MORPHOLOGICAL CHARACTERIZATION OF POTATO VARIETIES UNDER *IN VITRO* AND *IN VIVO* CONDITION

BY

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CERTIFICATE

This is to certify that the thesis entitled **'Morphological Characterization of Potato Varieties under *In vitro* and *In vivo* Condition'** submitted to the Department of Biotechnology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **Master of Science in Biotechnology,** embodies the results of a piece of bonafide research work carried out by **Khadija Akter,** Registration No. **08-02655** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

Dated: June, 2015
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Dedicated

to

My
Beloved Parents

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ABSTRACT

The experiment was conducted at the Biotechnology Laboratory and research farm of Sher-e-Bangla Agricultural University (SAU), Dhaka during the period from April 2015 to March 2016 to study the morphological characterization of potato varieties under *in vitro* and *in vivo* condition. The sprouts of potato varieties were used as explants and 4 times sub-culture were done to study the morphological parameter of plantlet. The highest length of shoot (5.19 cm) was in the variety Diamant and it was lowest in Asterix variety. The maximum diameter of shoot (1.16 mm) was recorded from Asterix, while the minimum diameter of shoot (0.82 mm) from Diamant. Due to the interaction effect of different varieties and number of explants, the highest length of shoot (6.00 cm) was found in Diamant variety when 8 explants used in each culture, while the lowest length (4.10 cm) in Cardinal variety used 3 explant per vial. *In vitro* regenerated plantlets were acclimatized under *in vivo* field condition. The variety Diamant showed the best performance in respect of all the parameters under this study. The weight of minituber and yield per plant were highest in Diamant (10.23 gm and 114.16 gm) variety whereas the lowest in Asterix (8.15 gm and 64.46 gm) variety.

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INTRODUCTION

CHAPTER I

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a member of the family Solanaceae and belongs to the genus *Solanum* and is the most important food crop of the world, with annual production approaching 300 million tons (CIP, 2007). It is used as a staple food in many countries of the world, but mainly as a vegetable in Bangladesh (Hussain, 1995). Potato is the rich source of starch, vitamins C and B, and minerals. It contains about 20.6% carbohydrate, 2.1% protein, 0.3% fat, 1.1% crude fiber and 0.9% ash. It contains a good amount of essential amino acids like leucine, tryptopan and isoleucine (Khurana and Naik, 2003). Potato also contains a variety of phytonutrients that have antioxidant activity. The Irish potato is one of the most widely grown tuber crops in the world and contributes immensely to human nutrition and food security (Steven, 1999; Karim *et al.*, 2010).

In Bangladesh, 462032 hectares of land were cultivated for potato and total production had been estimated as 8,950024 metric tons during 2013-2014 (BBS, 2014). Although it is contributing a major portion to our vegetable, its yield is too low in Bangladesh (19.07 t ha-1) when compared with that of other leading countries such as Netherlands (42.99 t ha-1), USA (38.40 t ha-1) and UK (39.64 t ha-1) (FAO, 2008). The main reasons for the low yield included use of varieties of low yield potential, use of poor quality seed tuber and inefficient management practices. In the last few decades, several dozens of high yielding varieties (HYV) of potato were brought to Bangladesh and tried experimentally under local conditions before being recommended for general cultivation (Islam *et al.*, 2003).

Most of the varieties of potato have been developed through ordinary selection and by conventional breeding which is very prolonged procedure. Recently, tissue culture or plant genetic engineering techniques have been providing a new opportunity for crop improvement. An efficient tissue culture system is thought to be crucial to the success of plant genetic transformation. The technique can be

used to add advantageous traits from the uncultivated relatives to the existing cultivars. However, for transferring genes into plants it is prerequisite to have efficient callus induction and plantlet regeneration system (Hamrick *et al.*, 2000). The regeneration of plants from cell and tissue culture represent an essential component of biotechnology and have the potentiality not only to improve the existing cultivars, but also for the generation of novel plants in a comparatively short time compared to conventional breeding (Khadiga *et al.*, 2009).

Now a days, tissue culture techniques are being applied all over the world. In our country, some universities *viz.* Dhaka University, Bangladesh Agricultural University, Tuber Crop Research Centre (TCRC) of Bangladesh Agricultural Research Institute (BARI) have been now producing microplants and microtubers for the national seed production program (Hossain and Sultana, 1995). Some NGOs and Private Companies like Bangladesh Rural Advancement Committee (BRAC), PROSHIKA, Institute of Integrated Rural Development (IIRD), Biotech Bangladesh Limited (BBL), Hitech Ltd., Square Agro Ltd. etc. are also in operation in producing mostly microplants and microtubers in a limited scale as supplementary propagules. Potato is susceptible to several fungus, virus and bacteria and cause heavy economic loss every year during the cultivation and storage. Conventional breeding through selection has a boundary of limitations. In the case of inbreeding, the improvement process is hampered due to lack of genetic variability. Potato breeding programs might be benefited through biotechnological techniques which may yield surpassing result over traditional plant breeding methods and open new avenues for crop improvement. The success of plant biotechnology relies on several factors which include an efficient tissue culture system for regeneration of plants from cultured cells and tissues (Pua *et al.*, 1996).

Shoot generation and rooting are important in the realization of the potential of the cell and tissue culture techniques for plant improvement (Purnhauser *et al.*, 1987). Plant regeneration in potato has been progressed a lot in recent years (Shirin *et al.*, 2007). Successful *in vitro* plant regeneration has been achieved from

explants of different organs and tissues of potato such as leaf, stem tuber discs and unripe zygotic embryos. Tissue culture technique has great potentiality which provides quick means of vegetative propagation in potato. It can produce thousand plants in a year. *In vitro* regeneration of potato has been reported from different explants on MS medium and different growth regulators for diseases free good quality plantlet or seed and pathogen free planting materials (Hossain, 1994; Rabbani *et al.*, 2001; Zaman *et al.*, 2001). Under Bangladesh condition, very few reports are available regarding the varietal effect and explants on plantlet multiplication under *in vitro* condition. The present research is to provide the information on high frequency plantlet production to meet the producer demand as well as morphological performances of different varieties under *in vivo* condition.

Considering the above facts, the present experiment has been undertaken with the following specific objectives:

- To study on morphological variation in potato cultivar on successive sub-culture generation.
- To observe varietal difference on sub culture stage using different number of explants.
- To study acclimatization potentiality of potato varieties in field condition;
- To study field performance of plantlet among different potato varieties.

CHAPTER-II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Potato is one of the leading staple food and most important vegetable crops throughout the Bangladesh as well as the whole world. In most cases potato are propagated by using seed tubers. But most of the countries of the world are now applying tissue culture and genetic engineering techniques for the development of this crop. Now a da ys, ma ny scientists of Bangladesh have carried out their research works in plant genetic engineering to open a new avenue for crop improvement. The present study was undertaken to provide information on high frequency plantlet multiplication as well field performances. The literatures, which are most relevant to the present study are reviewed here under the following headings-

2.1. Concept of potato tissue culture

This review was prepared as a survey of key articles presenting development, achievements and interconnection of various lines of potato biotechnology research united through the common use of *in vitro* culture techniques. Starting with the early researc h on the induction and differentiation of callus tissues, review sequentially and chronologically presents the advance of various *in vitro* culture techniques and their practical applications in clonal propagation, germplasm storage, production of healthy virus-free plants and breeding (Vinterhalter *et al.*, 2011).

Potato production with seed tuber is constrained by the accumulation of pathogen, physiological decline and low multiplication rates. Seed tuber is most expensive input in potato production. At least 35-40% total cost of potato production is covered by seed tuber. Now a days, plant cell tissue culture techniques are being applied for rapid multiplication of plantlet production of potato. Tissue culture or cell culture is the process where cells are grown and maintained in a controlled environment such as a laboratory, outside natural and original source. Cell culture is a vital technique in many branches of biological research. *In vitro* produced

disease free potato clones combined with conventional multiplication methods has become an integral part of seed production in many countries (Naik and Sarker, 2000)

2.2. Callus induction and plantlet regeneration

Shahab-ud-din *et al.* (2011) have conducted an experiment to investigate the effects of different concentrations of plant growth regulators and their combinations on callus induction of potato (*Solanum tuberosum* L.). The explants of potato tuber were cultured on Modified MS medium supplemented with different concentrations of 2,4-D, NAA and BA in combinations with BA and NAA in combination with BA for callus induction. The concentration of sucrose was 3% W/V level and the pH of the media was adjusted to 5.7 before the addition of agar 8% W/V. The explants were first dissected out aseptically and then inoculated to the media (with various levels of hormones), then incubated at 27±2°C in the culture room. Among the treatments 2,4-D at different concentrations produced different degree of calli but comparatively a massive amount of calli were formed on MS medium supplemented with 2,4-D alone at 3.0 mg/L. Also NAA and BA with different concentrations produced considerable degrees of callus but the degree of callus was best at higher concentrations of NAA and BA. 2,4-D in combination with BA at 2.0 mg/L both produced considerable amount of callus. In case of NAA in combination with BA the degree of callus formation was best at concentration 1.0 mg/L each. So according to the above findings it was concluded that 2,4-D is the best option for induction of callus among the other hormones used in the study.

Khalafalla *et al.* (2010) reported the procedure of plant regeneration from callus culture of potato (*Solanum tuberosum* L.). Calli were induced from 1.0 cm^2 tuber segment of potato cultivar Almera on MS medium supplemented with different levels (1.0-5.0 mg/L) of 2,4-D. The hundred percent explants produced nodular calli within 7- 12 days on MS medium when supplemented with 2.0-5.0 mg/L of 2, 4-D. Calli were differentiated into shoot-primordia when subcultured on MS medium supplemented with 1.5-5.0 mg/L of thidiazuron (TDZ) and 2.0-5.0 mg/L

of benzyladenine (BA). The best result for number of shoot per callus (3.3 ± 0.3) and longest shoot (0.8 ± 0.1) were obtained by using TDZ at 5.0 mg/L. Callus derived shoots were rooted most effectively in full-strength MS medium containing 1.0 mg/L IBA. The success of plant tissue culture for *in vitro* culture of potato was encouraged by acclimatization of the plantlets in the greenhouse conditions. Regenerated plants were morphologically uniform with normal leaf shape and growth pattern.

Hoque *et al.,* (2010) investigated i*n vitro* microtuber formation potentiality of potato was investigated to establish a rapid disease free seed production system in potato. MS medium supplemented with 4 mgL-1 of kinetin showed best performance in respect of multiple shoot regeneration and microtuber formation. Simple MS medium was not able to produce any micro tuber under *in vitro* condition. Dark condition better
responded to tuberization than light condition. Among the three different explants (nodal segment, sprout and shoot apex) nodal cutting showed the best performance on days to microtuber formation and average weight of microtuber. MS + 6% sucrose + 4 mgL-1 kinetin combination of treatment was the best for *in vitro* tuberization among the parameters under study.

Hussain, I., (2005) investigated i*n vitro* response and its relationship with different varieties, explants and medium were investigated in potato (*Solanum tuberosum*). Direct *In vitro* regeneration protocol from diverse explant source is a prerequisite for transformation studies. Three potato cultivars *viz.*, Cardinal, Altamash and Diamant were selected for *in vitro* responses. High regeneration and morphogenic potential of different explants i.e., shoot tips, leaf discs, nodes and internodes have been tested for direct regeneration.
Basal media was Murashige & Skoog and different hormonal combinations of benzyl adenine and indoleacetic acid were supplemented. Statistical analysis showed that explants source had significant effect on direct regeneration and the nodal explants had maximum regeneration. The number of shoots obtained from node was 17.6 from Cardinal followed by Diamant 14.3 and Altamash 9.0. Shoot

apices also resulted in shoot regeneration comparatively better than leaf discs and internodal explants but lesser than from nodes. Most suitable medium was MS with 2.0 mg/L BAP and IAA @ 0.5 mg/L giving maximum regeneration. It was also observed that interaction of cultivars with explant and media is highly significant at P 1.0%.

Yee *et al.* (2001) investigated the shoot regeneration *in vitro* of potato cultivars Chieftain, Desiree, Kennebec, Lenape, Niska, Russet Burbank and Shepody from petioles with intact leaflets was assessed using six treatment combinations a basal medium with or without silver thiosulphate or thidiazuron at two concentrations (2.0 or 0.5 mg/L) of IAA. The basal medium consisted of MS salts and vitamins supplemented with 3.0 mg/L BA, 1.0 mg/L gibberellic acid, 30 g/L sucrose and 7.0 g/L phyto-agar. Two full set repeat and one partial set repeat of independent experiments was conducted and all produced similar results. Silver thiosulfate decreased the regeneration frequency and number of shoots per callus. No significant changes were observed with thidiazuron. Regeneration rate of 100% with up to 20 shoots/plantlet per callus was achieved at 2.0 mg/L IAA with cultivars Desiree, Kennebec, Niska and Lenape. These cultivars still showed high regeneration rate (87-98%) on medium with 0.5 mg/L IAA and good regeneration rates were also achieved by the other three cultivars (48, 50 and 94% for Chieftain, Shepody and Russet Burbank, respectively). With the single medium protocol (0.5% IAA without thiosulfate or thidiazuron), Desiree, lenape and Niska exhibited a regeneration rate of 98%).

2.3. Field performances of different potato varieties

Chehaibi *et al.* (2013) conducted an experiment in the research station of the higher institute of agronomy of Chott-Mariem in the Sahel region of Tunisia with two varieties of potato: Alaska and Safrane, were mechanically planted at two different depths. The results showed that for tuber yield of Alaska variety was more productive than the Safrane variety with increasing yield of tubers.

Jatav *et al.* (2013) conducted an experiment was at Central Potato Research Station, Jalandhar to evaluate potato cultivars *viz.* Kufri Jyoti, Kufri Jawahar, Kufri Bahar, Kufri Sutlej, Kufri Pukhraj, Kufri Pushkar, Kufri Surya and Kufri Gaurav at four N levels and results revealed that Kufri Gaurav recorded maximum yield, agronomic efficiency and net return at all the levels of nitrogen followed by Kufri Pushkar and Kufri Pukhraj. Kufri Surya yielded minimum with least agronomic efficiency at all the levels of nitrogen.

Jovovic *et al.* (2012) conducted an experiment to find out the differences among genotypes for potato yield and recorded the highest yield was measured at variety Agria (30.0 t ha^{-1}), while the lowest at Riviera (24.6 t ha^{-1}) and recommended that Agria variety was favourable for yield of potato tuber.

Abbas *et al.* (2012) conducted an experiment on two potato genotypes for processing and yield quality traits. Significant differences in all the quality parameters and various characteristics were found, while the genotypes; 394021-120, 9625, Kiran, NARC 2002-1, NARC 1-2006/1 and VR 90-217 gave the highest results regarding yield and quality of potato tubers except Kiran, which has a high yield but low quality characters. The tuber sizes and weight was also significantly different among genotypes except weight of big size tubers.

Karim *et al.* (2011) studied with ten exotic potato varieties (var. All Blue, All Red, Cardinal, Diamant, Daisy, Granulla, Green Mountain, Japanese Red, Pontiac and Summerset) to established in the trial field for showing yield performance of tuber number per plant and tuber weight per plant from 10 randomly selected potato plants of each variety. The highest tuber number (57.52) per plant was recorded in var. Daisy and the lowest tuber number (8.82) per plant was recorded in red varieties. On the other hand, total tuber weight per plant was the highest (344.60 g) recorded in var. Diamant and total tuber weight per plant was the lowest (65.05 g) recorded in var Pontiac. All blue varieties showed the most potential yield in this experiment.

Sharma and Sarjeet (2010) was evaluated the production potential of small (10 g) seed tubers of four potato varieties of hills viz., Kufri Shailja, Kufri Kanchan, Kufri Giriraj and Kufri Jyoti at three plant densities viz., 83,333; 111,111 and 166,666 plants/ha. Plant vigour in respect of height, number of shoots and compound leaves was affected significantly by the varying plant densities as well as by the genotypes. Days to foliage maturity were not influenced by the plant densities but varieties differed significantly. Number of total and seed size tubers were maximum in Kufri Shailja (6.09 and 3.33 lac/ha, respectively) at the maximum plant density (166,666 plants/ha) and minimum in Kufri Jyoti (2.36 and 1.00 lac/ha respectively) at the minimum plant density. Similarly, total and seed size tuber yields were maximum in Kufri Shailja (369.0 and 175.8 q/ha) at the maximum plant density. Potato yields were minimum in Kufri Jyoti (242.6 q/ha) at minimum plant density, whereas seed size tuber yield was minimum (55.3 q/ha) in Kufri Giriraj but at par with Kufri Jyoti.

A study on the performance of different USA Potato Lines was conducted by Zulker (2009) and recorded the highest yield of tubers per hectare (35.24 t ha^{-1}) was recorded in the accession AC 10110 closely followed by AC 10076 (32.07 t ha^{-1} and Diamant (31.88 t ha^{-1}). The lowest yield was obtained from the accession AC 10124 (10.88 t ha^{-1}). The tallest plants were produced by the accession AC 10111 (81.70 cm) and the shortest plants came from the accession AC 10125 (5.79 cm). The accession AC 10081 required maximum days (54.69 days) to tuberization and the shortest duration by the variety Diamant (32.01 days).

A field experiment was conducted by Pandey *et al.* (2009) to evaluate the performance of 3 indigenous potato (*S. tuberosum*) processing cultivars (Kufri Chipsona 1, Kufri Chipsona 2 and Kufri Jyoti) and 2 exotic cultivars (Atlantic and FL 1533) in Agra, Indore, Jalandhar, Kufri, Modipuram and Ooty, Uttar Pradesh, India. The highest total tuber yield, processing-grade tuber yield, tuber dry matter content was recorded for two exotic cultivars.

Luthra *et al.* (2006) reported that Kufri Arun is a medium maturing, main season, high yielding table potato variety suitable for cultivation in north Indian plains. It is a clonal selection from the cross between Kufri Lalima and MS/82-797. Its plants are tall and vigorous with field resistance to late blight. Its tubers are red, oval with shallow to medium eyes and creamy-light yellow flesh, and having good keeping quality. It is fertilizer responsive and capable of yielding 350-400 q/ha under optimum agronomical practices.

Pandey *et al.* (2006) reported that Kufri Chipsona-3 is a medium maturing, late blight resistant potato variety with round oval tubers, white smooth skin and cream/pale yellow flesh. The total and process grade tuber yields of Kufri Chipsona-3 are higher than those of Kufri Chipsona-1 and Kufri Chipsona-2. The total tuber yields are higher than even the popular table variety Kufri Bahar. Kufri Chipsona-3 yields excellent defect free tubers. The chemical maturity of its tubers occurs at 110 days.

Alam *et al.* (2003) characterized fourteen exotic varieties of potato (*Solanum tuberosum*) namely Mondial, Granola, Cardinal, Ailsa, Petronese, Morene, Diamant, Cleopetra, Binella, Dheera, Multa, Kufri Sindhuri, Heera, Chamak and a local check (Lal Pakri) under Bangladesh condition. The yields ranged of exotic varieties were 19.44 to 46.67 t ha^{-1}. Variety Ailsa produced the maximum yield (46.67 t ha^{-1}) which was followed by Cardinal and Mondial.

Hossain *et al.* (2003) observed that the yield contributing characters of varieties differed significantly. Highest yield (27.31 t ha^{-1}) was obtained from the variety Akira and it was identical to Jaerla (26.30 t ha^{-1}) and these two varieties out yielded the check variety Diamant (22.81 t ha^{-1}). The varieties Baraka, Jaerla, Bintje, Midas, Ultra, Akira, Dura, Granola, Futuri and Diamant yielded more than 20.00 t ha^{-1}. Most of the varieties perhaps did not able to show their full yield potential due to the new environment of their first generation in Bangladesh.

Gregoriou and Onoufriou (2002) conducted an experiment on farmers fields in the main potato growing area of Cyprus (Kokkinochoria area). Emphasis was given to yield and earliness, and to tuber quality characters (keeping quality, size and shape, cooking, colour of skin and flesh, dry matter, appearance, physiological disorders, etc.). Considering the continuously changing production and marketing requirements and the fact that selection of a potato variety is a compromise among various factors, the following varieties were recommended for commercial production: Superstar, Burren, Ditta, Arinta, Cynthia, Filea, Othello, Armada, Akira, Fabula and Vivaldi.

Wu *et al.* (1997) conducted an experiment with potato variety Yushu 1 that was selected from the progeny of the cross Gaoyuan 7 × 762-93. Yushu 1 has early maturity, high yield, is resistant to disease and deterioration. It can be harvested 65 days after planting. Tuber yield is 33.7 t ha-1 in spring and 22.5 t ha-1 in autumn. The tubers have good quality and commercial characters, and are suitable for export and processing. Yushu 1 can be grown on the plains as part of a double cropping system or by single cropping in mountainous districts.

Reust (1997) studied yield formation in mid-early varieties Agria and Matilda, and mid-late Panda, grown. Tuber bulking rate was largely influenced by growing conditions. Mean daily yield increases varied from 500 to 1000 kg/ha, and high yielding variety Agria showed the highest rate of daily increase. Early in the season, starch content increased in proportion to the tuber bulking rate.

From the review of literature illustrated above, it may be concluded that the growth and yield of potato were greatly influenced by tuber and tuberlets generated from different variety. But the information must be different in our country from the other countries forward any conclusive recommendation for potato production. As a result they could not succeed in their endeavor. Therefore the present study will be carried out for a idea generation to improve the growth and yield of potato in Bangladesh.

CHAPTER -III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The materials and methods that were used for conducting the experiment have been presented in this chapter. It includes a short description of the experiment, materials used, design of the experiment, data collection procedure and data analysis methods.

Consequently following two experiments were conducted to fulfill the present objectives:

Experiment I : *In vitro* plantlet regeneration from three popular potato varieties using different number of explants.

Experiment II : Acclimatization and morphological characterization of plantlet under *in vivo* condition.

3.1. Experimental period

The experiment was conducted during the period from April 2015 to March 2016.

3.2. Description of experimental site

The experiment was conducted at the Biotechnology Laboratory and research farm of Sher-e-Bangla Agricultural University (SAU), Dhaka. It is located in 24.09°N latitude and 90.26°E longitude. The altitude of the location is 8 m from the sea level as per the Bangladesh Metrological Department, Agargaon, Dhaka-1207.

3.3. Climatic condition

The climate of experimental site is subtropical, characterized by three distinct seasons, the monsoon from November to February and the pre-monsoon period or hot season from March to April and the monsoon period from May to October. The monthly average temperature, humidity and rainfall during the crop growing period were collected from Weather Yard, Bangladesh Meteorological Department (climate & weather division) Agargaon, Dhaka-1212 and presented in the Appendix I.

3.4. Planting materials

The seed tuber of potato varieties Diamant, Cardinal and Asterix were used as planting materials for this experiment. The potato tubers were collected from Tuber Crop Research Centre (TCRC), BARI, Joydevpur, Gazipur.

3.5. Experiment-I:

3.5.1. Laboratory preparation

Laboratory preparation was started in April 2015 by collecting chemical and instruments and presented in Table 2.

Table 1. List of the chemicals and instruments used in the experiment

Chemicals		Instruments	
1	a) MS medium (powder) (Duchefa, Netherlands) MS medium ingredients	1	Autoclave
2	Sterilizing chemicals a) Sodium hypo chloride (NaOCl) b) Potassium hypochloride (KClO) Tween-20	2	Hotplate with magnetic stirrer
3	Sucrose	3	Freezers
4	Agar	4	Laminar Air Flow Chamber
5	NaOH (10 N, 1N)	5	Microwave oven
6	HCl (0.1 N)	6	Plant Growth Chamber
7	KCl (3M)	7	Water Purification System
8	Absolute Ethanol	8	pH meter
9	Ethanol (70%)	9	Top pan balance (500gm) (0.01gm) and Metlar balance (120gm)(0.0001gm)

Table 1. List of the chemicals and instruments used in the experiment

Chemicals		Instruments	
10		10	Scalpel, forceps, scissors etc.
11		11	Culture vials (petridishes, test tubes, culture bottles etc.)

3.5.2. Culture media

Success of any experiment depends on the culture media, hormone combination, tissue and employing cell. Murashige and Skoog (1962) medium was used as culture medium for *in vitro* regeneration.

3.5.3. Preparation of IN NaOH:

40 g NaOH pellets were weighed and added to the 800 ml of sterilized distilled water and stirred well until dissolved. Sterilized distilled water was added to make volume 1000ml and mixed the closed bottle.

3.5.4. Preparation of 70% Ethanol

In a 100 ml measuring cylinder 70 ml 99.9% ethanol was poured. Double distilled water was poured up to the level of 100 ml. Store the solution in a sterilized glass bottle. This solution was made fresh each time before use.

3.5.5. MS Media preparation from readymade MS powder

To prepare one liter of MS medium, the following steps were followed:

1. 700 ml double distilled water was taken into 1000 ml beaker
2. 5 gm of MS powder and 30 gm of sucrose were added and gently stirred to dissolve completely with the help of a hot plate magnetic stirrer.
3. The whole mixture was then made up to 1 liter with further addition of double distilled water.
4. pH of the medium was adjusted to 5.8 by pH meter with the addition of 1 N NaOH or 0.1 N HCl whichever was necessary.

5. Finally, 8gm agar was added to the mixture and heated for 8 minutes in an electric oven at 250°C for melting of agar.

The composition of MS medium is presented in Appendix I.

3.5.6. Sterilization

Sterilization of culture media

Prepared one liter MS medium was poured into two 1 liter conical flasks and capped with aluminum foil. Then the conical flasks were autoclaved at 15 kg/cm² pressure at 121°C for 20 minutes. The medium was then transferred into the culture room and cooled at 24°C temperature.

The medium was aliquot at around 25 ml into culture vials (diameter of vial was 25 cm). After dispensing the vials were covered with thin plastic caps and marked with different codes with the help of permanent marker to indicate specific treatment.

Sterilization of glassware and instruments

All types of glassware and instruments were washed properly by liquid detergent, cleaned with running tap water and finally washed with distilled water and dried in automatic drying oven. Glassware, culture vessels, beakers, petridishes, pipettes, slides, plastic caps, other instruments such as forceps, needles, scissor, spatula and surgical blades were sterilized in an autoclave at a temperature of 121°C for 20 minutes at 1.5 kg/cm² pressure.

Sterilization of culture room and transfer area

At the beginning, the culture room was sprayed with formaldehyde and then the room was kept closed for one day. Then the room was cleaned through gently washing the floor, wall and rakes with a detergent. This was followed by careful wiping with 70% ethanol. This process of sterilization of culture room was repeated at regular intervals. The transfer area was also cleaned with detergent and also sterilized twice in a month by 70% ethanol. Laminar air flow cabinet was sterilized by switching on the ultra violate ray to kill the microbes inside the

laminar airflow. It was switched on for 30 minutes before working keeping the instruments inside. The working surface was wiped with 70% ethanol, 30 minutes before starting the transfer work.

3.5.7. Preparation of explants

The sprouts of potato were used as explants. The sprouts were separated from the potato tuber and washed thoroughly with double distilled water inside laminar airflow cabinet for surface sterilization. Potato sprouts were first sterilized with 70% (v/v) ethanol for one minute. The sprouts were then rinsed twice with sterile distilled water. Afterwards the sprouts were surface sterilized by immersing in 0.1% $HgCl_2$ solution containing three drops of tween-20 solution and then finally rinsed and washed four times with sterilized distilled water. The surface sterilized disinfected sprouts were then cut into small segments and kept in distilled water of sterilized pestidishes to keep the sprout alive (Plate.1.). Then the explants were ready for inoculation.

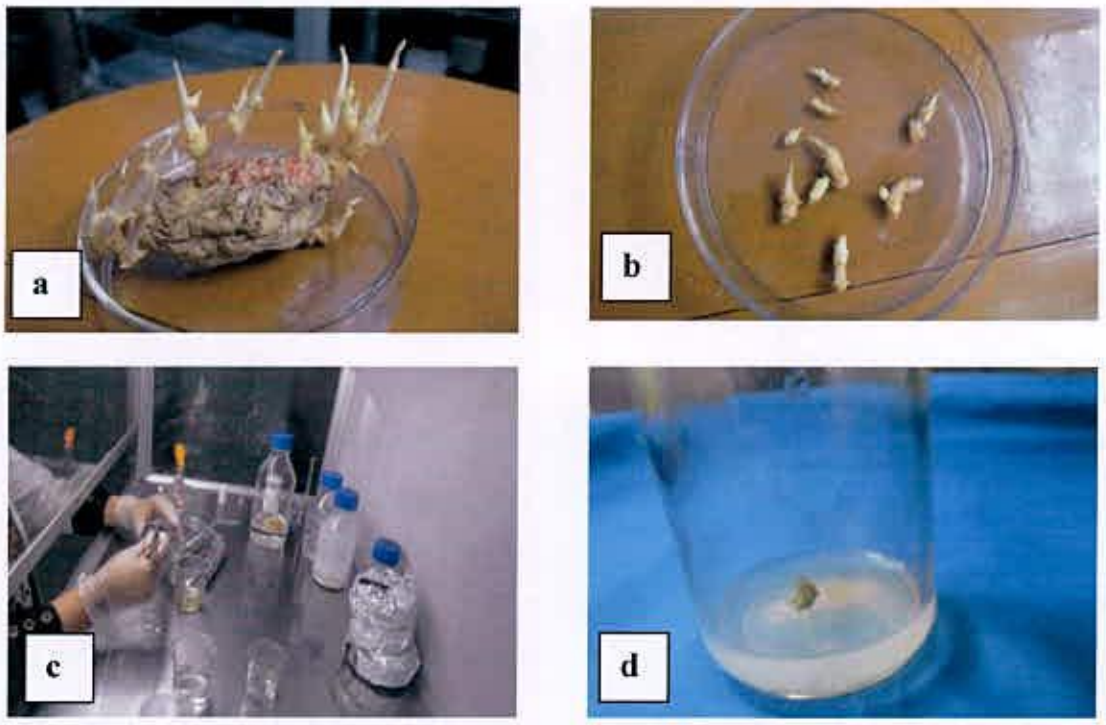


Plate.1. Explants preparation for *in vitro* regeneration

a) Potato tuber with sprout, b) Sprout used as explants,
c) Explants surface sterilization in laminar airflow hood,
d) Explants inoculation into culture vial

3.5.8. Inoculation of culture

The explants were prepared carefully under aseptic condition inside the laminar airflow cabinet. Explants were directly inoculated to each vial containing 25 ml of MS medium. The vials were capped and total operation was done in the laminar airflow cabinet in sterile condition.

3.5.9. Subculture

The regenerated plantlets were subcultured after 4 weeks of inoculation. A total of four subculture were done, each with different number of explants. Number of explants were 2, 3, 4 and 8 in subcultures I, II, III and IV respectively (plate.2.-plate.5.). The regenerated shoots were cut into small pieces and placed on prepared sterilized MS medium. The subcultured vials were then inoculated at 25±1°C with 16 h photoperid. Repeated subculture was attended at regular interval of 28 days. The observations and data collection were noted regularly.

3.5.10. Data collection

Data on the following parameters were recorded under *in vitro* condition.

In laboratory Condition

Number of internode

Total number of internode was recorded by visual observation at 28 days after inoculation (DAI) from each subculture. The mean value of the data provided the number of internode.

Length of internode (cm)

The length of internode was recorded by using a plastic scale in laminar airflow cabinet at 28 DAI from each subculture.

Length of shoot (cm)

The length of shoot was recorded by using a plastic scale in laminar airflow cabinet at 28 DAI from each subculture.

Diameter of shoot (mm)

The diameter of shoot was recorded by using a slide calipers in laminar airflow cabinet at 28 DAI from each subculture.

Number of branch/plant

Total number of branch/plant was recorded by visual observation at 28 days after inoculation (DAI) from each subculture.

Number of leaf/plant

Total number of leaf/plant was recorded by visual observation at 28 days after inoculation (DAI) from each subculture.

Length of the largest leaf (cm)

The length of largest leaf was recorded by using a plastic scale in laminar airflow cabinet at 28 DAI from each subculture.

Experiment II : Acclimatization and morphological characterization of plantlet under *in vivo* condition.

3.6. Transfer of plantlets to *in vivo* condition

When the regenerated plantlets were fully matured with well developed shoots, leaves and roots, they were removed from culture vials with the help of fine forceps. The medium attached with roots was washed with tap water carefully. Then the plantlets were transplanted to plastic pots containing autoclaved garden soil and cow dung ratio of 1:2 and covered with plastic paper. Plants were kept under culture room condition for 15-20 days, then transferred to net house and placed under shade until growth was observed. Transplantation of the plantlets was done in the afternoon. The plantlets were immediately irrigated after transplantation with a fine spray of water. Initially plants were kept in high humidity and with low light intensity. The humidity was gradually decreased by next 7-15 days and the light intensity was increased. It was irrigated regularly at an interval of 2 days.

3.7. Transfer of plantlets to the natural environment

After 10-15 days, the plantlets were established in the tray and root systems were developed and then they were transferred to the soil in open environment.

3.8. Experimental design

In laboratory condition, the two factors experiment was laid out in Completely Randomized Design (CRD) with three replications. In field condition single factor experiment was laid out in Randomized Complete Block Design (RCBD) with three replications.

3.9. Production Technology

3.9.1. Land Preparation

The soil was well prepared with good tilth to ensure proper potato crop production. The land of the experimental field was ploughed to a depth of 6-8 inch. After ploughing and laddering, all stubbles and uprooted weeds were removed. The *in vitro* plantlets were planted on 24th November 2015.

3.9.2. Manuring and Fertilizing

Urea, TSP and MoP fertilizers were applied in the experimental plot (3 kg, 2 kg, and 1 kg respectively). The half of urea, TSP and MoP were applied as basal during land preparation. The rest of urea and MoP used as top dressing in two installments at 35 and 60 days after planting. Admere and Ridomil Gold were applied at 30 DAP to prevent late blight.

3.9.3. Intercultural Operations

The *in vitro* plantlets were covered by net. Weeding and earthing up were done manually at 30 DAP. Irrigation was applied four times, first at one week after planting second at after earthing up, third at 45 days and last one at 60 days after planting. Admair and Ridomil Gold were applied to prevent late blight at 30 DAP.

3.9.4. Harvesting

The minitubers were harvested 4th March 2016. Data on minitubers were collected from the plot.

3.9.5. Data collection

Data on the following parameters were recorded under field condition.

In field condition

Plant height (cm)

The height of plant was recorded in centimeter (cm) at 7, 14, 21, 28, 35 and 42 DAP (Days after planting) in the experimental plots. Data were recorded as the average of 3 plants selected at random from the inner rows of each unit plot. The height was measured from the ground level to the tip of the growing point by a meter scale.

Diameter of stem (mm)

The diameter of stem was recorded in millimeter (mm) at 7, 14, 21, 28, 35 and 42 DAP in the experimental plots. Data were recorded as the average of 3 plants selected at random from the inner rows of each unit plot. The height was measured from in middle portion of a stem by a slide calipers.

Number of branch/plant

Total number of branch/plant was recorded at 7, 14, 21, 28, 35 and 42 DAP in the experimental plots as the average of 3 plants selected at random earlier from each unit plot.

Number of leaf/plant

Total number of leaf/plant was recorded at 7, 14, 21, 28, 35 and 42 DAP in the experimental plots as the average of 3 plants selected at random earlier from each unit plot.

Length of tuber (cm)

The length of tuber was recorded in centimeter (cm) from 10 selected tubers from the each experimental plot. The length of tubers was measured by using a meter scale.

Diameter of tuber (cm)

The diameter of tuber was recorded in centimeter (cm) from 10 selected tubers from the each experimental plot. The diameter of tubers was measured by using a slide calipers.

Number of tubers

The average number of tubers was recorded by counting total tubers from the each experimental plot.

Weight of tubers (gm)

The average weight of tubers was recorded by weighted total tubers from the each experimental plot and expressed in gram.

Weight of largest tubers (gm)

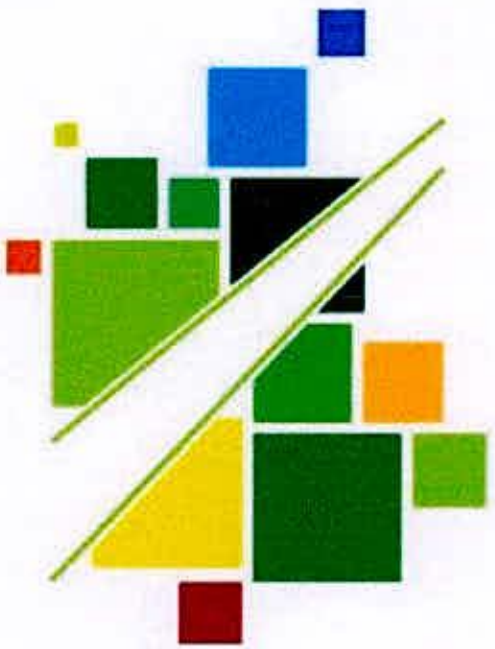
The weight of maximum size of tubers was recorded by weighted biggest size of tubers from the each experimental plot and expressed in gram.

Yield/plant

Total number of tubers from a plant was weighted from the each experimental plot and expressed in gram.

3.10. Statistical analysis

The data obtained for different characteristics were statistically analyzed to find out the significant difference among the treatments. The mean values of all the recorded characteristics were evaluated and analysis of variance was performed by the 'F' (variance ratio) test using MSTAT-C software. The significance of the difference among the treatment means was estimated by Duncan's Multiple Range Test (DMRT) at 5% level of probability (Gomez and Gomez, 1984). LSD was also calculated to compare the differences between two treatment means.



CHAPTER-IV

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

The present experiment was conducted to study the morphological characterization of potato varieties under *in vitro* and *in vivo* condition. Consequently two experiments were conducted under the laboratory and field condition. The analysis of variance (ANOVA) of the data has been presented in Appendix III-VIII. The results have been presented and discussed under the following headings:

4.1. Experiment I: *In vitro* plantlet regeneration from three potato varieties using different number of explants.

4.1.1. Number of internode

Number of internode among different plantlets of the potato varieties varied significantly from each other and presented in Table 2. The maximum number of internode (6.58) was recorded from Diamant, whereas the minimum number of internode (5.08) was observed in Asterix which was statistically similar (5.33) to Cardinal variety.

Statistically significant variation was recorded for number of internode in regenerated plantlet at different number of explants used per vial (Table 2.). Data revealed that the maximum number of internode (6.33) was found from 3 explants used in culture vial, while the minimum number of internode (5.33) was recorded from 2 explants (plate.2.) used in culture vial which was statistically similar (5.44 and 5.56) to that of 4 explants (plate.4.) and 8 explants (plate.5.) used in culture vial respectively.

Interaction effect of different varieties and number of explants showed significant variations on number of internode under *in vitro* condition (Table 3.). The maximum number of internode (7.00) was found in Diamant variety with both 3(plate.3.) and 4(plate.4.) no. of explants per vial, while the minimum number of internode (4.67) was found in Asterix variety when 4 (plate.4.) and 3 explants (plate.3.) were used in each culture vial.

Table 2. Effect of variety and number of explants on morphological parameters of plantlet under *in vitro* condition

Treatment	Number of internode	Length of internode (cm)	Length of shoot (cm)	Diameter of shoot (mm)	Number of branch/plantlet	Number of leaf/plantlet	Length of the largest leaf (cm)
Potato variety							
V_1	6.58 a	1.67 a	5.19 a	0.82 c	1.08 ab	6.50 b	1.28 a
V_2	5.33 b	1.37 b	4.75 b	0.97 b	1.42 a	8.25 a	1.00 b
V_3	5.08 b	1.18 c	4.53 b	1.16 a	0.83 b	5.92 b	0.79 c
$LSD_{(0.05)}$	0.444	0.088	0.244	0.080	0.421	0.628	0.119
Level of significance	0.01	0.01	0.01	0.01	0.05	0.01	0.01
Number of explants							
P_1	5.33 b	1.48 a	4.76 b	1.07 a	0.78 b	7.00 b	1.13 a
P_2	6.33 a	1.43 ab	4.19 c	1.00 ab	1.00 b	7.11 b	1.11 ab
P_3	5.44 b	1.38 ab	5.07 a	0.90 c	1.11 ab	5.44 c	.87 c
P_4	5.56 b	1.33 b	5.29 a	0.96 bc	1.56 a	8.00 a	0.99 bc
$LSD_{(0.05)}$	0.513	0.102	0.282	0.092	0.487	0.726	0.138
Level of significance	0.01	0.05	0.01	0.01	0.05	0.01	0.01

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability

V_1: Diamant
V_2: Cardinal
V_3: Asterix

P_1: 2 explants in a vial
P_2: 3 explants in a vial
P_3: 4 explants in a vial
P_4: 8 explants in a vial

Table 3. Interaction of variety and number of explants on morphological parameters of potato under laboratory condition

Treatment	Number of internode	Length of internode (cm)	Length of shoot (cm)	Diameter of shoot (mm)	Number of branch/plantlet	Number of leaf/plantlet	Length of the largest leaf (cm)
V_1P_1	6.00 bc	1.86 a	4.97 bcd	0.70 e	1.00 ab	8.67 ab	1.03 cd
V_1P_2	7.00 a	1.60 bc	4.37 ef	1.13 a	1.33 ab	8.33 abc	1.33 ab
V_1P_3	6.33 ab	1.70 ab	5.43 b	1.10 ab	1.00 ab	7.00 cdef	1.40 a
V_1P_4	7.00 a	1.53 bcd	6.00 a	1.20 a	1.67 a	7.67 abcd	1.37 a
V_2P_1	5.00 d	1.35 def	4.87 cde	1.10 ab	0.67 b	6.00 efg	0.77 de
V_2P_2	6.00 bc	1.40 de	4.10 f	0.93 bc	1.00 ab	7.33 bcde	1.10 bc
V_2P_3	5.33 cd	1.42 cde	4.97 bcd	0.90 cd	2.00 ab	5.00 gh	1.10 bc
V_2P_4	5.00 d	1.30 ef	5.07 bc	0.93 bc	1.67 a	9.00 a	1.03 cd
V_3P_1	5.00 d	1.24 ef	4.43 def	0.90 cd	0.67 b	6.33 defg	0.80 de
V_3P_2	6.00 bc	1.30 ef	4.10 f	0.93 bc	0.67 b	5.67 fg	0.90 cd
V_3P_3	4.67 d	1.03 g	4.80 cde	1.20 a	0.67 b	4.33 h	0.90 cd
V_3P_4	4.67 d	1.16 fg	4.80 cde	0.73 de	1.33 ab	7.33 bcde	0.57 e
$LSD_{(0.05)}$	0.889	0.177	0.488	0.160	0.843	1.257	0.238
Level of significance	0.05	0.05	0.05	0.01	0.05	0.05	0.05
CV(%)	9.30	7.44	6.02	9.62	15.00	10.82	13.80

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability

V_1: Diamant
V_2: Cardinal
V_3: Asterix

P_1: 2 explants in a vial
P_2: 3 explants in a vial
P_3: 4 explants in a vial
P_4: 8 explants in a vial

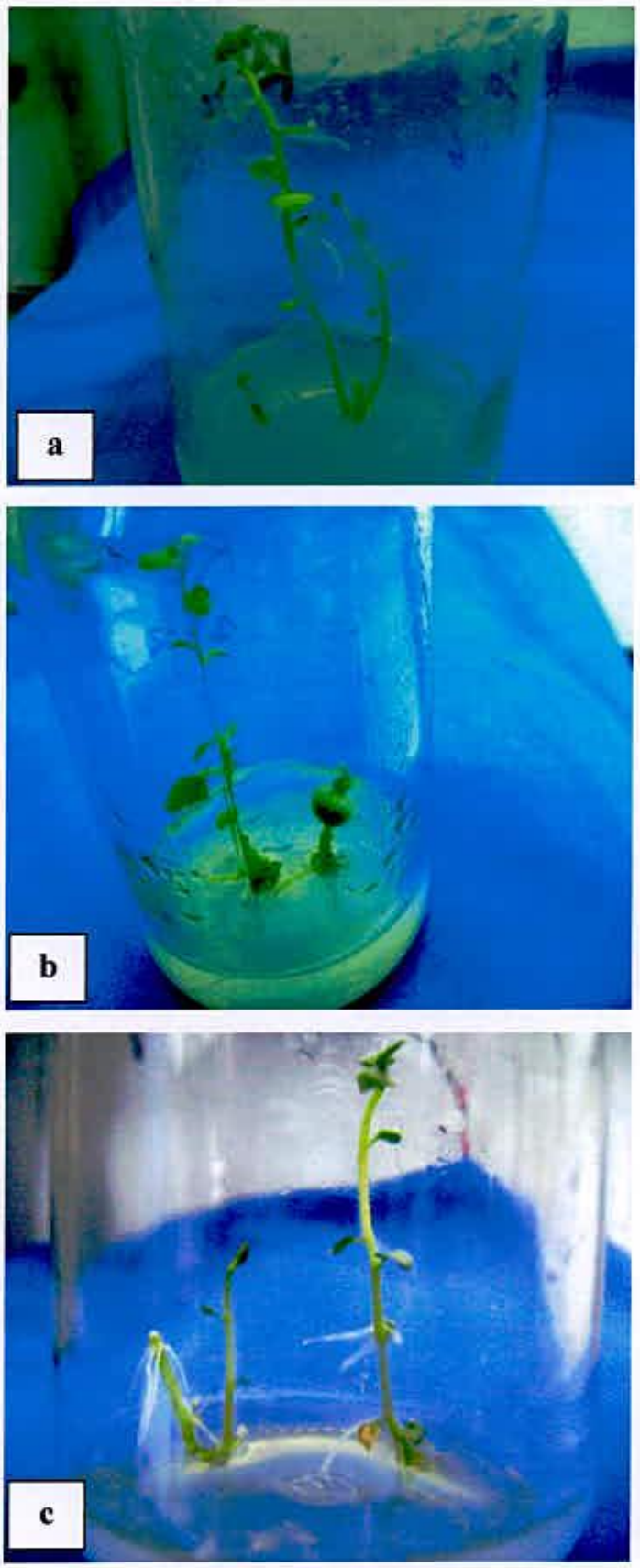


Plate.2. Plants regenerated from two explants used in a culture vial in subculture-I (a) Diamant (b) Cardinal and (c) Asterix

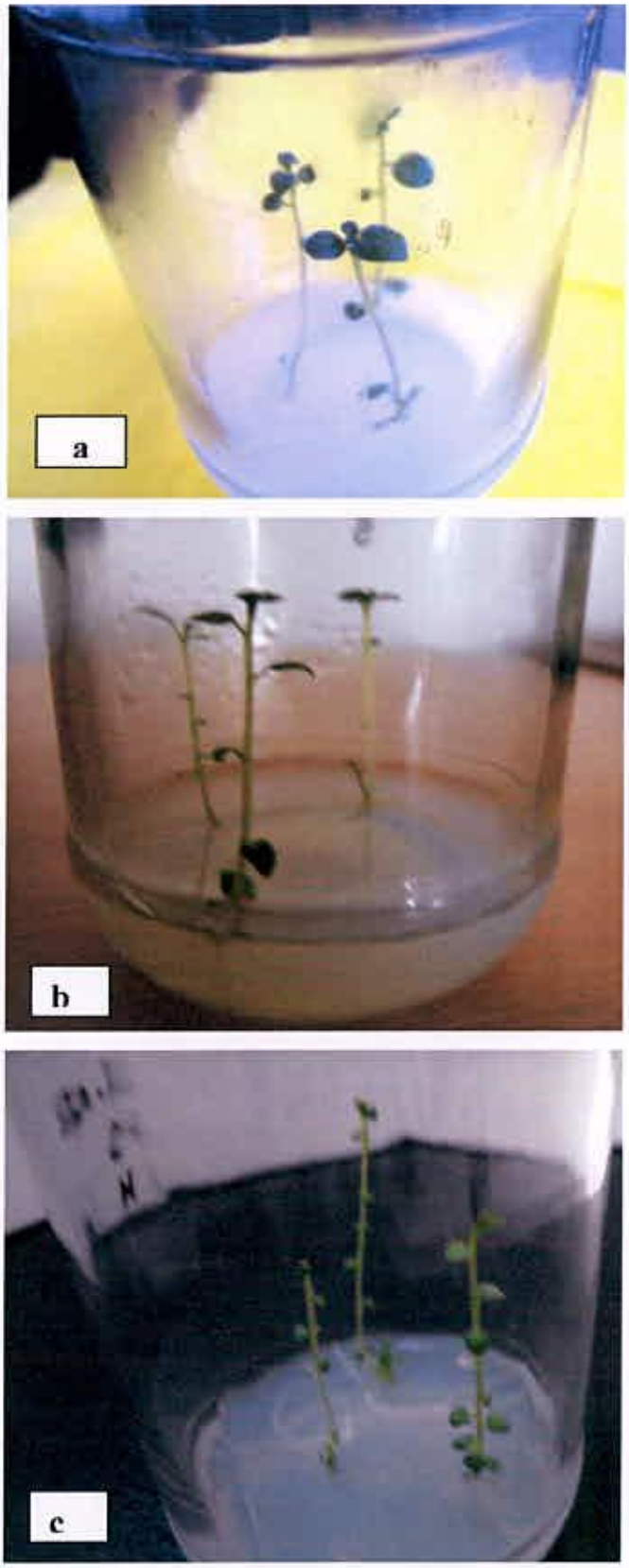


Plate.3.1 Plants regenerated from three explants used in a culture vial in subculture-II (a) Diamant (b) Cardinal and (c) Asterix

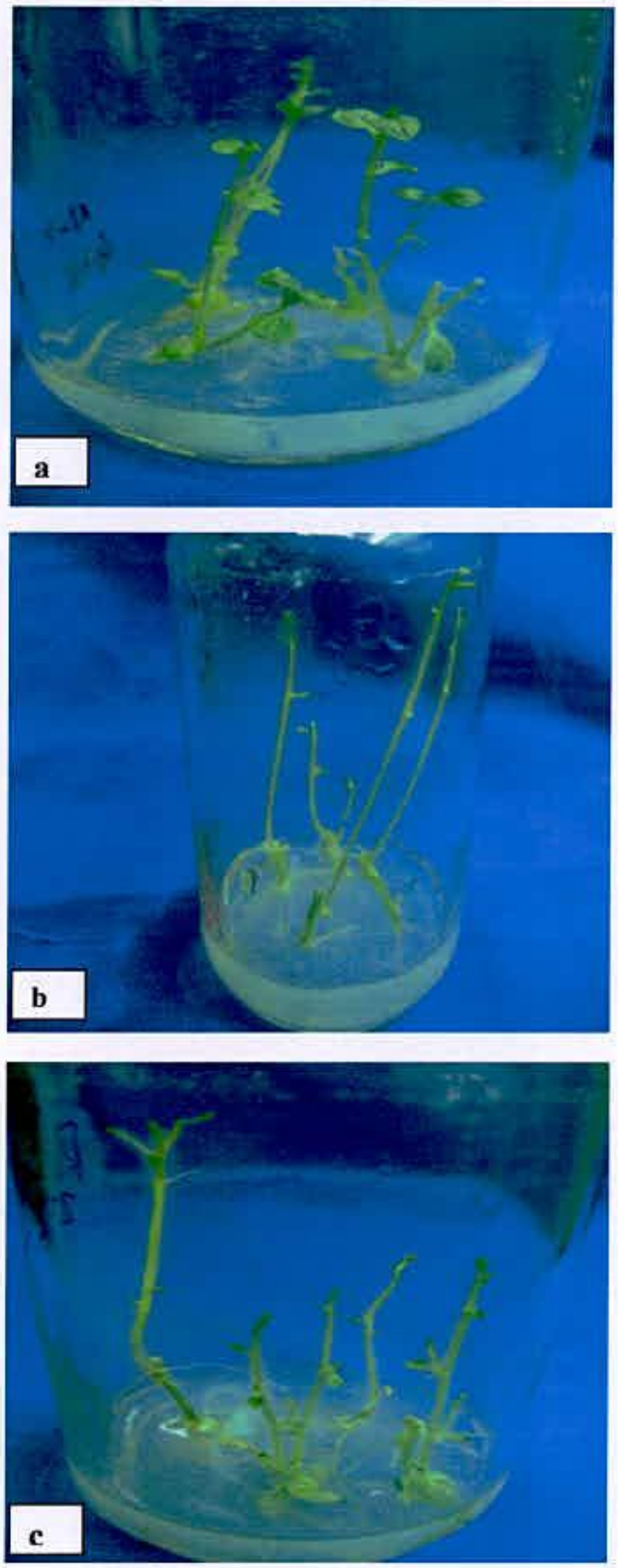


Plate.4. Plants regenerated from four explants used in a culture vial in subculture-III (a) Diamant (b) Cardinal and (c) Asterix

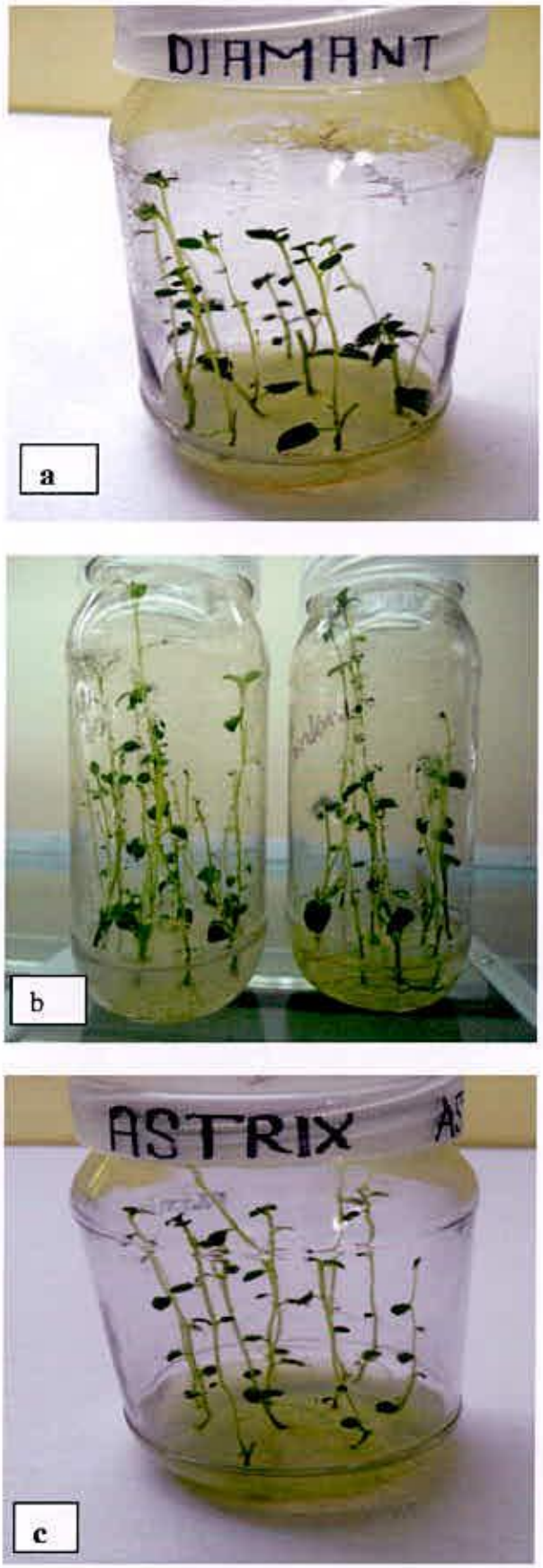


Plate.5. Plants regenerated from eight explants used in a culture vial in subculture-IV (a) Diamant (b) Cardinal and (c) Asterix

4.1.2. Length of internode (cm)

Length of internode of potato plantlet varied significantly with different varieties under the present study (Table 2.). The highest length of internode (1.67 cm) was recorded from Diamant which was closely followed (1.37 cm) by Cardinal, while the lowest length of internode (1.18 cm) was observed from Asterix.

Statistically significant variation was recorded for number of explants on length of internode (Table 2.). The highest length of internode (1.48 cm) was found from 2 explants (plate.2.) used in culture vial which was statistically similar (1.43 cm) and 1.38 cm) to 3 explants (plate.3.) in a vial.The lowest length of internode was found (1.33cm) when 8 explants (plate.5.) used in culture vial.

Interaction effect of different varieties and number of explants showed significant differences on length of internode (Table 3.). The highest length of internode (1.86 cm) was found from Diamant variety when 2 explants (plate.2.) were used in culture vial, while the lowest length of internode (1.03 cm) was found from Asterix variety when 4 explants (plate.4.) were used in culture vial.

4.1.3. Length of shoot (cm)

Length of shoot of potato plantlet varied significantly among the varieties under studty (Table 2.). The highest length of shoot (5.19 cm) was recorded from Diamant, while the lowest length of shoot (4.53 cm) was observed from Axterix which was statistically similar (4.75 cm) to Cardinal variety.

Statistically significant variation was also observed when number of explants variedy in different treatments for the parameter length of shoot. The highest length of shoot (5.29 cm) was found when 8 explants (plate.5.) per culture vial which was statistically similar (5.07 cm) to 4 explants (plate.4.) used in culture vial, while the lowest length of shoot (4.19 cm) was found from 3 explants (plate.3.) used in a culture vial.

Interaction effect of different varieties and number of explants showed significant differences on length of shoot (Table 3.). The highest length of shoot (6.00 cm) was found from Diamant variety when 8 explants (plate.5.) were used in culture

vial, while the lowest length of shoot (4.10 cm) was found in both Cardinal and Asterix variety with 3 explants in each (plate.3.) culture vial.

4.1.4. Diameter of shoot (mm)

Diameter of shoot of plantlets varied significantly for different varieties under the present trial (Table 2.). The highest diameter of shoot (1.16 mm) was recorded from Asterix, while the lowest diameter of shoot (0.82 mm) was observed from Diamant which was closely followed (0.97 mm) by Cardinal.

Statistically significant variation was recorded for number of explants on diameter of shoot (Table 2.). The highest diameter of shoot (1.07 mm) was found from 2 explants (plate.2.) per culture vial which was statistically similar (1.00 mm) to 3 explants (plate.3.) used in culture vial, while the lowest diameter of shoot (0.90 mm) was recorded from 4 explants (plate.4.) used in culture vial.

Interaction effect of different varieties and number of explants showed significant differences on diameter of shoot (Table 3.). The highest diameter of shoot (1.20 mm) was found in both Diamant and Asterix varieties when 8 explants (plate.5.) and 4 explants (plate.4.) were used in each culture vial, while the lowest diameter of shoot (0.70 mm) was found in Diamant variety when 2 explants (plate.2.) used in culture vial.

4.1.5. Number of branch/plantlet

Number of branch/plantlet under *in vitro* condition varied significantly within varieties (Table 2.). The maximum branch/plantlet (1.42) was recorded in Cardinal, which was statistically similar (1.08) in Diamant, while the minimum number of branch/plantlet (0.83) was observed from Asterix.

Number of branch/plantlet varied significantly when number of explants changed in a culture vial (Table 2.). The highest number of branch/plantlet (1.56) was found from 8 explants (plate.5.) used in culture vial which was statistically similar (1.11) to 4 explants (plate.4.) used in culture vial, while the lowest number of branch/plantlet (0.78) was recorded from 2 explants (plate.2.) used in a vial.

Interaction effect of different varieties and number of explants showed significant differences on number of branch/plantlet (Table 2.). The highest number of branch/plantlet (2.00) was found from Cardinal variety when 4 explants used in culture vial, while the lowest number of branch/plant (0.67) was found in Asterix variety using 2, 3 or 4 explants (plate .2.,plate .3. and plate .4.) in culture vials.

4.1.6. Number of leaf/plantlet

Number of leaf/plantlet of potato varieties under study varied significantly among each other (Table 2.). The highest number of leaf/plantlet (8.25) was recorded from Cardinal, while the lowest number of leaf/plant (5.92) was observed from Asterix which was statistically similar (6.50) to that of Diamant.

Statistically significant variation was recorded for number of explant per vial on number of leaf/plantlet (Table 2.). The highest number of leaf/plantlet (8.00) was found from 8 explants (plate.5.) in culture vial followed (7.11) by 3 explants (plate.3.) used in culture vial, while the lowest number of leaves/plant (5.44) was recorded from 4 explants (plate.4.) used in a vial.

Variety and number of explants interaction also showed significant differences on number of leaf/plantlet (Table 3.). The highest number of leaf/plant (9.00) was found in Cardinal variety when 8 explants (plate.5.) were used in culture vial, while the lowest number of leaf/plant (4.33) was found from Asterix variety with 4 explant (plate.4.) in each vial.

4.1.7. Length of the largest leaf (cm)

Length of the largest leaf among the potato verities under laboratory condition varied significantly among them (Table 2.). The highest length of largest leaf (1.28 cm) was recorded from Diamant, while the lowest leaf length (0.79 cm) was observed from Asterix which was followed (1.00 cm) by Cardinal.

Statistically significant variation was recorded for number of explants on length of largest leaf (Table 2.). The largest leaf (1.13 cm) was found from 2 explants (plate 2) used in a culture vial which was statistically similar (1.11 cm) to 3 explants

(plate.3.) used in culture vial, while the lowest leaf length (0.87 cm) was recorded from 4 explants (plate.4.) used in culture vial.

Interaction effect of different variety and number of explants showed significant differences on length of largest leaf in laboratory condition (Table 3.). The largest leaf length (1.40 cm) was found in Diamant variety when 4 explants (plate.4.) used in culture vial, while the lowest leaf length (0.57 cm) was found in Asterix variety when 8 explants (plate.5.) used in culture vial.

In vitro propagation and rapid multiplication for large scale plantlet production in potato is being practiced now a days in our country. Many government and private organizations are producing huge number of plantlets in each cropping season for disease free quality seed tuber production. Among the potato varieties Diamant, Cardinal and Asterix are more popular to the farmers.

One objective of the present experiment is to study the relative performance of plantlet in respect to morphological traits such as number of internodes, length of internodes, length of shoot, diameter of shoot, number of branch/plantlet, number of leaf/plantlet and length of the largest leaves within three popular potato varieties (Diamant, Cardinal and Asterix). The present study revealed that, Diamant variety showed better performance in most of the parameters under study. The parameters number of internodes, length of shoot, diameter of shoot, number of branches are directly correlated to rapid multiplication of potato plantlet. Hence, it notices that Diamant variety is more suitable for large scale production of potato *in vitro*.

Another objective of the present study is to identify the density of plantlet in each culture vial for proper growth and development of robust plantlet. Same numbers of explants in each culture vial were used for all the varieties. The number of explants per culture vial started from 2 and it was gradually increased up to 8 explants per vial. It was observed that, when population density of each culture vial decreased than the length of shoot and number of internode increased. It indicates that, there is a negative correlation between number of explants and length of shoot. Varietal difference with same number of population in a culture vial in this regard was negligible.

In vitro regeneration of potato has been practiced last for more than 60 years in all over the world and huge review of literature are available for references. But most of the works were with callus induction, *in vitro* regeneration, effect of phytohormone on shoot and root induction etc. Regarding population density in one culture vial and its effects on plantlet production is very less. The study revealed that, population density or number of explants has great effect on growth and development of plantlet.

4.2. Experiment II: Acclimatization and morphological characterization of plantlet under *in vivo* condition.

After completion of four subculture, huge numbers of culture vial with plantlet were produced in the culture racks (plate.6.) which were transferred in natural condition for acclimatization.

Rate of survival of regenerated varieties after transplanting is presented in Table 4. and plate.7. Acclimatization efficiency of the regenerated variants was recorded under natural field condition.

Table 4. Survival rate of in vitro regenerated plantlets of three potato varieties.

Acclimatization	Variety	No. of transplanted plants	No of plant survives	Survival rate (%)
Initially small earthen pot at growth chamber	Diamant	36	30	83.33
	Cardinal	36	28	77.78
	Asterix	36	28	77.78
In natural field condition under netting.	Diamant	30	25	83.33
	Cardinal	28	20	71.43
	Asterix	28	20	71.43

Preparation of plot and transplantation:

Potting mixture containing garden soil and cow dung in the ratio 1:2 was mixed properly and autoclaved one hour in 121°C for 20 minutes at 1.061 kg/cm². After cooling soil mixture was taken into 10 cm pots for growing in the pots *in vivo* condition. When the plantlet became 5-8 cm in height with sufficient shoot and root system, they were taken out from the vials without damaging any root. Medium attached to the roots was gently washed out running tap water to prevent further microbial infection. The plantlets were then transplanted to pot containing potting mixture mentioned above and immediately covered with polythene bag to prevent desiccation. To reduce sudden shock the pots were kept in growth room for 7-15 days under controlled environment. After 2-3 days polythene bags were gradually perforated to exposed the plants in natural environment. The polythene bags were completely removed after 10-15 days when the plantlets appeared to be self sustainable. The highest survival rate found in Diamant and lowest in Cardinal and Asterix.

The *in vivo* regenerated plantlets of three potato varieties were acclimatized under *in vivo* condition. The morphological traits of each variety were studied and presented in the following subheading-

4.2.1. Plant height (cm)

Plant height of three potato varieties at 7, 14, 21, 28, 35 and 42 DAP varied significantly under field condition (Table 5.). At 7, 14, 21, 28, 35 and 42 DAP the tallest plants (8.12, 12.62, 16.59, 27.87, 37.63 and 46.69 cm) were recorded in Diamant variety which was statistically similar (8.01, 11.95, 16.35, 27.66, 36.53 and 45.39 cm, respectively) to Cardinal, whereas the shortest plant (7.39, 10.99, 15.46, 25.30, 34.12 and 42.82 cm, respectively) was observed in Asterix.

Table 5. Plant height of three potato varieties at different days after planting (DAP) in field condition

Sl. No.	DAP	Plant height (cm)		
		Diamant	Cardinal	Asterix
01.	07	8.12 a	8.01 a	7.39 b
02.	14	12.62 a	11.95 a	10.99 b
03.	21	16.59 a	16.35 a	15.46 b
04.	28	27.87 a	27.66 a	25.30 b
05.	35	37.63 a	36.53 a	34.12 b
06.	42	46.69 a	45.39 a	42.82 b

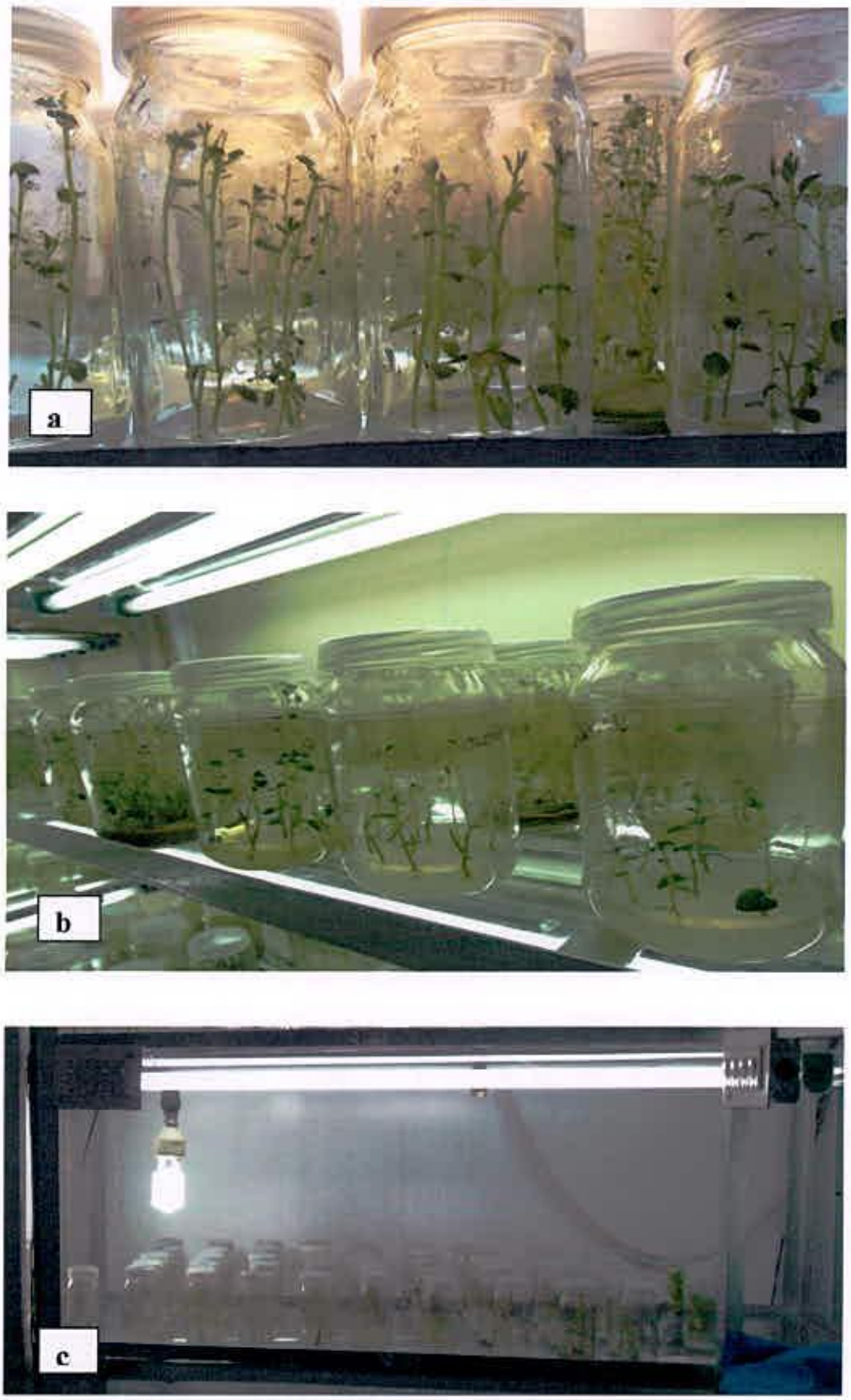


Plate.6. Large scale production of potato variety in culture rack (a) Diamant (b) Cardinal (c) Asterix

4.2.2. Diameter of stem(mm)

Diameter of stem among of potato at 7, 14, 21, 28, 35 and 42 DAP varied under *in vivo* condition (Table 6.). The highest diameter of stem 1.48, 3.28, 4.10, 4.97, 5.75 and 7.28 mm, respectively were recorded from variety Asterix at 7, 14, 21, 28, 35 and 42 days after planting (DAP), whereas the lowest diameter of stem 1.19, 2.55, 3.52, 4.77, 5.83 and6.72 mm, respectively were found from potato variety Diamant.

Table 6. Diameter of stem of three potato varieties at different days after planting (DAP) under field condition

Sl. No.	DAP	Diameter of stem (mm)		
		Diamant	Cardinal	Asterix
01.	07	1.19 c	1.40 b	1.48 a
02.	14	2.55 b	3.07 a	3.28 a
03.	21	3.52 c	3.85 b	4.10 a
04.	28	4.45 c	4.77 b	4.97 a
05.	35	5.83 a	5.47 b	5.75 a
06.	42	6.72 b	7.13 a	7.28 a

4.2.3. Number of branch/plant

Number of branch/plant of potato at 21, 28, 35 and 42 DAP varied significantly for different variety in field condition (Table 7.). At 21, 28, 35 and 42 DAP the highest number of branches/plant (1.45, 3.45, 5.02 and 5.60, respectively) was

recorded from Diamant which was statistically similar (1.43, 3.33, 4.87 and 5.35, respectively) to Cardinal, whereas the lowest diameter of stem (1.13, 3.05, 4.40 and 4.52, respectively) was found from Asterix.

Table 7. Number of branch/plant three potato varieties at different days after planting (DAP) under field condition

Sl. No.	DAP	Number of branch/plant		
		Diamant	Cardinal	Asterix
01.	21	1.45 a	1.43 a	1.13 b
02.	28	3.45 a	3.33 a	3.05 b
03.	35	5.02 a	4.87 a	4.40 b
04.	42	5.60 a	5.35 a	4.52 b

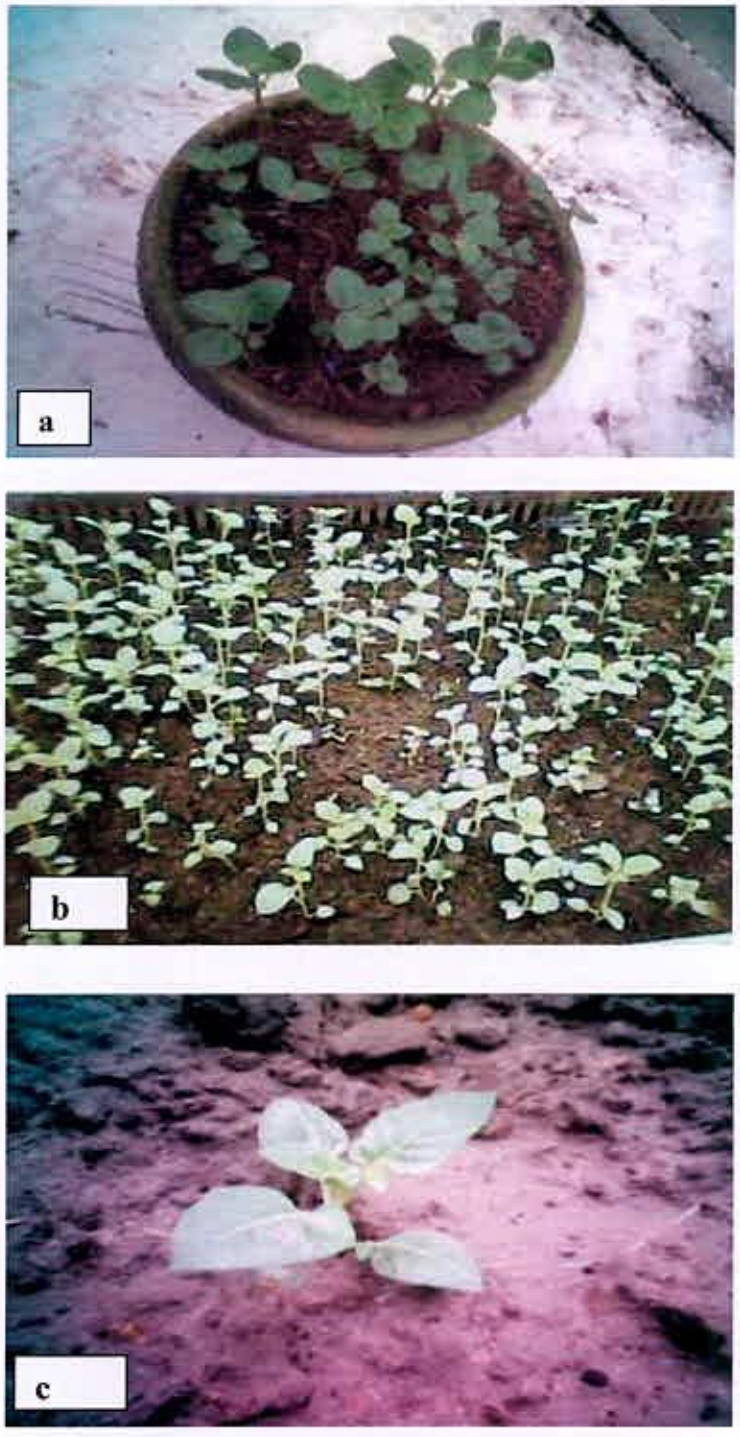


Plate.7. Acclimatization of plantlet

(a) In earthen pot

(b) In seed bed

(c) In main field

4.2.4. Number of leaf/plant

Number of leaf/plant of potato at 7, 14, 21, 28, 35 and 42 DAP varied significantly for different varieties (Table 8.). At 7, 14, 21, 28, 35 and 42 DAP the highest number of leaves/plant (5.65, 8.27, 15.72, 24.00, 30.85 and 39.30, respectively) was recorded from potato variety Cardinal which was statistically similar (5.37, 7.95, 14.95, 23.67, 29.40 and 35.87, respectively) to Diamant, whereas the lowest number of leaves/plant (3.93, 6.22, 13.50, 22.53, 27.68 and 30.65, respectively) was found from Asterix.

Table 8. Number of leaf/plant of three potato varieties at different days after planting (DAP) under field condition

Sl. No.	DAP	Number of leaf/plant		
		Diamant	Cardinal	Asterix
01.	07	5.37 a	5.65 a	3.93 b
02.	14	7.95 a	8.27 a	6.22 b
03.	21	14.95 b	15.72 a	13.50 c
04.	24	23.67 a	24.00 a	22.53 b
05.	35	29.40 b	30.85 a	27.68 c
06.	42	35.87 b	39.30 a	30.65 c

Morphology of mini tuber

The *in vitro* derived plantlets were cultivated under natural condition. All intercultural operation was successfully done for production of minituber from platlets. The minitubers of different varieties were harvested in time. Data were recorded for yield contributing traits and it is presented in the following sub-headings:

4.2.5. Length (cm) and diameter (mm) of minituber

Length of minituber varied significantly for different varieties (Table 9.). The highest length of tuber (4.56 cm) was recorded from Diamant which was closely followed (4.42 cm) by Cardinal, whereas the lowest length of tuber (4.02 cm) was found from Asterix.

Diameter of minituber varied significantly for different varieties in field condition (Table 9.). The highest diameter of tuber (3.07 cm) was recorded from Diamant which was closely followed (3.02 cm) by Cardinal, whereas the lowest diameter of tuber (2.83 cm) was found from Asterix.

4.2.6. Number of tubers/plant

Number of minituber per plant varied significantly in different varieties under study (Table 9.). The highest number of minituber (11.16) was recorded from Diamant which was followed (8.96) by Cardinal, whereas the lowest number of minituber (7.91) was found from Asterix.

4.2.7. Weight of tuber

Average weight of minituber varied significantly for different varieties in field condition (Table 9.). The maximum weight of tuber (10.23 g) was recorded from Diamant which was statistically similar (9.22 g) by Cardinal, whereas the lowest average weight of tuber (8.15 g) was found from Asterix.

Table 9. Yield contributing characters and yield of three potato varieties under field condition

Treatment	Length of tuber (cm)	Diameter of tuber (cm)	Number of tubers/plant	Weight of tuber (g)	Weight of largest tubers (g)	Yield (g/plant)
Diamant	4.56 a	3.07 a	11.16 a	10.23 a	35.28 a	114.16 a
Cardinal	4.42 b	3.02 a	8.96 b	9.22 a	32.85 a	82.05 b
Asterix	4.02 c	2.83 b	7.91 b	8.15 b	28.29 b	64.46 c
$LSD_{(0.05)}$	0.080	0.100	0.500	1.082	3.344	4.19
Level of significance	0.01	0.01	0.01	0.01	0.01	0.01
CV(%)	4.89	5.88	6.81	7.54	7.67	4.50

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly at 0.05 level of probability

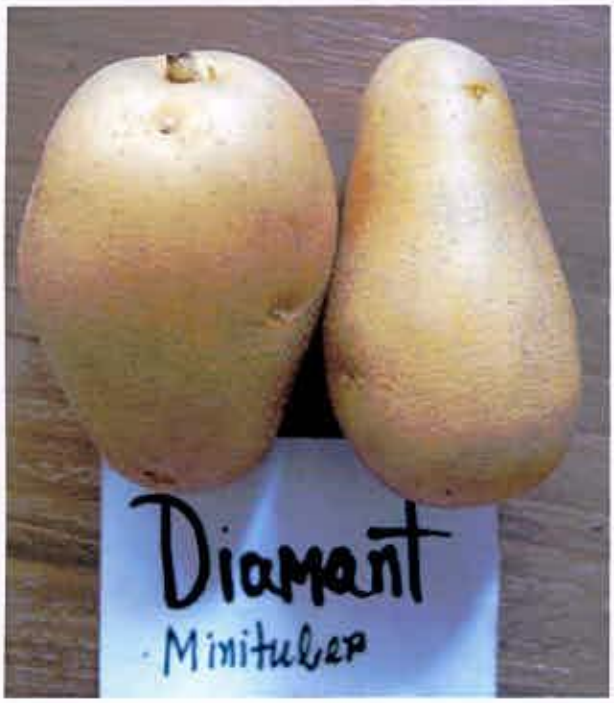


Plate.8. Potato mini tubers

4.2.8. Maximum weight of tuber

Maximum weight of tuber per variety varied significantly in field condition (Table 9.). The maximum size of tuber (35.28 g) was recorded from Diamant which was statistically similar (32.85 g) to Cardinal, whereas the minimum size of tuber (28.29 g) was found from Asterix (plate.8.).

4.2.9. Yield/plant

Yield per plant varied significantly for different varieties in field condition (Table 9.). The highest yield/plant (114.16 g) was recorded from Diamant which was closely followed (82.05 g) by Cardinal, whereas the lowest yield/plant (64.46 g) was found from Asterix.

In vitro regenerated plantlets were able to produce minituber. The yield performance among the varities revealed that, Diamant variety showed highest yield in respect of mintuber production.

CHAPTER-V

SUMMARY

CHAPTER V

SUMMARY

The experiment was conducted at the Biotechnology Laboratory and Research farm of Sher-e-Bangla Agricultural University (SAU), Dhaka during the period from April 2015 to March 2016 to study the morphological characterization of potato varieties under *in vitro* and *in vivo* condition. Data on different growth parameters in laboratory conditions and as well as in field condition were recorded. The key findings are given below.

The maximum number of internode (6.58) was recorded from Diamant, whereas the minimum number (5.08) from Asterix. The highest length internode (1.67 cm) was recorded from Diamant, while the lowest length (1.18 cm) was found in Asterix. The maximum number of branch/plant (1.42) was recorded in Diamant, while the minimum number (0.83) from Asterix.

The maximum number of internode (6.33) was found from the treatment which used 3 explants per vial. While the minimum number (5.33) was recorded when 2 explants were used in each culture vial. The maximum number of branch/plant (1.56) was found from 8 explants used in a culture vial, while the lowest number (0.78) was recorded from 2 explants used in a culture vial. The highest number of leaf/plant (8.00) was found from 8 explants used in a culture vial, while the lowest number (5.44) from 4 explants used in a culture vial.

Due to the interaction effect of different varieties and number of explants revealed that, the maximum number of internode (7.00) was found in Diamant variety when 3 explants used in a culture vial. The highest length of shoot (6.00 cm) was found from Diamant variety when 8 explants were used in a culture vial, while the lowest length (4.10 cm) from Cardinal variety when 3 explants were used in a culture vial. The highest diameter of shoot (1.20 mm) was found from Diamant variety when 2 explants were used in a culture vial, while the lowest diameter length (0.70 mm) from Asterix variety when 4 explants were used in a culture

vial. The highest number of branch/plant (2.00) was found from Diamant variety when 4 explants were used in a culture vial, while the lowest number (0.67) from Cardinal variety when 2 explants were used in a culture vial.

The tallest plant height (8.12, 12.62, 16.59, 27.87, 37.63 and 46.69 cm) was recorded at 7, 14, 21, 28, 35 and 42 DAP from Diamant, whereas the shortest plant (7.39, 10.99, 15.46, 25.30, 34.12 and 42.82 cm, respectively) was observed from Asterix. The highest length of tuber (4.56 cm) was recorded from Diamant, whereas the lowest length of tuber (4.02 cm) was found from Asterix. The maximum number of tuber (11.16) was recorded from Diamant, whereas the minimum number of tuber (7.91) was found from Asterix. The highest weight of tuber (10.23 g) was recorded from Diamant, whereas the lowest weight of tuber (8.15 g) was found from Asterix. The highest yield/plant (114.16 g) was recorded from Diamant, whereas the lowest yield/plant (64.46 g) was found from Asterix.

CHAPTER VI

CONCLUSION

CHAPTER VI

CONCLUSION

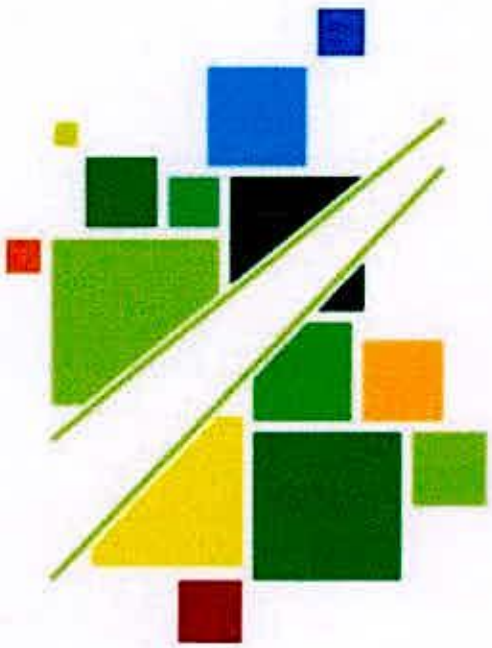
Based on the results and discussion it can be concluded as followed:

1. The variety Diamant showed better performance on most of the morphological traits under *in vitro* condition.

2. Number of explants per culture vial was negatively correlated with length of shoot, branches of plantlet and number of internodes.

3. Morphological parameters of *in vitro* regenerated plantlet were studied under field condition. Revealing that Diamant variety showed better performance in respect of yield contributing traits.

4. Number and weight of minituber per plant was also the highest in Diamant variety.



CHAPTER-VII

RECOMMENDATION

CHAPTER VII

RECOMMENDATION

The present investigation revealed that Diamant variety showed better performance under *in vitro* and *in vivo* condition. Further research may be carried out on the following mentioned points:

1. More potato varieties can be studied for *in vitro* regeneration following present research method.
2. Different auxin and cytokinin group of hormone can be used for more rapid multiplication focusing on explants number variation.
3. Population density per culture vial can be practiced with different number of explants.
4. More specific parameters for the effect of number of explants and duration of subculture can be studied.

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APPENDICES

APPENDICES

Appendix I: Composition of MS medium (Murashige and Skoog, 1962)

Components	Amount per liter (g)
Macronutrients	
KNO_3	1.900
NH_4NO_3	1.6500
$MgSO_4$	0.370
$CaCl_2.2H_2O$	0.440
KH_2PO_4	0.170
Micronutrients	**Amount per liter (mg)**
$MnSO_4.4H_2O$	22.3
H_3BO_3	6.2
$ZnSO_4.7H_2O$	8.6
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
KI	0.83
Iron Source	**Amount per liter (mg)**
$FeSO_4.7H_2O$	27.8
$Na_2EDTA.2H_2O$	37.3
Vitamins	**Amount per liter (mg)**
Nicotinic Acid	0.5
Pyridoxine HCl	0.5
Thiamin HCl	0.1
Glycine	2.0
Myo inositol	100 mg
Sucrose	30g
Agar	9g

Appendix II. Monthly record of air temperature, relative humidity, rainfall and sunshine hour of the experimental site during the period from June 2015 to March 2016

Month	*Air temperature (°c)		*Relative humidity (%)	Total Rainfall (mm)	*Sunshine (hr)
	Maximum	Minimum			
November, 2015	25.8	16.0	78	00	6.8
December, 2015	22.4	13.5	74	00	6.3
January, 2016	24.5	12.4	68	00	5.7
February, 2016	27.1	16.7	67	30	6.7
March, 2016	28.1	19.5	68	00	6.8

* Monthly average,

* Source: Bangladesh Meteorological Department (Climate & weather division) Agargoan, Dhaka – 1212

Appendix III. Analysis of variance of the data on morphological parameters of potato under laboratory condition as influenced by different variety and number of explants

Source of variation	Degrees of freedom	Mean square						
		Number of internode	Length of internode (cm)	Length of shoot (cm)	Diameter of shoot (mm)	Number of branches/ plant	Number of leaves/ plant	Length of largest leaf (cm)
Variety (A)	2	7.750**	0.740**	1.351**	0.352**	1.028*	17.694**	0.731**
Number of explants (B)	3	1.852**	0.039*	2.049**	0.045**	0.963*	10.148**	0.137**
Interaction (A×B)	6	2.046*	0.035*	0.159*	0.148**	0.769*	2.565*	0.049*
Error	24	0.278	0.011	0.084	0.009	0.250	0.556	0.020

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Appendix IV. Analysis of variance of the data on plant height of potato at different days after planting (DAP) under field condition as influenced by different variety and number of explants

Source of variation	Degrees of freedom	Mean square					
		Plant height (cm) at					
		7 DAP	14 DAP	21 DAP	28 DAP	35 DAP	42 DAP
Replication	2	0.004	0.138	0.461	2.543	0.186	0.495
Variety (A)	2	1.844**	8.091**	4.230**	24.380**	38.817**	46.630**
Number of explants (B)	3	3.109**	2.323*	3.626**	22.841**	43.590**	184.23 **
Interaction (A×B)	6	0.629**	2.529**	2.139*	7.087*	7.107*	14.722*
Error	22	0.188	0.730	0.670	2.808	2.774	5.511

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Appendix V. Analysis of variance of the data on diameter of stem of potato at different days after planting (DAP) under field condition as influenced by different variety and number of explants

Source of variation	Degrees of freedom	Mean square					
		Diameter of stem (mm) at					
		7 DAP	14 DAP	21 DAP	28 DAP	35 DAP	42 DAP
Replication	2	0.001	0.010	0.001	0.028	0.013	0.058
Variety (A)	2	0.285**	2.816**	1.135**	2.416**	2.158**	9.591**
Number of explants (B)	3	0.262**	1.703**	1.028**	0.814**	0.443**	1.034**
Interaction (A×B)	6	0.010*	0.417*	1.029**	0.607*	0.534*	0.168*
Error	22	0.003	0.137	0.085	0.033	0.069	0.118

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Appendix VI. Analysis of variance of the data on number of branches/plant of potato at different days after planting (DAP) under field condition as influenced by different variety and number of explants

Source of variation	Degrees of freedom	Mean square			
		Number of branches/plant at			
		21 DAP	28 DAP	35 DAP	42 DAP
Replication	2	0.001	0.001	0.021	0.088
Variety (A)	2	0.381**	0.508**	1.241**	3.861**
Number of explants (B)	3	0.363**	1.447**	1.213**	1.070**
Interaction (A×B)	6	0.031*	0.151**	0.142*	0.740*
Error	22	0.012	0.033	0.059	0.108

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Appendix VII. Analysis of variance of the data on number of leaves/plant of potato at different days after planting (DAP) under field condition as influenced by different variety and number of explants

Source of variation	Degrees of freedom	Mean square					
		Number of leaves/plant at					
		7 DAP	14 DAP	21 DAP	28 DAP	35 DAP	42 DAP
Replication	2	0.194	0.028	0.194	0.528	3.528	0.486
Variety (A)	2	17.444**	13.528**	11.444**	41.444**	46.528**	11.216**
Number of explants (B)	3	22.889**	21.880**	9.963**	15.741**	15.287**	26.166**
Interaction (A×B)	6	0.556*	4.491**	11.741**	4.630*	5.565*	4.816*
Error	22	0.194	0.755	1.073	1.710	1.710	1.828

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Appendix VIII. Analysis of variance of the data on number of leaves/plant of potato at different days after planting (DAP) under field condition as influenced by different variety and number of explants

Source of variation	Degrees of freedom	Mean square					
		Average length of tuber (cm)	Average diameter of tuber (cm)	Average number of tubers/plant	Average weight of tuber (g)	Weight of maximum size tubers (g)	Yield (g/plant)
Replication	2	0.002	0.001	0.076	0.004	15.502	175.764
Variety (A)	2	0.950**	0.194**	5.410**	30.884**	242.298**	7826.202**
Number of explants (B)	3	0.993**	0.254**	4.094**	7.903**	116.999**	5765.003**
Interaction (A×B)	6	0.082**	0.038*	0.886*	4.818*	65.983**	745.444*
Error	22	0.009	0.014	0.349	1.632	15.601	280.880

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability