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Article

Comparative study on meristem culture of three potato cultivars diamant, cardinal and granula and their shoot formation

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Abstract: The main aim of this study was to analyze the comparative study on meristem culture of three important potato cultivars, diamant, cardinal and granula and their shoot formation using meristem tips. Apical meristems were isolated from shoot tips of 25-35 days old field grown plants. After surface sterilization the meristems (0.3 mm) were isolated from the shoot tips. After isolation the apical meristems were placed quickly on "M" shaped filter paper bridge in culture tubes containing liquid MS medium supplemented with various concentrations and combinations of different plant growth regulators. Meristems showed their first growth response by increase in size and became greenish white in colour. They continued their growth and developed shoots with roots. In the present investigation, among the three cultivars of potato, cardinal were the best responsive cultivars for the resuming new growth of cultured meristems on MS medium supplemented with KIN (0.4 mg l^{-1}) + GA_3 (0.5 mg l^{-1}). Cultivar diamant showed comparatively better growth response in the primary culture and granula showed better results in shoot length formation in MS_0 medium. Cultivar cardinal proved to be best potato cultivar in case of meristem culture than diamant and granula cultivars.

Keywords: meristem culture; shoot formation; potato cultivars

1. Introduction

The potato (*Solanum tuberosum* L.) is a major world food crop. Potato ranks fourth in terms of total global food production. It comes after only wheat, rice and maize. Potato tubers give an exceptionally high yield per acre, many times that of any grain crop (Burton, 1969) and are used in a wide variety of table, processed, livestock feed and industrial uses. Starch is the predominant storage material in potato tubers. In addition to this, there is also a storage protein, substantial amounts of essential vitamins, minerals and trace elements present in potato tubers. Fresh potato tubers are consumed as vegetables in Bangladesh. To meet the increasing demands of the consumers, it has become imperative to develop varieties with higher yield and quality suitable for year round cultivation. But if a healthy plant is sown in the field, it is exposed to infections caused by pathogens mainly viruses and viroids, which can negatively affect yield and in some cases may kill the plants. The elimination ratio of viruses was higher when the sizes of isolated tips were smaller. About 20 kinds of viruses were eliminated by meristem tip culture. Meristem culture means the culture of excised meristems on suitable nutrient media under aseptic conditions. Meristem culture is one of the important methods to produce virus free stock plants (Uddin *et al*., 2004). Meristem culture provides a reproducible and economically viable method for producing pathogen free plants. As meristem tips are free from viruses, elimination and generation of virus free plants are possible through meristem culture (Jha and Ghosh, 2005). Though several workers have reported the use of MS medium without hormones during proliferation stage (Aburkhes *et al.,* 1984; Rosell *et al.,* 1987; Gopal *et al.,* 1980) but the growth was slow and it took 3-4 weeks to grow 30-50 cm height of shoots (Hussey and Stacey, 1981). Improvement has been made possible by addition of growth regulators to the medium.

Pennazio and Vecchiate (1976) used MS medium supplemented with GA_3 and NAA for proliferating meristem tip.

Potato varieties diamant, cardinal and granula are very popular in Bangladesh and widely cultivated in the country. But all of these three varieties are susceptible to viral diseases and virus free propagule can ensure a greater income to the farmers in the country. Protocol for quick growth of meristem culture holds the promise for enhancing plantlet development for better crop yield of potato. The present investigation was carried out with the objective to see the comparative study on meristem culture of three potato cultivars diamant, cardinal and granula and their shoot formation using meristem tips in order to short out the steady protocol.

2. Materials and Methods

The present experiment was carried out in the Biotechnology Labratory, Institute of Biological Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh. The meristem of three potato cultivars viz., diamant, cardinal and granula were used as explants. Apical meristems were isolated from shoot tips of 25-35 days old field grown plants. The explants were taken in a conical flask and thoroughly washed under running tap water for 30 min to reduce the level of surface microorganisms. Then the explants were taken in reagent bottle containing distilled water with few drops of tween-80 (wetting agent) and 2-3 drops of savlon for about 10-12 min and subsequently rinsed with constant shaking. This was followed by a second washing with distilled water to remove all traces of treated chemicals. Surface sterilization was carried out by 0.1% $HgCl_2$ with gentle shaking for 4 min. The sterilized explants were then washed 5-7 times with sterile distilled water immediately to remove all traces of $HgCl_2$. This procedure was carried out in aseptic condition of laminar airflow cabinet. After surface sterilization the meristems (0.3 mm) were isolated from the shoot tips. After isolation the apical meristems were placed quickly on "M" shaped filter paper bridge in culture tubes containing liquid MS medium supplemented with various concentrations of KIN, GA_3. The meristem responed after 4 weeks of inoculation and then subcultured in MS medium without growth regulators for shoot and root induction. Data were recorded on number of root developments/ explants, shoot length and root formation.

Before shoot formation, a serological identification was done in the cultured plants and to detected virus. In this detection the double antibody sandwitch enzyme-linked immunsorbent assay (DAS- ELISA) methods were followed. Virus free plantlets were used for shoot formation in MS semi-solid medium. After 4-5 weeks it was subcultured on MS_0 semisolid medium or MS semisolid medium having NAA, IBA singly or in combinations for root and shoot developments.

The explants were cultured on MS (Murashige and Skoog ,1962) medium with 3% (W/V) sucrose which was solidified with 0.7% (W/V) agar. The pH of the media was adjusted to 5.7 prior to autoclaving at 121^0C for 20 min. The cultures were incubated in a culture room at 25 ± 2^0C with a photoperiod of 16 hour at 3000 lux light intensity provided by cool white fluorescent tubes. For each treatment, 10 replications were used and all experiments were repeated thrice. Well developed plantlets were removed carefully from the culture vessels, washed gently under running tap water and planted in plastic pots containing a potting mixture of sand, soil and farmyard manure in the ratio of 1:1:1. The potted plantlets were covered by polythene sheet to maintain suitable humidity and then transferred to the fields.

3. Results and Discussion

3.1. Primary establishment of meristem

After surface sterilization (0.1% $HgCl_2$ for 4 min) the meristems (0.3 mm) were isolated from the shoot tips of diamant, cardinal and granula cultivars. The isolated apical meristems were placed quickly on "M" shaped filter paper bridge in culture tubes containing liquid MS_0 and liquid MS medium supplemented with various growth regulators. (Tables 1, 2 and Figure 1: A-I). The results on establishing primary meristem further showed that high percentage of growth response of meristem, use of growth regulators was found to be essential. Most quick response was found when the meristem were cultured MS medium supplemented with KIN (0.4 mg l^{-1}) + GA_3 (0.5 mg l^{-1}). Time taken to resume growth varied from 4 to 6 days in media supplemented with MS + 0.4 mg l^{-1} KIN + 0.5 mg l^{-1} GA_3. Quick growth was observed in cardinal cultivar, it took 4 days for shoot formation. They also showed the highest responding performance (100%). The cultivars diamant and granula showed the lowest performance (82.33%) and they took 6 and 5 days respectively for resuming growth. Respose of explants after 7 days, 21 days and 35 days of culture for cultivars of diamant, cardinal and granula are presented in Figure 1(A-I). Ahmmed (1999) used same growth regulators formulation and obtained satisfactory results for the

establishment of meristem culture in potato. Mohammad (2002) and Rahman (1999) also found the same results in potato.

After resuming growth the meristems were subcultured for elongation of shoots. In this experiment the primary established meristems of different cultivars cultured in MS semisolid medium supplemented with 0.4 mgl^{-1} KIN and 0.5 mgl^{-1} GA_3. The cultured explants showed different range of variation in length. Data was recorded on shoot length after 10, 20 and 30 days of culture and the results are presented in Table 3. The table shows that the maximum length of shoot was recorded 3.00 cm after 30 days of culture and it was obtained in diamant, Figure 2-a (A). The lowest length of shoot was 1.50 cm after 30 days of culture in media having 0.4 mgl^{-1} KIN with 0.5 mgl^{-1} GA_3 and it was obtained from granula, Figure 2-a (C) and cardinal was recorded 2.00 cm, Figure 2-a (B). Multiple shoot proliferation was not observed in any of the cultivars in this media formulation. However, in all cases, the explants were induced to develop single shoot.

Table 1. Primary establishment of meristems cultured on filter paper bridge of the different cultivars of potato in MS liquid medium without different concentrations of growth regulators.

Cultivars	Medium	Number of explants cultured	Days to response	Number of explants responded	% of explants showing growth response
Diamant	MS_0	10	9-21	3	30
Cardinal	MS_0	10	11-21	2	20
Granula	MS_0	10	8-21	3	30
Mean	MS_0	10	8-20	2.66	26.66
KIN (mg l^{-1})					
Diamant	0.1	10	21	2	16.76
Cardinal	0.1	10	23	2	16.67
Granula	0.1	10	-	-	-
Mean	0.1	10	17	1.33	33.43
Diamant	0.3	10	14	4	33.00
Cardinal	0.3	10	17	2	16.67
Granula	0.3	10	12	2	16.67
Mean	0.3	10	14	2.66	22.11
Diamant	0.4	10	9	4	35.00
Cardinal	0.4	10	9	3	33.00
Granula	0.4	10	13	4	33.33
Mean	0.4	10	10	3.66	33.77
Diamant	0.5	10	13	2	16.67
Cardinal	0.5	10	13	3	25.00
Granula	0.5	10	10	3	25.00
Mean	0.5	10	12	2.66	22.22
GA_3 (mg l^{-1})					
Diamant	0.1	10	24	2	16.60
Cardinal	0.1	10	29	1	8.00
Granula	0.1	10	-	-	-
Mean	0.1	10	18	1	8.2
Diamant	0.3	10	25	3	27.00
Cardinal	0.3	10	22	2	16.00
Granula	0.3	10	17	1	8.00
Mean	0.3	10	21	2	17.00
Diamant	0.4	10	14	2	16.00
Cardinal	0.4	10	10	4	33.33
Granula	0.4	10	15	1	8.33
Mean	0.4	10	13	2.33	19.11
Diamant	0.5	10	8	5	45.00
Cardinal	0.5	10	10	4	35.00
Granula	0.5	10	11	3	25.00
Mean	0.5	10	10	4	35

Table 2. Primary establishment of meristem cultures on filter paper bridge of different cultivars of potato in MS liquid medium with KIN (0.4 mg l^{-1}) + G A_3 (0.5 mg l^{-1}).

Cultivars	Number of explants cultured	Responding days	Number of explants responded	% of explants responded
Diamant	10	6	10	82.30
Cardinal	10	4	10	100.00
Granula	10	5	10	82.30
Mean	10	5	10	88.2

Table 3. Effects of KIN (0.4 mg l^{-1}) + GA_3 (0.5 mg l^{-1}) in MS semisolid medium on shoot length of different cultivars of potato (*Solanum tuberosum* L.).

Cultivars	No. of explants cultured	Shoot length (cm) after		
		10 days	20 days	30 days
Diamant	10	0.55	1.55	3.00
Cardinal	10	0.60	1.25	2.00
Granula	10	0.00	0.55	1.50
Mean	10	0.38	1.11	2.16

Table 4. Shoot induction on nodal segments obtained from primary established shoot cultures of different cultivars of potato (*Solanum tuberosum* L.) in MS_0 medium.

Cultivars	Mean no. of shoot with standard error (M ± S.E.)	Average shoot length (cm) with S.E. (M ± S.E.) after		
		10 days	20 days	30 days
Diamant	1.00±00	2.50±0.32	5.00±0.35	9.00±1.02
Cardinal	1.00±00	2.30±0.30	5.50±0.90	9.50±1.05
Granula	1.00±00	3.50±0.75	6.70±0.22	12.00±0.57
Mean	1.00±00	2.76±0.45	5.73±0.49	10.16±0.54

Table 5. Effects of MS_0, NAA and IBA either singly or in different combinations in MS_0 semisolid medium on mean number of roots per explant after 21 days.

Growth regulators and their different concentrations (mg l^{-1})	Cultivars		
	Diamant	Cardinal	Granula
	M ±S.E.	M±S.E.	M±S.E.
MS_0	4.80±0.03	5.20±0.02	3.445±0.02
NAA			
0.1	2.80±0.02	3.03±0.01	2.11±0.01
0.5	2.03±0.02	2.82±0.02	2.40±0.6
1.0	2.10±0.05	2.24±0.21	2.29±0.01
2.0	1.87±0.09	1.29±0.02	1.02±0.11
IBA			
0.1	4.08±0.01	4.90±0.01	3.19±0.01
0.5	4.49±0.01	5.08±0.01	4.79±0.01
1.0	4.13±0.02	4.55±0.03	4.19±0.01
2.0	3.74±0.02	4.28±0.02	3.42±0.03
NAA+ IBA			
0.1 + 0.1	3.57±0.03	4.18±0.03	3.22±0.13
0.5 + 0.5	4.05±0.01	4.84±0.02	3.62±0.01
1.0 + 1.0	3.72±0.01	4.12±0.01	3.02±0.01
2.0 + 2.0	3.13±0.01	3.70±0.02	2.51±0.02

Here, each value represents an average of 10 replicates and each experiment was replicated thrice and M = Mean and S.E. = Standard error

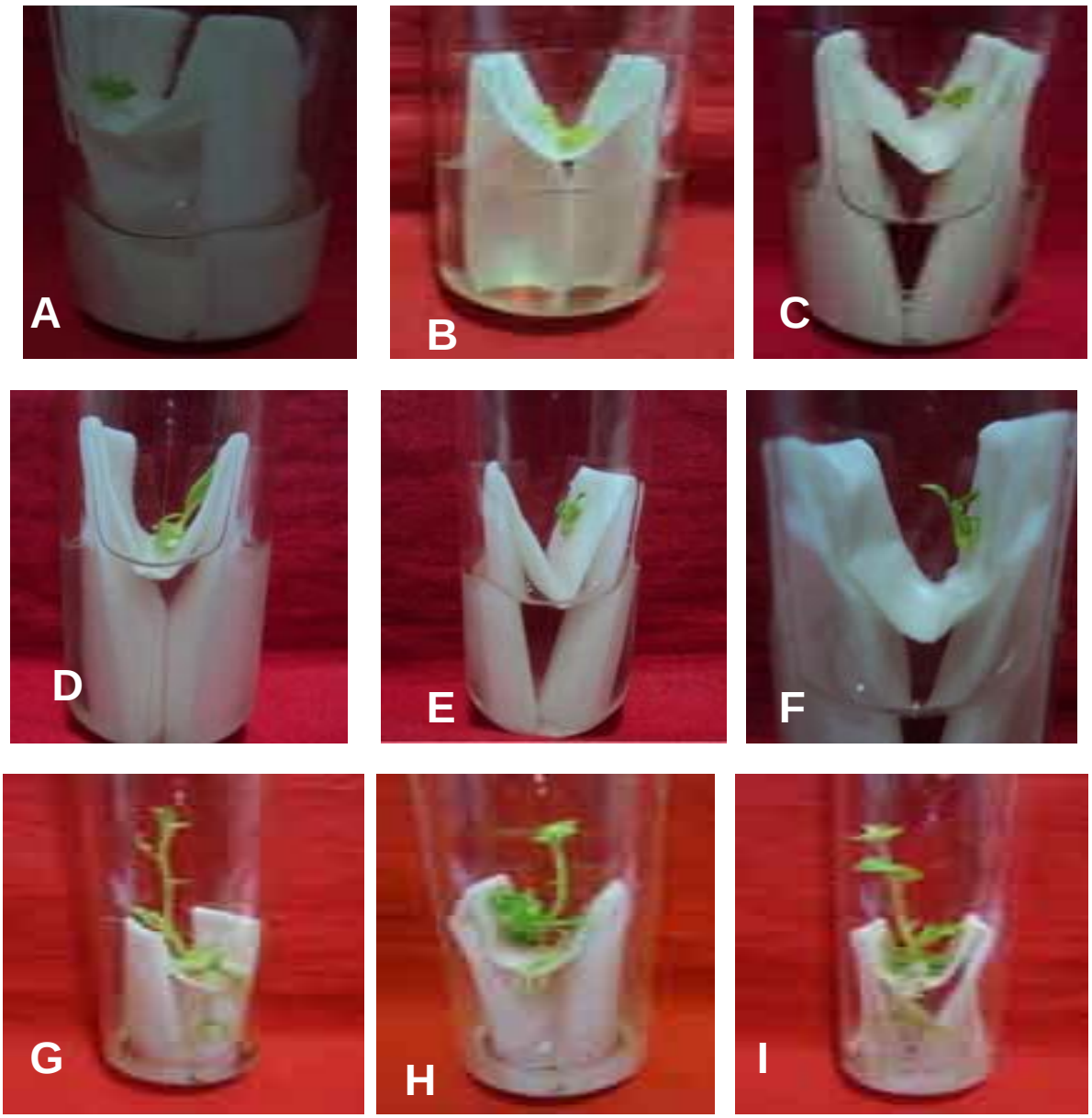

Figure 1 (A-I).
Primary establishment of meristem culture on MS liquid medium supplemented with 0.4 mg l^{-1}KIN + 0.5 mg l^{-1} GA_3 of three potato cultivars.
(A-C) primary response of explants after seven days, (D-F) response of explants after 21 days and (G-I) Primary response of explants after 35 days of culture in the cultivars of diamant, cardinal and granula respectively.

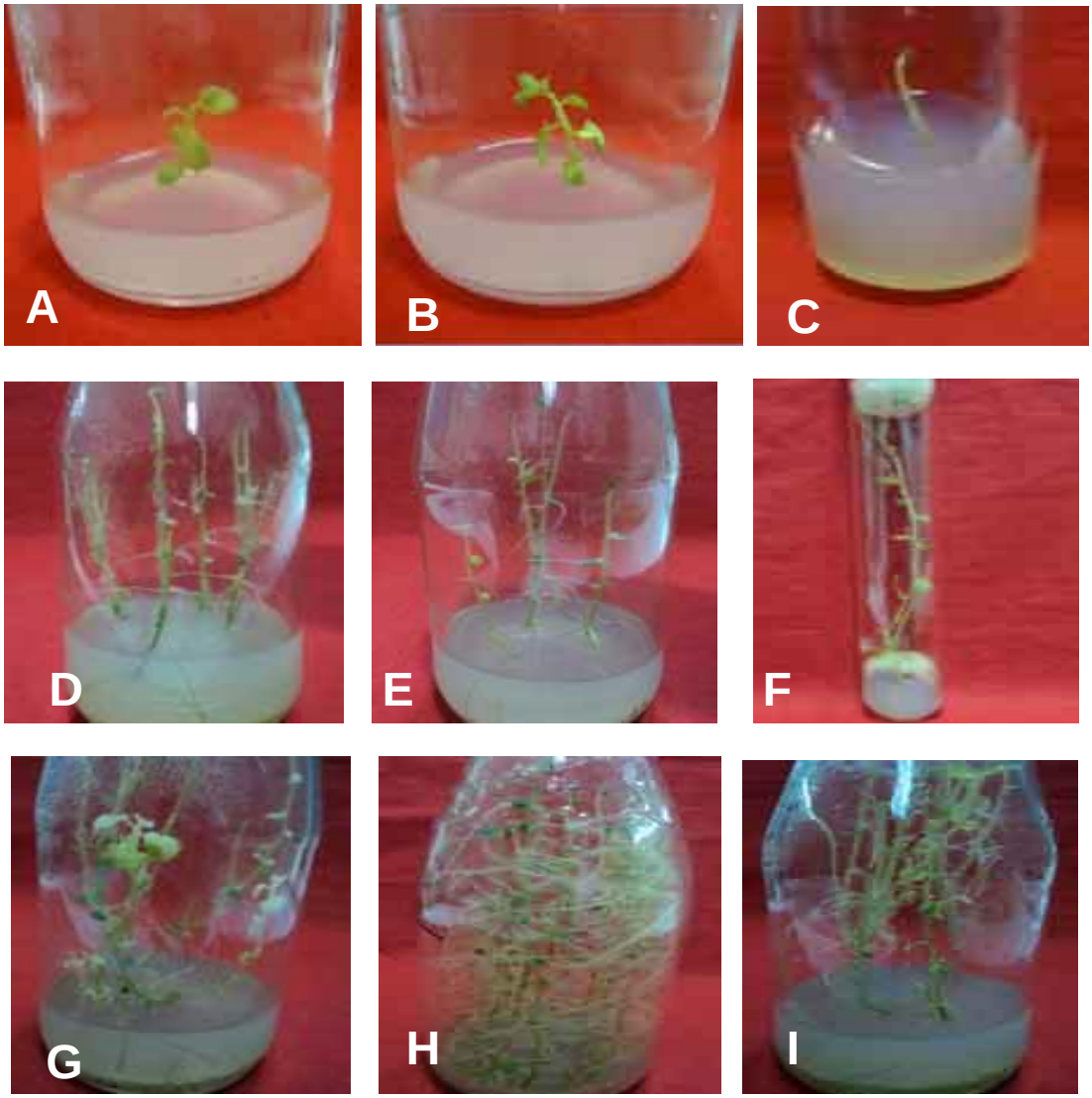

Figure 2 (a). Primary establishment of meristem culture on MS semisolid medium supplemented with 0.4 mg/l KIN + 0.5 mg/l GA_3 of three potato cultivars.
(A-C) primary response of explants after 30 days of culture in cultivars of diamant, cardinal and granula respectively.
Figure 2 (b). Shoot induction on nodal segments obtained from primary established shoot cultures of three cultivars.
D. Diamant, E.Cardinal and F. Granula respectively of potato in MS_0 medium and rooting of virus free *in vitro* plantlets of three potato varieties, G. Diamant, H. Cardinal, I. Granula respectively in MS_0 medium

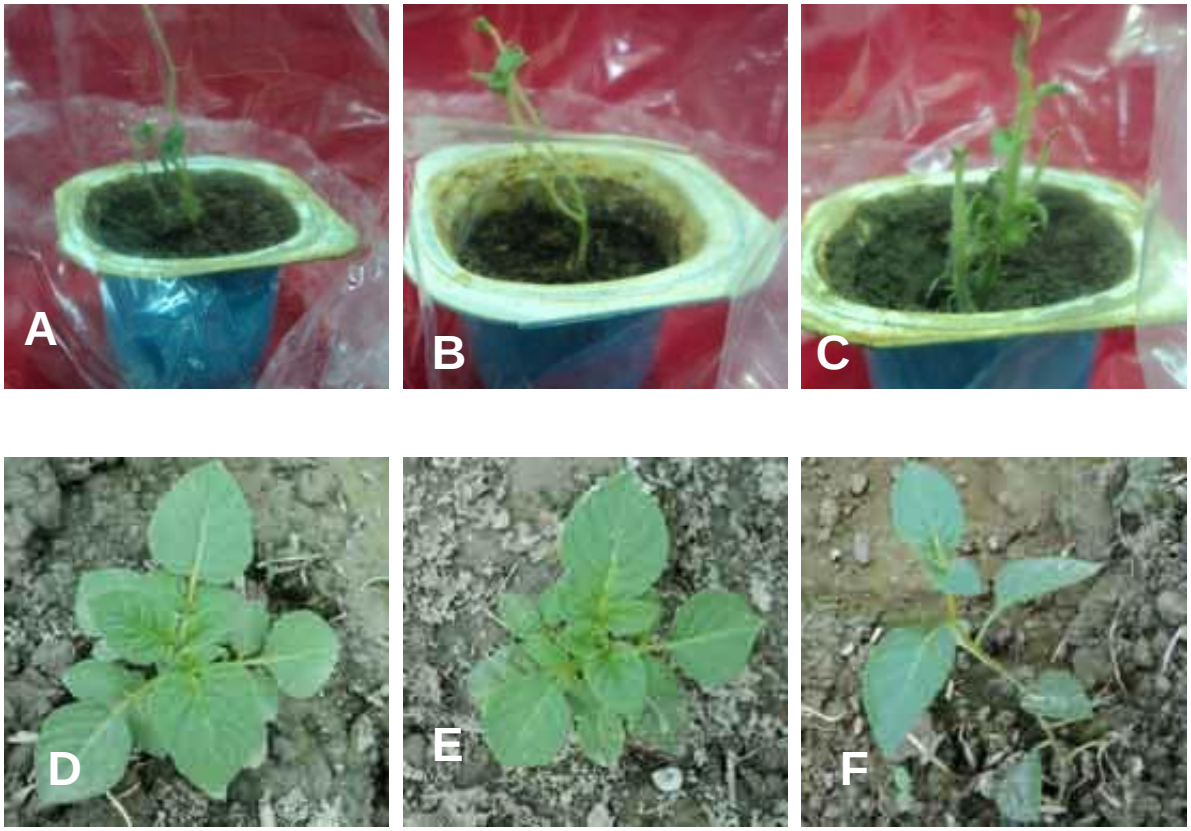

Figure 3 (A-F).
Acclimatization of virus free *in vitro* plantlets under natural condition.
(A-C) virus free *in vitro* derived plantlets poly bag under natural condition in diamant, cardinal and granula respectively.
(D-F) virus free *in vitro* derived plantlets under field condition in diamant, cardinal and granula respectively.

3.2. Shoot formation and root developments from established meristem

In this experiment the primary shoots developed from meristem cultures were aseptically taken out and cut into nodal segments. The individual nodal segments were cultured on MS semi solid medium without any growth regulators. Data were recorded on average shoot length after 10 days, 20 days, 30 days of culture and the results are presented in Table 4 and Figure 2-b. (D-F). The results showed that average shoot length of potato cultivars increased with the advancement of time and within one month the shoots attained up to 9-12.00 cm. The table showed that the cultivar granula produced the average longest shoot (12.00±0.57cm) after 30 days of culture (Figure 2-b. F). The cultivars cardinal also showed moderate performance in their average shoot length (9.50±1.05cm) on MS_0 medium after 30 days of culture (Figure 2-b. E). The lowest average shoot length was recorded 9.00±1.02 cm after 30 days of culture in cultivar diamant (Figure 2-b. D).
The shoots raised through *in vitro* technique of three potato varieties were sub cultured into MS_0 medium and MS with different concentrations and combinations of NAA and IBA to observe the effect on root induction (Figure 2-b. G-I). The summarized results of this experiment are shown in Table 5. In cultivar diamant, the mean number of roots per explant was the highest (4.80±0.03) in MS_0 medium (Figure 2-b. G), where as, cardinal was recorded (5.20±0.02) (Figure 2-b. H) and granula was (4.79±0.01) (Figure 2-b. I).
In the present finding, MS_0 and IBA (0.5 mgl^{-1}) were proved most efficient for rooting. Mean number of roots per explant and root length were highly influenced by the concentration of IBA. Efficient effect of IBA in root induction was also observed in better gourd (Rahman, 1998). Roy and De (1986) also reported faster growth of root in *Calotropis gigantea* and *Prunus* spp. respectively.

4. Conclusions

The main aim of our study to minimize this limitation associated with meristem culture and plantlet elongation for commercial production of disease free potato seeds. The potato crop has been emerged as an important

commercial crop for the farmers particularly in the lean period of the country. Protocol for rapid production of meristem culture and disease free potato seed would be a mile stone for our farmers in their socio-economic development.

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Conflict of interest
None to declare

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