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Effect of different growth regulators on *in vitro* micro-propagation of Kufri Frysona

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Abstract: In the present investigation, experiment was conducted for *in vitro* micro-propagation with different concentration of growth regulators in different explants Sprouts and Shoot tips of potato cultivar Kufri Frysona. The maximum survival percentage (40) of sprouts and (100%) of shoot tips were obtained when the explants were surface sterilized with 0.2% bavistin & 0.4% streptocyclin (45minutes) and 0.1% mercuric chloride (60seconds). Sterilized explants were inoculated on MS basal supplemented with various growth regulators and established successfully. The maximum shoot induction (62.5±1.44%) in 11.3±0.33 days and (74.0 ± 2.13 %) in 10.0 ± 0.50 days were reported on medium PM_1 (BAP 0.25 mg/l) in sprouts and shoot tip explants respectively. The sprouted explants were further sub-cultured on MS media supplemented with various growth regulator alone and in combination for *in vitro* multiplication. In Kufri Frysona (11.2) shoots were obtained on MS medium fortified with 0.25mg/l BAP + 0.01mg/l IAA on 42th day of subculture. *In vitro* rooting was observed on MS basal medium supplemented with 2.0 mg/l NAA in Kufri Frysona after 10 days. Rooted plantlets were successfully hardened in green house using different types of potting mixture and finally transferred to field. The protocol will be very useful for large-scale production of disease free planting material of potato (*S. tuberosum*) in future.

Keywords: Growth regulators, *In vitro*, Kufri Frysona, Micro-propagation, Murashige and Skoog media

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the world's most important tuber crop, belongs to family Solanaceae. It is found throughout the world having about 90 genera and 2800 species. Potato is the fourth most cultivated food crop after wheat, rice and maize (Moeini *et al*., 2011). This crop gives an exceptionally high yield and produces more edible energy and protein per unit area and time than many other crops. It is being used as a staple food in many countries. Conventional seed multiplication methods take a long time and are prone to virus infection (Biniam and Tadesse, 2008). The viruses are transmitted through different ways including planting infected tubers. If the seed stock is ill maintained or frequently replaced with fresh ones, the virus infiltration can reach up to 100% in 3-4 successive crop seasons resulting in almost half or one third yields (Khurana *et al*., 2001).

Micro-propagation is the most effective method and is the alternative to conventional propagation of potatoes. It has been proved to be very efficient technique to speed-up the production of high quality disease-free plantlets, in terms of genetic and physiological uniformities (Sathish *et al*., 2011). Plantlets are produced *in vitro* that can be used for further rapid multiplication, microtuber production (*in vitro*), minituber production in the greenhouse (Struik and Lommen, 1991), or transplant production followed by transfer to the field. This method allows quick and round-the-year production of disease-free good quality seed and thus is a way out to supplement the ever increased demand of quality potato seed. Due to the problems associated with conventional propagation of potato there is a need to develop an alternate method for propagation and production of quality planting material. The aim of the present study was the effect of different growth regulators on *in vitro* micro-propagation of Kufri Frysona.

MATERIALS AND METHODS

The present study "Effect of different growth regulators on *in vitro* micro-propagation of Kufri Frysona" was conducted in the Department of Vegetable science and Plant Tissue Culture Laboratory of the Centre for Plant Biotechnology, Government of Haryana, CCS HAU Campus, Hisar during 2013 to 2014 growth seasons. The Potato cultivar Kufri Frysona were selected for the present investigation as a source of explants.

Collection of explants: From healthy plants grown in

green house of Centre for Plant Biotechnology Hisar, young shoot tips explants were collected were brought to the laboratory. The shoot tips explants of 2-3cm size were used. Sprouts were taken from Potato tuber collected from farm of Vegetable Science.

Inoculation: Excised explants were first soaked in mild detergent for 5-10 min. followed by washing in running tap water, so that all detergent from the explant could be removed. The explants were then treated with 0.2% bavistin and 0.2-0.4% streptocyclin for 45 min followed by 4-6 washing with double distilled water. The explants were treated with 0.1 % $HgCl_2$ treatments for 45sec to 120 seconds. Finally the explants were washed with sterile water for 4-5times to remove toxic $HgCl_2$. The sterilized explants were then inoculated in various media fortified with different composition of growth regulators.

Culture conditions: The experiments for *in vitro* studies were conducted under controlled light and temperature and the culture room was fitted with photoperiodic controller and sequential timer. Temperature was maintained at 25 ± 1^0C and light intensity of 1000 lux was provided using florescent tubes. Photoperiod of 16 hrs/8hrs of light and dark was provided.

Data analysis: The data related to various characters were recorded in replicated form using complete randomized design (CRD). Data were analyzed statistically with one factor analysis using OPSTAT software on CCS HAU website. To judge the significant difference between means of two treatments, the critical difference (C.D.) was used.

RESULTS AND DISCUSSION

The present investigation revealed that $HgCl_2$ alone at lower concentration was not sufficient for sterilization of the explants. Increasing the duration (90 seconds) of $HgCl_2$ subsequently decreases the survival. The sterilization treatment (ST_4) bavistin (0.2%) + streptocyclin (0.4%) for 45 minutes followed by $HgCl_2$ (0.1%) for 60 seconds was found best amongst all the sterilization treatment used. Similarly Gami *et al.* (2013) treated sprouts with 0.1% $HgCl_2$ (Mercury chloride) for 30 seconds and then washed with sterile distilled water for surface sterilization. Bhuiyan (2013) decontaminated the sprouts by 0.1% $HgCl_2$ for 7 minute of surface sterilization and increased percentage of responsive explants (survivability) was noted with 4 minutes of surface sterilization in case of potato cv. Esprit, Lady Rosseta and Meridian.

Data was significantly influenced by different treatments. Maximum shoot induction response in sprouts (62.5%) was obtained on medium PM1 (MS basal salt + BAP 0.25 mg/l) in 11.3 days and it was found significantly superior to all other treatments and followed by PM9 medium (MS basal salt + KIN 1.00 mg/l) in 13.0 days. Minimum shoot induction response (47.6%) was observed on medium PM0 having MS Basal in 14.0 days. Results obtained for percent shoot induction response from shoot tip explants in potato cv. Kufri Frysona have been presented. Different media used for shoot induction response contains MS basal medium supplemented with different growth regulators. Maximum shoot induction response (74.0%) was obtained on medium PM_1 (MS basal salt + BAP 0.25 mg/l) in 10.0 days and it was significantly superior to all other treatments. Minimum shoot induction response (45.6%) was observed on medium PM_7 having MS Basal in 14.0 days.

BAP was found more effective for *in vitro* establishment of both cultivars of potato. Similar results were also observed by Bhuiyan (2013). He used the different concentrations of BAP (0.5- 2.5 mg/l) and KIN (0.5 -2.5 mg/l) and found that BAP showed better response in terms of number of shoots per explants as well as shoot length. He observed that MS medium supplemented with 1.0 mg/l BAP was found best for *in vitro* establishment of cv. Esprit and Meridian using shoot apex and nodal segment while MS medium fortified with 0.5 mg/l BAP was found more effective for cv. Lady Rosseta.

Shoot multiplication response in cv. Kufri Frysona: The effect of cytokinin (BAP, Kin), auxin (NAA, IAA, IBA), and GA_3 alone and in combinations were studied on *in vitro* multiplication of potato cv. Kufri Frysona. Amongst all the media used medium PM_{13} supplemented with MS basal + 0.25mg/l BAP + 0.01mg/l IAA was

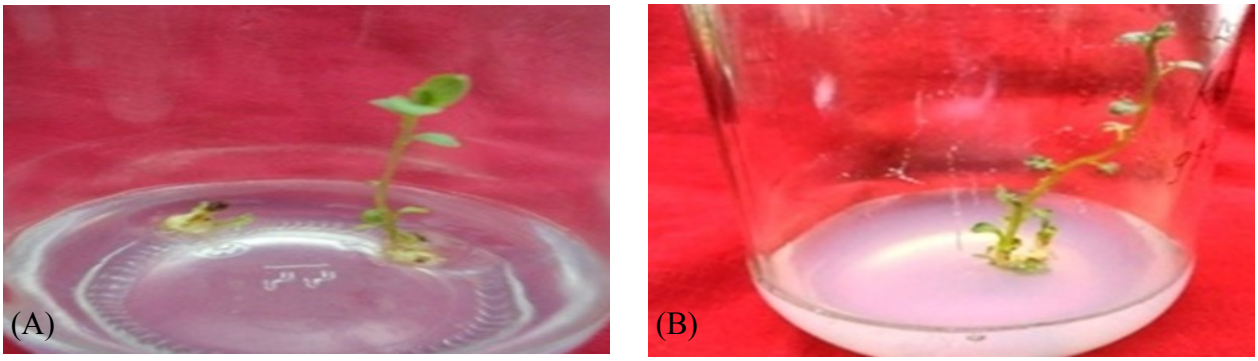

Fig. 1. (A&B): *In vitro Establishment of sprouts of potato on ST_4 treatment (bavistin 0.2% + streptocyclin 0.4% for 45 min + $HgCl_2$ 0.1% for 60 sec) on (A) 7th Day and (B) 14th Day.*

Table 1. Effect of sterilization treatments on survival percentage of explants (sprouts and shoot tips) using three replications in cv. Kufri Frysona.

S. N.	Code ST*	Duration of treatments			Sprouts (Explants)		Shoot tips (Explants)	
		$HgCl_2$ (0.1%) in sec	Bavistin (0.2%) + Strep-tocyclin (0.2%) min	Bavistin (0.2%) + Strep-tocyclin (0.4%) min	Survival %	Contamin -ation %	Survival %	Contami-nation %
1	ST_0	0	-	-	0.0	100.0	0.0	100.0
2	ST_1	45	45	-	5.3	94.7	7.0	93.0
3	ST_2	55	45	-	5.5	94.5	10.0	90.0
4	ST_3	45	-	45	10.0	90.0	33.4	66.6
5	ST_4	60	-	45	40.0	60.0	100.0	0.0
6	ST_5	90	-	45	20.0	20.0	-	-

*ST=sterilization treatment

Table 2. Percent shoot induction response and number of days taken for shoot induction response with three replications using sprouts and shoot tips as explants in cv. Kufri Frysona.

Medium Code	Sprouts Concentration of growth regulators (mg/l)	Percent shoot induction re-sponse	Number of days taken for shoot induction	shoot tips Percent shoot induction response	Number of days taken for shoot induction
PM_0	MS Basal	47.6±1.45	14.6±0.33	50.6±1.23	14.0±0.19
PM_1	BAP 0.25	62.5±1.44	11.3±0.33	74.0±2.13	10.0±0.50
PM_2	BAP 0.50	51.8±0.92	12.7±0.23	61.7±1.23	12.3±0.19
PM_3	BAP 0.75	55.0±1.15	12.1±0.44	61.7±2.46	11.0±0.38
PM_4	BAP 1.00	55.0±0.57	11.6±0.33	62.9±2.13	10.4±0.48
PM_5	BAP 1.25	52.3±0.33	12.6±0.33	59.2±2.13	12.5±0.11
PM_6	KIN 0.25	49.0±0.57	13.6±0.33	56.7±1.23	12.6±0.77
PM_7	KIN 0.50	52.0±0.57	13.3±0.66	45.6±1.23	14.0±0.00
PM_8	KIN 0.75	54.1±0.44	12.3±0.333	54.3±1.23	12.7±0.29
PM_9	KIN 1.00	55.6±0.33	13.0±0.00	60.4±1.23	12.2±0.11
PM_{10}	KIN 1.25	55.0±0.57	13.3±0.33	60.4±1.23	10.3±0.33
	CD at 5%	2.53	1.07	4.91	1.10

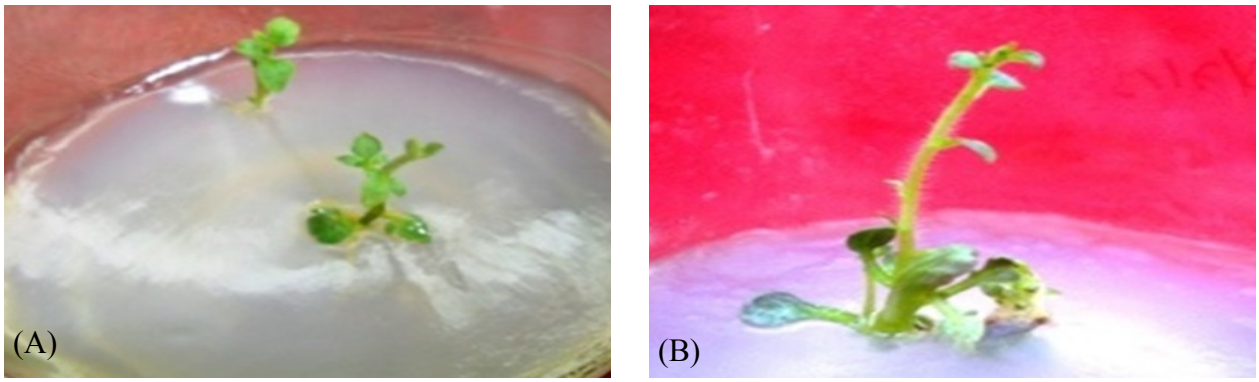

Fig. 2. *(A &B): In vitro establishment of shoot tips on ST_4 treatment (bavistin 0.2% + streptocyclin 0.4% for 45 min + $HgCl_2$ 0.1% for 60 sec) on (A) 7^{th} Day and (B) 14^{th} Day.*

found to be most effective for shoot multiplication with 11.2 shoots/explant in potato cv. Kufri Frysona. On the contrary Asma *et al*. (2001) reported maximum number of shoots (14) on MS medium supplemented with 2.0 mg/l BAP while Shibli *et al*. (2001) observed an increase in number of proliferating shoots and nodes per culture flask on MS medium supplemented with BA 1.0 and 1.5 mg/l. Similarly, Hoque (2010) observed that MS medium supplemented with 4mg/l of KIN showed best performance for multiple shoot regeneration. While Badoni and Chauhan, (2010) found shoots with 9.4 nodes in medium MSH1 (0.25 mg/l GA3 and 0.01 mg/l NAA) reached 8.28 cm and 11.9 cm root length. Gami *et al*. (2013) found 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 responsive for multiple shoots as well as roots.

Percent rooting response in cv. Kufri Frysona: The data on rooting percentage and days required for rooting as influenced by ½ MS salt supplemented with various

Table 3. Effect of different growth regulators on shoot multiplication in cv. Kufri Frysona with three replications using single node cuttings as explants.

Medium Code	Concentration of growth regulators (mg/l)	Average number of shoots			
		7th day	14th day	21st day	2nd subcultulture 45days
PM_0	MS Basal	1.2 ± 0.11	1.5 ± 0.11	1.7 ± 0.11	5.6±0.50
PM_1	BAP 0.25	1.0 ± 0.00	1.0 ± 0.00	1.5 ± 0.11	5.3±0.33
PM_2	BAP 0.5	1.2 ± 0.22	1.4 ±0.22	1.5 ± 0.11	6.0±0.19
PM_3	BAP 0.75	1.2±0.22	1.2 ±0.22	1.3 ± 0.19	6.1±0.77
PM_4	BAP 1.0	1.0±0.00	1.1 ± 0.11	1.4 ± 0.22	7.0±0.19
PM_5	BAP 1.25	1.1±0.11	1.5 ± 0.29	1.6 ± 0.38	7.7±0.55
PM_6	KIN 0.25	1.0±0.00	1.3 ± 0.19	1.4± 0.22	6.7±0.22
PM_7	KIN 0.5	1.0±0.00	1.2± 0.22	1.4 ± 0.22	6.1±0.48
PM_8	KIN 0.75	1.2±0.22	1.2 ± 0.22	2.0± 0.38	5.6±0.19
PM_9	KIN 1.0	1.1±0.11	1.3 ± 0.19	1.5 ± 0.29	7.0±0.19
PM_{10}	KIN 1.25	1.0±0.00	1.1 ± 0.11	1.2 ± 0.11	6.7±0.77
PM_{11}	0.25 BAP + 0.25 KIN	1.6±0.19	1.8 ± 0.29	2.0 ± 0.19	7.7±0.58
PM_{12}	0.25 BAP + 0.01 NAA	1.3 ±0.00	1.6 ± 0.19	2.1± 0.29	8.6±0.19
PM_{13}	0.25 BAP + 0.01 IAA	1.3±0.00	1.6 ± 0.00	2.0 ± 0.19	11.2±0.67
PM_{14}	0.25 BAP + 0.01 IBA	1.7±0.11	1.7± 0.22	2.0 ± 0.19	10.4±0.22
PM_{15}	0.25 KIN + 0.01 NAA	1.3± 0.33	1.3 ± 0.33	1.5 ± 0.29	7.1±0.96
PM_{16}	0.25 KIN + 0.01 IAA	1.6 ± 0.33	1.6 ± 0.33	2.3 ± 0.88	8.4±0.58
PM_{17}	0.25 KIN + 0.01 IBA	1.3 ± 0.00	1.7 ± 0.22	3.2 ± 0.29	10.6±1.07
PM_{18}	0.01 BAP + 0.25 GA_3 + 0.01 NAA	1.6±0.19	2.2 ± 0.11	2.6 ± 0.19	8.7±0.72
PM_{19}	0.25 BAP + 0.25 GA_3 + 0.01NAA	1.1±0.11	1.3 ± 0.19	1.5± 0.22	7.8±0.48
PM_{20}	0.25 BAP + 0.25 GA_3 + 0.01 IAA	1.5±0.11	1.8± 0.294	2.2 ± 0.11	7.1±0.11
PM_{21}	0.25 BAP + 0.25 GA_3 + 0.01 IBA	1.1±0.11	1.6 ± 0.00	2.2 ± 0.29	9.5±0.00
PM_{22}	0.25 BAP + 0.25 GA_3 + 0.02 NAA	1.1±0.11	1.5 ± 0.29	2.0 ± 0.19	9.1±0.40
PM_{23}	0.25 BAP + 0.25 GA_3 + 0.02 IAA	1.3±0.33	1.4 ± 0.22	1.7 ± 0.11	7.7±0.44
PM_{24}	0.25 BAP + 0.25 GA_3 + 0.02 IBA	1.1±0.11	1.1 ± 0.11	1.2 ± 0.22	6.0±0.57
	CD at 5%	0.43	0.60	0.81	1.51

Table 4. Effect of different growth regulators with three replications on percent rooting response of potato cv. Kufri Frysona.

S. N.	Medium code (mg/l)	Rooting Percentage	Days to be required for Rooting
1	PR_1 (IBA 0.5)	100	13.5±0.77
2	PR_2 (IBA 1.0)	100	12.4±0.22
3	PR_3 (IBA 1.5)	100	12.6±0.33
4	PR_4 (IBA 2.0)	100	11.0±0.19
5	PR_5 (IBA 2.5)	100	12.1 ±0.11
6	PR_6 (NAA 0.5)	100	12.4±0.22
7	PR_7 (NAA 1.0)	100	12.3±0.50
8	PR_8 (NAA 1.5)	100	10.2±0.29
9	PR_9 (NAA 2.0)	100	10.1±0.61
10	PR_{10}(NAA 2.5)	100	11.4 ±0.11
	CD at 5%		1.19

growth regulators are presented in (Table 4). Maximum rooting (100 %) were observed in medium PR_9 having (½ MS salt supplemented with NAA 2.0 mg/l) with minimum 10.1 days followed by 100% rooting in (PR_4) medium having ½ MS salt + IBA 2.00 mg/l with 11.0 days. It is clear from the table that as the concentration of the IBA is increases days required for rooting decreases. Similar results were also reported by Badawi *et al*. (1996) on MS medium supplemented with 2.0 mg/l of IBA using nodal cutting culture of potato cv. Cara. While in present study, the rooting percentage response was 100% in media PR_4 and PR_9 fortified with 2.0mg/l IBA and 2.0 mg/l NAA, respectively. The minor variation in results may be due to varietal difference.

Hardening: In the present investigation different potting mixture i.e. sand, soil, FYM and vermicompost were used alone and in combinations. The maximum (100%) survival percentage was found in potting mixture POM_4 (sand + soil + vermicompost in 1:1:1)

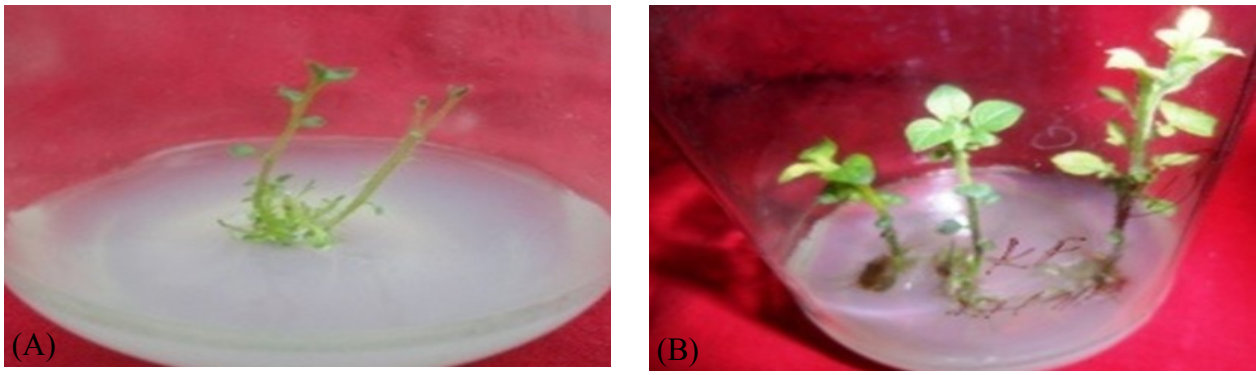

Fig. 3. *(A-B): Showing regeneration of potato in in vitro regeneration medium (PM1) BAP 0.25 mg/l on (A) 21th Day in sprouts and (B) 21th Day in shoot tips.*

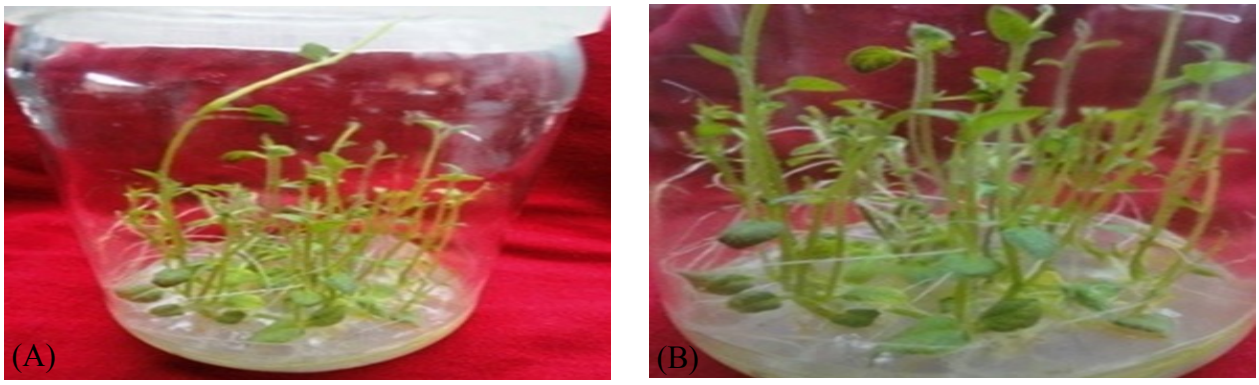

Fig. 4. *(A-B): Showing in vitro multiplication of potato cv.Kufri Frysona on medium (PM_{13}) 0.25 mg/l BAP + 0.01 mg/l IAA) on 42th day of inoculation of (A) Sprouts and (B) Shoot tips.*

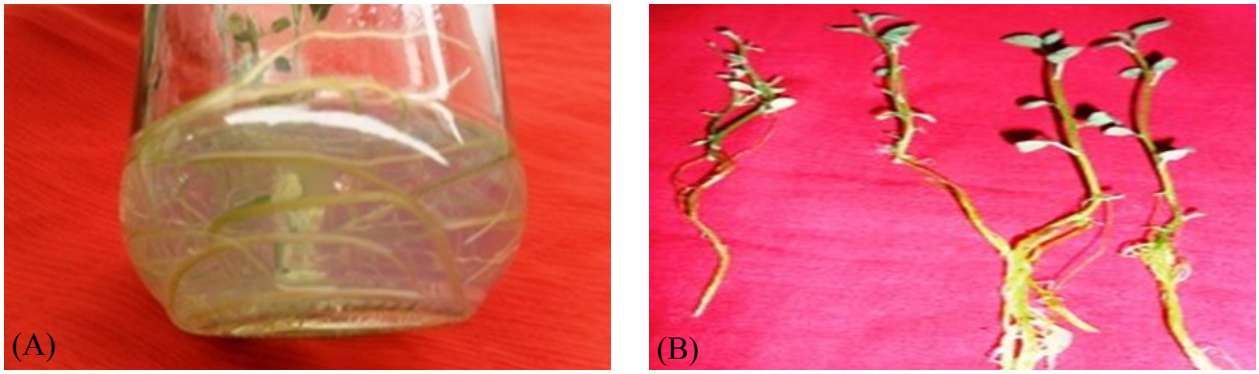

Fig. 5. *A-B: Showing in vitro rooting of potato on medium (PR_9) NAA 2.0 mg/l in 10.1 day of inoculation in fig 5(A)Inside Glass bottle rooting and (B) Established rooted plant.*

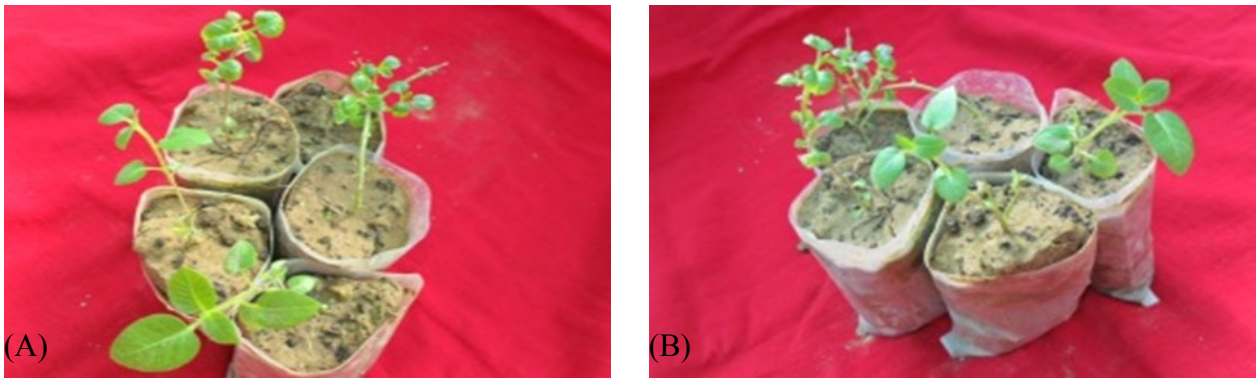

Fig. 6. *A-B: Showing hardening of potato plants in green house in potting mixture (sand: soil: vermicompost in 1:1:1) from (A) Sprouts and (B)Shoot tips.*

of *in vitro* raised plantlets of both the potato cultivars under green house conditions. Similarly Badoni and Chauhan (2009) acclimatized potato plants after rooting. For hardening of plants, they used mixture of soil, sand and vermicompost (2:1:1) and kept under poly house condition for survival and growth. The plantlets were survived well as about 70-80%. While Vargas *et al.* (2005) hardened the potato plants (forty plantlets 8cm long) in a potted mixture of soil and river sand (3:1) and placed under high humidity (80-93% relative humidity), and low light conditions (10µmol·m- 2·s-1). Ten days later plantlets were transferred to a greenhouse.

Conclusion

In this experiment the maximum (100.0%) survival was recorded on the treatment (bavistin (0.2%) and streptocyclin (0.4%) for 45 minutes followed by $HgCl_2$ (0.1%) for 60 sec) amongst the various treatment used using when stoot tips as explant. The maximum number of shoots (11.2) in cv. Kufri Frysona were observed on medium PM13 (0.25 mg/l BAP + 0.01 mg/l IAA) on 42th day of inaculation followed by PM17 (0.25 mg/l KIN + 0.01 mg/l IBA) and PM14 (0.25 mg/l BAP + 0.01 mg/l IBA) i.e. 10.6 and 10.4 shoots respectively. Profused rooting was observed (100 %) on MS medium supplemented with PR_9 (NAA 2.0 mg/l) in 10.1±0.61. The tissue culture raised plants were successfully transferred to potting mixture (sand: soil: vermicompost in 1:1:1) in with 100% survival in green house. So micro-propagation is the answer for mass production of true-to-type, disease free quality planting material of potato.

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