditions (as above) were maintained for the inoculated cultures kept in culture room.

$$\text{Rooting \% regeneration} = \frac{\text{Number of in vitro rooted adventitious shoots regenerated}}{\text{Total number of inoculations}} \times 100$$

Hardening of potato cultivar Kufri Pushkar. The *in vitro* plants of the cultivar were obtained after few days and washed under running tap water to remove adhering 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 green house conditions (Table 5).

DNA isolation 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 Pushkar) 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 five gram young leaves of *in vitro* raised plants and mother plants of potato cultivar Kufri Pushkar following modified Cetyl Trimethyl Ammonium Bromide (CTAB) extraction method modified by Sagai-Maroof *et al.* (1984); Xu *et al.* (1994). 20 µl reaction volume containing 2 µl of 20ng/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.

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, 21stand 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 Pushkar were inoculated on MS medium fortified with different concentrations and combinations of growth regulators. The data for *in vitro* establishment of both the cultivars 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.

The Table 2 reveals that the maximum buds (3.6±0.07) were reported on shoot tip explants of cv. Kufri Pushkar on medium PM_6 (MS medium + 0.25 BAP + 0.50 KIN mg/l) in minimum (3.3±0.08) days. The least number of buds (2.5±0.07) were observed on medium PM_4(MS medium + KIN 0.50 mg/l) 0.25 mg/l) in (4.3±0.09) days. It was observed that the BAP was found to be more effective than kinetin at the concentration of 0.25 mg/l for shoot bud initiation in cv. KufriPushkar. The medium PM_6 (MS medium + 0.25 BAP + 0.50 KIN mg/l) containing BAP and kinetin was found most effective amongst the array of media used for *in vitro* establishment of shoot tip explant of cv. Kufri Pushkar (Fig. 1).

***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 presented in the Table 3.

The data presented in the Table 3 recorded the maximum number of shoots (4.4±0.15) were observed on medium PM_{13} (MS medium + 0.25 mg/l BAP + 0.01 mg/l IAA) on 28th day of subculture. The minimum shoots (2.3±0.05) were observed on medium PM_1 (MS medium + 0.25mg/l BAP) on 28th day of subculture. It is clear from the table that the MS medium supplemented with different concentrations of growth regulators in combination were found more effective than individual concentration of BAP and kinetin on *in vitro* multiplication of potato cv. Kufri Pushkar. This may be due to the synergetic effect of growth regulators. Hence it is better to use the combination of growth regulators i.e. BAP and KIN for *in vitro* multiplication of potato cv. Kufri Pushkar (Fig. 2).

***In vitro* rooting.** After twenty eight days, the elongated shoots of cultivar Kufri Pushkar were transferred to MS

medium supplemented with different concentrations of auxins for rooting. Data were recorded for rooting percentage, number of days required for root initiation and number of roots formed per shoot (Table 4).

The table 4 reveals that 100% rooting was achieved on all media used in the present investigation. On 21th day of sub culturing, the maximum number of roots per shoot (14.0±0.37) in potato cv. Kufri Pushkar were reported on medium PR_4 (MS medium + 2.0 mg/l IBA). The medium PR_8 (MS medium + 1.5 mg/l NAA) and PR_{10} (MS medium + 2.5 mg/l NAA) takes least number of days (2.3±0.10) and (2.3±0.12) respectively for root initiation. It was observed that the IBA at the concentration of 2.0 mg/l was more effective for *in vitro* rooting in potato cv. Kufri Pushkar (Fig. 3).

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

The table 5 depicted the effect of different potting mixture on survival percentage of *in vitro* raised plants of cv. Kufri Pushkar. 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. Maximum survival percentage (100%) was recorded on potting mixture POM_7 (coco peat + vermiculite + perlite in 3:1:1) in 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 regenerated plantlets (60%) was recorded in potting mixture POM_1 (Sand). In the present study different potting mixture i.e. sand, soil, FYM and vermicompost were used alone and in combinations for better results (Fig. 4).

Genetic assessment of *in vitro* raised plants using RAPD markers. To study the genetic fidelity the DNA was isolated from mother plant of cultivar Kufri Pushkar and 150 tissue culture raised plants were randomly selected from hardened plants of the cultivar. Total 20Random Amplified Polymorphic DNA (RAPD) primers (Table1) were screened for genetic fidelity testing. Out of 20 primers screened, four primers produced amplification (Table 6) in the cultivar Kufri Pushkar. 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 Pushkar using RAPD primers. The maximum three bands were reported in primers OPB-01, OPB-04 (Fig. 5) and OPB-11 while two bands were reported in primer OPB-12. The banding pattern of mother plant and five tissue culture raised plants were compared. It was observed that all bands were monomorphic. Hence, the plants produced using the present tissue culture protocols were true to type in nature (Fig. 5).

DISCUSSION

Potato is an excellent example of a crop where biotechnology methods have been widely used in several nations. The meristem culture method was used to effectively create virus-free potato clones as the first biotechnology approach. In recent years, the problem-driven application of biotechnological techniques to the potato crop has proven to be effective in addressing many of the crop's inherent difficulties. In many countries, combining in vitro potato clone propagation with traditional multiplication methods has become a major aspect of seed production (Naik and Buckseth 2018). Explant is crucial in their response to the in vitro propagation method for shoot regeneration and multiplication. Various forms of explants, such as axillary buds, shoot tips, and nodal stems, are extensively utilised for efficient micropropagation and germplasm conservation (Zhang *et al*., 2017). Because a virus-free plant can be propagated from a little (0.1–0.3 mm) piece of meristematic tissue, many viruses are unable to infect the apical/axillary meristem of a growing plant using the shoot tip culture method. It is also beneficial in simultaneous exclusion of other pathogens such as mycoplasmas, bacteria and fungi (Millam and Sharma 2007).

Plant growth regulators (PGRs) play a vital role in *in vitro* propagation of potato (Fabian, 2010). Our results reflected that the PGR applications were efficient to shorten days to shoot bud initiation compared to regeneration on control medium. The results are not in agreement with Bhuiyan (2013) reported that BAP showed better response in terms of number of shoots per explants along with shoot length among different concentrations of BAP (0.5- 2.5 mg/l) and KIN (0.5-2.5 mg/l) used. He noted that for *in vitro* establishment of cvs. Esprit and Meridian best result was obtained on MS medium supplemented with 1.0 mg/l BAP using shoot apex and nodal segment and in cv. Lady Rosseta, MS medium fortified with 0.5 mg/l BAP was found more effective. The outcome of the present results were in consensus with other researchers like Islam (1990); Mila (1991), Sarker and Mustafa (2002) who found that MS medium along with BAP showed better response in shoot bud initiation. The response of BAP alone on bud initiation may be due to varietal difference of potato. This may be due to the synergetic effect of growth regulators.

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 partial harmony with Gami *et al.* (2013) used different combinations of growth regulators for multiplication of shoots and found that the combinations of IBA 1.0 mg/l + NAA 1.0 mg/l + kinetin 2.0 mg/l and 2.0 mg/l IBA + 2.0 mg/l kinetin + 2.0 mg/l NAA + 1.0 mg /l 2,4-D mg/l were more effective than others. Our results are not in agreement with Asma *et al*. (2001) who used MS medium fortified with 2.0 mg/l BAP and showed maximum number of shoots (14). Furthermore, the

results were not similar to the findings of Shibli *et al*. (2001) who used MS medium supplemented with BA 1.0 and 1.5 mg/l and showed gradual increase in number of vigorous shoots and nodes per culture flask. Similarly, Badawi *et al*. (1996) used MS medium fortified with 2.0 mg/l of IBA and showed better results by taking nodal cuttings as explants in potato cv. Cara. The varietal difference may be reason for minor variation in results. Badoni and Chauhan (2009) used mixture of soil, sand and vermicompost (2:1:1) for acclimatization of potato plants after rooting and recorded good results. Furthermore, a mixture of soil and river sand (3:1) was used by Vargas *et al.* (2005) for hardening of the potato plants. In addition, Nasiruddin (2006) showed better results with the use of one part sterile garden soil, one part sand and compost mixture for *in vivo* hardening of plants. Panicker *et al*. (2007) suggested the transplanting of *in vitro* raised plants into well drained sterile growing medium to overcome these limitations. Salem and Hassanein (2017) detected that cv. Agria and Hermes are closer to each other when compared with cv. Spunta through RAPD techniques. Thus, it can be concluded that the genetic variation can be observed among different cultivars but high genetic fidelity is maintained when tissue raised plants are regenerated from mother plants and can be used for micropropagation at commercial level.

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

Sr. 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

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

Medium Code *PM	Concentration of growth regulators (mg/l)	Average number of buds per explant	Average number of days taken for bud initiation
PM_0	MS Medium	2.4±0.12g	5.2±0.14abc
PM_1	BAP 0.25	3.0±0.12b	3.4±0.12bdc
PM_2	BAP 0.50	2.8±0.09e	3.9±0.10cde
PM_3	KIN 0.25	2.5±0.09d	4.0±0.10cd
PM_4	KIN 0.50	2.5±0.07f	4.3±0.09ab
PM_5	0.25 BAP + 0.25 KIN	2.9±0.14c	3.8±0.13cdf
PM_6	0.25 BAP + 0.50 KIN	3.6±0.07a	3.3±0.08acd

*PM-Potato Medium

Table 3: Effect of different concentrations of growth regulators on *in vitro* multiplication of potato cv. Kufri Pushkar: 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±0.03u	1.5±0.03tr	1.8±0.08zp	2.2±0.06poj
PM_1	BAP 0.25	1.2±0.03u	1.4±0.08rw	1.5±0.03phk	2.3±0.05sty
PM_2	BAP 0.5	1.1±0.03v	1.5±0.05yu	1.5±0.06ouy	2.4±0.12hur
PM_3	BAP 0.75	1.1±0.08r	1.3±0.03po	1.6±0.03yp	2.4±0.03ryo
PM_4	BAP 1.0	1.2±0.05	1.4±0.06tp	1.5±0.11yre	2.7±0.18yjo
PM_5	BAP 1.25	1.2±0.03u	1.4±0.03hr	1.5±0.07yph	2.8±0.09ple
PM_6	KIN 0.25	1.0±0.03ok	1.4±0.03hr	1.6±0.06lur	2.6±0.06trd
PM_7	KIN 0.5	1.1±0.08r	1.4±0.03hr	1.6±0.06lur	3.1±0.12bgt
PM_8	KIN 0.75	1.2±0.03u	1.5±0.03tr	1.6±0.03yp	3.1±0.14yfw
PM_9	KIN 1.0	1.1±0.03v	1.4±0.08rw	1.7±0.12iuj	3.0±0.12pqz
PM_{10}	KIN 1.25	1.4±0.14q	1.6±0.08wx	2.0±0.12kil	3.2±0.28zwp
PM_{11}	0.25 BAP + 0.25 KIN	1.5±0.08p	1.7±0.08zd	2.2±0.06poj	3.7±0.09rdw
PM_{12}	0.25 BAP + 0.01 NAA	1.8±0.12vc	2.1±0.12dr	2.8±0.31gnh	4.2±0.12frs
PM_{13}	0.25 BAP + 0.01 IAA	1.4±0.03hr	2.0±0.11dt	2.9±0.08frm	4.4±0.15rdi
PM_{14}	0.25 BAP + 0.01 IBA	1.6±0.05t	1.9±0.08uk	2.9±0.06vgt	4.0±0.06hts
PM_{15}	0.25 KIN + 0.01 NAA	2.0±0.03yg	2.3±0.08jy	3.3±0.18rqd	4.3±0.09irf
PM_{16}	0.25 KIN + 0.01 IAA	1.6±0.05t	1.9±0.08uk	2.6±0.12lrg	3.4±0.08wbm
PM_{17}	0.25 KIN + 0.01 IBA	1.9±0.05z	2.2±0.03gf	3.0±0.15srl	3.8±0.14mhy
PM_{18}	0.01 BAP + 0.25 GA_3 + 0.01 NAA	1.9±0.12rt	2.3±0.11gn	2.8±0.15pyl	4.0±0.08olf
PM_{19}	0.25 BAP + 0.25 GA_3 + 0.01NAA	1.8±0.15ts	2.1±0.03rf	2.6±0.06trd	3.8±0.18bhq
PM_{20}	0.25 BAP + 0.25 GA_3 + 0.01 IAA	1.6±0.03yp	1.9±0.05tm	2.4±0.03qpr	3.6±0.06pdh
PM_{21}	0.25 BAP + 0.25 GA_3 + 0.01 IBA	1.3±0.06ar	1.7±0.08gu	2.7±0.06fyu	3.9±0.15hgq
PM_{22}	0.25 BAP + 0.25 GA_3 + 0.02 NAA	1.3±0.11x	1.6±0.08hl	2.3±0.27ujg	3.6±0.03pzk
PM_{23}	0.25 BAP + 0.25 GA_3 + 0.02 IAA	1.5±0.07y	2.0±0.03yg	2.7±0.15kyt	4.1±0.03lfj
PM_{24}	0.25 BAP + 0.25 GA_3 + 0.02 IBA	1.4±0.08w	1.6±0.03yp	2.2±0.18ydr	3.4±0.15ejk

*PM-Potato Medium

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

Sr. No.	Medium code (mg/l)*PR	Rooting percentage	Days required for root initiation	Average number of roots per shoot (on 21st day)
1.	PR_1 (IBA 0.5)	100	3.2±0.16ade	10.7±0.33htg
2.	PR_2 (IBA 1.0)	100	2.5±0.11sqb	10.7±0.33htg
3.	PR_3 (IBA 1.5)	100	2.8±0.14uyl	11.5±0.34gki
4.	PR_4 (IBA 2.0)	100	2.4±0.14hwd	14.0±0.37yhb
5.	PR_5 (IBA 2.5)	100	2.8±0.13frv	10.3±0.49rfa
6.	PR_6 (NAA 0.5)	100	2.6±0.16rhw	10.7±0.61wdt
7.	PR_7 (NAA 1.0)	100	3.5±0.10dfg	11.2±0.40svc
8.	PR_8 (NAA 1.5)	100	2.3±0.10yil	10.3±0.42ijz
9.	PR_9 (NAA 2.0)	100	2.5±0.14kjw	13.2±0.48lwm
10.	PR_{10} (NAA 2.5)	100	2.3±0.12qsl	10.7±0.67dbz

*PR-Potato Rooting

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

Sr. No.	Potting mixture (*POM)	Percent survival of Kufri Pushkar
1.	POM_1 (sand)	60.0
2.	POM_2 (sand+FYM in 1:1)	70.0
3.	POM_3 (sand+soil+ FYM in 1:1:1)	80.0
4.	POM_4(sand+soil+vermicompost in 1:1:1)	90.0
5.	POM_5(sand + soil+ FYM+ vermicompost in 1:1:1:1)	85.0
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 Pushkar.

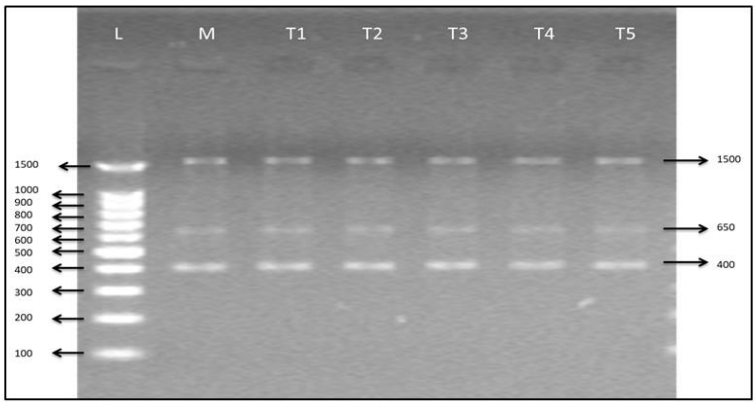
Sr. No.	Primer Sequence (5'-3')	Number of bands
1.	OPB-01 (GTTTCGCTCC)	3
2.	OPB-04 (GGACTGGAGT)	3
3.	OPB-11 (GTAGACCCGT)	3
4.	OPB-12 (CCTTGACGCA)	2

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

Fig. 2. *In vitro* multiplication of Potato cv. Kufri Pushkar on medium PM_{13} (MS Medium + 0.25 mg/l BAP + 0.01 mg/l IAA)

Fig. 3. *In vitro* rooting of Potato cv. Kufri Pushkar on medium PR_4 (MS Medium + 2.0 mg/l IBA) Survival percentage of regenerated plantlets.

Fig. 4. Hardened plants of Potato cv. Kufri Pushkar.



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

Fig. 5. RAPD profiles of mother plant and *in vitro* raised plants of Potato cv. Kufri Pushkar using primer OPB-04.

CONCLUSIONS

An efficient micropropagation via shoot tips as initial explants along with its genetic fidelity was described for potato cv. Kufri Pushkar. 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 for the production of high-quality virus-free potato planting material, to enhance the yield and productivity of the crop.

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