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In vitro propagation of *Solanum tuberosum* cultivar kufri chipsona 1

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Abstract
Present investigation presents fast and reproducible *in vitro* propagation method for Solanum tuberosum cultivar Kufri Chipsona 1, which can be utilized for *in vitro* propagation of potato plants. Aseptic cultures were established on Murashige and Skoog (MS) medium supplemented with 6-benzyle adenine (BA, 0.1µM). For shoot multiplication, BA and Kinetin (KIN) were utilized. The BA was seen as better for shoot multiplication as compared with KIN. Maximum shoot multiplication was seen on MS medium containing 0.5 µM of BA, which enhances further by addition of 1.0 µM of α-naphthalene acetic acid (NAA). Indole 3-butyric acid (IBA) was found to be better auxin for root induction and maximum rooting frequency was seen on MS medium supplemented with 5.0 µM of IBA. Micropropagated plants showed healthy growth and survival during polyhouse and nursery conditions.

Keywords: auxins, cytokinins, shoot multiplication, potato, micropropagation

Introduction
Potato (*Solanum tuberosum* L.) is viewed as the world's most significant non-grain food yield and one half of the worldwide root and tuber crop usefulness is contributed by potato. Further it is considered as third most significant food harvest of world (FAOSTAT 2019) [1]. Potato production of around 388,190,674 tons was accounted for in 2017, of which 12% offer was contributed by India, the second biggest producer (FAOSTAT 2019) [1]. The potato tubers are an all around the world significant dietary source of various nutrients and assumes a significant part as to worldwide food security (Barrell *et al.*, 2013) [2].
Potato propagated vegetatively utilizing pieces or entire potato tubers, still a various infection diseases cause's crop decrease to practically half, which changes from one place to another and from season to another (Anoop and Chauhan 2009) [3]. Customarily, plant breeding is considered as a compelling and suitable methodology for crop improvement (Aggarwal *et al.*, 2020) [4]. However, a restricted hereditary base combined with a restricted space of sexual generation, makes breeding a difficult task in potato (Barrell *et al.*, 2013) [2]. Further, the tetraploid status of the harvest, inbreeding gloom and necessity to screen enormous offspring of populaces confounds regular breeding practices (Molla *et al.*, 2011) [5]. Over many years, plant biotechnology including *in vitro* propagation techniques has been furnishing an answer for issues related with conventional breeding of different plants including potato. Potato being a significant crop has wide scope for different various trait-specific improvements, especially identified with nutritional improvement and disease resistance (Ahmad *et al.*, 2012) [6]. Genetic transformation is enthusiastically suggested as strategy for such quality improvement programs (Aggarwal *et al.*, 2012) [7].
For any effective genetic transformation, improvement of a high-recurrence *in vitro* propagation protocols utilizing different techniques like enhanced-axillary branching and additionally somatic embryogenesis is a pre-imperative (Aggarwal *et al.*, 2020a) [8].
Despite the fact that there are many reports of micropropagation of potato (Saker *et al.*, 2012, Ibrahim *et al.*, 2017; Salem and Hassanein 2017, Kaur *et al.*, 2017) [9-12], however challenges are experienced w.r.t reproducibility.
Hence emerges the requirement for advancement proficient and reproducible *in vitro* propagation protocols for potato. Along these lines, the current examination was endeavored to foster an effective micropropagation convention for *Solanum tuberosum* cultivar Kufri Chipsona 1.

Materials and Methods
Plant material, chemicals, glassware
The sprouted tubers (Figure 1A) of Solanum tuberosum cultivar Kufri Chipsona 1 were obtained from Potato Technology Center, Shamgarh District Karnal (Haryana), used for establishment of aseptic cultures using standard tissue culture protocols.
All chemicals utilized were from HiMedia Laboratory unless specified. The experiments were carried out in 300ml glass bottles (Kasablanka, Mumbai) containing 30ml of medium. Medium pH was settled at 5.8 before autoclaving at 21ºC for 20min. Cultures were raised using sprouts as explants utilizing methodology after Aggarwal *et al*., (2012) [13].
The shoot cultures were kept on Murashige and Skoog medium (MS medium) (Murashige and Skoog 1962) [14] containing 58mM sucrose and gelled with 0.7% (w/v) agar supplemented with 0.1 µM BA (benzyl adenine).

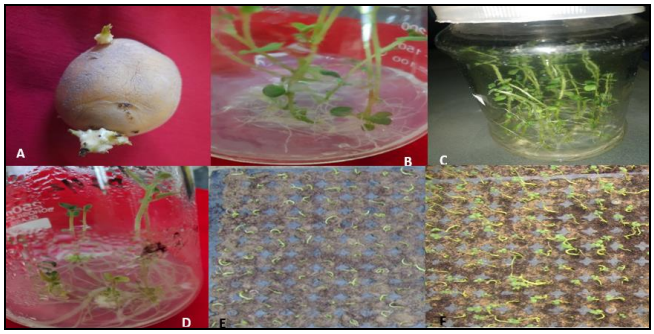

Fig 1: Micropropagation of Potato cultivar Kufri Chipsona 1. Sprouts used for establishment of Aseptic cultures (A), Aseptic cultures on MS medium supplemented with 0.1 µM BA (B), Shoot Multiplication on MS medium supplemented with 0.5 µM BA in combination with 1.0 µM NAA (C), Rooting of microshoots on MS medium supplemented with 5.0 µM IBA (D), Rooted microshoots before acclimatization (E), Acclimatized plantlets (F).

Effect of PGRS on shoot proliferation

The impact of various plant growth regulators (PGRs) in particular, BA (6-benzyl adenine), KIN (Kinetin, 6-furfurylaminopurine) in concentration of 0-2.5 µM either alone or in combination with 0–2.5 µM of α- naphthalene acetic acid (NAA), indole-3-acetic acid (IAA) on shoot multiplication was examined. The data for shoot multiplication was recorded after three weeks of culture.

Rooting of micro-shoots and acclimatization of plantlets

Micro-shoots were excised from clumps just below the node, leaves were removed from lower nodes and were cultured on MS medium supplemented with different concentrations (0-5 µM) of NAA, or indole-3-butyric acid (IBA) for the induction of roots. Acclimatization of plantlets was carried out in polyhouse with controlled temperature (25-28^{o}C) and humidity (90-95%). Plantlets were planted in a mixture of soil and vermicompost in equal ratio in polybags and kept in polyhouse. During the initial periods 90% relative humidity was maintained and slowly it was reduced to 40% over a period of one month.

Statistical analysis

Unless otherwise stated, all experiments were conducted using four replicates with three explants in each culture vessel and repeated four times. The data were recorded after 4 week of subculture. Data were analyzed by analysis of variance and the means were compared with Duncan's multiple range test.

Results

Disinfection protocol followed (10 % Sodium Hypochlorite for 7-8 minutes) was found to be satisfactory and aseptic cultures were established successfully within 2-3 weeks of inoculation on MS medium supplemented with 0.1 µM BA (Figure 1B). Shoots start to emerge from the sprouts and maintained on same medium for further experimentation.

Effect of PGRS on shoot multiplication

After four weeks of culturing, aseptic cultures were utilized for checking the effect of various PGRS on shoot multiplication as mentioned in the Table 1. In the first set of experiments, effect of BA and KIN (0.0-2.5 µM) in MS medium was analyzed (Table 1). It was observed that media supplemented with 0.5 µM of BA have recorded maximum shoot multiplication (10.6) as compared to other media combinations (Table 1). Maximum shoot length was also recorded on same medium (Table 1) as compared with other media formulations. BA was found to be better cytokine as compared with KIN.

Table 1: The effect of different cytokinins on shoot proliferation of microshoots of Potato cultivar Kufri Chipsona 1 propagated on MS medium.

Cytokinins (µM)		Morphogenic Responses	
BA	**KIN**	**Average no. of shoots multiplied**	**Average shoot length(cm)**
0.0	0.0	4.5	3.5
0.1	0.0	7.5	6.4
0.5	0.0	10.6	8.1
1.0	0.0	9.1	7.1
1.5	0.0	8.5	6.5
2.0	0.0	7.2	6.1
2.5	0.0	6.3	5.8
0.0	0.1	4.6	4.1
0.0	0.5	5.8	4.6
0.0	1.0	5.4	4.1
0.0	1.5	4.9	3.9
0.0	2.0	4.3	3.4
0.0	2.5	3.9	3.1

Further effect of NAA and IAA (0.0-2.5 µM) in combination with 0.5 µM of BA was analyzed. It was observed that addition of small amount of auxins in the medium resulted in enhanced shoot proliferation as compared to cytokinins alone. Addition of 1.0 µM of NAA resulted in maximum shoot proliferation (13.9) (Figure 1C, Table 2). Out of the two auxins i.e. IAA and NAA (0.0- 2.5 µM), NAA was found to be better auxin as compared with IAA in combination with 0.5 µM BA.

Table 2: The effect of plant growth regulators on shoot proliferation of microshoots of Potato cultivar Kufri Chipsona 1 propagated on MS medium supplemented with 0.5 µM of BA.

Plant growth regulators (µM)		Morphogenic Responses	
NAA	**IAA**	**Average no. of shoots multiplied**	**Average shoot length(cm)**
0.0	0.0	9.4	6.8
0.5	0.0	11.6	7.4
1.0	0.0	13.9	7.9
1.5	0.0	13.1	7.1
2.0	0.0	12.4	6.8
2.5	0.0	10.9	6.2
0.0	0.5	9.1	5.9
0.0	1.0	8.7	5.7
0.0	1.5	8.4	5.3
0.0	2.0	8.0	4.9
0.0	2.5	7.9	4.5

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Rooting of micro-shoots and acclimatization of plantlets

The effects of various auxins *i.e.* NAA, IBA and IAA (0.0–10.0 µM), was examined on rooting efficiency of micro-shoots. The IBA was found to be most effective amongst all auxins (Table 3). Maximum rooting frequency of micro-shoots (79.8%) was observed on MS medium supplemented with 5.0 µM IBA (Figure 1D; Table 3). The IBA was found to be best for induction followed by IAA and NAA. Rooted microshoots were successfully acclimatized under polyhouse conditions (80%, Figure). Later acclimatized

plants were successfully established in their natural conditions (Figure 1E, F).

Table 3: The effect of different auxins on rooting of Potato cultivar Kufri Chipsona 1 micropropagated shoots on MS medium.

Auxin (μM)	Percentage of shoots showing rooting	Average no. of roots per shoot
0.0	40.3	1.8
1.0 NAA	50.2	2.3
2.5 NAA	56.7	2.7
5.0 NAA	60.3	3.1
10.0 NAA	57.8	2.8
1.0 IAA	61.2	3.4
2.5 IAA	65.4	3.2
5.0 IAA	70.4	2.9
10.0 IAA	66.8	3.4
1.0 IBA	68.5	3.7
2.5 IBA	73.6	4.1
5.0 IBA	79.8	4.3
10.0 IBA	67.4	3.9

Discussion

Plant tissue culture is considered as elective procedure to ordinary vegetative propagation strategies used for plant propagation as it offers quick and enormous scope for establishing material for commercial scale production of elite plants with in short span of time (Aggarwal *et al.*, 2020a)[8]. Diverse tissue culture frameworks, for instance, axillary and extrinsic shoot augmentation, shoot organogenesis and somatic embryogenesis are used for the propagation of valuable plants (Kaur and Nautiyal 2013) [15]. Thusly the current examination was looked in towards the improvement of a capable and reproducible micropropagation technique of *S. tuberosum* cultivar Kufri Chipsona 1.

Surface disinfection of explants is possibly the major step towards establishment of aseptic culture. A couple of surface disinfecting agents are recommended for expulsion of surface microorganisms from explants (Sauer and Burroughs 1986) [16]. In the current assessment 10 % solution of sodium hypochlorite (NaOCl) at was utilized as surface disinfecting agent and was found to be satisfactory for establishment of aseptic cultures. Sodium hypochlorite was choice surface disinfecting agent due to its less phytoxicity and therefore used successfully for establishments of aseptic cultures for various plant species (Badoni and Chauhan 2009, Teixeira da Silva *et al.*, 2016) [17, 18]. Viable micropropagation of any plant under *in vitro* conditions depends on use of right sort of plant growth regulator and in right amount as PGRs are known to advance cell division alongside shoot proliferation and axillary bud improvement (Chuntale 2018) [19]. Thusly, in present assessment additionally PGRs in different amounts were tested for maximum production of plantlets of potato. Out of the two attempted cytokinins for example BA and KIN for *in vitro* shoot increase, most reliable recurrence for shoot initiation was seen on MS medium supplemented with 2.5 μM of BA (Table 1), maximum shoot proliferation was seen with also with BA (Table 1) followed by kinetin. BA is adenine type cytokinin and offered an explanation to be used as regularly as feasible for development, improvement and expansion in many plant species including potato (Abd Elaleem *et al.*, 2009, Kaur *et al.*, 2017, Aggarwal *et al.*, 2020) [20, 12, 4].

Further impact of auxins (IAA or NAA) along cytokinins were tried for additional upgrade of shoot multiplication effectiveness (Table 2). Shoot multiplication further upgraded by use of auxins alongside cytokinins as contrasted with cytokinins alone (table 2). Suitable auxin and cytokinins proportion is needed for the ideal shoot proliferation in many plants. Further suitable auxin and cytokinins proportion is accounted to control shoot proliferation through regulation of intercellular auxin distribution (Bishopp *et al.*, 2011) [21]. Further NAA is offered an explanation to play play significant job in cytokinin metabolism and stability which might have helped in gaining higher shoot multiplication frequency (Palani *et al.*, 1988) [22].

Rooting of micro-shoots is considered as one of the most important steps for any micropropagation protocol to be successful. It helps in foundation of plantlets in soil under ordinary conditions. Auxins are for the most part used for induction of roots in microshoots (Aggarwal *et al.*, 2012) [13]. Out of the various auxins attempted (IBA, NAA and IAA), IBA at concentration of 5.0μM was found most suitable for *in vitro* root induction of mi croshoots (Table 3). Usage of IBA for root enlistment of miniature goes for various plant species has been all around overviewed and offered an explanation to be more useful than various auxins like NAA and IAA (Aggarwal *et al.*, 2020, Kumar *et al.*, 2010) [4, 23]. Rooted plantlets were successfully acclimatized upon transfer to pots containing soil and farmyard manure in equal ratio under poly house conditions (85 % humidity/ 25-28 ºC Temperature). Conclusively, present study presents the fast, reliable and most importantly reproducible micropropagation protocol for the plants of *S. tuberosum* cultivar Kufri Chipsona 1.

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References

1. FAOSTAT. Food and Agriculture organisation of the United Nations, 2019. (www.fao.org/faostat/en/#data/QC/;2019),: Accessed 20th of August 2021
2. Barrell PJ, Meiyalaghan S, Jacobs JME, Conner AJ. Applications of biotechnology and genomics in potato improvement. Plant Biotechnology Journal,2013:11:907-920.
3. Anoop B, Chauhan JS. A note on microtuber seed production of potato: necessitate step for Uttarakhand Hills. Report and Opinion,2009:1:9-11.
4. Aggarwal D, Neeti N, Reddy MS, Kumar A. Shoot organogenesis and assessment of clonal fidelity of regenerated plants of *Ocimum tenuiflorum* L.: Queen of Herbs. Vegetos,2020:33:420-429.
5. Molla MMH, Nasiruddin KM, Al-Amin M, Khanam D, Salam MA. Effect of growth regulators on direct regeneration of potato. International Conference on Environment and Industrial Innovation,2011:12:205-210.
6. Ahmad MZ, Hussain I, Muhammad A, Ali S, Ali GM, Roomi S *et al*. Factor affecting *Agrobacterium*-mediated transformation of rice chitinase gene in

Solanum tuberosum L. African Journal of Biotechnology,2012:11(141):9716-9723.
7. Aggarwal D, Jaiswal N, Kumar A, Reddy MS. Factors affecting shoot regeneration and genetic transformation of *Bacopa monnieri* (L.) Wettst: a popular medicinal plant. Journal of Plant Biochemistry and Biotechnology,2012:22(4):382-391
8. Aggarwal D, Upadhyay SK, Singh R, Sehrawat N, Yadav M, Singh M *et al*. Tissue culture propagation of a medicinal plant *Bacopa monnieri* (L.) Pennell. Advances in Bioresearch,2020a:11(05):97-103.
9. Saker MM, Moussa TAA, Heikal NZ, Abo ELLil AHA, Abdel-Rahman RMH. Selection of an efficient *in vitro* micropropagation and regeneration system for potato (*Solanum tuberosum* L.) cultivar Desirée. African Journal of Biotechnology,2012:11(98):16388-16404.
10. Ibrahim IA, Emara HA, Nower AA, Abodiab AY. *In vitro* cultivation of potato plants. International Journal of Current Microbiology and Applied Sciences,2017:5:858-868.
11. Salem J, Hassanein AM. *In vitro* propagation, microtuberization, and molecular characterization of three potato cultivars. Biologia Plantarum,2017:61:427-437.
12. Kaur A, Reddy MS, Kumar A. Efficient one step and cultivar independent shoot organogenesis of potato. Physiology and Molecular Biology of Plants,2017:23:461-469.
13. Aggarwal D, Kumar A, Sharma J, Reddy MS. Factors Effecting micro propagation and acclimatization of an elite clone of *Eucalyptus tereticornis*. *In vitro* Cellular and Developmental Biology-Plant,2012a:48:521-529
14. Murashige T, Skoog FA. Revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum,1962:15:473-497.
15. Kaur J, Nautiyal K. *In vitro* propagation of *Bacopa monnieri* (L.) Wettst A medicinally priced herb International Journal of Current Microbiology and Applied Sciences,2013:2:131-8.
16. Sauer DB, Burroughs R. Disinfection of seed surfaces with sodium hypochlorite. Phytopathology,1986:76:745-749.
17. Badoni A, Chauhan JS. *In vitro* sterilization protocol for micropropagation of *Solanum tuberosum* cv. 'Kufri Himalini'. Academia Arena,2009:1:5-8.
18. Teixeira da Silva JA, Kulus D, Zhang X, Zeng S, Ma G, Piqueras A. Disinfection of explants for saffron (*Crocus sativus* L.) tissue culture. Environmental and Experimental Biology,2016:14:183-198.
19. Chuntale K. Biotechnological approaches to improve potato: review article. Food Science and Quality Management,2018:77:15-23.
20. Abd Elaleem KG, Modawai RS, Khalafalla MM. Effect of plant growth regulators on callus induction and plant regeneration in tuber segment culture of potato (*Solanum tuberosum* L.) cultivar Diamant. African Journal of Biotechnology,2009:8:2529-2534.
21. Bishopp A, Benkova E, Helariutta Y. Sending mixed messages: Auxin-cytokinin crosstalk in roots. Current Opinion in Plant Biology,2011:14:10-16.
22. Palni LMS, Burch L, Horgan R. The effect of auxin concentration on cytokinin stability and metabolism. Planta,1988:174(2):231-4.
23. Kumar A, Aggarwal D, Gupta P, Reddy MS. Factor affecting *in vitro* propagation and field establishment of *Chlorophytum borivilianum*. Biologia Plantarum,2010:54:601-606.
24. Raj S, Verma S, Bisen RK, Sharma G, Agrawal HP. Effect of growth parameters as influenced by nutrient sources of Potato (*Solanum tuberosum* L.).